A Phase 2b randomized, open-label, controlled, single center study in *Plasmodium falciparum*-infected and uninfected adults age 18-55 years old in Kenya to evaluate the efficacy of the delayed, fractional dose RTS,S/AS01$_E$ malaria vaccine in subjects treated with artemisinin combination therapy plus primaquine

Protocol Identifying Number: CVIA 078

Principal Investigator: [redacted]

Co-Principal Investigator: [redacted]

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LIST OF ABBREVIATIONS

ACT	Artemisinin combination therapy
ADI	Active detection of infection
AE	Adverse event
AESI	Adverse event of special interest
A/L	Artemether/lumefantrine
ALT	Alanine aminotransferase
anti-CS	Antibody to *P. falciparum* circumsporozoite protein repeat region
anti-HBs	Antibody to hepatitis B surface antigen
AS01	GSK's proprietary adjuvant system containing QS21, MPL, and liposomes
ATP	According to protocol
BMGF	Bill and Melinda Gates Foundation
BP	Blood pressure
CRF	Case Report Form
CBC	Complete Blood Count
CDMS	Clinical Data Management System
CHW	Community Health Worker
CI	Confidence Interval
CMI	Cell-mediated Immunity
CMP	Clinical Monitoring Plan
CRF	Case Report Form
CRO	Contract Research Organization
CS	Circumsporozoite protein
DBS	Dried blood spot
DHA/Pip	Dihydroartemisinin-piperaquine
dL	Deciliter
DMP	Data Management Plan
DNA	Deoxyribonucleic acid
DOD	Department of Defense
DOT	Directly observed therapy
ECCT	Expert Committee on Clinical Trials (ECCT)
EDC	Electronic date capture
ELISA	Enzyme-linked immunosorbent assay
FDA	Food and Drug Administration
FV	Field visit
G6PD	Glucose-6-Phosphate Dehydrogenase
GCP	Good Clinical Practice
GHVAP	Global Health Vaccine Accelerator Platform
GMT	Geometric Mean Titer
GSK	GlaxoSmithKline
H_0	Null hypothesis
H_1	Alternative hypothesis

Hb	Haemoglobin
HBsAb	Hepatitis B surface antibody
HBsAg	Hepatitis B surface antigen
HDSS	Health and Demographics Surveillance System
HIV	Human immunodeficiency virus
HJFMRI	Henry Jackson Foundation Medical Research International
HSPB	Human Subject Protection Branch
IAVI-HIL	International AIDS Vaccine Initiative Human Immunology Laboratory
IB	Investigator's Brochure
IEC	Institutional Ethics Committee
IMP	Investigational Medical Product
IRB	Institutional Review Board
ICH	International Council for Harmonisation
IM	Intramuscular
ITN	Insecticide-treated bed net
IU	International Unit
IVRS	Interactive Voice Response System
IWRS	Interactive Web Response System
JOOTRH	Jaramogi Oginga Odinga Teaching and Referral Hospital
KEMRI	Kenya Medical Research Institute
KEMRI SERU	KEMRI Scientific and Ethical Review Unit
KC	KCRC Clinic visit
KCRC	Kombewa Clinical Research Center
L	Liter
LD PQ	Low dose primaquine
MBF	Malaria Blood Film
MedDRA	Medical Dictionary for Regulatory Activities
mIU	Milli-international units
mL	Milliliter
MOP	Manual of Procedures
MPL®	Monophosphoryl lipid A
mRNA	Messenger ribonucleic acid
P. falciparum	*Plasmodium falciparum*
PATH REC	PATH's Research Ethics Committee
PBMCs	Peripheral Blood Mononuclear Cells
PCR	Polymerase chain reaction
PI	Principal Investigator
PII	Personally identifying information
pIMD	Potential immune-mediated disease
PLT	Platelet
PPB	Pharmacy and Poisons Board
PPB ECCT	PPB Expert Committee on Clinical Trials
QA	Quality Assurance

QC	Quality Control
QTc	Corrected QT interval
QS21	*Quillaja saponaria* 21
RDT	Rapid diagnostic test for malaria
rRNA	Ribosomal ribonucleic acid
RTS,S	Protein comprising CS and hepatitis B surface antigen
Rx	Treatment
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SERU	Scientific and Ethics Review Unit
SOP	Standard Operating Procedure
TVC	Total vaccine cohort
µg	Microgram
µL	Microliter
µmol	Micromole
USAMRD-A	US Army Medical Research Directorate-Africa
USAMRDC	U.S. Army Medical Research and Development Command
VE	Vaccine efficacy
Vx	Vaccine
WBC	White blood count
WHO	World Health Organization
WRAIR	Walter Reed Army Institute of Research

STATEMENT OF COMPLIANCE

The signature below constitutes the approval of this protocol and the attachments and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and ICH-GCP (E6) guidelines.

[redacted]

[redacted]

Signed:

Date:

PROTOCOL SUMMARY

<table>
<tr><td>Title</td><td>A Phase 2b randomized, open-label, controlled, single center study in Plasmodium falciparum-infected and uninfected adults age 18-55 years old in Kenya to evaluate the efficacy of the delayed, fractional dose RTS,S/AS01$_E$ malaria vaccine in subjects treated with artemisinin combination therapy plus primaquine</td></tr>
<tr><td>Design</td><td>The proposed trial design has been developed to answer several questions related to the nature of RTS,S vaccine efficacy in African adults that may be influenced by concurrent and/or past P. falciparum infection leading to a state of immunologic hypo-responsiveness. The proposed study design encompasses five groups. Three groups (Groups 1, 2, and 3) will be administered RTS,S/AS01$_E$ on a 0, 1, 7 month schedule with Dose 3 delivered as a 1/5th fractional dose shown below. Two groups (Groups 4 and 5) will be administered a comparator vaccine on a 0, 1, 7 month schedule.
<table>
<tr><th>Group # [1]</th><th>Vaccine</th><th>Sample size</th><th>Baseline Parasitemia</th><th>Anti-malarial Rx</th><th>Anti-malarial Rx rationale</th></tr>
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* The first 50 subjects from Groups 1 and 2, and all subjects in Group 3 will have solicited local and systemic AEs captured for 7 days after each immunization

Vaccine efficacy rationale
Groups of adults that are to be administered RTS,S/AS01$_E$ include individuals that differ with the presence/absence of baseline PCR-positive parasitemia that also receive anti-malarial treatment to clear parasites. A rabies comparator vaccine (Abhayrab) is included in order to assess absolute vaccine efficacy (VE) against P. falciparum parasitemia and to investigate whether sub-optimal immune responses in previously infected or concurrently</td></tr>
</table>

	infected adults is a general phenomenon independent of the vaccine antigen (RTS,S/AS01$_E$ versus rabies vaccine). The primary endpoint aims to elicit vaccine efficacy against *P. falciparum* infection in African adults with sub-clinical PCR-positive parasitemia and treated to clear parasites prior to RTS,S/AS01$_E$ administration (Group 1) compared to adults with sub-clinical PCR-positive parasitemia and treated to clear parasites prior to administration of a comparator rabies vaccine (Group 4). The vaccine efficacy secondary endpoint assesses whether African adults administered RTS,S, negative for *P. falciparum* infection at baseline and given anti-malaria chemoprophylaxis (Group 2) can elicit high VE compared to adults administered a comparator rabies vaccine that are without infection at baseline and given anti-malaria chemoprophylaxis (Group 5). A smaller group of RTS,S-vaccinated subjects (Group 3) will be enrolled and followed only for immunological endpoints. This group serves as the 'positive control' for immune dysregulation as a result of infection with *P. falciparum*, it is not powered to assess VE against *P. falciparum* parasitemia and subjects will not be treated with anti-malarial chemoprophylaxis in conjunction with vaccinations. Adults living in areas of moderate to high malaria transmission often have co-existing asymptomatic circulating blood stage *P. falciparum* parasites that results from multiple exposures to infective mosquito bites. This has been recognized as inducing a level of immunologic hypo-responsiveness that may impede the development of a protective immune response following immunization. We hypothesize that treatment of malaria infection in individuals prior to immunization with the RTS,S/AS01 vaccine will reset the immune response to the vaccine and result in an increased vaccine efficacy. **Immune profiling rationale** In-depth immunological monitoring of CS-specific and HBsAg-specific antibodies will be assayed in all subjects from Groups 1, 2, and 3 (secondary objective). Note that the RTS,S antigen consists of sequences of both the *P. falciparum* circumsporozoite protein and Hepatitis B surface antigen and thus the testing for antibodies to both these antigens is included. In-depth immunological monitoring of rabies-specific antibodies will be assayed in the first 50 subjects from both groups 4 and 5 (exploratory objective). Anti-CS assays will include evaluating both the levels and avidity of antibody responses at PATH's reference center at Walter Reed Army Institute of Research (WRAIR). Anti-Hepatitis B surface antigen antibodies will be assessed at the International AIDS Vaccine Initiative Human Immunology Laboratory (IAVI-HIL) at Imperial College, London, UK, using a commercially available ELISA kit. Anti-rabies antibodies will be assessed at the Kansas State Veterinary Diagnostic Laboratory, Manhattan, Kansas. Cell-mediated immune (CMI) responses will be assessed in subjects from Groups 1, 2, and 3 only (RTS,S-vaccinated cohort) and will be assayed at Stanford University, a Global Health Vaccine Accelerator Platform (GHVAP) partner of the Bill and Melinda Gates Foundation (BMGF). Other exploratory immunological assays evaluating the CS and HBsAg immune

	responses, including but not limited to antibody isotype titer and isotype avidity, might be performed at additional BMGF partner organizations. HLA typing as an exploratory endpoint may be performed to associate CS-specific immune responses with protection from *P. falciparum* infection. The results will inform our understanding on immune dysregulation in the context of antecedent or active *P. falciparum* infection.
Objectives	**Primary Objective**: To assess vaccine efficacy assessed by time to first *P. falciparum* infection in RTS,S vaccinated adults (Group 1) **positive** for *P. falciparum* by PCR at baseline and treated to clear parasites compared to adults administered a comparator vaccine (Group 4) **positive** for *P. falciparum* by PCR at baseline and treated to clear parasites. **Secondary Objectives:** Efficacy: • To assess vaccine efficacy by time to first *P. falciparum* infection in RTS,S vaccinated adults (Group 2) **negative** for *P. falciparum* by PCR at baseline and provided anti-malarial chemo-prophylaxis versus comparator group (Group 5) **negative** for *P. falciparum* by PCR at baseline and provided anti-malarial chemo-prophylaxis. Safety: • To assess the safety of RTS,S in terms of serious adverse events (SAEs) during the whole study period (from Dose 1 to study conclusion) • To assess the safety of RTS,S in terms of solicited adverse events within 7 days after each vaccination • To assess the safety of RTS,S in terms of unsolicited adverse events within 28 days after each vaccination Immunogenicity: • To assess anti-circumsporozoite (CS) antibody levels & avidity, hepatitis B surface antibodies (HBsAb), from select groups as shown in Appendix F. **Exploratory Objectives:** • To assess in subjects immunized with RTS,S/AS01$_E$ the relationship between HLA Class 1 and Class 2 alleles with protection against *P. falciparum* infection. • To assess anti-rabies antibodies in a subset of subjects at time points from groups 4 and 5 as shown in Appendix F. • To assess cell-mediated immune responses and transcriptomes in subjects from Groups 1 – 3. • To assess malaria cross-sectional prevalence in all study participants at the end of the study. Note: additional immunological assays evaluating the immune response to CS and HBsAg might be performed.
Endpoints	**Primary Endpoint:** The time to first malaria infection by PCR after completing vaccination in Groups 1 and 4 **Secondary Endpoints:** Efficacy: • The time to first malaria infection by PCR after completing vaccination in Groups 2 and 5.

	Safety: • Frequency count and proportion of subjects reporting serious adverse events (SAEs) during the whole study period (from Dose 1 to study conclusion) • Frequency count and proportion of subjects reporting solicited local and systemic adverse events within 7 days after each vaccination • Frequency count and proportion of subjects reporting unsolicited adverse events within 28 days after each vaccination Immunogenicity: • Anti-circumsporozoite (CS) antibody levels & avidity and hepatitis B surface antibody (HBsAb) antibodies from subjects in Groups 1, 2, and 3 at time points indicated in Appendix F as measured by ELISA assays. **Exploratory Endpoints:** • Anti-rabies antibodies at timepoints indicated in Appendix F in subset of subjects in Groups 4 and 5. • Cell-mediated immune responses in subjects administered RTS,S/AS01$_E$ (Groups 1, 2, and 3) will be descriptive in nature. • RNA transcriptional responses for all subjects in Groups 1 – 5. • HLA typing in subjects administered RTS,S/AS01$_E$ (Groups 1, 2, and 3). • Cross-sectional malaria PCR/DBS at Study Termination visit in Groups 1 – 5. Note: additional immunological assays evaluating the immune response to CS and HBsAg might be performed.
Population	Adults 18 to 55 years old at enrollment, inclusive.
Phase	Phase 2b
Number of sites enrolling participants	One site; Kombewa Clinical Research Center, Kenya
Description of study agent	• RTS,S/AS01$_E$ vaccine 0.5mL, containing 25 µg RTS,S, 25 µg MPLs, 25 µg QS21 in a liposomal formulation) for the first two immunizations. One-fifth dose RTS,S/AS01$_E$ vaccine for third immunization. • Comparator Abhayrab rabies vaccine (one dose, 0.5 mL, contains 2.5 IU rabies antigen). • Dihydroartemisinin-piperaquine (DHA/Pip) is a long acting anti-malarial. • Coartem®(artemether/lumefantrine); Coartem is a short-acting ACT that is used in this study to provide clearance of blood stage parasites before assessment of vaccine efficacy. It is used in this study to remove any confounding anti-malarial effects when analyzing time to infection and vaccine efficacy. • Primaquine; Low dose Primaquine (LD PQ) is an 8-aminoquinoline that clears stage 5 gametocytes of *P. falciparum*.
Study duration	Estimated 20.5 months from when the study opens to enrollment until completion of last subject last visit. If ADI is extended to 12 months, then study duration will be an estimated 26.5 months.
Participant Duration	Estimated 20.5 months it will take for each individual participant to complete all participant visits over the full 12-month ADI. If the number of events satisfying the primary and secondary endpoints has been achieved after at least 6 months of ADI, the trial will be truncated thus shortening the duration of participant participation.

SCHEMATIC OF STUDY DESIGN

Table 1. Group Description

Group # [1]	Vaccine	Sample size	Baseline Parasitemia	Anti-malarial Rx	Anti-malarial Rx rationale
Group 1*	RTS,S/AS01$_E$	164	+	+	Clear parasites
Group 2*	RTS,S/AS01$_E$	128	-	+	Prophylaxis
Group 3*	RTS,S/AS01$_E$	35	+	-	-
Group 4	Rabies	164	+	+	Clear parasites
Group 5	Rabies	128	-	+	Prophylaxis
	total	**619**			

Table 2. Group Anti-malarial and Vaccine Schedule

Group #[1]	Month -1	Month 0 (Vaccine 1)	1-2 wks before Vx dose 2	Month 1 (Vaccine 2)	1 wk before Vx dose 3	Month 7 (Vaccine 3)	Month 8 to 14
Group 1*	DHA/Pip + LD PQ	RTS,S/AS01$_E$	DHA/Pip + LD PQ	RTS,S/AS01$_E$	A/L + LD PQ	1/5th dose RTS,S/AS01$_E$	ADI
Group 2*	DHA/Pip + LD PQ	RTS,S/AS01$_E$	DHA/Pip + LD PQ	RTS,S/AS01$_E$	A/L + LD PQ	1/5th dose RTS,S/AS01$_E$	ADI
Group 3*	-	RTS,S/AS01$_E$	-	RTS,S/AS01$_E$	-	1/5th dose RTS,S/AS01$_E$	-
Group 4	DHA/Pip + LD PQ	Rabies	DHA/Pip + LD PQ	Rabies	A/L + LD PQ	Rabies	ADI
Group 5	DHA/Pip + LD PQ	Rabies	DHA/Pip + LD PQ	Rabies	A/L + LD PQ	Rabies	ADI

[1] Unsolicited AEs will be captured from all subjects in groups 1 to 5 for 28 days post anti-malarial treatment (DHA/Pip + LD PQ or A/L + LD PQ), as applicable, and following each immunization

* The first 50 subjects from Groups 1 and 2, and all subjects in Group 3 will have solicited local and systemic AEs captured for 7 days after each immunization

ABSTRACT

PATH and GlaxoSmithKline (GSK) are committed to developing a malaria vaccine to help reduce the burden of malaria disease in children and contribute to malaria elimination. GSK has developed a candidate vaccine against malaria caused by *Plasmodium falciparum*, RTS,S/AS01. The vaccine has been shown to be safe in multiple trials and efficacy data in pediatric populations has led to a pilot implementation program in three African countries including Kenya. The RTS,S/AS01 vaccine mechanism of action is presumed to work on the initial sporozoite and liver stages of *P. falciparum* infection through neutralization of the circumsporozoite (CS) antigen on parasites invading after a mosquito bite in individuals immunized with the RTS,S/AS01 vaccine. In order to inform whether a vaccine such as RTS,S/AS01 may have a future role in malaria elimination, it will be important to establish vaccine efficacy in adults in Sub-Saharan Africa who are reservoirs of parasites and who contribute to ongoing malaria transmission. However, in previous trials, the vaccine has been less effective in adults in endemic regions compared to challenge studies. While the cause of this is likely multi-factorial, there is a degree of immunologic hypo-responsiveness that occurs in endemic regions that may impede the development of a protective immune response following immunization that is presumed to be related

to chronic infection. This study postulates that treatment of infection prior to immunization can reset the immune response leading to an improved vaccine efficacy. To evaluate this hypothesis, the study will recruit 5 groups. Groups 1 and 4 will have asymptomatic infection with *P. falciparum* as measured by a highly sensitive PCR assay (planned assay is an RNA-based PCR though the backup utilizes both RNA and DNA PCR, see protocol for details) and will be treated with antimalarial medications prior to immunization with RTS,S/AS01 or the comparator rabies vaccine, respectively, with the primary objective of evaluating the vaccine efficacy of RTS,S/AS01 relative to the rabies vaccine in this context. Groups 2 and 5 will be negative for asymptomatic infection with *P. falciparum* as measured by a highly sensitive PCR assay and will be treated with antimalarial medications prior to immunization with RTS,S/AS01 or the comparator rabies vaccine, respectively, with the secondary objective of evaluating the vaccine efficacy of RTS,S/AS01 relative to the rabies vaccine in this context. Group 3 will have asymptomatic infection with *P. falciparum* as measured by a highly sensitive PCR assay but will not be treated with antimalarial medications prior to immunization with the RTS,S/AS01 vaccine; the immunological profile (including anti-CS and cell-mediated immune responses) of this group and groups 1 and 2 will be evaluated as part of secondary and exploratory objectives. Other secondary objectives include safety assessments and other exploratory objectives defined in this protocol. A total of 619 subjects will be enrolled (164 in groups 1 and 4, 128 in groups 2 and 5, and 35 in group 3) over a period of 6 months and will participate in the study for an initial immunization period (vaccine given on 0, 1, and 7 month schedule with the final dose being 1/5 of the dose of the first two immunizations) followed by 6-12 months of follow-up (varying based on the number of events), with the primary and secondary efficacy endpoints of time to first malaria infection by PCR. Total duration expected to be 20.5-26.5 months. Those groups receiving antimalarial medications (1, 2, 3, 4) will receive either dihydroartemisinin-piperaquine or artemether/lumefantrine and low-dose primaquine as described in the protocol.

LAY SUMMARY

PATH and GlaxoSmithKline (GSK) are committed to developing a malaria vaccine to help reduce the burden of malaria disease in children and contribute to malaria elimination. GSK has developed a candidate vaccine called RTS,S/AS01 which helps the immune system of people vaccinated with RTS,S/AS01 prevent infection with the parasite that causes malaria. The RTS,S/AS01 vaccine has been shown to be safe in multiple studies around the world and is currently beginning a pilot implementation program in children in sub-Saharan Africa. The vaccine has been shown to be effective in adults who have never had malaria when they are challenged with malaria infection in the United States, but has been less effective when given to adults in Africa who have had malaria before. There are probably multiple reasons for this, but one possible reason that is probably very important is that prior infection with malaria or an infection with malaria for long periods, even without symptoms of the disease, can prevent the vaccine from working properly. This study is testing the question that if we treat individuals with antimalarial medications before giving the vaccine, will that make it more effective in people who have a history of malaria infection. The RTS,S/AS01 malaria vaccine will be compared to a rabies vaccine to see if this will improve the effectiveness. We will also be looking at the safety of the vaccine and the response of the immune system to this vaccine.

MAIN PROTOCOL

1. KEY ROLES

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CRO -1	Triclinium Clinical Development (Pty) Ltd (TCD) Design House, 121 Amkor Road, Centurion 0157, Gauteng, South Africa

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CRO -2	DFnet Research, Inc. (DFnet) 140 Lakeside Ave, Seattle, WA 98122, United States

The Principal Investigator, will be responsible for the overall conduct of the trial at this study site, administrative actions between USAMRD-A, Institutional Review Boards, and the sponsor, oversight of field activities and patient care. Both the Principal Investigator and Co-Principal investigator contributed to the protocol development and will contribute to the statistical analysis and writing of the scientific report(s).

All Clinical Investigators listed above will be responsible for the clinical conduct of the trial, the care provided to patients, oversight of field activities, and will contribute to the statistical analysis and writing of scientific report(s). Dr. Ben Andagalu also contributed to the protocol development. See Appendix I for detailed roles and responsibilities for this study.

2. INTRODUCTION: BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1. Background Information

PATH and GlaxoSmithKline (GSK) are committed to developing a malaria vaccine to help reduce the burden of malaria disease in children and contribute to malaria elimination. The latter will ultimately require extension of the target group for the vaccine beyond children to adults who contribute to malaria parasite transmission in endemic countries. GSK has developed a candidate antigen against malaria caused by *Plasmodium falciparum*, RTS,S/AS01. The RTS,S antigen consists of sequences of the circumsporozoite (CS) protein and hepatitis B surface antigen (HBsAg) and is formulated with an adjuvant AS01 (liposome formulation with 3-O-desacyl-4'-monophosphoryl lipid A (MPL) and *Quillaja saponaria* Molina, fraction 21 (QS21) immunostimulants).

Vaccines containing the RTS,S antigen are being developed by GSK primarily for the prevention of disease in children in malaria-endemic countries, and therefore most of the data referring to the safety, immunogenicity and reactogenicity has, to date, been collected in the pediatric population in Africa [1-4]. The RTS,S/AS01 vaccine has progressed through sequential stages of evaluation in trials in both adults, young children, and infants in Africa to evaluate safety and proof-of-concept efficacy trials. The vaccine has been shown to be safe, with acceptable reactogenicity. Efficacy against malaria in both controlled human challenge trials [5,6] and in field trials have been demonstrated [1-4]. Refer to the Investigators Brochure for details on the safety, reactogenicity, and efficacy.

The mechanism by which the RTS,S malaria vaccine works to reduce the frequency and severity of clinical disease episodes is presumed to be through its effect on the initial sporozoite and liver stage parasite burden, leading to a significant reduction of the infectious inoculum of liver stage merozoites released into the blood stream. This hypothesis is fully consistent with the data observed in clinical trials of RTS,S/AS01 to date. Biologically, this occurs through the neutralization of the CS antigen on the surface of sporozoites after the bite of an infected anopheline mosquito by antibodies elicited after

RTS,S immunization [7]. Sterile protection in a person, as defined by the absence of blood stage parasites, prevents onward sexual-stage gametocyte transmission to mosquitoes.

RTS,S/AS01 has been found to be highly immunogenic and in human challenge studies performed in Europe and the United States the RTS,S/AS01 vaccine prevents or delays infection as measured by sterile protection against peripheral blood stage parasitemia [5,6]. Depending upon dose and schedule, vaccine efficacy (VE) varies widely. While the Phase 3 evaluation of the RTS,S/AS01 candidate malaria vaccine was ongoing, PATH and GSK continued to investigate ways to further improve VE and duration of protection. Higher VE levels may lead to improved malaria control and contribute to the malaria elimination goal set as a long-term target by the global health community. An important milestone was achieved in 2014–2015, when an alternative regimen of RTS,S/AS01—in which the third dose is delayed by six months and fractionated to one-fifth ($1/5^{th}$) of the standard dose (thus the term "FxRTS,S") achieved 87% protection (95% CI: 67–95), compared to 63% (95% CI: 20–80) for the standard full-dose regimen, in a controlled human malaria infection (CHMI) study [6]. Based on these results, the GSK/PATH partnership decided to proceed to a Phase 2b field study (MAL094), in young African children (ages 5 to 17 months), to evaluate the potential of FxRTS,S to prevent naturally acquired *P. falciparum* infection.

In order to inform whether a vaccine such as RTS,S/AS01 may have a future role in malaria elimination, it will be important to establish vaccine efficacy in adults in Sub-Saharan Africa who are reservoirs of parasites and who contribute to ongoing malaria transmission. When the RTS,S/AS01 vaccine was evaluated in adults in an area of Western Kenya with moderate *P. falciparum* malaria transmission, VE was modest compared to individuals receiving a comparator rabies vaccine (VE 4 months of follow-up; 29.5% (95% CI: -15.4 to 56.9, p = 0.164 vs control) and parasite prevalence at 16 weeks after the third vaccine dose was similar in subjects administered RTS,S/AS01 8.6% (95% CI 3.2 to 17.7) or the rabies comparator vaccine 4.2% (95% CI 0.9 to 11.9) [8].

The reason for the differences in VE between adults in USA following CHMI and adults in Kenya following natural infection are unknown but may be attributable to various factors, including host genetic differences, parasite differences between challenge strain and naturally occurring parasites, and the presence of concurrent malaria infection during the immunization period. Additionally, significant and pronounced differences in anti-CS repeat antibody titers were observed between adults in the US and Kenya where anti-CS NANP repeat titers in US adults administered RTS,S/AS01 were approximately 3 times higher than antibody titers of African adults.

Despite a general decline in malaria in Kenya over the past 10-15 years, the study area in the western part of Kisumu County, Kenya remains holoendemic for malaria with moderate to high malaria transmission throughout the year, peaking during the rainy seasons. Adults living in areas of moderate to high malaria transmission, such as the study site catchment area, often have co-existing asymptomatic circulating blood stage *P. falciparum* parasites that results from multiple exposures to infective mosquito bites. In 2017, Dr. Andagalu completed the malaria Transmission Dynamic Study that showed in our study catchment area we have a 36% point-prevalence of asymptomatic parasitemia (vs. 0.6% point-prevalence of symptomatic malaria during the same time period, resulting in 98.5% of individuals with detectable *Plasmodium* being asymptomatic) in adults over the course of the 1-year study [data unpublished]. Chronic infection has been recognized as inducing a level of immunologic hypo-responsiveness that may impede the development of a protective immune response following

immunization [9-15]. It is recognized that immunologic hypo-responsiveness as measured by decreased immune response to malaria and other infectious pathogens is influenced by concurrent asymptomatic sub-microscopic parasitemia and/or by prior malaria infection inducing both T cell exhaustion and B cell memory dysfunction. Preclinical models reveal an impaired ability to generate anti-CS antibody levels compared to animals whose parasitemia was cleared with anti-malarials [10]. Since the RTS,S/AS01 vaccine does not elicit immune responses that kill existing blood stage parasites, but rather acts by neutralizing the infectious sporozoites delivered after an Anopheline mosquito bite, it may be necessary to clear blood stage infections with anti-malarial medications either prior to, or at the time of immunization to optimize vaccine take and induce higher and more potent antibodies. We hypothesize that treatment of malaria infection in individuals prior to immunization with the RTS,S/AS01 vaccine will reset the immune response to the vaccine and result in an increased vaccine efficacy compared to a rabies comparator.

2.2. Rationale

A vaccine such as RTS,S/AS01, which may be considered in malaria elimination strategies, would presumably have limited use if vaccine efficacy is compromised by co-existing sub-clinical malaria infections. The minimal threshold of vaccine efficacy needed to demonstrate an effect on reducing transmission is not known. We hypothesize that anti-malarial pre-treatment of individuals with coexisting malaria infections or prophylactic treatment to prevent malaria infection during the immunization period would elicit favorable vaccine efficacy in RTS,S/AS01-vaccinated individuals compared to individuals administered a comparator vaccine (i.e. rabies) thereby restoring the immunologic setpoint. We also hypothesize that RTS,S/AS01 vaccine efficacy against a comparator vaccine would elicit favorable efficacy in individuals negative for *P. falciparum* infection and under malaria chemoprophylaxis during the immunization period (secondary objective).

2.2.1. Study design rationale

The underlying premise is that vaccines that may be considered in novel malaria control and/or malaria elimination initiatives in Sub-Saharan Africa would include anti-malarial drug administration in conjunction with other tools (ITNs, vector control). This study addresses the hypothesis with a design that is cost-effective and efficient (single study center, appropriate sample size, study completion within 26.5 months, and also provides actionable knowledge based on both vaccine efficacy and immunological readouts likely to contribute to our understanding of immune exhaustion and correlates of protection.

The study design below is not intended to represent the optimal dosage and vaccine schedule for RTS,S/AS01 (indicated for malaria elimination) under investigation elsewhere. There are several operating assumptions:

a) It is assumed that RTS,S/AS01$_E$ formulation is appropriate in adults based on unpublished data from MAL-092, see Table 3.
b) It assumes that a 0, 1, 7 month immunization schedule will elicit protective levels of anti-CS antibody
c) It assumes that fractional 1/5th dose of RTS,S/AS01$_E$ delivered as the 3rd dose enhances immunogenicity

d) It assumes that a one-month window following clearance of asexual stage parasites in groups with baseline positive *P. falciparum* parasitemia is sufficient to reset the immunological set-point
e) It assumes an incidence rate of 40% per 6 months of observation with 50% VE as determined from Cox proportional hazard model for time to 1st infection identified by PCR (planned to be RNA-based PCR throughout protocol, though see section 2.2.4 for details) for sample size estimates (assumption of incidence rate based on unpublished data from the Transmission Dynamic Study by Dr. Ben Andagalu performed in our study catchment area).

The practical implications for understanding whether concurrent *P. falciparum* malaria infection affects vaccine-take may likely influence if and how to best deliver RTS,S/AS01 or 2nd generation CS-based vaccines through strategies that employ anti-malarial medications that clear infection thereby resetting the immunological set point for optimal vaccine take.

2.2.2. Rationale for RTS,S/AS01$_E$ and rabies vaccine

The rationale to immunize with RTS,S/AS01$_E$ in African adults was based on comparable vaccine efficacy in CHMI challenge models in the U.S. comparing RTS,S/AS01$_B$ (a vaccine formulation in which a 0.5mL dose contains twice the active ingredients as RTS,S AS01$_E$; 50 µg RTS,S, 50 µg MPL, 50 µg QS21 in a liposomal formulation) to the efficacy observed with the pediatric formulation, RTS,S/AS01$_E$ on a 0, 1, 7 month immunization schedule where the 3rd vaccine dose was delivered as a 1/5th dose (FxRTS,S) (Table 3).

Table 3. Malaria-092 CHMI trial in US

RTS,S/AS01 formulation	RTS,S µg/immunization*	Schedule (months)	# Enrolled	# Challenged	# Uninfected (% uninfected)	VE (95%CI)
RTS,S/AS01$_B$	50-50-10	0,1,7	26	20	11 (55%)	51% (19,70)
RTS,S/AS01$_E$	25-25-5	0,1,7	26	22	14 (64%)	60% (30,77)
Infectivity controls			24	24	2 (8%)	-

GSK data on file, Malaria-092 Clinical Study Protocol

Rabies vaccine has been selected as the comparator because the vaccine has been used in previous studies of RTS,S-based vaccines in Africa, there is a validated serological marker for protection, and the subjects may benefit from receiving rabies vaccine as rabid animals occur in the study area.

2.2.3. Rationale for antimalarials for treatment and prophylaxis

We selected dihydroartemisin-piperaquine (DHA/Pip) for its pK properties with extended half-life of approximately 22 days in adults. We designed the study to optimize parasite clearance in subjects that are positive for baseline parasitemia (Groups 1 and 4) at enrollment and that would be predicted to be "parasite-free" for an extended period of time between immunizations due to the ACT's extended half-life. For those subjects that are parasite-negative at enrollment (Groups 2 and 5), we chose DHA/Pip for its prophylaxis effects.

We selected artemether/lumefantrine (A/L; Coartem®) to be given prior to 3rd vaccine dose for its short half-life and to clear any volunteer subjects of blood stage parasites in order to establish a clean baseline for determination of vaccine efficacy during the active detection of infection (ADI) period of observation.

We selected low dose Primaquine (LDPQ, 15 mg) so as to clear circulating mature sexual stage gametocytes.

2.2.4. Rationale for using PCR as endpoint for vaccine efficacy

As this is an experimental medicine trial, we sought to use a molecular-based PCR assay to detect the presence of malaria parasites after exposure to infectious Anopheline mosquito bites. Since the majority of adults residing in the study area (western Kisumu County, see 5.3.2 for full description) may be semi-immune to malaria infection due to frequent prior infections, a highly sensitive PCR is critical to detect sub-clinical parasitemia in subjects who otherwise may be negative when tested using more traditional methods of diagnosis such as malaria blood film (MBF) or rapid diagnostic tests (RDTs). The PCR assay to be used in this trial is a qualified assay, *Plasmodium falciparum*/ Pan-*Plasmodium* 18S rRNA LDT, used routinely in the US Army Medical Research Directorate-Africa (USAMRD-A) Laboratories in Kisumu, Kenya where the SOP is maintained. A positive PCR result from blood samples collected at any of the time points during the trial will be recorded as a positive event for the presence of *P. falciparum* blood stage infection, and the time to positive first event for each subject will determine the vaccine efficacy between the different groups.

In the event that a subject at any time during the trial experiences symptoms such as fever, chills, headache, and myalgia consistent with clinical malaria (prompting an unscheduled visit), clinicians at the Kombewa Clinical Research Center (KCRC) will collect a blood sample for routine diagnosis for malaria (MBF and/or RDT) along with a sample for PCR and a dry blood spot (DBS). If the diagnostic test is positive for the detection of malaria, the subject will be treated with standard of care anti-malarial medication. A PCR will also be done on a sample of blood collected among those currently undergoing ADI (groups 1, 2, 4, and 5; during ADI phases: Epoch 2 and 3; see Appendices C & D), and the results (positive or negative for *P. falciparum*) will be recorded and used for endpoint definitions both in ADI and passive case detection.

2.2.5. Rationale for immunology assays and timepoints

For secondary and exploratory immunogenicity endpoints, we chose to collect serum samples from all subjects in Groups 1 - 5 before and after each immunization.

CS-repeat and C-term antibody levels and avidity will be quantified in groups 1-3 using a qualified ELISA-based assay at WRAIR. Anti-CS antibody levels and avidity have previously been associated with protection against infection in CHMI trials and against clinical malaria in field trials in Sub-Saharan African children. Anti-HBsAg will be measured from subjects in groups 1-3.

A primary exploratory objective is to assess whether immune hypo-responsiveness in cellular-based assays using PBMCs can be reversed with anti-malarial treatment in subjects that have been administered the RTS,S malaria vaccine. As an exploratory endpoint, serologic assays that measure antibody responses to the comparator rabies vaccine will be measured in the first 50 subjects from Groups 4 and 5 at the timepoints shown in Appendix F.

PBMC collection and the assays for evaluating the cellular immune response are exploratory in nature; they include multi-color flow cytometry in excess of 20 different cellular markers. Such exploratory assays will evaluate vaccine antigen-specific immune responses elicited by the RTS,S vaccine in order to evaluate whether immune exhaustion is a prominent feature related to the presence or absence of

asymptomatic baseline parasitemia and how reversal of immune exhaustion affects the RTS,S-elicited immune response and protection against *P. falciparum* infection. As such, the requirement to draw additional blood for PBMC from the comparator vaccine groups is not justified.

PBMC will be collected at four timepoints from subjects administered the RTS,S malaria vaccine (Groups 1, 2, and 3). The nature of the exploratory cellular assays is based on measuring cell surface and/or intracellular markers on peripheral blood mononuclear cells using either multi-color flow cytometric panels or CYTOF panels to be conducted at the GHVAP Laboratories in the U.S.

Exploratory analysis using whole blood that analyzes mRNA transcripts from all subjects may be analyzed. Such analysis would measure transcriptomes that change as a result of anti-malarial chemotherapy or change the innate immune transcriptional responses immediately before and after vaccination. Such transcriptional analysis will be limited to understanding the immune responses related to immunization and will not be used to identify specific genetic traits or diseases.

HLA typing as an additional exploratory endpoint from subjects administered RTS,S/AS01$_E$ may be performed in order to associate immune responses to CS with protection from *P. falciparum* infection. Because RTS,S/AS01$_E$ is a vaccine composed of the hepatitis B surface antigen in which the CS antigen is co-expressed, and the strong association of the immune response to Hepatitis B surface antigen that is influenced by a persons' MHC background, it is reasonable to ask whether there is any linkage between protection after malaria challenge in African subjects administered RTS,S/AS01$_E$ as has been demonstrated previously in U.S. based RTS,S/AS01-vaccinated subjects undergoing CHMI [16]. HLA typing will be limited to understanding the immune responses related to immunization and will not be used to identify specific genetic traits or diseases.

The timepoints for all immunological assays are indicated in Appendices B, C, D and E and are summarized in Appendix F.

2.3. Potential Risks and Benefits

Details of potential risks are provided in the Investigator's Brochure for RTS,S/AS01. Potential risks for licensed products to include DHA/Pip, Coartem®, Primaquine, and Abhayrab® are included below and details may be found in the package insert for the product.

As indicated in section 5.2, pregnant women are excluded from participating in the vaccination portion of this study. While pregnant women and their fetuses are particularly susceptible to the effects of *P. falciparum* malaria, this study will exclude women who are pregnant at the time of screening, or who become pregnant during the immunization phase of the study for two reasons. First and foremost, the safety of the RTS,S/AS01$_E$ vaccine (intended for immunization of young children 5-17 months of age only) has not been previously evaluated in nonclinical reproductive toxicology studies by GSK. This requirement for nonclinical toxicology is recommended in the draft FDA guidance document on the inclusion of pregnant women in clinical trials (https://www.fda.gov/regulatory-information/search-fda-guidance-documents/pregnant-women-scientific-and-ethical-considerations-inclusion-clinical-trials). Secondly, the current study is an experimental medicine study to examine whether immune hyporesponsiveness to a model CS-based vaccine (in this case RTS,S/AS01$_E$) is observed in adults who may or may not be infected with *P. falciparum* parasites at the time of immunization. The study is not intended to support a clinical development plan for regulatory approval in either adult men or women.

2.3.1. Known Potential Risks and Risk Management

2.3.1.1. Risks of receipt of RTS,S/AS01$_E$ or rabies vaccine

Recipients of any of the investigational (RTS,S/AS01$_E$) or licensed products in this study (Abhayrab rabies vaccine) may experience pain and/or swelling at the injection site, fever, headache, fatigue, nausea, vomiting and/or abdominal pain, joint pain and/or muscle aches. As with any vaccination, there is the potential for an anaphylactic reaction. Additionally, there may be other reactions that at this time are not known. To facilitate management of these potential problems, medical staff experienced in the management of anaphylactic reaction will observe patients for at least 30 minutes following each vaccination to ensure that should any anaphylactic reaction occur it is managed in a timely manner. Subjects will be provided a follow up by study staff 7 days after each immunization of investigational product in order to assess solicited and unsolicited adverse events that may have occurred on the day of vaccination plus 6 additional days. In the large Phase III study, MALARIA-055, an imbalance of meningitis cases of any etiology (i.e. including cases with confirmed etiology and cases with no etiology found), with no cluster in time-to-onset, has been observed in children 5-17 months of age at first dose [reference 1]. Meningitis has not been a safety concern in RTS,S studies in adults. Potential immune-mediated disease (pIMD) is a theoretical concern with adjuvanted vaccines as no evidence of autoimmune disease caused by RTS,S/AS01 has been observed. Nonetheless, both meningitis and pIMDs will be monitored as AEs of specific interest for the duration of the study.

2.3.1.2. Risks of receipt of dihydroartemisin-piperaquine

The ADRs noted for DHA/Pip were generally mild in severity, and the majority were non-serious. Reactions such as cough, pyrexia, headache, *P. falciparum* infection, anaemia, asthenia, anorexia and the observed changes in blood cell parameters are consistent with those expected in patients with acute malaria. The effect on prolongation of the QTc interval has been observed on Day 2, but had resolved by Day 7 (the next time point at which ECGs were performed). To ensure compliance, Dose 1 of DHA/Pip will be given under the supervision of a medical provider at KCRC. Dose 2 and Dose 3 of DHA/Pip will be given under the supervision of a fieldworker. Any subjects who are unwell at these visits will be referred to a study clinician at KCRC for evaluation and treatment.

2.3.1.3. Risks of receipt of Coartem®

Subjects who receive Coartem® may experience abdominal pain, anorexia, nausea, vomiting, diarrhea, headache and/or dizziness. To ensure compliance, subjects will receive the first dose of Coartem® under the supervision of a medical provider at KCRC. Dose 2 and Dose 3 of Coartem® will be given under the supervision of a fieldworker. Any subjects who are unwell at these visits will be referred to a study clinician for evaluation and treatment.

2.3.1.4. Risks of receipt of Primaquine

Subjects who receive primaquine may experience abdominal pain, nausea, vomiting, diarrhea, dizziness, pruritis, and anemia. Standard dose primaquine is contraindicated in subjects with severe glucose-6 phosphate dehydrogenase (G6PD) deficiency due to the risk of hemolytic anemia. However, a single low dose (15 mg primaquine base) is safe in individuals with G6PD deficiency and recommended by the WHO for the clearance of sexual stage gametocytes of *P. falciparum* without specific testing for G6PD in

adults (except in pregnant or breastfeeding women) [https://www.who.int/malaria/publications/atoz/who_htm_gmp_2015.1.pdf?ua=1].

Primaquine is contraindicated in pregnant women. A single low dose 15 mg base of primaquine will be given under the supervision of a medical provider at KCRC. Any subjects who are unwell at these visits will be referred to a study clinician for evaluation and treatment. G6PD status will not be considered in the context of low dose primaquine administration though testing will be performed in the context of assessing the role of hemoglobinopathies that may impact time to infection detection after an infective mosquito bite.

2.3.1.5. **Risks of accidental disclosure of private medical information**

In order to ensure that all information collected on study volunteers is kept confidential, the following safeguards will be applied: Access to study files and personal information will be limited to study personnel, ethics committees, regulatory authorities, and sponsor. Study information will be kept in locked rooms when not in use. All information or samples that leave USAMRD-A will be labeled with a unique study identification number and have no personal identifying information (PII). Any link between individual study identification number and an individual's PII (e.g., an individual's study file and associated documents) will be maintained at KCRC in accordance with site SOPs to maintain each individual's confidentiality.

There may be additional risks associated with disclosure of subjects' HIV status as well as disclosure of any abnormal lab tests found during screening. In the case of HIV, subjects who are tested will undergo pre- and post-test counseling in accordance with Kenyan Ministry of Health Guidelines. There is risk of disclosure of the subjects' HIV status, and careful measures will be taken to ensure confidentiality is maintained. Standard pre-testing and post-testing counseling and linking to care will be followed. To the greatest extent possible, abnormal tests will be communicated to participants and screen failures in simplified language and referrals made to their regular health care provider as appropriate.

2.3.1.6. **Risks of participation in the study**

If a participant is hurt as a direct result of participating in this study, the medical care will be provided by the study team at the respective research centers and the study will pay for the expenses. If the injury requires hospital admission, the participants will be admitted at the Kombewa Sub-County Hospital or the Jaramogi Oginga Odinga Teaching and Referral Hospital (JOOTRH) as appropriate. If specialized treatment is required, they will be referred to relevant hospitals for management and the study will pay for medical expenses.

2.3.1.7. **Risks of phlebotomy**

Venipuncture is a routine clinical procedure the medical community commonly uses to obtain blood samples. Immediate complications may be slight pain during the entry of the needle into the skin, very rarely possible dizziness and syncope. Additionally, a hematoma may result from the venipuncture, but this has minimal risk. Infection of the skin/soft tissue at the puncture site, vein, or blood stream can all occur, though are very rare with both finger sticks and venous blood draws. Late complications might include thrombosis of the vein due to trauma or infection. These complications are extremely rare. Participant monitoring, aseptic technique, including sterile disposable blood collection apparatus and adherence to standard medical precautions reduce any risk to a minimum. A credentialed phlebotomist

or member of the clinical team experienced in venipuncture techniques will perform all venipunctures. The amount of blood to be taken for sampling will not be harmful to the subject's health.

2.3.2. Known Potential Benefits

All volunteers for this study will receive the following benefits for their participation:

- All volunteers will undergo a medical examination at screening free of charge. All volunteers, whether accepted for enrolment into the trial or not will benefit from this free health check-up. The results of all tests will be communicated to all volunteers. Where illnesses are newly-diagnosed, a referral to an appropriate health provider will be made for the volunteer.
- For the duration of their participation in the trial, all randomized subjects will receive free health care for acute conditions. In case of chronic conditions, subjects will be referred to appropriate health providers.
- All enrolled subjects in RTS,S groups will be offered free of charge a full three-dose course of vaccinations against rabies to occur following the completion of the study. The vaccine offered will be either the same Abhayrab rabies control vaccine received by participants randomized to groups 4 or 5, or another licensed rabies vaccine available in Kenya at the time of completion of the study. Subjects who are randomized to receive the rabies control vaccine will receive these vaccinations during the course of the trial. Rabies is present among animals in Kisumu County, Kenya, and vaccination will provide pre-exposure protection against rabies to participants.
- The care that is provided is by KEMRI/USAMRD-A staff working in collaboration with staff at the Kombewa Sub-County Hospital, the JOOTRH or other hospitals depending on the condition of the patient. This will include treatment for symptoms caused by the study products (drugs/vaccines) or participation in the study as well as treatment for any acute illnesses during the study period. Individuals with acute medical problems requiring specialist attention not available on site will be referred appropriately and this cost will be covered by the study. The site does not provide routine medical care for non-study participants. However, poor access to appropriate diagnostics and medications (and personnel) at Ministry of Health facilities is well documented. The sites have clinicians involved in the study and the study participants will be followed closely throughout the duration of the study and will have access to medical personnel, basic diagnostic evaluation including laboratory and radiologic evaluation and medications in accordance with Kenyan MOH guidelines. Thus, study participants will be managed in accordance with the Kenyan standard of care. All home visits for this protocol will be conducted by designated KEMRI/USAMRD-A staff who will be known to the participants and who will identify themselves using an identification card if necessary.

3. OBJECTIVES AND PURPOSE

Primary Objective:

To assess vaccine efficacy assessed by time to first *P. falciparum* infection in RTS,S/AS01$_E$ vaccinated adults (Group 1) positive for *P. falciparum* by PCR at baseline and treated to clear parasites compared to adults administered a comparator vaccine (Group 4) positive for *P. falciparum* by PCR at baseline and treated to clear parasites.

Secondary Objectives:

Efficacy:

To assess vaccine efficacy by time to first *P. falciparum* infection by PCR in RTS,S/AS01$_E$ vaccinated adults (Group 2) negative for *P. falciparum* at baseline and provided anti-malarial chemo-prophylaxis versus comparator group (Group 5) negative for *P. falciparum* at baseline and provided anti-malarial chemo-prophylaxis.

Safety:

- To assess the safety of RTS,S/AS01$_E$ in terms of serious adverse events (SAEs) during the whole study period (from Dose 1 to study conclusion)
- To assess the safety and reactogenicity of RTS,S/AS01$_E$ in terms solicited local and systemic adverse events within 7 days after each vaccination in the first 50 subjects in groups 1 and 2, and in all 35 subjects from Group 3.
- To assess the safety of RTS,S/AS01$_E$ in terms of unsolicited adverse events within 28 days after each vaccination

Immunogenicity:

- To assess anti-circumsporozoite (CS) antibody levels & avidity, hepatitis B surface antibodies (HBsAb), from groups 1, 2 and 3 as detailed in Appendix F.

Exploratory Objectives:

- To assess in subjects immunized with RTS,S/AS01$_E$ the relationship between HLA Class 1 and Class 2 alleles with protection against *P. falciparum* infection.
- To assess anti-rabies antibodies in subset of subjects at time points from groups 4 and 5 as shown in Appendix F.
- To assess cell-mediated immune responses and transcriptomes in Groups 1 – 3.
- Other immunological assays evaluating the immune response to CS and HBsAg might be performed.
- To assess malaria cross-sectional prevalence in all study participants at the end of the study.

4. STUDY DESIGN AND ENDPOINTS

4.1. Description of the Study Design

Tables 1 and 2 above show the groups, sample size, and descriptors.

The study design includes five groups.

Group 1. Group 1 subjects have detectable *P. falciparum* parasitemia at baseline measured by PCR (note any positive result from PCR, per our lab derived cutoff in accordance to the PCR SOP, will be considered positive for purposes of group selection and study endpoints). Anti-malarial treatment with DHA/Pip to clear asexual stage and young gametocyte parasites plus LD PQ to clear mature gametocytes will be given 4 weeks prior to immunization with RTS,S/AS01$_E$. A 2nd course of DHA/Pip plus Primaquine will be given 2 weeks before second RTS,S/AS01$_E$ immunization. One week before 3rd RTS,S/AS01$_E$ immunization, a three-day course of A/L plus Primaquine will be administered to

clear infection. Rationale for administration of A/L is its preferred shortened half-life allowing for evaluation of vaccine efficacy thereby excluding any confounder effect due to prolonged anti-malarial effect of drug.

Group 2. Group 2 subjects have no detectable *P. falciparum* parasitemia as measured by PCR at enrolment. It is proposed to initiate anti-malarial chemoprevention to subjects (prophylaxis effect) with DHA/Pip plus LD PQ 4 weeks prior to immunization with RTS,S/AS01$_E$. A 2nd course of DHA/Pip plus Primaquine will be given 2 weeks before second RTS,S/AS01$_E$ immunization. One week before 3rd RTS,S/AS01$_E$ immunization, a three-day course of A/L plus Primaquine will be administered to clear infection.

Group 3. Group 3 subjects have detectable *P. falciparum* parasitemia at baseline measured by PCR but will not receive any anti-malarial medications to clear PCR-positive parasites. This group includes 35 subjects and is included only for immunological assessment and not for vaccine efficacy. Subjects in Group 3 will be administered RTS,S/AS01$_E$ three times on a 0, 1, 7 month schedule.

Group 4. Group 4 subjects have detectable *P. falciparum* parasitemia at baseline measured by PCR (note any positive result from PCR will be considered positive for purposes of group selection and study endpoints as with Group 1) and will receive DHA/Pip , Primaquine, and A/L on the same schedule as subjects in group 1. Subjects in Group 4 will be administered Abhayrab rabies vaccine on a 0, 1, 7 month schedule.

Group 5. Group 5 subjects have no detectable *P. falciparum* parasitemia as measured by PCR at enrolment and will receive DHA/Pip, Primaquine, and A/L on the same schedule as subjects in group 2. Subjects in Group 5 will be administered Abhayrab rabies vaccine on a 0, 1, 7 month schedule.

4.2. Study Endpoints

The impact of the event-driven design is that we will continue to follow up until 92 first infection events have been observed in aggregate between Groups 1 and 4, and 72 first infection events have been observed in the aggregate of Groups 2 and 5 up to a maximum of 12 months of ADI. This clinical study was designed to capture positive *P. falciparum* infection events by PCR for at least 6 months of ADI (see section 10.5 for sample size calculation and presumed attack rate) but no longer than 12 months. If the aggregate numbers of first infection events have been reached between 6 and 12 months of ADI, the study endpoints will have been reached. The study will then be concluded. A final cross-sectional PCR (along with DBS and serum sampling) will be performed at study end. This will require the PCR lab to provide timely aggregated reports of first infection event counts (across Group 1 plus 4 and Group 2 plus 5) and may result in more than the required first infection events for either the primary or secondary endpoint.

4.2.1. Primary Endpoint

The primary endpoint is defined as the time to first PCR-detectable malaria infection in Groups 1 and 4 during the ADI phase of the study.

Vaccine efficacy in African adults (Group 1) administered RTS,S with sub-clinical PCR-positive parasitemia at baseline and treated to clear parasites before immunization will be compared to

volunteers in Group 4 with sub-clinical PCR-positive parasitemia at baseline and treated to clear parasites before immunization with a comparator rabies vaccine.

4.2.2. Secondary Endpoints

Secondary endpoint for vaccine efficacy:

The secondary endpoint is defined as the time to first PCR-positive malaria infection in Groups 2 and 5 during the ADI phase of the study. Vaccine efficacy in African adults administered RTS,S, and who are negative for *P. falciparum* infection at baseline and given anti-malaria chemoprophylaxis (Group 2) are compared to rabies vaccinated adult subjects who are negative for *P. falciparum* infection at baseline and given anti-malaria chemoprophylaxis (Group 5).

Secondary endpoint for safety:

- Frequency count and proportion of subjects reporting serious adverse events (SAEs) during the whole study period
- Frequency count and proportion of subjects reporting solicited local and systemic adverse events within 7 days after each vaccination
- Frequency count and proportion of subjects reporting unsolicited adverse events within 28 days after each vaccination

Secondary endpoint for immunogenicity:

- Anti-circumsporozoite (CS) antibody levels & avidity at time points shown in Appendix F.
- Hepatitis B surface antibody (HBsAb) levels at time points shown in Appendix F.

4.2.3. Exploratory Endpoints

Immune profiling of cell-mediated immune (CMI) responses will be assessed only in subjects administered RTS,S (Groups 1, 2, 3). Subjects in Groups 4 and 5 that are administered the comparator rabies vaccine will not have PBMC collected. The flow cytometric assays will measure T cell, B cell and innate cell markers associated with immune exhaustion. The specific flow cytometric assays to be measured may include but are not limited to T cell markers CD3, CD4, CD8, TCRgD, CD56, CD45RA, CD27, CD25, CD127, CTLA4, PD-1 associated with immune exhaustion. Flow cytometric assays specific for B cells, Monocyte, NK subsets may include but are not limited to, CD27, HLA-DR, CD16, CD14, CD123, PD-1, CD56, CD21.

Whole blood samples will be collected for future use to measure RNA transcriptional responses related to innate immune responses before and after immunization as shown in Appendix F. While PAXgene tube samples will be collected from all subjects in Groups 1 through 5, it is anticipated that a small cross-sectional case-control subset of samples may be selected to measure transcriptional immune responses. No genetic testing related to risk of disease will be performed from these samples.

Anti-rabies antibody levels in the first 50 subjects in Groups 4 and 5 will be assayed at timepoints indicated in Appendix F.

HLA typing from buccal swabs or from PBMC will be collected for determination of an association of RTS,S elicited immune responses and protection from *P. falciparum* infection.

The results from the immunological and transcriptional analyses will inform whether the presence or absence of *P. falciparum* infection is associated with immune exhaustion and will serve as a knowledge base for the development of improved second-generation CS-based vaccines.

Malaria parasitemia will be assessed in Groups 1 through 5 at the Study Termination Visit within a 6 week period to get a cross-sectional malaria prevalence.

5. STUDY ENROLLMENT AND WITHDRAWAL

5.1. Participant Inclusion Criteria

In order to be eligible to participate in this study, an individual must meet all of the following criteria:

- Provision of signed or thumb printed and dated informed consent form
- Stated willingness to comply with all study procedures and availability for the duration of the study
- Male or female between 18 and 55 years of age, inclusive
- In good general health as evidenced by medical history and clinical examination before entering the study
- Ability to take oral medication and be willing to adhere to the medication regimen
- For females, she must be of non-childbearing potential or use appropriate measures to prevent pregnancy for 30 days prior to vaccination through 2 months after completion of the vaccine series. Non-childbearing potential means she is surgically sterilized or at least one year post-menopausal. Appropriate measures to prevent pregnancy include abstinence or adequate contraceptive precautions (e.g. intrauterine contraceptive device; oral contraceptives; diaphragm or condom in combination with contraceptive jelly, cream or foam; Norplant or Depo-Provera). Kombewa clinical staff will assist with provision of acceptable birth control for study entry and will discuss with volunteer at screening visit.

5.2. Participant Exclusion Criteria

An individual who meets any of the following criteria will be excluded from participation in this study:

- Planned administration/administration of a vaccine not foreseen by the study protocol from within 30 days before the first dose of study vaccine until 30 days after the last dose of study vaccine.†

  † In the context of the COVID-19 pandemic, the administration of the COVID-19 vaccine will be allowed as an exception to this exclusion criteria as follows. The study team will work with the participant to attempt to have any COVID-19 vaccine administration occur 30 days or more before or after study vaccinations. When this is not possible, COVID-19 vaccination will be allowed 10 days or more before or after study vaccination. Intervals shorter than 10 days can be allowed on a case-by-case basis in discussion with the sponsor.
- Any prior receipt of any rabies vaccine or experimental malaria vaccine.
- Confirmed or suspected significant immunosuppressive or immunodeficient condition as determined by the investigator, including clinical stage 3 or 4 human immunodeficiency virus (HIV) infection (Appendix H).
- A family history of congenital or hereditary immunodeficiency.

- History of allergic reactions, significant IgE-mediated events or anaphylaxis to previous immunizations.
- History of any neurologic disorders.
- Acute disease (defined as the presence of a moderate or severe illness with or without fever), including acute malaria, at the time of enrolment. All vaccines can be administered to persons with a minor illness, such as diarrhea or mild upper respiratory infection without fever, i.e. temperature < 37.5°C*. Individuals excluded with acute disease, including acute malaria, can become eligible again after complete recovery of the illness, including appropriate treatment as applicable, and can be rescreened at a later date.
  * Temperature readings may be taken by site staff either using either oral, axillary, or infrared thermal thermometers during clinic or field visits, while subjects enrolled in the reactogenicity cohort will be supplied with oral thermometers for the purposes of recording their own temperature measurements in the memory aid over 7 days after each vaccination.
- Acute or chronic, clinically significant pulmonary, cardiovascular (including cardiac arrythmias), hepatic or renal functional abnormality, as determined by medical history, physical examination or laboratory screening tests.
- History of homozygous sickle cell disease (Hgb SS).
- Any clinically significant laboratory abnormalities as determined by the investigator on screening labs (site reference ranges, Appendix G).
- History of splenectomy.
- Administration of immunoglobulins, blood transfusions or other blood products within the three months preceding the first dose of study vaccine or planned administration during the study period.
- Pregnant (i.e. a positive pregnancy test) or lactating female during immunization phase of the study (refer to section 2.3 for rationale). If a woman becomes pregnant after all vaccinations are complete, she will not be excluded from the remainder of the study.
- Female planning to become pregnant or planning to discontinue contraceptive precautions during the vaccination phase.
- History of chronic alcohol consumption and/or drug abuse.
- Chronic administration (defined as more than 14 days) of immunosuppressants or other immune-modifying drugs within six months prior to the first vaccine dose (for corticosteroids, this will mean prednisone, or equivalent, ≥ 0.5 mg/kg/day. Inhaled and topical steroids are allowed).
- Major congenital defects or serious chronic illness.
- Simultaneous participation in any other clinical trial (apart from participation in the HDSS network).
- Any other findings that the investigator feels would increase the risk of having an adverse outcome from participation in the trial.

5.3. Strategies for Recruitment and Retention

5.3.1. Community information

The community in which the study will take place will be informed about the nature and design of the study. Community leaders (chiefs, local village elders, and opinion leaders) and local health authorities

will be formally briefed in their own language on the nature and purpose of the study. They will have the opportunity to ask questions of the PI or his designees.

5.3.2. Recruitment

Adults age 18 to 55 years will be recruited from the villages in the Kombewa Health and Demographics Surveillance System (HDSS) consisting of half of Kisumu West and all of Seme Sub-Counties of Kisumu County, Kenya. Potential participants identified from within the HDSS will be approached in the community using a recruitment script by study staff delegated to carry out recruitment. Informed consent will be obtained in accordance with section 13.3. Language and illiteracy will not be impediments in the recruitment process, all briefings and explanations will be in the understandable language of the participant. It is expected that approximately 1240 adult volunteers will have to be screened to achieve the 619 subject enrolment number. HIV-infected participants will be allowed to participate in this trial, assuming they meet all eligibility criteria (less than stage 3 HIV and in the opinion of the PI they are healthy enough to participate). The study is not seeking to actively enroll HIV-positive individuals. However, given the fact that the study area has a high prevalence of HIV, it is expected that there will be HIV-positive individuals in the screening population. Therefore, the screening for HIV and staging will ensure that only healthy HIV-positive individuals are included in the study. The proportion of HIV-positive subjects enrolled in the trial is intended to reflect the same proportionate make-up of HIV-positives in the recruitment catchment areas, estimated to be approximately 15%. As such, we intend to cap HIV-positive enrollees at approximately 20-25% to avoid an over-representation by HIV-positive subjects. Final determination for enrolment of HIV-positive subjects will be determined by the Principal Investigator.

5.4. Participant Withdrawal or Termination

5.4.1. Reasons for Withdrawal or Termination

Subjects can leave the study at any time for any reason if they wish to do so without any penalty or loss of benefits to which they are otherwise entitled. Subjects can also be withdrawn from the study procedures at the discretion of the clinical investigator. The following reasons may lead to withdrawal of individual subjects:

- Withdrawal of informed consent by volunteer;
- Any serious adverse event;
- Any adverse event that, according to clinical judgment of the investigator, is considered as a definite contraindication to proceeding with the study procedures;
- Completely lost to follow-up (unable to contact after at least 3 phone or physical attempts by the study staff to locate the participant and 1 in-person attempt by a field supervisor to contact the individual; additional attempts may be made at the discretion of the PI);
- Pregnancy during immunization phase
- Ineligibility (arising during the study or retrospectively, having been overlooked at screening);
- If the investigator or DoD Research Monitor believes that continuation would be detrimental to the subject's well-being;
- Volunteer non-compliance with study requirements;
- Any other protocol deviation that results in a significant risk to the subject's safety.

5.4.2. Handling of Participant withdrawals or termination

Investigators, or designee, will follow subjects who are withdrawn as result of a SAE/AE until resolution of the event and/or chronicity is established. Study staff will make an attempt to contact those subjects who do not return for scheduled visits or follow-up. Information relative to the withdrawal will be documented on the CRF. The investigator will document whether the decision to withdraw from the study was made by the subject or the investigator and which of the following possible reasons was responsible for withdrawal:

- serious adverse event
- non-serious adverse event
- protocol violation (specify)
- consent withdrawal, not due to an adverse event
- moved from the study area
- lost to follow-up

If a subject withdraws or is terminated from the study after enrolment and receipt of investigational product (including anti-malarial medications), including lost to follow-up, they will not be replaced. If a subject withdraws or is terminated from the study after enrolment but prior to receipt of investigational product, including lost to follow-up, (e.g., participant in group 3 who withdraws or is terminated between study visits 2 and 5) that participant may be replaced.

If the reason for withdrawal from the study is pregnancy during the immunization phase of the study, the participant will be withdrawn from any further immunizations, but will be followed for safety until delivery or termination of the pregnancy.

5.5. Premature Termination or Suspensions of Study

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause as determined by the sponsor, PATH. Written notification, documenting the reason for study suspension or termination, will be provided to the investigator, GSK, WRAIR IRB, and to Kenyan regulatory authorities. If the study is prematurely terminated or suspended, the PI will promptly inform the IRB and will provide the reason(s) for the termination or suspension.

6. STUDY VACCINE

6.1. Study Vaccine and Antimalarial Medications

Refer to the IB for details of the description of RTS,S/AS01$_E$. The IB for RTS,S and the package inserts for Abhayrab®, DHA/Pip, A/L, and Primaquine will be provided to the ethical and regulatory review committees. The candidate RTS,S/AS01 vaccine to be used has been developed and manufactured by GSK Biologicals. The vaccine is labelled and packed according to applicable regulatory requirements.

The RTS,S/AS01$_E$ vaccine presentation is provided as a clipped two-vial combination in 3 mL vials, one vial of RTS,S antigen and a second vial of AS01$_E$ adjuvant. RTS,S antigen is a lyophilized pellet containing

50 µg of RTS,S per vial. The pellet is reconstituted with 1mL adjuvant in liquid form and 0.5 mL dose of reconstituted vaccine contains 25 µg RTS,S. The $AS01_E$ adjuvant contains 25 µg of MPL®, 25 µg QS21 (QS21 is a triterpene glycoside purified from the bark of *Quillaja saponaria*) in a suspension of liposomes in phosphate buffered saline per 0.5 mL and is presented in 3 mL vials. A dose of 0.5 mL (first two immunizations) or 0.1 mL (third immunization) will be delivered as appropriate. The presentation of the reconstituted $RTS,S/AS01_E$ candidate malaria vaccine is an opalescent liquid.

Table 4. Vaccine Formulation and Presentation

Treatment name	Vaccine/product name	Formulation	Presentation	Volume to be administered
$RTS,S/AS01_E$ (Full dose)	RTS,S	RTS,S=25µg	Lyophilized pellet in a glass vial	0.5 mL
	$AS01_E$	MPL=25µg; QS21=25µg; Liposomes	Liquid solution in a glass vial	
$RTS,S/AS01_E$ ($1/5^{th}$ dose)	RTS,S	RTS,S=5µg	Lyophilized pellet in a glass vial	0.1 mL
	$AS01_E$	MPL=5µg; QS21=5µg; Liposomes	Liquid solution in a glass vial	
Rabies vaccine	Abhayrab	Rabies virus = 2.5 IU	Powder and solvent for solution for injection. After dissolution of the white lyophilizate (powder), a clear colorless solution is obtained.	0.5 mL

The Abhayrab vaccine is a sterile freeze-dried vaccine obtained by growing the fixed-virus strain Flury LEP in primary cultures of chicken fibroblasts. The potency of one dose (0.5 mL) Abhayrab is at least 2.5 IU of rabies antigen. Abhayrab is a white, freeze-dried vaccine for reconstitution with the diluent prior to use; the reconstituted vaccine is a clear to slightly opaque, colorless suspension. A dose of 0.5 mL will be delivered. The presentation of the reconstituted vaccine is as a clear or slightly opaque suspension.

6.1.1. Acquisition

$RTS,S/AS01_E$ will be provided by GSK and sent directly to the study site in Kisumu, Kenya. Abhayrab, DHA/Pip, Coartem®, and Primaquine will be procured by USAMRD-A.

6.1.2. Formulation, Appearance, Packaging, and Labeling

See IB and package inserts for details.

6.1.3. Product Storage and Handling

All vaccine vials (RTS,S/AS01$_E$ and Abhayrab rabies vaccines) must be stored in the refrigerator (+2°C to +8°C) and must not be frozen. All vaccine/adjuvant/water for injection vials will be stored in a safe and locked place with no access for unauthorized personnel. The storage conditions will be assessed during pre-study activities under the responsibility of the sponsor study contact. The storage temperature should be continuously monitored with calibrated (if not validated) temperature monitoring device(s) and recorded according to SOPs at the investigator's site. An alarm system and a back-up refrigerator will be available in case of power failure/breakdown. Whether it occurs prior to receipt of RTS,S vaccine at site or after, GSK and the study monitor must be contacted if the cold chain is broken (e.g. vaccines become frozen or refrigeration fails).

GSK will manage temperature deviations during the primary shipment until the reception at the clinical trial site. Temperature excursions are the responsibility of PATH upon receipt of RTS,S/AS01 study product. Data will be provided to PATH that will allow PATH to decide whether the IP can still be administered or should be destroyed following a temperature excursion. Any temperature excursion outside the range of 0.0 to +8.0°C (for +2 to +8°C/+36 to +46°F label storage condition) impacting investigational medicinal products (IMPs) must be reported to PATH. In case of temperature excursion below +2.0°C down to 0.0°C impacting IMP(s) there is no need to report, but adequate actions must be taken to restore the +2 to +8°C/+36 to +46°F label storage temperature conditions. The impacted IMP(s) may still be administered, but the site should avoid re-occurrence of such temperature excursion.

All anti-malarial medications should be stored below 30°C.

6.1.4. Preparation

In this study, the RTS,S/AS01$_E$ will be supplied in clipped vials, i.e. a two-dose glass vial of lyophilized RTS,S antigen (50 µg) to be reconstituted with a two-dose glass vial of AS01$_E$ Adjuvant System (1.0 ml). The final product for administration will be prepared by reconstitution of the lyophilized antigen with the liquid adjuvant. From the reconstituted vaccine vial, 0.5 mL will be administered for RTS,S/AS01$_E$ full doses or 0.1 mL will be administered for RTS,S/AS01$_E$ fractional doses (1/5th dose). All vials of vaccine provided in this study are intended for single use only.

6.1.5. Dosing and Administration

Refer to dosing and administration of DHA/Pip, Coartem®, and Primaquine in Table 5.

Table 5. Anti-malarial Medications

Study Agents	Dosage forms and strength	Body weight	Day 1 (AM)	Day 1 (+8hr)	Day 2 (AM)	Day 2 (PM)	Day 3 (AM)	Day 3 (PM)
Dihydroartemisinin-piperaquine	Dihydroartemisinin (40 mg) and piperaquine tetraphosphate (320 mg)	36 to <75 kg	3 tabs	-	3 tabs	-	3 tabs	-
		75 to 100 kg	4 tabs	-	4 tabs	-	4 tabs	-
COARTEM **	artemether (20 mg) and lumefantrine (120 mg)	> 35 kg	4 tabs	4 tabs @ 8 hrs	4 tabs	4 tabs	4 tabs	4 tabs

PRIMAQUINE***	primaquine phosphate tablets 26.3 mg primaquine phosphate (equivalent to 15 mg primaquine base)	> 35 kg	1 tab	-	-	-	-	-

*Dihydroartemisin-piperaquine should be taken at approximately the same time each day – refer to the Manual of Operating Procedures for further details. Dihydroartemisin-piperaquine should be taken orally with water. No food should be taken within 3 hours before and after each dose.

** Coartem should be taken with food

***Primaquine may be taken with food

6.1.6. Route of Administration

Route of administration for vaccines RTS,S/AS01$_E$ and Abhayrab will be by the intramuscular route to the deltoid of the arm.

DHA/Pip, A/L, and Primaquine will be administered orally according to the dosages above by directly observed therapy (DOT) by study staff or field workers and documented in the CRF.

6.1.7. Vaccination Schedule

Refer to Appendix B for vaccination schedule.

6.1.8. Tracking of Dose

Adherence to and tracking doses of vaccines and anti-malarial study agents will be documented in the CRF for each participant.

6.2. Study Vaccine Accountability Procedures

The study vaccines will be distributed to and maintained by the KCRC pharmacy and will include amount of product shipped, documentation of adequate and safe handling and use, temperature log for vaccine product and plans for returning or destroying of unused product.

7. STUDY PROCEDURES

7.1. Study procedures and evaluations

7.1.1. Study specific procedures

This is a Phase 2b, single-center, open label, randomized controlled trial with five groups in one study site. 619 adults will be enrolled. It is assumed that 90% will complete study procedures and be evaluable. Prior to study start, a community information program will inform the local population of the study and study information will be presented at community centers. Screening procedure timelines for any single subject will include a 12-day window period before randomization. Data collection will be by CRF. Following the Screening Phase, trial-specific procedures are sub-divided into 3 separate Epochs. Epoch 1 describes the procedures during the Treatment and Immunization Phase (Appendix B). Epoch 2 describes the procedures during the Active Detection of Infection Phase (Appendix C). If insufficient number of PCR-positive events to meet both the primary and secondary endpoints occur during Epoch 2, it will be necessary to extend the ADI for an additional 24 weeks (Epoch 3, Extension Phase, Appendix D) (refer to Statistical Section 10).

A. Study-specific Screening Procedures (Appendix A)

Screening procedures are to be completed as shown in Appendix A. Screening (day -39 to day -28) may be done on a separate visit from recruiting. Only adults aged 18 to 55 years with a signed/thumb printed and dated Informed Consent document will be eligible to be screened for the trial. At the screening visit each subject will be given a Subject Screening Number (only to be used for screening) and each will have a clinic record prepared.

Volunteers will provide a medical history through a one-on-one interview with a clinical officer. Volunteers will also undergo physical examination and standard laboratory screening tests, which include complete blood count (CBC), creatinine, and ALT, and HIV infection. An additional sample of blood (350 µL to 500 µL) will be collected from each subject for determination of *P. falciparum* infection by PCR and to make a DBS card for a back-up sample. This finger-stick blood sampling is in addition to venous blood collected for safety laboratories and HIV screening. A urine pregnancy test will be performed on all female subjects. Volunteers may be screened one additional time if re- screening is determined to be necessary. Volunteers will be excluded from participation if they meet any of the exclusion criteria. Volunteers excluded from this study because of significant abnormalities will be referred to an appropriate health provider for evaluation and treatment. All screening tests will be completed within 12 days prior to entry into the study. Information gathered during screening (medical history, physical examination, and laboratory analysis) will be recorded in the Source Documents and the CRFs. Laboratory references values for the KCRC are shown in Appendix G.

A photograph will be taken of each volunteer who is screened and stored securely in a computer at KCRC.

Note: HIV screening will be obtained to identify subjects positive for HIV. Subjects found to be HIV positive will have a confirmatory laboratory test as per local standard of care procedures. A positive HIV test does NOT exclude the subject from participation in the trial by itself, though a cap on enrolment of HIV positive participants will be implemented as needed by the Principal Investigator, as discussed above. Subjects found to be HIV positive (stage 1 and 2) will be equally randomized among all study groups to the extent possible and within the limits of the cap. Subjects will be verbally consented for HIV testing according to the local standard of care. If a participant has been previously screened for this study and is being rescreened outside of the window, HIV-testing will only be repeated if it is >90 days from their initial negative screening HIV testing at the clinic. In this same scenario but where they initially screened HIV-positive at the clinic while screening for this study, they will not require any repeat HIV screening. In the cases where HIV screening will not be repeated, the prior HIV test result from their initial screening attempt will be transcribed to the CRF for their new screening ID.

B. Epoch 1 - The Treatment and Immunization Phase (Appendix B)

Appendix B shows the visit number, study day, window periods, and blood sampling volumes for all study procedures for Visits 2 to 18. When screening and review of inclusion/exclusion criteria are complete, a picture ID card of each eligible volunteer will be created using the stored photo. In addition to the subject's photo, subject ID number and the study number and name, the card will contain contact information for KCRC. Subjects will be assigned a subject ID number sequentially upon randomization; example: CVIA078-001. This will ensure that the subject can contact an investigator, and the clinic can be contacted if medical care is received outside the study health facilities. Copies of these pictures will

be kept in the subjects' records to aid the study staff in confirmation of the volunteers' identification for future visits. The photos of subjects who are determined ineligible to participate during screening will be deleted from the computer.

The subject is randomized to one of the 5 groups as determined by block randomization (section 10.5.1). A study subject ID number will be assigned. This number will be used throughout the study to identify every document and blood sample associated with the volunteer. The clinic records will contain the Subject ID Number, the subject's date of birth, medical history, findings of the physical examination, the date of screening visit, whether the subject was enrolled, and (where applicable) reasons for exclusion from the study. The name of the study will be written on both the ID card and clinic records of enrolled volunteers.

A subject is considered enrolled once they have been randomized and any Visit 2 laboratory test, excluding pregnancy test, is performed or any investigational product is administered.

At enrollment, visit 2, subjects will have a blood sample for hemoglobinopathy panel (G6PD, alpha thalassemia, and sickle cell) collected. A urine pregnancy test will be performed on all female subjects.

Subjects from Groups 1, 2, 4, and 5 that receive anti-malarial medications, will receive DHA/Pip plus LD PQ before the first 2 vaccinations. They will have the first dose of a 3-day regimen administered by DOT at the KCRC. The 2nd and 3rd doses will be administered by field workers by DOT. Prior to the 3rd vaccination, subjects in groups 1, 2, 4, and 5 will be treated with A/L plus LD PQ for presumptive clearance of malaria parasitemia and to ensure a clean baseline for determination of vaccine efficacy. They will receive the first dose of A/L and LD PQ by DOT at the KCRC and be sent home with their second dose to be taken 8 hours later. A field worker will visit the next morning and verify the second dose was taken and administer the 3rd dose by DOT and leave the 4th dose to be taken in the evening. The same will happen again the next day for the 5th dose by DOT and give the final dose to be taken in the evening. Compliance with this final dose will be verified at the next visit (vaccination visit) before vaccine administration. Any failure to follow the exact schedule for either antimalarial treatment will be reported as a deviation, but allowance can be made for completion of missed doses to complete the full treatment regimen outside this schedule prior to vaccination at the discretion of the PI and documented in a progress note.

All subjects that are administered RTS,S or rabies vaccine will be observed for at least 30 minutes after vaccination to evaluate and treat any acute adverse events.

The first 50 subjects in groups 1 and 2 and all subjects in group 3 will constitute the Reactogenicity Cohort, which will be evaluated for solicited local and general adverse effects seven days after each immunization. Following each injection, the Reactogenicity Cohort will be provided a memory aid, instructed on its use, and encouraged to fill it out as directed. When field workers visit subjects on day 7 after each immunization, the field worker will review the memory aid document with the subject. The memory aid and/or the interview with the subject will be used to complete the CRF entries for solicited local and general AEs that have occurred on days 1 through day 7 post immunization. If the memory aid is not completed or not brought to a study visit by Day 28 following each dose, this will be documented in the CRF. Specifically, the Reactogenicity Cohort will be asked to:

- Record any local or general symptoms that they experience on a daily basis during the 7-day period after each injection in the memory aid.
- Record their temperatures daily in the memory aid for the first 7 days after each injection to help assess for possible fevers. To more accurately account for possible fevers, subjects will be given an oral thermometer and instructed on its use.
- Record local reactions at the injection sites in the memory aid for 7 days after each injection.

Subjects in Groups 4 and 5 will not have solicited AEs captured.

There will be a 28-day follow-up period after each dose of vaccine for reporting unsolicited symptoms in all subjects from all Groups and, where applicable, following each course of per protocol scheduled anti-malarial treatment (i.e. Groups 1, 2, 4 and 5).

Blood sampling for immunologic assays in selected groups of subjects at pre-defined timepoints will be collected as shown in Appendix F. For the determination of anti-rabies antibody levels, only the first 50 subjects each in Groups 4 and 5 will have blood samples collected for rabies antibody determination.

A urine pregnancy test will be performed prior to administering any vaccine or before each course of anti-malarial drug begins according to Appendix B.

Any subjects presenting to KCRC with medical complaints (either during unscheduled or scheduled visits) will be evaluated thoroughly by a study provider and care or referral will be provided as needed. If a subject has medical complaints during a scheduled visit with study product administration, the investigator will determine if it is safe to proceed with the scheduled intervention. If there is clinical concern for malaria during either a scheduled or unscheduled visit during Epoch 1, appropriate testing and treatment according to local standard of care will be completed regardless of the group allocation (including testing with MBF or RDT as clinically indicated), with the malaria results recorded in the CRF. If the subject is within the window to receive scheduled anti-malarial medication at this same visit, then the subject may receive the study anti-malarial treatment unless deemed inappropriate by the investigator and an alternative anti-malarial drug is required. In addition to the diagnosis and management of suspect malaria described above, if there is concern for malaria during Epoch 1 that occurs during an unscheduled visit or a visit where a sample for parasitemia assessment is not scheduled according to the schedule in Appendix B, a sample of whole blood for DBS will be collected (by finger stick or venous blood). If this occurs on a visit where a parasitemia assessment is scheduled according to the schedule in Appendix B, then a sample of whole blood for PCR and DBS will be collected (optimally by finger stick) as scheduled. Malaria positivity on any testing during Epoch 1 will not be considered a study endpoint.

C. Epoch 2 – The Active Detection of Infection Phase (Appendix C)

Appendix C presents the visit number, study day, and blood sampling for detection of infection by PCR for Visits 19 to 27. Surveillance for Active Detection of Infection (ADI) will begin 2 weeks after Dose 3 of vaccine. The total period of surveillance for ADI will be 24 to 48 weeks, with Epoch 2 covering the initial 24 weeks. All subjects will complete the entirety of Epoch 2 according to the description in this section, but whether individual subjects continue into Epoch 3 is determined by the aggregate number of events that have occurred among all subjects upon that individual's completion of Epoch 2. An event is

designated as the first positive PCR for *P. falciparum* malaria during an ADI phase (either Epoch 2 or 3). If all events are achieved while all subjects are in Epoch 2, then visit 27 will be the final study visit and subjects will not continue into Epoch 3.

Subjects will be seen at KCRC or visited by field workers or community health workers (CHW) every 21 days during the ADI period per the schedule in Appendix C. At each contact for ADI a sample of whole blood will be collected by finger stick into EDTA-coated tubes (at KCRC a venous blood sample can be collected in cases where a finger prick sample cannot be collected). In the case of field visits, samples are then transported back to KCRC for processing. Approximately 350 to 500 µL of whole blood will be collected at each ADI visit. For all subjects an aliquot (approximately 250 µL) will be placed on DBS cards. If the subject has not yet had a positive PCR sample obtained during the ADI phase, then approximately 100 to 250 µL blood will be sent for PCR testing. Subjects with a positive PCR during the ADI phase will have their results recorded in the CRF. Subjects with a prior positive *P. falciparum* PCR will continue to have finger prick blood samples for DBS collected every three weeks until the end of Epoch 2. Such samples may be used in a future exploratory study under a separate protocol in order to genotype parasites that are identical to or dissimilar from the *P. falciparum* parasite 3D7 strain used in the RTS,S vaccine. All subjects will return for visit 27 where groups 1 – 3 will have serology assessment and all subjects will have a whole blood sample collected (optimally by finger stick) for parasitemia assessment (both DBS and PCR regardless of if they have had prior positive PCR during ADI). Note: only a participant's first positive PCR during ADI will be counted as an endpoint.

At each contact for ADI, history of fever will be queried, and temperature taken. At any ADI contact in the field, if a subject has a history of subjective fever within the previous 24 hours, temperature ≥37.5°C, or reports concern for having clinical malaria, arrangements will be made for that subject to present to KCRC for an unscheduled clinic visit (in addition to their scheduled field visit that day). Attempts should be made by the field team to collect the finger stick sample for PCR and DBS during the field visit according to protocol, but if not feasible for safety reasons, this will be reported to the clinic so the sample can be collected during the unscheduled visit.

All subjects who present to KCRC for a scheduled or unscheduled visit during Epoch 2 and who have symptoms that are consistent with malaria in the clinical judgement of a study provider (including but not limited to: history of fever within previous 24 hours or temperature ≥37.5°C) will have blood collected for a MBF and/or RDT. Subjects with confirmed malaria by lab testing will be treated with appropriate anti-malarial chemotherapy within the same day. Subjects with negative testing or if there is a problem that prevents prompt testing, and for whom the clinical suspicion for malaria remains/is high may be treated with appropriate anti-malarial chemotherapy, at the discretion of the investigator, for the wellbeing of the participant. For all subjects who are seen for confirmed or suspected malaria during an unscheduled visit, a sample of whole blood for DBS and PCR (the latter if appropriate per previous discussion regarding PCR testing during ADI) will be collected (optimally by finger stick) during the unscheduled visit (unless already collected during a field ADI visit that same day). The results from the PCR, if collected, will be the only official determination of *P. falciparum* positivity for the study endpoint and will be entered into the CRF.

Subjects in group 3 will not participate in ADI, but will continue to participate in immunological testing during Epoch 2. The blood sampling for immunologic assays in selected groups of subjects at pre-defined

timepoints will be collected as shown in Appendix F. For assessment of rabies antibodies, only the first 50 subjects each in Groups 4 and 5 will have blood samples collected for rabies antibody determination.

D. Epoch 3 Extension Phase (Appendix D) – if required

Appendix D presents the visit number, study day, and blood sampling for detection of infection by PCR for Visits 28 to 35.

Due to rolling enrollment at the site (planned enrollment of ~103 subjects per month over 6 months), some subjects recruited early will reach the end of Epoch 2 at the same time as other subjects recruited later on in the study are just entering Epoch 2. As such, the target number of events required to meet study endpoints may not have been achieved by the time the first subjects enrolled complete Epoch 2. If the total number of events (see section 10.5/Table 9) has not been reached for the primary and secondary endpoints by the time an individual in an ADI group (groups 1, 2, 4, and 5) reaches the end of Epoch 2, and that individual has yet to have an event (positive malaria PCR during an ADI phase), that individual will begin Epoch 3. Prior to the total number of required events being achieved among all subjects, any individual progressing into Epoch 3 will perform each visit per to the schedule in Appendix D and according to the guidance in the protocol. Once the total number of events required for the primary and secondary endpoints is reached among the ADI groups, then the study will move into completion as discussed in the next section regarding the Study Termination Visit. As such, it is not required for any individual to complete any or all study visits in Epoch 3 once the primary and secondary endpoints have been reached (though all visits in Epoch 2 must be completed). Once all required primary and secondary endpoints have been reached, all Epoch 3 visits, if applicable, will cease (this will have no impact on anyone still in Epoch 2 as they will be required to complete that Epoch through visit 27 regardless).

Subjects entering the extension phase will continue with ADI every 3 weeks as described for Epoch 2 until study completion as described above. However, in Epoch 3, if an individual has had an event (positive malaria PCR during the ADI phase) then that individual will no longer have field visits to collect blood draws for PCR and DBS every 3 weeks, and will only complete the Study Termination Visit, as below.

Concern for clinical malaria will be assessed and managed the same in Epoch 3 as was outlined in Epoch 2.

Subjects in group 3 will not participate in the extension of ADI.

Blood sampling for immunologic assays in selected groups of subjects at pre-defined timepoints will be collected as shown in Appendix F. Immunological testing will only be performed in Epoch 3 at visit 35, if required.

E. Study Termination Visit

All participants who have not been withdrawn from the study will complete a Study Termination Visit. The Study Termination Visit will be scheduled once the following criteria have been met:

1. The primary and secondary endpoints have been met; AND
2. All participants have completed Epoch 2.

OR

3. All participants have completed all scheduled visits in Epochs 2 and 3, as applicable (through Visit 35 for Groups 1, 2, 4, and 5; through Visit 27 for Group 3).

The Study Termination Visit will occur within 6 weeks of the above criteria being met. During the Study Termination visit, a whole blood sample for a final cross-sectional parasitemia assessment for PCR and DBS will be collected. This assessment will not contribute to the primary or secondary endpoints.

If the primary and secondary endpoints are not met during the first 6 months ADI and the study has continued into Epoch 3, an additional serology timepoint will be collected at the Study Termination Visit for those subjects in Groups 1, 2, and 3 only. This will be an exploratory endpoint to evaluate the immune response to CS.

The Study Termination Visit may occur at KCRC or as a field visit - see Appendices E and F.

7.1.2. Standard of Care Study Procedures

For the duration of their participation in the trial, all randomized subjects will receive free health care from study medical personnel in accordance with Kenya Ministry of Health guidelines for acute illnesses. The capabilities of the KCRC have been developed to ensure any acute illness or outpatient medical need of anyone involved in a study can be addressed. The KCRC is staffed during the study with at least a clinical officer, community nurse and a driver with an evacuation vehicle. All outpatient services for study participants will be provided by the staff at KCRC or an appropriate provider in the community.

7.2. Laboratory Procedures and Evaluations

All laboratory samples will be collected either in the field or at KCRC as per the study procedures outlined in Appendices A - E. The clinical laboratory at KCRC will perform initial processing of all samples according to local SOPs as appropriate for each specimen type. All biological materials collected will be handled according to established SOPs for the protection of participants and study staff.

7.2.1. Clinical Laboratory Evaluations

The clinical laboratories of USAMRD-A will conduct the following testing: CBC (Hb, WBC, PLT), creatinine, ALT, HIV, hemoglobinopathy panel (sickle cell, G6PD, alpha thalassemia), and urine pregnancy testing. These labs will be primarily performed at KCRC clinical labs or the USAMRD-A Basic Science Lab in Kisumu, but other USAMRD-A labs (e.g., Kericho Field Station) or commercial laboratories (e.g., Pathologists Lancet Kenya or PathCare Kenya) are available for back-up testing if the primary laboratories are not available.

A urine pregnancy test must be done in all females of child-bearing potential no more than 24 hours prior to study intervention as indicated in Appendices A & B and the results must be available prior to administration of study products (DHA/Pip, Coartem, Primaquine, RTS,S, Abhayrab).

7.2.2. Other Assays or Procedures

Ultrasensitive quantitative PCR will be performed under the supervision of USAMRD-A personnel at the Kisian KEMRI Campus. The PCR assay SOP is maintained at USAMRD-A.

Serological, cell-mediated immunity, and other special testing will be performed at non-USAMRD-A laboratories in the United Kingdom and United States. The primary labs are:

- Anti-CS antibody levels and avidity testing as described in reference 6: WRAIR, Silver Spring, MD, United States
- Anti-HBs antibody levels: IAVI-HIL, Imperial College, London, United Kingdom
- Rabies serology: Kansas State Veterinary Diagnostic Laboratory, Manhattan, KA, United States
- Cell mediated immune testing: BMGF GHVAP partner, Stanford University, Palo Alto, CA, United States
- Transcriptomics: BMGF GHVAP partner, Stanford University, Palo Alto, CA, United States. Future use to be determined if transcriptomics are performed at another Sponsor-designated laboratory.
- HLA typing: C.W. Bill Young DoD Bone Marrow Program at Georgetown University

Other commercial or academic labs may be utilized for exploratory assays.

7.2.3. Specimen Preparation, Handling, and Storage

Samples will be prepared, handled, and stored according to USAMRD-A SOPs at Kombewa CRC prior to assay performance or shipment, as applicable. All samples will be labeled with the subject ID number, date/time of collection, study designator, and bar code; no PII will be included on sample labels. Samples being shipped locally to another USAMRD-A or commercial lab for performance of an assay will be sent from the KCRC clinical lab to the performing lab maintaining appropriate temperatures according to local SOPs. A chain of custody will be maintained both at the sending lab and the receiving lab. All specimens being shipped internationally, except buccal swabs for HLA typing, will be sent to a PATH-designated central laboratory which will arrange for further aliquoting and shipping, as needed. The PATH-designated central laboratory will be the long-term storage facility for these specimens. Buccal swabs for HLA typing will be shipped to the C.W. Bill Young DoD Bone Marrow Program at Georgetown University directly (11333 Woodglen Drive, Rockville, MD 20852) with no long-term storage planned after testing is complete. Local storage of specimens (e.g., local back-up serological or PBMC samples, DBS) will be at appropriate temperatures and according to local SOP and will be maintained until at least study close-out.

7.2.4. Specimen Shipment

Serum and PBMC samples will be shipped to a PATH-designated central laboratory which will further distribute samples to the testing laboratories, according to the discussion in 7.2.2.

7.3. Study Schedule

Refer to Appendices A, B, C, D for details on study schedule. All visits completed according to this schedule are considered scheduled visits. If a scheduled visit occurs outside the permissible window, it will be reported as a protocol deviation.

7.3.1. Final Study Visit

The final study visit for a subject is defined as the visit at the conclusion of the ADI phase of the trial as discussed in 7.1.1 (either visit 27 or 35).

7.3.2. Unscheduled Visit

Any subject visit to KCRC or evaluation by study staff at a health facility that takes place outside of the schedule in Appendices A – D will be considered an unscheduled visit. Any medical issues discovered at an unscheduled visit will be evaluated, treated, and/or referred as appropriate and their encounter documented on source documents and entered into the CRF as an unscheduled visit. Additionally, unsolicited AEs/SAEs will be collected at all unscheduled visits. Any suspected malaria cases will be handled as per procedures in 7.1.1 according to the Epoch.

7.3.3. Schedule of Events Table

Refer to the following Appendices for details:

Appendix A. Screening Visit Procedures

Appendix B. Epoch 1

Appendix C. Epoch 2

Appendix D. Epoch 3

Appendix E. Study Termination Visit

Appendix F. Biological sampling schedule

7.4. Concomitant Medications, Treatments, and Procedures

Concomitant medications, treatments and procedures will be recorded on the CRF.

8. ASSESSMENT OF SAFETY

8.1. Specification of Safety Parameters

The investigator is responsible for the detection and documentation of events meeting the criteria and definition of an adverse event (AE) or serious adverse event (SAE) as provided in this protocol. During the study, when there is a safety evaluation, the investigator or site staff will be responsible for detecting AEs and SAEs. Each subject will be instructed to contact the investigator immediately should they manifest any signs or symptoms they perceive as serious.

8.1.1. Definition of Adverse Events (AE)

An AE is any untoward medical occurrence in a study participant, temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product, or temporally associated with a study procedure.

An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of a medicinal product. For marketed medicinal products, this also includes failure to produce expected benefits (i.e., lack of efficacy), abuse or misuse.

8.1.2. Definition of Serious Adverse Event

A Serious Adverse Event (SAE) is any adverse event that:

- Results in death.
- Is life-threatening.
  Note: The term 'life-threatening' in the definition of 'serious' refers to an event in which the study participant was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, had it been more severe.
- Requires hospitalization or prolongation of existing hospitalization.
  Note: In general, hospitalization signifies that the study participant has been admitted at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or outpatient setting.
  Complications that occur during hospitalization are also considered AEs. If a complication prolongs hospitalization or fulfils any other serious criteria, the event will also be considered serious. When in doubt as to whether 'hospitalization' occurred or was necessary, the adverse event should be considered serious.
  Hospitalization for elective treatment of a pre-existing condition (known/diagnosed prior to informed consent signature) that did not worsen from baseline is NOT considered an SAE.
- Results in disability/incapacity,
  Note: The term disability means a substantial disruption of a person's ability to conduct normal life functions. This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, and accidental trauma (e.g. sprained ankle) which may interfere or prevent everyday life functions but do not constitute a substantial disruption.
- Results in a congenital anomaly and / or birth defect.

Medical or scientific judgement should be exercised in deciding whether reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the study participant or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These should also be considered serious. Examples of such events are invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization.

8.1.3. Definition of Unanticipated Problems

Unanticipated problems are those problems that are not specifically described in the protocol. For example, misplacing a subject's study records containing identifiable private information results in the risk of breach of confidentiality. Another example would be administering the wrong agent to a subject

at one time point in a series of vaccinations. Risks to others must also be reported. Appropriate supporting documents should be submitted with the unanticipated problem report.

8.1.4. Definition of Adverse Events of Special Interest

AEs of specific interest for safety monitoring include all seizures occurring within 30 days post-vaccination, meningitis and pIMDs and will be recorded in the CRF.

For the further evaluation of the safety signal of meningitis in the investigational vaccine groups all cases of meningitis occurring during the study will be reported as a SAE.

pIMDs are a subset of AEs that include autoimmune diseases and other inflammatory and/or neurologic disorders of interest which may or may not have an autoimmune etiology. AEs that need to be recorded and reported as pIMDs include those listed in Table 6.

Table 6. List of Potential Immune-mediated Diseases:

Neuroinflammatory disorders	**Musculoskeletal disorders**	**Skin disorders**
• Cranial nerve disorders, including paralyses/paresis (e.g. Bell's palsy) • Optic neuritis • Multiple sclerosis • Transverse myelitis • Guillain-Barré syndrome, including Miller Fisher syndrome and other variants • Acute disseminated encephalomyelitis, including site specific variants: e.g. non-infectious encephalitis, encephalomyelitis, myelitis, myeloradiculoneuritis • Myasthenia gravis, including Lambert-Eaton myasthenic syndrome • Immune-mediated peripheral neuropathies and plexopathies, (including chronic inflammatory demyelinating polyneuropathy, multifocal motor neuropathy and polyneuropathies associated with monoclonal gammopathy).	• Systemic lupus erythematosus and associated conditions • Systemic scleroderma (Systemic sclerosis), including diffuse systemic form and CREST syndrome • Idiopathic inflammatory myopathies, including dermatomyositis • Polymyositis • Antisynthetase syndrome • Rheumatoid arthritis, and associated conditions including juvenile chronic arthritis and Still's disease • Polymyalgia rheumatica • Spondyloarthritis, including ankylosing spondylitis, reactive arthritis (Reiter's Syndrome) and undifferentiated spondyloarthritis • Psoriatic arthropathy • Relapsing polychondritis	• Psoriasis • Vitiligo • Erythema nodosum • Autoimmune bullous skin diseases (including pemphigus, pemphigoid and dermatitis herpetiformis) • Alopecia areata • Lichen planus • Sweet's syndrome • Localised Scleroderma (Morphoea)

• Narcolepsy	• Mixed connective tissue disorder	

Vasculitides	**Blood disorders**	**Others**
• 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 anti-neutrophil cytoplasmic antibody (ANCA) positive vasculitis (type unspecified), Henoch-Schonlein purpura, Behcet's syndrome, leukocytoclastic vasculitis.	• Autoimmune hemolytic anemia • Autoimmune thrombocytopenia • Antiphospholipid syndrome • Pernicious anemia • Autoimmune aplastic anaemia • Autoimmune neutropenia • Autoimmune pancytopenia	• Autoimmune glomerulonephritis (including IgA nephropathy, glomerulonephritis rapidly progressive, membranous glomerulonephritis, membranoproliferative glomerulonephritis, and mesangioproliferative glomerulonephritis) • Ocular autoimmune diseases (including autoimmune uveitis and autoimmune retinopathy) • Autoimmune myocarditis/cardiomyopathySarcoidosis • Stevens-Johnson syndrome • Sjögren's syndrome • Idiopathic pulmonary fibrosis • Goodpasture syndrome • Raynaud's phenomenon

Liver disorders	**Gastrointestinal disorders**	**Endocrine disorders**
• Autoimmune hepatitis • Primary biliary cirrhosis • Primary sclerosing cholangitis • Autoimmune cholangitis	• Inflammatory Bowel disease, including Crohn's disease, ulcerative colitis, microscopic colitis, ulcerative proctitis • Celiac disease • Autoimmune pancreatitis	• Autoimmune thyroiditis (including Hashimoto thyroiditis) • Grave's or Basedow's disease • Diabetes mellitus type I • Addison's disease • Polyglandular autoimmune syndrome • Autoimmune hypophysitis

8.2. Classification of An Adverse Event

All AE/SAEs either observed by the investigator or one of his clinical collaborators or reported by the subject spontaneously or in response to a direct question will be evaluated by the investigator. The

nature of each event, date and time (where appropriate) of onset, outcome, intensity and relationship to vaccination should be established. Details of any corrective treatment should be recorded on the appropriate page of the CRF.

When an AE/SAE occurs, it is the responsibility of the investigator to review all documentation relative to the event. The investigator will then record all relevant information regarding an AE/SAE on the CRF or SAE Report Form as applicable.

The investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. In such cases, the diagnosis should be documented as the AE/SAE and not the individual signs/symptoms.

8.2.1. Severity of Event

For AEs, the following guidelines will be used to describe severity.

- Grade 1; Mild – Events require minimal or no treatment and do not interfere with the participant's daily activities.
- Grade 2; Moderate – Events result in a low level of inconvenience or concern with therapeutic measures. Moderate events may cause some interference with functioning.
- Grade 3; Severe – Events prevents a participant's usual daily activity.

8.2.2. Relationship to Study Vaccine or Study scheduled anti-malarial treatment

All AEs must have their relationship to study agent assessed. In a clinical trial, the study product must always be suspect. To help assess, the following guidelines are used.

- Related –The AE is considered related when there is a reasonable possibility that the study agent caused the AE
- Not Related – There is not a reasonable possibility that the administration of the study agent caused the event and/or an alternate etiology has been established.

8.3. Time Period and Frequency of Event Assessment and Follow-up

All solicited local and systemic AEs occurring within 7 days following administration of each dose of vaccine must be recorded on the CRF for the first 50 subjects in groups 1 and 2 and from all subjects in group 3 (Reactogenicity Cohort). All subjects being followed for solicited AEs will be offered a memory aid to assist in recall of AEs. All unsolicited AEs occurring within 28 days following administration of each dose of vaccine and, where applicable, course of per protocol scheduled anti-malarial treatment, must be recorded on the CRF for all subjects. All SAEs that occur will be collected and recorded from the time the subject consents to participate in the study until she/he is discharged. The occurrence of an AE or SAE may come to the attention of study personnel during study visits and field worker visits. Information to be collected includes event description, date of onset, clinician's assessment of severity, relationship to study product, and date of resolution/stabilization of the event. All AEs occurring while on study must be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution.

Any medical condition that is present at the time that the participant is screened will be considered as baseline and not reported as an AE. However, if the study participant's condition deteriorates at any time during the study, it will be recorded as an AE.

After the initial AE/SAE report, the investigator is required to proactively follow each subject and provide further information per study reporting procedures (section 8.4). All AEs and SAEs documented at a previous visit/contact and designated as not recovered/not resolved or recovering/resolving will be followed-up.

Investigators will follow-up subjects:

- with SAEs or who are withdrawn from the study as a result of an AE, until the event has resolved, subsided, stabilized, disappeared, the event is otherwise explained, or the subject is lost to follow-up;
- or, in the case of other non-serious AEs, until the AE resolves, they complete the study, or they are lost to follow-up.

PATH or the KEMRI Scientific and Ethics Review Unit may request that the investigator perform or arrange for the conduct of supplemental measurements and/or evaluations to elucidate as fully as possible the nature and/or causality of the AE or SAE. The investigator is obliged to assist. If a subject dies during participation in the study or during a recognized follow-up period, study contacts for reporting of a serious adverse event will be provided with a copy of post-mortem finding, including histopathology, if available.

New or updated information for SAEs will be recorded on the originally completed SAE Report Form, with all changes signed and dated by the investigator.

Outcome of any non-serious local or general solicited AE occurring within 7 days post-vaccination or unsolicited AE occurring within 28 days post-vaccination or 28 days of a scheduled course of anti-malarials as per the protocol (i.e. unsolicited AE), or any SAE reported during the entire study will be assessed as:

- Recovered/resolved
- Not recovered/not resolved
- Recovering/resolving
- Recovered with sequelae/resolved with sequelae
- Fatal (SAEs only).

8.4. Reporting Procedures

8.4.1. Serious Adverse Event Reporting

All serious adverse events (SAEs) will be promptly (within 48 hours) reported to the KEMRI SERU and to the WRAIR IRB. Prompt (within 48 hours) reports will be submitted to the KEMRI SERU by email (kemriseru18@gmail.com; seru@kemri.go.ke) and to the WRAIR IRB by phone (301) 319-9940, or by email (usarmy.detrick.medcom-wrair.mbx.hspb@health.mil), or by facsimile (301) 319-9961. The Principal Investigator will then submit written reports within 10 working days to the WRAIR IRB at the following address: Walter Reed Army Institute of Research, ATTN: Human Subject Protection Branch (HSPB), 503 Robert Grant Ave, Silver Spring, MD 20910.

In addition, all SAEs and Suspected Unexpected Serious Adverse Reactions will be reported to the Kenya Pharmacy and Poisons Board as soon as possible but within 7 calendar days of the notification of the

event and with follow-up reporting provided within 8 calendar days of the initial report. These reports can be submitted to the Board through the online system at www.pv.pharmacyboardkenya.org.

Any SAEs or other events that meet PATH Research Ethics Committee's (REC) expedited reporting requirements will be promptly (within 24 hours) reported to the PATH Study Representative by email. The PATH Study Representative will then promptly (within 48 hours) submit the report to PATH REC using their online submission and reporting website (www.irbnet.org).

Follow up reports will be submitted as additional information becomes available. A summary of the non-serious adverse events and the SAEs (both related and unrelated) that occurred during the reporting period should also be included in the continuing review report to the KEMRI SERU, and the WRAIR IRB. The WRAIR HSPB will report SAEs to the USAMRMC ORP HRPO as per WRAIR SOP UWZ-C-636.

SAEs will be reported promptly to GSK within the timeframes described in Table 7, once the investigator determines that the event meets the protocol definition of a SAE.

Table 7. Timeframes for submitting serious adverse event and other events reports to GSK Biologicals

Type of Event	Initial Reports		Follow-up of Relevant Information on a Previous Report	
	Timeframe	Documents	Timeframe	Documents
SAEs	24 hours*‡	electronic Expedited Adverse Events Report	24 hours*	electronic Expedited Adverse Events Report
AEs of specific interest**	24 hours*‡	electronic Expedited Adverse Events Report	24 hours*	electronic Expedited Adverse Events Report

* Timeframe allowed after receipt or awareness of the information.

** AEs of specific interest include meningitis and pIMDs.

‡ The investigator will be required to confirm review of the SAE/AEs of specific interest causality by ticking the 'reviewed' box in the GSK electronic Expedited Adverse Events Report within 72 hours of submission of the SAE/AEs of specific interest.

8.4.2. Reporting Events of Special Interest

AEs of specific interest (all seizures occurring within 30 days post-vaccination, pIMDs, and meningitis) will be reported promptly to GSK within the timeframes described in Table 7, once the investigator determines that the event meets the protocol definition of an AEs of specific interest (all seizures occurring within 30 days post-vaccination, pIMDs and meningitis).

Contact information for reporting serious adverse events and AEs of specific interest:

Study Contact for Reporting SAEs and AEs of specific interest*
Refer to the local study contact information document.
Back-up Study Contact for Reporting SAEs and AEs of specific interest*
24/24 hour and 7/7 day availability: **GSK Biologicals Clinical Safety & Pharmacovigilance** Outside US & Canada sites: Fax: +32 2 656 51 16 or +32 2 656 80 09 Email address: Rix.CT-safety-vac@gsk.com

8.4.3. Unanticipated Problem Reporting

All non-serious unanticipated problems (events not involving risk to participants or others) will be reported in the continuing review report to the KEMRI Scientific and Ethics Review Unit (SERU), and the WRAIR IRB.

All serious unanticipated problems involving risk to participants or others should promptly (within 48 hours) reported by telephone, by email, or by facsimile to the KEMRI SERU (kemriseru@gmail.com; seru@kemri.go.ke) and by phone (301) 319-9940, or by email (Usarmy.detrick.medcom-wrair.mbx.hspb@health.mil), or by facsimile (301) 319-9961 to the WRAIR IRB. The Principal Investigator will then submit written reports within 10 working days to the WRAIR IRB at the following address: Walter Reed Army Institute of Research, ATTN: Human Subject Protection Branch, 503 Robert Grant Ave, Silver Spring, MD 20910. The WRAIR HSPB will report to USAMRMC ORP HRPO as per UWZ-C-636.

In addition, all serious unanticipated problems will be reported to the Kenya PPB within 7 days. These reports can be submitted to the Board through the online system at www.pv.pharmacyboardkenya.org.

Any adverse drug reactions considered as possibly related to DHA-Pip anti-malarial treatment will also be reported to the Pharmacovigilance department of Tridem Pharma SAS, who supplied the DHA-Pip to the Sponsor (PATH) for the purposes of this clinical study, within 24 hours.

Any unanticipated problems that meet PATH Research Ethics Committee's (REC) expedited reporting requirements will be promptly (within 24 hours) reported to the PATH Study Representative by email. The PATH Study Representative will then promptly (within 48 hours) submit the report to PATH REC using their online submission and reporting website (www.irbnet.org).

Follow up reports should be submitted as soon as additional information becomes available. A summary of the serious unanticipated problems should also be included in the continuing review report submitted to the KEMRI SERU, and the WRAIR IRB.

8.4.4. Reporting of Pregnancy

Subjects who become pregnant during the study must not receive additional doses of investigational study vaccines but may continue other study procedures at the discretion of the investigator. The investigator, or his designee, will collect pregnancy information on any subject who becomes pregnant while participating in this study. The investigator, or his designee, will record pregnancy information on the Pregnancy Report Form and submit it to the WRAIR IRB, KEMRI SERU, Kenya PPB, PATH, and GSK. In

the event a woman becomes pregnant after completing all three vaccinations she will be eligible to continue in the ADI phase. If a pregnant female develops asymptomatic malaria infection discovered by a positive malaria PCR during ADI, she will be treated with standard of care malaria therapy.

The subject will be followed to determine the outcome of the pregnancy. At the end of the pregnancy, whether that be full-term or premature, information on the status of the mother and child will be forwarded to the above ethical committees, regulatory authorities and sponsor for reporting.

While pregnancy itself is not considered an AE or SAE, any pregnancy complication or elective termination of a pregnancy for medical reasons will be recorded as an AE or a SAE. A spontaneous abortion is always considered to be a SAE and will be reported.

8.5. Safety Oversight

8.5.1. DoD Research Monitor

The DoD Research Monitor or their approved alternate are required to review all unanticipated problems involving risks to participants or others, serious adverse event (SAE) reports, and all participant deaths. The DoD Research Monitor will provide an unbiased written report of all unanticipated problems involving risks to subjects or others, and related SAEs, and all deaths, promptly within 10 working days to the WRAIR IRB by phone (301) 319-9940 or by email (usarmy.detrick.medcom-wrair.mbx.hspb@health.mil), by facsimile (301) 319-9961 or at the following address: Walter Reed Army Institute of Research, ATTN: Human Subjects Protection Branch, 503 Robert Grant Ave, Silver Spring, MD 20910. In addition to submission of the DoD Research Monitor reports to the WRAIR IRB, a copy of the same report will be sent to the Sponsor team. All DoD Research Monitor reports for unrelated SAEs should be kept with the corresponding SAE reports at the study site.

The WRAIR HSPB will submit copies of these reports to the USAMRMC ORP HRPO as per WRAIR SOP UWZ-C-636. The DoD Research Monitor or their approved alternate at a minimum must comment on the outcomes of the event or problem and in case of a serious adverse event or death, comment on the relationship to participation in the study. They must also indicate whether he/she concurs with the details of the report provided by the principal investigator.

The DoD Research Monitor or their approved alternate should review all initial, follow up, and final reports for SAEs, unanticipated problems involving risks to participants or others, and all participant deaths in a timely manner, and provide their own independent report.

The DoD Research Monitor will also:

1. Discuss research progress with the PI, interview participants, consult on individual cases, or evaluate suspected adverse reaction reports on behalf of the IRB.

2. Perform, at the direction of the IRB, oversight functions (e.g., observe recruitment, enrolment procedures, and the consent process for individuals, groups or units; oversee study interventions and interactions; review monitoring plans and (Unanticipated Problem Involving Risk to Subjects or Others) UPIRTSO reports; and oversee data matching, data collection, and analysis)

3. Promptly report discrepancies or problems to the IRB.

4. Have the authority to stop a research study in progress, remove individual participants from a study, and take whatever steps are necessary to protect the safety and well-being of research participants until the IRB can assess the DoD Research Monitor's report

8.6. Solicited adverse events

Solicited local and general AEs will be collected among RTS,S vaccinated groups in the first 50 subjects enrolled in Groups 1 and 2 and all subjects enrolled in group 3 (Reactogenicity Cohort) for seven days (day of vaccination and six subsequent days) after each dose of vaccine. On the day of vaccination for each vaccine dose, the evaluation will be carried out by the study clinician at the KCRC. Seven days after each vaccination a field worker will visit each subject and review the memory aid for solicited local and systemic AEs and unsolicited AEs that have occurred since vaccination and per protocol scheduled anti-malarial treatment, if applicable.

Local (injection site) adverse events

- Pain at injection site
- Swelling at injection site

Systemic adverse events

- Fever (temperature ≥37.5°C)
- Headache
- Gastrointestinal problems
- Fatigue
- Muscle ache

The visiting field worker will record these adverse events during the field worker visits.

8.6.1. Assessment of intensity

Table 8. Intensity Scales for Solicited Symptoms in Adults

Pain at injection site	0	Absent
	1	Painful on touch
	2	Painful when limb is moved
	3	Pain that prevents normal activity
Swelling at injection site	0	Absent
	1	Present and is easily tolerated
	2	Present and interferes with normal activity
	3	Present and prevents normal activity
Fever	0	<37.5°C (99.5°F)
	1	37.5°C (99.5°F) to 38.0°C (100.4°F)
	2	>38.0°C (>100.4°F) to 39.0°C (102.1°F)
	3	>39.0°C (102.1°F)
Headache	0	Normal
	1	Headache feeling is easily tolerated
	2	Headache feeling interferes with normal activity
	3	Headache feeling prevents normal activity
	0	Gastrointestinal symptoms normal
	1	Gastrointestinal symptoms that are easily tolerated

Gastrointestinal symptoms (nausea, vomiting, diarrhea	2	Gastrointestinal symptoms that interfere with normal activity
	3	Gastrointestinal symptoms that prevent normal activity
Fatigue	0	Normal
	1	Fatigue that is easily tolerated
	2	Fatigue that interferes with normal activity
	3	Fatigue that prevents normal activity
Muscle ache	0	Absent
	1	Muscle ache that is easily tolerated
	2	Muscle ache that interferes with normal activity
	3	Muscle ache that prevents normal activity

8.6.2. Assessment of causality

The investigator is obligated to assess the relationship between investigational product and the occurrence of each solicited AE/SAE. The investigator will use clinical judgment to determine the relationship. Alternative causes, such as natural history of the underlying diseases, concomitant therapy, other risk factors and the temporal relationship of the event to the investigational product will be considered and investigated. The investigator may also consult the Investigator Brochure and/or Product Information, for marketed products, in the determination of his/her assessment. The investigator may change his/her opinion of causality in light of follow-up information.

All solicited local (injection site) reactions will be considered causally related to vaccination.

Relatedness to the study product of all other solicited AE/SAEs will be classified by the same criteria established in 8.2.2.

8.6.3. Follow-up and management of adverse events

Follow-up of Solicited AEs/SAEs will be in accordance with section 8.3. AE/SAEs will be managed by the clinical team at KCRC, if possible, until resolution or study completion with nursing and clinical staff providing care under the supervision of the PI. Anything that requires treatment beyond what the KCRC can manage will be referred to an appropriate medical facility, but the staff will continue to follow-up as per section 8.3. At the end of the study, the KCRC staff will refer all subjects with unresolved/chronic AE/SAEs to an appropriate provider for continued management, but will continue to follow-up and document the AE/SAEs as appropriate per section 8.3.

9. CLINICAL MONITORING

Site monitoring is conducted to ensure that the rights and well-being of human subjects are protected, that the reported trial data are accurate, complete, and verifiable, and that the conduct of the trial is in compliance with the currently approved protocol/amendment(s), with GCP, and with applicable regulatory requirement(s).

A separate clinical monitoring plan (CMP) prepared by the CRO will describe:

- Person who will conduct the monitoring,
- Frequency monitoring will be done,
- Level of detail monitoring will be performed, and
- Distribution of monitoring reports.

10. STATISTICAL CONSIDERATIONS

10.1. Statistical Analysis Plan

The statistical analysis will be conducted following the principles as specified in the International Conference on Harmonization (ICH) Topic E9 (ICH, 1998). The final statistical analyses will be performed after database lock.

All data will be collected and verified prior to analysis. Detailed statistical procedures, listings, table shells, and figures will be provided in a statistical analysis plan (SAP) prior to analysis. The SAP will be finalized before study close-out and database lock. The following key statistical components and a detailed description will be documented in the SAP:

- Primary and secondary endpoints and how they will be measured
- Statistical methods and tests that will be used to analyze the endpoints
- Strategy that will be used if the statistical test assumptions are not satisfied
- Indication of whether the comparisons will be using one-tailed or two-tailed tests (with justification of the choice) and the level of significance to be used
- Identification of whether any adjustments to the significance level or the overall p value will be made to account for any planned or unplanned subgroup analyses or multiple testing
- Specification of potential adjusted analyses and a statement with which covariates or factors will be included
- Planned exploratory analyses and justification of their importance

The number of subjects enrolled, vaccinated, completed, or withdrawn will be summarized. Reasons for withdrawal, when known, will be provided. Demographic data will be summarized by descriptive statistics and will include total number of observations (n), mean, standard deviation (SD) and range for continuous variables, and number and percentages for dichotomous variables.

10.2. Statistical Hypotheses

The primary and secondary hypotheses will be assessed using Cox proportional hazards regression (time to first PCR-positive infection). These hypotheses tests will be supplemented with two-sided confidence intervals for the hazard ratios as described below.

The null (H_0) and alternative (H_1) hypotheses for the primary analysis of time to first malaria infection in Groups 1 and 4 is as follows:

H_0: Among adults positive for *P. falciparum* at baseline the hazard rate for first malaria infection for RTS,S/AS01$_E$ vaccinated adults (Group 1) is not different than that of adults administered the comparator rabies vaccine (Group 4) as measured by the hazard ratio.

H_1: Among adults positive for *P. falciparum* at baseline the hazard rate for malaria infection for RTS,S/AS01$_E$ vaccinated adults (Group 1) is different than that of adults administered the comparator rabies vaccine (Group 4) as measured by the hazard ratio.

The null and alternative hypotheses for the secondary efficacy analysis of time to first malaria infection in Groups 2 and 5 is as follows:

H_0: Among adults negative for *P. falciparum* at baseline the hazard rate for malaria infection for RTS,S/AS01$_E$ vaccinated adults (Group 2) is not different than that of adults administered the comparator rabies vaccine (Group 5) as measured by the hazard ratio.

H_1: Among adults negative for *P. falciparum* at baseline the hazard rate for first malaria infection for RTS,S/AS01$_E$ vaccinated adults (Group 2) is different than that of adults administered the comparator rabies vaccine (Group 5) as measured by the hazard ratio.

10.3. Analysis Datasets

10.3.1. Enrolled population

All subjects who provide written informed consent, regardless of the subject's screening, randomization and vaccination status in the study.

10.3.2. Total vaccinated cohort (TVC)

All subjects in the enrolled population who were randomized and received at least one vaccination.

10.3.3. Safety analysis population

All subjects in the TVC for which any safety data is available. All safety analyses will be performed using this population. The denominators for different safety endpoints may vary according to the number of subjects with available data for the specific endpoint.

10.3.4. According to protocol (ATP) cohort for efficacy

The ATP cohort for efficacy will include all subjects included in the TVC with no major protocol deviations that are determined to potentially interfere with the efficacy assessment of the study vaccine and contributed to the time at risk starting 14 days after the third dose. The membership in this study population will be determined in a blinded fashion at a data review meeting attended by the sponsor Medical Officer, the site PI/Co-PI, and the sponsor Statistician, and the list of criteria used to exclude subjects from the ATP cohort for efficacy, including vaccine or anti-malarial administration and other relevant analysis protocol deviations, will be finalized before database freeze.

10.3.5. ATP cohort for immunogenicity

The ATP cohort for immunogenicity will include all subjects included in the TVC who received all vaccinations according to protocol procedures and within the protocol specified intervals, performed blood samplings for immunogenicity according to protocol intervals, and did not use any medication or blood products forbidden by the protocol and did not have any reported underlying medical condition influencing immune responses.

10.3.6. Total cohort for efficacy

The total cohort for efficacy includes all TVC subjects who receive all three doses of RTS,S/AS01$_E$ vaccine or comparator and contributed to the time at risk starting 14 days after the third dose.

10.4. Description of Statistical Methods

10.4.1. General Approach

A positive PCR for the presence of *P. falciparum* parasites meets the definition of an event if the result is obtained from a blood sample collected at any time during the ADI phase of the trial (Epoch 2 or 3).

10.4.2. Analysis of the Primary Efficacy Endpoint

Vaccine efficacy against the first PCR-positive *P. falciparum* infection among adults who were *P. falciparum* positive at baseline will be assessed using Cox proportional hazards regression with a covariate for group assignment to compare Groups 1 and 4. The vaccine efficacy estimates (1-hazard ratio), 95% CI, and p-values will be calculated from this model. Cumulative incidence graphs will also be provided. This analysis will be conducted in the ATP for efficacy cohort.

10.4.3. Analysis of the Secondary Endpoint(s)

Efficacy:

Vaccine efficacy against the first PCR-positive *P. falciparum* infection among adults who were *P. falciparum* negative at baseline will be assessed using Cox proportional hazards regression with a covariate for group assignment to compare Groups 2 and 5. The vaccine efficacy estimates (1-hazard ratio), 95% CI, and p-values will be calculated from this model. Cumulative incidence graphs will also be provided. This analysis will be conducted in the ATP for efficacy cohort.

Immunogenicity:

The primary analysis will be based on the ATP for immunogenicity cohort for analysis of immunogenicity. A secondary analysis based on the TVC will be performed to complement the ATP analysis.

Anti-CS antibodies:

The percentage of subjects with sero-positive levels of anti-CS (proportion of subjects with anti-CS antibody levels with 95% CI will be determined as shown in Appendix F. Antibody levels after the third dose will also be investigated using reverse cumulative curves.

Anti-HBs antibodies:

The percentage of subjects with sero-positive levels of anti-HBs (proportion of subjects with anti-HBs antibody levels greater than the lower limit of detection/assay dependent will be determined prior to vaccination, and 14 days post Dose 3. Antibody levels after the third dose will also be investigated using reverse cumulative curves.

Anti-rabies antibodies:

The percentage of subjects in Groups 4 and 5 with sero-positive levels of anti-rabies antibodies will be determined prior to vaccination, and 28 days post Dose 3. Antibody levels after the third dose will also be investigated using reverse cumulative curves.

10.4.4. Safety Analyses

For the safety analysis, data from all subjects from the safety analysis population will be included. All analyses will be descriptive. Data will be presented by dose, overall/dose and overall/subject. Results

will be summarized by study group. The percentage of subjects with at least one AE during the follow-up period will be tabulated with exact 95% CI (two-sided). No multiplicity adjustment will be implemented in analysis. The reports of solicited and unsolicited AEs will be reviewed by a physician and will be categorized by the MedDRA dictionary. The percentage of subjects with at least one report of unsolicited AE and reported up to 28 days after each anti-malarial course will be tabulated with exact 95% CI. The percentage of subjects with at least one report of unsolicited AE and reported up to 28 days after each vaccine dose will be tabulated with exact 95% CI.

The occurrence of SAEs will be determined on the safety analysis population. The proportion of subjects with an SAE, classified by the MedDRA preferred term level, reported from study start until study conclusion will be tabulated with exact 95% CI. Comparisons between groups will be done using Fisher's Exact Test for each preferred term. Serious adverse events (SAEs) occurring at any point during the trial will be summarized and relatedness to vaccine will be assessed.

Withdrawals due to AEs/SAEs will also be summarized. Vital signs which are outside of the normal range and clinically significant will also be listed in tables. The frequency of signs and symptoms will be compared between groups with the chi-square test or Fisher's exact test.

The occurrence of Grade 3 solicited and unsolicited general reactions will be determined on the Total Vaccinated Cohort. The proportion of subjects with a Grade 3 solicited and unsolicited reaction, reported from study start until study conclusion will be tabulated with exact 95% CI. Comparisons between groups will be done using Fisher's Exact Test.

The occurrence of adverse events will be determined on the Total Vaccinated Cohort. The proportion of subjects with an AE, classified by the MedDRA preferred term level, reported from study start until study conclusion will be tabulated with exact 95% CI. Comparisons between groups will be done using Fisher's Exact Test.

10.4.5. Adherence and Retention Analyses

Participants who prematurely discontinue from the study will not be included in analyses/summary statistics beyond the time of discontinuation.

Rates of discontinuation and reason for discontinuation will be summarized in data listings or summary tables.

10.4.6. Baseline Descriptive Statistics

Baseline demographics and characteristics, including age, height, weight, sex, race and HIV status will be summarized for both the TVC and ATP populations by vaccine group using descriptive statistics (mean, standard deviation, median, minimum and maximum for continuous and rates for categorical).

For the exposed population, medical history will be listed and summarized by category. Using the WHO Drug Dictionary, concomitant medications will be tabulated by anatomical therapeutic chemical (ATC) classification, preferred drug name and treatment group. Medical history will be tabulated by MedDRA System Organ Class (SOC), Preferred Term (PT) and vaccine group.

Summaries of subject disposition will be prepared for all subjects, including the number and percent enrolled, screened, randomized, and administered vaccine, as well as a CONSORT diagram describing

study participation and discontinuation. The reasons for screen failures and discontinuations will be summarized and listed.

A summary and listing of visit attendance will be prepared, in addition to a summary and listing of vaccine administration and sample collection/availability for each sample.

10.4.7. Planned Interim Analyses

There are no planned interim analyses.

10.4.8. Multiple Comparison/Multiplicity

No adjustment for multiplicity will be performed.

10.4.9. Exploratory Analyses

Exploratory analyses will be described in a separate Exploratory Statistical and Analysis Plan when the exploratory assays have been finalized.

10.5. Sample Size

This is a Phase 2b study to assess the vaccine efficacy of RTS,S in adults in a malaria endemic region. The statistical analysis will measure vaccine efficacy using Cox proportional hazards regression (time to first PCR-positive infection). An attack rate of 40% over 6 months has been assumed as a conservative estimate in an epidemiologic setting of perennial transmission in Western Kenya. A fixed vaccine efficacy of 50% is a conservative assumption according to the estimated vaccine efficacy.

Using an event-based design and assuming 10% drop out of the enrolled population and a one-sided α=0.025, the following are power and required number of events to conduct the analysis:

Table 9. Sample Size Calculations

Power	N to enroll per group	Total events required to conduct analysis	Endpoint
90%	164	92	Primary (Group 1 vs 4)
80%	128	72	Secondary (Group 2 vs 5)

The impact of the event-driven design is that we will continue to follow up until 92 events have been observed in aggregate between Groups 1 and 4 of the ATP cohort for efficacy, and 72 events have been observed in the aggregate of Groups 2 and 5 of the ATP cohort for efficacy. If the aggregate numbers of events have been reached prior to 24 weeks of ADI, study actives will continue to occur through the end of Epoch 2 only (though some subjects may have begun Epoch 3 as discussed in 7.1.1). If the aggregate number of events during Epoch 2 does not reach the cutoff indicated in Table 9, then the ADI will be extended up to 48 weeks or until the total number of events is reached, whichever occurs first. This will require the PCR lab to provide timely aggregated reports of event counts (across Group 1 plus 4; and Group 2 plus 5) and may result in more than the required events for either the primary or secondary endpoint.

An additional cross-sectional PCR will be performed at the Study Termination Visit as described in section 7.1.1, but these PCR results will not count toward the study endpoints.

Based on these assumptions we estimate a greater than 90% probability of observing the required number of events for the primary and secondary analyses by 6.9 months. However, larger dropout rates and/or a lower attack rate will extend the amount of follow-up time necessary to accumulate the required number of events.

The immunologic sub-cohort for CMI assays in all subjects in RTS,S groups (groups 1, 2, and 3). This sample size has been chosen based on logistical considerations and the feasibility of the collection and processing of PBMCs. Analysis of these exploratory endpoints and inter-group differences will be descriptive in nature.

10.5.1. Randomization Procedures

Eligible subjects will be randomized to their assigned group with secure IWRS (or in the absence of web connectivity, IVRS), which will be developed and managed by the PATH designated CRO. The IWRS/IVRS allows authorized staff from the study site to perform subject randomization and treatment assignment 24 hours a day, 7 days a week.

Subjects will be stratified by baseline parasitemia status and then block randomized. Among those positive for parasitemia at baseline a total of 363 will be randomized. The first 105 will be randomized in a 1:1:1 ratio with 35 subjects assigned to each of Groups 1, 3 and 4. The next 258 subjects with baseline parasitemia will be randomized in a 1:1 ratio to Groups 1 and 4 with a total of 129 additional subjects per group. Two hundred fifty-six subjects that are negative for parasitemia at baseline will be randomized with a 1:1 ratio to Groups 2 and 5. This randomization schedule will result in a total of 164, 128, 35, 164, and 128 for Groups 1 through 5, respectively. Additional details of the randomization procedure, such as block size, will be described in the Randomization Plan. HIV infection status will be noted prior to randomization, and individuals will be stratified across the groups to the extent possible. The proportion of subjects per group that are HIV positive will be capped near 20-25% for each group.

11. SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS

The KCRC site will maintain appropriate medical and research records for this trial. Source data are all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial.

12. QUALITY ASSURANCE AND QUALITY CONTROL

PATH has a Quality Assurance Standard for Document Management in Essential Medicines. SOPs for quality management to ensure compliance with the protocol, ethical standards, and regulatory requirements for the study are on file at PATH. Having the highest quality data and studies are essential aspects of vaccine development. For the purpose of compliance with Good Clinical Practice it may be necessary for PATH to conduct a site audit. This may occur at any time from start to after conclusion of the study. When an investigator signs the protocol, he agrees to permit PATH audits, providing direct access to source data/ documents to include:

- IRB/IEC approval
- Vaccine accountability
- Approved study protocol and amendments
- Informed consent of the subjects (written consent [or witnessed oral if applicable])
- Medical records and other source documents supportive of CRF data
- Reports to the IRB/IEC the sponsor, PATH, GSK and WRAIR
- Record retention

The KCRC site has SOPs for quality management that include staff training methods, product accountability records, specimen tracking logs, questionnaires, audio or video recordings.

The Quality Control Standards and Requirements for the candidate RTS,S vaccine are described in separate release documents maintained by GSK.

QC procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated by the CRO selected by PATH. Any missing data or data anomalies will be communicated to the site(s) for clarification/resolution. Following written SOPs, the monitors will verify that the clinical trial is conducted, data are generated, documented, and reported in compliance with the protocol, GCP, and the applicable regulatory requirements.

13. ETHICS/PROTECTION OF HUMAN SUBJECTS

13.1. Ethical Standard

This study will be conducted in accordance with the latest revision of the Declaration of Helsinki (2013), the Medical research Involving Human Subjects act (WMO), the ICH Good Clinical Practice, Department of Defense (DoD) requirements as outlined in 32 CFR 219, local rules of KEMRI SERU, and regulations of the Kenya Pharmacy and Poisons Board. The investigators are responsible for obtaining all relevant ethical approvals of the protocol and any subsequent amendments in compliance with local law before the start of the study.

13.2. Institutional Review Board

13.2.1. Ethics Approval

Submission of the protocol and supporting documents will occur in accordance with local regulatory requirements. KEMRI SERU is the IRB of record for the Kenyan Site, Kombewa in Kisumu. This protocol will be submitted to KEMRI Center Scientific Committee for review and approval. It will also be submitted to the KEMRI SERU where ethical aspects of the protocol will be reviewed before the protocol can be approved.

The Walter Reed Army Institute of Research IRB will also be involved in the approval process for this protocol. WRAIR IRB is the IRB of record for US Army Medical Research Directorate-Africa (USAMRD-A). For this reason, approval from the WRAIR IRB will be sought alongside the KEMRI SERU approval prior to implementation of the protocol or protocol amendments for this study.

Headquarters-level oversight of the WRAIR Human Research Protection Program (HRPP) is provided by the USAMRDC ORP HRPO for studies involving outside collaborators. HRPO will review all protocol life cycle actions as outlined in UWZ-C-636.

Regulatory Approval will also be obtained from the Kenya Pharmacy and Poisons Board (PPB) Expert Committee on Clinical Trials (ECCT) for the initial protocol and any subsequent protocol amendments, as well, as any amendments that increase the risks to subjects or others.

PATH will delegate ethical review and oversight to KEMRI SERU.

13.2.2. Protocol Amendments

All amendments and modifications will be submitted to the WRAIR IRB, KEMRI SERU and PPB ECCT for review and approval. No changes in protocol conduct will be implemented until approval is obtained from the WRAIR IRB, KEMRI SERU as well as USAMRMC ORP HRPO, and PPB, as applicable, unless required to eliminate apparent immediate hazards to the study subjects. Amendments and modifications cannot be implemented until the WRAIR Commander's Memorandum has been issued. The WRAIR HSPB will submit amendments to USAMRMC ORP HRPO as per WRAIR SOP UWZ-C-636.

Non-significant amendments from the sponsor that are defined as amendments that do not affect the safety and wellbeing of the subjects at the site, and that are more administrative will also require both WRAIR IRB and KEMRI SERU approval prior to implementation.

13.2.3. Reporting requirements

13.2.3.1. **Continuing Review Reporting**

The PI or designee will be responsible for submitting the required continuing review report and supporting documentation to the WRAIR IRB, KEMRI SERU and PPB ECCT, allowing sufficient time for review and continuation determination prior to the established continuing review date. A closeout report will be submitted at the end of the study upon completion of all the study activities or 5 years, whichever comes first. The HSPB will submit the continuing review and closeout reports to the USAMRMC ORP HRPO as per SOP UWZ-C-636.

PATH will submit a safety report to the PPB annually through the clinical trial or on request. The report will take into account all new available safety information received during the reporting period. The aim is to describe concisely all new safety information relevant for the trial and assess the safety conditions of subjects included in the trial

13.2.3.2. **Protocol Deviation Reporting**

Major deviations will be promptly (within 48 hours) reported by email to the KEMRI SERU (kemriseru18@gmail.com; seru@kemri.go.ke) and by phone (301) 319-9940, or by email (usarmy.detrick.medcom-usamrmc.other.hrpo@health.mil), or by facsimile (301) 319-9961, to the WRAIR IRB. The Principal Investigator will then submit written reports within 10 working days to the WRAIR IRB at the following address: Walter Reed Army Institute of Research, ATTN: Human Subjects Protection Branch, 503 Robert Grant Avenue, RM 1W30, Silver Spring, Maryland, 20910-7500. All deviations will be summarized in the continuing review reports that are submitted to the WRAIR IRB and KEMRI SERU. The Principal Investigator is responsible for reporting the major and minor deviations to the WRAIR IRB and the KEMRI SERU appropriately.

In addition, all major deviations will be reported to the Kenya PPB within 7 days. These reports can be submitted to the Board through the online system at www.pv.pharmacyboardkenya.org.

Any deviations that meet PATH Research Ethics Committee's (REC) expedited reporting requirements will be promptly (within 24 hours) reported to the PATH Study Representative by email. The PATH Study Representative will then promptly (within 48 hours) submit the report to PATH REC using their online submission and reporting website (www.irbnet.org).

Any suspensions (to include continuing review lapses), clinical holds (voluntary or involuntary), or terminations of this research by an IRB, the institution, the Sponsor, or regulatory agencies will be promptly reported to the WRAIR IRB, KEMRI SERU, PPB, and PATH REC.

The WRAIR HSPB will submit deviations, suspensions, and terminations to USAMRMC ORP HRPO as per WRAIR SOP UWZ-C-636.

In addition to the requirements listed above, minor deviations will be reported to WRAIR IRB and KEMRI SERU with the continuing review reports.

13.2.3.3. **Reporting of Inspections or Audits**

Knowledge of any pending compliance inspections/visits by Office for Human Research Protections (OHRP) or other government agency concerning clinical investigation or research, the issuance of Inspection Reports, warning letters or actions taken by any Regulatory Agencies including legal or medical actions and any instances of serious or continuing noncompliance with the regulations or requirements will be reported immediately to the KEMRI SERU (kemriseru18@gmail.com; seru@kemri.go.ke) and WRAIR IRB by phone (301) 319-9940, or by email (Usarmy.detrick.medcom-wrair.mbx.hspb@health.mil), or by facsimile (301) 319-9961. The WRAIR HSPB will report to USAMRMC ORP HRPO as per WRAIR SOP UWZ-C-636.

13.3. Informed Consent Process

13.3.1. Consent and Other Informational Documents Provided to Participants

Consent forms describing in detail the study agent, study procedures, and risks and benefits are given to the participant and written documentation of informed consent is required prior to starting intervention/administering study products.

13.3.2. Consent Procedures and Documentation

At the beginning of the study, potential participants selected from within the HDSS will be approached in the community using a recruitment script by study staff delegated to carry out recruitment. Prior to any volunteer joining the study or undergoing any screening procedures, an informed consent process will be carried out. The study staff will brief the participant about the study using the informed consent document. This briefing may be done individually or in groups with the aid of Study Briefing Slides (which will be submitted to ethical review along with this protocol) either done live or as recorded audio with the slides by the principle investigator or delegate. At the end of the briefing, there will be a question and answer session (when a group briefing is completed, this will be both as a group and as individual participants). This will enable participant to understand the purposes of the study as well as the study procedures. They will be given adequate time to think about participating, ask any questions and decide whether they want to participate or not. Subjects may take time to talk with their family and/or friends prior to deciding to consent for participation. Thereafter, signature of the written informed consent will be obtained from each participant who wants to participate in the study. The

participant and the study staff conducting informed consent explanation will sign and date the consent form. The participant signature confirms that (s)he has understood the information. For illiterate individuals, the informed consent process will be conducted in the presence of a literate impartial witness selected by the participant but not a member of the KCRC staff. The participant will thumb print the consent form and the witness will write the participant's name, sign and date the consent form. Each volunteer's consent form will be kept in their study folder and a copy will be given to the participant to take home on the same day. In case of any amendments to the protocol resulting in changes in study procedures, the participant will be informed and additional informed consent will be obtained if necessary. In accordance with Good Clinical Practice, the volunteer may terminate participation in the study at any time for any reason without penalty. Additionally, if the participant is unable or unwilling to adhere to the protocol design, the investigator may terminate volunteer's participation. The study team will use the participant's preferred language usually English, Kiswahili or Luo for informed consent.

13.4. Participant and Data Confidentiality

Participant confidentiality is strictly held in trust by the participating investigators, their staff, and the sponsor(s). This confidentiality is extended to cover testing of biological samples in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of PATH. The study monitor, other authorized representatives of the sponsor, may inspect all documents and records required to be maintained by the investigator.

The study participant's contact information will be securely stored at the clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location.

13.4.1. Future Use of Stored Specimens

After the study is complete, sera, PBMC, and whole blood mRNA specimens will be maintained in a biorepository managed by PATH and will be stored for a period of up to 15 years. Dried blood spots of whole blood collected during the study will be maintained at the study site and/or shipped to a repository in the United States selected by PATH with the concurrence and approval by the Kenya Medical Research Institute (KEMRI)/US Army Medical Research Directorate-Africa (USAMRD-A) for a period of up to 15 years. Participants will be asked to consent for the future use of these specimens beyond the scope of the testing outlined in this protocol, but consent for future use will not be required for participation in this study and an individual participant can choose to withdraw consent to have biological specimens stored for future research. However, withdrawal of consent with regard to biosample storage will not be possible after the study is completed. The future use of biosamples obtained during this study includes further PCR or genetic characterization of malaria parasites or further characterization of participants' serum, PBMC or mRNA samples (to potentially include further serological testing, additional cell-mediated immune response assays, HLA typing or further evaluation of transcriptomics profile). HLA typing will be limited to understanding the immune responses related to immunization and will not be used to identify specific genetic traits or diseases. The potential transcriptomics assays will assess the mRNA transcription profiles in response to vaccination and do not evaluate for hereditary genetic conditions in the subjects' DNA.

13.5. Compensation

13.5.1. Compensation for injury

Participants will be insured against injury caused by the study according to legal requirements and compensation for research related injury (to include costs of long term and future medical care needs) is available, should it occur. If a participant suffers injury or death directly attributable to participation in this study, the participant is asked to contact the PI using the emergency contacts that will be provided on the consent forms and emergency number in the participant ID card. They will also be provided the contacts of KEMRI SERU secretariat in the event that they would like to speak to someone independent of the trial. Appropriate treatment during the trial will be provided by the site personnel and paid by PATH.

13.5.2. Compensation for subject participation

The Kisumu Site will compensate participants for time, inconvenience and lost wages at the rate of 500 KES per scheduled visit. Kisumu (specifically Kombewa) does not have commercial agriculture and jobs are quite limited; most people practice subsistence farming or fishing and minimum wage for unskilled labor ranges from 300-500 KES per day. To be equitable, therefore, Kisumu will compensate for lost wages, time and inconvenience at approximately a day's work at minimum wage (500 KES).

Participants taking part in this study will receive transport reimbursement (if they are not utilizing the site's transport) in accordance with local SOPs, taking into consideration average cost of travel to the clinic between 500 and 1,000 Kenya shillings (US $ 5 and $ 10) for scheduled visits, and 300 and 500 Kenya Shillings (US $ 3 to $ 5) for unscheduled visits as determined by the study team on a case by case basis. Unit transport may also be used as necessary.

14. DATA HANDLING AND RECORD KEEPING

Data collection is the responsibility of the clinical study staff at the site under the supervision of the site Principal Investigator (PI). The PI is responsible for assuring that the data collected is complete, legible, attributable, accurate, and recorded in a timely manner. Data recorded in the CRF should be consistent with the data recorded on the source documents. All source documents and laboratory reports must be reviewed by the site team, who will ensure that they are accurate and complete. The PATH designated CRO is responsible for data management activities, including quality and accuracy review, analysis, and reporting of the study data according to Standard Operation Procedures (SOPs).

14.1. Data Collection and Management Responsibilities

14.1.1. Source Data

All information in original records and certified copies of original records or clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents (original records or certified copies). [ICH E6 section 1.51].

14.1.2. Source documents

Original documents, data and records (e.g., hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries of evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial are considered Source Documents. [ICH E6 section 1.52]

This study is using a barcoded paper Case Report Form (CRF) which is also considered as Source Documents with a few exceptions where the CRF are not source and other documents are considered source. These include the following CRF pages:

- The Informed Consent CRF, where the signed Informed Consent Form is considered source
- The results sections of the Local Laboratory Results CRF, HIV Test Results CRF, Parasitemia Assessment CRFs and Hemoglobinopathy CRF, where the lab reports are considered source. (The urine pregnancy test results CRF is considered source.)
- The PCR results section of the Parasitemia Assessment CRFs, where reports from the MDR laboratory are considered source.
- The IP Administration CRF, where pharmacy IP dispensing and accountability records are considered.
- The SAE CRF, where in case of hospitalizations the hospital records are considered as source.

Printed copies of the barcoded CRF are completed by site staff and field staff during subject visits and then scanned and sent to the PATH designated data management CRO, DFnet.

14.1.3. Data Capture Methods (CRF Development and Completion)

Clinical data recorded in source documents, including the barcoded CRF pages, should be completed in a neat, legible manner to ensure accurate interpretation of data. Source documentation supporting the CRF data should document the dates and details of study procedures, AEs and subject status. The PI will ensure that all information in the CRFs and all other source documents that support the data collected from each subject are maintained in a secure area and treated as confidential material.

Data reported in the CRF that is derived from other source documents should be consistent. It is the PI's responsibility to ensure the accuracy, completeness, and timeliness of the data reported in the subject's CRF and any supporting documentation.

Clinical data entered in the barcoded CRFs and other source documents (as listed above) are uploaded to a 21 CFR Part 11 compliant EDC system using intelligent character recognition software. The scanned pages are then verified against data captured in the database by the data management staff at DFnet. For randomization, data confirming eligibility of the subject, *P.falciparum* infection status and HIV status at screening (based on source data as listed above), is entered directly into the same EDC system by site staff at Visit 2.

The database includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate.

Additionally, a description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by U.S. Law. The clinicaltrials.gov registry will not identify any individual participants and will include a summary of the results.

14.1.4. Data Management

The PATH designated Data Management CRO, DFnet, will develop a Data Management Plan (DMP) for review and approval by PATH. The DMP will describe the roles of the stakeholders and specific procedures to ensure appropriate handling of data at all steps of the data management process, to assure a valid and high-quality database at the end of the study, ready for analysis.

14.1.5. Data Storage

All study documentation containing personal information relating to study subjects like consent forms and other documents that might link subject ID with other subject personal details will be kept in a secure locked area with limited access at the KCRC. Such documentation will only be made available to authorized personnel. These study documents will be made available to the investigators, clinical personnel who require this information to treat the subjects and the above-mentioned personnel for inspection or auditing reasons. In addition, access to subjects' medical records will be granted to representatives of the Sponsor, regulatory agencies, ethics committees, and the USAMRDC for the purpose of validating data.

14.2. Study Records Retention and Disposal

Study documents should be retained for the duration required by PATH, applicable local regulations, and in according to U.S. DoD requirements, in a safe and secure facility. No records will be destroyed without the written consent of PATH. Upon study completion, all records will be stored safely at one of USAMRD-A's archiving facilities or at a secure contracted storage facility for at least 15 years. If storage at an alternative facility is considered, this will be documented, and the documentation will be filed in the study's regulatory file. Should longer storage be recommended by the sponsor or relevant authorities, the site will contact the reviewing IRBs for consideration and approval. Study files can be stored either as paper or digitally. Digitization, if done, will consist of the scanning of all paper records according to site SOPs to ensure accurate and complete representation of the same. This may be done by the site staff of a contracted third-party. All digitized records will be maintained on a secure, password protected computer system with access limited to those with access to study as previously listed in this protocol as well as USAMRD-A's archivist(s) and IT personnel who will maintain the database. Upon completion of digitization of study records, and receipt of a sponsor's approval, paper copies may be destroyed by incineration according to site SOPs.

Paper or digitized records may be disposed of after storage duration has been met (15 years or as otherwise specified as above) and with the sponsor's approval. Any remaining paper records will be incinerated and/or digitized records will be securely deleted. The final disposal of any remaining paper or digitized records will be witnessed by a representative of USAMRD-A's regulatory affairs department and will be documented. The documentation of records disposal will be provided to the sponsor and a copy will be stored at the Regulatory Affairs department.

14.3. Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol, or GCP requirements.

The noncompliance may be either on the part of the participant, the investigator, or the study site staff.

As a result of deviations, corrective actions are to be developed by the site and implemented promptly. It is the responsibility of the site to use continuous vigilance to identify and report deviations.

14.4. Publication and Data Sharing Policy

This study will comply with PATH and U.S. DoD policies. PATH policy ensures that the public has open access to the published results of Gates Foundation funded research. The International Committee of Medical Journal Editors (ICMJE) member journals have adopted a clinical trials registration policy as a condition for publication.

The first publication or disclosure of study results shall be a complete publication or disclosure coordinated by PATH.

15. CONFLICT OF INTEREST POLICY

The independence of this study from any actual or perceived influence is critical. The sponsor, PATH has no conflict of interest to disclose.

16. STUDY LIMITATIONS AND POTENTIAL BIASES

Limitations of this study include that the ideal correlate of protection for a malaria vaccine is unknown given that antibody responses do not always correlate with protection. This study is exploring additional immunological responses to have a better understanding of the correlate of protection. Additionally, while the PCR assay used here tests for the presence of a blood stage infection, incubating liver stage parasites may be missed during screening resulting in misallocation of a subject to a group other than originally intended. We believe that such an occurrence would be rare and there is no way to mitigate should such an event would occur. The selection methods and randomization were designed to limit bias, but we may potentially have other confounding factors that were not considered and will fail to be randomly distributed despite our best efforts.

17. MILITARY RELEVANCE

US Service Members conduct operations in regions of the world where they are at risk of malaria acquisition. Acquiring malaria is significantly detrimental to individual Service Members and their units resulting in lost duty hours, increased utilization of medical resources in the field (often in austere settings), potential requirement for medical evacuation, risk of death to individual Service Members, and risk to a unit's mission if a significant proportion or key individuals become ill. As such medical countermeasures to malaria are essential in malaria endemic regions and include effective treatment, effective prophylaxis, and immunoprophylaxis (such as a vaccine). Malaria vaccines have the advantage of potentially eliminating the need for other prophylaxis (if high enough efficacy) and/or decreasing the severity of disease if an individual does become ill. To date, the RTS,S malaria vaccine is the most advanced in development and has shown reasonable efficacy in adults in challenge studies, but less so in field settings. This study attempts to optimize the RTS,S vaccines protective efficacy in endemic areas

and also further understand the immunology of a protective response. What is learned will help in advancing the understanding and application of this vaccine, and in the future development of malaria immunoprophylaxis. Additionally, an effective malaria vaccine may contribute to disease reduction in the long-term goals of malaria elimination. In the short term, reduced disease burden can improve the health of individuals in endemic regions which can improve regional stability. Also, any declines in malaria burden as we work toward elimination decreases the risk to service members deployed in endemic areas.

18. LITERATURE REFERENCES

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19. APPENDICES

APPENDIX A. SCREENING VISIT PROCEDURES

Screening Visit	**1**
Type of Visit (KC = KCRC Clinic Visit or FV = Field visit)	KC
Study day	-39 to -28
Informed consent	●
Photo for ID card	●
Demographics	●
Medical History	●
Physical Examination	●
Concomitant medications	●
Check inclusion/exclusion criteria	●
Vital Signs (BP, pulse, temperature)	●
Urine Pregnancy test	●
Complete blood count (Hb, WBC, PLT; (4 mL)	●
Creatinine, ALT (4 mL)	●
HIV (4 mL)	●
Blood sampling for assessment of parasitemia (PCR and DBS) (0.35 to 0.5 mL) by finger stick	●
Record any SAEs	●
CUMULATIVE BLOOD VOLUME	13 mL

APPENDIX B. EPOCH 1: TREATMENT AND IMMUNIZATION PHASE

	Treatment and Immunization Phase (Epoch 1)																
Visit #	**2**	**3^**	**4^**	**5**	**6**	**7**	**8^**	**9^**	**10^**	**11**	**12[1]**	**13**	**14^**	**15^**	**16^**	**17**	**18[1]**
Type of Visit (KC or FV) preferred	**KC**	**FV**	**FV**	**KC**	**KC**	**KC**	**KC**	**FV**	**FV**	**KC**	**FV**	**KC**	**KC**	**FV**	**FV**	**KC**	**FV**
Study day	**-27**	**-26**	**-25**	**1**	**2**	**8**	**15**	**16**	**17**	**29**	**36**	**57**	**190**	**191**	**192**	**197**	**204**
Permissible window (+/- days)*	**2**	**0**	**0**	**3**	**0**	**+2**	**2**	**0**	**0**	**3**	**+2**	**3**	**2**	**0**	**0**	**3**	**+2**
Issue subject's identification card	●																
Check inclusion/exclusion criteria	●			●			●			●			●			●	
Record any intercurrent medical conditions	●			●			●			●			●			●	
Record any concomitant medications/vaccinations	●			●	●	●	●			●		●	●			●	
Record if subject belongs to reactogenicity sub-cohort[1]	●																
Check contraindications to vaccinations/anti-malarial medications	●			●			●			●			●			●	
History of fever/Record body temperature	●			●			●			●			●			●	
Focused/Symptom & physical exam as needed				●						●						●	
DHA/piperaquine	●	●	●				●	●	●								
Primaquine (low dose)	●						●						●				
Coartem													●	●	●		
Record vital signs (BP, pulse, temperature)				●						●						●	
Randomization upon completion of parasite PCR	●																
Vaccination				●						●						●	
LABS																	
Complete blood count (Hb, WBC, PLT; 4 mL)				●													
Creatinine, ALT (4 mL)				●													
Hemoglobinopathy screen (4 mL)	●																
Urine Pregnancy test	●			●			●			●			●			●	
Buccal swab- HLA typing (Groups 1, 2, 3 only)	●[3]																

	Treatment and Immunization Phase (Epoch 1)																
Visit #	**2**	**3^**	**4^**	**5**	**6**	**7**	**8^**	**9^**	**10^**	**11**	**12[1]**	**13**	**14^**	**15^**	**16^**	**17**	**18[1]**
Type of Visit (KC or FV) preferred	**KC**	**FV**	**FV**	**KC**	**KC**	**KC**	**KC**	**FV**	**FV**	**KC**	**FV**	**KC**	**KC**	**FV**	**FV**	**KC**	**FV**
Study day	**-27**	**-26**	**-25**	**1**	**2**	**8**	**15**	**16**	**17**	**29**	**36**	**57**	**190**	**191**	**192**	**197**	**204**
SAFETY DATA COLLECTION [1]																	
Record solicited local & systemic AEs				●	●	●				●	●					●	●
Record unsolicited AEs post anti-malarial treatment/ post vaccination	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Record AEs/SAEs (fatal; AESI)	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
INVESTIGATIONAL ASSAYS																	
Blood serum sampling (10 ml) -refer to Appendix F for Group-specific timepoints [2]	●			●						●		●				●	
CMI- PBMC from all RTS,S vaccinated subjects Groups 1,2,3 (30 mL)	●			●		●											
Whole blood transcriptomics all subjects (Paxgene; < 3 mL)	●			●	●	●											
PARASITEMIA ASSESSMENT																	
Blood sampling for PCR and DBS (0.35 to 0.5 ml)				●						●						●	
CUMULATIVE BLOOD VOLUME (mL)	60			112	115	158				169		179				190	

* Rationale for window period: Anti-malarial treatment does not allow for missed doses (no permissible window period). Total 6-day window period on either side of optimal vaccination scheduled day (+/- 3d). total 8-day window period on either side of ADI scheduled days (+/- 4 days)

[1] Reactogenicity cohort for solicited local and general AEs are the first 50 subjects per group in Groups 1 and 2, and all subjects in Group 3 (Field Visits 12 and 18 are only applicable to this cohort). All subjects (Groups 1 to 5) will have unsolicited AEs captured through day 28 after each immunization and post anti-malarial treatment, as applicable (i.e. Groups 1, 2, 4 and 5).

[2] Only the first 50 subjects each in Groups 4 and 5 will have blood samples collected for rabies antibody determination. Subjects in Groups 4 and 5 who do not require serology samples or follow up for solicited AEs post anti-malarial treatment or vaccination may have these visits done in the field instead of at the clinic.

[3] Buccal swab samples for HLA typing should ideally be collected (for all subjects in Groups 1, 2, and 3) at Visit 2, but can be collected at a later study visit.

^ While subjects in Group 3 are required to attend Visit 2 for enrolment and randomization, Visits 8 and 14 for start of anti-malarial treatment, and Visits 3, 4, 9, 10, 15, and 16 for DOT of anti-malarial treatment by FW or CHW are not applicable for subjects assigned to this group.

APPENDIX C. EPOCH 2: ACTIVE DETECTION OF INFECTION PHASE

	Active Detection of Infection (Epoch 2)								
Visit #	**19**	**20**	**21‡**	**22‡**	**23‡**	**24‡**	**25‡**	**26‡**	**27**
Type of Visit (KC or FV) preferred	**FV**	**KC**	**FV**	**FV**	**FV**	**FV**	**FV**	**FV**	**KC**
Study day	**211**	**225**	**246**	**267**	**288**	**309**	**330**	**351**	**372**
Permissible window (+/- days)*	**3**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**
Issue subject's identification card									
Check inclusion/exclusion criteria									
Record any intercurrent medical conditions									
Record any concomitant medications/vaccinations		•							
Record if subject belongs to reactogenicity sub-cohort[1]									
Check contraindications to vaccinations/anti-malarial medications									
History of fever/Record body temperature	•	•	•	•	•	•	•	•	•
Focused/Symptom & physical exam as needed									
DHA/piperaquine ; Primaquine (low dose)									
Coartem									
Record vital signs (BP, pulse, temperature)									
Vaccination									
SAFETY LABS									
Complete blood count (Hb, WBC, PLT; 4 mL)									
Creatinine, ALT (4 mL)									
Urine Pregnancy test									
SAFETY DATA COLLECTION [1]									
Record solicited local & systemic AEs									
Record unsolicited AEs post anti-malarial treatment/ post vaccination	•	•							

	Active Detection of Infection (Epoch 2)								
Visit #	**19**	**20**	**21‡**	**22‡**	**23‡**	**24‡**	**25‡**	**26‡**	**27**
Type of Visit (KC or FV) preferred	**FV**	**KC**	**FV**	**FV**	**FV**	**FV**	**FV**	**FV**	**KC**
Study day	**211**	**225**	**246**	**267**	**288**	**309**	**330**	**351**	**372**
Record AEs/SAEs (fatal; AESI)	●	●	●	●	●	●	●	●	●
INVESTIGATIONAL ASSAYS									
Blood serum sampling (10 ml) -refer to Appendix F for Group-specific timepoints [2]		●							●
CMI (PBMC from RTS,S vaccinated subjects (Gps 1,2,3) only (30 mL)		●							
Whole blood transcriptomics all subjects (Paxgene; < 3 mL)		●							
PARASITEMIA ASSESSMENT									
Blood sampling for PCR and DBS (0.35 to 0.5 ml) [3]	●	●	●	●	●	●	●	●	●
CUMULATIVE BLOOD VOLUME (mL)	191	235	236	237	238	239	240	241	252

* Rationale for window period: Anti-malarial treatment does not allow for missed doses (no permissible window period). Total 6-day window period on either side of optimal vaccination scheduled day (+/- 3d). total 8-day window period on either side of ADI scheduled days (+/- 4 days)

[1] Reactogenicity cohort for solicited local and general AEs are the first 50 subjects per group in Groups 1 and 2, and all subjects in Group 3. All subjects (Groups 1 to 5) will have unsolicited AEs captured through day 28 after each immunization and post anti-malarial treatment, as applicable (i.e. Groups 1, 2, 4 and 5).

[2] Only the first 50 subjects each in Groups 4 and 5 will have blood samples collected for rabies antibody determination.

[3] Subjects in Group 3 will not participate in ADI. Parasitemia assessment is therefore not applicable for this group (with the exception of the cross-sectional sampling at Visit 27 and Study Termination).

‡ Subjects in Group 3 will not participate in ADI (as above), but will be followed up 7 and 28 days post dose 3, as per protocol i.e. Visit 18 (per Appendix B, Epoch 2) and Visit 20. Visits 19, and 21 to 26 are therefore not required for this group Visit 27 is required for group 3.

APPENDIX D. EPOCH 3: EXTENSION DETECTION OF INFECTION PHASE (IF REQUIRED)

	Active Detection of Infection-Extension (Epoch 3)							
Visit #	**28**	**29**	**30**	**31**	**32**	**33**	**34**	**35**
Type of Visit (KC or FV) preferred	**FV**	**FV**	**FV**	**FV**	**FV**	**FV**	**FV**	**FV**
Study day	**393**	**414**	**435**	**456**	**477**	**498**	**519**	**540**
Permissible window (+/- days)*	4	4	4	4	4	4	4	4
Issue subject's identification card								
Check inclusion/exclusion criteria								
Record any intercurrent medical conditions								
Record any concomitant medications/vaccinations								
Record if subject belongs to reactogenicity sub-cohort[1]								
Check contraindications to vaccinations/anti-malarial medications								
History of fever/Record body temperature	•	•	•	•	•	•	•	•
Focused/Symptom & physical exam as needed								
DHA/piperaquine ; Primaquine (low dose)								
Coartem								
Record vital signs (BP, pulse, temperature)								
Vaccination								
SAFETY LABS								
Complete blood count (Hb, WBC, PLT; 4 mL)								
Creatinine, ALT (4 mL)								
Urine Pregnancy test								
SAFETY DATA COLLECTION								
Record solicited local & systemic AEs								

	Active Detection of Infection-Extension (Epoch 3)							
Visit #	**28**	**29**	**30**	**31**	**32**	**33**	**34**	**35**
Type of Visit (KC or FV) preferred	**FV**	**FV**	**FV**	**FV**	**FV**	**FV**	**FV**	**FV**
Study day	**393**	**414**	**435**	**456**	**477**	**498**	**519**	**540**
Record unsolicited AEs post anti-malarial treatment/ post vaccination								
Record AEs/SAEs (fatal; AESI)	●	●	●	●	●	●	●	●
PARASITEMIA ASSESSMENT								
Blood sampling for PCR and DBS (0.35 to 0.5 ml)– Groups 1, 2, 4 and 5 only.	●	●	●	●	●	●	●	●
CUMULATIVE BLOOD VOLUME (mL)	253	254	256	257	258	259	260	281

* Rationale for window period: Anti-malarial treatment does not allow for missed doses (no permissible window period). Total 6-day window period on either side of optimal vaccination scheduled day (+/- 3d). total 8-day window period on either side of ADI scheduled days (+/- 4 days)

APPENDIX E. STUDY TERMINATION VISIT

Visit #	STUDY TERMINATION VISIT
Type of Visit (KC or FV) preferred	**KC or FV[1]**
Study day	**TBD [2]**
Permissible window (+/- days)	+56 [2]
Record AEs/SAEs (fatal; AESI)	●
Blood sampling for PCR and DBS (0.35 to 0.5 ml) (all subjects, all groups)	●
Blood serum sampling (10 ml) [3]: - Groups 1 to 3	●
Offer and schedule post-study course of rabies vaccination – Groups 1 to 3 only [4]	●

[1] Either acceptable, though visits for Groups 1 to 3 will need to be conducted at KC for serology sample collection.

[2] Date for visit will be the day according to the criteria as specified in section 7.1.1, part E of the protocol being met; and will be completed for all volunteers within 56 days of the set calendar date.

[3] Serology timepoint at Study Termination only applies to Groups 1 to 3 - see sections 7.1.1, above, and Appendix F for further details.

[4] Rabies immunization for subjects in Groups 1, 2, and 3 when scheduled at termination visit may include any rabies vaccine approved for use in Kenya. If participants are willing/able, rabies immunization may start at this study termination visit after all study specific procedures are completed.

APPENDIX F. BIOLOGICAL SAMPLES FOR IMMUNOGENICITY TESTING

SAMPLES COLLECTED	Visit #	2	5	6	7	11	13	17	20	27	Study Termination Visit
	Study day	-27	1	2	8	29	57	197	225	372	TBD*
Secondary endpoint -Groups 1, 2, 3											
Serum	anti-CS NANP levels	●	●			●	●	●	●	●	●
	anti-CS NANP avidity	●	●			●	●	●	●	●	●
	anti-CS C-term levels	●	●			●	●	●	●	●	●
	anti-CS C-term avidity	●	●			●	●	●	●	●	●
	anti-HbsAb antibodies	●	●			●			●		
Exploratory											
Serum	Anti-rabies antibodies -(first 50 subjects in Group 4 and Group 5)		●						●		
PBMC	Flow cytometry from all RTS,S vaccinated subjects (Gps 1,2,3)	●	●		●				●		
Transcriptomics	Transcriptomics Groups 1, 2, 3, 4, 5	●	●	●	●				●		

* Study Day for the Study Termination Visit will be the day according to the criteria as specified in section 7.1.1, part E of the protocol being met.

APPENDIX G. ADULT NORMAL REFERENCE VALUES – KEMRI & USAMRD-A KISUMU CLINICAL LABORATORIES

Analyte	Adult Male	Adult Female	Unit	Critical Values	
				Low	High
Creatinine	54.2 - 137.8	52.4 - 96.8	µmol/L	N/A	Male:>248; Female:>174.2
ALT	12.0 - 80.6	10.7 - 61.3	U/L	N/A	Male:>201.5; Female:>153.3
WBC	3.3 - 9.6	3.7 - 9.1	$x10^3/\mu L$	< 2.0	$\geq$ 40.0
HGB	12.6 - 17.2	9.0 - 14.9	g/dL	Male: <8.0; Female: <7.0	>20.0
Platelets	126 - 356	147 - 454	$x10^3/\mu L$	<80	>1000

APPENDIX H. WHO CLINICAL STAGING OF HIV INFECTION IN KENYA

Guidelines on Use of Antiretroviral Drugs for Treating and Preventing HIV Infection in Kenya – 2018 Edition

Annex 2: WHO Clinical Staging of HIV Infection in Adolescents and Adults

Stage 1 • Asymptomatic • Persistent Generalized Lymphadenopathy (PGL)	Stage 2 • Moderate unexplained weight loss (< 10% of presumed or measured body weight) • Minor mucocutaneous manifestations (seborrheic dermatitis, papular pruritic eruptions, fungal nail infections, recurrent oral ulcerations, angular cheilitis) • Herpes zoster • Recurrent upper respiratory tract infections (sinusitis, tonsillitis, bronchitis, otitis media, pharyngitis)
Stage 3 • Unexplained severe weight loss (over 10% of presumed or measured body weight) • Unexplained chronic diarrhoea for longer than one month • Unexplained persistent fever (intermittent or constant for longer than one month) • Persistent oral candidiasis • Oral hairy leukoplakia • Pulmonary tuberculosis • Severe bacterial infections (e.g. pneumonia, empyema, pyomyositis, bone or joint infection, meningitis, bacteraemia) • Acute necrotizing ulcerative stomatitis, gingivitis or periodontitis • Unexplained anaemia (below 8 g/dl), neutropenia (below 0.5 x 109/l) and/or chronic thrombocytopenia (below 50 x 109 /l)	Stage 4 Conditions where a presumptive diagnosis can be made using clinical signs or simple investigations: • HIV wasting syndrome • Pneumocystis jirovecipneumonia (PCP) • Recurrent severe bacterial pneumonia (≥ 2 episodes within 1 year) • Cryptococcal meningitis • Toxoplasmosis of the brain • Chronic orolabial, genital or ano-rectal herpes simplex infection for > 1 month • Kaposi's sarcoma (KS) • HIV encephalopathy • Extra pulmonary tuberculosis (EPTB) Conditions where confirmatory diagnostic testing is necessary: • Cryptosporidiosis, with diarrhoea > 1 month • Isosporiasis • Cryptococcosis (extra pulmonary) • Disseminated non-tuberculous mycobacterial infection • Cytomegalovirus (CMV) retinitis or infection of the organs (other than liver, spleen, or lymph nodes) • Progressive multifocal leucoencephalopathy (PML) • Any disseminated mycosis (e.g. histoplasmosis, coccidiomycosis) • Candidiasis of the oesophagus or airways • Non-typhoid salmonella (NTS) septicaemia • Lymphoma cerebral or B cell Non-Hodgkin's Lymphoma • Invasive cervical cancer • Visceral leishmaniasis • Symptomatic HIV-associated nephropathy or HIV associated cardiomyopathy

APPENDIX I. DETAILED ROLES AND RESPONSIBILITIES

The **Principal Investigator (PI)** will be responsible for ensuring the protocol is executed as written, obtaining consent, conducting physical examinations and medical histories, determining eligibility of an individual to participate in the study, collecting data, data analysis, protocol design, administrative support, adverse event reporting, observation of participants, progress/continuing review reporting, briefing volunteers, clinical care of participants, review of source documents, randomization, investigational product accountability, overseeing all other research activities, documenting protocol deviations, training and supervising study staff, and delegating responsibilities as appropriate. The **Co-Principle Investigator (Co-PI)** will have the same responsibilities as the PI and can serve as an alternate in decision making if the PI is unavailable. However, if there are any disagreement between the Co-PI and the PI, the ultimate authority for a decision will rest with the PI.

The **Associate Clinical Investigators**, as delegated by the PI, may be responsible for executing the protocol as written, obtaining consent, conducting physical examinations and medical histories, determining eligibility of an individual to participate in the study, collecting data, data analysis, protocol design, assist in supervising study staff, administrative support, adverse event reporting, observation of participants, progress/continuing review reporting, briefing volunteers, clinical care of participants, review of source documents, overseeing research activities, and any other study activities as delegated by the PI.

The **Clinical Research Coordinator**, as delegated by the PI, may be responsible for executing the protocol as written, collecting data, data analysis, protocol design, administrative support, coordinating all protocol activities, maintaining essential trial documents, adverse event reporting, scheduling participant appointments, reviewing source documents, drug accountability, randomization (though will not have access to manual randomization envelops), quality assurance/control, overseeing research activities, and any other study activities as delegated by the PI.

The **Clinicians**, as delegated by the PI, may be responsible for obtaining consent, conducting physical examinations and medical histories, executing the protocol as written, adverse event reporting, observation of participants, progress reporting, recruiting study participants, briefing volunteers, appointing follow-up visits, clinical care of participants, reviewing source documents, and any other study activities as delegated by the PI.

The **Study Nurses**, as delegated by the PI, may be responsible for obtaining consent, recording demographics, obtaining vital sign measurements, specimen collection, administering study vaccinations, observation of participants, briefing volunteers, appointing follow-up visits, clinical care of participants, dispensing of medications, and any other study activities as delegated by the PI.

The **Pharmacist**, as delegated by the PI, may be responsible for drug accountability, vaccine preparation, randomization, dispensing of medications, and any other study activities as delegated by the PI.

The **Laboratory Staff**, as delegated by the PI, may be responsible for specimen collection, specimen processing, transportation/shipping of samples, sample management, protocol design, quality assurance/control, malaria slide coordination, and any other study activities as delegated by the PI.

The **Records Staff** and **Data Management Team**, as delegated by the PI, may be responsible for photo taking and ID preparation, data entry, quality assurance/control, and any other study activities as delegated by the PI.

The **Community Relations** and **Field Team**, as delegated by the PI, may be responsible for recruitment of participants, appointing follow-up visits, community education and outreach, and any other study activities as delegated by the PI.