Phase 1, Dose-Escalating, Randomized, Comparator-Controlled Trial of the Safety, Tolerability, and Immunogenicity of the Co-administration of Transmission-Blocking Vaccines (R0.6C-AlOH/ Matrix-M™ with Pfs230D1-EPA/Matrix-M™) and individual comparators (R0.6C-AlOH/ Matrix-M™ ; ProC6C-AlOH/ Matrix-M™ and Pfs230D1-EPA/Matrix-M™) against *Plasmodium falciparum* in Adults in Mali (TBVax2)

FMPOS Protocol Number:
FMPOS Project Federalwide Assurance (FWA): #00001769

Conducted by:
Laboratory of Malaria Immunology and Vaccinology (LMIV)
National Institute of Allergy and Infectious Diseases (NIAID)
National Institutes of Health (NIH)
And
Statens Serum Institute (SSI)
and
Faculté de Médecine Pharmacie d'Odonto Stomatologie (FMPOS)
University of Sciences, Techniques, & Technologies of Bamako (USTTB)

Principal Investigator:
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CONFIDENTIAL

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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'Odonto Stomatologie
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
MAAEs	Medically Attended Adverse Events
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
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
PIMMCs	Potentially Immune-Mediated Medical Conditions
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	thick blood smear
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 Synopsis

Full Title:	Phase 1, Dose-Escalating, Randomized, Comparator-Controlled Trial of the Safety, Tolerability, and Immunogenicity of the Co-administration of Transmission-Blocking Vaccines (R0.6C-AlOH/ Matrix-M™ with Pfs230D1 EPA/Matrix-M™) and individual comparators (R0.6C-AlOH/ Matrix-M™; ProC6C-AlOH/ Matrix-M™ and Pfs230D1 EPA/Matrix-M™) against Plasmodium falciparum in Adults in Mali (TBVax2)
Short Title:	TBV Antigen Study in Mali (TBVax2)
Clinical Phase:	Phase 1
Clinical Sponsor:	USTTB, Bamako, Mali
Conducted by:	MRTC, in collaboration with LMIV and SSI
Supported by:	PfTBV European & Developing Countries Clinical Trials Partnership (EDCTP) Consortium (University of Bamako)
Principal Investigator:	MRTC: Abdoulaye Katile, MD, MSPH(MRTC/DEAP/FMPOS)
Study Agent Description:	**R0.6C-AlOH/Matrix-M™:** Recombinant Pfs48/45 6C domain fused with glutamate rich protein (GLURP, R0) and absorbed to Alhydrogel® and adjuvanted with Matrix-M **ProC6C/Matrix-M™:** Recombinant Pfs48/45 6C domain fused with Pfs230-Pro domain using a Circumsporozoite protein linked and absorbed to Alhydrogel® and adjuvanted with Matrix-M **Pfs230D1-EPA/Matrix-M™:** Recombinant Pfs230 domain 1 (Pfs230D1M; a subdomain of a surface antigen of gametocytes, gametes, and zygotes, in the mosquito stage of *Plasmodium falciparum [Pf]*) conjugated to a recombinant *Pseudomonas aeruginosa* ExoProtein A (EPA) and adjuvanted with Matrix-M. **R0.6C-AlOH/Matrix-M™ Co-administered with Pfs230D1-EPA/Matrix-M™:** Co-administration of the individual R0.6C-AlOH/Matrix-M and Pfs230D1-EPA/MatrixM vaccines. **Verorab Rabies Vaccine:** One dose consists of the administration of 0.5 mL of vaccine via the intramuscular route and contains rabies virus, WISTAR Rabies PM/WI38 1503-3M strain (inactivated) ≥ 2.5 IU.
Subject Sample Size:	N=125-130
Accrual Ceiling:	N=260
Accrual Period:	Estimated July 2022 – September 2022

Study Duration:

Estimated Start Date: July 2022

Estimated End Date: July 2024 (includes data analysis)

Study participants will be enrolled for a total of approximately 12 to 15 months depending upon timing of screening

Study Population:

Healthy male and female adults (≥18 to ≤50 years of age) who reside in Doneguebougou and surrounding villages, Mali

Study Design:

This is a Phase 1, dose-escalating, randomized, comparator-controlled study to assess the safety, tolerability, immunogenicity and transmission-blocking activity (TBA) of a 3-dose regimen of Study Agents (four total) versus rabies vaccine in healthy adults. This will be a first-in-human assessment of the co-administration of R0.6C-AlOH/Matrix-M with Pfs230D1-EPA/Matrix-M. Participants will be randomized to one of the study arms. Participants will be followed for 12 months from the last dose of study vaccine for safety and tolerability, as well as immunogenicity and functional antibody responses.

The study groups and arms are as follows. For all participants in Arms 1 and 2 the assigned study vaccine will be administered at Days 1, 29, and 57. For participants in Arm 3 the study vaccine will be administered on Days 1, 29, and 169 (0, 1, and 6 months).

Group 1: Pilot Group

- **Arm 1a** (n=5): 30 µg ProC6C-AlOH/15 µg Matrix-M and normal saline (this arm to be enrolled only if safety has not already been demonstrated in ongoing first-in-human trial PACTR202201848463189)
- **Arm 1b** (n=5): 30 µg R0.6C-AlOH/15 µg Matrix-M co-administered with 12.5 µg Pfs230D1-EPA/25 µg Matrix-M
- **Arm 1c** (n=5): rabies vaccine (standard dose) and normal saline

Group 2: Main Group

- **Arm 2a** (n=20): 100 µg R0.6C-AlOH/50 µg Matrix-M and normal saline
- **Arm 2b** (n=20): 100 µg ProC6C-AlOH/50 µg Matrix-M and normal saline

- **Arm 2c** (n=20): 40 µg Pfs230D1-EPA/50 µg Matrix-M and normal saline (Pfs230D1-EPA regimen may be adjusted based on results of ongoing clinical trial NCT05135273)
- **Arm 2d** (n=20): 100 µg R0.6C-AlOH/25 µg Matrix-M co-administered with 40 µg Pfs230D1-EPA/25 µg Matrix-M (Pfs230D1-EPA regimen may be adjusted based on ongoing clinical trial PACTR202201848463189)
- **Arm 2e** (n=20): rabies vaccine (standard dose) and normal saline
- **Arm 3** (n=5-10): 40 µg Pfs230D1-EPA/50 µg Matrix-M given on a 0, 1, and 6 month schedule (Pfs230D1-EPA regimen may be adjusted based on results of ongoing clinical trial NCT05135273)

Study Objectives:

Primary Objective:

- To assess in African adults the safety and the reactogenicity of the co-administration of R0.6C-AlOH/Matrix-M™ and Pfs230D1-EPA/Matrix-M™ (first-in-human) as compared to the rabies vaccine control

Secondary Objectives:

- To assess the dynamics of transmission reducing activity in the standard membrane feeding assay of sera collected during and after Study Agent immunizations
- To assess the dynamics of Study Agent antibody quantities during and after Study Agent immunizations
- To assess in African adults the safety and the reactogenicity of R0.6C-AlOH/Matrix-M™, ProC6C/Matrix-M™, and Pfs230D1-EPA/Matrix-M™ immunizations as compared to the rabies vaccine control

Exploratory Objectives:

- To explore parasite, host genetics and functional antibody responses to Study Agents
- To assess differences in immunological responses (such as standard membrane feeding assays and antibody responses measured by ELISA) to Pfs230/Matrix-M given on a 0, 1 and 2

month schedule compared to a 0, 1, and 6 month schedule (Arm 3)

Study Endpoints:

Primary Endpoint:

- Incidence of serious adverse events and solicited grade 3 local and systemic adverse events (AEs) possibly, probably or definitely related to co-administered vaccinations in the period from first vaccinations up to 1 month after the last immunization.

Secondary Endpoints:

- The functional transmission reducing activity in the standard membrane feeding assay of volunteer sera collected two weeks after the third immunizations, compared to baseline within each of the Study Agent Groups
- The TRA at other timepoints (2 weeks after first and second immunizations and 4 months post third vaccination) compared to baseline (D0) in each of the Study Agent Groups
- The Study Agent antibody quantity in volunteer sera collected two weeks after each dose and at 4 months post dose 3) compared to baseline (D0) in each of the three dose-adjuvant combinations, as determined by ELISA.
- Incidence of adverse events possibly, probably or definitely related to any investigational vaccines

Exploratory Endpoints:

- Study Agent antibody decay rate following Study Agent Immunization
- Cellular immune responses and antibody repertoire of functional antibody responses to vaccination
- RNA transcriptome quantification as detected by RNA sequencing comparing vaccinees to controls
- Estimation of interaction between host factors including but not limited to hemoglobinopathies, immune signatures, co-infections, and environmental, demographic, and socioeconomic characteristics and primary and secondary endpoints

- Antibody quantity in volunteer sera collected two weeks after each dose and at 4 months post dose 3 compared to baseline by ELISA and functional transmission reducing activity in standard membrane feeding assays of volunteer sera collected two weeks after the third immunizations compared to baseline (Arm 3)

Cohort	Group	n= 115	Candidate vaccine dose and adjuvant combination
Pilot Phase	1A	n=5	3x 30µg ProC6C-AlOH+15µg Matrix-M and normal saline (Arm will only be included if safety not yet shown in prior clinical trial)
	1B	n=5	3X (co-administration) 30µg R0.6C-AlOH+15µg Matrix-M and 12.5µg Pfs230D1-EPA+25µg Matrix-M
	1C	n=5	3x Comparator Vaccine (Rabies) and normal saline
Main Phase	2A	n=20	3x 100µg R0.6C-AlOH+50µg Matrix-M and normal saline
	2B	n=20	3x 100µg ProC6C-AlOH+50µg Matrix-M and normal saline
	2C	n=20	3X 40µg Pfs230D1-EPA+50µg Matrix-M and normal saline (Pfs230D1-EPA regimen may be adjusted based on ongoing clinical trial NCT05135273)
	2D	n=20	3X (co-administration) 100µg R0.6C-AlOH+25µg Matrix-M and 40µg Pfs230D1-EPA+ 25µg Matrix-M (Pfs230D1-EPA regimen may be adjusted based on ongoing clinical trial Protocol #2021/000105/MS/SESRS/CERS registered at: www.pactr.org)
	2E	n=20	3x Comparator Vaccine (Rabies) and normal saline
	3	n=5-10	3X 40µg Pfs230D1-EPA+50µg Matrix-M given on a 0, 1, and 6 month schedule (Pfs230D1-EPA regimen may be adjusted based on ongoing clinical trial NCT05135273)

1 Background Information and Scientific Rationale

1.1 Background Information

According to the World Health Organization (WHO), progress in malaria control has recently stalled, with no reductions in the number of malaria cases worldwide in the past several years (WHO 2020). In 2019, there were 229 million cases and 409,000 deaths. 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 (Sachs and Malaney 2002). These factors—as well as growing drug resistance of the causative parasite *P. falciparum*, widespread resistance of mosquitoes to insecticides, 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 (Duffy and Gorres 2020)

Malaria is transmitted by *Anopheles* mosquitoes. **Erreur ! Source du renvoi introuvable.** shows the life cycle of the malaria parasite. During a blood meal from an infected person, the female mosquito ingests parasites, including gametocytes, the sexual forms of the parasite. Inside the mosquito midgut, male and female gametes fertilize to form a zygote, which further develops into an elongated ookinete form. The ookinete migrates to the outer surface of the midgut and develops into an oocyst, which undergoes multiple rounds of nuclear division to produce thousands of sporozoites that then migrate to the salivary glands. When the mosquito takes another blood meal, it injects sporozoites with its saliva, transmitting the malaria parasite to another person.

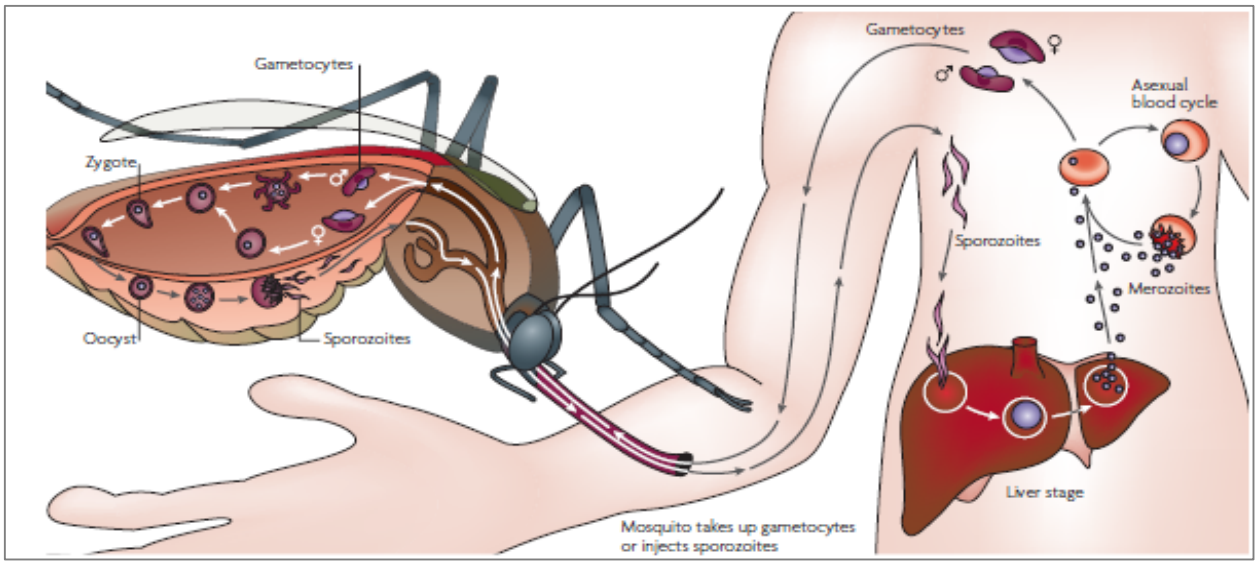


Figure 1: Life Cycle of Malaria Parasite Plasmodium ssp. (Su, Hayton et al. 2007)

Since transmission by mosquitoes is a biological bottleneck for malaria, measures to block transmission have been integral components of the malaria control strategy. 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 TBA in SMFAs (Kaushal, Carter et al. 1983, Williamson, Keister et al. 1995, MacDonald, Nguyen et al. 2016, Coelho, Tang et al. 2021). 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 (Williamson, Criscio et al. 1993). Malaria-exposed populations acquire antibody against Pfs230, which suggests that a Pfs230-based vaccine may be boosted by natural malaria infection.

The sexual stage Pfs48/45 antigen is a well-established lead candidate for a *P. falciparum* transmission blocking vaccine because of its critical role in parasite fertilisation. Male gametes lacking Pfs48/45 are unable to bind female gametes in the mosquito midgut, thus preventing ookinete, oocyst and ultimately sporozoite development. The R0.6C fusion protein is a chimera consisting of the 6-cysteine C-terminal fragment of PfS48/45 (6C) coupled to the N-terminal region of asexual stage Glutamate Rich Protein GLURP (R0) produced in *Lactococcus lactis*. Immunisation with R0.6C in rodents induces functional antibodies against the 6C subunit [7]. Anti-6C antibodies (Abs) are ingested during the blood meal and can bind male sexual forms in the mosquito gut, preventing their fertilisation of female gametes and thus ookinete and oocyst development. Sera of vaccinated animals were able to reduce transmission in the standard membrane feeding assay (SMFA) with cultured gametocytes. Anti-6C antibody titres were further increased by immunising with R0.6C adjuvanted with Alhydrogel or Alhydrogel and Matrix-M.

1.1.1 Development of the Four Study Agents

1.1.1.1 R0.6C-AlOH/Matrix-M™

The R0.6C fusion protein is a chimera consisting of the 6-cysteine C-terminal fragment of Pfs48/45 (6C) coupled to the N-terminal region of asexual stage Glutamate Rich Protein GLURP (R0) produced in *Lactococcus lactis* (Singh et al. 2017; Singh et al. 2015). The carrier protein R0 helps to enhance the immune response against Pfs48/45 (Theisen et al. 2014). The hybrid protein consists of $GLURP_{27–500}$ and $Pfs48/45_{291-428}$ regions, and the vector-encoded amino acid residues A-E-R-S at the N-terminal end. R0.6C and the position of the Pfs48/45-6C domain is shown in **Figure 2.** The cloning of R0.6C is described (Singh et al. 2017).

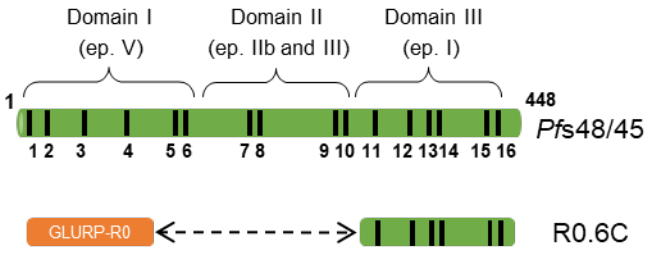


Figure 2: Schematic representation of Pfs45/48 and R0.6C

Figure2 shows the schematic representation of Pfs45/48 and the fragment cloned.

The Amino acid sequence of R0.6C recombinant protein is presented below.

AERSTSENRNKRIGGPKLRGNVTSNIKFPSDNKGKIIRGSNDKLNKNSEDVLEQSEKSL
VSENVPSGLDIDDIPKESIFIQEDQEGQTHSELNPETSEHSKDLNNNGSKNESSDIISEN
NKSNKVQNHFESLSDLELLENSSQDNLDKDTISTEPFPNQKHKDLQQDLNDEPLEPFP
TQIHKDYKEKNLINEEDSEPFPRQKHKKVDNHNEEKNVFHENGSANGNQGSLKLKSFD
EHLKDEKIENEPLVHENLSIPNDPIEQILNQPEQETNIQEQLYNEKQNVEEKQNSQIPSL
DLKEPTNEDILPNHNPLENIKQSESEINHVQDHALPKENIIDKLDNQKEHIDQSQHNINVL
QENNINNHQLEPQEKPNIESFEPKNIDSEIILPENVETEEIIDDVPSPKHSNHETFEEETS
ESEHEEAVSEKNAHETVEHEETVSQESNPEKADNDGNVSQNSNNELNENEFVESEKS
EHEARSEKKVIHGCNFSSNVSSKHTFTDSLDISLVDDSAHISCNVHLSEPKYNHLVGLN
CPGDIIPDCFFQVYQPESEELEPSNIVYLDSQINIGDIEYYEDAEGDDKIKLFGIVGSIPKT
TSFTCICKKDKKSAYMTVTIDSA

L. lactis signal peptide carryover

GLURP-R0

Pfs48/45-6C

Cloning sites

R0.6C has a deduced mass of approximately 70263.90 Da based on the amino acid sequence derived from the DNA sequence. The relative molecular mass is approximately 105 kDa as determined by SDS-PAGE, and by western blotting.

The correct folding of R0.6C is dependent on the formation of three disulfide bonds. The monoclonal antibody (mAb) 45.1 recognizes R0.6C when the *Pf*s48/45-6C domain of this hybrid protein adopts its native fold with respect to the cysteine connectivity.

1.1.1.2 ProC6C-AlOH/Matrix-M™

The ProC6C fusion protein is a chimera consisting of the 6-cysteine C-terminal fragment of PfS48/45 (6C) coupled to the Pro domain of Pfs230 produced in *Lactococcus lactis* (Singh et al. 2021, manuscript submitted). ProC6C is a recombinant hybrid protein derived from Plasmodium falciparum 3D7, Pfs230Pro (Pro) genetically coupled to *P. falciparum* Pfs48/45 (6C). The hybrid protein consists of the $Pro_{443–590}$ and $Pfs48/45_{291-428}$ regions joined by a linker region, $PfCSP_{105-140}$, and the vector-encoded amino acid residues A-E-R-S at the N-terminal end and a C-tag (E-P-E-A) in its C-terminus. ProC6C and the position of the Pfs48/45-6C domain is shown in Figure

3. The cloning of ProC6C is described below. **Figure 3** shows the schematic representation of ProC6C and its constituent antigens.

Figure 3: Schematic representation of ProC6C.

The Amino acid sequence of ProC6C recombinant protein is presented below.

AERSEYVDEKERQGEIYPFGDEEEKDEGGESFTYEKSEVDKTDLFKFIEGGEGDDVYK
VDGSKVLLDDDTISRVSKKHTARDGEYGEYGEAVEDGENVIKIIRSVLQSGALPSVGVD
ELDKIDLSYETTESGDTAVSEDSYDKYASNNTNKENANPNVDPNANPNVDPNANPNV
DPNANPNANPNANPEKKVIHGCNFSSNVSSKHTFTDSLDISLVDDSAHISCNVHLSEPK
YNHLVGLNCPGDIIPDCFFQVYQPESEELEPSNIVYLDSQINIGDIEYYEDAEGDDKIKLF
GIVGSIPKTTSFTCICKKDKKSAYMTVTIDSAEPEA

L. lactis signal peptide carryover

Cloning sites

Pfs230-Pro

Linker derived from PfCSP

Pfs48/45-6C

C-tag

ProC6C has a deduced mass of approximately 36315.33Da based on the amino acid sequence derived from the DNA sequence. The relative molecular mass is approximately 45 kDa as determined by SDS-PAGE, and by western blotting. The appearance of ProC6C at higher molecular weight during biochemical evaluation (Such as SDS-PAGE or SEC) is largely due to the intrinsically unstructured nature of the “Pro” Pfs230 domain.

The correct folding of ProC6C is dependent on the formation of three disulfide bonds. The monoclonal antibody (mAb) 45.1 recognizes ProC6C when the *Pf*s48/45-6C domain of this hybrid protein adopts its native fold with respect to the cysteine connectivity.

1.1.1.3 Pfs230D1-EPA/ Matrix-M™

The recombinant protein Pfs230D1M was developed at 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 (MacDonald, Nguyen et al. 2016). A comparative analysis with 11 Malian isolates shows two points 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 (**Erreur ! Source du renvoi introuvable.**4). These same mutations were also observed in an analysis of over 2,000 parasite isolates (MacDonald, Nguyen et al. 2016). In West Africa, the minor allelic frequency was reported to be 0.111 and 0.339 for G605S and K661N, respectively, shown in **Erreur ! Source du renvoi introuvable.**4. 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.

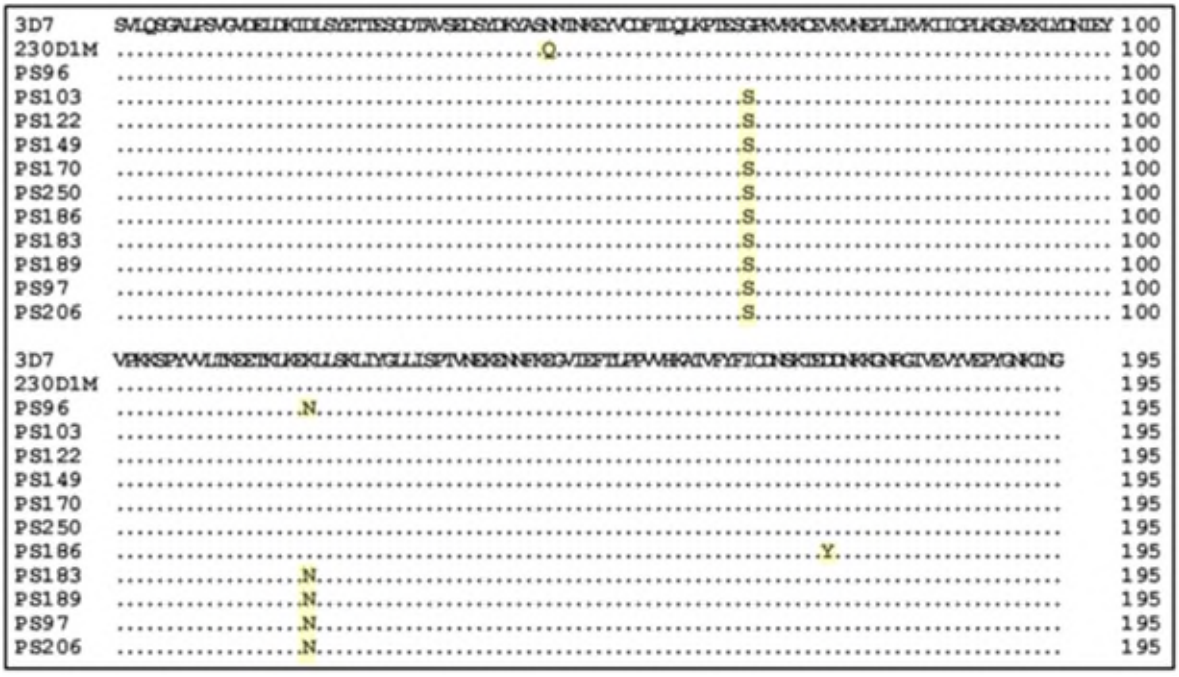


Figure 4: Protein Alignments of Recombinant Pfs230D1M to its Respective Native Protein or Protein Fragment.

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 (Farrance, Rhee et al. 2011, Tachibana, Wu et al. 2011). 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 (Lin, Ho et al. 2001, Passwell, Ashkenzi et al. 2010, Thiem, Lin et al. 2011) 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 NIAID protocol #15-I-0044 and was demonstrated to be safe and immunogenic both in malaria-naïve and malaria-exposed adults (see Section 3.13).

Pfs230D1M-EPA formulated in the more potent adjuvant AS01 has completed recent evaluation over two years in NIAID protocol #17-I-N006, with initial results supporting higher potency (ELISA titers and antibody activity) for AS01 (see Section 3.3.4). 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).

Pfs230D1-EPA formulated in the adjuvant Matrix-M™ is currently being evaluated in NIAID protocol #17-I-N006, in a Phase 1, dose-escalating, randomized, double-blind, comparator-controlled study in Mali with Matrix-M™.

1.1.1.4 R0.C6-AlOH/Matrix-M™ co-administered with Pfs230D1-EPA/ Matrix-M™

Given the R0.6C candidate encompasses the Pfs48/45 TBV target protein alone, and Pfs230D1 candidate encompasses the Pfs230 TBV protein alone, co-administration of the two individual products will be evaluated for added benefit and efficacy by eliciting antibodies to two transmission block proteins. Further this co-administration can be further compared to the ProC6C vaccine candidate, which includes a different region of Pfs230 (Pro-domain) than that of Pfs230D1 (D1 domain). The individual products (R0.6C-AlOH) and (Pfs230D1) will be handled as separate independent properties (without co-mixing or co-formulation) in this First in Human evaluation. Each individual product will be mixed with Matrix-M and administered to a separate immunization site (although same draining lymph node). Matrix-M doses will be adjusted in the individual formulations to provide a maximum dose of 50 ug total Matrix-M across the two products per volunteer, per dosing day.

1.2 Rationale for Trial and Study Design

Pfs230D1 has become one of the leading transmission-blocking antigens for consideration as a licensed TBV to be used either alone or in combination with other transmission-blocking antigens. Clinical trials with this antigen adjuvanted with either Alhydrogel or AS01 have provided very encouraging results (Section 3). With the execution of the recent age de-escalation trial with Pfs230D1M-EPA adjuvanted with AS01 (Section 3), it is clear that research efforts with this vaccine should proceed forward. Pfs230D1-EPA formulated with the Matrix-M

adjuvant is currently undergoing evaluation in Mali (NIAID Number is 000576 under IND27670).

Another lead TBV candidate R0.6C has shown good transmission reducing efficacy in rodent models. Also, a monoclonal antibody TB31F against the same Pfs48/46 6C region has shown excellent safety and transmission reducing activity in humans in a recent clinical trial (van der Boor et al, manuscript in preparation). Efficacious TBVs would be a critical step on the path towards malaria elimination and their safety and preliminary efficacy data can be acquired in malaria-endemic countries volunteers. The R0.6C-AlOH vaccine is currently undergoing Phase I evaluation in The Netherlands and Burkina Faso.

As background, the R0.6C fusion protein is a chimera consisting of the 6-cysteine C-terminal fragment of PfS48/45 (6C) coupled to the N-terminal region of asexual stage Glutamate Rich Protein GLURP (R0) produced in *Lactococcus lactis*. The sexual stage Pfs48/45 antigen is a well-established lead candidate for a *P. falciparum* TBV because of its critical role in parasite fertilization. Male gametes lacking Pfs48/45 are unable to bind female gametes in the mosquito midgut, thus preventing ookinete, oocyst, and ultimately sporozoite development. Immunization with R0.6C in rodents induces functional antibodies against the 6C subunit (Singh, Roeffen et al. 2017). Anti-6C antibodies are ingested during the blood meal and can bind male sexual forms in the mosquito gut, preventing their fertilization of female gametes and thus ookinete and oocyst development. Sera of vaccinated animals were able to reduce transmission in the SMFA with cultured gametocytes. Anti-6C antibody titres were further increased by immunizing with R0.6C adjuvanted with Alhydrogel or Alhydrogel and Matrix-M.

The other vaccine which will be tested alongside these vaccines will be a “chimera” vaccine known as PRO.C.6C, a recombinant Pfs48/45-Pfs230 fusion protein expressed in *L. lactis*, also manufactured by SSI and being tested in a “first-in-humans” trial during the second half of 2021. This chimera contains the pro-domain of Pfs230 (at the n-terminus), a 23 native amino acid linker potion of the circumsporozoite protein and the 48/45 antigen.
The ProC6C TBV candidate is a novel chimeric antigen that includes both Pfs230 and Pfs48/45 proteins, while also containing an efficacious CSP sequence which may provide infection inhibition to the immunized individual given previous preclinical trials (Ref). The ProC6C-AlOH vaccine is planned to start Phase I evaluation in Burkina Faso in March 2022.

Several factors have spurred the programmatic decision to proceed with a trial evaluating Pfs230D1-EPA in combination with Matrix-M™, rather than focusing the next trial on further assessment of Pfs230D1-EPA adjuvanted with AS01, as follows:

1. *Inaccessibility of AS01 as an adjuvant* – Although the combination of Pfs230D1M-EPA with AS01 has proven to be safe, well-tolerated, immunogenic and efficacious in terms of preventing transmission of *P. falciparum* in the field, the adjuvant is not available for combination with Pfs230D1M-EPA unless it is purchased on the open market. GlaxoSmithKline, the manufacturer of AS01, has declined any additional collaborations using AS01 in malaria vaccine trials pending the completion of trials that test new

regimens of their RTSS/AS01B malaria vaccine. Because of this inaccessibility, LMIV has had to seek an alternative adjuvant to combine with Pfs230D1M-EPA.

2. *Data from non-human primates indicating that Matrix-M has a similar immunogenicity profile to AS01 as an adjuvant for Pfs230D1M* – In recent pre-clinical studies conducted by Dr. David Narum and his laboratory team at LMIV in non-human primates, Matrix-M appeared to have a very similar immunogenicity profile to AS01. **Erreur ! Source du renvoi introuvable.**5 shows a comparison of rhesus antibody responses (IgG) by ELISA (A) and TRA by SMFA (B) following immunization with conjugated Pfs230D1-EPA in AS01 or Matrix-M. Groups of 6 non-human primates were immunized with 40 ug Pfs230D1-EPA/50 µg Matrix-M on days 0, 28, and 112. Multiple bleeds were performed through day 301 or 308 for AS01 or Matrix-M, respectively. No marked differences were observed in the IgG responses between AS01 and Matrix-M. The conjugated Pfs230D1M-EPA antisera were evaluated by SMFA in presence of human complement two weeks following the primary and secondary boost and at the end of the study, approximately 6 months following the final boost. AS01 and Matrix-M ELISA titers and % TRA were compared on days 42, 140 and 301/308 using a Wilcoxon test. No significant differences were observed (ELISAs days 42, 140 and 301/308, p = 0.48 for all; % TRA days 42, 140 and 301/308, p = 0.17, 0.86 and 0.81, respectively). Historical antibody responses by ELISA for a conjugated Pfs230D1-EPA formulated with Alhydrogel are also included (in panel A) for reference. The study with Alhydrogel as an adjuvant was performed independently but with the same procedures, a slight variation in immunization schedule (days 0, 28, and 168), and the final bleed at day 308.

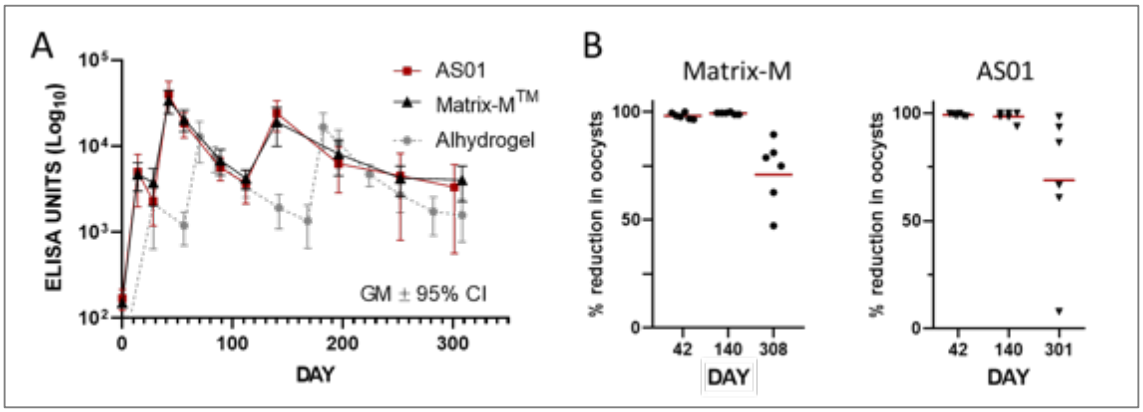


Figure 5: Comparison of Rhesus Antibody Responses (IgG) by ELISA (A) and TRA by SMFA (B) Following Immunization with Conjugated Pfs230D1-EPA in AS01 (GlaxoSmithKline) or Matrix-M™ (Novavax).

3. *Potential for antigen dose-sparing* – Immunization with Matrix-M has been shown to lead to increased numbers of CD169+ macrophages and activated dendritic cells (Magnusson, Altenburg et al. 2018) in draining lymph nodes, which may help to increase

antigen presentation. CD169+ macrophages have been shown to have a role in transporting antigens to B lymphocytes and facilitating cross-presentation of antigen to CD8+ T lymphocytes (Carrasco and Batista 2007, Gray and Cyster 2012). Subsequent generation of cross-reactive antibodies and multi-functional CD4+ T lymphocytes (Bengtsson, Song et al. 2016, Shinde, Cai et al. 2020) may lead to increased antibody and cellular responses with the potential for antigen dose-sparing. For this reason, the current clinical trial evaluating Pfs230D1-EPA/Matrix-M includes an arm with only 20 µg of Pfs230D1M in addition to the arm with 40 µg of Pfs230D1M.

Note on plan for Arms 1A, 2C, and 2D

The plan for several arms on this trial may be modified as follows based on information not currently available:

Arm 1A: 30µg ProC6C-AlOH+15µg Matrix-M:

A clinical trial that includes ProC6C-AlOH+ Matrix-M in Africa adults is planned to begin enrollments in March 2022 in Burkina Faso (PACTR202201848463189, see section 3.1). If safety results from this antigen/adjuvant regimen do not indicate any safety concerns in the judgment of the MRTC PI then the purpose of Arm 1A will be considered fulfilled and will not enroll.

Arm 2C: 40µg Pfs230D1-EPA+50µg Matrix-M

The dosing of Pfs230D1-EPA and/or Matrix M may be adjusted based on results from an ongoing clinical trial of this antigen/adjuvant combination in Malian adults (NCT05135273, see Section 3.6). If immunogenicity measured by ELISA titers warrant an adjustment to the dose of Pfs230D1-EPA and/or of Matrix-M in the judgement of the MRTC PI then Arm 2C will proceed with the determined dose, and the FMPOS EC will be notified of the adjustment in writing.

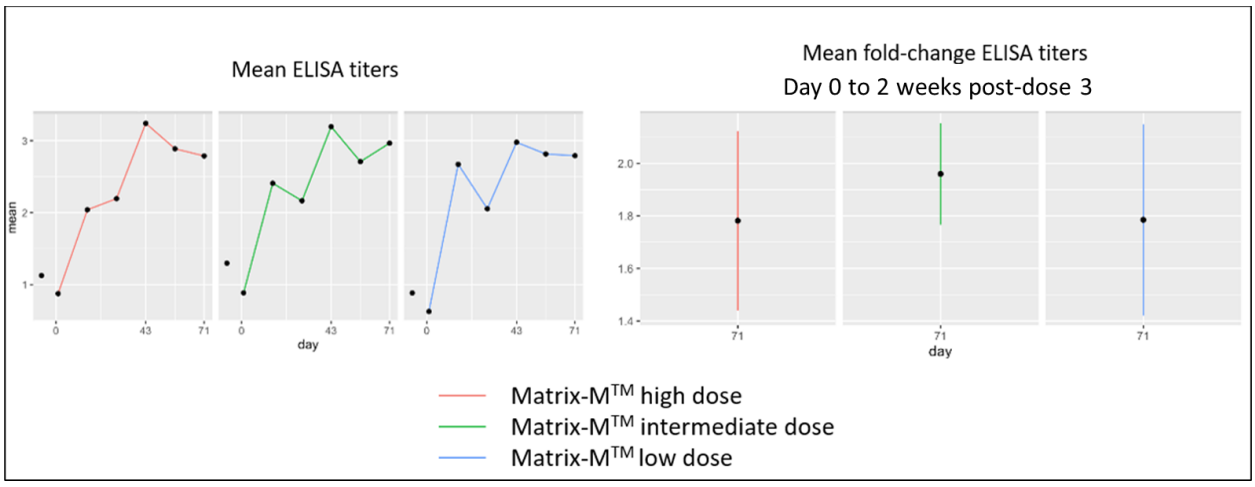
Arm 2D: co-administration of 100µg R0.6C-AlOH+25µg Matrix-M and 40µg Pfs230D1-EPA+ 25µg Matrix-M

Likewise, if results of the two ongoing trials mentioned above warrant adjustment to the Pfs230D1-EPA in the judgment of the PI then Arm 2D will proceed with an adjusted regimen and FMPOS will be notified in writing.

Note on plan for Arm 3 (extended schedule for Pfs230/Matrix-M)

Interim analyses of ELISA titers of an ongoing trial of several doses of Pfs230 paired with Matrix-M (NCT05135273, see Section 3.6 for more details) indicates that there is little to no effective change in mean titers post-dose 3 compared to post-dose 2 measured 2 weeks after each vaccination. This trial was conducted on a 0, 1, and 2 month dosing schedule, and it may be the case that additional time between the second and third vaccinations could facilitate a boosting effect. The trial is therefore being amended to

add a small cohort which will receive the Pfs230/Matrix-M alone (same dose as Arm 2c) administered on a 0, 1, and 6 month schedule rather than a 0, 1, and 2 month schedule to assess any differences in immunological responses evident between the two schedules.



The high dose arm is 40 ug of Pfs230 paired with Matrix-M, the intermediate dose arm is 20 ug, and low dose arm is 12.5 ug.

1.2.1 Study Plan

This is a Phase 1, dose-escalating, randomized, comparator-controlled study to assess the safety, tolerability, immunogenicity and transmission-blocking activity (TBA) of a 3-dose regimen of Study Agents (four total) versus rabies vaccine in healthy adults. This will be a first-in-human assessment of the co-administration of R0.6C-AlOH/Matrix-M with Pfs230D1-EPA/Matrix-M. Participants will be randomized to one of the study arms. Participants will be followed for 12 months from the last dose of study vaccine for safety and tolerability, as well as immunogenicity and functional antibody responses.

For the Pilot Groups, ProC6C-AlOH/Matrix-M and R0.6C-AlOH/Matrix-M co-administered will contain 30 µg and Pfs230D1-M/Matrix-M co-adminstered will contain 12.5 µg protein dosages (respectively) which correspond to previously evaluated independent Fractional/Low-dose administration of study agents (

). After the first injections to 5 participants, a report will be prepared for the Data Safety and Monitoring Board (DSMB) which will include trial safety data through 72 hours after the second injections in the pilot group. If the DSMB concurs, immunization of the Main Group can proceed with 20 subjects from each arm plus the rabies control subjects as shown in

. (

is provided as an example of what may occur.) A DSMB meeting will also occur after the first immunizations in the main phase. In order to lessen the chance for errors when handling different dosages and to ease the workload on the staff on any given day, we will attempt to divide the immunizations for the Main Group over 3 days. We will do the same for the second and final immunizations for the Main Group.

Table 1: Immunization schedule

	Day1	Day29		Day 57		28 days after Dose 1 for Main Group	56 (or 168) days after Dose 1 for Main Group
Low Dose: Pilot Phase 30µg R0.6C-AlOH+15µg Matrix-M and 12.5µg Pfs230D1-EPA+25µg Matrix-M	Dose 1	Dose 2	DSMB Meeting	Dose 3	DSMB Meeting		
Rabies Vaccine	Dose 1						
High Dose: Main Phase 100µg R0.6C-AlOH+50µg Matrix-M				Dose 1		Dose 2	Dose 3
100µg ProC6C-AlOH+50µg Matrix-M							
40µg Pfs230D1-EPA+50µg Matrix-M							
100µg R0.6C-AlOH+25µg Matrix-M and 40µg Pfs230D1-EPA+ 25µg Matrix-M							
Rabies Vaccine				Dose 1			

* **For simplicity, the table shows only the first of 3 doses of the Rabies Vaccine in the Control Group.**

2 Previous Preclinical Experience with the Four Experimental Vaccines

2.1 R0.6C-AlOH/Matrix-M™

Preclinical studies focused on safety, immunogenicity, and functional activity of the R0.6C vaccine candidate. **Table 1** summarizes the preclinical studies conducted in various animal species.

Table 2: Summary of Preclinical Animal Studies for R0.6C candidate Vaccines

Animal Species	Study Protocol	Material	Purpose	Dose, Route, & Regimen	Summary
Rat	Radboud UMC under approval number 2016-0020 [12]	Small scale lab-produced R0.6C	Perform dose-titration studies within anticipated dose range to demonstrate that R0.6C elicit TB antibodies and to provide data for potency evaluation.	0.03, 1.2, 4.7, 9.4, 18.9, and 37.7µg of R0.6C adjuvanted on Alhydrogel, 3 SC injections on D0, D14, and D28. Terminal bleed on SD43.	Doses are within the anticipated range for potency evaluation. Pfs48/45-6C antibody titers are associated with TRA.
Rat	MVI-01-2015/2016 under P 20-204 [7]	Up-scaled lab-produced R0.6C	Perform dose-titration studies to demonstrate that up-scaled R0.6C product elicit TB antibodies.	2.5, 10, 25µg of R0.6C adjuvanted in Montanide ISA720, 3 SC injections on D0, D21, and D42. Terminal bleed on D63.	Doses are within the anticipated range for potency evaluation. Pfs48/45-6C antibody titers are

					associated with TRA.
Mice	Radboud UMC under approval number 2016-0020	Up-scaled R0.6C reference material	Perform two studies within anticipated dose ranges and at the intended route of administration to confirm immunogenicity of DP formulations.	0.4, 2, 10µg of R0.6C adjuvanted on Alhydrogel or Alhydrogel + Matrix-M, 2 IM injections on D0, and D28. Terminal bleed on D63.	Both R0.6C/AlOH and R0.6C/AlOH + Matrix-M elicit high levels of functional antibodies when injected twice by the intended route of immunization.
Rabbits	CRL Study no. 78813	Tox Grade Material	Toxicity of repeated dose of R0.6C/AlOH and R0.6C/AlOH + Matrix-M	A total of 4 doses by IM, with 100 µg R0.6 with or without 50 µg Matrix-M	No systemic toxicological changes. Local test item related histopathology changes were observed at the injection sites and consisted of an up to marked focal inflammatory response. Partial recovery was recorded when comparing the site dosed 3 days prior to necropsy with the site dosed 46 days prior to necropsy

2.1.1 Immunogenicity of R0.6C/AlOH and R0.6C/AlOH + Matrix-M™ adjuvant in Rats

The preliminary study used preclinical R0.6C/Alhydrogel vaccines to determine the appropriate dose response range in rats for the products. To determine the appropriate dosing range, a preliminary study was conducted with six groups of rats, 5 rats per group, with each animals receiving 3 SC injections on Study Days 0, 14, and 28 according to Table 9 with research grade R0.6C adjuvanted on Alhydrogel.

Table 3: Design for rat Study of R0.6C/AlOH

Group	Number of Rats per Group	Antigen	Dose level (µg/50µL)	Adjuvant
1	5	R0.6C	0.03	Alhydrogel®
2	5		1.2	
3	5		4.7	
4	5		9.4	

5	5		18.9	
6	5		37.7	

Six groups of 5 rats were immunized on days 0, 14, and 28 by subcutaneous injection (SC) with 50 µL of the indicated formulation.

The rats were also observed for general health. The rats were terminated on Day 43, and anti-Pfs48/45 titers as well as TRA were determined. Figure 5 shows the TRA and anti-Pfs48/45 IgG levels measured by ELISA on plates coated with a gametocyte extract. In general, R0.6C elicited high levels of gametocyte-specific antibodies. At the optimal dose of 9.4 µg R0.6C, 3 out of 5 animals showed ≥90% TRA and one animal showed ≥85% TRA. Sera which exhibits a high level of TRA also has a low mosquito infection rate **(Table 3).**

There was a statistically significant correlation (Spearman rank, $p < 0.001$, $r = 0.5652$) between biological activity in SMFA and anti-gametocyte antibody levels.

Table 4: Summary of Transmission-Blocking activity of sera from rat immunized with R0.6C/AlOH

Dose		GCT	SMFA		
µg	Rat	Titre	% infected (n/N)	Mean oocysts	% TRA (95% CI)
37.7	1	644	90 (18/20)	11	58 (37.1-72.2)
	2	362	100 (20/20)	17	37 (8.1-57.0)
	3	235	100 (20/20)	16	39 (9.9-58.5)
	4	907	50 (10/20)	1.1	96 (92.5-97.7)
	5	581	55 (11/20)	1.9	93 (87.7-95.8)
18.9	1	195	100 (20/20)	19	26 (-8.7-49.4)
	2	122	100 (20/20)	15	44 (18.8-60.9)
	3	194	10 (2/20)	0.1	100 (98.4-99.9)
	4	200	95 (19/20)	24	10 (-36.1-41.0)
	5	360	95 (19/29)	22	18 (-23.3-45.1)
9.4	1	197	75 (15/20)	3.7	86 (77.3-91.7)
	2	171	85 (17/20)	8	70 (51.7-81.2)
	3	650	10 (2/20)	0.2	99 (97.8-99.7)
	4	468	90 (18/20)	2.6	90 (84.1-94)
	5	258	70 (14/20)	1.4	95 (91.2-97.1)
4.7	1	257	90 (18/20)	22.6	15 (-42.4-49.4)
	2	357	86 (18/22)	10.5	60 (38.1-74.4)
	3	138	80 (16/20)	16.4	38 (-2.7-62.9)
	4	175	95 (19/20)	15.2	43 (14.6-61.9)
	5	309	80 (16/20)	2.2	92 (96.8-95.0)
1.2	1	129	100 (20/20)	19.7	0 (-133.6- -2.8)
	2	123	95 (19/20)	12.3	1 (-59.1-38.6)
	3	527	90 (18/20)	14.7	0 (-59.1-38.6)
	4	209	95 (19/20)	16.6	0 (-96.2-28.3)
	5	511	75 (15/20)	5.2	58 (22.4-77)
0.3	1	130	100 (17/17)	19.4	0 (-144.2- -1.5)
	2	163	80 (16/20)	9.4	24 (-30.7-56.0)
	3	95	100 (19/19)	22.2	0 (-180.4- -14.0)
	4	41	93 (13/14)	13.4	0 (-96.3-40.8)
	5	17	100 (8/8)	19.8	0 (-197.5-14.6)

Individual final bleed (1:9 diluted) were tested in the SMFA. Seven days after membrane feed, the number of infected mosquitoes and oocyst density were determined. Rat: Rat serum from the R0.6Cc-immunized group; GCT: antibody titer in the gametocyte ELISA; % Infected (n/N): percentage of infected mosquitoes, n/N total infected mosquitoes/total dissected mosquitoes;

%TRA: percentage of Transmission Reducing Activity compared with the mean of the S0 samples, IC: interquartile rang; P-value: sample of the control S0 (pre-bleed serum) was used for calculation.

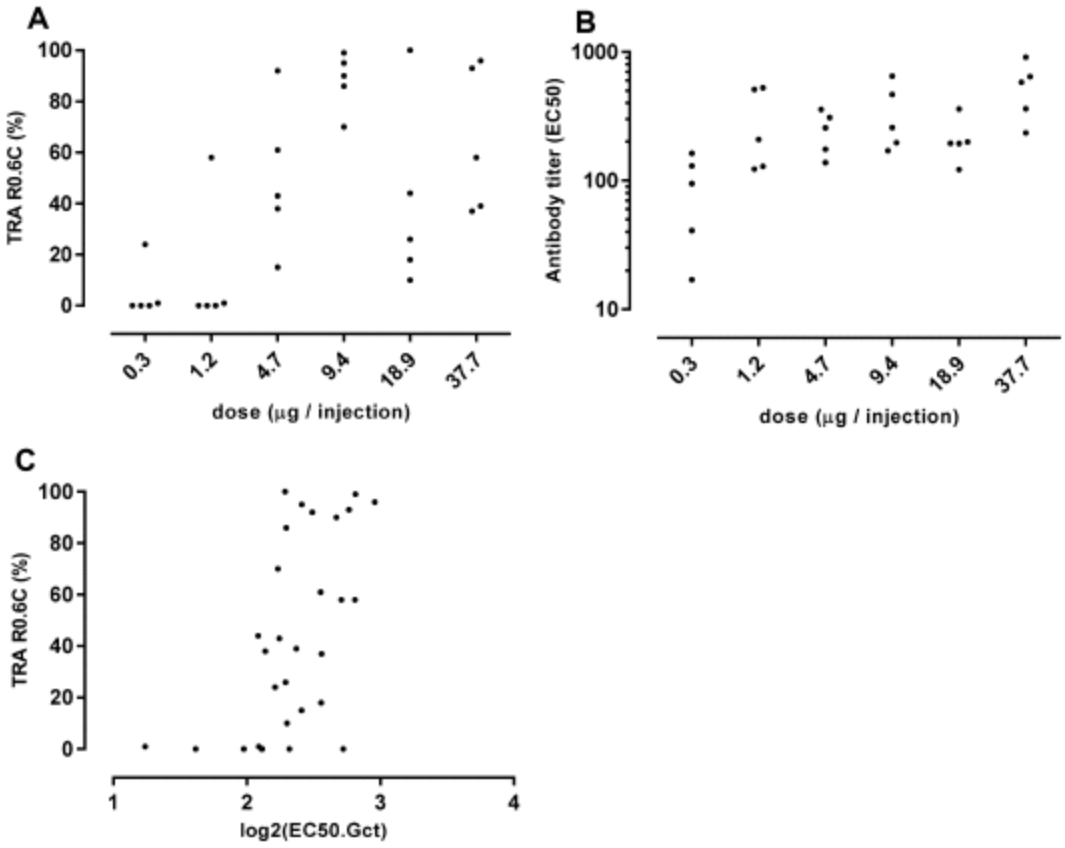


Figure 6: Functional activity and immune recognition of R0.6C/AlOH in rats

Figure 6 Functional activity and immune recognition of vaccination antigens. (**A**) Individual sera from groups of rats (n = 5) immunized with different doses of R0.6C in $Al(OH)_3$ were assessed for functional activity in the SMFA. (**B**) Recognition of gametocytes (●) by ELISA. Horizontal lines represent median values. (**C**) The relationship between functional activity and antibody level for individual rats is shown.

2.1.2 Immunogenicity of R0.6C/AlOH and R0.6C/AlOH + Matrix-M™ adjuvant in Mice

Formulations were prepared to confirm immunogenicity of DP formulations. Outbreed CD-1 mice were immunized with research grade R0.6C and sera were taken on day 42 for immune analysis. Mice were immunized intramuscularly in the right thigh with 50 µl vaccine, two times on days 0 and 28 according to **Table 5.** Alhydrogel formulations contained 75 micrograms of Alhydrogel and were mixed by pipetting for 5 minutes. Matrix-MTM adjuvant (Novavax AB, Uppsala, Sweden) formulations contained 5 micrograms of Matrix-M adjuvant per injection and were mixed by pipetting for a short period of time. 70% Montanide ISA720 (Seppic, France) formulations were prepared following the manufacturer's instructions. Formulations that contained both Alhydrogel and Matrix-M adjuvant were prepared by first adsorbing R0.6C to Alhydrogel as described above and then adding Matrix-M adjuvant. Fourteen days after the second immunization, mice were sacrificed, and serum was collected for ELISA and SMFA analysis. **Figure 7** shows the anti-Pfs48/45 ELISA titers in individual rats and their association with TRA.

Table 5: Design for Mouse Study of R0.6C/AlOH

Group	Number of Mice per Group	Antigen	Dose level (µg/50µL)	Adjuvant
1	5	R0.6C	10	Montanide ISA720
2	5	R0.6C	0.2	Alhydrogel®
3	5		4	
4	5		10	
5	5	R0.6C	0.2	Alhydrogel® + Matrix-M™ adjuvant
6	5		4	
7	5		10	

To confirm the immunogenicity of the R0.6C DP configurations, R0.6C with Matrix-M™ adjuvant, which is a saponin-based adjuvant composed of purified saponin from the tree Quillaja Saponaria Molina [13], was evaluated in mice. Outbred CD-1 mice were immunized intramuscularly on days 0 and 28 with different dosages of R0.6C adjuvanted in either Alhydrogel® or Matrix-M™ adjuvant. Two weeks after the last injection, mice were bled, and antibody titers and functionality of antibodies were assessed by ELISA and the SMFA, respectively. We investigated whether addition of Matrix-M adjuvant to an Alhydrogel formulation could enhance immunogenicity in mice. 6C-specific antibody titers were significantly higher when mice were immunized with Alhydrogel/Matrix-M and 2 or 10 µg R0.6C, compared to groups that received equal amounts of R0.6C with Alhydrogel only (Figure 3A). Interestingly, pooled sera from groups that received Alhydrogel + Matrix-M and 0.4, 2 or

10 µg R0.6C reduced transmission >80% (Figure 3B). Altogether we show that a formulation of R0.6C on Alhydrogel/Matrix-M induces high levels of transmission reducing antibodies.

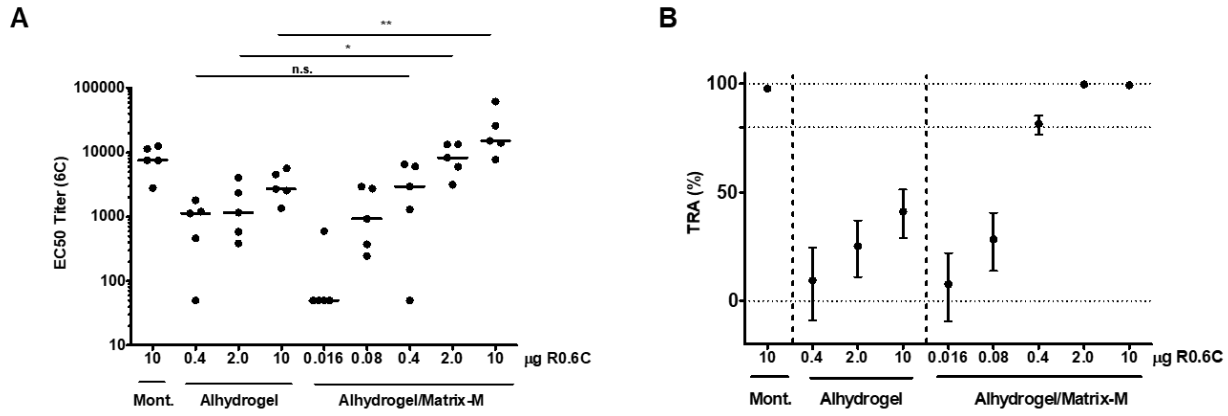


Figure 7: Functional activity and immune recognition of R0.6C/AlOH with or without Matrix-M™ adjuvant in mice

Figure 7 Immunogenicity of R0.6C vaccine formulations. Outbred CD-1 mice were immunized intramuscularly on days 0 and 28 with different dosages of R0.6C adjuvanted in Alhydrogel® with or without Matrix-M adjuvant. Two weeks after the last injection, mice were bled, and antibody titers and functionality of antibodies were assessed by ELISA and the SMFA, respectively. **A**) 6C-specific antibody titers for individual mice are shown as mid-point titers. Mid-point titers below 50 are reported as 50. Bars represent median values. Statistical difference between same-dose groups is determined by Mann-Whitney test, and reported p-values are two-sided (n.s. not significant, * $p<0.05$, ** $p<0.01$). B) Pooled sera were tested in SMFA at 1:9 dilution. Reported values and 95% confidence intervals (bars) are determined by General Linearized Mixed Models and used oocyst count data from two independent SMFA experiments with 20 mosquitoes per condition and experiment. Transmission reducing activity is calculated by comparing to a non-serum control included in each SMFA.

In summary, R0.6C /AlOH with and without Matrix-M adjuvant elicit high levels of vaccine-specific antibodies with the capacity to control parasite development in the SMFA. No toxicity in rats and mice was observed.

2.1.3 Repeated Dose Toxicity Study by Intramuscular Administration in Rabbits

The objective of this study was to assess the potential toxicity and local tolerance of a Malaria Vaccine antigen (R0.6C) and the associated adjuvant (Alhydrogel® (AlOH)) administrated either in combination with Matrix-M or without in NZW rabbits. The groups, dose levels, and animal numbers are detailed in **Table 6**.

The vaccine was administered four times by intramuscular injection over a period of 43 days. The injections were administered with an interval of 14 days between each treatment. A 4-weeks recovery period will be allowed.

The main animals were terminated on Day 46 and the recovery animals on Day 71.

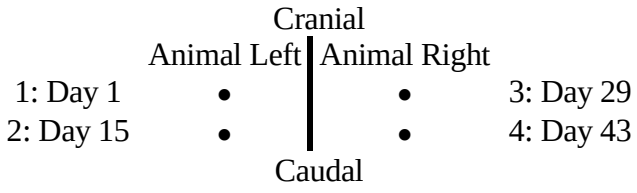
Table 6: Design for Rabbit Study of R0.6C/Alhydrogel® with or Matrix-M™ adjuvant

Group	Test article	Dose (µg)	Dose volume (mL)	Animal Nos			
				Main		Recovery	
				Male	Female	Male	Female
1	Placebo	0	0.5	1-5	11-15	6-10	16-20
2	R0.6C-AIOH	100	0.5	21-25	31-35	26-30	36-40
3	R0.6C-AIOH + Matrix-M	100	0.5 + 0.130	41-45	51-55	46-50	56-60

- The doses were given as intramuscular injections.
- The intramuscular dosing was performed on Days 1, 15, 29 and 43.
- The dose volume was a total volume of 0.5 mL/injection for Group 1 -2.
- The dose volume was a total volume of 0.630 mL/injection for Group 3.

The control group (Group 1) was treated with 0.9% NaCl whereas Group 2 was treated with R0.6C-AlOH which contains recombinant R0.6C (0.2 mg/mL) in HEPES buffer, EDTA, Glucose, NaCl, water and Alhydrogel® (1.6 mg/mL). Group 3 received R0.6C-AIOH + Matrix-M which is a mixture of recombinant R0.6C (0.2 mg/mL) in HEPES buffer, EDTA, Glucose, NaCl, water and Alhydrogel® (1.6 mg/mL) and Matrix-M (0.375 mg/mL) in PBS buffer. The dose volume was 0.5 mL/injection for Groups 1 -2 and 0.630 mL/injection for Group 3.

The injections were placed in the following way:



The following parameters were evaluated in the study: Clinical signs, local reactions at the injection sites, body weight, food consumption, body temperature, clinical pathology (haematology, coagulation and clinical chemistry) and antibody analysis. At necropsy, a full

pathological evaluation was performed including organ weights and macroscopic and microscopic examination.

2.1.3.1 Serology Assessments by ELISA

Antibody ELISA was conducted on bleeds collected prior to immunization (pre-bleed) and on Days 46 for the main group animals and on Day 71 for the recovery animals. **Figure 8** shows anti-R0.6C antibody levels in each group.

Both pre-bleed samples from study groups as well as pre-bleed and terminal bleed from the placebo group did not show antibody responses above that of background and negative controls. The antibody titer of negative controls, pre-bleeds, and placebo group were <400-fold dilution. Such findings indicate the preclinical toxicology assessment of R0.6C/AlOH and R0.6C/AlOH + Matrix-M is valid as the host and immunization regimen induced a valid antibody response in the preclinical toxicology model. It should be noted that no differentiation between the two formulations (R0.6C/AlOH and R0.6C/AlOH+Matrix-M) could be made based on the assay method limitations.

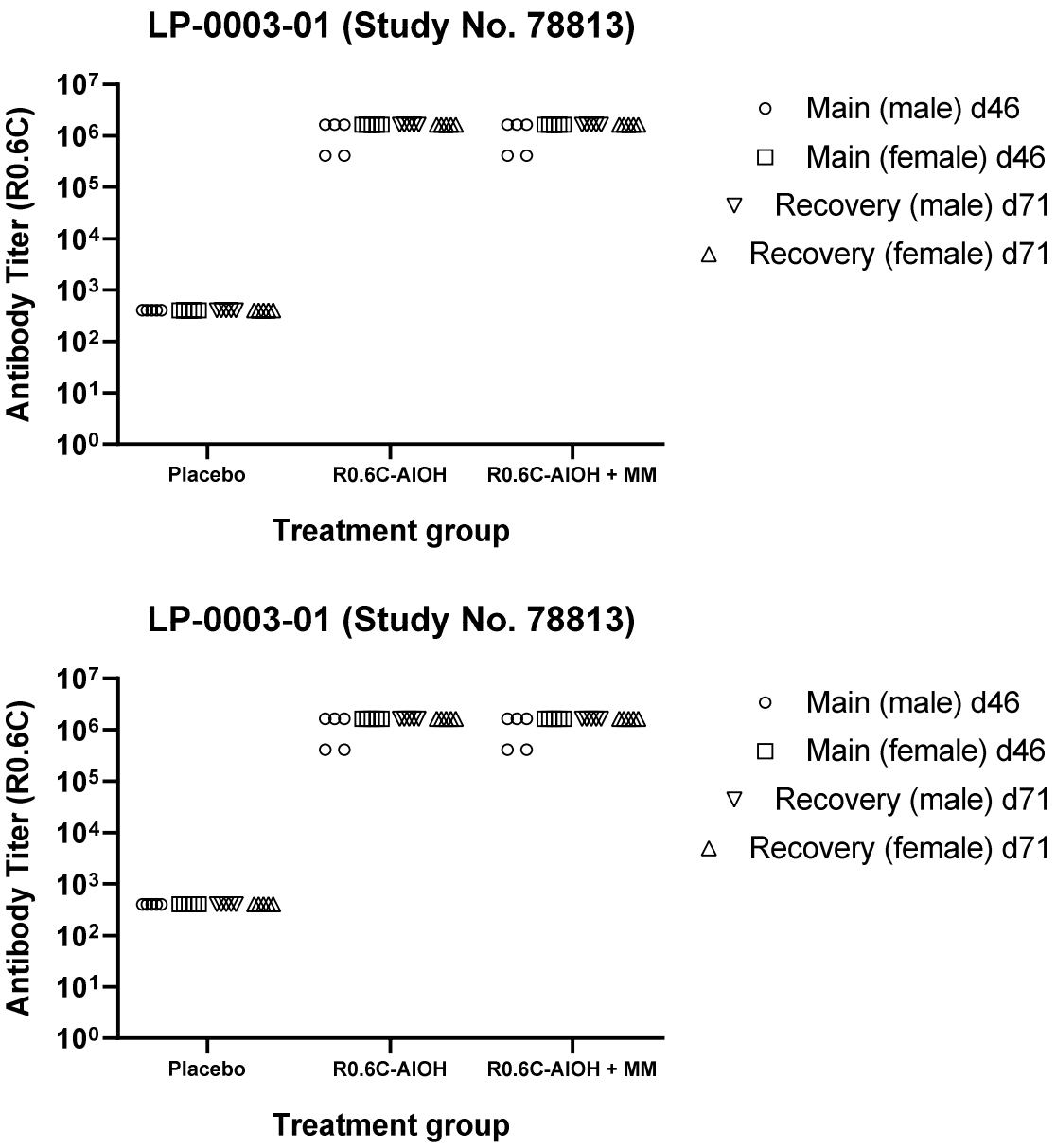


Figure 8: Immune recognition of R0.6C/AlOH with or without Matrix-M™ adjuvant in Rabbits

Figure 8 Immunogenicity of R0.6C vaccine formulations. NZW rabbits were immunized intramuscularly on days Days 1, 15, 29 and 43 with the full human dose of R0.6C adjuvanted on Alhydrogel® with or without Matrix-M adjuvant. The main animals were terminated on Day 46 and the recovery animals on Day 71. The antibody titer for each serum sample was assessed by ELISA on plates coated with R0.6C and were displayed on a log scale. Antibody titer (Reciprocal dilution) for a test sample is defined as the last serum dilution at which the optical density reading at 450 nm (OD450) of the sample is greater than the mean OD450 of the pre-bleed sera plus 3 times the standard deviation utilizing GrapPad Prism Version 8.3.0.

2.1.3.2 Toxicology assessment in Rabbits

SSI has received a fully QA audited final GLP Toxicological Report from the CRO, from which the conclusions in this section are taken.

No test item related adverse findings were observed in relation to clinical signs including the injection site observations, body weight, food consumption, body temperature, clinical pathology or at necropsy including macroscopic findings as well as organ weights. Further, no signs of systemic toxicity were recorded at the histopathological examination.

A slight increased mean body temperature was seen especially in the treated males following some of the dosing occasions, in both genders of Group 3 an elevation in the fibrinogen level was observed and in males of Group 2 and 3 an increase in the level of globulin was seen. These findings were considered either linked to an inflammatory process which is a normal physiological effect of a vaccine or incidental based on the inconsistency of the findings (only seen in single dose group or single sex).

The histopathological evaluation revealed that the injection sites 4 (treated on Day 43) of both R0.6C–AlOH treated groups showed similar up to marked focal inflammatory response compared to mild inflammation at placebo injection sites. For injection site 1 (treated Day 1) of both main and recovery animals, the sites treated with placebo, showed no microscopic changes. In comparison, the inflammatory response recorded in Groups 2 and 3, included degeneration/necrosis of muscle tissue and infiltration of inflammatory cells, mainly of an up to mild degree. Thus, partial and comparable recovery was recorded at the injection sites 1 (treated on Day 1) of the R0.6C–AlOH treated groups compared to complete recovery of changes at placebo injection sites. The 4 week recovery period did not add further to the regression of treatment related changes at injection sites 1 compared to main groups.

In conclusion, four intramuscular injections at 14 day intervals with the Malaria Vaccine antigen (R0.6C) and the associated adjuvant (Aluminium hydroxide (AlOH) without or with Matrix-M1 adjuvant in NZW rabbits caused no systemic toxicological changes. Local test item related changes were observed at the injection sites and consisted of an up to marked focal inflammatory response at the sites dosed 3 days prior to necropsy. At the sites dosed 46 and 71 days prior to necropsy the response was mainly of an up to mild degree indicating recovery is ongoing.

2.2 ProC6C-AlOH/Matrix-M™

Preclinical studies focused on safety, immunogenicity, and functional activity of the ProC6C vaccine candidate. **Table 5** summarizes the preclinical studies conducted in various animal species.

Table 7: Summary of Preclinical Animal Studies for ProC6C candidate Vaccines

Animal Species	Study Protocol	Material	Purpose	Dose, Route, & Regimen	Summary
Mice	Radboud UMC under approval number 2016-0020	Up-scaled ProC6C reference material	Perform two studies within anticipated dose ranges and at the intended route of administration to confirm immunogenicity of DP formulations.	0.4, 2, 10µg of ProC6C adjuvanted on Alhydrogel or Alhydrogel + Matrix-M, 2 IM injections on D0, and D28. Terminal bleed on D63.	Both ProC6C/AlOH and ProC6C/AlOH + Matrix-M elicit high levels of functional antibodies when injected twice by the intended route of immunization.
Rabbits	CRL Study no. 48599	Tox Grade Material	Toxicity of repeated dose of ProC6C/AlOH and ProC6C/AlOH + Matrix-M	A total of 4 doses by IM, with 100 µg R0.6 with or without 50 µg Matrix-M	No systemic toxicological changes. Local test item related histopathology changes were observed at the injection sites and consisted of an up to marked focal inflammatory response. Partial recovery was recorded when comparing the site dosed 3 days prior to necropsy with the site dosed 46 days prior to necropsy

2.2.1 Immunogenicity of ProC6C-AlOH in Mice

Formulations were prepared to confirm immunogenicity of DP formulations. Outbreed CD-1 mice were immunized with research grade ProC6C and sera were taken on day 42 for immune analysis. Mice were immunized intramuscularly in the right thigh with 50 µl vaccine, two times on days 0 and 28 according to Table 6. Alhydrogel formulations contained 75 micrograms of Alhydrogel and were mixed by pipetting for 5 minutes. Matrix-MTM adjuvant (Novavax AB, Uppsala, Sweden) formulations contained 5 micrograms of Matrix-M adjuvant per injection and were mixed by pipetting for a short period of time. 70% Montanide ISA720 (Seppic, France) formulations were prepared following the manufacturer's instructions. Formulations that contained both Alhydrogel and Matrix-M adjuvant were prepared by first adsorbing ProC6C to Alhydrogel as described above and then adding Matrix-M adjuvant. Fourteen days after the second immunization, mice were sacrificed, and serum was collected for ELISA and SMFA analysis. Figure 4 shows the anti-Pfs48/45 ELISA titers in individual mice and their association with TRA.

Table 8: Design for Mouse Study of ProC6C/AlOH

Group	Number of Mice per Group	Antigen	Dose level (µg/50µL)	Adjuvant
2	5	ProC6C	0.2	Alhydrogel®
3	5		4	
4	5		10	
5	5	ProC6C	0.2	Matrix-M™ adjuvant
6	5		4	
7	5		10	
8	5	ProC6C	0.2	Alhydrogel® + Matrix-M™ adjuvant
9	5		4	
10	5		10	

To confirm the immunogenicity of the ProC6C DP configurations, ProC6C with Matrix-M™ adjuvant, which is a saponin-based adjuvant composed of purified saponin from the tree Quillaja Saponaria Molina [13], was evaluated in mice. Outbred CD-1 mice were immunized intramuscularly on days 0 and 28 with different dosages of ProC6C adjuvanted in either Alhydrogel® or Matrix-M adjuvant. Two weeks after the last injection, mice were bled, and antibody titers and functionality of antibodies were assessed by ELISA and the SMFA, respectively. We investigated whether addition of Matrix-M adjuvant to an Alhydrogel formulation could enhance immunogenicity in mice. 6C-specific antibody titers were higher when mice were immunized with Alhydrogel/Matrix-M and 0.4, 2 or 10 µg ProC6C, compared to groups that received equal amounts of ProC6C with Matrix-M only (Figure 4A). Interestingly, pooled sera from groups that received Alhydrogel + Matrix-M and 0.4, 2 or 10 µg ProC6C reduced transmission >80% (Figure 4B). Altogether we show that a formulation of ProC6C on Alhydrogel/Matrix-M induces high levels of transmission reducing antibodies than ProC6C formulated on Alhydrogel, or Matrix-M only.

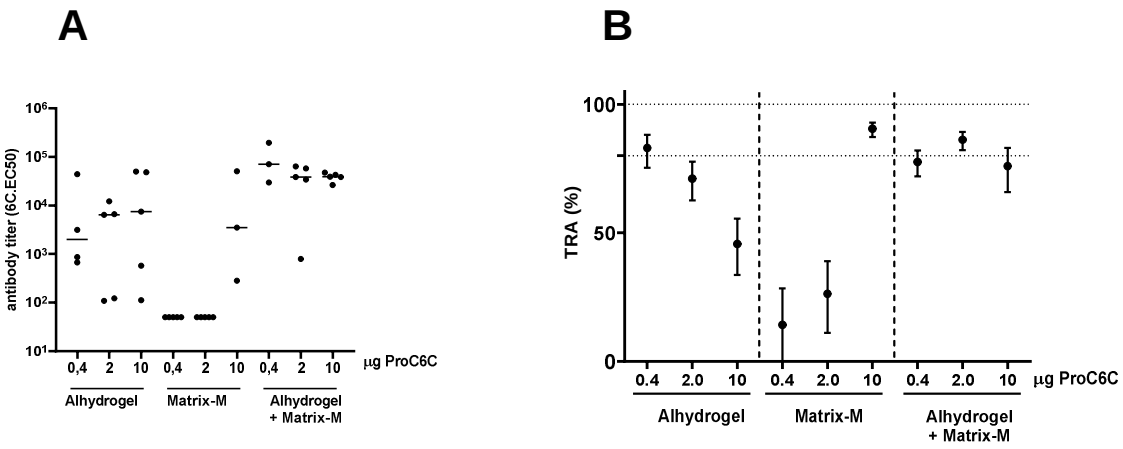


Figure 9: Functional activity and immune recognition of ProC6C/AlOH with or without Matrix-M™ adjuvant in mice

Figure 9 Immunogenicity of ProC6C vaccine formulations. Outbred CD-1 mice were immunized intramuscularly on days 0 and 28 with different dosages of ProC6C adjuvanted in Alhydrogel® with or without Matrix-M adjuvant and in Matrix-M only. Two weeks after the last injection, mice were bled, and antibody titers and functionality of antibodies were assessed by ELISA and the SMFA, respectively. **A**) 6C-specific antibody titers for individual mice are shown as mid-point titers. Mid-point titers below 50 are reported as 50. Bars represent median values. B) Pooled sera were tested in SMFA at 1:9 dilution. Reported values and 95% confidence intervals (bars) are determined by General Linearized Mixed Models and used oocyst count data from two independent SMFA experiments with 20 mosquitoes per condition and experiment. Transmission reducing activity is calculated by comparing to a non-serum control included in each SMFA.

In summary, ProC6C /AlOH with Matrix-M adjuvant consistently elicit high levels of vaccine-specific antibodies with the capacity to control parasite development in the SMFA. No toxicity in rats and mice was observed.

2.2.2 Repeated Dose Toxicity Study by Intramuscular Administration in Rabbits

The objective of this study was to assess the potential toxicity and local tolerance of a Malaria Vaccine antigen (ProC6C) and the associated adjuvant (Alhydrogel® (AlOH)) administrated either in combination with Matrix-M or without in NZW rabbits. The groups, dose levels, and animal numbers are detailed in **Table 9**.

The vaccine was administered four times by intramuscular injection over a period of 43 days. The injections were administered with an interval of 14 days between each treatment. A 4-weeks recovery period will be allowed.

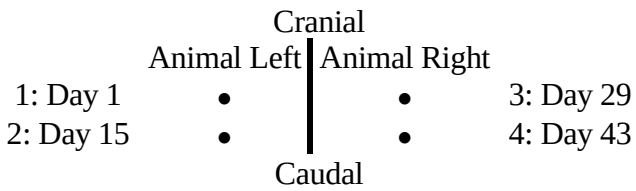
The main animals were terminated on Day 46 and the recovery animals on Day 71.

Table 9: Design for Rabbit Study of ProC6C-Alhydrogel® with or Matrix-M™ adjuvant

TOX STUDY DESIGN (STUDY NO. 48599)									
Grp	**Test Article**	**Dose**	**Route**	**Vaccination**	**Dose volume (ml)**	**Number of animals**			
						Main D=46		**Recovery D=71**	
						Male	**Female**	**Male**	**Female**
1	Placebo (Saline)		i.m.	0, 14, 28, 42	0.5	5 1001 to 1005	5 1501 to 1505	5 1006 to 1010	5 1506 to 1510
2	ProC6C+AlOH	100	i.m.	0, 14, 28, 42	0.5	5 2001 to 2005	5 2501 to 2505	5 2006 to 2010	5 2506 to 2510
3	ProC6C+AlOH + Matrix-M™	100	i.m.	0, 14, 28, 42	0.630	5 3001 to 3005	5 3501 to 3505	5 3006 to 3010	5 3506 to 3610

The control group (Group 1) was treated with 0.9% NaCl whereas Group 2 was treated with ProC6C-AlOH which contains recombinant ProC6C (0.2 mg/mL) in HEPES buffer, EDTA, Glucose, NaCl, water and Alhydrogel® (1.6 mg/mL). Group 3 received ProC6C-AIOH + Matrix-M which is a mixture of recombinant ProC6C (0.2 mg/mL) in HEPES buffer, EDTA, Glucose, NaCl, water and Alhydrogel® (1.6 mg/mL) and Matrix-M (0.375 mg/mL) in PBS buffer. The dose volume was 0.5 mL/injection for Groups 1 -2 and 0.630 mL/injection for Group 3.

The injections were placed in the following way:



- Site 1: intramuscular injection on Day 1: right hind leg (posterior thigh muscle).
- Site 2: intramuscular injection on Day 15: left hind leg (posterior thigh muscle).
- Site 3: intramuscular injection on Day 29: right hind leg (anterior thigh muscle).
- Site 4: intramuscular injection on Day 43: left hind leg (anterior thigh muscle).

The following parameters were evaluated in the study: Clinical signs, local reactions at the injection sites, body weight, food consumption, body temperature, clinical pathology (haematology, coagulation and clinical chemistry) and antibody analysis. At necropsy, a full pathological evaluation was performed including organ weights and macroscopic and microscopic examination.

2.2.2.1 Serology Assessments by ELISA

Antibody ELISA was conducted on bleeds collected prior to immunization (pre-bleed) and on Days 46 for the main group animals and on Day 71 for the recovery animals. **Figure 8** shows anti-ProC6C antibody levels in each group.

Both pre-bleed samples from study groups as well as pre-bleed and terminal bleed from the placebo group did not show antibody responses above that of background and negative controls. The antibody titer of negative controls, pre-bleeds, and placebo group were <400-fold dilution. Such findings indicate the preclinical toxicology assessment of ProC6C/AlOH and ProC6C/AlOH + Matrix-M is valid as the host and immunization regimen induced a valid antibody response in the preclinical toxicology model. It should be noted that no differentiation between the two formulations (ProC6C/AlOH and ProC6C/AlOH+Matrix-M) could be made based on the assay method limitations.

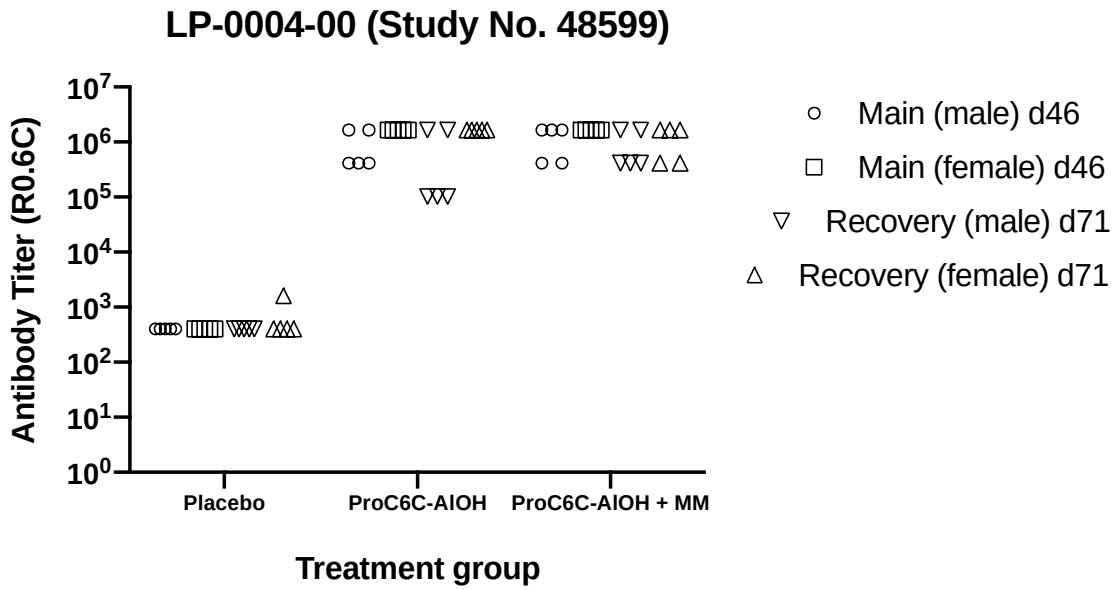


Figure 10: Immune recognition of ProC6C/AlOH with or without Matrix-M™ adjuvant in Rabbits

Figure 10 Immunogenicity of ProC6C vaccine formulations. NZW rabbits were immunized intramuscularly on days Days 0, 14, 28 and 42 with the full human dose of ProC6C adjuvanted on Alhydrogel® with or without Matrix-M adjuvant. The main animals were terminated on Day 46 and the recovery animals on Day 71. The antibody titer for each serum sample was assessed

by ELISA on plates coated with ProC6C and were displayed on a log scale. Antibody titer (Reciprocal dilution) for a test sample is defined as the last serum dilution at which the optical density reading at 450 nm (OD450) of the sample is greater than the mean OD450 of the pre-bleed sera plus 3 times the standard deviation utilizing GrapPad Prism Version 8.3.0.

2.2.2.2 Toxicology assessment in Rabbits

No unscheduled deaths and no clinical signs indicative of systemic toxicity were reported during the study. There were no vaccine-related local reactions, no ophthalmological findings and no effects on body weight, food consumption or rectal temperature.

Slight changes observed in the clinical pathology parameters were suggestive of a transient systemic inflammatory reaction following vaccine administration and consisted of increases in fibrinogen and/or CRP concentrations.

All test item groups showed a significant antibody response. The placebo test article (Group 1) did not induce an immune response in any animals.

Various findings consistent with the expected immune response to injection of immunogenic material and adjuvants (Sellers et al. 2020) were present at injection sites in groups dosed with the candidate vaccines. These included mixed inflammatory cell (heterophils and lymphocytes) infiltrates for all candidate vaccines, in association of variably degenerated macrophages/multinucleated giant cells typical for AlOH-adjuvanted vaccines (ProC6C-AlOH, ProC6C-AlOH + Matrix-M1™), and increased edema and/or hemorrhage for ProC6C-AlOH + Matrix-M1™.

Infiltrates of macrophages and multinucleated giant cells were still observed at late euthanasia, 4 weeks after the injection, which is classically observed after injection of AlOH-adjuvanted adjuvants (Verdier et al. 2005). Mixed inflammatory infiltrates related to AlOH-adjuvanted vaccines were decreased in incidence and/or severity at early euthanasia at sites dosed on Day 1 relative to sites dosed on Day 43, and at late euthanasia, indicating ongoing recovery, whereas edema and hemorrhage were seen only at early euthanasia at sites dosed on Day 43 and were not present at late euthanasia i.e. full recovery of the acute changes. Macrophage degeneration/necrosis was also fully reversible.

In lymphoid tissues, partially reversible increased lymphoid cellularity, mainly characterized by increased size and/or number of germinal centers, was present in the spleen and lymph nodes draining the sites (iliac, popliteal and inguinal, both sides) and variably correlated with macroscopic enlargement and increased weights of the spleen and lymph nodes. These data demonstrated systemic and local immune responses to the candidates vaccines (Sellers et al. 2020). Minor other associated changes present in the draining lymph nodes with all candidate vaccines consisted of infiltrates of macrophages/multinucleated giant cells, infiltrates of heterophils and intrasinusoidal erythrocytes.

In conclusion: Four intramuscular injections at 14 day intervals with the Malaria Vaccine antigen (ProC6C) and the associated adjuvant (Aluminium hydroxide (AlOH) without or with

Matrix-M1 adjuvant in NZW rabbits caused no systemic toxicological changes and was well tolerated. All changes were expected after vaccine administration and were indicative of a local and systemic inflammatory process and the initiation of an innate and adaptive immune response.

None of these changes were considered to be adverse.

2.3 Pfs230D1-EPA/Matrix-M™

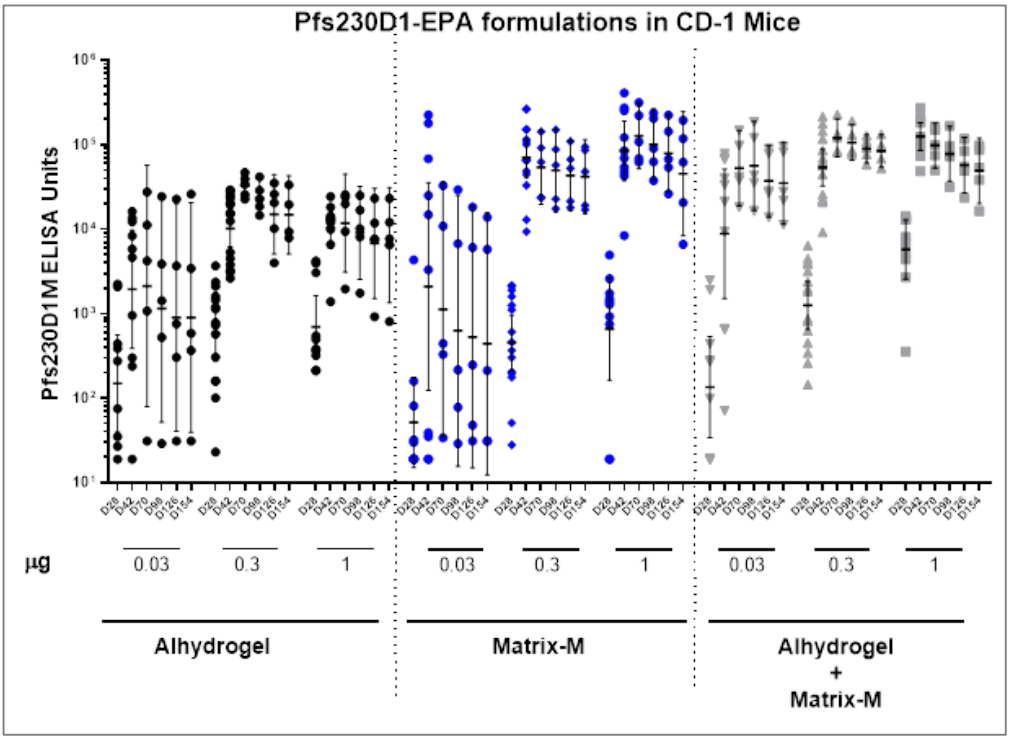
2.3.1 Immunogenicity of Pfs230D1-EPA with Alhydrogel and Matrix-M™ in CD-1 Mice

Nine groups of CD-1 mice, 3 groups of 15 animals and 6 groups with 10 animals, were immunized on Days 0 and 28 by IM injection with 50 µL (Groups 1-6) or 80 µL (2 sites x 40 µL for Groups 7-9), of formulation per vaccination day. The formulations and dosing concentrations/volumes are shown in **Erreur ! Source du renvoi introuvable.**10 below. Immune sera were collected on Days 0, 28, 42, 70, 98, 126 and 154.

Table 10: Immunogenicity of Pfs230D1-EPA Formulated with Alhydrogel or Matrix-M™ in CD-1 Mice

Group No.	Formulation	Antigen Dose (µg Pfs230D1M)	Group Size	Dose Volume (mL)	Vaccinations (Days)	Bleed Days (ELISA)	Termination	
							Day 42	Day 154
1	**Pfs230D1-EPA/Alhydrogel**	1	10	0.05	0, 28	0, 28, 42, 70, 98, 126, 154	5	5
2		0.3	15				10	5
3		0.03	10				5	5
4	**Pfs230D1-EPA/Matrix-M**	1	10				5	5
5		0.3	15				10	5
6		0.03	10				5	5
7	***Pfs230D1-EPA/Alhydrogel + Matrix-M**	1	10	** 2 sites x 0.04			5	5
8		0.3	15				10	5
9		0.03	10				5	5

* Pfs230D1-EPA/Alhydrogel (1600 µg/mL Alhydrogel) was formulated on Day -1 and mixed at bedside with Matrix-M adjuvant on Day 0 and Day 28.
** Formulations were prepared for dosing 80 µL per animal and delivered in two sites with 40 µL per site.



Error bars and line represent geometric mean (GM) and 95% confidence intervals.

Figure 11: Dose dependent Pfs230D1M specific IgG responses by ELISA in CD-1 mice on multiple bleed days (28-154) following IM immunization on days 0 and 28.

Erreur ! Source du renvoi introuvable. shows the dose dependent Pfs230D1M specific IgG responses by ELISA.

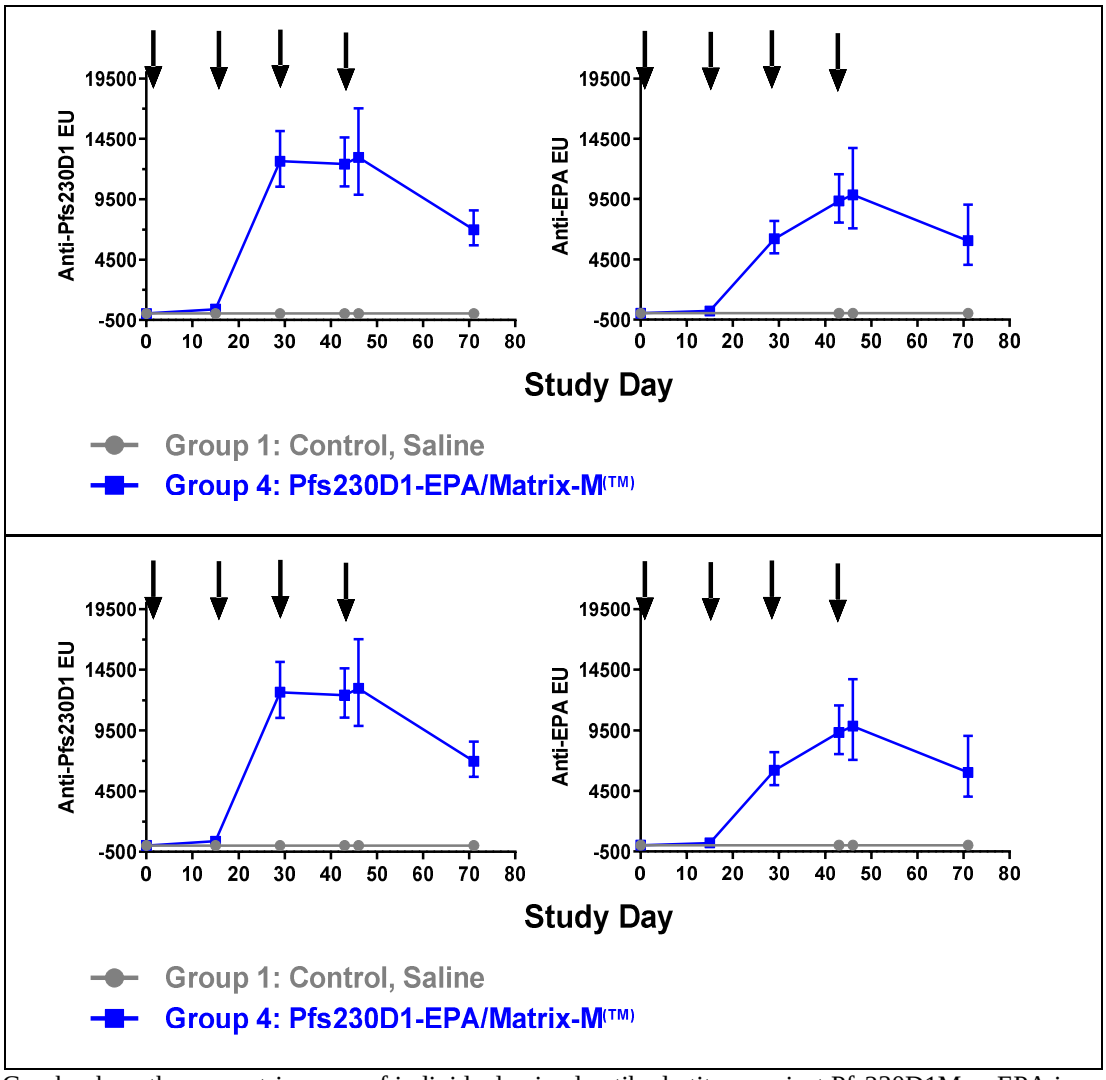
Conclusion: CD-1 mice immunized with Pfs230D1-EPA/Matrix-M or Pfs230D1-EPA/ Alhydrogel+Matrix-M had greater Pfs230D1M specific IgG titers than Pfs230D1-EPA/ Alhydrogel groups at 0.3 and 1 µg dose levels for up to 154 study days. The Pfs230D1-EPA/ Alhydrogel+Matrix-M group at 0.03 µg maintained higher titers than either Alhydrogel or Matrix-M alone groups.

2.3.2 Repeated Dose Toxicity Study by Intramuscular Administration in Rabbits

2.3.2.1 Pfs230D1-EPA/Matrix-M™ Serology Assessments by ELISA

A repeat dose toxicity study was performed on rabbits with administration of 40 µg Pfs230D1-EPA/50 µg of Matrix-M, or saline controls. Immunizations occurred on Days 0, 15, 29 and 43. Antibody responses were assessed as shown in **Erreur ! Source du renvoi introuvable.** below. Bleed days included the pre-bleed (Day 0), Day 15, Day 29, Day 43 and final bleeds (Day 46 or

71) for Pfs230D1M, and pre-bleed pools, Day 15, Day 29, Day 43 and final bleeds (Day 46 or 71) for EPA. Arrows represent days of vaccination (Day 0, 15, 29, and 43).



Graphs show the geometric mean of individual animal antibody titers against Pfs230D1M or EPA in vaccine groups as a function of time; error bars represent the Geomean with 95% CI.

Figure 12: Rabbit antibody responses following immunization with Control (Saline), 40 µg Pfs230D1-EPA/50 µg Matrix-M™ (Group 4).

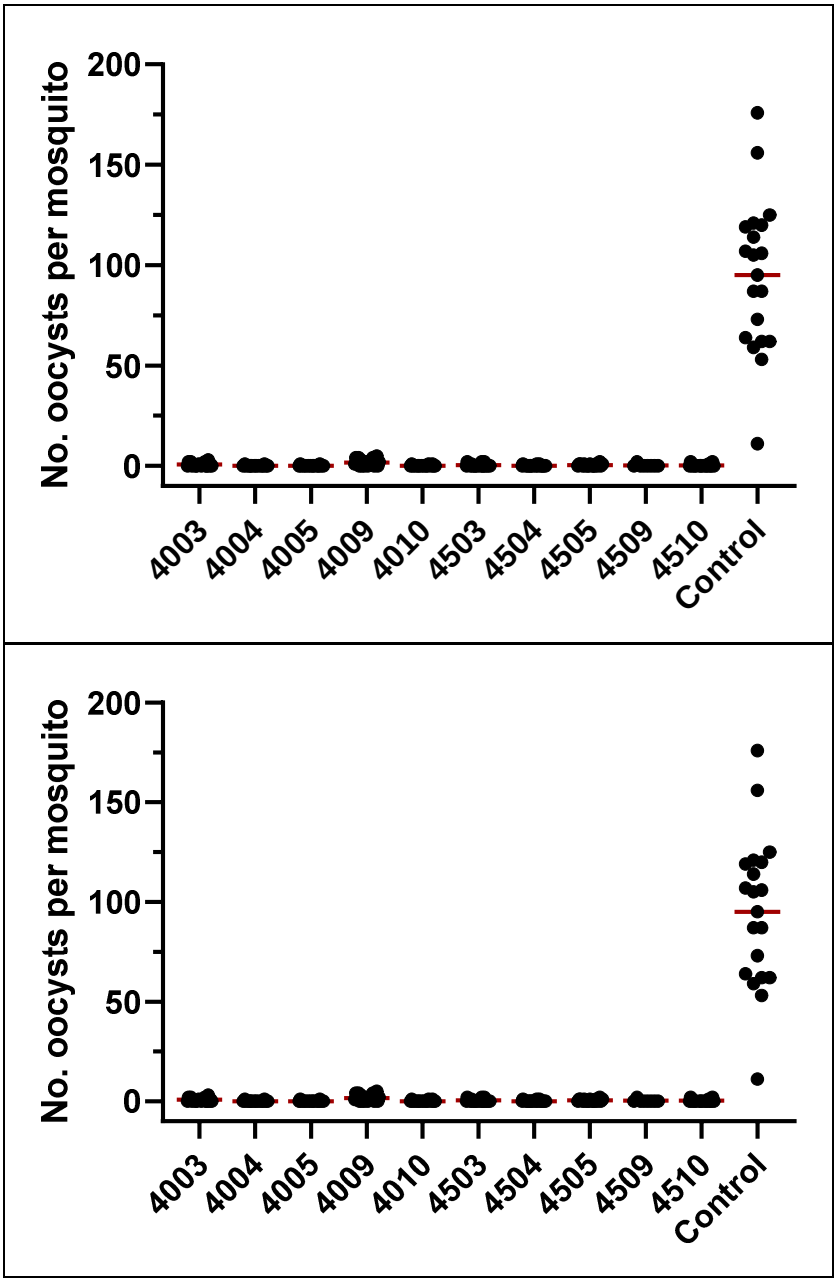
Conclusion:

All immunized animals in Group 4 responded to the Pfs230D1-EPA vaccine, as evaluated by ELISA against Pfs230D1M and EPA for specific antibody (IgG) responses. No animals in the Control group (Group 1) had detectable antibodies against Pfs230D1M or EPA. The vaccine was immunogenic in Group 4.

2.3.2.2 Pfs230D1-EPA/Matrix-M™ Serology Assessments by ELISA

The terminal sera collected from individual rabbits on D71 (28 days after the 4th vaccination) were tested for transmission blocking activities by a SMFA. Each assay sample was prepared by mixing 60 µL of heat-inactivated rabbit sera with 100 µL of non-heat-inactivated human serum pool and added to 100 µL gametocyte-infected RBCs. A pool of D71 sera from 10 Group 1 (control) rabbits was used as control for calculation of transmission reducing activity (TRA, defined as reduction of oocyst count in individual midgut in comparison to that in controls) and transmission blocking activity (TBA, defined as reduction of infected mosquito in comparison to the controls). The results are summarized in **Erreur ! Source du renvoi introuvable.**.

Transmission blocking activity by SMFA in rabbits



Evaluation of the transmission reducing activity of rabbit antisera from

Group 4 of the rabbit toxicology study 48599. The average transmission reducing activity is shown (red line) and the individual number of oocysts per mosquito, using in general 20 mosquitoes. Rabbits 4004 and 4509 had a reduced number of total mosquitoes evaluated (18/20 and 8/20, respectively) so those antisera were used in a repeat study using the same negative control. The results for % TRA were similar to those shown here (% TRA > 99.5%).

Figure 13: Transmission blocking activity by SMFA in rabbits

Conclusion: Pfs230D1-EPA/Matrix-M is immunogenic in rabbits. The antisera induced by the vaccine induced strong activity with an average level of transmission reducing activity of 99.6% and the reduction in prevalence of 75.2%.

2.3.2.3 Toxicology Study in Rabbits

The toxicology study was done to evaluate the local tolerance and the potential toxicity in rabbits induced by four intramuscular injections of Pfs230D1M-EPA formulated with Matrix-M. On completion of the treatment period, designated animals were euthanized after the last injection (early euthanasia) or after a 4-week treatment-free period (late euthanasia) in order to evaluate the reversibility of any findings and potential delayed adverse effects. The toxicology study has been completed and an initial draft report has been received with no marked indications noted.

2.4 R0.6C-AlOH/Matrix-M™ Co-administered with Pfs230D1-EPA/Matrix-M™

2.4.1 Immunogenicity of R0.6C-AlOH with Pfs230D1-EPA/Matrix-M™

The objective of this study was to evaluate antibody titers and duration of functional antibody to Pfs230D1M as assessed by standard membrane-feeding assay (SMFA) to assess any change in responses when Pfs230D1-EPA and R0.6C are combined by either co-adminstration or coformulation, using the adjuvant Matrix-M. The groups, dose levels, and animal numbers are detailed in Table X. The vaccines were administered two times by intramuscular injection on Days 0 and 28, with antibody responses assayed for titer and duration on Days 0, 28, 42, 70, 98, 126 and 154 and for function on Days 42 and 154.

Table 11: Design for Mouse Study of R0.6C-Alhydrogel® co-administered with Pfs230D1-EPA with Matrix-M™ adjuvant

Group	Number of Mice per Group	Formulation/s	Antigen Dose Level (µg)	Dose volume (µL)	Adjuvant
1	10	Pfs230D1-EPA/5 µg Matrix-M	0.3	0.05	Alhydrogel (1.6 mg/mL) Matrix-M = 5 µg/0.05 mL total in specified vaccines
2	10	R0.6C/Alhydrogel+5 µg Matrix-M	2	**0.063	
3	10	G3A) Pfs230D1-EPA/2.5 µg Matrix-M + G3B) R0.6C/Alhydrogel+2.5 µg Matrix M Co-Administered, Each vaccine dosed in the same limb	0.3 + 2	0.05 + **0.055	

4	10	G4A) Pfs230D1-EPA/5 µg Matrix-M + G4B) R0.6C/Alhydrogel Each vaccine dosed in the same limb	0.3 + 2	0.05 + 0.05	for Groups 1, 2, 4, and 5 or 2.5 µg + 2.5 µg for 5 µg total per mouse in Group 3
5	10	*Pfs230D1-EPA+R0.6C/Alhydrogel+5 µg Matrix-M Coformulated	0.3 + 2	0.05	

Note: Groups 3 and 4 require vaccines be administered to the SAME LIMB on each vaccination day.

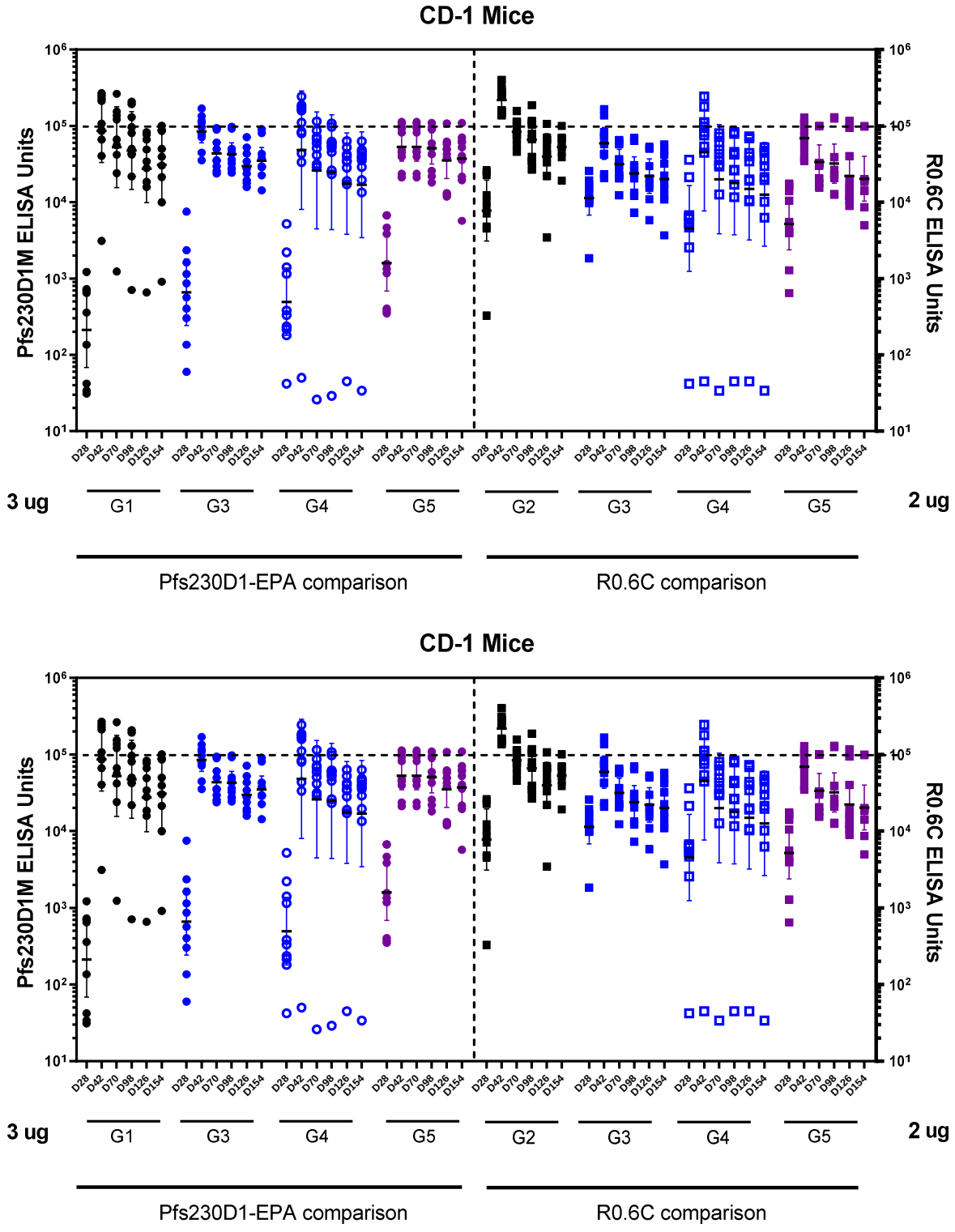
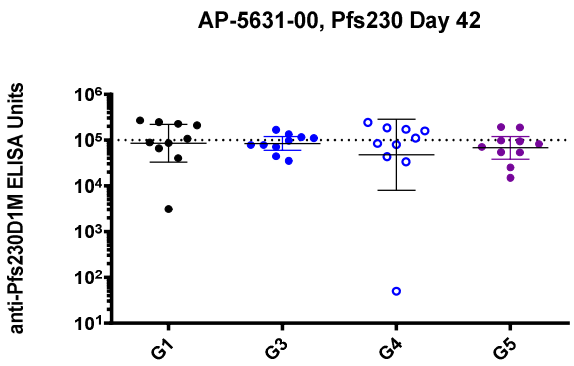
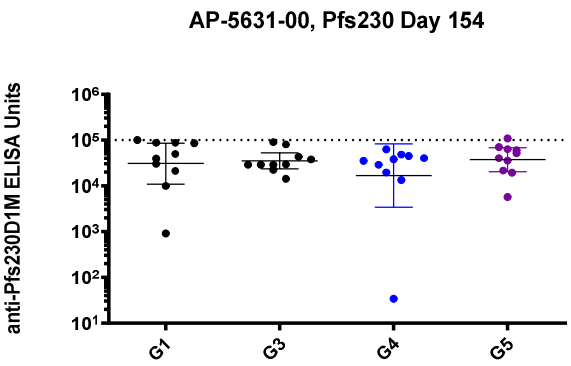


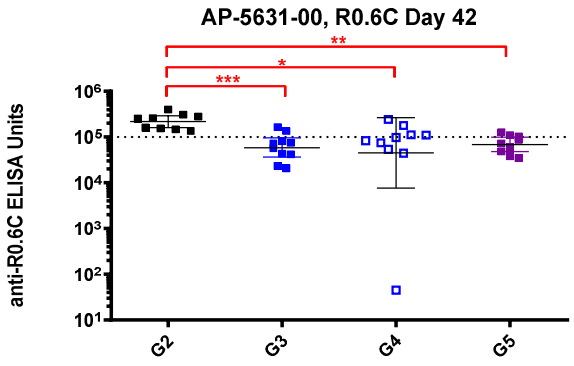
Figure 14: Graph of all animals in Study on All study days

G1	G2	G3	G4	G5
Pfs230D1-EPA/5 µg Matrix-M	R0.6C/Alhydrogel+5 µg Matrix-M	Pfs230D1-EPA/2.5 µg Matrix-M + R0.6C/Alhydrogel+2.5 µg Matrix M Co-Administered Each vaccine dosed in the same limb	Pfs230D1-EPA/5 µg Matrix-M + R0.6C/Alhydrogel Each vaccine dosed in the same limb	Pfs230D1-EPA+R0.6C/Alhydrogel+5 µg Matrix-M Coformulated

Figure 14: Dose dependent Pfs230D1M and R0.6C specific IgG responses by ELISA in CD-1 mice on multiple bleed days (28-154) following IM immunization on days 0 and 28. Error bars and line represent geometric mean (GM) and 95% confidence intervals.


AP-5631-00, Pfs230 Day 42
anti-Pfs230D1M ELISA Units
G1
G3
G4
G5


AP-5631-00, Pfs230 Day 154
anti-Pfs230D1M ELISA Units
G1
G3
G4
G5


AP-5631-00, R0.6C Day 42
anti-R0.6C ELISA Units
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*
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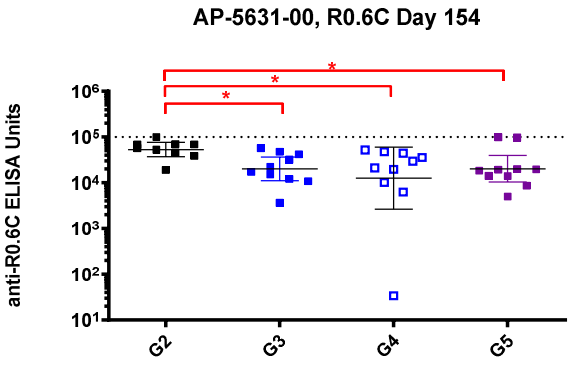
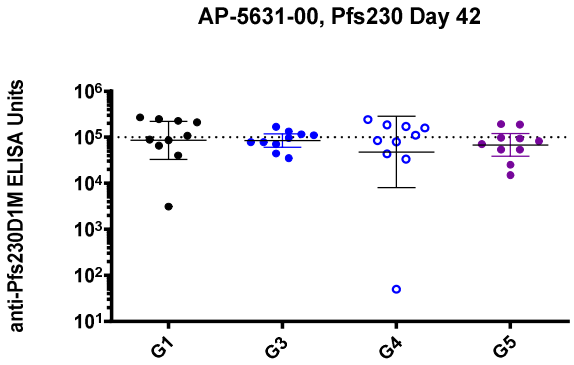
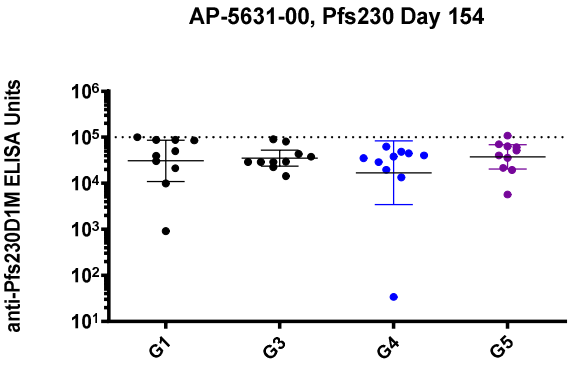
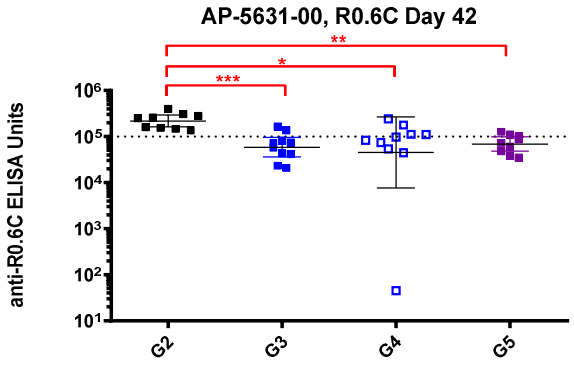
G2
G3
G4
G5


AP-5631-00, R0.6C Day 154
anti-R0.6C ELISA Units
*
*
*
G2
G3
G4
G5


AP-5631-00, Pfs230 Day 42
anti-Pfs230D1M ELISA Units
G1
G3
G4
G5


AP-5631-00, Pfs230 Day 154
anti-Pfs230D1M ELISA Units
G1
G3
G4
G5


AP-5631-00, R0.6C Day 42
anti-R0.6C ELISA Units
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*
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G2
G3
G4
G5

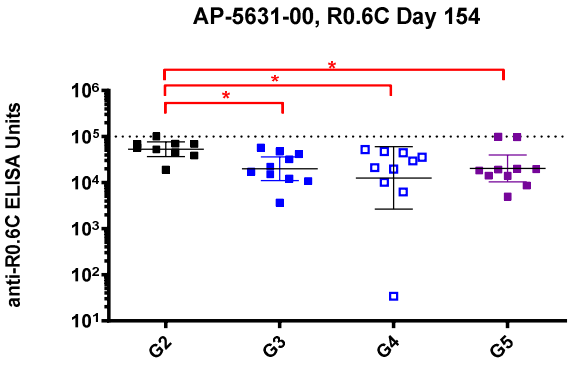

AP-5631-00, R0.6C Day 154
anti-R0.6C ELISA Units
*
*
*
G2
G3
G4
G5

Figure 15: Graph of Pfs230D1M and R0.6C antibody responses on Days 42 and 154

G1	G2	G3	G4	G5
Pfs230D1-EPA/5 µg Matrix-M	R0.6C/Alhydrogel+5 µg Matrix-M	Pfs230D1-EPA/2.5 µg Matrix-M + R0.6C/Alhydrogel+2.5 µg Matrix M Co-Administered Each vaccine dosed in the same limb	Pfs230D1-EPA/5 µg Matrix-M + R0.6C/Alhydrogel Each vaccine dosed in the same limb	Pfs230D1-EPA+R0.6C/Alhydrogel+5 µg Matrix-M Coformulated

Figure 15: Antibody kinetics of the Pfs230D1M and R0.6C specific IgG responses in CD-1 mice on Days 42 and 154. Error bars and line represent geometric mean (GM) and 95% confidence intervals. Demarcation at 10^5 is for ease of visual comparison between groups. Red lines (–) indicate significant differences between groups. For R0.6C responses, significant differences were observed by Kruskal-Wallace test followed by a Dunn's multiple comparison test, between G2 and G3 ((***) p = 0.0005), G2 and G4 ((*) p = 0.0167) and between G2 and G5 ((**) p= 0.0019) on Day 42, and between G2 and G3 ((*) p=0.0330), G2 and G4 ((*) p=0.0431) and G2 and G5 ((*) p=0.0264) on Day 154. There were no significant differences seen for Pfs230D1M IgG responses between groups on Days 42 or 154.

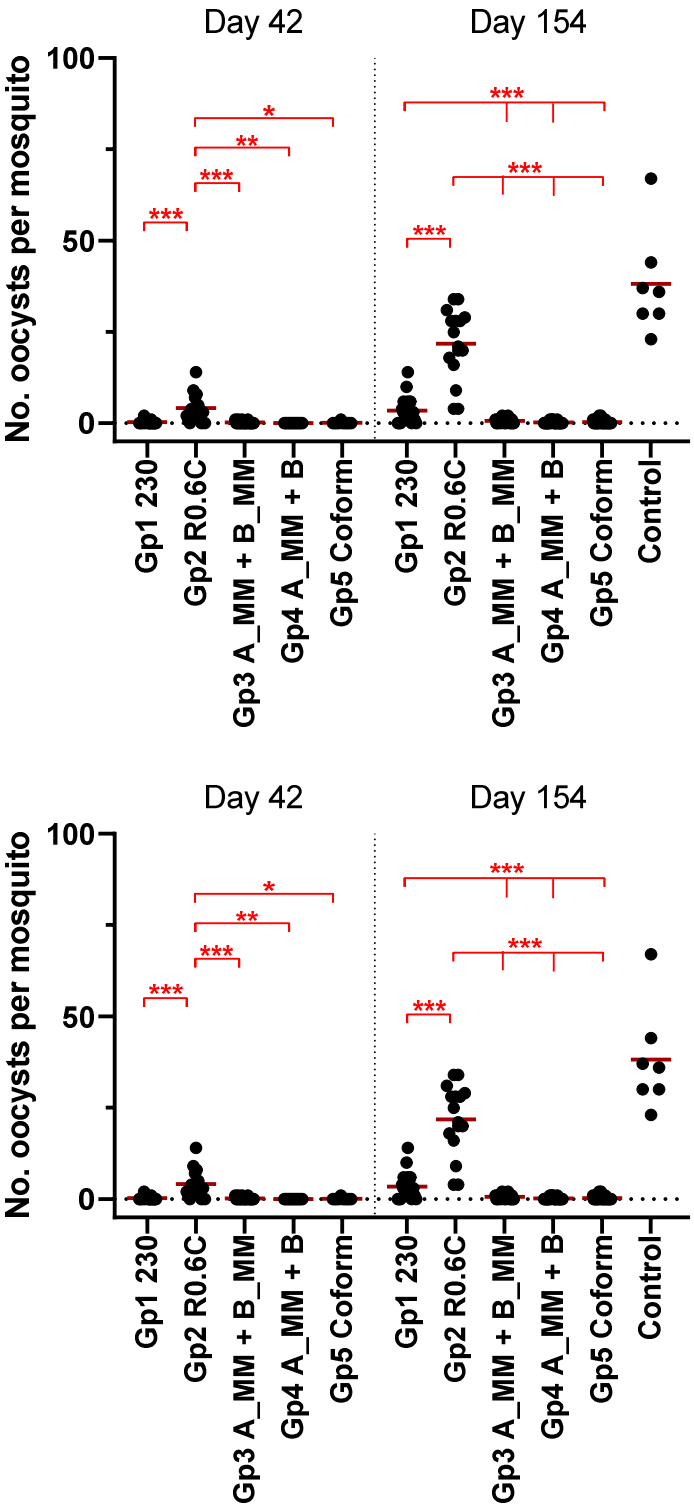


Figure 16: Standard membrane feeding assay (SMFA) results of Pfs230D1M and R0.6C

G1	G2	G3	G4	G5

Pfs230D1-EPA/5 µg Matrix-M	R0.6C/Alhydrogel+5 µg Matrix-M	Pfs230D1-EPA/2.5 µg Matrix-M + R0.6C/Alhydrogel+2.5 µg Matrix M Co-Administered Each vaccine dosed in the same limb	Pfs230D1-EPA/5 µg Matrix-M + R0.6C/Alhydrogel Each vaccine dosed in the same limb	Pfs230D1-EPA+R0.6C/Alhydrogel+5 µg Matrix-M Coformulated

Figure 16: Standard membrane feeding assay (SMFA) results of Pfs230D1M and R0.6C administered alone and in various combinations as described using pooled sera from CD-1 mice. One pool per Group was analyzed on Days 42 and 154 against a pre-immune pool as the negative control. Each feed included 30 µL of test serum diluted with 30 µL of a human serum pool that included complement and tested for the ability to block oocyst development in the mosquito midgut. Serum was mixed with *in vitro* cultured *P. falciparum* parasitized RBCs including mature sexual stages and fed to mosquitos that were dissected 7 days later. Significant differences in a pairwise comparison using a Bonferroni adjustment of 0.05/10 are noted: (*** = p <0.00001; ** = p = 0.000673; * = p=0.00215).
CD-1 mice immunized with Pfs230D1-EPA, R0.6C/Alhydrogel, or both in various combinations with the adjuvant Matrix-M, delivered in either a co-administration of two vaccines to the same limb or as a coformulation on Alhydrogel + Matrix-M did not significantly affect the Pfs230D1M specific IgG responses. The R0.6C specific IgG responses were significantly reduced by three to four-fold geometric mean titer between the combination groups and the single R0.6C group. The functional activity as assessed for TRA using a SMFA showed no significant differences at Day 42 between the Pfs230D1M single or combination groups, with significance noted between the R0.6C and all of combination groups. On Day 154, there was high significance ($p < 0.00001$) for each antigen between the single and all combination groups, respectively. The level of TRA observed for all three combination groups at Day 154 compared to the vaccines alone shows the presence of a combined activity of antibodies against Pfs230D1M and R0.6C to reduce transmission ($p<0.0005$). The results are shown in **Figure 16**.

2.4.2 Repeated Dose Toxicity Study by Intramuscular Administration in Rabbits

The objective of this study was to assess the potential toxicity and local tolerance of a Malaria Vaccine antigens (R0.6C) and the associated adjuvant (Alhydrogel® (AlOH)) administrated in combination with Pfs230D1-EPA, both with the Matrix-M adjuvant in NZW rabbits. The groups, dose levels, and animal numbers are detailed in **Table 12**.

The vaccine was administered four times by intramuscular injection over a period of 43 days. The injections were administered with an interval of 14 days between each treatment. A 4-weeks recovery period will be allowed.

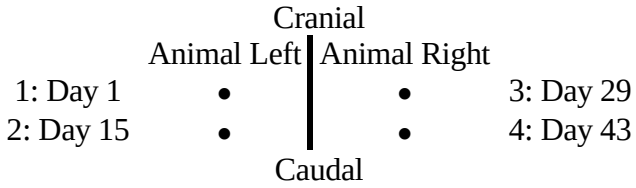
The main animals were terminated on Day 46 and the recovery animals on Day 71.

Table 12: Design for Rabbit Study of R0.6C-Alhydrogel® co-administered with Pfs230D1-EPA with or Matrix-M™ adjuvant

TOX STUDY DESIGN (STUDY NO. 48599)

Grp	Test Article	Dose	Route	Vaccination	Dose volume (ml)	Number of animals			
						Main D=46		Recovery D=71	
						Male	Female	Male	Female
1	Placebo (Saline)		i.m.	0, 14, 28, 42	0.5	5 1001 to 1005	5 1501 to 1505	5 1006 to 1010	5 1506 to 1510
2	Pfs230D1- EPA + Diluent + Matrix-M™ co-administered w/ R0.6C-AlOH+Matrix-M	40 100	i.m.	0, 14, 28, 42	0.4 0.57	5 5001 to 5003; 5006 to 5007	5 5501 to 5503; 5506 to 5507	5 5004 to 5005; 5008 to 5010	5 5504 to 5505; 5508 to 5510

The injections were placed in the following way:



- Site 1: intramuscular injection on Day 1: right hind leg (posterior thigh muscle).
- Site 2: intramuscular injection on Day 15: left hind leg (posterior thigh muscle).
- Site 3: intramuscular injection on Day 29: right hind leg (anterior thigh muscle).
- Site 4: intramuscular injection on Day 43: left hind leg (anterior thigh muscle).

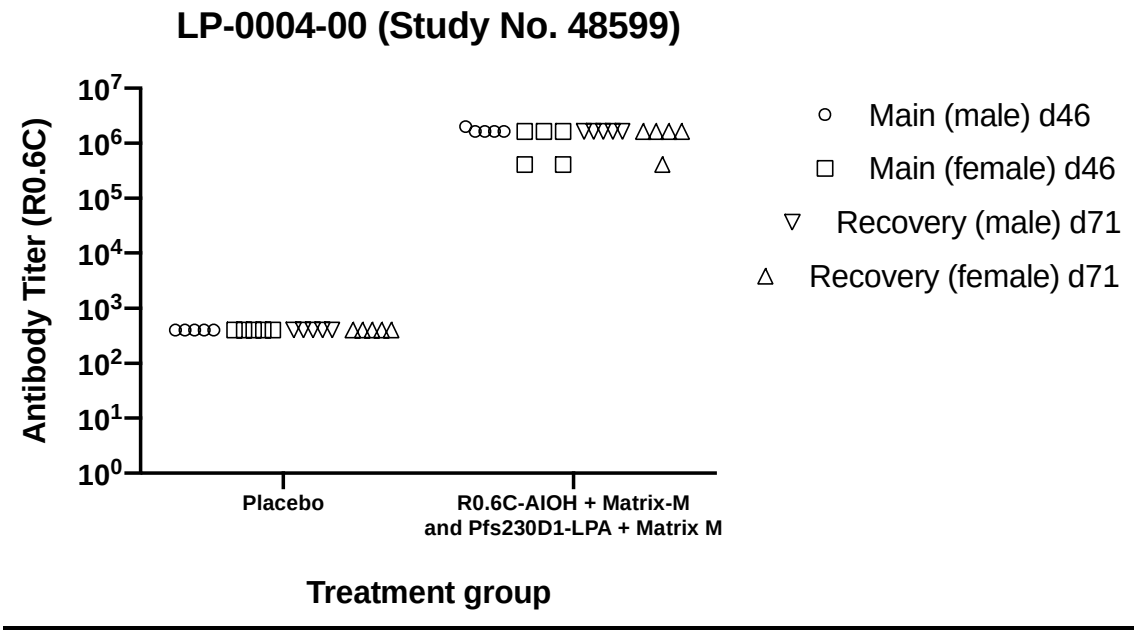
The following parameters were evaluated in the study: Clinical signs, local reactions at the injection sites, body weight, food consumption, body temperature, clinical pathology (haematology, coagulation and clinical chemistry) and antibody analysis. At necropsy, a full pathological evaluation was performed including organ weights and macroscopic and microscopic examination.

2.4.2.1 Serology Assessments by ELISA

The Serology assessment for antibodies elicited by the co-administration of the two test articles were performed independent (e.g. ELISA for Pfs48/45 R0.6C antigen and ELISA for Pfs230D1) by the respective manufacturing sites (SSI and NIH).

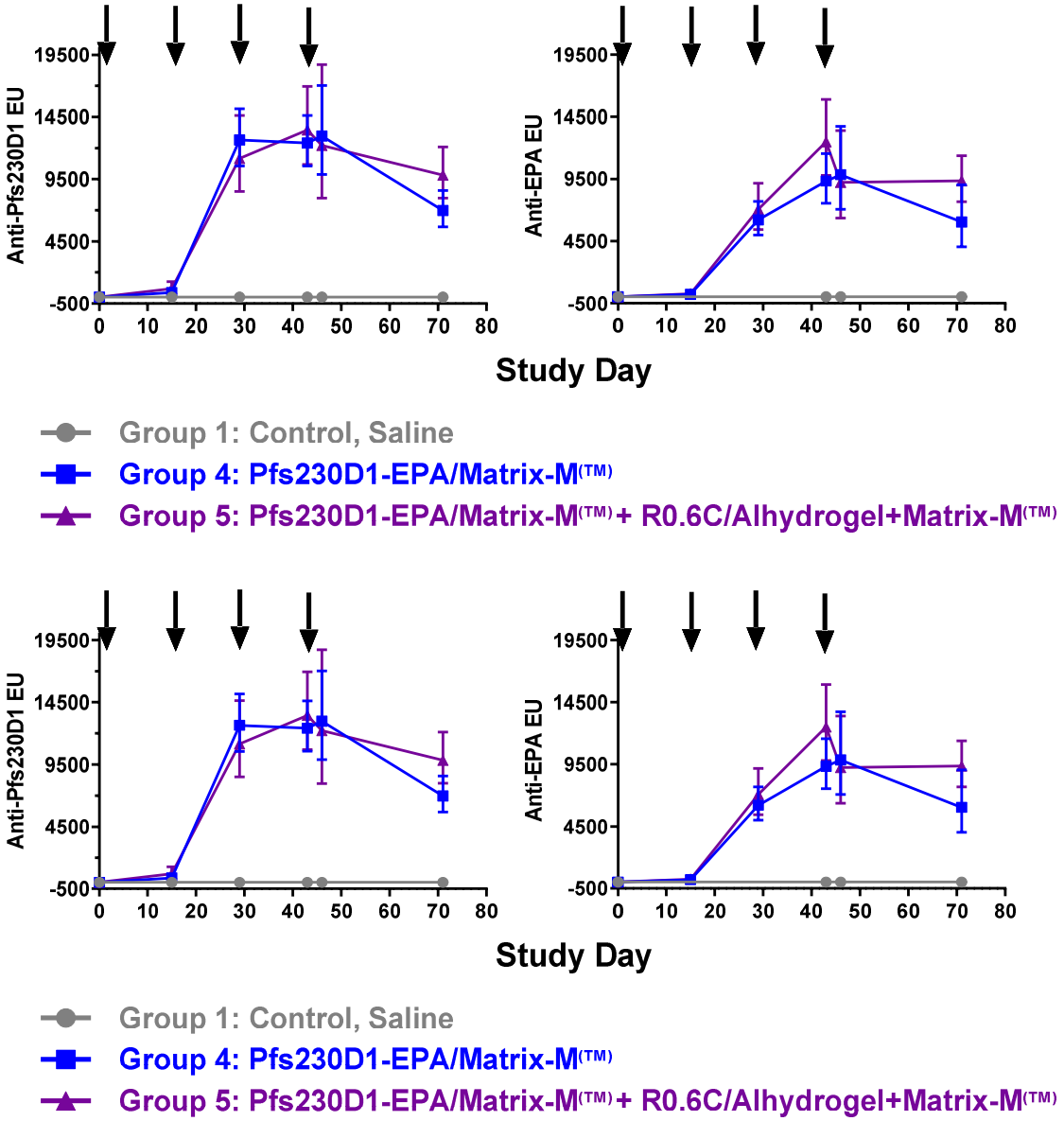
Pfs48/45 serology

The test article group R0.6C-AlOH + Matrix-M when co-administered with Pfs230D1-EPA showed a significant Pfs48/45 antibody response with antibody titers as calculated (> 102,400 fold dilution) with the majority of animals' demonstrating a response > 1,638,400. The placebo test article (Group 1) did not induce an immune response for all animals as detected here, above those values of background, negative control and pre-bleed specimens.



Pfs230D1 Serology

All individual samples were evaluated for Pfs230D1M specific antibody responses. The results are shown in Figure17. A pool of pre-bleed sera from all samples was assessed for EPA specific antibody responses, and all individual samples were evaluated for EPA specific antibody responses. Terminal (Day 46 and Day 71) titers are reported n Figure 17. All immunized animals responded to the Pfs230D1-EPA vaccine, as evaluated by ELISA against Pfs230D1M and EPA for specific antibody (IgG) responses. No animals in the Control group (Group 1) had detectable antibodies against Pfs230D1M or EPA. The vaccine was immunogenic.



Anti-Pfs230D1 EU
Anti-EPA EU
19500
14500
9500
4500
-500
0 10 20 30 40 50 60 70 80
Study Day
Group 1: Control, Saline
Group 4: Pfs230D1-EPA/Matrix-M™
Group 5: Pfs230D1-EPA/Matrix-M™+ R0.6C/Alhydrogel+Matrix-M™

Figure 17: Graph of Anti-Pfs230D1M and Anti-EPA ELISA titers for Group 1 (Saline Control), Group 4 (Pfs230D1-EPA/Matrix-M™) and Group 5 (Pfs230D1-EPA/Matrix-M™ + R0.6C/Alhydrogel+Matrix-M™)

Figure 17. Rabbit antibody responses following immunization with 40 µg Pfs230D1-EPA/50 µg Matrix-M™ (Group 4)or 40 µg Pfs230D1-EPA/ 25 µg Matrix-M™ in a coadministration with 100 µg R0.6C/Alhydrogel + 25 µg Matrix-M™ (Group 5) or saline as a Control (Group 1). Graphs show the geometric mean of individual animal antibody titers against Pfs230D1M or EPA in vaccine groups as a function of time; error bars represent the Geomean with 95% CI. Bleed days include the pre-bleed (Day 0), and final bleeds (Day 46 or 71) for Pfs230D1M, and pre-bleed pools, and final bleeds (Day 46 or 71) for EPA.

2.4.2.2 Toxicology assessment in Rabbits

No unscheduled deaths and no clinical signs indicative of systemic toxicity were reported during the study. There were no vaccine-related local reactions, no ophthalmological findings and no effects on body weight, food consumption or rectal temperature.

Slight changes observed in the clinical pathology parameters were suggestive of a transient systemic inflammatory reaction following vaccine administration and consisted of increases in fibrinogen and/or CRP concentrations.

All test item groups showed a significant antibody response. The placebo test article (group 1) did not induce an immune response in any animals.

Various findings consistent with the expected immune response to injection of immunogenic material and adjuvants (Sellers et al., 2020) were present at injection sites in groups dosed with the candidate vaccines. These included mixed inflammatory cell (heterophils and lymphocytes) infiltrates for all candidate vaccines, in association of variably degenerated macrophages/multinucleated giant cells typical for AlOH-adjuvanted vaccines (Pfs230D1-EPA + Diluent + Matrix-M1™ co-administered with R0.6C-AlOH + Matrix-M1™), and increased edema and/or hemorrhage for Pfs230D1-EPA + Diluent + Matrix-M1™ co-administered with R0.6C-AlOH + Matrix-M1™.

Infiltrates of macrophages and multinucleated giant cells were still observed at late euthanasia, 4 weeks after the injection, which is classically observed after injection of AlOH-adjuvanted adjuvants (Verdier et al, 2005). Mixed inflammatory infiltrates related to AlOH-adjuvanted vaccines were decreased in incidence and/or severity at early euthanasia at sites dosed on Day 1 relative to sites dosed on Day 43, and at late euthanasia, indicating ongoing recovery, whereas edema and hemorrhage were seen only at early euthanasia at sites dosed on Day 43 and were not present at late euthanasia i.e. full recovery of the acute changes. Macrophage degeneration/necrosis was also fully reversible. Microscopic findings related to Pfs230D1-EPA + Matrix-M1™ were seen at early euthanasia only at sites dosed on Day 43, and not on sites dosed on Day 1, and were not observed at late euthanasia.

In lymphoid tissues, partially reversible increased lymphoid cellularity, mainly characterized by increased size and/or number of germinal centers, was present in the spleen and lymph nodes draining the sites (iliac, popliteal and inguinal, both sides) and variably correlated with macroscopic enlargement and increased weights of the spleen and lymph nodes. These data demonstrated systemic and local immune responses to the candidates vaccines (Sellers et al., 2020). Minor other associated changes present in the draining lymph nodes with all candidate vaccines consisted of infiltrates of macrophages/multinucleated giant cells, infiltrates of heterophils and intrasinusoidal erythrocytes.

In Conclusion
Four intramuscular administrations of Malaria vaccine candidates to New Zealand White rabbits were clinically well tolerated.

Microscopic findings at administration sites were consisted with the expected local immune

response to injection of immunogenic material and included partially reversible mixed inflammatory cell infiltrates, macrophage/multinucleated giant cell infiltrates typical for AlOH-adjuvanted vaccines (Pfs230D1-EPA + Diluent + Matrix-M1™ co-administered with R0.6C-AlOH + Matrix-M1™), and fully reversible increased edema and/or hemorrhage (Pfs230D1-EPA + Diluent + Matrix-M1™ co-administered with R0.6C-AlOH + Matrix-M1™).

Partially reversible increased lymphoid cellularity was present in the spleen, iliac, popliteal and inguinal lymph nodes, demonstrating systemic and local immune responses to the candidate vaccines.

All changes were expected after vaccine administration and were indicative of a local and systemic inflammatory process and the initiation of an innate and adaptive immune response. None of these changes were considered to be adverse.

3 Previous Clinical Experience

3.1 R0.6C-AlOH/Matrix-M™

The R0.6C-AlOH/Matrix-M™ vaccine is currently being evaluated for safety and tolerability in humans through two clinical trials. A Phase I in The Netherlands (STOP-TRANS, NCT04862416) initiated in May 2021 study is on-going with subjects receiving both a 30µg or 100µg of R0.6C adjuvanted with Alhydrogel alone, or combined with Matrix-M1without any vaccine-related SAEs. A Phase I (TBVax1) study is expected to start in March 2022 in Burkina Faso evaluating both 30µg or 100µg of R0.6C or ProC6C adjuvanted with Alhydrogel alone, or combined with Matrix-M1.

RUMC (OptiMalVax Horizon2020 funding) NCT04862416: Safety, Tolerability and Plasmodium falciparum transmission-reducing activity of R0.6C vaccine adjuvanted with Alhydrogel alone or combined with Matrix-M in healthy malaria-naïve adults in the Netherlands (STOP-TRANS). Thirty-two healthy adult volunteers will be recruited and divided over the study arms that will receive four vaccinations on days 0, 28, 56 and 168 with either 30µg or 100µg of R0.6C adjuvanted with Alhydrogel alone or combined with Matrix-M1. All volunteers will be followed up for adverse events until 84 days after the last immunisation. Total trial duration is approximately 8 months for each subject. Immunogenicity and serum SMFA activity will be assessed.

USTTB (Burkina Faso) through PfTBV EDCTP RIA2018SV-2311: 2019-2024: Phase 1 Dose Escalating, Double-Blind, Randomised Comparator Controlled Trial of Different Adjuvant Formulations of R0.6C and ProC6C transmission blocking vaccines against Plasmodium falciparum in Adults in Burkina Faso (TBVax1). The study is a first-in-human phase Ib, double blind randomized controlled, dose escalation study in healthy, malaria exposed adults aged 20 – 45 years who receive three intramuscular vaccinations on days 0, 28, 56 with R0.6C or ProC6C adsorbed to AlOH alone or combined with an additional adjuvant Matrix-M. Dose escalation with the two adjuvant arms will be staggered starting with the lower dose group.

In addition, the GLURP-R0 region, which constitute 76.7% of R0.6C, forms part of the malaria vaccine candidate, GMZ2, which has been tested in both European and African clinical trials **Table 14**. Notably, GMZ2, adjuvanted with aluminum hydroxide (GMZ2/AlOH) was tested in 880 children 12-60 month of age in Burkina Faso (Banfora; n=580, Sapone; n=300).

To date 994 individuals, of whom the majority are African children below 5 years of age, have been immunized with GMZ2/AlOH. The GMZ2/AlOH formulation has shown excellent safety and tolerability confirming the safety of the expression system and the safety of the GLURP-R0 portion of R0.6C.

Table 13: Clinical experience of GMZ2/AlOH.

Trial registration number	Title	Conditions	Interventions	Characteristics	Population	Reference
NCT00397449	Safety and immunogenicity of GMZ2 - a MSP3-GLURP fusion protein malaria vaccine candidate	Malaria, *P. falciparum*	Biological: 10µ GMZ2/AlOH 30µ GMZ2/AlOH 100µg GMZ2/AlOH	Phase 1a	30 healthy malaria-naïve German adults; 10 / group	(Esen et al. 2009)
NCT00424944	Safety and immunogenicity of the malaria vaccine candidate GMZ2 in malaria-exposed, adult individuals from Lambaréné, Gabon	Malaria, *P. falciparum*	Biological: 100µg GMZ2/AlOH VerorabTM	Phase 1b	40 healthy malaria-exposed Gaboneese adults; 20 / group	(Mordmuller et al. 2010)
NCT00703066	A Randomized Controlled Phase Ib Trial of the Malaria Vaccine Candidate GMZ2 in African Children	Malaria, *P. falciparum*	Biological: 30µ GMZ2/AlOH 100µg GMZ2/AlOH VerorabTM	Phase 1b	30 healthy malaria-exposed Gaboneese children one to five years of age; 10 / group	(Belard et al. 2011)
PACTR20100 60002033537	A phase 2b randomized, controlled trial of the efficacy of the GMZ2 malaria vaccine in African children	Malaria, *P. falciparum*	Biological: 100µg GMZ2/AlOH VerorabTM	Phase 2b	1735 healthy malaria-exposed Gaboneese children received three doses of vaccine (868 GMZ2, 867 control-vaccine).	(Sirima et al. 2016)

GMZ2/AlOH

GMZ2, adjuvanted with aluminum hydroxide (GMZ2/AlOH) is under clinical development since October 2006, when the first volunteer was injected with 10 µg GMZ2 in Tübingen, Germany (Esen et al. 2009). In this trial, GMZ2 was injected subcutaneously three times in 4-week intervals at a dose of 10, 30, or 100 µg. Each dosing group consisted of 10 healthy, malaria-naïve individuals. All doses elicited an adequate immune response, although a trend towards better immunogenicity of the higher doses was observed. After this first dose-escalation trial, GMZ2 has been tested in two clinical trials performed in Gabon. The first trial was a randomized controlled trial involving malaria-exposed Gabonese adults comparing the higher dose of GMZ2 (100 µg) to a control vaccine (rabies vaccine). In this study, the vaccine was well tolerated and boosted preexisting immune responses against the vaccine antigen (Mordmuller et al. 2010). Second, a randomized controlled trial involving malaria-exposed African children 1–5 years of age was performed in which 3 doses of either 30 µg or 100 µg of GMZ2 was compared to a control rabies vaccine (Belard et al. 2011). Generally, GMZ2 was immunogenic, safe, and well tolerated in all 3 clinical trials.

The GMZ2/AlOH formulation has subsequently been tested in a multi-center Phase IIb efficacy trial involving 1849 children living in Burkina Faso, Gabo, Ghana, and Uganda.

To date 994 individuals, of whom the majority are African children below 5 years of age, have been immunized with GMZ2/AlOH. The GMZ2/AlOH formulation has shown excellent safety and tolerability confirming the safety of the expression system.

3.1.1 Clinical Phase I trial, subcutaneous administration of GMZ2 and AlOH

The first-in-man Phase Ia clinical trial with three different doses (10 µg, 30 µg, or 100 µg) of GMZ2, adjuvanted with AlOH, were studied in Tübingen Germany. GMZ2 showed good safety and immunogenicity (Esen et al. 2009). Immunogenicity was evaluated by the analysis of total IgG against GLURP, MSP3 and the combined vaccine antigen (GMZ2) by ELISA. The quality of the humoral immune response was assessed by measuring IgG1, IgG2, IgG3, and IgG4 responses to GMZ2 by ELISA. At all vaccine doses there was an increase of IgG against all antigens four weeks after the second and third doses. On Day 84 vaccine-induced IgG against the vaccine antigen reached levels comparable to randomly chosen, adult, semi-immune individuals from Lambaréné. One year after the first dose (Day 365) specific antibody-levels and B cell memory was still detectable.

GMZ2 was generally well tolerated. Grade 3 local reactions were present after the third dose in four individuals within 24 hours after vaccine administration. They consisted of erythema and indurations in all cases; in addition one individual in the 10 µg group presented with oedema. One serious adverse event was reported on 185 days after the third administration (fracture of the 12th thoracic spine with hospitalization). The adverse event was judged not to be related to the investigational product. The volunteer recovered with sequelae. All but one individual received all vaccinations. The volunteer was excluded from receiving the third vaccination in the 100 µg group for reasons unrelated to investigational product. One pregnancy was recorded after the third vaccination; unfortunately, this individual was lost to the subsequent follow-up. The other loss to follow-up was due to the relocation of one participant.

3.1.2 Clinical Phase I trial, intramuscular administration of GMZ2 and AlOH

A randomized controlled trial involving malaria-exposed Gabonese adults compared the higher dose of GMZ2 (100 µg) to a control vaccine (rabies vaccine) (Mordmuller et al. 2010). After correction for baseline values, a similar response-pattern as in non-exposed Europeans was present: (i) GMZ2 boosted antigen-specific IgG responses against GMZ2 and its constituents GLURP and MSP3, whereby the anti-MSP3 response was weakest, (ii) GMZ2-induced antigen-specific IgG subclasses were of cytophilic nature and, in contrast to the previous trial, included IgG3 and (iii) the number of GMZ2-specific memory B-cells one month after the last vaccination (Day 84) was higher in GMZ2 compared to rabies vaccinated individuals. Six individuals experienced seven malarial episodes during the trial. The individuals were equally distributed

among the two interventional groups and no distinctive pattern was observed. Since all individuals in the trial have naturally acquired immunity to malaria and the vaccine is intended to induce exactly this type of immunity, it was not surprising that no difference between the groups was present.

In the Phase Ib study in healthy adults a total of 10 serious adverse events (SAE) in seven participants were recorded: six SAE from four subjects in the rabies vaccine group and four SAE in three individuals in the GMZ2 group. One SAE was initially considered possibly related to GMZ2 but was subsequently found to be due to a persistent Loa loa infection. All other SAE were judged not to be related to the intervention. One death due to hepatocellular carcinoma occurred in the rabies vaccine group. A total of seven malarial episodes in six individuals occurred: three episodes in three participants in the rabies vaccine and four episodes in three participants in the GMZ2 group. After the third vaccination four participants experienced four malaria episodes (two in the rabies vaccine and two in the GMZ2 group). No grade 3 solicited adverse events was reported throughout the study. Within 30 minutes after injection, grade 2 pain at the injection site was reported after rabies vaccination on four and after GMZ2 administration on nine occasions with no increase in frequency or severity due to previous vaccination. Only one grade 1 immediate systemic reaction was encountered (headache).

3.1.3 Clinical Phase I trial, intramuscular administration of GMZ2 and AlOH

A randomized controlled trial involving malaria-exposed healthy African children 1–5 years of age was performed in which 3 doses of either 30 μg or 100 μg of GMZ2 was compared to a control rabies vaccine (Belard et al. 2011). Local reactions at the injection sites, including pain on touch or pain when the limb was moved, swelling, induration, erythema, pruritus were the most common. Induration was the most important (50.5 % of local reactions) and was mostly observed in the GMZ2 vaccine groups (71.4 % of induration). All of these reactions were of mild intensity, short duration and well tolerated by the participants in the three vaccine groups and all disappeared after 3 days. Of the solicited systemic reactions occurring 30 minutes to 14 days after the vaccine dose the most important were loss of appetite (37.5%) mainly in both GMZ2 vaccine groups (30 μg and 100 μg) and fever (25%). These were of mild intensity and tolerated by the participants. There were no serious adverse events. There were four grade 3 solicited adverse events including fever and loss of appetite. All of these adverse events were considered not to be related to vaccination.

3.1.4 Clinical Phase II trial of intramuscular administration of GMZ2 and AlOH

A randomized, controlled, multicenter, double blind phase 2 trial to measure VE of GMZ2/AlOH in African children (Sirima et al. 2016). Participants were allocated in a 1:1 ratio to receive three doses of either GMZ2/AlOH or the control vaccine (rabies, Verorab) four weeks apart, and were followed for six months.

Between November 2010 and September 2011, 1849 children were enrolled to receive GMZ2/AlOH or the control Rabies vaccines. Demographics and other baseline characteristics were similar in the two groups although there was a slightly higher proportion of older children in the rabies vaccine group.

Vaccine doses were well tolerated. There were 17 individuals in the rabies group and 29 in the GMZ2/AlOH group with solicited, grade 3 AE within seven days of a dose. Of these, four in the rabies group (two induration and two fever ≥39 ºC) and five in the GMZ2/AlOH group (one swelling and four fever ≥39 ºC) were reported as related to the study vaccine. Three grade 3 unsolicited vaccine related AEs were reported within 28 days of a dose, two in the rabies group and one in the GMZ2/AlOH group. During the six months follow-up period post dose 3, five children died, two in the GMZ2/AlOH group (Pneumonia and severe malaria) and three in the control group (sudden death, drowning, and malaria convulsions). There were 68 other SAEs (35 in the rabies group and 33 in the GMZ2/AlOH group) most of these were malaria. Two of the SAEs were considered related to vaccination, both events in one individual who had received rabies vaccine.

3.2 ProC6C-AlOH/Matrix-M™

The ProC6C-AlOH/Matrix-M™ vaccine is currently being evaluated for safety and tolerability in humans through a clinical trial, expected to start in March 2022 in Burkina Faso. The Phase I (TBVax1) study is on-going in Burkina Faso evaluating both 30µg or 100µg of R0.6C or ProC6C adjuvanted with Alhydrogel alone, or combined with Matrix-M1.

USTTB (Burkina Faso) through PfTBV EDCTP RIA2018SV-2311: 2019-2024: Phase 1 Dose Escalating, Double-Blind, Randomised Comparator Controlled Trial of Different Adjuvant Formulations of R0.6C and ProC6C transmission blocking vaccines against Plasmodium falciparum in Adults in Burkina Faso (TBVax1). The study is a first-in-human phase Ib, double blind randomized controlled, dose escalation study in healthy, malaria exposed adults aged 20 – 45 years who receive three intramuscular vaccinations on days 0, 28, 56 with R0.6C or ProC6C adsorbed to AlOH alone or combined with an additional adjuvant Matrix-M. Dose escalation with the two adjuvant arms will be staggered starting with the lower dose group.

3.3 Pfs230D1M-EPA

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

Summary: 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 US and Bancoumana, Mali (NIAID protocol #15-I-0044; clinicaltrials.gov: NCT02334462). Another TBV candidate, Pfs25M-EPA/Alhydrogel, was also assessed as a stand-alone vaccine and also co-administered with Pfs230D1M-EPA/Alhydrogel (Erreur ! Source du renvoi introuvable.**14**). In summary, the studies in the US and Mali established that Pfs230 vaccine was superior to the Pfs25 vaccine for inducing functional activity, and that the

combination of Pfs230 and Pfs25 was not superior to Pfs230 vaccine alone (Healy, Anderson et al. 2021).

Table 14: 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.

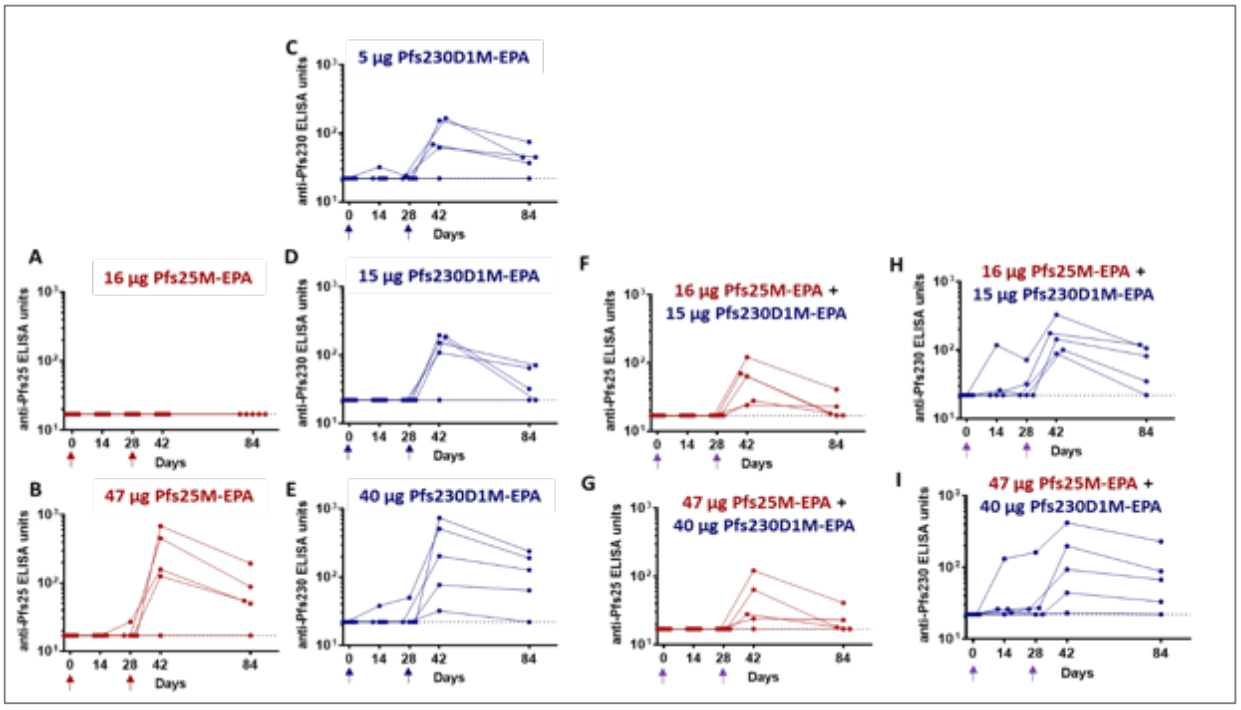
3.3.2 Safety of Pfs230D1M-EPA/Alhydrogel in Healthy Adults

In both the US and Mali, 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). Safety analysis of the high dose (40 µg) of Pfs230D1M-EPA/Alhydrogel showed that many reported AEs were mild (Grade 1; 708/1431; 49%), with the most commonly reported AEs being injection site pain, headache, malaria, neutropenia, nasopharyngitis, and rhinitis. The majority of laboratory abnormalities were Grade 1. The most commonly reported related AEs were injection site reactogenicity (pain, induration, pruritus, and edema), leukopenia, neutropenia, and headache, which were all Grade 1 or 2. The related AE reported with the highest frequency was injection site pain, which did not increase in frequency with subsequent vaccination. However, overall, reported local reactogenicity, but not solicited systemic symptoms, appeared to increase (in frequency and duration of symptoms) with increasing antigen dose of Pfs230. In comparison to the comparator arms, more related AEs were reported for the Pfs230D1M vaccinees, alone or in combination, and the majority of these were Grade 1 or 2 local reactogenicity.

In a single Pfs230-vaccinated subject in Mali, a Grade 3 gastroenteritis was reported with associated Grade 4 laboratory abnormalities (leukocytosis, increased blood creatinine), all deemed unlikely related to vaccination and all of which resolved shortly after resolution of the gastroenteritis symptoms. Other than this case, there were no Grade 3 or 4 AEs. There were 3 unrelated SAEs, one of which was a cerebrovascular accident that led to death. No SAEs were reported in the Pfs230-vaccinated arms. No participants were removed from study participation due to a related AE of any severity.

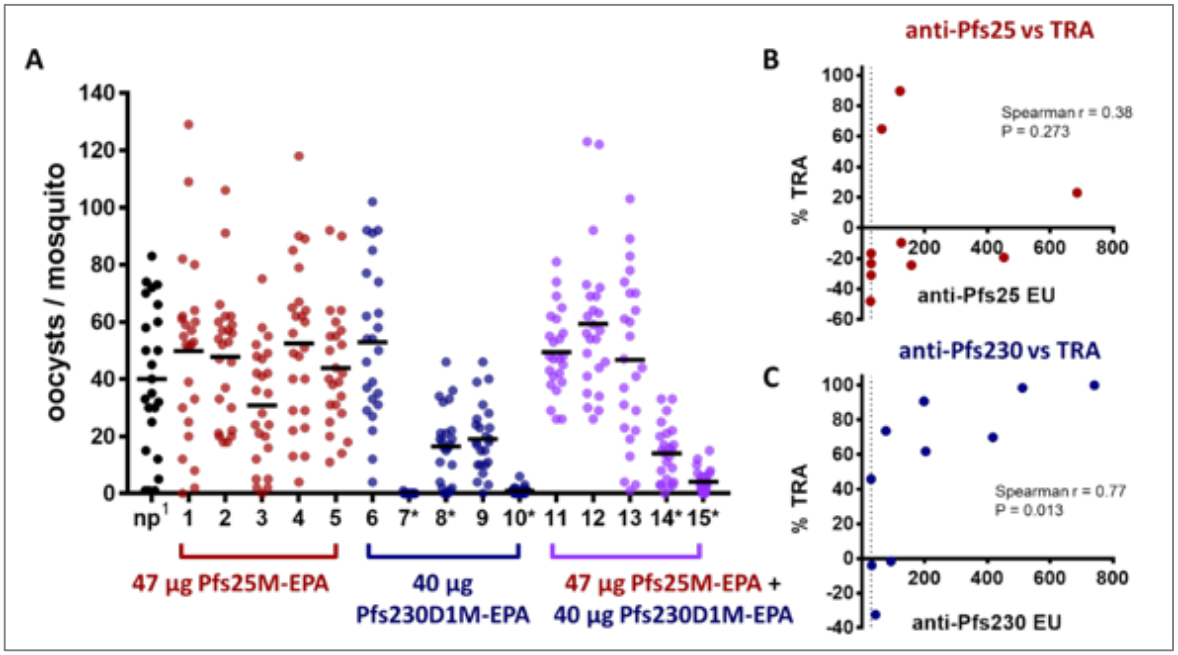
3.3.3 Immunogenicity and Functional Activity of Pfs230D1M-EPA/Alhydrogel in Healthy Adults

Pfs230D1M induced antibody responses in most US vaccinees (**18**C-E) and resulted in high TRA (100%, 98% TRA) in 2 out of 5 individuals and significant TRA (73%, 62% TRA) in 2 others (Erreur ! Source du renvoi introuvable.**19**A) after just 2 vaccine doses. In the Pfs25M + Pfs230D1M combination group, antibody responses were similar to the individual antigen arms (18F-I), and 2 individuals had appreciable functional activity after 2 vaccinations (one had 90% and the other had 68% (19A). The activity correlated well with anti-Pfs230D1M titers, demonstrating that the functional activity was due to the vaccine (**Erreur ! Source du renvoi introuvable.**C). 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 (**Erreur ! Source du renvoi introuvable.**).



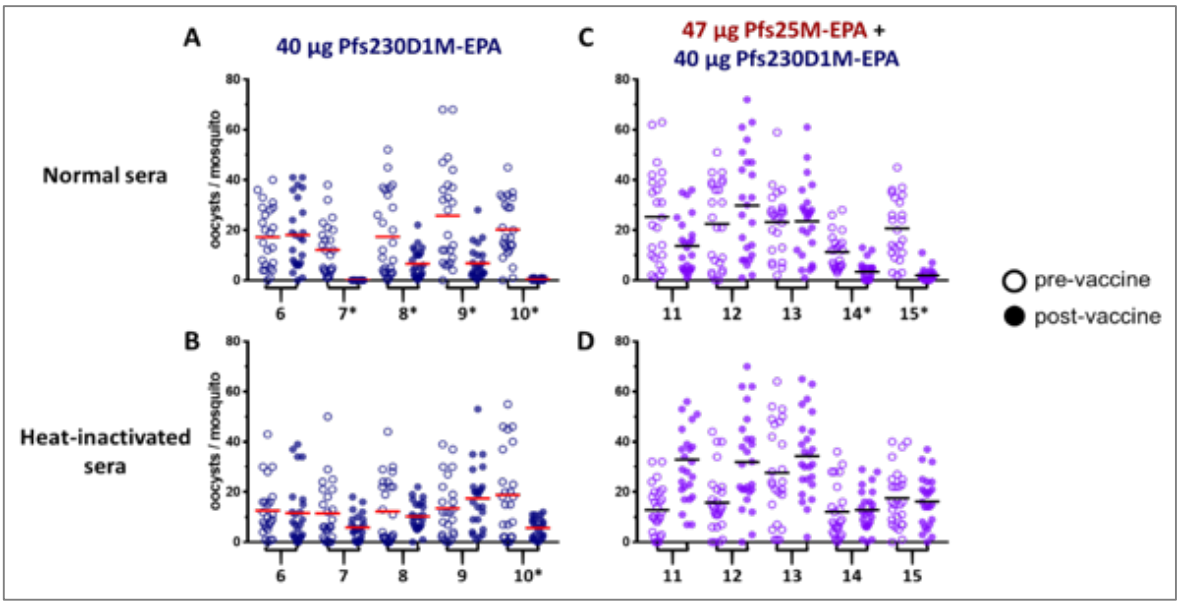
Results presented in enzyme-linked immunosorbent assay (ELISA) units for each arm. Vaccinations occurred on Days 0, 28. Day 0 was drawn pre-vaccination; Day 42 is 14 days post Vaccination #2. Each individual datapoint represents an individual subject anti-Pfs230 ELISA response.

Figure 18: Pfs230-specific Antibody Responses in Subjects Receiving Pfs230D1M, US Cohort (#15-I-0044).



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.

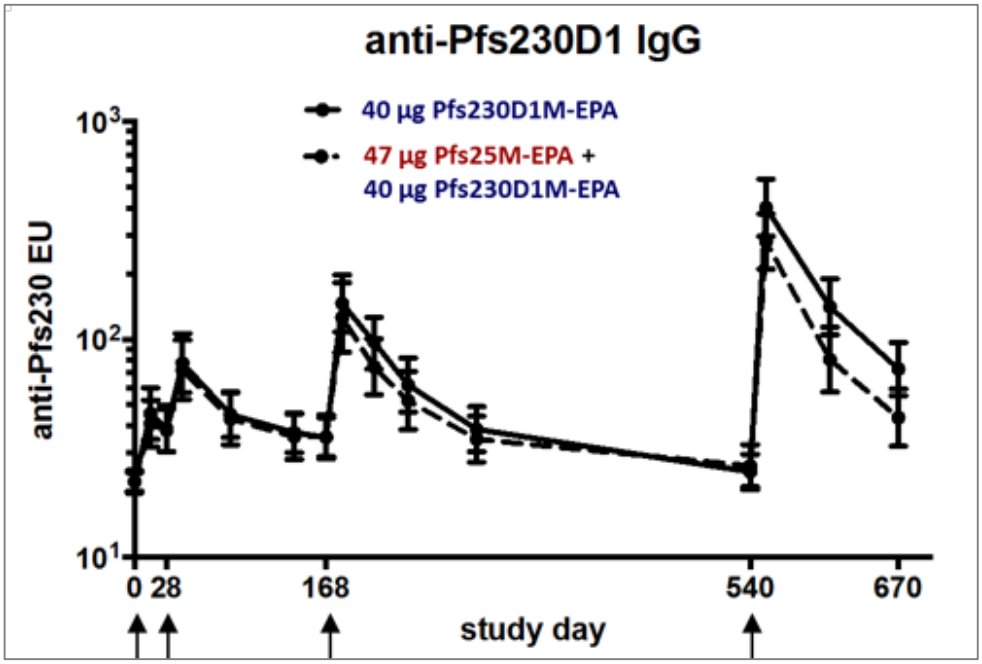
Figure 19: Pfs25 and Pfs230 Functional Activity by Standard Membrane Feeding Assay.



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.

Figure 20: Pfs230 Functional Activity by Standard Membrane Feeding Assay in the Presence or Absence of Complement.

Evaluation of immunogenicity by ELISA in healthy Malian 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 (**Erreur ! Source du renvoi introuvable.**). 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; **Erreur ! Source du renvoi introuvable.**) and percentage of responders (detectable antibody responses).



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.

Figure 21: Pfs230-specific Antibody Responses in Subjects Receiving Pfs230D1M, 40 µg, in Mali (#15-I-0044).

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.

3.3.4 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.

In summary, preliminary ELISA and SMFA results suggested that the two doses induced similar activity. In the main phase of the study, the 40-µg dosage was used for vaccination. Unblinding of a larger cohort of this trial, with 60 subjects in the full-dose arm (3 doses of 40 µg at 0, 1, and

6 months) and 60 subjects in a fractional-dosing arm (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), occurred in March of 2018. The results of the trial established the full 3-dose regimen using 40-ug dosage as the benchmark for future trials, and demonstrated safety and tolerability of the regimen. A booster dose of Pfs230D1M (40 µg) was administered approximately 12 months after the third immunization.

3.3.5 Safety of Pfs230D1M-EPA/AS01 in Healthy Adults

Pilot Phase
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 were mild (Grade 1; 13 µg: 23/29, 79%; 40 µg: 42/51, 82%), with the majority being injection site pain. Laboratory abnormalities were also observed, with the majority being transient, asymptomatic Grade 1 (mild) and Grade 2 (moderate) neutropenias. 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.

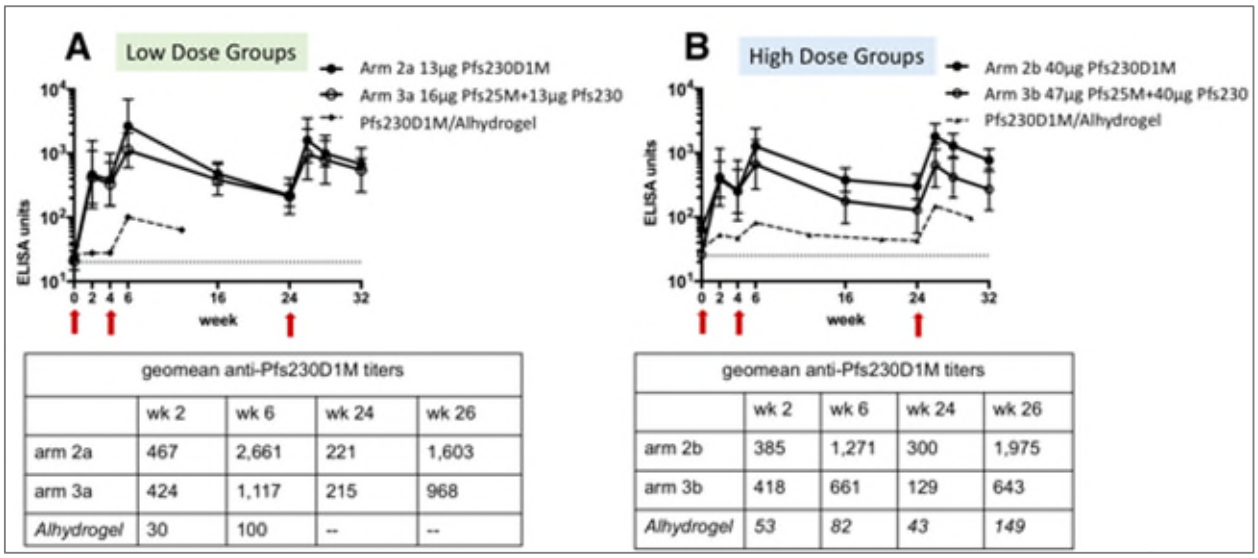
Main Phase
In the main phase of the trial, there was a full-dose arm (n=56) and a fractional-dose arm (n=61). The full-dose arm received Pfs230D1M-EPA/AS01 at 40 µg at 0, 1, 6, and 18 months. The fractional-dose arm received Pfs230D1M-EPA/AS01 at 40 µg at 0, 1, and 18 months and received a fractional dose of 8 µg of Pfs230D1M-EPA/AS01 at dose #3 at 6 months. Because there is no fractional dosing in the proposed trial, full details of the fractional dosing arm are not provided here; overall, the AE profile was similar to that of the full-dose arm, and further details can be found in the Investigator Brochure.

First 3 vaccinations: In the full-dose arm (n=56), the first three vaccinations were well tolerated with 66% (252/383) of the total AEs being mild. The most common Grade 1 AEs were injection site pain (95/252; 38%) followed by headache and malaria. 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. 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. Grade 2 injection site pain was also significantly greater in the full-dose and fractional-dose arms compared to the control. Grade 1 headaches were significantly greater in the full-dose arm than the control. Pyrexia was reported more frequently in the full-dose arm than the control arm.

Booster dose: The fourth dose of Pfs230D1M-EPA/AS01 was well tolerated; most AEs 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. Of the related Grade 2 AEs, injection site pain was most common (7/11, 63%) followed by headache, fatigue, and injection site movement impairment in one subject. There were no related Grade 3 AEs. Participants in the Pfs230D1M booster arm experienced significantly more Grade 1 injection site pain and headache than the comparator arm. Grade 2 injection site pain was also reported more frequently in the Pfs230D1M arm but was not significantly different from the comparator.

3.3.6 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 (**Erreur ! Source du renvoi introuvable.**). 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 (**Erreur ! Source du renvoi introuvable.**). 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 post dose 2. The combination of Pfs25 and Pfs230 was not superior to Pfs230 alone for inducing functional serum activity.

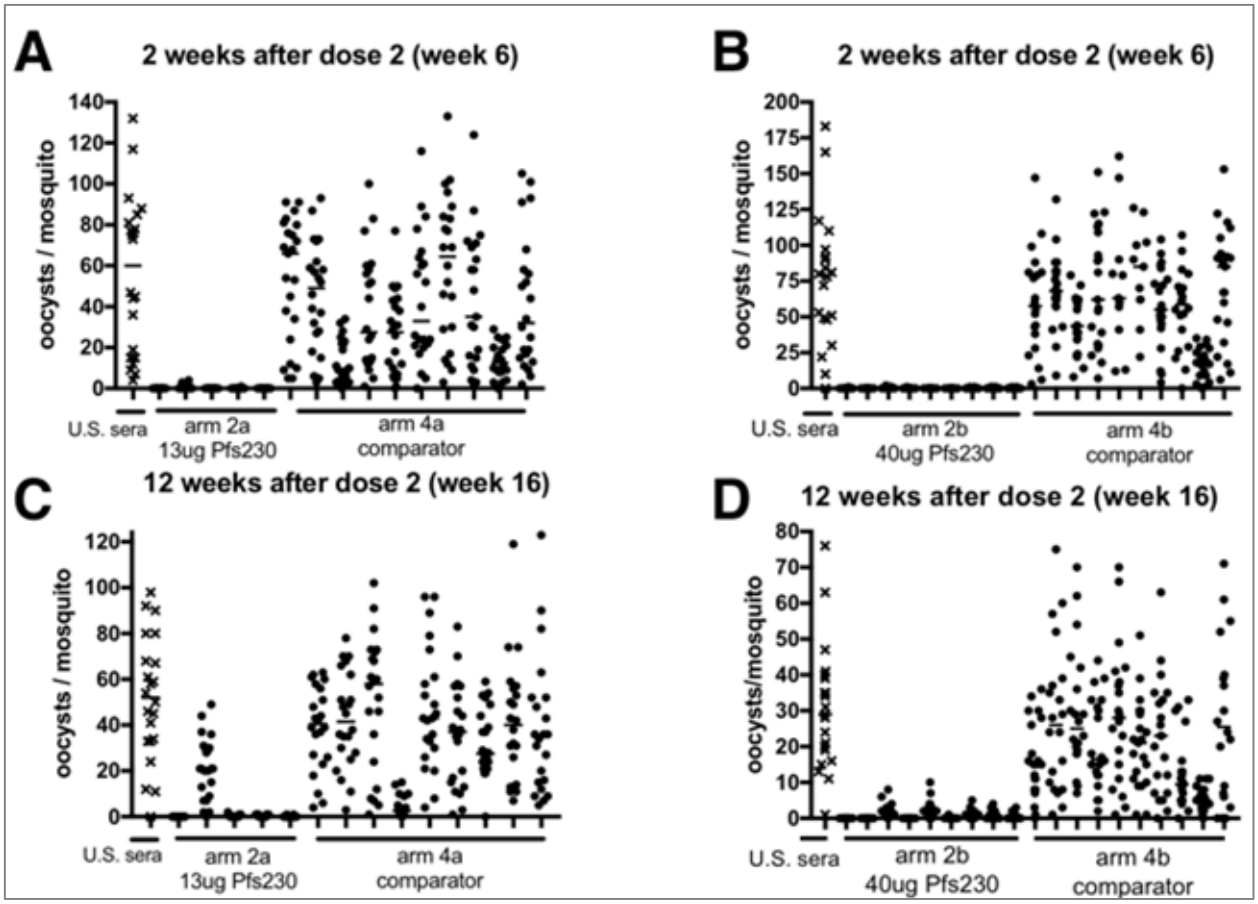


geomean anti-Pfs230D1M titers				
	wk 2	wk 6	wk 24	wk 26
arm 2a	467	2,661	221	1,603
arm 3a	424	1,117	215	968
Alhydrogel	30	100	--	--

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*

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.

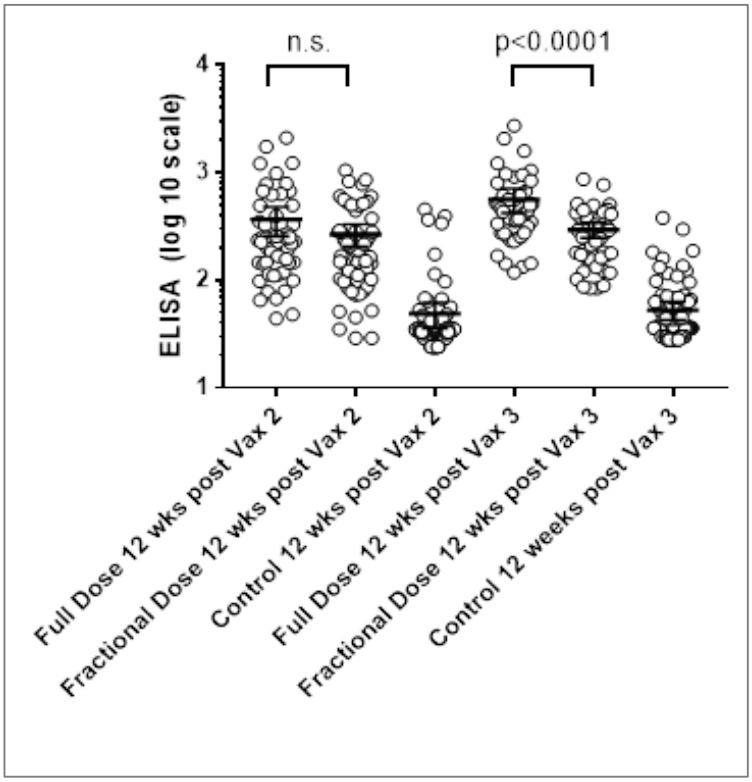
Figure 22: Antibody Responses to Pfs230D1M-EPA/AS01 after Low Dose and High Dose Vaccinations of Adults in Sotuba, Mali during Pilot Phase trial.



(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 US 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.

Figure 23: 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.

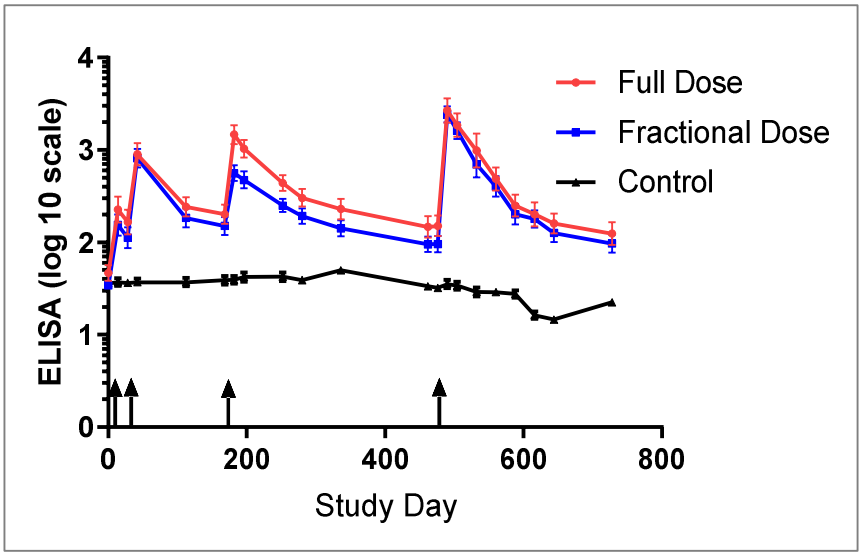
As was seen with the pilot phase, higher antibody titers were seen with AS01 adjuvant compared with what has been observed previously with other adjuvants. A significantly higher antibody titer measured by ELISA against Pfs230 was observed in both the full-dose and fractional-dose arms compared to the control arm after only 2 doses given at 0 and 1 months ($p<0.01$ in both; **Erreur ! Source du renvoi introuvable.**). Antibody titers were also assessed after the third vaccination, and as expected, the full-dose 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 third vaccination compared to second vaccination ($p=0.20$ for full dose group; $p=>0.99$ for fractional dose group; **Erreur ! Source du renvoi introuvable.**). Notably, Pfs230D1 titers were significantly higher in the full versus fractional dosing regimen at 12 weeks post dose 3.



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

Immune responses, as defined by antibody titers and functional activity in SMFA, 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 (**Erreur ! Source du renvoi introuvable.**).

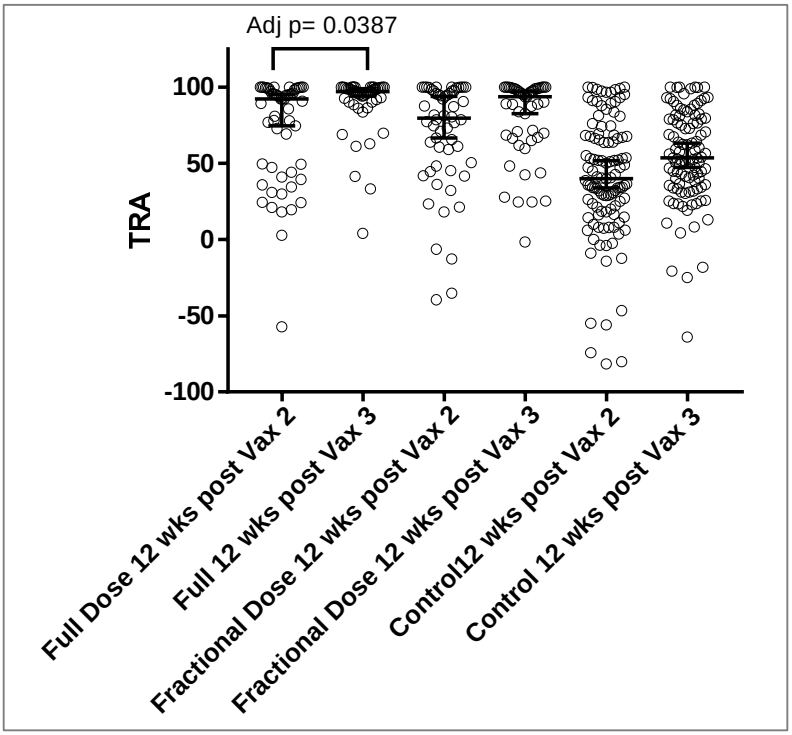
Figure 24: Antibodies Against Pfs230D1M Measured by ELISA 12 Weeks Post Second and Third Vaccination in Bancoumana/Doneguebougou, Mali During Main Phase Trial.



Titer increase was greater with full versus fractional regimen after the third 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.

Figure 25: ELISA Against Pfs230D1 Antigen.

The functional activity was also measured by SMFA at 12 weeks post second and third vaccinations. Both fractional and full dose arms were found to have significantly higher TRA (**Erreur ! Source du renvoi introuvable.**) (the decrease in the number of oocysts per infected mosquito) after both second and third 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 the third vaccination compared to the second vaccination; this difference was not observed in the fractional-dose arms (**Erreur ! Source du renvoi introuvable.**). Together with the ELISA data (**Erreur ! Source du renvoi introuvable.**), these results indicate that the full dose arm regimen may be inducing better antibody responses than the fractional dose regimen.



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

Functional activity, measured by SMFA by TRA, was maintained after the fourth dose at a high level of activity. TBA (reduction in infected mosquitoes) by SMFA was significantly higher in both Pfs230D1M vaccine arms compared to the comparator at 3 months post dose 4 but did not significantly differ between full and fractional dosing regimens.

Figure 26: Transmission-Reducing Activity Measured by SMFA 12 Weeks Post Second and Third Vaccinations in Bancoumana/Doneguebougou, Mali during Main Phase trial.

3.3.6.1 Functional Activity by Direct Skin Feeds

Direct skin feeds (DSFs) 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 5065 DSFs were completed in 2018. There were 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 (Erreur ! Source du renvoi introuvable.**15**). A trend of lower infections in the full-dose regimen was observed in 2017 but was not significant. Positive DSFs were significantly less frequent in the full-dose regimen in 2018, a first and extremely important achievement in the field of malaria TBVs – in vivo functional activity.

Table 15: Summary of DSF Results.

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%

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.

3.3.6.2 DSF Analysis as an endpoint

Results of vaccine efficacy have been calculated to be similar to the Age De-escalation study below for biostatistical purposes only. **Erreur ! Source du renvoi introuvable.** provides the preliminary results of DSFs for Year 1 and Year 2 of the study, and both years combined for the full dose arm of the study, showing strong vaccine efficacy in the adult trial.

Table 16: Vaccine efficacy based on DSFs for Year 1 and Year 2, full dose arm.

	Vaccine Efficacy	95% CI		p value
Year 1	0.4972	-1.0672	0.8777	0.341
Year 2	0.8119	0.3875	0.9423	0.006
Year 1 and 2	0.7250	0.3041	0.8913	0.006

3.3.7 Age De-Escalation/Family Compound Trial of Pfs230D1M-EPA/AS01 Vaccine (#19-I-N086)

This phase 2 study of the safety, immunogenicity, vaccine activity, and vaccine efficacy of Pfs230D1M-EPA/AS01 against *P. falciparum* malaria in vaccine units (VU) in Doneguebougou, Mali and surrounding villages began in April 2019. The trial is currently undergoing full unblinding and analysis so only partial results are available, described below.

The trial has been conducted to assess Pfs230D1M-EPA/AS01 versus comparators (Havrix, Typhim Vi, and Menactra) at a community level. The sample was drawn using a census of compounds in Doneguebougou, Mali and an adjacent village. Family compounds were aggregated by proximity and mosquito habitat into VUs (n=137), which were randomly assigned to receive Pfs230D1M-EPA/AS01, 40 µg, or comparators at 0, 1, and 2 months in all eligible subjects 5 years of age or older. Unvaccinated children 1-4 years of age received AL treatment prior to their VU receipt of dose #3, for parasitemia endpoints. All vaccinated subjects were treated with AL prior to dose #1, and children 5-8 years of age were treated with AL prior to dose #3. The trial began with age de-escalation pilot phases (to ensure safety) with smaller numbers of subjects prior to moving to main phases of the trial. In June 2020, available subjects

were re-enrolled to receive a fourth vaccine dose approximately 1 year post dose #3. Subjects aged 1-4 years were also re-enrolled in the corresponding VU.

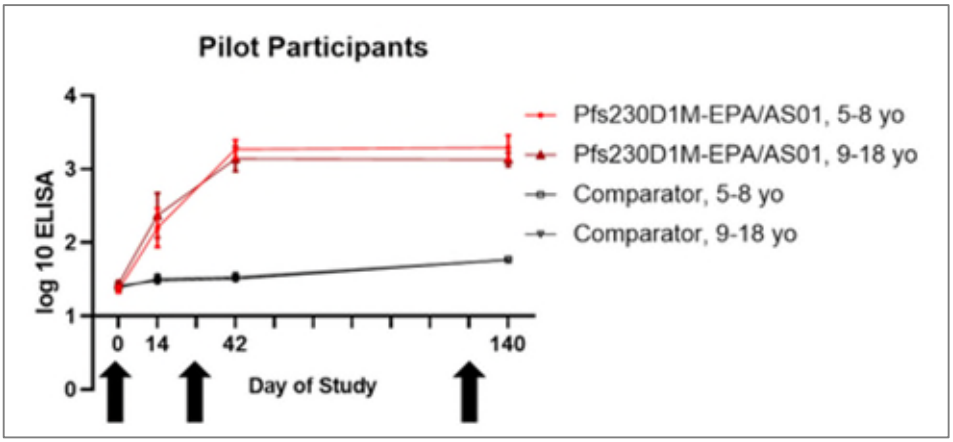
To assess vaccine efficacy, DSFs (for 9- to 18-year-old subjects only) were performed by feeding 60 mosquitoes directly on the subject's skin for approximately 15 minutes.

3.3.8 Safety

Thus far, AEs and laboratory abnormalities have been reported in an unblinded manner only. Analysis of the data is in progress. Overall, no safety signals have been observed in this trial. The majority of AEs have been mild injection site pain, headache, and pyrexia. A trend previously noted with the adjuvant AS01, increased frequency and severity of fever episodes post dose #2, has been seen, particularly in the youngest participants. The majority of laboratory abnormalities have been Grade 1. There have been no related SAEs noted. A more detailed description can be found in the Investigator's Brochure accompanying this submission.

3.3.9 Immunogenicity in Healthy Malian Children

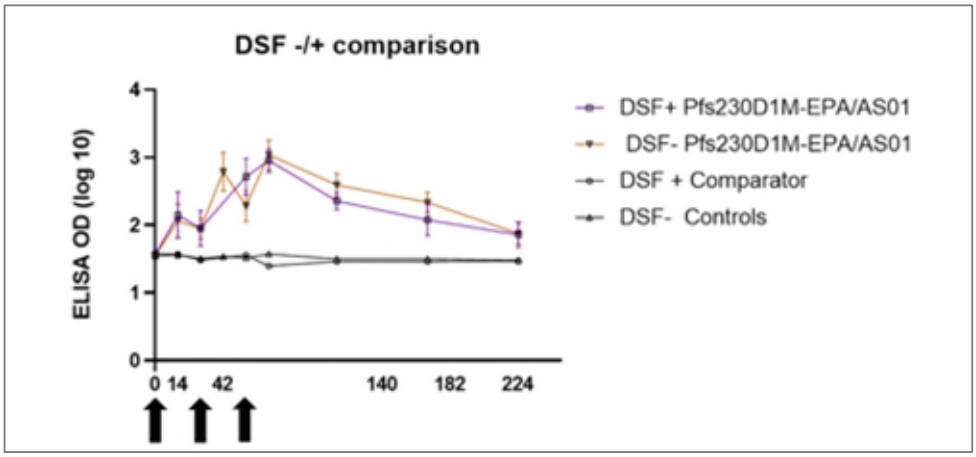
Initial ELISA results have been generated for Year 1 as shown in **Erreur ! Source du renvoi introuvable.**. ELISA assays from the pilot phase (pediatric arms, n=30 9-18 years old, n=30 5-8 years old; 1:1 randomization to Pfs230D1M-EPA/AS0, n=15/age arm and comparator, n=15/age arm; represented by group vaccine assignment) appear to have similar antibody responses to Pfs230D1M-EPA/AS01 vaccine as healthy adults receiving a similar vaccine regimen and same Pfs230D1M-EPA/AS01 vaccine dose. Also similar to what was seen in protocol #17-I-N006 (adult AS01-only study), children had significantly higher antibody titer measured by ELISA against Pfs230 compared to the control arm after only 2 doses given at 0 and 28 days. Antibody titers were also assessed after the third vaccination, and as expected, the Pfs230 vaccine arms had significantly higher titers compared to control arms, but again similar to the adult-only study (protocol #17-I-N006), the antibody titer was not significantly higher after the third vaccination compared to the second vaccination.



Vaccinations administered on day 0, 28, 126 (black arrows). ELISA timepoints = day 0 (pre-vaccination), day 14 (14 days post dose 1), day 42 (14 days post dose 2), and day 140 (14 days post dose 3).

Figure 27: Pfs230 ELISA Results During Year 1 in Pilot Subjects.

In an initial comparison of Pfs230 antibody responses in Pfs230D1M-EPA/AS01-vaccinated children at 12 weeks post dose #3, children who were found to have a DSF-positive feed (at least 1 mosquito with ≥1 oocyst) had a statistically significant ($p<0.0001$) lower Pfs230 antibody level than children with a negative DSF feed (**Erreur ! Source du renvoi introuvable.**). No differences between DSF+ and DSF- were seen in antibody responses to Pfs230 in the comparator arm ($p=0.9998$).



Vaccinations administered on day 0, 28, 56 (black arrows).

Figure 28: Comparison of Subset of DSF Cohort (9- to 18-Year-Old Subjects) by Vaccine Arm and DSF Positivity.

3.3.10 Vaccine Efficacy in Healthy Malian Children

Results of vaccine efficacy have been unblinded for biostatistical purposes only. **Erreur ! Source du renvoi introuvable.** provides the preliminary results of DSFs for Year 1 and Year 2 of the study. It is important to emphasize that these are preliminary results only.

Table 17: Vaccine Efficacy Based on DSFs for Year 1 and Year 2.

	Vaccine Efficacy	95% CI	p value
Year 1	72%	42; 86	< 0.001
Year 2	75%	42; 89	< 0.001

These preliminary efficacy results are extremely promising and support the idea that Pfs230D1M-EPA is an excellent candidate antigen for a TBV. Future plans for additional analyses of these positive DSFs include oocyst speciation, sieving analysis of Pfs230, and parasite genotyping.

3.3.11 Phase 1 study of Pfs230D1-EPA/Matrix-M™ against *Plasmodium falciparum* in Adults in Mali (NCT05135273)

This is a Phase 1, dose-escalating, randomized, double-blind, comparator-controlled study to assess the safety, tolerability, immunogenicity and transmission-blocking activity (TBA) of a

3-dose regimen of Pfs230D1-EPA/Matrix-M versus rabies vaccine in healthy adults. This ongoing trial began screening participants in October 2021 and is a first-in-human assessment of Pfs230D1-EPA/Matrix-M. Participants from 18 to 50 years old were randomized to one of the study arms and will be followed for 12 months from the last dose of study vaccine for safety and tolerability, as well as immunogenicity and functional antibody responses. A total of 80 participants have been enrolled and vaccinated, beginning in November 2021. The study consists of two groups: a pilot group with four arms as follows: arm 1a (n=5): 12.5 µg Pfs230D1-EPA/25 µg Matrix-M, arm 1b (n=5): 20 µg Pfs230D1-EPA/50 µg Matrix-M, arm 1c (n=5): 40 µg Pfs230D1-EPA/50 µg Matrix-M, and arm 1d (n=4): rabies vaccine (standard dose) while the main group consists of four corresponding (but larger) arms as follows: arm 2a (n=15): 12.5 µg Pfs230D1-EPA/25 µg Matrix-M, arm 2b (n=15): 20 µg Pfs230D1-EPA/50 µg Matrix-M, arm 2c (n=15): 40 µg Pfs230D1-EPA/50 µg Matrix-M, and the arm 2d (n=16): rabies vaccine (standard dose).

As of 21 December 2021, all arms of the pilot group had received at least one vaccination and safety data was available for at least 72 hours after each groups' most recent vaccination. There have been a total of 19 AEs reported amongst the 19 participants. Four of these occurred in the high dose group: Grade 1 myalgia and malaise in a single participant on the day of injection, one instance of Grade 1 neutropenia 3 days after injection, and one episode of Grade 2 injection site pain in two other individuals. All AEs were considered either probably or definitely related to study interventions. Three AEs have occurred in the middle dose group, all Grade 1: two episodes of injection site pain and one cough all in unique individuals. Twelves AEs were recorded in the low dose group amongst 5 subjects: 5 episodes of injection site pain (1 Grade 2, 4 Grade 1), 2 instances of neutropenia (1 Grade 2, 1 Grade 1), 4 episodes of rhinitis or sinobronchitis (all Grade 1), and 1 episode of malaria (Grade 1). The most common AE overall has been injection site pain (8/19 AEs). No SAEs have been reported to date.

3.4 R0.6C-AlOH/Matrix-M™ co-administered with Pfs230-EPA/Matrix-M™

The Co-administration of R0.6C-AlOH/Matrix-M with Pfs230-EPA/Matrix-M has not been evaluated previously in humans. However the individual vaccines R0.6C-AlOH/Matrix-M and Pfs230-EPA/Matrix-M have been evaluated independently as outlined earlier.

3.5 EPA

Recombinant EPA is not a component of any licensed vaccines, but has been extensively studied as a carrier for polysaccharide conjugate vaccines. These include a typhoid vaccine containing 22-µg recombinant EPA tested in children as young as 2 months old (Lin, Ho et al. 2001, Thiem, Lin et al. 2011), and a shigellosis vaccine containing 75-µg recombinant EPA tested in children 1-7 years old (Ashkenazi, Passwell et al. 1999, Passwell, Ashkenazi et al. 2003, Passwell, Ashkenzi et al. 2010). No safety issues have been identified to date with the use of the recombinant EPA.

3.6 MATRIX-M™

Matrix-M is a saponin-based adjuvant manufactured by Novavax AB (Uppsala, Sweden). Although it is not yet fully understood how the Matrix-M adjuvant achieves its stimulatory effects, this adjuvant is known to transiently enhance the number of activated immune cells in the draining lymph nodes which may in turn lead to increased uptake and presentation of vaccine antigens to elicit a competent immune response (Reimer, Karlsson et al. 2012). Specifically, it has been shown that there is an increase of CD169+ macrophages, as well as activated dendritic cells, to the draining lymph nodes after immunization with Matrix-M adjuvanted vaccines, which may help to increase antigen presentation (Magnusson, Altenburg et al. 2018). Hence, CD169+ macrophages have previously been shown to have a role in transporting antigens to B lymphocytes by trapping them in the draining lymph node and to facilitate cross-presentation of the antigen to CD8+ T lymphocytes (Carrasco and Batista 2007, Gray and Cyster 2012). This may lead to increased humoral and cellular immune responses, manifested by cross-reactive antibodies and multi-functional CD4+ T lymphocytes (Bengtsson, Song et al. 2016, Shinde, Cai et al. 2020). Consequently, Matrix-M has been shown to contribute to antigen dose-sparing and increased duration of humoral and cellular vaccine responses.

3.6.1 Summary of Clinical Experience with Matrix-M™

The Matrix-M adjuvant technology (Bengtsson, Karlsson et al. 2013) is a promising technology which has been explored for various infectious diseases and has a good safety profile in humans (Shinde, Fries et al. 2018). To date, Matrix-M has been utilized in more than 25 clinical trials, including multiple Phase III trials (**Erreur ! Source du renvoi introuvable.**). In these studies, Matrix-M has been combined with multiple malaria vaccine candidates, influenza, COVID-19, and other disease indications. The most common dosage of Matrix-M has been 50 µg in adults, with a maximum dosage of 75 µg.

Datoo et al reported on a double-blind, randomized, controlled trial (NCT02925403) of a low-dose circumsporozoite protein-based 37 vaccine, R21, with two different doses of adjuvant, Matrix-M (25 µg and 50 µg), in 450 children aged 5-17 months in Nanoro, 38 Burkina Faso, a highly seasonal malaria transmission setting. Three vaccinations were administered at 4-week 39 intervals prior to the malaria season with a fourth dose one year later. R21/MM had a favorable safety profile and was well-tolerated. Vaccine efficacy (VE) was 74% (95% CI, 63-82) and 46 77% (95% CI, 67-84) in the low- and high-dose adjuvant groups, respectively. At 1 year, VE remained high at 47 77% (95% CI, 67-84) in the high-dose adjuvant group (Datoo, Natama et al. 2021).

Keech et al (Keech, Albert et al. 2020) published the results of a randomized, placebo-controlled, phase 1–2 trial to evaluate the safety and immunogenicity of the rSARS-CoV-2 vaccine (in 5-µg and 25-µg doses, with or without Matrix-M1 adjuvant in 131 healthy adults (NCT04368988). The vaccine, NVX-CoV2373 appeared to be safe and was shown to elicit immune responses that exceeded levels in Covid-19 convalescent serum. The addition of Matrix-M resulted in enhanced immune responses, was antigen dose–sparing, and induced a T helper 1 (Th1) response.

Shinde et al (Shinde, Bhikha et al. 2021) reported on rSARS-CoV-2 vaccine (containing 50 µg of Matrix-M) administered intramuscularly to adult subjects in South Africa (NCT04533399).

Among 2,684 baseline seronegative participants (94% HIV-negative and 6% HIV-positive), predominantly mild-to-moderate COVID-19 developed in 15 participants in the vaccine group and in 29 in the placebo group (vaccine efficacy, 49.4%; 95% confidence interval [CI], 6.1 to 72.8). Among the vaccine recipients, the most common solicited systemic adverse events after the first dose and second dose were headache, muscle pain, and fatigue. The mean duration of such events was slightly longer after the second dose but generally less than 3 days.

In yet another recent example (NCT01444482), an influenza vaccine containing Matrix-M was tested in a randomized, observer-blinded, active comparator-controlled trial during the 2019-2020 influenza season (Shinde, Cho et al. 2020). In brief, 2,654 clinically stable, community-dwelling adults ≥65 years of age were randomized to receive a single IM dose of either Matrix-M-adjuvanted quadrivalent nanoparticle influenza vaccine (qNIV) or a licensed inactivated influenza vaccine (IIV4). Local reactogenicity, primarily mild to moderate and transient pain, was higher in the qNIV group. qNIV was generally well tolerated and produced a qualitatively and quantitatively enhanced humoral and cellular immune response in older adults.

Table 18: Key Clinical Experience of Matrix-M™ with Various Vaccine Antigens.

NCT Number	Title	Conditions	Interventions	Characteristics	Population
NCT04201431	Safety, Immunogenicity and Efficacy of the Blood-stage Plasmodium Vivax Malaria Vaccine Candidate PvDBPII in Matrix M1	Malaria, Vivax	• Biological: PvDBPII/Matrix M1	Phase 1 Phase 2	18 Years to 45 Years (Adult)
NCT01669512	Adjuvanting Viral Vectored Malaria Vaccines With Matrix M	Malaria	• Biological: Low Dose Matrix M Regimen • Biological: Standard Dose Matrix M Regimen	Phase 1	18 Years to 50 Years (Adult)
NCT04130282	VAC077: Safety and Immunogenicity of the Pfs25-IMX313/Matrix-M Vaccine	Malaria	• Biological: Pfs25-IMX313/ Matrix-M1	Phase 1	18 Years to 45 Years (Adult)
NCT04318002	Safety and Immunogenicity of RH5.1/ Matrix-M in Adults and Infants Living in Tanzania	Malaria	• Biological: RH5.1/Matrix-M	Phase 1	6 Months to 45 Years (Child, Adult)
NCT03896724	Safety, Immunogenicity and Efficacy of R21 Matrix-M in 5-17 Month Old Children in Nanoro, Burkina Faso	Malaria	• Biological: R21 adjuvanted with 25mcg Matrix-M • Biological: R21 adjuvanted with 50mcg Matrix-M	Phase 1 Phase 2	5 Months to 17 Months (Child)
NCT04271306	Safety, Immunogenicity and ex Vivo Efficacy of Pfs25-IMX313/Matrix-M in Healthy Volunteers in Bagamoyo, Tanzania.	Malaria	• Biological: Pfs25-IMX313 (10ug)/Matrix-M (50ug) • Biological: Pfs25-IMX313 (50ug)/Matrix-M (50ug) • Biological: Pfs25-IMX313 (50ug)/Matrix-M (50ug) & Pfs25-IMX313 (10ug)/ Matrix-M (50ug)	Phase 1	5 Years to 45 Years (Child, Adult)
NCT02572388	A Study to Assess the Safety and Immunogenicity of the Malaria Vaccine, R21, Administered With and Without Matrix-M1	Malaria	• Biological: R21 • Biological: Matrix-M1	Phase 1	18 Years to 50 Years (Adult)
NCT02925403	A Study to Assess the Safety and Immunogenicity of the Malaria Vaccine, R21, With Matrix-M1 Adjuvant	Malaria	• Biological: R21/Matrix-M1 • Other: Saline	Phase 1 Phase 2	18 Years to 45 Years (Adult)
NCT01444482	Study of Parenterally Administrated Adjuvanted Seasonal Influenza Vaccine in Healthy Elderly Volunteers	Influenza	• Biological: Matrix M • Biological: Seasonal influenza vaccine	Phase 1	65 Years to 75 Years (Older Adult)
NCT04611802	A Study Looking at the Efficacy, Immune Response, and Safety of a COVID-19 Vaccine in Adults at Risk for SARS-CoV-2	COVID-19	• Biological: SARS-CoV-2 rS/ Matrix-M1 Adjuvant • Other: Placebo	Phase 3	18 Years and older (Adult, Older Adult)
NCT04368988	Evaluation of the Safety and Immunogenicity of a SARS-CoV-2 rS Nanoparticle Vaccine With/Without Matrix-M Adjuvant	COVID-19	• Biological: SARS-CoV-2 rS - Phase 1 • Biological: SARS-CoV-2 rS/ Matrix-M Adjuvant, Days 0 and 21 - Phase 2 • And more	Phase 1 Phase 2	18 Years to 84 Years (Adult)
NCT04533399	A Study Looking at the Effectiveness and Safety of a COVID-19 Vaccine in South African Adults	COVID-19	• Biological: SARS-CoV-2 rS/ Matrix-M1 Adjuvant • Other: Placebo	Phase 2	18 Years to 84 Years (Adult)
NCT03580824	A Study to Determine if a New Malaria Vaccine is Safe and Induces Immunity Among Kenyan Adults, Young Children and Infants	Malaria	• Biological: R21 in Matrix- M adjuvant vaccine	Phase 1 Phase 2	5 Months to 45 Years (Child, Adult)
NCT04583995	A Study Looking at the Effectiveness, Immune Response, and Safety of a COVID-19 Vaccine in Adults in the United Kingdom	COVID-19	• Biological: SARS-CoV-2 rS/ Matrix M1-Adjuvant • Other: Placebo • Biological: Licensed seasonal influenza vaccine	Phase 3	18 Years to 84 Years (Adult, Older Adult)
NCT02078674	A(H7N9) VLP Antigen Dose-Ranging Study With Matrix-M1™ Adjuvant	Influenza (Pandemic)	• Biological: Monovalent Avian Influenza VLP (H7N9) • Biological: Matrix-M1™ adjuvant	Phase 1 Phase 2	18 Years to 64 Years (Adult)

NCT Number	Title	Conditions	Interventions	Characteristics	Population
NCT02300142	Rollover Trial for Placebo Subjects Previously Enrolled Into GEN-003-002 Study	Genital Herpes	• Biological: GEN-003 Vaccine (30-60µg of each antigen) • Biological: Matrix-M2 Adjuvant (25-75µg)	Phase 2	18 Years to 50 Years (Adult)
NCT01667341	Safety and Immunogenicity Study of Therapeutic HSV-2 Vaccine	Genital Herpes	• Biological: GEN-003 with Matrix M-2 • Biological: GEN-003	Phase 1 Phase 2	18 Years to 50 Years (Adult)
NCT03026348	Safety and Immunogenicity Study to Evaluate Single- or Two-Dose Regimens Of RSV F Vaccine With and Without Aluminum Phosphate or Matrix-M1™ Adjuvants In Clinically-Stable Older Adults	Respiratory Syncytial Viruses	• Biological: RSV F Vaccine with Aluminum Phosphate Adjuvant • Biological: RSV F Vaccine • Biological: Matrix-M1 Adjuvant	Phase 2	60 Years to 80 Years (Adult, Older Adult)
NCT02114060	Dose Ranging Safety and Efficacy of Therapeutic HSV-2 Vaccine	Genital Herpes	• Biological: GEN-003 Vaccine (30µg of each antigen) • Biological: Matrix-M2 Adjuvant (75µg) • And more	Phase 2	18 Years to 50 Years (Adult)
NCT03947190	A Study to Determine if New Types of Malaria Vaccines Are Safe, Effective and Lead to Immunity in Kenyan Adults	Malaria	• Biological: R21/Matrix-M • Biological: ChAd63/MVA ME-TRAP • Biological: intradermal injection (ID) or direct venous injection (DVI) of PfSPZ Challenge	Phase 2	18 Years to 45 Years (Adult)
NCT03293498	Evaluation of the Safety and Immunogenicity of a Recombinant Trivalent Nanoparticle Influenza Vaccine With Matrix M-1 Adjuvant (NanoFlu)	Influenza	• Biological: NanoFlu • Biological: Fluzone HD - Day 0 • Biological: Fluzone HD - Day 21 • Other: Saline - Day 21	Phase 1 Phase 2	60 Years and older (Adult, Older Adult)
NCT03658629	Phase 2 Dose and Formulation Confirmation of Quad-NIV in Older Adults	Influenza	• Biological: NanoFlu (Quad-NIV) • Other: Matrix-M Adjuvant • Biological: Fluzone HD • Biological:Flublok	Phase 2	65 Years and older (Older Adult)
NCT03970993	VAC 072-An Efficacy Study of R21/MM in Different Dose Schedules	Malaria	• Biological: R21 Matrix-M vaccination • Biological: R21 Matrix-M vaccination and CHMI	Phase 1 Phase 2	18 Years to 45 Years (Adult)
NCT02370589	Study to Evaluate the Immunogenicity and Safety of an Ebola Virus (EBOV) Glycoprotein (GP) Vaccine in Healthy Subjects	Ebola	• Biological: Base Dose EBOV GP Vaccine • Biological: 2-8x Base Dose EBOV GP Vaccine • Biological: Matrix-M Adjuvant	Phase 1	18 Years to 50 Years (Adult)
NCT02515175	Evaluating New Formulation of Therapeutic HSV-2 Vaccine	Genital Herpes	• Biological: Matrix-M2 • Biological: GEN-003	Phase 2	18 Years to 50 Years (Adult)
NCT04120194	Phase 3 Pivotal Trial of NanoFlu™ in Older Adults	Influenza	• Biological: NanoFlu • Biological: Fluzone Quadrivalent	Phase 3	65 Years and older (Older Adult)
NCT04645147	Safety and Immunogenicity of an Epstein-Barr Virus (EBV) gp350-Ferritin Nanoparticle Vaccine in Healthy Adults With or Without EBV Infection	EBV	• Biological: EBV gp350- Ferritin Vaccine • Other: Matrix-M1	Phase 1	18 Years to 29 Years (Adult)
NCT03146403	Maintenance Dose Study of GEN-003 in Subjects With Genital Herpes Infection	Genital Herpes	• Biological: GEN-003 • Biological: Matrix-M	Phase 2	Child, Adult, Older Adult
NCT02905019	A Safety and Efficacy Study of R21 +/- ChAd63/MVA ME-TRAP	Malaria	• Biological: R21 with Matrix- M1 • Biological: ChAd63 ME-TRAP • Biological: MVA ME-TRAP	Phase 1 Phase 2	18 Years to 45 Years (Adult)

4 Study Objectives

Primary Objective:

- To assess in African adults the safety and the reactogenicity of the co-administration of R0.6C-AlOH/Matrix-M™ and Pfs230D1-EPA/Matrix-M™ (first-in-human) as compared to the rabies vaccine control

Secondary Objectives:

- To assess the dynamics of transmission reducing activity in the standard membrane feeding assay of sera collected during and after Study Agent immunizations
- To assess the dynamics of Study Agent antibody quantities during and after Study Agent immunizations
- To assess in African adults the safety and the reactogenicity of R0.6C-AlOH/Matrix-M™, ProC6C/Matrix-M™, and Pfs230D1-EPA/Matrix-M™ immunizations as compared to the rabies vaccine control

Exploratory Objectives:

- To explore parasite, host genetics and functional antibody responses to Study Agents
- To assess differences in immunological responses (such as standard membrane feeding assays and antibody responses measured by ELISA) to Pfs230/Matrix-M given on a 0, 1 and 2 month schedule compared to a 0, 1, and 6 month schedule (Arm 3)

5 Study Design

This is a Phase 1, dose-escalating, randomized, comparator-controlled study to assess the safety, tolerability, immunogenicity and transmission-blocking activity (TBA) of a 3-dose regimen of Study Agents (four total) versus rabies vaccine in healthy adults. This will be a first-in-human assessment of the co-administration of R0.6C-AlOH/Matrix-M with Pfs230D1-EPA/Matrix-M. Participants will be randomized to one of the study arms. Participants will be followed for 12 months from the last dose of study vaccine for safety and tolerability, as well as immunogenicity and functional antibody responses.

The study groups and arms are as follows. For all participants in Arms 1 and 2, the assigned study vaccine will be administered at Days 1, 29, and 57. For participants in Arm 3 the study vaccine will be administered on Days 1, 29, and 169 (0, 1, and 6 months).

Group 1: Pilot Group

- **Arm 1a** (n=5): 30 µg ProC6C/15 µg Matrix-M and normal saline
- **Arm 1b** (n=5): 30 µg R0.6C-AlOH/15 µg Matrix-M co-administered with 12.5 µg Pfs230D1-EPA/25 µg Matrix-M
- **Arm 1c** (n=5): rabies vaccine (standard dose) and normal saline

Group 2: Main Group

- **Arm 2a** (n=20): 100 µg R0.6C-AlOH/50 µg Matrix-M and normal saline
- **Arm 2b** (n=20): 100 µg ProC6C-AlOH/50 µg Matrix-M and normal saline
- **Arm 2c** (n=20): 40 µg Pfs230D1-EPA/50 µg Matrix-M and normal saline
- **Arm 2d** (n=20): 100 µg R0.6C-AlOH/25 µg Matrix-M co-administered with 40 µg Pfs230D1-EPA/25 µg Matrix-M
- **Arm 2e** (n=20): rabies vaccine (standard dose) and normal saline
- **Arm 3** (n=5-10): 40 µg Pfs230D1-EPA/50 µg Matrix-M given on a 0, 1, and 6 month schedule (Pfs230D1-EPA regimen may be adjusted based on results of ongoing clinical trial NCT05135273)

5.1 Study Endpoints

Primary Endpoint:

- Incidence of serious adverse events and solicited grade 3 local and systemic adverse events (AEs) possibly, probably or definitely related to co-administered vaccinations in the period from first vaccinations up to 1 month after the last immunization.

Secondary Endpoints:

- The functional transmission reducing activity in the standard membrane feeding assay of volunteer sera collected two weeks after the third immunizations, compared to baseline within each of the Study Agent Groups
- The TRA at other timepoints (2 weeks after first and second immunizations and 4 months post third vaccination) compared to baseline (D0) in each of the Study Agent Groups
- The Study Agent antibody quantity in volunteer sera collected two weeks after each dose and at 4 months post dose 3) compared to baseline (D0) in each of the three dose-adjuvant combinations, as determined by ELISA.
- Incidence of adverse events possibly, probably or definitely related to any investigational vaccines

Exploratory Endpoints:

- Study Agent antibody decay rate following Study Agent Immunization
- Cellular immune responses and antibody repertoire of functional antibody responses to vaccination
- RNA transcriptome quantification as detected by RNA sequencing comparing vaccinees to controls

- Estimation of interaction between host factors including but not limited to hemoglobinopathies, immune signatures, co-infections, and environmental, demographic, and socioeconomic characteristics and primary and secondary endpoints

5.2 Sample Size and Estimated Duration of Study

A total of 125-130 subjects will be vaccinated with either one of the four Test Articles or the rabies vaccine comparator. The last study visit will occur approximately 12 months after the final immunization). Up to 260 subjects will be screened to accommodate possible screening failures.

The sample size was derived to be able to demonstrate safety, tolerability, and immunogenicity of the study vaccine as described in Section 14.1.

5.3 Study Definitions

Screened: Subjects will receive a screening identification number when the informed consent is signed, and will either be determined as “enrolled” or “screen failures” as noted below.

- Screening may be completed over the course of multiple visits.
- Screening will occur within 56 days prior to enrollment into the study.
- If screening laboratories are obtained >56 days prior to planned enrollment (Day -7), then the subject will need to have a repeat physical exam, medical history review and all laboratories outside of the window will need to be collected again (inclusive of safety labs, human immunodeficiency virus [HIV],) to confirm whether the subject may proceed to enrollment

Enrolled: Subjects will be considered enrolled beginning on Day -7, and the final study number will be assigned at this point.

Randomized: Participants will be randomized to one of the study groups. See Section 14.44 for details.

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 8.1
- Subject withdraws consent before Day -7 (i.e., enrollment).

Withdrawn: Participants will be considered to be withdrawn from the study if they meet any of the withdrawal criteria in Section 12.11 prior to completing the final study visit.

Completed: Subjects are considered completed when they complete the final study visit for their arm.

Lost to follow-up: A participant will be considered lost to follow-up if he or she fails to attend a required study visit and cannot be located. Study site staff will make at least 3 attempts to contact participants to complete the remaining study visits. These contact attempts should be documented in the participant's study file. Should the participant continue to be unreachable, they will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

6 Study Population

6.1 Study Site

The study will be carried out in collaboration between the LMIV/National Institutes of Health (NIH), Statens Serum Institute (SSI) and the MRTC headquartered in Bamako, Mali. The study will be conducted by the MRTC at one location in Mali, West Africa (see Erreur ! Source du renvoi introuvable.). 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.

Figure 29: Maps Showing the Location of Doneguebougou, Mali.

6.2 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 about the study will be made at the time of community permission, using local radio or any traditional channel of communication. The announcement will include general information about the study as well as contact information for the study site/staff for those interested in participating.

6.3 Inclusion Criteria

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

1. Age: $\geq$ 18 years old and $\leq$ 50 years old.
2. Available for the duration of the trial.
3. Known resident or long-term resident (more than 1 year) of Doneguebougou 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 **one** of the following:
        - Confirmed pharmacologic contraceptives (parenteral) delivery.
        - Intrauterine or implantable device.
    - EXCEPTIONS to required pregnancy prevention includes the following:
        - Postmenopausal state: defined as no menses for 12 months without an alternative medical cause.
        - Surgical sterilization.
7. Willing to have blood samples stored for future research.

6.4 Exclusion Criteria

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. 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.
3. 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.)
4. 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.)
5. Infected with HIV.
6. Evidence of clinically significant neurologic, cardiac, pulmonary, hepatic, endocrine, rheumatologic, autoimmune, hematological, oncologic, or renal disease by history, physical examination, and/or laboratory studies.
7. History of receiving any investigational product within the past 30 days.
8. Current or planned participation in an investigational vaccine study until the time period of the last required study visit under this protocol.
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.
12. Receipt of:

- Live vaccine within 4 weeks prior to enrollment or a killed vaccine within 2 weeks prior to enrollment.
- Immunoglobulins and/or blood products within the past 6 months.
- Investigational malaria vaccine in the last 2 years.

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.

Co-enrollment guidelines: Co-enrollment in other trials is limited. 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. Enrolled subjects will be informed that they may be invited to participate in other subsequent studies.

6.5 Justification for Exclusion of Special Populations

6.5.1 Justification of Exclusion of Pregnant Women

Pregnant women are excluded from participation in this study. The effects of the co-administration of R0.6C-AlOH/Matrix-M with Pfs230D1-EPA/Matrix-M on the developing human fetus are unknown with the potential for teratogenic or abortifacient effects.

6.5.2 Justification for Exclusion of Children

This is a “first-in-human” trial with the co-administration of R0.6C-AlOH/Matrix-M with Pfs230D1-EPA/Matrix-M vaccine. As such, safety and tolerability should be established in adults prior to testing in children.

7 Study Agents

7.1 Matrix-M™ adjuvant

The Matrix-M™ adjuvant is supplied and stored separately. The Matrix-M adjuvant is mixed with other test articles immediately prior to administration.

7.1.1 Manufacturing

Matrix-M™: The Matrix-M™ adjuvant is manufactured by Novavax AB (Uppsala, Sweden), a subsidiary of Novavax, Inc. The adjuvant contains purified saponin components derived from an extract of the bark of the Quillaja saponaria tree; a phospholipid, egg-derived phosphatidyl-choline (PC); and semi-synthetic cholesterol of non-animal origin. The manufacturing process starts with extraction of the Quillaja bark to provide the Quillaja extract which is fractionated by chromatography into two distinct fractions (Fraction-A and Fraction-C). The individual fractions are each formulated together with cholesterol and PC into nanoparticles (Matrix-A™ and Matrix-C™).

Matrix-M™ was manufactured in compliance with cGMP regulations.

7.1.2 Disposition and Dispensation

The Matrix-M™ adjuvant will be supplied under refrigerated conditions (2°C to 8°C) for mix with Test Articles at the site. Vials and cartons containing the adjuvant will be labelled as appropriate. See Section 7.122 for information about study agent accountability.

7.1.3 Formulation, Packaging, and Labeling

The active ingredient in Matrix-M™, specifically Matrix-M1, are the adjuvant components Matrix-A and Matrix-CMatrix-M1 has a ratio of Matrix-A and Matrix-C of 85:15 (by weight). Both Matrix-A and Matrix-C are nanoparticles formulated from individual fractions (separated by chromatography) derived from extracts from the *Quillaja saponaria* tree. Each vial of Matrix-M1 contains saponin content of 0.375 mg/mL in PBS, at a pH of 7.2, in a final volume of 0.75 mL. The Matrix-M1 adjuvant is analyzed according to set specifications for saponin content (Matrix-A and Matrix-C) by reversed-phase high-performance liquid chromatography (RP-HPLC), appearance, turbidity, endotoxins, and sterility. The adjuvant is supplied for on-site reconstitution under refrigerated conditions (+2-8°C).

7.1.4 Storage, Shipping, and Stability

The container with Matrix M1 is stored at +2-8°C until use.

Vials will be transported and stored at temperature-controlled conditions, according to 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 indicated temperature conditions until usage approval has been obtained from the Sponsor.

7.1.5 Preparation and Dosage

Refer to individual sections for test article preparation with Matrix-M™ adjuvant

7.2 R0.6C-AlOH/Matrix

7.2.1 Manufacturing

The R0.6C fusion protein is a chimera consisting of the 6-cysteine C-terminal fragment of PfS48/45 (6C) coupled to the N-terminal region of asexual stage Glutamate Rich Protein GLURP (R0) produced in Lactococcus lactis [7] (Singh et al. 2020; Singh et al. 2015). The carrier protein R0 helps to enhance the immune response against Pfs48/45 (Theisen et al. 2014).

R0.6C is fermented in SSI medium supplemented with glucose. The harvest supernatant is clarified by centrifugation followed by filtration and concentration. Following expression and harvest, the R0.6C recombinant protein is purified by three different steps (Capturing on Q HP column, HCP removal on SP HP column and Isolation of monomer on Q HP column). The final process yields

about 20-25mg pure monomeric non-his tagged R0.6C, which reacts strongly with mAb45.1 indicating that it is properly folded.

The bulk drug substance, recombinant R0.6C is stored at <-50 °C until subsequent formulation for Drug Product.

The Master Cell Bank (MCB01VUA20) were produced under cGMP at SSI in Copenhagen, Denmark. The bulk drug substance K-0120 was manufactured at SSI in Copenhagen, Denmark. The Bulk drug substance was then transferred to Baccinex for subsequent formulation/fill/finish as the R0.6C-AlOH Drug product.

Drug Product R0.6C/Alhydrogel was manufactured in compliance with cGMP regulations. Drug Product R0.6C/Alhydrogel + Matrix-M adjuvant will be admixed at bedside.

7.2.2 Disposition and Dispensation

The vaccines will be shipped to the trial centre according to the pre-determined schedule which will be based on the estimated study start date, and only after the written Burkinabe ethics approval for the trial and import licenses has been received by the sponsor's representative. At the clinical trial site, a staff member (investigator, designated site staff or pharmacist) will be in charge of product management and will be responsible for receipt, storage and accountability of the products. The person in charge of investigational product management will be expected to return to the sponsor's representative a completed dispatch note, which will be attached to the package, as acknowledgement of receipt. The person in charge of product receipt will check that the cold chain was maintained during shipment. In case of a problem, (s)he should alert the clinical trial monitor (and sponsor's representative) immediately. The acknowledgement of receipt will be dated and signed by the person in charge of product management. A scanned copy is sent to the sponsor's representative.

7.2.3 Formulation, Packaging, and Labeling

The recombinant R0.6C protein is formulated at 200 ug/m in 10 mM HEPES, 2.5% glucose, 0.5 mM EDTA, 155 mM NaCl and absorbed to 1.6 mg/mL Alhydrogel [Al(OH)3], at a fill volume of 0.8 mL in a 2 mL borosilicate glass vial and stored at 2-8°C.

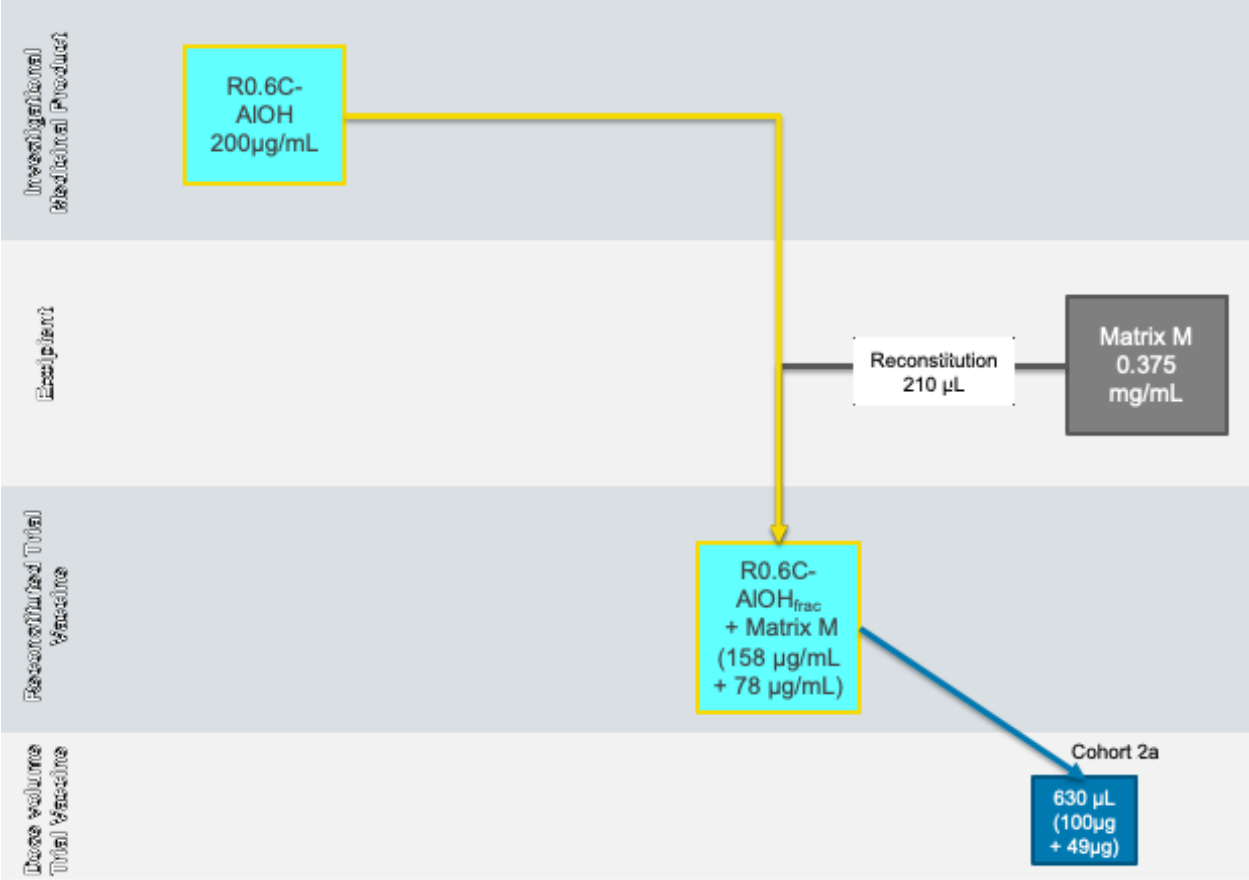
Example of label for R0.6C-AlOH:

200 µg R0.6C - 1.60 mg $Al(OH)_3$/mL for reconstitution and IM injection
Single Use according to Pharmacy Manual
For clinical trial use only

Store cold at 2°C to 8°C
For Clinical Trial Use Only
Batch: xxxxxx

Full dose R0.6C-AlOH + Matrix-M
The full dose R0.6C-AlOH + Matrix-M used for intramuscular administration is composed of one vial R0.6C-AlOH which is on-site reconstituted with 210 µL Matrix-M. The contents of the vial should be mixed by inversion (10x) to ensure re-suspension and assure homogeneity before withdrawing into a syringe for administration. Once the two components are mixed, the

reconstituted Trial Vaccine will be used immediately in a volume of 630 µL which correspond to 100 µg of R0.6C and 49 µg of Matrix-M.



7.2.4 Storage, Shipping, and Stability

Vials of R0.6C-AlOH must be stored at 2ºC to 8ºC. Freezing destroys the integrity of aluminium hydroxide suspensions. Thus, any vials that have been frozen must not be used for administration to humans.

Vials will be transported and stored at temperature-controlled conditions, according to 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 indicated temperature conditions until usage approval has been obtained from the Sponsor.

An in-use Point-of-Injection (POI) stability study has been performed to support the Point-of-Use mixture of R0.6C-AlOH with an aqueous form of the adjuvant Matrix-M, for preclinical studies. The materials R0.6C-AlOH and Matrix-M were those supplied for toxicology.

In summary, under all test conditions with R0.6C-AlOH, the drug product (Either with or without Matrix-M addition) showed no adverse changes during the stability analysis. Each product (R0.6C-AlOH alone or R0.6C-AlOH with Matrix-M added) was within specification when stored at 2-8°C or room temperature (18-25°C) for up to 24 hours. Indicating the two products are compatible as analyzed by the methods here and stable for use during the preclinical toxicology manipulations intended.

A second 24 hour study for the trial vaccine with Matrix-M was performed on the DP for use in the clinical trial (GMP material of R0.6C-AlOH and of Matrix-M). The results showed no change over 24 hours storage at room temperature of the trial vaccine, based on analysis of appearance, protein identity (WB) and degree of R0.6C-AlOH adsorption to aluminum hydroxide. This study together with the Pharmacy Manual will support the "Point-of-use mixture."

7.2.5 Preparation and Dosage

The final vaccine for administration is obtained by admixing R0.6CC-AlOH with Matrix-M as appropriate for each dose level and must be administered within 4 hours of reconstitution.

Prior to dose administration, vial must be mixed by hand (invert 10x by hand) to ensure homogeneity. If the vial is left to settle for > 1 minute, the vial must be mixed immediately again prior to dosing/filling of syringe.

7.3 ProC6C-AlOH/Matrix

7.3.1 Manufacturing

The ProC6C fusion protein is a chimera consisting of the 6-cysteine C-terminal fragment of PfS48/45 (6C) coupled to the Pro domain of Pfs230 produced in *Lactococcus lactis* (Singh et al. 2021, manuscript submitted). ProC6C is a recombinant hybrid protein derived from *Plasmodium falciparum* 3D7, Pfs230Pro (Pro) genetically coupled to *P. falciparum* Pfs48/45 (6C). The hybrid protein consists of the $Pro_{443–590}$ and $Pfs48/45_{291-428}$ regions joined by a linker region, $PfCSP_{105-140}$, and the vector-encoded amino acid residues A-E-R-S at the N-terminal end and a C-tag (E-P-E-A) in its C-terminus.

ProC6C is fermented in SSI medium supplemented with glucose. The harvest supernatant is clarified by centrifugation followed by filtration and concentration. Following expression and harvest, the ProC6C recombinant protein is purified by three different steps (Capturing on Q HP column, HCP removal on CaptureSelect™ C-tagXL column and Isolation of monomer on Q HP column). The final process yields about 540 mg pure monomeric ProC6C (for K0220) , which reacts strongly with mAb45.1 indicating that it is properly folded.

The bulk drug substance, recombinant ProC6C is stored at <-50 °C until subsequent formulation for Drug Product.

The Master Cell Bank (MCB01VUA21) were produced under cGMP at SSI in Copenhagen, Denmark. The bulk drug substance K-0220 was manufactured at SSI in Copenhagen, Denmark. The Bulk drug substance was then transferred to Baccinex for subsequent formulation/fill/finish as the ProC6C-AlOH Drug product.

Drug Product ProC6C/Alhydrogel was manufactured in compliance with cGMP regulations. Drug Product ProC6C/Alhydrogel + Matrix-M adjuvant will be admixed at bedside.

7.3.2 Disposition and Dispensation

The vaccines will be shipped to the trial centre according to the pre-determined schedule which will be based on the estimated study start date, and only after the written Burkinabe ethics approval for the trial and import licenses has been received by the sponsor's representative. At the clinical trial site, a staff member (investigator, designated site staff or pharmacist) will be in charge of product management and will be responsible for receipt, storage and accountability of the products. The person in charge of investigational product management will be expected to return to the sponsor's representative a completed dispatch note, which will be attached to the package, as acknowledgement of receipt. The person in charge of product receipt will check that the cold chain was maintained during shipment. In case of a problem, (s)he should alert the clinical trial monitor (and sponsor's representative) immediately. The acknowledgement of receipt will be dated and signed by the person in charge of product management. A scanned copy is sent to the sponsor's representative.

7.3.3 Formulation, Packaging, and Labeling

The ProC6C-AlOH Vaccine is composed of ProC6C recombinant protein (0.2 mg/ml of the recombinant protein together with 1.6 mg/ml Alhydrogel®. The vaccine is supplied in sterile 2 mL Type I glass vials. Each vial contains a total volume of 0.8 mL

Example of label for ProC6C-AlOH:

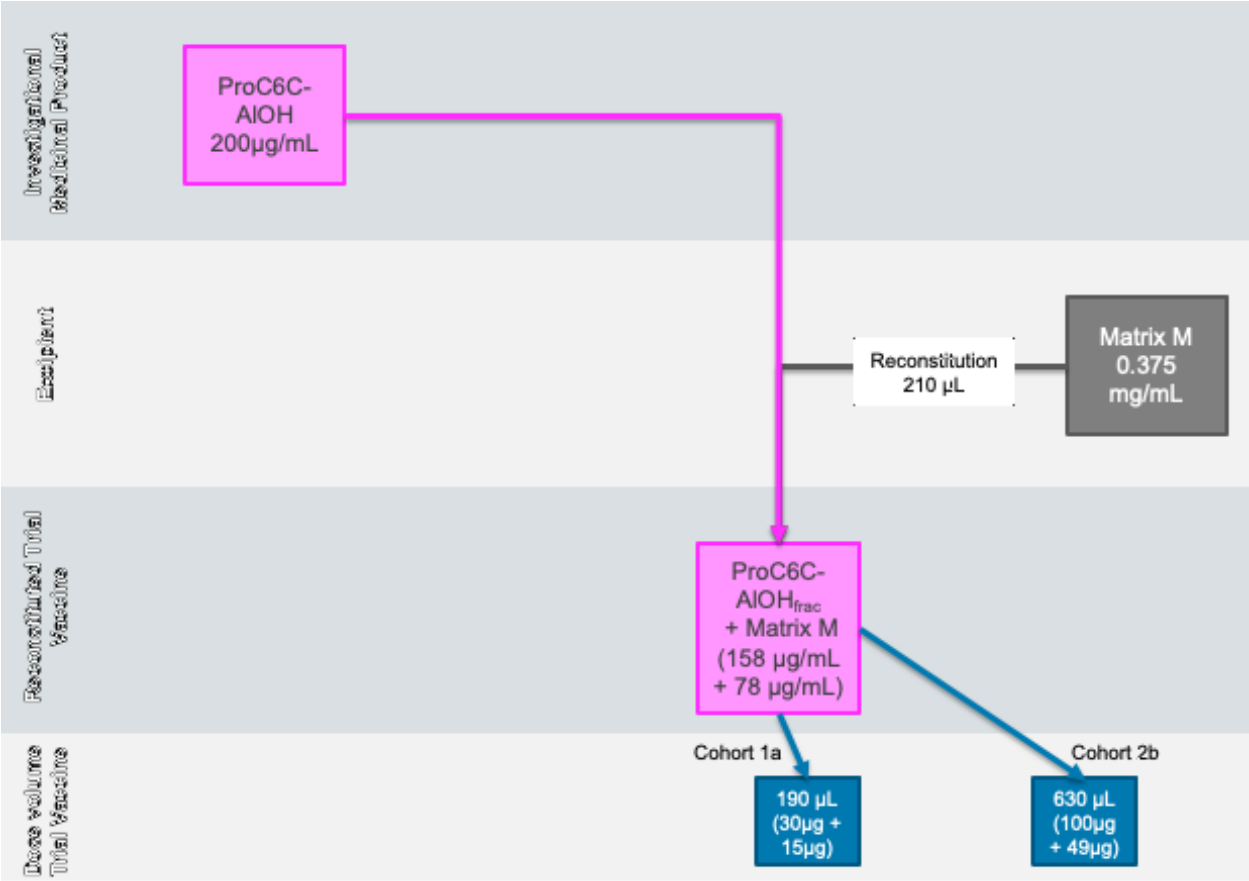
200 µg ProC6C - 1.60 mg $Al(OH)_3$/mL for reconstitution and IM injection
Single Use according to Pharmacy Manual
For clinical trial use only

Store cold at 2ºC to 8°C
For Clinical Trial Use Only
Batch: xxxxxx

Fractioned dose ProC6C-AlOH + Matrix-M
The fractioned dose ProC6C-AlOHfrac + Matrix-M used for intramuscular administration is composed of one vial ProC6C-AlOH which is on-site reconstituted with 210 µL Matrix-M. The contents of the vial should be mixed by inversion (10x) to ensure re-suspension and assure homogeneity before withdrawing into a syringe for administration. Once the two components are mixed, the reconstituted Trial Vaccine will be used immediately in a volume of 190 µL which correspond to 30 µg of ProC6C and 15 µg of Matrix-M.

Full dose ProC6C-AlOH + Matrix-M
The full dose ProC6C-AlOH + Matrix-M used for intramuscular administration is composed of one vial ProC6C-AlOH which is on-site reconstituted with 210 µL Matrix-M. The contents of the

vial should be mixed by inversion (10x) to ensure re-suspension and assure homogeneity before withdrawing into a syringe for administration. Once the two components are mixed, the reconstituted Trial Vaccine will be used immediately in a volume of 630 µL which correspond to 100 µg of ProC6C and 49 µg of Matrix-M.



7.3.4 Storage, Shipping, and Stability

Vials of ProC6C-AlOH must be stored at 2ºC to 8ºC. Freezing destroys the integrity of aluminium hydroxide suspensions. Thus, any vials that have been frozen must not be used for administration to humans.

Vials will be transported and stored at temperature-controlled conditions, according to 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 indicated temperature conditions until usage approval has been obtained from the Sponsor.

An in-use Point-of-Injection (POI) stability study has been performed to support the Point-of-Use mixture of ProC6C-AlOH with an aqueous form of the adjuvant Matrix-M, for preclinical studies. The materials ProC6C-AlOH and Matrix-M were those supplied for toxicology.

In summary, under all test conditions with ProC6C-AlOH, the drug product (Either with or without Matrix-M addition) showed no adverse changes during the stability analysis. Each product (ProC6CAlOH alone or ProC6C-AlOH with Matrix-M added) was within specification when stored at 2-8°C or room temperature (18-25°C) for up to 24 hours. Indicating the two products are compatible as analyzed by the methods here and stable for use during the preclinical toxicology manipulations intended.

A second 24 hour study for the trial vaccine with Matrix-M was performed on the DP for use in the clinical trial (GMP material of ProC6C-AlOH and of Matrix-M). The results showed no change over 24 hours storage at room temperature of the trial vaccine, based on analysis of appearance, protein identity (WB) and degree of ProC6C-AlOH adsorption to aluminum hydroxide. This study together with the Pharmacy Manual will support the “Point-of-use mixture.”

7.3.5 Preparation and Dosage

The final vaccine for administration is obtained by admixing ProC6C-AlOH with Matrix-M as appropriate for each dose level and must be administered within 4 hours of reconstitution.

Prior to dose administration, vial must be mixed by hand (invert 10x by hand) to ensure homogeneity. If the vial is left to settle for > 1 minute, the vial must be mixed immediately again prior to dosing/filling of syringe.

7.4 Pfs230D1-EPA/Matrix-M™

7.4.1 Manufacturing

Pfs230D1M-EPA: PpPfs230D1M and EcEPA lots, both manufactured at the Pilot Bioproduction Facility, Walter Reed Army Institute of Research (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 the Walter Reed Army Institute of Research Pilot Bioproduction Facility in compliance with cGMP standards.

7.4.2 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 ThermoFisher BioServices (Gaithersburg, Maryland) where the materials are formulated and packaged, or from

ThermoFisher BioServices (Gaithersburg, Maryland) where additional product manufactured at the Biopharmaceutical Development Program at the Frederick National Laboratory of Research is stored.

7.4.3 Formulation, Packaging, and Labeling

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

Formulation for each dose level

Erreur ! Source du renvoi introuvable. provides an illustration of the formulation of Pfs230D1M-EPA and Matrix-M for each of the two preparations (single administration or coadministration.

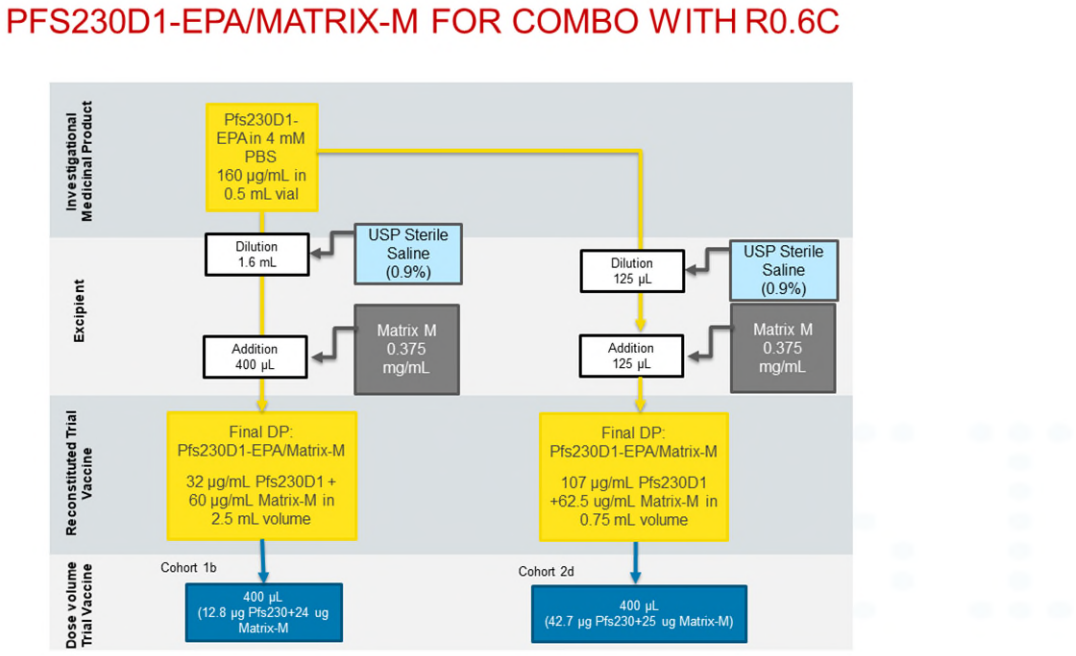


Figure 30: Formulation of Pfs230D1M-EPA and Matrix-M™ for each dose level of Pfs230D1-EPA/Matrix-M™ vaccine.

7.4.4 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. However, the long-term storage in a freezer cannot exceed -60°C. Vaccines are always shipped on dry ice, and generally remain below -70°C.

Vials will be transported and stored at temperature-controlled conditions, according to 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 indicated temperature conditions until usage approval has been obtained from the Sponsor.

7.4.5 Preparation and Dosage

The Pfs230D1M-EPA conjugates are stored at -60°C to -90°C until just before transport to the field for use, then stored at 2-8°C as described above. The final vaccine for administration is obtained by admixing Pfs230D1M-EPA with Matrix-M as appropriate for each dose level (see **Erreur ! Source du renvoi introuvable.**) and must be administered within 4 hours of reconstitution. See Section 7.77 for administration information.

7.5 R0.6C-AlOH/Matrix Co-administered with Pfs230D1M-EPA/Matrix-M™

7.5.1 Manufacturing

The individual test article manufacturing are detailed in individual sections.

7.5.2 Disposition and Dispensation

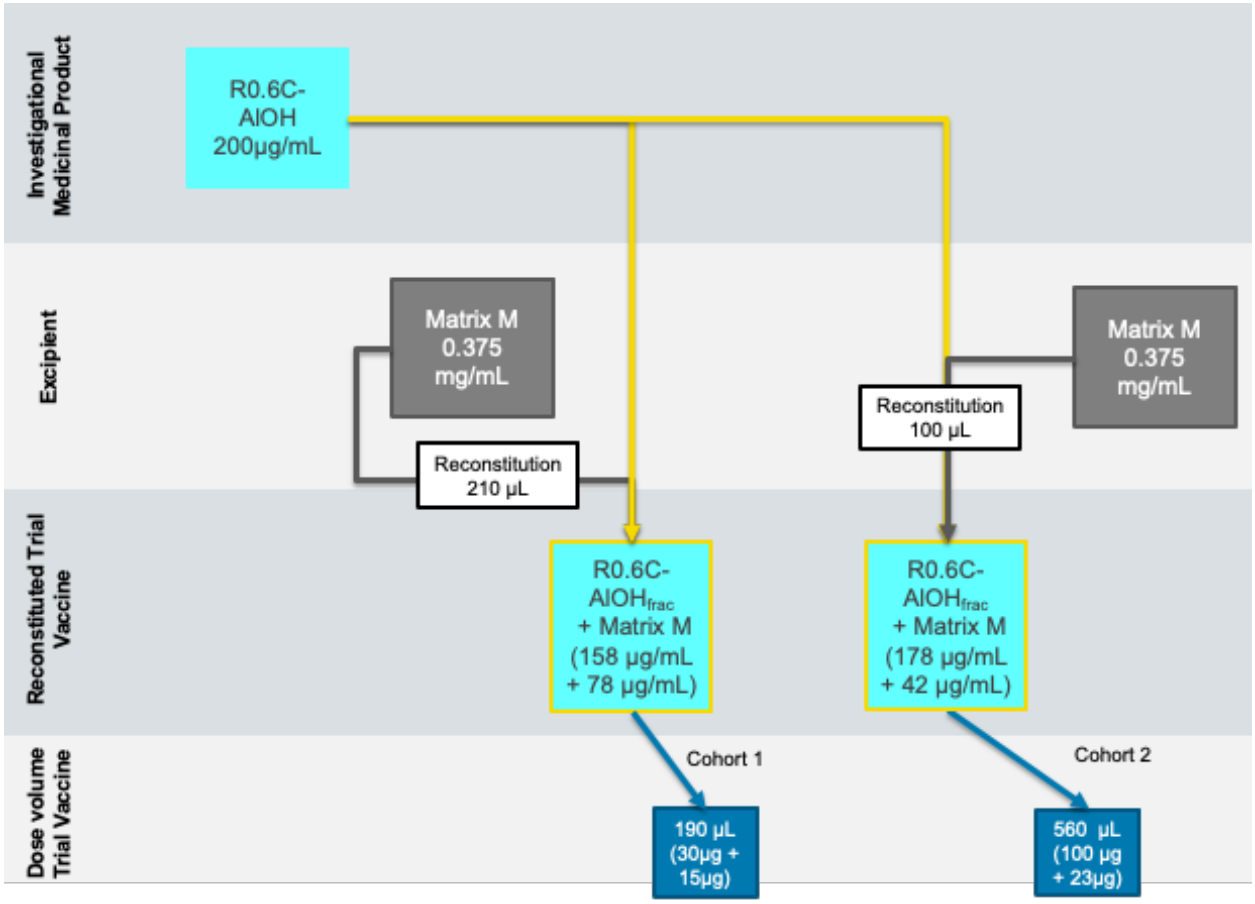
The individual test article vials will be supplied as detailed in individual sections.

7.5.3 Formulation, Packaging, and Labeling

Co-Administration of the two test articles will evaluate two individual dosage levels of the each individual component.
Fractioned dose R0.6C-AlOH + Matrix-M
The fractioned dose R0.6C-AlOHfrac + Matrix-M used for intramuscular administration is composed of one vial R0.6C-AlOH which is on-site reconstituted with 210 µL Matrix-M. The contents of the vial should be mixed by inversion (10x) to ensure re-suspension and assure homogeneity before withdrawing into a syringe for administration. Once the two components are mixed, the reconstituted Trial Vaccine will be used immediately in a volume of 190 µL which correspond to 30 µg of R0.6C and 15 µg of Matrix-M.

Full dose R0.6C-AlOH + Matrix-M
The full dose R0.6C-AlOH + Matrix-M used for intramuscular administration is composed of one vial R0.6C-AlOH which is on-site reconstituted with 210 µL Matrix-M. The contents of the vial should be mixed by inversion (10x) to ensure re-suspension and assure homogeneity before withdrawing into a syringe for administration. Once the two components are mixed, the

reconstituted Trial Vaccine will be used immediately in a volume of 630 µL which correspond to 100 µg of R0.6C and 49 µg of Matrix-M.



Lower dose of Pfs230D1-EPA + Matrix-M

The Lower dose - of Pfs230D1-EPA + Matrix-M for co-administration with Fractioned dose R0.6C-AlOH + Matrix-M.

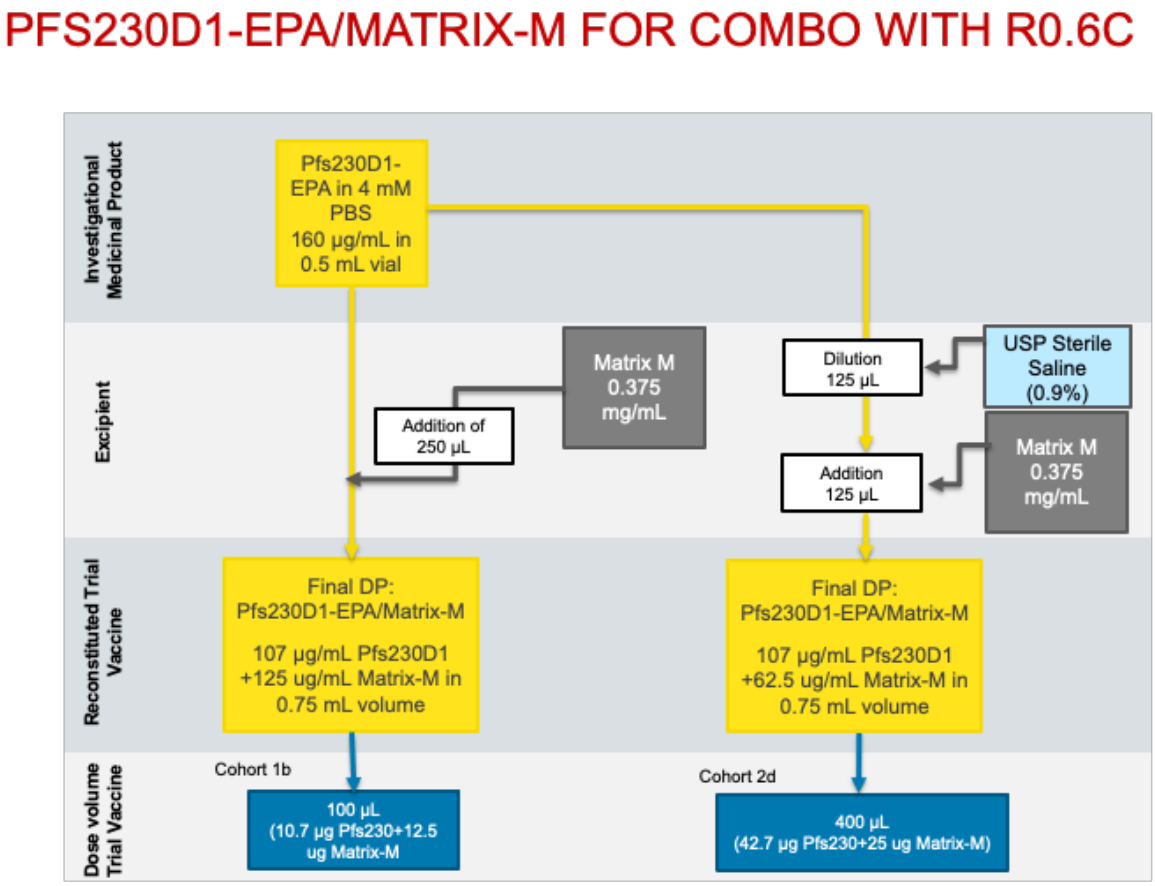
The lower dose of Pfs230D1-EPA + Matrix-M used for intramuscular administration is composed of one vial Pfs230D1-EPA which is on-site mixed with 1.6 mL USP Sterile Saline for Injection and 400 µL Matrix-M. The contents of the vial should be mixed by inversion (10x) to ensure re-suspension and assure homogeneity before withdrawing into a syringe for administration. Once the three components are mixed, the reconstituted Trial Vaccine will be used immediately in a volume of 400 µL which correspond to 12.5 µg of Pfs230D1 and 25 µg of Matrix-M.

Full dose of Pfs230D1-EPA+ Matrix-M

Full dose of Pfs230D1-EPA+ Matrix-M for co-administration with Full dose R0.6C-AlOH + Matrix-M.

The full dose of Pfs230D1-EPA + Matrix-M used for intramuscular administration is composed of one vial Pfs230D1-EPA which is mixed on-site with 125 uL USP Sterile Saline for Injection and 125 µL Matrix-M. The contents of the vial should be mixed by inversion (10x) to ensure re-suspension and assure homogeneity before withdrawing into a syringe for administration. Once

the three components are mixed, the reconstituted Trial Vaccine will be used immediately in a volume of 400 µL which correspond to 40 µg of Pfs230D1M and 25 µg of Matrix-M.



7.5.4 Storage, Shipping, and Stability

The individual test article details are detailed in each individual section.

7.5.5 Preparation and Dosage

The final vaccine for administration is obtained through preparing two syringes as detailed above. The first syringe is prepared by admixing R0.6C-AlOH with Matrix-M as appropriate for each dose level and must be administered within 4 hours of reconstitution. The Second syringe is prepared by admixing Pfs230D1M-EPA with Matrix-M as appropriate for each dose level and must be administered within 4 hours of reconstitution.

Prior to dose administration, vial must be mixed by hand (invert 10x by hand) to ensure homogeneity. If the vial is left to settle for > 1 minute, the vial must be mixed immediately again prior to dosing/filling of syringe.

7.6 Comparator vaccine

7.6.1 Manufacturing

Verorab Rabies Vaccine is manufactured by Sanofi Pasteur.

7.6.2 Disposition and Dispensation

Verorab Rabies Vaccine will be supplied to the study site according to the manufacturer's recommendations. See Section 7.122 for information about study agent accountability.

7.6.3 Formulation, Packaging, and Labeling

Verorab Rabies Vaccine is a purified inactivated rabies vaccine (Wistar rabies PM/WI 38 1503-3M strain) prepared on Vero cells. It is supplied as a powder and solvent for suspension for injection in a prefilled syringe. Before reconstitution, the powder is a white and homogeneous pellet. The solvent is a limpid solution.

7.6.4 Storage, Shipping, and Stability

The product should be stored in the original outer package, refrigerated (2°C - 8°C), and protected from light. It should not be frozen. After reconstitution, the vaccine may be used up to 8 hours after reconstitution provided it is maintained at 2°C to 8°C and protected from light. Unused vaccine must be discarded after 8 hours.

7.6.5 Preparation and Dosage

Verorab Rabies Vaccine should be reconstituted immediately prior to use, according to instructions provided in the package insert. After reconstitution, 1 dose (0.5 mL) contains rabies virus, WISTAR Rabies PM/WI38 1503-3M strain (inactivated) ≥ 2.5 IU.

7.7 Administration

Prior to dose administration, vial must be mixed by hand (invert 10x by hand) to ensure homogeneity. If the vial is left to settle for > 1 minute, the vial must be mixed immediately again prior to dosing/filling of syringe.

Each dose of the Test Articles or Verorab (or other rabies vaccine approved by Malian health regulatory) is administered as an IM injection into the deltoid muscle. For Groups 1B and 2D, two separate administrations of the prepared individual test articles are administered to the same deltoid muscle approximately 2cm apart. 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.

7.8 Contraindications to Vaccination

The following criteria should be checked prior to each study vaccination and are contraindications to further vaccination with either the experimental vaccine or the rabies vaccine:

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

Subjects who receive at least 1 dose of study vaccine under this protocol prior to developing a contraindication 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. Subjects who have a positive β-hCG test prior to their first study vaccination may be withdrawn from the study and replaced.

7.9 Indications for Deferral of Vaccination

If any of the following criteria are met at the time of the scheduled study vaccination, the vaccination will be deferred pending resolution of the issue:

- Oral temperature >38.0ºC at the time of vaccination.
- Receipt of a prohibited medication/procedure as described in Section 7.11.
- 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.

Symptomatic individuals may 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.

If the subject meets any of the above criteria for deferral on the day of first immunization, the investigator may elect to withdraw 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, they 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. Subjects who miss vaccinations after the first vaccination cannot be replaced.

7.10 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.

7.11 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.

7.12 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.

8 Study Schedule

The study schedule and approximate amounts of blood drawn are detailed in Appendix A. Schedule of Assessments and Day-to-Day Schedule.

At all visits during the trial, appropriate measures will be taken to prevent transmission of SARS-CoV-2, the organism which causes COVID-19. This will include proper use of Personal Protective Equipment (PPE) such as face masks and gloves, social distancing, good hand hygiene and other control and prevention measures as outlined in procedures.

8.1 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.

If a subject is found to be HIV positive at screening, local counseling will be provided first and then the subject will be referred to the national management system for further follow-up. 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.

The following screening evaluations must be completed for all subjects within the 56 days prior to enrollment (Day -7 visit):

- Explain the study and informed consent/assent documents to the subject
- Ensure the subject has correctly answered ≥80% of the questions on the Malaria Comprehension Exam.
- 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, and medication use.
- Females of childbearing potential must be willing to use reliable contraception from at least 21 days prior to first vaccination through 1 month after the last vaccination.
    - EXCEPTIONS to required pregnancy prevention includes the following:
        - Postmenopausal state: defined as no menses for 12 months without an alternative medical cause
        - Surgical sterilization
- Administer a complete physical examination, including vital signs (blood pressure, temperature, and heart rate), height/length, and weight.
- HIV pre- and post-test counseling as indicated including follow-up contact with subject to report the results and referral for appropriate medical care if indicated.
- Obtain blood for complete blood count with differential (CBC w/diff) and platelet count, ALT, Creatinine, Hepatitis B, Hepatitis C, HIV antibody and hemoglobin typing.
- For females of childbearing potential, obtain urine (or serum) for pregnancy testing.

If screening laboratories are completed within ≤2 days prior to Study Day -7, 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 -7, the following screening procedures will need to be repeated before enrollment: updated medical history, repeat physical exam and all laboratories outside of the window (inclusive of safety labs and human

immunodeficiency virus [HIV]). Subjects will then be reassessed for eligibility based on the re-screening information.

8.2 Enrollment and On-Study Visits

Individuals who are deemed eligible for study participation will be enrolled and begin study participation with Day -7 procedures. See Appendix A. Schedule of Assessments and Day-to-Day Schedule for a detailed study schedule.

The study visits scheduled for Study Days 281, 337, 393, 449, and 505 (8, 10 and 12 months after the final vaccination) may be conducted as phone calls if the participant is not able to come to the study site in person.

8.3 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 end-of-study visit procedures as possible.

9 Study Procedures/Evaluations

9.1 Photographs of Rash or Injection Site Reactions

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

9.2 Blood Draw

The total amount of blood collected is well within the American Association of Blood Banks recommendations and the current NIH guidelines, and will not compromise these otherwise healthy subjects (Howie 2011). Blood will be used for evaluations and assays described below and may be stored for future research.

9.3 Clinical Laboratory Testing

Using standard techniques, the clinical laboratory will perform the following tests. Laboratory reference ranges are provided in Appendix C. Mali Adult Institutional Normal Laboratory Values.

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 is collected for research purposes
2. Serum Creatinine

3. ALT
4. Hepatitis B and C
5. HIV antibody test (can include rapid diagnostics, ELISA, western blot if indicated)
6. Urine and/or serum pregnancy testing (β-hCG) in females of childbearing potential

9.4 Malaria Diagnostics

9.4.1 Blood Smears

Blood Smears:
Blood smears (BS) will also be collected periodically and will be read in real time.
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.

Blood BS Reading: Giemsa-stained thick and thin films will be examined for asexual and sexual parasites in the MRTC clinical laboratory. 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.

9.4.1.1 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.

For clinical diagnostics, RDTs will be utilized for determination of an acute malarial illness. The RDT will be paired with collection and reading of a thick BS.

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

9.5 Immunologic Laboratory Testing

9.5.1 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.

9.5.2 Transmission Assays

The transmission-blocking assay which will be conducted is summarized in **Erreur ! Source du renvoi introuvable.**19.

Table 19: 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

Feeding assays demonstrate biologic activity of transmission-blocking antibodies and are critical to selection of TBV candidates. Subjects will be screened periodically by BS (see Appendix A. Schedule of Assessments and Day-to-Day Schedule) for the presence of asexual parasites and gametocytes (slides read retrospectively).

9.5.3 Standard Membrane Feeding Assays

Membrane-feeding assays demonstrate biologic activity of TBA 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. Schedule of Assessments and Day-to-Day Schedule. 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 (Cheru, Wu et al. 2010). The SMFAs will be conducted at LMIV and LMVR in Rockville, Maryland 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.

9.6 Immunology Assays

9.6.1 Antibody Assay

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

For Pfs230, briefly, microwell plates are coated with antigen solution. Plates are washed with TBS containing Tween-20 (T-TBS) and blocked with TBS containing skim milk powder. After washing with T-TBS, diluted serum samples are added in triplicate and incubated at room temperature for 2 hours. After incubation, unbound antibodies are removed by washing the plates with T-TBS, and alkaline phosphatase-conjugated goat anti- human IgG solution is added to each well and incubated for 2 hours at room temperature. Plates are then washed with T-TBS, followed by adding phosphatase substrate solution to each well; the plates are then covered and incubated for 20 minutes at room temperature for color development. The plates are read immediately at 405 nm with a microplate reader. The optical density values are used to determine antibody levels by comparing to a standard curve generated from a known positive-control plasma included on each ELISA plate.

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

9.6.2 B-Cell and T-Cell Assays

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

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

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

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

9.6.3 Transcriptional Profiling

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

Transcriptional analyses will be performed on whole blood collected as outlined in Appendix A. Schedule of Assessments and Day-to-Day ScheduleBlood will be collected via venous puncture and placed in PAXGene tubes to preserve RNA integrity until the RNA is extracted. Specimens will be analyzed at the Research Technologies Branch, NIAID, the NIH Intramural Sequencing

Center, and/or by other LMIV laboratory collaborators. The molecular profiling encompasses the identification of RNA transcripts present in all humans, which are induced or repressed after each vaccination. This does not represent genetic testing of individuals or their DNA.

9.7 Other Laboratory Assays

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

- 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 (Fanello, Santolamazza et al. 2002).
- Cytokine levels may be evaluated during and following vaccination.

Study physicians may ask for additional laboratory exams related to subject care. Results of clinically indicated laboratory evaluations may be collected for research use.

9.8 Collection of Malaria Prevention Measures During the Transmission Season

Enrolled subjects may be asked at one or more of their study visits about other malaria prevention measures being utilized by the individual, including use of bed nets, indoor residual spraying, seasonal malaria chemoprophylaxis, personal use of insecticide, and/or intermittent preventive treatment.

10 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.

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 (with no personally identifiable information) will be shipped to LMIV during and after the study as appropriate. These samples will be stored at the LMIV in Bethesda, 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. The code key will be maintained by the Malian investigators. 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 16.1.

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. In that case, the principal investigators will review the request. If the planned research falls within the category of "human subjects research" on the part of the investigators, FMPOS EC review and approval will be obtained. This includes the investigators sending out coded and linked specimens or data and getting results that they can link back to their subjects.

Data will be archived by the study team in compliance with requirements for retention of research records; alternatively, after FMPOS 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 EC:
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 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 FMPOS EC. This decision will not affect the individual's participation in any other protocols at MRTC.

11 Data Sharing Plan

In 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 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 institutional policies and guidelines. In general, we expect de-identified data will be available through an approved public repository, speaking engagements and publications, and 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.

12 Assessment of Safety

12.1 Definitions

AE: 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 a study agent. ARs are a subset of all suspected adverse reactions (defined below) where there is reason to conclude that the study agent caused the event.

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

SARs are the subset of all AEs for which there is a reasonable possibility that the study agent caused (see "Causality" below) the event. Inherent in this definition, and in the requirement to report SARs, is the need for the sponsor to evaluate the available evidence and make a judgment about the likelihood that the study agent actually caused the AE.

The sponsor is responsible for making the causality judgment.

SAE: An SAE:

- is an AE that results in death
- is an AE that is life-threatening (places the participant at immediate risk of death from the event as it occurred)
- is an AE that requires inpatient hospitalization or prolongs an existing hospitalization
  NOTE:
  - Hospitalization is considered required if outpatient treatment would generally be considered inappropriate.
  - Same-day surgical procedures that are required to address an AE are considered hospitalizations, even if they do not involve an overnight admission.
  - Hospitalization due to a condition that has not worsened and that pre-dates study participation (e.g., elective correction of an unchanged baseline skin lesion), or due to social circumstance (e.g., prolonged stay to arrange aftercare), or that is planned/required "per protocol" and that proceeds without prolongation or complication, is not considered an SAE by this criterion.
- is, or results in, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- is a congenital anomaly/birth defect/miscarriage/stillbirth

NOTE: This definition is more inclusive than some commonly published definitions. It includes an affected conceptus/neonate whose:

- biological mother was exposed to a study agent at any point from conception through the end of the pregnancy, and/or, if breastfeeding, the 30-day neonatal period; or
- biological father was exposed to a study agent at any point during the 90 days prior to conception.
- This is separate from, and in addition to, general reporting of pregnancy in a study participant or female partner of a male participant (see Section 12.5.4 below).
- a medically important event
  NOTE: Medical and scientific judgment should be exercised. Events that significantly jeopardize the subject and/or require intervention to prevent one of the SAE outcomes listed above are generally considered medically important, and are thus SAEs.

Unexpected AE: An AE is unexpected if it is not listed in the investigator's brochure or package insert (for marketed products) at the frequency, specificity, and severity that has been observed.

NOTE:

- Such events should also be evaluated for possible reporting as unanticipated problems (UPs) (see Section 12.5.3 below).
- Unexpected, as used in this definition, also refers to AEs or SARs that are mentioned in the investigator's brochure as occurring with a class of drugs/biologics, or as anticipated from the pharmacological properties of the study agent but are not specifically mentioned as occurring with the particular study agent under investigation.

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

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

- unexpected in terms of nature, severity, or frequency in relation to
  - the research (including but not limited to risks) as described in the EC-approved research protocol and informed consent document, investigator's brochure, or other study documents; and
  - the characteristics of the subject population being studied; and is
- possibly, probably, or definitely related to participation in the research; and
- suggests the research 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 documents currently approved by the EC.

  NOTE:

  - Per the sponsor, an SAE always meets this "greater risk" criterion.
  - An incident, experience, or outcome that meets the definition of a UP generally will warrant consideration of changes to the protocol or informed

consent form, or to study procedures (e.g., the manual of procedures [MOP] for the study), in order to protect the safety, welfare, or rights of participants or others. Some UPs may warrant a corrective and preventive action plan (CAPA) at the discretion of the sponsor or other oversight entities.

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

UP that is not an AE (UPnonAE): A UPnonAE belongs to a subset of UPs that

- meets the definition of a UP, and
- does not meet the definition of an AE or SAE

  NOTE: Examples of UPnonAEs include, but are not limited to:
  - a breach of confidentiality
  - prolonged shedding of a vaccine virus beyond the anticipated timeline
  - unexpectedly large number of pregnancies on a study
  - subject departure from an isolation unit prior to meeting all discharge criteria
  - accidental destruction of study records
  - unaccounted-for study agent
  - overdosage, underdosage, or other significant error in administration or use of study agent or intervention, even if there is no AE/SAE
  - development of an actual or possible concern for study agent purity, sterility, potency, dosage, etc.

    NOTE: A decision to temporarily quarantine, or to permanently not use all or part of study agent supply due to an unexpected finding or event (e.g., particulate, cloudiness, temperature excursion), even if there is no known or proven issue (i.e., out of an “abundance of caution”), is considered a UPnonAE.

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

12.2 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 AE Case Report Form (CRF) or electronic database, and
- reported as outlined below (e.g., IND sponsor, FMPOS EC).

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 below.

12.3 Investigator Assessment of Adverse Events

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

All solicited (see Erreur ! Source du renvoi introuvable. 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 (i.e., asymptomatic parasitemia) will not be reported as an AE. Clinical or symptomatic malaria will be reported as an AE.

Table 20: Solicited Adverse Events

Systemic adverse events
Fever (temperature ≥38.0 °C)
Headache
Nausea/Vomiting
Diarrhea
Abdominal pain
Fatigue
Malaise
Myalgia
Arthralgia
Urticaria
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, all laboratory AEs will be collected and graded through 14 days after the first and second vaccination and 28 days after the third 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.

12.3.1 Severity

Severity of AEs will be assessed by the investigator according to the toxicity tables provided in

Appendix B. Toxicity Tables. AEs not included in the Appendices will be graded for severity using the definitions provided in **Erreur ! Source du renvoi introuvable.**.

Table 21: Definitions for Severity of AE Grading

Severity	Definition
Grade 1 (Mild)	No interference with activity, may use 1 dose of an over-the-counter medication
Grade 2 (Moderate)	Repeated use of non-narcotic pain reliever >24 hours or some interference with activity
Grade 3 (Severe)	Activities of daily living limited to <50% of baseline, medical evaluation/therapy required
Grade 4 (Potentially Life-Threatening)	Extreme limitation in activity, significant assistance required; immediate medical intervention or therapy required to prevent death
Grade 5	Death

12.3.2 Causality

Causality (likelihood that the event is 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 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.

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.

Causality assessment will be provided by the Principal Investigator or designee and reviewed by the sponsor. The sponsor may make a separate and final determination on the “reasonable

possibility" that the event was "related" or "unrelated" to the study agent, in keeping with applicable (US FDA) guidance on sponsor IND safety reporting.

12.4 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 last known status and 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 by REDCap or on the Safety Expedited Report Form (SERF).

12.5 Investigator Reporting Responsibilities to the Sponsor

12.5.1 Adverse Events

AE data will be entered into the research database no less than every other week and will include all data through one week prior to database entry. Line listings, frequency tables and other summary AE data will be submitted to the sponsor when requested for periodic safety assessments and preparation of final study reports.

12.5.2 Serious Adverse Events

All SAEs (regardless of relationship and whether or not they are also UPs) must be reported to the DPM/Mali Ministry of Health 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.

Sponsor Medical Monitor (SMM)
*** Contact Info ***

DPM/Mali Ministry of Health Contact Information
Direction de la Pharmacie et du Médicament (DPM), BP E-5202, Bamako, Mali
Tel fixe: 00223 20 22 65 70
Emails: couliyaya2003@yahoo.fr; chehyaicha@gmail.com; aradomother@hotmail.com

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 DPM/Mali Ministry of Health.

The clinical site investigator in Mali will also notify LMIV and the ISM 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

12.5.3 Unanticipated Problems

UPs that are also AEs or SAEs must be reported to the FMPOS EC as specified above.

UPnonAEs must be reported to FMPOS according to their requirements and preferred methods. If the UPnonAE raises a significant potential subject safety concern, the SMM should be consulted by email or phone no later than when reports are made to FMPOS.

12.5.4 Pregnancy

Pregnancy itself is not an AE. However, complications of pregnancies are AEs and may be SAEs. Events that meet AE or SAE criteria in relation to pregnancy, delivery, or the conceptus/neonate (see Section 12.1) are reportable. All pregnancies occurring up until 1 month after last vaccination will be reported to the SMM within 1 business day from site awareness. Pregnancies after that timepoint will be reported in a non-expedited manner.

Pregnancy outcome data (e.g., delivery outcome, spontaneous or elective termination of the pregnancy) will be reported to the SMM and LMIV within 3 business days of the site's awareness.

In the event of pregnancy in a study subject who has possibly been exposed to study agent, the following steps will be taken:

- Unblind the study subject
- 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) and clinical visits.
- Report to FMPOS EC as an informational item.
- Report to the DSMB, SMM, and Malian ISM.
- Advise research participant to notify the obstetrician of study participation and potential study agent exposure.

12.5.5 Medically Attended Adverse Events (MAAEs) that are Potential Immune-Mediated Medical Conditions (PIMMCs)

This trial includes plans for collection and analysis of data relating to medically attended adverse events (MAAEs) among subjects in all treatment groups through 12 months following the last study vaccination, due to the theoretical potential for induction of autoimmune or auto-inflammatory diseases due to Matrix-M.

Collection of MAAE data beyond 6 months after the last study vaccination will not delay submission of clinical trial reports and initiation of subsequent clinical trials. Final analyses of MAAEs will be submitted in clinical trial report addenda.

As part of our analyses of MAAEs in all annual reports, clinical trial reports, and clinical trial report addenda, we will include tabulated summaries of available adverse event data for potentially immune-mediated medical conditions reported during the trial, categorized by MedDRA term. These summaries will include any MedDRA preferred term included in the Immune-mediated/Autoimmune disorders Standard MedDRA Query (SMQ). Conditions reported during the trial that match the terms included in the SMQ (or list of terms provided by FDA) as well as any other potentially immune-mediated medical conditions reported during the trial that do not appear in the SMQ will be included. For each adverse event included in the summaries, we will provide a case narrative, along with an assessment of the seriousness of the event and causal relationship to study vaccine, as required by 21 CFR 312.32.

Because the request for this information by the FDA is based on a theoretical potential, the occurrence of a potentially vaccine-related immune-mediated medical condition will be considered unexpected. We will adhere to expedited reporting requirements as provided in 21 CFR 312.32 for any adverse event assessed as a serious and unexpected suspected adverse reaction (SUSAR).

Based on the theoretical concern for the development of autoimmune diseases after vaccination with new vaccines containing novel adjuvants, a list of Adverse Events of Special Interest (AESI) specific to potential immune-mediated medical conditions (PIMMCs) is provided in Appendix D (Integrated Summary of Safety of Other Novavax Recombinant Nanoparticle Vaccine Antigens with Matrix-M1^{TM} Adjuvant, Version 1.0 dated 20 April 2021).

MAAEs (which are PIMMCs) will be reported to the SMM as per Section 12.5.2 (SAE Reporting).

In order to be certain that the study team is alerted to any PIMMCs as quickly as possible, study participants will be asked to report to the study team for any medical issues which arise from the start of the study through 12 months after the final dose. They will also be asked to provide information about any Medically Attended Adverse Events (MAAEs) where the participant was seen by a Health Care Provider outside of the study (including visits to doctors, nurse practitioners, traditional healers and any other local practitioners). In this way, the study team can determine if any of these complaints may be considered PIMMCs.

12.6 Investigator Reporting Procedures to FMPOS EC

12.6.1 Definitions

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

- **Major Deviations:** Deviations from the EC-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 EC, 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 affects 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 EC).

12.6.2 Expedited Reporting to FMPOS EC

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

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.

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: 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.

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

12.6.3 Annual Reporting to FMPOS EC

Investigators must provide the following information to the EC in summary format at the time of continuing review: minor protocol deviations; AEs and SAEs that do not meet the definition of an UP.

12.7 Sponsor's Reporting Responsibilities

Events reported to the sponsor will be promptly evaluated.

12.8 Pausing Rules for the Protocol

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

12.8.1 Pausing Rules for an Individual Subject

The pausing criteria for a SINGLE subject in this study include any of the following:

- A subject experiences one SAE that is determined as possibly, probably, or definitely related to a study agent
- A subject experiences a hypersensitivity reaction (e.g. anaphylaxis, diffuse urticaria) that is determined to be possibly, probably, or definitely related to the vaccine
- A subject experiences ≥1 Grade 3 or greater AEs **or** ≥1 Grade 3 laboratory abnormalities that are deemed not to be a SAE (solicited local/systemic or unsolicited; lasting 72 hours or more) that are possibly, probably, or definitely related to a study agent, 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 SMM, in collaboration with the PI, may also pause for an individual subject for any safety issue. The study safety oversight bodies (i.e., DSMB and/or ISM) may recommend a pause to the PI.

12.8.2 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 1 business day to the SMM and to the FMPOS EC according to their requirements. The PI will also notify the DSMB and ISM through the specified pathway.

12.8.3 Resumption of a Paused Study

The SMM, in collaboration with the PI, DSMB, and ISM, will determine whether or not it is safe to resume administration of the study agent to the subject. The PI will notify the FMPOS EC of the decision on resumption of the study agent. A subject who does not resume study agent administration will continue to be followed for safety and may provide samples for immunology assays.

12.9 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):

- 1 or more subject(s) experience an SAE as defined in Section 12.1 of this protocol that is determined to be possibly, probably, or definitely related to the vaccine, **or**
- 1 or more subjects experience a hypersensitivity reaction (e.g. anaphylaxis, diffuse urticaria) that is determined to be possibly, probably, or definitely related to the vaccine, **or**
- Pilot Group: > 20% (2 or more of n=5) of vaccinees assigned to an experimental vaccine dosage (either low, middle or high dose) experience any Grade 3 AE (solicited or unsolicited) lasting 48 hours or more or any Grade 3 laboratory abnormality with either the AE or lab abnormality determined to be possibly, probably, or definitely related to the vaccine as defined in this protocol, within the 7 days post vaccination
- Main Group*: ≥20% (4 or more of n=20) of vaccinees assigned to an experimental vaccine dosage (either low, middle or high dose) experience any Grade 3 AE (solicited or unsolicited) with signs or symptoms lasting for 48 hours or more or any Grade 3 laboratory abnormality with either the AE or lab abnormality determined to be possibly, probably, or definitely related to the vaccine as defined in this protocol, within the 7 days post vaccination

  or
- Any safety issue that the study PI or CSO determines should halt the study.

**Once the Main Group starts, Halting Rules will be based on review of data from all 20 participants receiving a specific experimental vaccine dosage (n=20)*

If halting criteria are met based on blinded data, the list of research participants with the Grade 3 AEs or laboratories will be sent to the DSMB Executive Secretary. A DSMB Meeting could be convened on short notice at the occurrence of such an event.

In addition, the FMPOS EC and/or SMM may halt the study at any time.

12.9.1 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 SMM and to the FMPOS EC according to their requirements. The PI will also notify the DSMB and ISM through the specified pathway.

12.9.2 Resumption of a Halted Study

The SMM, in collaboration with the PI, DSMB, and ISM, 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 EC of the decision on resumption of the study. Subjects who do not resume study agent administration will continue to be followed for safety. They may also provide samples for immunology assays if the PI agrees that this is appropriate.

12.10 Early Termination of Study

The USTTB as the study sponsor, or the FMPOS EC may terminate this study at any time. Reasons for terminating the study may include, but are not limited to, the following:

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

12.11 Withdrawal Criteria for an Individual Participant

A subject will be withdrawn from the study for any of the following reasons:

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 after being enrolled (see Section 5.3). 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. *At the PI's discretion* - for any event that may pose a safety risk to the subject or jeopardize data collected for the study
5. *Other* – is used when previous categories do not apply and a written explanation is required.

A withdrawn subject will not have any further study visits, safety evaluations, or research procedures for this protocol; however, any data collected from that subject prior to the withdrawal will be included in the safety and immunogenicity analysis if the subject completed at least 1 study vaccination.

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. If the reason is not immediately clear, the investigator will make a reasonable effort to determine it.

12.11.1 Replacement of Withdrawn Participants

Subjects who withdraw or are withdrawn from the study after receiving at least 1 study vaccination will not be replaced. Subjects withdrawn before the first vaccination may be replaced.

12.12 Safety Oversight

12.12.1 Sponsor Medical Monitor

A medical monitor, representing the trial sponsor (USTTB), 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 protocol.

12.12.2 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. The ISM will remain blinded throughout the study.

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 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 the EC on a biannual basis or more frequently if a safety concern is raised.

12.12.3 Data and Safety Monitoring Board (DSMB)

The DSMB includes independent experts that do not have direct involvement in the conduct of the study and have no significant conflicts of interests. The DSMB will review the study prior to initiation, during the pilot and main phases as outlined in Section 1.2.1 and Table 1, 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. All SAEs and all UPs will be reported by the PI to the DSMB at the same time they are submitted to FMPOS. 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 FMPOS EC.

13 Site Monitoring Plan

According to the International Conference on Harmonisation (ICH) E6(R2) Good Clinical Practice (GCP) guidelines, section 5.18, clinical protocols are required to be adequately monitored by the study sponsor. Monitors will visit the clinical research site (or conduct virtual site visits) 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 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, the site monitors, representatives of the PfTBV EDCTP Consortium 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.

14 Statistical Considerations and Sample Size

14.1 Sample Size

Safety:
The arms are sufficiently sized for safety. For each main phase arm (n=20), vaccination of 20 subjects gives a power of at least **0.80** for detecting 1 or more serious or severe AEs that occur with a probability of **0.077** or more per subject (**or 0.90** for an event with a probability of **0.109**).

If we combine all treated groups in the main phase (arms 2a, 2b, 2c, and 2d), with a total of 80 subjects, we have **80% power** to detect 1 or more serious or severe AEs that occur with a probability of **0.026** or more per subject (or **0.90** for an event with a probability of .037).

The aforementioned calculations do not assume any historical data; they are statistical consequences of the binomial distribution.

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

Immunogenicity:
Using historical data from our prior AS01 trial (NIAID Protocol Number: 17-I-N006), we are well powered to detect a difference in TRAs between groups for functional immunogenicity measured by SMFA (see section 14.2.2 below).

Primary Objective
To assess safety and reactogenicity of administration of Pfs230D1-EPA/Matrix-M, R0.6C-AlOH/Matrix-M with Pfs230D1-EPA/Matrix-M, ProC6C-AlOH/Matrix-M and R0.6C - AlOH/Matrix-M (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 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.

14.2 Secondary Objectives

14.2.1 ELISA Analysis

We plan to do four tests, one for each treatment arm versus the control. Using historical data from the AS01 trial and adjusting for multiplicity (Bonferroni corrected alpha of 0.05/4), we are well powered to detect a log difference (>99.9% power) and ½ log difference (85.1% power).

These calculations were made assuming normal distributions with mean and standard deviations from the AS01 trial. 1000 simulations were run to see how often a significant result came from a Wilcoxon-Mann-Whitney test (WMW).

There are several questions of interest related to antibody response, which include the change in ELISA values from baseline after a given number of doses of vaccine and the change in ELISA values between doses. To address these questions, we will use an arm-specific WSRT within the 20 subjects receiving a given vaccine dose.

The data from subjects who had Pfs230 ELISA measurements after receiving two doses on protocol #17-I-N006 (Pfs230/AS01 trial) allow us to estimate the 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 protocol #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 protocol #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.

ELISA results will be analyzed by Wilcoxon-Mann-Whitney test, as the simulated power calculations were conducted.

14.2.2 SMFA Analysis

For a group of 20 participants for each vaccine dosage, we anticipate about 90% power to detect a difference in TRAs of 32% (the observed difference in protocol #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 previously established methods (Swihart, Fay et al. 2018). A nice feature of the standardized TBA is that its power is identical to those of the TRA.

SMFA results will be analyzed by Wilcoxon-Mann-Whitney test, as the simulated power calculations were conducted.

14.3 Exploratory Objectives

14.3.1 Analysis

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, as appropriate.

14.4 Measures to Minimize Bias: Randomization and Blinding

14.4.1 Randomization

The randomization list will be prepared and the code maintained by the study biostatistician. Randomization will be assigned at the time of first vaccination with the next available subject. Once a subject has received their first vaccination, they cannot be replaced.

During the study, the list linking randomization numbers to study product (Pfs230D1-EPA/ Matrix-M or Verorab or other Malian health regulatory approved rabies vaccine) will be made available only to the study statistician and associated team members, pharmacy team/syringe preparers (at the start of the study), ISM (if needed to review), and DSMB chair (if needed for closed session unblinded review). On vaccination days, the vaccines associated with each randomization number will be obtained from the pharmacist.

14.4.2 Blinding

The study is double-blind (clinical staff and participants). Blinding extends to laboratory staff conducting clinical chemistry, hematology, and parasitology tests, and to laboratory staff conducting research assays such as antibody and cellular immunity assays. Blinding will continue until completion of the last study visit and the cleaning and locking of the data set. The principal investigator will be responsible for strict maintenance of the blinding on site.

14.4.3 Unblinding

Details of unblinding procedures are provided in the unblinding SOP.

After unblinding, subjects who received the investigational vaccines will be offered the opportunity to receive the Rabies Vaccine on the same dosing schedule as the Controls (3 doses given at 1-month intervals).

Unblinding of individual participants: If knowledge of the treatment assignment is needed to provide appropriate medical care, and unblinding is recommended by the principal investigator, the ISM, or the DSMB, the treatment assignment of that research subject may be unblinded and provided to the treating clinician and other clinical staff on a need-to-know basis by the head of Pharmaceutical Operations at the study site or other designated unblinded staff who have access to the unblinded randomization list and pharmaceutical team records. The principal investigator must contact the Sponsor and provide documentation of the event and the reasons for unblinding.

See Section 12.9 regarding unblinding if pausing or halting criteria are met based on blinded data.

Unintentional unblinding: If unintentional unblinding of study agent assignment occurs, the principal investigator will create a plan for ongoing management of the participant(s) involved and for preventing the recurrence of a similar incident, as appropriate. If the protocol team determines that the unintentional unblinding may have a significant impact on the study plan (e.g., if the randomization codes for multiple participants or an entire cohort were accidentally broken), the need for a protocol amendment will be addressed as soon as possible.

Reporting: The PI will report all cases of intentional and unintentional unscheduled unblinding to the DSMB in writing within 1 business day after site awareness via email to the DSMB mailbox (niaiddsmbia@niaid.nih.gov) outlining the reason for the unblinding and the date it occurred. The report will also be submitted to the SMM.

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

15 Ethics/Protection of Human Subjects

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

15.1 FMPOS USTTB EC

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

15.2 Informed Consent Process

Informed consent is a process where information is presented to enable persons to voluntarily decide whether or not to participate as a research subject. It is an 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 forms will be approved by the FMPOS EC. The subject will sign the informed consent document prior to undergoing any research procedures. The subjects may withdraw consent at any time throughout the course of the trial. The informed consent process will be documented in the subject's research chart, as required by 21 CFR 312.62. The informed consent form will be signed (or fingerprinted) and personally dated by the subject and the person who conducted the informed consent discussion. The original signed informed consent form will be retained in the subject's chart and a signed and dated copy will be provided to the subject. The rights and welfare of the subjects will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

15.2.1 Mali Site Community Permission and Individual Informed Consent Process

15.2.1.1 Community Permission

Community permission will be obtained from village elders, family heads, and other community members after explanation and discussion of the study (Diallo, Doumbo et al. 2005). 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's 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.

15.2.1.2 Individual Informed Consent

Potential subjects will be invited to come to the study clinic for review of the informed consent.

At the consenting visit, the potential subject will read the consent form, or have it explained in cases of illiteracy. Subjects 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.

15.3 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 FMPOS EC or the sponsor’s designee.

15.4 Potential Risks

Risks to the subjects are associated with vaccination and other study procedures. These risks are outlined below.

15.4.1 Study Vaccines

15.4.1.1 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.

15.4.1.2 General Study Vaccine Risks

The study vaccine can cause non-specific inflammation and may be harmful while pregnant (Section 6.5). Therefore, pregnant women are excluded from study participation, and women of

reproductive potential will be required to agree to use birth control as outlined in Section 6.3 and will be tested for pregnancy prior to each study vaccine administration.

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

15.4.1.3 Pfs230D1-EPA/Matrix-M™

15.4.1.4 Pfs230D1M

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

While several subjects in studies conducted in the US and Mali experienced changes in hematologic parameters (such as anemia, leukopenia, and neutropenia), some of which were moderate (Grade 2) in severity, an examination of trends in the US study and the unblinded portion of the Mali study 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, SMM, 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.

15.4.1.5 Matrix-M™

Common solicited AEs during past administrations of other vaccines containing the adjuvant Matrix-M (including the COVID Vaccine candidate, NVX-CoV2373) are: injection site pain, redness, induration, and swelling and the following systemic AEs: fever, nausea/vomiting, headache, fatigue, malaise, myalgia and arthralgia.

15.4.1.6 EPA

EPA has been studied in both malaria transmission vaccine studies (as noted with Pfs230D1M) and other vaccination studies (Ashkenazi, Passwell et al. 1999, Lin, Ho et al. 2001, Passwell, Ashkenazi et al. 2003, Passwell, Ashkenzi et al. 2010, Thiem, Lin et al. 2011). The use of EPA has identified no safety issues to date.

R0.6C is a transmission blocking vaccine (TBV) candidate produced in the Lactococcus lactis expression system. It is a highly purified recombinant protein containing a subdomain (6C) of Pfs48/45 on the surface of gametes of P. falciparum, fused in frame to a carrier protein (R0) derived from the P. falciparum glutamate rich protein. While various recombinant subdomains of the native Pfs48/45 have been shown to elicit functional antibodies in multiple animal species (Theisen et al. 2014; Outchkourov et al. 2008), R0.6C is a candidate TBV which contains the main target of transmission blocking antibodies - the Pfs48/45-6C domain.

The carrier protein (R0) also forms part of the malaria vaccine candidate GMZ2 which has been tested in both European and African clinical trials. Notably, GMZ2, adjuvanted with aluminum hydroxide (GMZ2/AlOH) was tested in 880 children 12-60 month of age in Burkina Faso (Banfora; n=580, Sapone; n=300). The GMZ2/AlOH formulation has shown excellent safety and tolerability confirming the safety of the expression system and the safety of the GLURP-R0 portion of R0.6C.

Drug Product R0.6C/Alhydrogel was manufactured in compliance with cGMP regulations. Matrix-MTM was manufactured in compliance with cGMP regulations. Drug Product R0.6C/Alhydrogel + Matrix-M adjuvant will be admixed at bedside.

Both the R0.6C antigen and the R0.6C/Alhydrogel and R0.6C/Alhydrogel + Matrix-M adjuvant candidate vaccines were tested in toxicology studies in Rabbits. On the basis of the results of these studies, it is considered safe to enter clinical phase I with R0.6C/Alhydrogel and R0.6C/Alhydrogel + Matrix-M adjuvant candidate vaccines. R0.6C/Alhydrogel with and without Matrix-M is currently being evaluated in health adult volunteers (The Netherlands). To date there have been no safety issues observed.

R0.6C/Alhydrogel+Matrix-M may be co-administered with Pfs230D1-EPA (ProC6C) and Matrix-M. The two were evaluated in co-administration in toxicology studies in Rabbits and were considered safe to enter clinical Phase 1 together.

15.4.1.7 Detailed information is available into the investigator brochure. Verorab Rabies Vaccine

The following adverse reactions have been associated with Verorab Rabies Vaccine:

Very common (≥ 1/10): adenopathy/lymphadenopathy; myalgia; injection-site pain; fever; malaise.

Common (≥ 1/100): cutaneous allergic reactions such as rash, pruritus, and oedema; headache; dizziness; abdominal pain; nausea; somnolence; arthralgia; shivering; injection-site erythema, pruritus, hematoma, and induration; asthenia; influenza-like syndrome.

Uncommon (≥ 1/1000): injection-site swelling; urticaria; angioedema; dyspnea; diarrhea.

Verorab Rabies Vaccine is considered a Category B vaccine. One animal toxicity study on reproduction and development led with another inactivated rabies vaccine produced in VERO cells, did not evidence any deleterious effect on female fertility and on pre- and post-natal

development. Clinical use of rabies vaccines (inactivated "WISTAR Rabies PM/WI38 1503-3M strain") during a limited number of pregnancies did not show any malformative or fetotoxic effects to date.

For complete safety details, please refer to the package insert provided for Verorab Rabies Vaccine.

15.4.2 Treatment for Malaria

Participants who are diagnosed with clinical malaria will be treated with the standard of care as per the Mali Ministry of Health.

15.4.3 Venipuncture

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

15.5 Potential Benefits

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

15.6 Photography

Taking pictures of the body or face may be embarrassing to some people. These photographs may be published in medical journals, without identifying the participant. We will attempt to preserve the anonymity of the participant as much as possible, while providing the information needed to support the research being published. Participants may decline photographs or place any restrictions on their use. Participants will be given the opportunity to discuss this with the principal or associate investigators.

15.7 Compensation

Subjects will be given in kind (such as rice and/or millet) or cash equivalent payments, in multiple installments as outlined in **Erreur ! Source du renvoi introuvable.**, 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 of compensation is 3000 CFA for each scheduled visit with laboratory procedures and is 1500 CFA for each scheduled visit without laboratory procedures.

Table 22: Estimated Compensation Schedule 1

Study Activity	Number of Visits	Local Currency (CFA)[2]
Screening (with blood draw)	1	3000
Day -7 (with blood draw)	1	3000
Vaccination #1 and follow-up visits with blood draw	4	12,000
Vaccination #1- related visits without blood draw	1	1,500
Vaccination #2 and follow-up visits with blood draw	4	12,000
Vaccination #2 - related visits without blood draw	1	1,500
Vaccination #3 and follow-up visits with blood draw	4	12,000
Vaccination #3 - related visits without blood draw	1	1,500
Visits at 2, 3 and 6 months after last vaccination (with blood draws)	4	12,000
Visits at 8, 10 and 12 months after last vaccination (without blood draws)	3	4,500
Total	**24**	
Unscheduled visits	TBD	**1500-3000**

16 Data Handling and Record Keeping

16.1 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 and 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 access coded data only, and will not have access to personally identifiable information.

Collection and Storage of Biometric Data: In this study we are planning on using biometric data to identify study participants. Biometrics is a science that measures certain physical characteristics in order to uniquely identify a person. Common biometric measurements are fingerprints, photographs, DNA and face recognition. In this study we will take fingerprint measurements and a photograph of each enrolled participant; these will be stored in a secure biometric database and kept separate from all other study data. Only study personnel will have access to this database.

16.2 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 for a minimum of 5 years, and in compliance with institutional and IRB/EC medical records retention requirements, whichever is longest. All stored records will be kept confidential to the extent required by 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 the FMPOS EC with the name of the person who will accept responsibility for the transferred records and/or their new location. The FMPOS EC will be notified in writing and written FMPOS EC permission shall be obtained by the site prior to destruction or relocation of research records.

16.3 Protocol Revisions

No revisions to this protocol will be permitted without documented approval from the FMPOS EC that granted the original approval for the study. Any change to the protocol will be submitted to the Sponsor and to the FMPOS 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 sponsor of all such occurrences.

17 Role of the NIH & SSI Collaborators/Investigators

The MRTC and the LMIV/NIAID/NIH clinical research teams will work collaboratively on this research. However, the NIH research staff will not be directly engaged in any clinical research activities (such as recruiting, obtaining informed consent of individuals to be study participants, making decisions about subject eligibility, administering the study product or assessing adverse events). The NIH study team will not have access to any identifiable private information from the study participants. The NIH collaborators will primarily be studying, interpreting, and analyzing unlinked data and specimens for purposes of this research.

Appendix A. Schedule of Assessments and Day-to-Day Schedule

Arms: 1a, 1b, 1c, 2a, 2b, 2c, 2d, 2e		Months			0					1					2					3	4	5	8	10	12	14
		Visits		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
		Study Day (4)		-7	1	2	4	8	15	29	30	32	36	43	57	58	60	64	71	85	113	141	225	281	337	393
Procedures	Collection Tube	Days Post Vac	Screening	-7	0	1	3	7	14	0	1	3	7	14	0	1	3	7	14	28	56	84	168	224	280	336
		Visit Windows (days) (1)		+/-7	0	0	±1	±2	±3	±7	0	±1	±2	±3	±7	±0	±1	±2	±3	±14	±14	±28	±28	±28	±28	±28
Clinical Procedures																										
Complete medical history physical (2)			X																						
Informed consent			X																						
Pre-test/Post-test HIV counseling			X																						
Interim clinical evaluation				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
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						
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											
Unblinding																										X
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						
Hepatitis B, C, HIV testing	SST		5																							
Urine/Serum pregnancy test (females only)	Urine Container or SST		X	X	X					X					X											
TBS (# indicates collect for PCR as well)	TBD			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			20					10	10				10	10				10			10	20		10	20
SMFA																										
Transcriptional analysis	PAXGene			1	1												1						1			
Daily blood draw volume in mL (3)			10	26	6	0	5	0	16	15.5	0	5	0	15.5	15.5	0	6	0	15.5	5.5	0.5	10.5	21.5	0.5	10.5	20.5
Cumulative blood volume in mL			10	36	42	42.0	47	47	63	78	78	83	83	98.5	114	114	120	120	136	141	142	152	173.5	174	185	205.0
Compensation			6	6	6	3	6	6	6	6	3	6	6	6	6	3	6	6	6	6	6	6	6	6	6	6

(1) Visit windows are based off timing of days post the preceding vaccination; (2) A complete physical exam will be performed at the screening visit. (3) Blood draw amounts are "up to" the mL listed for each laboratory. If less than that amount is collected, it will not be considered a deviation. In addition, listed tube types may be substituted as appropriate at the PI's discretion. (4) As per FDA guidelines, study days are listed with Day of Immunization #1 as Day 1 (rather than Day 0).
(5) The study visits scheduled for study day 224, 28 and 336 (8, 10 and 12 months after the final vaccination) may be conducted as phone calls if the participants is not able to come to the study site in person. * Samples for hemoglobin typing may

Arm 3		Months			0					1					6					7	8	9	12	14	16	20
		Visits		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Procedures	Collection Tube	Study Day (4)	Screening	-7	1	2	4	8	15	29	30	32	36	43	169	170	172	176	183	197	225	253	337	393	449	505
		Days Post Vac		-7	0	1	3	7	14	0	1	3	7	14	0	1	3	7	14	28	56	84	168	224	280	336
		Visit Windows (days) (1)		+/-7	0	0	±1	±2	±3	±7	0	±1	±2	±3	±56	±0	±1	±2	±3	±14	±14	±28	±28	±28	±28	±28
Clinical Procedures																										
Complete medical history physical (2)			X																						
Informed consent			X																						
Pre-test/Post-test HIV counseling			X																						
Interim clinical evaluation				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
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						
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											
Unblinding																										X
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						
Hepatitis B, C, HIV testing	SST		5																							
Urine/Serum pregnancy test (females only)	Urine Container or SST		X	X	X					X					X											
TBS (# indicates collect for PCR as well)	TBD			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 SMFA	SST			20					10	10				10	10				10			10	20		10	20
Transcriptional analysis	PAXGene			1	1												1						1			
Daily blood draw volume in mL (3)			10	26	6	0	5	0	16	15.5	0	5	0	15.5	15.5	0	6	0	15.5	5.5	0.5	10.5	21.5	0.5	10.5	20.5
Cumulative blood volume in mL			10	36	42	42.0	47	47	63	78	78	83	83	98.5	114	114	120	120	136	141	142	152	173.5	174	185	205.0
Compensation			6	6	6	3	6	6	6	6	3	6	6	6	6	3	6	6	6	6	6	6	6	6	6	6

(1) Visit windows are based off timing of days post the preceding vaccination; (2) A complete physical exam will be performed at the screening visit. (3) Blood draw amounts are "up to" the mL listed for each laboratory. If less than that amount is collected, it will not be considered a deviation. In addition, listed tube types may be substituted as appropriate at the PI's discretion. (4) As per FDA guidelines, study days are listed with Day of Immunization #1 as Day 1 (rather than Day 0).
(5) The study visits scheduled for study day 393, 449 and 505 (8, 10 and 12 months after the final vaccination) may be conducted as phone calls if the participants is not able to come to the study site in person. * Samples for hemoglobin typing may

Day to Day Schedule

Study Day -7 (±7 days)

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 56 mL of blood for CBC with differential and platelet count, ALT, creatinine, malaria blood smear and PCR, hemoglobin typing, anti-Pfs230 antibody ELISA, standard membrane feeding assay (SMFA), cellular assays, transcriptional analysis.
   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. For females, obtain a urine or serum sample for β-hCG testing. Ensure that the test is negative before proceeding.
5. For females, ensure agreement and compliance with pregnancy prevention before AL dosing.

Study Day 1 (±0 days; 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 46.5 mL of blood for CBC with differential and platelet count, ALT, creatinine, malaria blood smear and PCR, anti-Pfs230 antibody ELISA, cellular and ex vivo assays, SMFA, and transcriptional analysis.
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 2 (±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 4 (±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 8 (±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 15 (±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 37 mL of blood for CBC with differential and platelet count, ALT, creatinine, malaria blood smear, anti-Pfs230 antibody ELISA, cellular and ex vivo assays, SMFA and transcriptional analysis.

Study Day 29 (±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. 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 16.5 mL of blood for CBC with differential and platelet count, ALT, creatinine, malaria blood smear, anti-Pfs230 antibody ELISA, SMFA and transcriptional analysis.
7. Confirm continued eligibility to receive vaccination.
8. Administer the vaccine.

The following evaluations/procedures will be completed following vaccination:

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

Study Day 30 (±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 32 (±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 36 (±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 43 (±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 36.5 mL of blood for CBC with differential and platelet count, ALT, creatinine, malaria blood smear, anti-Pfs230 antibody ELISA, cellular and ex vivo assays, SMFA and transcriptional analysis.

Study Day 57 (±7 days; day of Vaccination #3 for Arms 1 and 2) and Study Day 169 (±56 days; day of Vaccination #3 for Arm 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, ensure agreement and compliance with pregnancy prevention before vaccination

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
5. Obtain approximately 16.5 mL of blood for CBC with differential and platelet count, ALT, creatinine, malaria blood smear, anti-Pfs230 anti body ELISA and transcriptional analysis.

Study Day 58 for Arms 1 and 2 and Study Day 170 for Arm 3 (±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 60 for Arms 1 and 2 and Study Day 172 for Arm 3 (±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, malaria smear, and transcriptional analysis.

Study Day 64 for Arms 1 and 2 and Study Day 176 for Arm 3 (±2 day; 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 71 for Arms 1 and 2 and Study Day 183 for Arm 3 (±3 days; 14 days after Vaccination #3)

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

4. Obtain approximately 16.5 mL of blood for CBC with differential and platelet count, ALT, creatinine, malaria blood smear, anti-Pfs230 antibody ELISA, SMFA and transcriptional analysis.

Study Day 85 for Arms 1 and 2 and Study Day 197 for Arm 3 (±14 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 5.5 mL of blood for CBC with differential and platelet count, ALT, creatinine, and blood smear.

Study Day 113 for Arms 1 and 2 and Study Day 225 for Arm 3 (±14 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 30.5 mL of blood for malaria blood smear and for cellular and ex-vivo assays.

Study Day 141 for Arms 1 and 2 and Study Day 253 for Arm 3 (±28 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. For females, obtain a urine or serum sample for β-hCG testing. Ensure that the test is negative before proceeding.
5. Obtain approximately 11.5 mL of blood for malaria blood smear, anti-Pfs230 antibody ELISA, standard membrane feeding assay (SMFA) and transcriptional analysis.

Study Day 225 for Arms 1 and 2 and Study Day 337 for Arm 3 (±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 21.5 mL of blood for malaria blood smear, anti-Pfs230 antibody ELISA, standard membrane feeding assay (SMFA) and transcriptional analysis.

Study Day 281 for Arms 1 and 2 and Study Day 393 for Arm 3 (±28 days; 224 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 337 for Arms 1 and 2 and Study Day 449 for Arm 3 (±28 days; 280 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 393 for Arms 1 and 2 and Study Day 505 for Arm 3 (±28 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. Scheduled unblinding of VU
    a. If received investigational vaccines offer Rabies Vaccine comparator vaccination

Appendix B. 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]	2.5 – 5 cm	5.1 – 10 cm	> 10 cm	Necrosis or exfoliative dermatitis
Induration/Swelling at site[3]	2.5 – 5 cm and does not interfere with activity	5.1 – 10 cm or interferes with activity	> 10 cm or prevents daily activity	Necrosis
Bruising at site[2]	2.5 – 5 cm	5.1 – 10 cm	> 10 cm	Necrosis or exfoliative dermatitis

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.

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	101 – 115	116 – 130	> 130	ER visit or hospitalization for arrhythmia
Bradycardia - beats per minute[4]; at rest + calm	50 – 54	45 – 49	< 45	ER visit or hospitalization for arrhythmia
Hypertension (systolic) -mm Hg; at rest + calm	141 – 150	151 – 155	> 155	ER visit or hospitalization for malignant hypertension
Hypertension (diastolic) -mm Hg; at rest + calm	91 – 95	96 – 100	> 100	ER visit or hospitalization for malignant hypertension
Hypotension (systolic) -mm Hg; at rest + calm	85 – 89	80 – 84	< 80	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.
[3] Oral temperature; no recent hot or cold beverages or smoking.
[4] 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)
Fever (°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
Headache	No interference with activity	Repeated use of non-narcoti c pain reliever > 24 hours or some interference with activity	Significant; any use of narcotic pain reliever or 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
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
Abdominal Pain	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization
Fatigue	No interference with activity	Some interference with activity	Significant; 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
Urticaria	No interference with activity	Requiring PO or topical treatment > 24 hours or IV medications or steroids for ≤24 hours	Requiring IV medication or steroids for >24 hours	ER visit or hospitalization

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: Adults

Hematology and Biochemistry Values[1, 2]	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Hemoglobin (Male) - gm/dL	9.5 – 10.3	8.0 – 9.4	6.5 – 7.9	< 6.5 and/or requiring transfusion
Hemoglobin (Female) - gm/dL	8.0 – 9.3	7.0 – 7.9	6.0 – 6.9	< 6 and/or requiring transfusion
WBC Increase - 10^3/µL	11.5 – 15.0	15.1 – 20.0	20.1 – 25.0	> 25.0
WBC Decrease - 10^3/µL	2.5 – 3.3	1.5 – 2.4	1.0 – 1.4	< 1.0 with fever
Neutrophil/Granulocyte Decrease[3] - 10^3/µL	0.80 – 0.90	0.50 – 0.79	< 0.50	< 0.50 with fever
Platelets Decreased - 10^3/µL	100 – 110	70 – 99	25 – 69	< 25
Creatinine (Male) - µmol/L	130.00 – 150.99	151.00 – 176.99	177.00 – 221.00	> 221.00 and requires dialysis
Creatinine (Female) - µmol/L	110.00 – 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 (Bain 1996, Haddy, Rana et al. 1999).

Appendix C. Mali Adult Institutional Normal Laboratory Values

Chemistry

Chemistry[1]	Reference Range
Creatinine (Female) - µmol/L	57.4 – 96.2
Creatinine (Male) -µmol/L	59.4 – 119.4
ALT - U/L	< 45.5

Abbreviations: ALT, alanine transaminase.
[1] The laboratory values provided in the table are based on Bancoumana, Malian adults (age 18-45 years old). [Doucoure 2021, submitted]

Hematology

Hematology[1]	Reference Range
Hemoglobin (Female) - gm/dL	9.6 – 14.6
Hemoglobin (Male) - gm/dL	10.8 – 16.6
WBC - 10^3/µL	3.7 – 9.4
Absolute Neutrophil/Granulocyte Count - 10^3/µL	1.0 – 4.8
Absolute Lymphocyte Count - 10^3/µL	1.5 – 4.5
Platelet Count (Female) - 10^3/µL	163.9 – 429.1
Platelet Count (Male) - 10^3/µL	119.7 – 368.5

Abbreviations: WBC, white blood cell.
[1] The laboratory values provided in the table are based on Bancoumana, Malian adults (age 18-45 years old). [Doucoure 2021, submitted]

Appendix D. Potential Immune-Mediated Medical Conditions

Categories	Diagnoses (as MedDRA Preferred Terms)
Neuroinflammatory disorders:	Acute disseminated encephalomyelitis (including site-specific variants: eg, non-infectious encephalitis, encephalomyelitis, myelitis, myeloradiculomyelitis), cranial nerve disorders including paralyses/paresis (eg, Bell's palsy), generalized convulsion, Guillain-Barre syndrome (including Miller Fisher syndrome and other variants), immune-mediated peripheral neuropathies and plexopathies (including chronic inflammatory demyelinating polyneuropathy, multifocal motor neuropathy and polyneuropathies associated with monoclonal gammopathy), myasthenia gravis, multiple sclerosis, narcolepsy, optic neuritis, transverse myelitis, uveitis.
Musculoskeletal and connective tissue disorders:	Antisynthetase syndrome, dermatomyositis, juvenile chronic arthritis (including Still's disease), mixed connective tissue disorder, polymyalgia rheumatic, polymyositis, psoriatic arthropathy, relapsing polychondritis, rheumatoid arthritis, scleroderma (including diffuse systemic form and CREST syndrome), spondyloarthritis (including ankylosing spondylitis, reactive arthritis [Reiter's Syndrome] and undifferentiated spondyloarthritis), systemic lupus erythematosus, systemic sclerosis, Sjogren's syndrome.
Vasculitides:	Large vessels vasculitis (including giant cell arteritis such as Takayasu's arteritis and temporal arteritis), medium sized and/or small vessels vasculitis (including polyarteritis nodosa, Kawasaki's disease, microscopic polyangiitis, Wegener's granulomatosis, Churg–Strauss syndrome [allergic granulomatous angiitis], Buerger's disease [thromboangiitis obliterans], necrotizing vasculitis and ANCA-positive vasculitis [type unspecified], Henoch-Schonlein purpura, Behcet's syndrome, leukocytoclastic vasculitis).
Gastrointestinal disorders:	Crohn's disease, celiac disease, ulcerative colitis, ulcerative proctitis.
Hepatic disorders:	Autoimmune hepatitis, autoimmune cholangitis, primary sclerosing cholangitis, primary biliary cirrhosis.
Renal disorders:	Autoimmune glomerulonephritis (including IgA nephropathy, glomerulonephritis rapidly progressive, membranous glomerulonephritis, membranoproliferative glomerulonephritis, and mesangioproliferative glomerulonephritis).
Cardiac disorders:	Autoimmune myocarditis/cardiomyopathy.
Skin disorders:	Alopecia areata, psoriasis, vitiligo, Raynaud's phenomenon, erythema nodosum, autoimmune bullous skin diseases (including pemphigus, pemphigoid and dermatitis herpetiformis), cutaneous lupus erythematosus, morphoea, lichen planus, Stevens-Johnson syndrome, Sweet's syndrome.
Hematologic disorders:	Autoimmune hemolytic anemia, autoimmune thrombocytopenia, antiphospholipid syndrome, thrombocytopenia.
Metabolic disorders:	Autoimmune thyroiditis, Grave's or Basedow's disease, new onset Hashimoto thyroiditis, diabetes mellitus type 1, Addison's disease.
Other disorders:	Goodpasture syndrome, idiopathic pulmonary fibrosis, pernicious anemia, sarcoidosis.

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