SAFETY AND IMMUNOGENICITY EVALUATION OF THE MALARIA VACCINE, RTS,S/AS01, IN HEALTHY THAI ADULTS

Protocol Number: BAKMAL 1605

OxTREC: 54 - 16

Date and Version No: 2/11/2017 version 5.0

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Investigator Agreement and Conflict of Interest

"I have read this protocol and:

- agree to abide by all provisions set forth therein.
- agree to comply with the principles of the International Conference on Harmonisation Tripartite Guideline on Good Clinical Practice.
- and declare no conflict of interest, according to the current version of the Declaration of Helsinki"

Principal Investigator	Principal Investigator Signature	Date

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AMENDMENT HISTORY

Amendment No.	Protocol Version No.	Date issued	Author(s) of changes	Details of Changes made
1	V 2.0	6/02/2017	Lorenz von Seidlein	- Increase of blood volume for the immunology panel in order to have sample leftover for future use - Revision of antimalarial drugs dosing schedule and drugs level tests - Addition of a biorepository sample management - The changes to address ECs comments ▪ Subject compensation to be 1,000 THB/visit ▪ Addition of appropriate contraceptive methods ▪ Addition of informed consent process for pre-post counselling for HIV screening, report and treatment
2	V 3.0	14/03/2017	Lorenz von Seidlein	- Revision of study procedures and outcome analysis for gr. 7 participant at M1D0 and M1D7
3	V 4.0	11/05/2017	Lorenz von Seidlein	- Revision to allow the use of screening lab tests for enrollment evaluation if interval between and screening and enrolment is three days or less.
4	V 5.0	2/11/2017	Lorenz von Seidlein	- Revision to allow additional PK assessments if additional data are required.

LIST OF ABREVIATIONS:

AE	Adverse event
ALT	Alanine aminotransferase
anti-CS	Antibody to the *P. falciparum* circumsporozoite (CS) repeat domain
anti-HBs	Antibody to the hepatitis B surface antigen
AS01B	GSK's proprietary Adjuvant System containing MPL, QS-21 and liposome (50 µg MPL and 50 µg QS-21)
AS01E	GSK's proprietary Adjuvant System containing MPL, QS-21 and liposome (25 µg MPL and 25 µg QS-21)
AST	Aspartate aminotransferase
CI	Confidence interval
CREA	Creatinine
CRF	Case report form
CS	Circumsporozoite protein of *P. falciparum*
DHA-PIP	Dihydroartemisinin–piperaquine
DSMB	Data safety monitoring board
ECG	Electrocardiogram
ELISA	Enzyme linked immunosorbent assay
EPT	Endpoint titre
FDA	Food and Drug Administration, United States
GCP	Good clinical practice
GSK	GlaxoSmithKline
Hb	Haemoglobin
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
HCV	Hepatitis C virus
Hep B	Hepatitis B
HIV	Human immunodeficiency virus
IB	Investigator's brochure
ICF	Informed consent form
ICH	International Committee on Harmonization
IEC	Independent ethics committee
IgG	Immunoglobulin G
IM	Intramuscular
IRB	Institutional review board

IU	International unit
kg	Kilogram
MedDRA	Medical Dictionary for Regulatory Activities
mg	Milligram
mL	Milliliter
ms	Millisecond
MPL®	3-deacylated monophosphoryl lipid A
MVI	Malaria Vaccine Initiative
P. falciparum	*Plasmodium falciparum*
PFS	Pre-filled syringe
PI	Principal Investigator
PLT	Platelets
PQ	Primaquine
QS-21	'*Quillaja saponaria* 21': a triterpene glycoside purified from the bark of the soap bark tree, *Quillaja saponaria*
RTS	Hybrid protein comprising HBs (hepatitis B surface antigen) and CSP portions
RTS,S	Particulate antigen, containing both RTS and HBs proteins
SAE	Serious adverse event
SLD-PQ	Single low dose Primaquine
SOP	Standard operating procedure
ULN	Upper limit of normal
WBC	White blood cells
WRAIR	Walter Reed Army Institute for Research

1 SYNOPSIS

Study Title	Safety and immunogenicity evaluation of the malaria vaccine, RTS,S/AS01, in healthy Thai adults
Study Design	Randomized, open label, single centre, Phase 2 study
Study Participants	• 190 healthy non-pregnant Thai adults, aged 18-55 years, inclusive, will be recruited.
Study arms	**See Table 3 for dosing table** 1. RTS,S/AS01B Fractional dose group (Group 1, n=20) will receive RTS,S/AS01B standard dose at Month 0 and Month 1 + RTS,S/AS01B fractional dose (1/5th dose) at Month 2. 2. Double RTS,S/AS01E Fractional dose group (Group 2, n=20) will receive a double dose of RTS,S/AS01E standard dose at Month 0 and Month 1 + a double dose of RTS,S/AS01E fractional dose (1/5th dose) at Month 2. 3. RTS,S/AS01E Standard dose group (Group 3, n=30) will receive RTS,S/AS01E standard dose at Month 0, Month 1 and Month 2. 4. RTS,S/AS01E + DHA-PIP+PQ Standard dose group (Group 4, n=30) will receive RTS,S/AS01E standard dose + DHA-PIP+PQ at Month 0, Month 1 and Month 2 5. RTS,S/AS01E Fractional dose group (Group 5, n=30) will receive RTS,S/AS01E standard dose at Month 0 and Month 1 + RTS,S/AS01E fractional dose (1/5th dose) at Month 2. 6. RTS,S/AS01E + DHA-PIP+PQ Fractional dose group (Group 6, n=30) will receive RTS,S/AS01E standard dose + DHA-PIP+PQ at Month 0 and Month 1 + RTS,S/AS01E fractional dose (1/5th dose) + DHA-PIP+PQ at Month 2. 7. RTS,S/AS01E + DHA-PIP+PQ Fractional two-dose group (Group 7, n=30) will receive RTS,S/AS01E standard dose + DHA-PIP+PQ at Month 0 + RTS,S/AS01E fractional dose (1/5th dose) + DHA-PIP+PQ at Month 2.

	Standard dose RTS,S/AS01B: 50µg RTS,S + standard dose AS01B (0.5ml containing 50µg MPL + 50µg QS21) Standard dose RTS,S/AS01E: 25µg RTS,S + standard dose AS01E (0.5ml containing 25µg MPL + 25µg QS21) Fractional dose RTS,S/AS01B (1/5th dose): 10µg RTS,S + 1/5 standard dose AS01B (0.1ml containing 10µg MPL + 10µg QS21) Fractional dose RTS,S/AS01E (1/5th dose): 2.5µg RTS,S + 1/5 standard dose AS01E (0.1ml containing 2.5µg MPL + 2.5µg QS21) DHA-PIP: Dihydroartemisinin–piperaquine co-formulated, given on day of vaccination (day 0) and each of the subsequent two days (days 1 and 2 post-vaccination). PQ: Primaquine, given on day of vaccination (day 0).
Number of vaccine vials needed	• 60 vials RTS,S/AS01B (adult formulation) • 540 vials RTS,S/AS01E (standard formulation)
Planned Study Period	6 months for each participant, from receipt of first vaccination 3 months to recruit 190 eligible volunteers Total 9 months
Objectives	• 1) To assess the safety and immunogenicity of the standard formulation of the RTS,S vaccine (RTS,S/AS01E) in Thai adults. • 2) To assess the safety and immunogenicity of the adult formulation of the RTS,S vaccine (RTS,S/AS01B) in Thai adults. • 3) To compare the safety and immunogenicity of a fractional dose RTS,S regime (where the third dose is a 1/5th of the standard dose) using the adult formulation (RTS,S/AS01B) to the safety and immunogenicity of fractional dose RTS,S regimes using either single or double doses of the standard formulation (RTS,S/AS01E), in Thai adults. G1 vs G2 vs G5 • 4) To compare the safety and immunogenicity of fractional dose regime (where the third dose is a 1/5th of the standard dose) using a single dose of the standard formulation (RTS,S/AS01E) to the safety and immunogenicity of fractional

	dose RTS,S regime using a double dose of the standard formulation (RTS,S/AS01E), in Thai adults. G2 vs G 5) • 5) To compare the serologic response to RTS,S/AS01E vaccine when co-administered with DHA-PIP + SLD-PQ with RTS,S/AS01E vaccine alone in adults, when RTS,S/AS01E is given in either a fractional dose regime (where the third dose is a 1/5th of the standard dose) or a full standard dose regime. G2 vs G3 and G4 vs G6 • 6) To compare the serologic response to RTS,S/AS01E vaccine co-administered with DHA-PIP + SLD-PQ, when RTS,S/AS01E is given in a fractional two-dose regimen (Month 0, standard dose and Month 2, fractional (1/5th) dose) with RTS,S/AS01E vaccine co-administered with DHA-PIP + SLD-PQ, when RTS,S/AS01E is given in a fractional three-dose regimen (Month 0 and Month 1, standard doses and Month 2, fractional (1/5th) dose) G6 vs G7
Primary Outcomes	• The safety of the investigational vaccine in each group: o Occurrence of serious adverse events (SAEs) from the date of the first vaccination to 29 days after the last vaccination, according to the MedRA classification. o Occurrence of SAEs during the whole study period, i.e. during a 6 month follow up period from the receipt of first vaccination, according to the MedRA classification. • For groups 1 to 6, the concentration of antibodies against *Plasmodium falciparum* circumsporozoite (anti-CS antibody), one month after the first dose (at Study month 1), one month after the second dose (at Study month 2), one month after the third dose (at Study Month 3) and six months after the first dose (at Study Month 6). • For group 7, the concentration of antibodies against *Plasmodium falciparum* circumsporozoite (anti-CS antibody), one month after the first dose (at Study month 1), two months after the first dose (at Study month 2), one month after the second dose (at Study month 3), and six months after the first dose (at Study Month 6).

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2 BACKGROUND AND RATIONALE

The malaria vaccine, RTS,S/AS01, consists of hepatitis B virus surface antigen virus-like particles incorporating a portion of the *Plasmodium falciparum*-derived circumsporozoite protein and a liposome-based adjuvant (AS01) containing two immune enhancers MPL (3'-O-desacyl-4'-monophosphoryl-lipid A) and QS-21 (*Quillaja saponaria* Molina, fraction 21) [1]. In a large Phase 3 trial of RTS,S/AS01E at 11 sites in seven sub-Saharan African countries, efficacy against clinical malaria in children aged 5–17 months following three primary doses was 45.1% (CI 41.4 to 48.7), 35.2% (CI 30.5 to 39.5) and 28.3% (CI 23.3 to 32.9) during 20, 32 and 48 months of follow-up, respectively[2]. In July 2015, RTS,S/AS01E received a positive scientific opinion by the European Medicines Agency for active immunization of children aged 6 weeks to 17 months against malaria[3].

Other than considering RTS,S/AS01E for inclusion in the expanded programme of immunisations (EPI) in sub-Saharan Africa, the vaccine could be used as an innovative and potentially critical additional tool in the effort to stop the spread of artemisinin-resistant falciparum malaria in Southeast Asia. Multidrug-resistant *P. falciparum* strains have replaced the susceptible wild type in many parts of Cambodia[4], where chloroquine, pyrimethamine, and sulphadoxine resistance originated, and from where these strains spread to Africa at a cost of millions of children's lives[5, 6]. With the subsequent development of resistance to artemisinin combination therapy (ACT), the

usefulness of ACTs in Cambodia has been compromised[7] and malaria incidence in northeast Cambodia is again on the rise. These resistant *P. falciparum* strains have spread across the Greater Mekong Subregion (GMS), compromising ACT efficacy, and they are now encroaching on the Indian subcontinent, the traditional gateway of malaria resistance to Africa[8]. There is a large and growing sub-Saharan African population that is immunologically naïve to malaria due to the dramatic reductions in malaria transmission during the last decade. If artemisinin-resistant *P. falciparum* strains are introduced and spread in sub-Saharan Africa, this could give rise to a surge in malaria-related illness and death, as was seen during the spread of chloroquine resistance in the 1980s and 1990s[9]. The only way to stop the spread of artemisinin-resistant malaria is through the elimination of *P. falciparum* in the GMS while this is still possible.

Targeted malaria elimination (TME), which comprises appropriate case management by village health workers, vector control and mass drug administration, is currently being implemented through pilot projects in selected villages in the Greater Mekong Subregion (GMS) and the scale-up of the intervention to the regional level are underway. TME is a time-consuming, demanding and difficult process requiring several months for regional coverage. Importantly, the elimination of individual *P. falciparum* parasitaemia using anti-malarial drugs is short-term; untreated parasitaemic travellers and migrants can readily re-introduce infections into treated villages. There is a concern that TME can achieve only a transient reduction in malaria followed by resurgence. Based on mathematical modelling, extending the post-TME parasitaemia-free period in the majority of villagers for as short as 200 days will substantially increase the chances of achieving the interruption of malaria transmission.

The mass administration of antimalarials like DHA/piperaquine can interrupt malaria transmission for limited time periods. To achieve longer lasting interruptions it would be critical to prevent re-infections, and the addition of mass vaccination campaigns integrated in mass drug administration campaigns may achieve this goal.

Immunogenicity of RTS,S is greater in older children, and the short term malaria protective effect is stronger than the overall effect assessed over 1-2 years. Addition of mass RTS,S/AS01E vaccination to the TME arsenal could provide this much needed additional protection.

Prior to the roll out of the combination of antimalarials with a vaccine it is mandatory to assess and confirm safety and vaccine immunogenicity.

RTS,S has not been used in combination with antimalarial drugs in the past. There is no practical or theoretical reason why the antimalarial drugs dihydroartemisinin/piperaquine and primaquine should

have any influence on the immunogenicity or safety of the RTS,S/AS01 vaccine. The only potential reason why there could be interference is the observation that the immunogenicity of live rabies vaccine is reduced by co-administration with chloroquine. The WHO therefore has warned that the concurrent use of chloroquine can reduce the antibody response to intradermal application of cell-culture rabies vaccines. People who are currently receiving malaria prophylaxis or who are unable to complete the entire three-dose rabies vaccine pre-exposure series before starting malarial prophylaxis should receive pre-exposure vaccination by the intramuscular route. In the past there have been no safety concerns regarding the co-administrations of vaccines with antimalarials.

Currently there are no safety and immunogenicity data for the use of RTS,S/AS01 in Asian populations. This trial will generate the required data for the use of this vaccine in Asian populations. For integration with the current TME activities, which provide mass drug administrations at months M0, M1, and M2, it would be most efficient and practical to provide the vaccine at the same intervals. To address a two round intervention (M0, M2) where a three round intervention is not feasible, one study arm will look at the immune response generated by only two doses of vaccine and antimalarial medications. Recent evidence suggests that a vaccination schedule which includes a fractional dose of RTS,S/AS01 (1/5th of the standard dose) could be similarly or more protective than a schedule with three standard doses, while requiring less vaccine and resources[10]. The evidence demonstrates that a change in immunization schedule that includes a dose reduction of the third RTS,S/AS01 vaccination, results in a significant increase in protection against controlled human malaria infection (CHMI) without an increase in the absolute antibody concentration after vaccination. Qualitative evaluation of immune responses showed that the third dose spacing and dose reduction increased somatic hypermutation in immunoglobulin genes and avidity. The trial therefore includes study arms which will assess the safety and immunogenicity of fractional dose schedules.

In summary: We propose to conduct a safety and immunogenicity trial of RTS,S/AS01 in Thai adults. The two major aims of this study are to 1) confirm that the co-administration of antimalarial drugs with the malaria vaccine RTS,S/AS01 does not reduce the immunogenicity of the vaccine, 2) assess the safety and immunogenicity of RTS,S/AS01 in Thai adults.

3 OBJECTIVES

The trial has the following objectives:

- To assess the safety and immunogenicity of the standard formulation of the RTS,S vaccine (RTS,S/AS01E) in Thai adults (Group 3 alone).
- To assess the safety and immunogenicity of the adult formulation of the RTS,S vaccine (RTS,S/AS01B) in Thai adults (Group 1 alone).
- To compare the safety and immunogenicity of a fractional dose RTS,S regime (where the third dose is a 1/5th of the standard dose) using the adult formulation (RTS,S/AS01B) to the safety and immunogenicity of fractional dose RTS,S regimes using either single or double doses of the standard formulation (RTS,S/AS01E), in Thai adults (Group 1 vs Group 2 and Group 1 vs Group 5).
- To compare the safety and immunogenicity of fractional dose regime (where the third dose is a 1/5th of the standard dose) using a single dose of the standard formulation (RTS,S/AS01E) to the safety and immunogenicity of fractional dose RTS,S regime using a double dose of the standard formulation (RTS,S/AS01E), in Thai adults (Group 2 vs Group 5).
- To compare the serologic response to RTS,S/AS01E vaccine when co-administered with DHA-PIP + SLD-PQ with RTS,S/AS01E vaccine alone in adults, when RTS,S/AS01E is given in either a fractional dose regime (where the third dose is a 1/5th of the standard dose) or a full standard dose regime (Group 3 vs. Group 4 and Group 5 vs Group 6).
- To compare the serologic response to RTS,S/AS01E vaccine co-administered with DHA-PIP + SLD-PQ, when RTS,S/AS01E is given in a fractional two-dose regimen (Month 0, standard dose and Month 2, fractional (1/5th) dose) with RTS,S/AS01E vaccine co-administered with DHA-PIP + SLD-PQ, when RTS,S/AS01E is given in a fractional three-dose regimen (Month 0 and Month 1, standard doses and Month 2, fractional (1/5th) dose) (Group 6 vs Group 7)

4 TRIAL DESIGN

4.1 Summary of Trial Design

This is a randomized, open-label, single centre, Phase 2 trial of RTS,S/AS01 in healthy Thai adults.

4.2 Primary Outcome Measures

The primary outcome measures will be:

- The safety of the investigational vaccine in each group:
  - Occurrence of serious adverse events (SAEs) from the date of the first vaccination to 29 days after the last vaccination, according to the MedRA classification.
  - Occurrence of SAEs during the whole study period, i.e. during a 6 month follow up period from the receipt of first vaccination, according to the MedRA classification.

- For groups 1 to 6, the concentration of antibodies against *Plasmodium falciparum* circumsporozoite (anti-CS antibody), one month after the first dose (at Study month 1), one month after the second dose (at Study month 2), one month after the third dose (at Study Month 3) and six months after the first dose (at Study Month 6).
- For group 7, the concentration of antibodies against *Plasmodium falciparum* circumsporozoite (anti-CS antibody), one month after the first dose (at Study month 1), two months after the first dose (at Study month 2), one month after the second dose (at Study month 3), and six months after the first dose (at Study Month 6).

4.3 Trial Participants

4.3.1 Overall Description of Trial Participants

190 healthy, non-pregnant Thai adults (aged 18 to 55 years, inclusive) will be recruited into the study.

4.3.2 Inclusion Criteria

The participant may enter the study if all of the following apply:

- Participant is a healthy male or non-pregnant female, aged 18 to 55 years (inclusive), of Thai origin.
- Participant is willing and able to give informed consent to participate in the trial
- Able, in the investigators opinion, and willing to comply with the study requirements and follow-up.

4.3.3 Exclusion Criteria

The following criteria should be checked at the time of study entry. If ANY exclusion criterion applies, the subject must not be included in the study:

- Female participant who is pregnant, lactating or planning pregnancy during the course of the study.
- Presence of any condition which in the judgment of the investigator would place the participant at undue risk or interfere with the results of the study (e.g. serious underlying cardiac, renal, hepatic or neurological disease; severe malnutrition; congenital defects or febrile condition).
- Hepatitis B surface antigen (HBsAg) detected in serum.
- Screening ECG demonstrates a QTc interval ≥ 450 ms

- Seropositive for hepatitis C virus (antibodies to HCV) at screening (unless has taken part in a prior hepatitis C vaccine study with confirmed negative HCV antibodies prior to participation in that study, and negative HCV RNA PCR at screening for this study).
- Anaemia (Hb < 10 g/dL)
- Positive malaria parasitaemia at screening or baseline (Month 0, Day 0).
- Use of any investigational or non-registered product or investigational use of a registered product (drug or vaccine), other than the study vaccines, during the period from the date of screening to the first vaccination, or planned use during the study period.
- Any medical condition that in the judgment of the investigator would make intramuscular (IM) injection unsafe.
- Any medical condition that in the judgment of the investigator would make the administration of the antimalarial treatment unsafe, such as prior allergic reactions to one or more component of the drug regimen: artemisinins, piperaquine or primaquine.
- Contraindications to the use of artemisinins, piperaquine or primaquine or use of medications known to have a potentially clinically significant interaction with these medications.
- Chronic administration (defined as more than 14 days in total) of immunosuppressants or other immune-modifying drugs during the period starting six months prior to the first vaccine dose. For corticosteroids, this will mean prednisone > 20 mg/day (for adult subjects), or equivalent. Inhaled and topical steroids are allowed.
- Planned administration/administration of a vaccine not foreseen by the study protocol in the period starting seven days before the first dose.
- Concurrently participating in another clinical study, at any time during the study period, in which the subject has been or will be exposed to an investigational or a non-investigational vaccine/product.
- History of splenectomy.
- Any confirmed or suspected immunosuppressive or immunodeficient condition, including HIV infection or based on medical history and physical examination.
- History of anaphylaxis post-vaccination.
- Serious chronic illness.
- Any abnormal baseline laboratory screening tests: ALT, AST, creatinine, haemoglobin, platelet count, total WBC, out of normal range as defined in the protocol.
- Hepatomegaly, right upper quadrant abdominal pain or tenderness.
- Personal history of autoimmune disease.
- Administration of immunoglobulins and/or any blood products during the period starting three months before the first dose of study vaccine or planned administration during the study period.

4.4 Indications for deferral of vaccination

The following events constitute contraindications to administration of RTS,S/AS01 at that point in time. If any one of these adverse events (AEs) occurs at the time scheduled for vaccination, the participant may be vaccinated at a later date, or withdrawn at the discretion of the investigator.

- Acute disease at the time of vaccination (acute disease is defined as the presence of a moderate or severe illness with or without fever). All vaccines can be administered to persons with a minor illness such as diarrhoea, mild upper respiratory infection with or without low-grade febrile illness, i.e. Axillary temperature <37.5°C).
- Axillary temperature of ≥ 37.5°C at time of vaccination.
- Administration of a vaccine not foreseen by the study protocol within 30 days of any dose of RTS,S/AS01 with the exception of vaccines against polio, diphtheria, tetanus, pertussis, hepatitis B, *Haemophilus influenzae* type b, BCG, measles, influenza, pneumococcal disease or yellow fever which may not be given within one week of vaccination.

4.5 Absolute contraindications to further vaccination

The following AEs constitute absolute contraindications to further administration of RTS,S/AS01. If any of these AEs occur during the study, the participant must not receive additional doses of RTS,S/AS01, but may continue other study procedures at the discretion of the investigator. It is expected that the participant would continue full safety monitoring procedures, as per protocol.

- Acute allergic reaction or anaphylactic shock following the administration of vaccine investigational product.
- Pregnancy
- Any other findings that the investigator feels would increase the risk of having an adverse outcome from participation in the trial.

4.6 Expenses and Benefits

Reasonable compensation (e.g. for transport, parking, and lost working time) for any study visits will be reimbursed as appropriate. Subjects will receive compensation of 1,000 Baht for each scheduled study visit.

	group 1, 2, 3, 5	group 4, 6	group 7
screening	1,000	1,000	1,000
vaccination	3,000	3,000	2,000
antimalarials day 2 and 3		6,000	4,000
follow up	5,000	5,000	4,000

total	9,000	15,000	11,000

No direct benefit is foreseen from study participation. Meals to be provided to study participants at some study visits might be considered as a direct benefit and other possible indirect benefits may be possible since subjects will be screened for human immunodeficiency virus (HIV), hepatitis B and C and will receive a medical check-up. Knowledge gained from this study is may assist with the development of a vaccine against *P.falciparum* malaria which could potentially be used to help stop the spread of malaria and anti-malarial resistance in the region.

4.7 Potential Risks for Study Participants

4.7.1 Phlebotomy

There may be minor bruising, local tenderness or pre-syncopal symptoms associated with venepuncture, which will not be documented as AEs if they occur.

4.7.2 RTS,S/AS01 vaccination

The most frequent adverse reactions observed in previous clinical trials using the RTS,S antigen and AS01 adjuvant system include pain, swelling, erythema, and tenderness at the site of injection, and systemic symptoms such as low-grade fever and short-term flu-like symptoms: fatigue, myalgia, headache, malaise. As with any vaccine, unexpected serious adverse events, including severe allergic reactions to the vaccine components, may occur, although this has not been reported in prior studies with RTS,S or the adjuvant in adults. In order to mitigate this risk, volunteers will be vaccinated in a clinical area where Advanced Life Support trained physicians, equipment and drugs are immediately available for the management of any immediate post-vaccination serious adverse reactions. Subjects will be observed closely for at least 30 minutes following administration of the vaccine.

Febrile seizures assessed as related to RTS,S/AS01 vaccination have been reported in children, but not in adults.

In the large Phase III study of RTS,S in infants and children in sub-Sahara Africa, an imbalance of meningitis cases of any aetiology (i.e. including cases with confirmed aetiology and cases with no aetiology found), with no cluster in time-to-onset, has been observed in children 5-17 months of age at first dose. 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. Some individuals vaccinated with adjuvant components identical to those that will be used in this study have reported autoimmune diseases (AID). A causal association between the adjuvant components and occurrence of autoimmune diseases has however not been established, as these can occur in people

who get other vaccines, or no vaccines at all. A meta-analysis of a GSK Vaccines adjuvanted vaccine showed no increased risk of AID associated to the adjuvant.
Meningitis and pIMDs are adverse events (AE) of specific interest.

4.7.3 Antimalarial medications

Dihydroartemisinin/piperaquine (DHA-PIP)

DHA-PIP is the first line treatment for uncomplicated malaria in many malaria endemic countries in Asia and Africa and has an extraordinary robust safety profile [11-34]. DHA-PIP is also very well tolerated. The main reported side effects are gastrointestinal upset (nausea, vomiting, abdominal pain and diarrhoea) as well as dizziness, headache and disturbed sleep. Rates of these side effects are generally < 10% and often <5% except for dizziness (~12%). There is no evidence that DHA-PIP can cause clinically significant QTc prolongation at therapeutic doses. To exclude the possibility that pre-existing QTc prolongation results in AEs, the QTc of all volunteers will be measured and candidates with a QTc≥450ms will not be enrolled.

Primaquine (PQ)

Like all 8-aminoquinolones, primaquine is associated with haemolysis in G6PD deficient individuals in a dose-related relationship. To avoid any potential risk for haemolysis, participants will receive just a single low dose of primaquine (approximately 0.25mg/kg) on the day of vaccination, at each vaccine dose (groups 4, 6, and 7, only). This dose is safe even when administered to individuals with a genotype associated with severe G6PD deficiency. Other known side effects with primaquine administration include symptoms of gastritis and enteric complaints when administered for 14 days at large doses for the treatment of *Plasmodium vivax* malaria. Such abdominal complaints have not been observed when a single low dose primaquine is administered[35, 36].

Dosing Schedule

	Number of tablets	
Bodyweight in kilograms	(1 tablet contains 40mg DHA and 320 mg PIP)	(1 tablet contains 15mg primaquine)
41 - 50	2.5	0.5
51 - 60	3	1.0
61 - 70	3.5	1.0
71 - 84	4	1.5
85 - 100	5	2.0

4.8 Study Procedures

Procedures will be performed at the time points indicated in the schedule of procedures (Appendix A). Additional procedures or laboratory tests may be performed, at the discretion of the investigators if clinically necessary (e.g. follow up repeat haematology or biochemistry laboratory tests in the event of clinically significant abnormal results).

4.8.1 Recruitment of volunteers

Volunteers will be recruited using methods traditionally employed by the healthy volunteer unit of the Mahidol-Oxford Tropical Medicine Research Unit. The volunteer unit maintains a register of candidates who expressed interest in the past to participate in clinical trials. In addition volunteers will be recruited via flyers displayed and distributed in the University Hospital and among students attending the university.

4.8.2 Informed Consent

The informed consent process: trained study personnel will explain the purpose of the study according to the information sheet prior to any study related procedures being undertaken. The participant must personally sign and date the latest approved version of the informed consent form before any study specific procedures are performed. In such cases where the participant is illiterate, informed consent will be conducted in the presence of an impartial witness and the impartial witness would sign and date the informed consent form, while the participant provides their thumbprint.

The information sheet and informed consent form will be explained to the participants detailing no less than: the exact nature of the study; the implications and constraints of the protocol; the known side effects and any risks involved in taking part. The aims of the study and all tests to be carried out will be explained. It will be clearly stated that participation is entirely voluntary and that refusing to participate will not involve any penalty or affect the patients' right to receive standard medical care at the healthcare post. It will also be emphasized that if they do consent to participate and are enrolled, that they are free to withdraw from the study at any time, for any reason, without any penalty or prejudice to future care, and with no obligation to give the reason for withdrawal. The volunteers will have the opportunity to question the Investigator, or other independent parties to decide whether or not they will participate in the study.

Written informed consent will then be obtained by means of participant dated signature or thumbprint and witness dated signature, in the case of an illiterate participant, and dated signature of the person who presented and obtained the informed consent. The person who obtained the

consent must be suitably qualified and experienced, and have been authorized to do so by the study site Principal Investigator. A copy of the signed Informed Consent and Information Sheet will be given to the participants. The original signed form will be kept in the investigator's site file and retained at the study site.

Hospital consent for HIV test will be obtained prior to counselling and HIV test. Pre and post counselling for HIV screening, report and treatment for volunteer will be conducted with the support of certified staff from the Bangkok Hospital of Tropical Diseases. In case a participant is found to be HIV positive, follow up measures including but not limited to providing counselling and treatment according to standard hospital procedure will be arranged through certified staff from the Hospital for Tropical Diseases.

4.8.3 Screening and eligibility assessment (Screening visit)

All potential volunteers will have a screening visit, which may take place up to 30 days prior to enrolment. Once informed consent is given, a screening number will be assigned in sequential order. Screening numbers will be issued consecutively (e.g. RTS-SC-001, RTS-SC-002, RTS-SC-003,…). This will be recorded in the Case Screening Form, together with the demographic information and procedures performed to determine eligibility. Screening procedures indicated in the schedule of procedures (Appendix A) will be undertaken. If a screening haematology or biochemistry test result is deemed clinically significant it may be repeated to ensure it is not a single occurrence. If an abnormal finding at screening is deemed to be clinically significant, the volunteer will be informed and appropriate medical care arranged with the permission of the volunteer. In the unlikely event that a volunteer is found to have malaria or anaemia the volunteer will be treated according to national guidelines and will be excluded from participating in the study.

4.8.4 Enrolment, baseline assessment, regimen allocation, and first vaccination (Month 0 / Day 0 visit; Baseline visit)

All inclusion and exclusion criteria will be checked before enrolment in the study. Physical examination will be performed. Any new medical issues or symptoms that have arisen will be assessed. Blood will be collected for baseline parasite microscopy, haemoglobin and biochemistry. Participants with parasitaemia or anaemia will be treated according to national guidelines. Blood will be collected and stored for measurement of antibodies against *P. falciparum* circumsporozoite (anti-CS antibody) until shipment to the reference laboratory. Urine will be collected from women of child-bearing age for immediate pregnancy test.
If interval between screening and enrolment is three days or less, the screening test results (parasite microscopy, haemoglobin, biochemistry, and urine pregnancy test) can be used for

enrolment evaluation. In such cases, these tests (parasite microscopy, haemoglobin, biochemistry, and urine pregnancy test) would not need to be repeated at the baseline (Month 0 / Day 0) visit.
If all inclusion criteria are fulfilled and none of the exclusion criteria apply, the patient will be enrolled into the study and a CRF specific to each participant completed. Regimen allocation and administration of the vaccine(s) will be on Day 0. The randomization lists will be prepared by MORU.

Randomization numbers will be generated in blocks, for the 7 intervention arms in a ratio of 20:20:30:30:30:30:30, as follows:

- RTS,S/AS01B Fractional dose group (Group 1)
- Double RTS,S /AS01E Fractional dose group (Group 2)
- RTS,S/AS01E Standard dose group (Group 3)
- RTS,S/AS01E + DHA-PIP+PQ Standard dose group (Group 4)
- RTS,S/AS01E Fractional dose group (Group 5)
- RTS,S/AS01E + DHA-PIP+PQ Fractional dose group (Group 6)
- RTS,S/AS01E + DHA-PIP+PQ Fractional two-dose group (Group 7)

Study participants will be assigned the next available randomization number on the list, and thus will be randomly allocated to Group 1, 2, 3, 4, 5, 6 or 7. This is an open-label study and participants and clinical investigators will not be blinded to group allocation.
Subjects will then be vaccinated by intramuscular (IM) needle injection into the deltoid region of the arm. Subjects in Groups 4, 6 and 7 will also receive anti-malarial medications.
The study participants will be observed closely for at least 30 minutes following the administration of each study vaccine, with appropriate medical treatment readily available in case of an anaphylactic reaction.
An oral thermometer, tape measure and a paper diary card for solicited AEs will be given to each participant along with the emergency 24 hour telephone number to contact the on call study physician if needed. Diary cards will collect information on the timing and severity of the following solicited AEs:

Local solicited AEs	**Systemic solicited AEs**
Pain at injection site	Fever
Redness at injection site	Gastrointestinal symptoms (nausea, vomiting, diarrhoea and/or abdominal pain)
Swelling at injection site	Fatigue
	Headache

Participants will be instructed on how to self-assess the severity of these AEs. There will also be space on the diary card to self-document unsolicited AEs, and whether medication was taken to relieve the symptoms.

4.8.5 Subsequent vaccination visits (Month 1 / Day 0 and Month 2 / Day 0 visits)

Subsequent vaccination visits will be done according to the schedule of procedures (Appendix A). Physical examination will be performed. Any new medical issues or symptoms that have arisen will be assessed. Blood will be collected for parasite microscopy. Participants with parasitaemia or anaemia will be treated according to national guidelines. Blood will be collected and stored for measurement of antibodies against *P. falciparum* circumsporozoite (anti-CS antibody) until shipment to the reference laboratory. Urine will be collected from women of child-bearing age for immediate pregnancy test.

Before vaccination, the on-going eligibility of the volunteer will be reviewed. All participants will attend the clinic for vaccination visits, will be observed closely for at least 30 minutes following the administration of each study vaccine, and will receive a paper diary card for recording solicited AEs, as described above. Information will be recorded in the CRF for subsequent vaccination visits.

4.8.6 Follow up assessments

Follow-up assessments (visits not involving vaccination) will be done according to the schedule of procedures (Appendix A). Information will be recorded in the CRF for follow-up visits. All participants will be reviewed according to the schedule, either by attending the clinic or being visited at home by a health visitor.

Diary cards will be reviewed for details of solicited and unsolicited AEs, and any new or undocumented medical issues or symptoms that have arisen will also be assessed and recorded in the adverse event CRF. Further clinical assessment in the form of detailed history and physical examination will be undertaken if appropriate.

4.8.7 Blood tests

Blood will be drawn at the time points indicated in the schedule of procedures (Appendix A) and the following laboratory assays performed:

At screening:

- Haematology: haemoglobin [Hb], leukocytes [white blood cells; WBC] and platelets [PLT].
- Biochemistry: alanine aminotransferase [ALT], aspartate aminotransferase [AST] and creatinine [CREA].

- Diagnostic serology: HBsAg, HCV antibodies, HIV antibodies (Counselling will be given prior to testing blood for these blood-borne viruses)
- Parasite microscopy*

At M0, D0: Parasite microscopy*, Haematology: Hb, WBC, PLT, Biochemistry: ALT, AST, CREA, anti-CS antibody; Pharmacokinetics** (piperaquine and primaquine drug levels will be assessed if additional data are required)
At M0, D1: Pharmacokinetics** (piperaquine and primaquine drug levels)
At M0, D2: Pharmacokinetics** (piperaquine drug levels)
At M0, D7: Haematology: Hb, WBC, PLT; Biochemistry: ALT, AST, CREA; Pharmacokinetics** (piperaquine drug levels)
At M1, D0: Parasite microscopy*, anti-CS antibody; Pharmacokinetics** (piperaquine drug levels)
At M1, D1: Pharmacokinetics** (piperaquine and primaquine drug levels)
At M1, D2: Pharmacokinetics** (piperaquine drug levels)
At M1, D7: Haematology: Hb, WBC, PLT; Biochemistry: ALT, AST, CREA; Pharmacokinetics** (piperaquine drug levels)
At M2, D0: Parasite microscopy*, anti-CS antibody; Pharmacokinetics** (piperaquine drug levels)
At M2, D1: Pharmacokinetics** (piperaquine and primaquine drug levels)
At M2, D2: Pharmacokinetics** (piperaquine drug levels)
At M2, D7: Haematology: Hb, WBC, PLT; Biochemistry: ALT, AST, CREA; Pharmacokinetics** (piperaquine drug levels)
At M3: Parasite microscopy*, Haematology: Hb, WBC, PLT; Biochemistry: ALT, AST, CREA; anti-CS antibody
At M6: Parasite microscopy*, anti-CS antibody

* In the unexpected event that volunteers are found to be parasitaemic during screening, baseline or during vaccination or follow-up visits they will be treated according to the most up-to-date government guidelines. If at screening or baseline, these volunteers will be excluded from participation in the study. If at Month 1 or Month 2 vaccination days, these volunteers will be withdrawn from further study vaccination.
** Samples for pharmacokinetics will only be taken for subjects in Groups 4 and 6. Additional drug level assessment for Primaquine will be done if additional data are required

Samples collected for screening assays including haematology, biochemistry, diagnostic serology and parasite microscopy will be tested at Bangkok Hospital for Tropical Disease Laboratory, Faculty of Tropical Medicine, Mahidol University.

Samples collected for drug levels assessment (piperaquine and primaquine) will be tested at the Department of Clinical Pharmacology Laboratories, Faculty of Tropical Medicine, Mahidol University.

Samples collected for assessment of antibody responses will be processed at the site and serum samples will be stored at -20C or below until shipment to a biorepository sample management laboratory (Precision) and will then be shipped to the Walter Reed Army Institute of Research (WRAIR) for testing. The following assays will be conducted at the WRAIR laboratories in Silver Spring, United States:

Component	Method	Kit / Manufacturer	Unit	Cut-off	Laboratory
Plasmodium falciparum. Circumsporozoite Protein.(NANP)6 Ab.IgG	ELISA	In house	EPT or ug/ml	100	WRAIR
Plasmodium falciparum. Circumsporozoide Protein.(NANP)6 Ab.IgG Avidity	ELISA	In house	Avidity index	Not applicable	WRAIR
Plasmodium falciparum. anti-C-Term Circumsporozoide Ab.IgG	ELISA	In house	EPT	100	WRAIR
Plasmodium falciparum. anti-C-Term Circumsporozoide Ab.IgG avidity	ELISA	In house	Avidity Index	Not applicable	WRAIR
Plasmodium falciparum. Circumsporozoite Full length (N+C-Terminal)	ELISA	In house	EPT	100	WRAIR
P falciparum. anti-full length CSP Ab.IgG avidity	ELISA	In house	Avidity Index	Not applicable	WRAIR

ELISA: Enzyme-linked immunosorbent assay; EPT: endpoint titer; IgG: Immunoglobulin G; WRAIR: Walter Reed Army Institute of Research

These assays allow the characterisation of B cell responses elicited by RTS,S/AS01, quantification of anti-CSP titers and avidity index (NANP repeat region, C-terminus, and full length). This will enable bridging back to earlier trials with RTS,S/AS01.

With the volunteers' informed consent, any leftover serum will be frozen for up to 5 years for future immunological analysis of malaria-specific responses. This may include human DNA and RNA analysis to search for correlates of vaccine immunogenicity and efficacy.

4.8.8 Drug levels

The study investigates two questions regarding the potential interactions between antimalarial drugs and vaccines.

1) Does the co-administration of antimalarial drugs with vaccines influence vaccine antibody levels

2) Does the co-administration of antimalarial drugs with vaccines influence drug levels

The second question will be addressed for piperaquine and primaquine. Piperaquine levels will be assessed during each round (M0, M1, and M2) on Day 0, 1, 2, and 7 (M0D0 drug level will be

assessed if additional data are required). Primaquine levels will be assessed during each round 24hrs after drug administration. Additional drug level assessment for Primaquine will be done if additional data are required.
The drug levels will be assessed in the 60 participants who receive vaccine and drugs (groups 4 and 6). The drug levels will be compared to historic controls.

4.9 Definition of Start and End of Trial

The start of the trial is defined as the date of the first vaccination of the first participant. The end of trial is the date of the last visit of the last participant, which would be a maximum of 6 months after recruitment and first vaccination of the last participant.

4.10 Participant withdrawal from the study

Each participant has the right to withdraw from the study at any time and for any reason, and is not obliged to give his or her reasons for doing so. Participants who are withdrawn because of AEs must be clearly distinguished from participants who are withdrawn for other reasons. For all withdrawals due to SAE/AEs, appropriate follow-up visits or medical care will be arranged, with the agreement of the participant, until the SAE/AE has resolved and stabilized

From an analysis perspective, a 'withdrawal' from the study is any participant who did not come back for the concluding visit or was not available for the concluding contact foreseen in the protocol. A participant qualifies as a 'withdrawal' from the study when no study procedure has occurred, no follow-up has been performed and no further information has been collected for this participant from the date of withdrawal/last contact. Investigators will make an attempt to contact those participants who do not return for scheduled visits or follow-up.

In addition participant may be withdrawn from the study by the investigator at any time, if it is in the best interests of the participant's health and well-being, or for any of the following reasons:

- Ineligibility (either arising during the study or retrospectively, having been overlooked at screening).
- Significant protocol deviation.
- Participant non-compliance with study requirements.
- An AE, which requires discontinuation of the study involvement or results in inability to continue to comply with study procedures.

If a participant withdraws from the study, blood samples collected before their withdrawal from the trial will be used/stored unless the participant specifically requests otherwise. Data collected prior to the withdrawal of a participant will be used.

In all cases of participant withdrawal or discontinuation, excepting those of complete consent withdrawal, long-term safety data collection will continue as appropriate if subjects have received one or more vaccine doses.

4.11 Participant withdrawal from the study agent

Participants withdrawn from the study agent are those who do not receive the complete number of vaccine doses. A participant withdrawn from the study agent may not necessarily be withdrawn from the study, as further procedures or follow-up may be performed (safety or immunogenicity) if planned in the study protocol. Information relative to premature discontinuation of the study agent will be documented in the CRF. The investigator will document whether the decision to discontinue further vaccination was made by the participant or the investigator and which of the following possible reasons was responsible for withdrawal:

- serious adverse event,
- non-serious adverse event,
- other (specify).

Subjects with positive microscopy result indicating malaria on vaccination days at Month 1 or Month 2 will be treated according to national guidelines and the volunteer will be withdrawn from further vaccination.

4.12 Pregnancy

Participants are informed that the safety of the vaccine in pregnancy is unknown. Female study participants are asked to use appropriate contraceptive methods to prevent pregnancy while they receive vaccinations. Appropriate contraceptive methods include:
- Established use of oral, injected or implanted hormonal contraceptives
- Intrauterine Device or Intrauterine System
- Barrier methods (condoms or diaphragm with additional spermicide)
- Male sterilisation (with appropriate post-vasectomy documentation of absence of sperm in the ejaculate)

- True abstinence, when this is in line with the preferred and usual lifestyle of the subject. Periodic abstinence (e.g., calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal are not acceptable methods of contraception.
Female participants will be tested for pregnancy immediately prior to each vaccination.
Should a participant become pregnant during the trial, she will be followed up as other volunteers and in addition will be followed until pregnancy outcome, with the participant's permission. We will not routinely perform venepuncture on such participants.

4.13 Source Data

Source documents are original documents, data, and records from which participants' CRF data are obtained. These include, but are not limited to, hospital records (from which medical history and previous and concurrent medication may be summarized into the CRF), clinical and office charts, registration logbook, laboratory and pharmacy records, diaries, radiographs, referral notes and correspondence.

All documents will be stored safely in confidential conditions. On all study-specific documents, other than the signed consent, the participant will be referred to by the study participant number/code, not by name.

5 TREATMENT OF TRIAL PARTICIPANTS

5.1 Description and administration of the study agents

5.1.1 Vaccines

The candidate RTS,S/AS01 vaccine to be used has been developed and manufactured by GSK Vaccines. The Quality Control Standards and Requirements for each candidate vaccine are described in separate Quality Assurance documents (e.g. release protocols, certificate of analysis) and the required approvals have been obtained. The vaccines are labelled and packed according to applicable regulatory requirements.

RTS,S/AS01E

RTS,S/AS01E is the standard formulation of the vaccine. The RTS,S antigen is presented as a lyophilized pellet with sucrose as cryoprotectant, which is reconstituted in the liquid adjuvant, AS01E. Once prepared, a standard dose of RTS,S/AS01E contains approximately 25µg of RTS,S antigen, in 0.5mL of liquid adjuvant containing 25µg MPL and 25µg QS-21. In this

study, the commercial presentation of RTS,S/AS01E will be used, i.e. a two-doses glass vial of lyophilized RTS,S antigen (50µg) to be reconstituted with a two-doses glass vial of AS01E 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 withdrawn to administer RTS,S/AS01E standard doses, or
- 0.1 ml will be withdrawn to administered RTS,S/AS01E fractional doses (1/5th dose), or
- 1.0 ml will be withdrawn to administer RTS,S/AS01E double doses, or
- 0.2 ml will be withdrawn to administered double dose of RTS,S/AS01E fractional doses (1/5th dose).

All vials of vaccine provided in this study are intended for single use only.

RTS,S/AS01B (0.5 mL dose)

RTS,S/AS01B is the adult formulation of the vaccine. The RTS,S antigen is presented as a lyophilized pellet with sucrose as cryoprotectant, which is reconstituted in the liquid adjuvant, AS01B. Once prepared, a standard dose of RTS,S/AS01B contains approximately 50µg of RTS,S antigen, in 0.5mL of liquid adjuvant containing 50µg MPL and 50µg QS-21. In this study, the clinical presentation of RTS,S/AS01B will be used, i.e. a monodose glass vial of lyophilized RTS,S antigen (50µg) to be reconstituted with a mono-dose glass vial of AS01B Adjuvant System (0.5 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 withdrawn to administer RTS,S/AS01B standard doses, or
- 0.1 ml will be withdrawn to administered RTS,S/AS01B fractional doses (1/5th dose).

All vials of vaccine provided in this study are intended for single use only.

Administration of RTS,S/AS01

The procedure for administration is as follows. Disinfect the top of vaccine vial and adjuvant vial with alcohol swabs and let dry. Withdraw the contents of the adjuvant vial in a syringe and inject adjuvant into the vial of lyophilized antigen. The pellet is then dissolved by gently shaking the vial. Wait for 1 minute to ensure complete dissolution of vial contents before withdrawing the correct volume (see above). The reconstituted vaccine should be administered by slow IM injection, using a fresh 25G needle with length of 1 inch (25 mm), preferably into the non-dominant arm. Vaccine should be injected within 4 hours of reconstitution (storage at +2°C to +8°C). The study participants will be observed closely for at least 30 minutes following the administration of study vaccine, with appropriate medical treatment readily available in case of an anaphylactic reaction.

5.1.2 Anti-malarial therapy

Participants in Groups 4 and 6 will receive three rounds of antimalarial drugs - each round starting on the day of vaccination (Day 0) at Study Months 0, 1 and 2. Participants in Group 7 will receive two rounds of antimalarial drugs – each round starting on the day of vaccination (Day 0) at Study months 0 and 2. Each round consists of three daily doses of co-formulated dihydroartemisinin/piperaquine on Day 0, 1, and 2 (i.e. the day of vaccination and each day for 2 days after). Dihydroartemisinin/piperaquine tablets (Guilin Pharmaceutical Company or Sigma Tau Pharmaceuticals) for adult patients each contain 40mg dihydroartemisinin and 320mg piperaquine with a therapeutic dose range between 2–10 mg/kg/day dihydroartemisinin and 16–26 mg/kg/dose piperaquine. In addition each participant will receive a single low dose primaquine on the day of vaccination (Day 0). One single low dose primaquine of approximately 0.25mg/kg (Thai Government Pharmaceutical Organisation) will be administered. This drug regimen has been used in mass drug administrations in more than 10,000 participants which have established that this antimalarial drug regimen is safe and effective.

In the unexpected event that volunteers are found to be infected with malaria during screening, baseline or during follow-up examination they will be treated according to the most up-to-date government guidelines.

5.2 Storage of vaccines and anti-malarial medications

The RTS,S/AS01 vaccines must be stored according to the manufacturer's recommendations at +2°C to +8°C. Any temperature deviation outside the range 0 to 8°C must be reported to the GSK Vaccines as soon as detected. Following an exposure to such a temperature deviation, vaccines will not be used until approval has been given by GSK Vaccines. In case of temperature deviation between 0 and 2°C/32 to 36°F the impacted study vaccines can still be administered, but the site must take adequate actions to go back to the defined range +2 to +8°C/36 to 46°F and avoid re-occurrence of such a temperature deviation.

Study anti-malarial medications must be stored at room temperature below 30 degrees Celsius.

All vaccines and study medications will be stored in a safe and locked place, with no access to unauthorized personnel. Only trained, authorized study staff will have access to the vaccines and study medications. Storage temperatures will be monitored daily, according to SOPs at the investigator's site. In addition, for vaccine storage refrigerator(s), an alarm system and a back-up refrigerator will be available in case of power failure/breakdown. All movements of the study vaccines will be documented and cold boxes, with temperature monitoring, will be used to transfer vaccines to the area where it will be administered. Vaccine and study medication accountability, storage, and handling will be in accordance with local SOPs.

This study will not be blinded; i.e. it is an open-label study.

6 SAFETY REPORTING

6.1 SAFETY

The investigator or site staff is/are responsible for the detection, documentation and reporting of events meeting the criteria and definition of an AE or SAE as provided in this protocol. Each subject will be instructed to contact the investigator immediately should the subject manifest any signs or symptoms they perceive as serious.

REPORTING PROCEDURES FOR SERIOUS AEs

In order to comply with current regulations on serious adverse event reporting to regulatory authorities, the event will be documented accurately and notification deadlines respected.

SAEs will be reported to a safety group (specified members of the study team) and medical monitor immediately (within 24 hours) of the investigators being aware of their occurrence. This safety group includes the Principal Investigator, who acts on behalf of the sponsor for notification of SAEs.

SAEs will also be reported to ethics committees and the regulatory authority, in accordance with reporting requirements and according to required timelines.

6.2 Safety definitions

6.3 Definition of an adverse event

An AE is any untoward medical occurrence in a clinical investigation subject, temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product. An AE can therefore be any unfavourable 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.

Examples of an AE include:

- Significant or unexpected worsening or exacerbation of the condition/indication under study.
- Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition.
- New conditions detected or diagnosed after investigational vaccines administration even though they may have been present prior to the start of the study.
- Signs, symptoms, or the clinical sequelae of a suspected interaction.
- Signs, symptoms, or the clinical sequelae of a suspected overdose of either investigational vaccines/ antimalarial medications or a concurrent medication (overdose per se should not be reported as an AE/SAE).
- Signs, symptoms temporally associated with vaccines/antimalarial drug administration.

- Pre- or post-treatment events that occur as a result of protocol-mandated procedures (i.e. invasive procedures, modification of subject's previous therapeutic regimen).

Examples of an AE DO NOT include:

- Medical or surgical procedures (e.g. endoscopy, appendectomy); the condition that leads to the procedure is an AE/SAE.
- Situations where an untoward medical occurrence did not occur (e.g. social and/or convenience admission to a hospital, admission for routine examination).
- Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen.
- Pre-existing conditions or signs and/or symptoms present in a subject prior to the first study vaccination. These events will be recorded in the medical history section of the CRF.

6.4 Definition of a serious adverse event

A SAE is any untoward medical occurrence that:

a. Results in death,
b. Is life-threatening,

   Note: The term 'life-threatening' in the definition of 'serious' refers to an event in which the subject 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.
c. Requires hospitalization or prolongation of existing hospitalization,

   Note: In general, hospitalization signifies that the subject 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 in an out-patient 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 AE should be considered serious.

   Hospitalization for elective treatment of a pre-existing condition (known or diagnosed prior to informed consent signature) that did not worsen from baseline is NOT considered an AE.
d. Results in disability/incapacity, OR

   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, diarrhoea, influenza like illness, and accidental trauma (e.g. sprained ankle) which may interfere or prevent everyday life functions but do not constitute a substantial disruption.
e. Is a congenital anomaly/birth defect in the offspring of a study subject.
f. Spontaneous pregnancy loss

g. Medical or scientific judgment 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 subject 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.

6.5 Solicited adverse events

6.6 Solicited local (injection-site) adverse events

The following local (injection-site) AEs will be solicited:

All age groups
Pain at injection site
Redness at injection site
Swelling at injection site

6.7 Solicited general adverse events

The following general AEs will be solicited:

Adult
Fatigue
Fever
Gastrointestinal symptoms †
Headache

† Gastrointestinal symptoms include nausea, vomiting, diarrhoea and/or abdominal pain.

Note: Temperature will be recorded in the evening. Should additional temperature measurements be performed at other times of day, the highest temperature will be recorded in the CRF.

6.8 Clinical laboratory parameters and other abnormal assessments qualifying as adverse events or serious adverse events

In absence of diagnosis, abnormal laboratory findings (e.g. clinical chemistry, haematology, urinalysis) or other abnormal assessments that are judged by the investigator to be clinically significant will be recorded as AE or SAE if they meet the definition of an AE or SAE. Clinically significant abnormal laboratory findings or other abnormal assessments that are present at baseline and significantly worsen following the start of the study will also be reported as AEs or SAEs.

The investigator will exercise his or her medical and scientific judgment in deciding whether an abnormal laboratory finding or other abnormal assessment is clinically significant.

6.9 Adverse events of specific interest

AEs of specific interest for safety monitoring include meningitis and potential Immune-Mediated Diseases pIMDs.

6.10 Potential immune-mediated diseases (pIMD)

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 aetiology. AEs that need to be recorded and reported as pIMDs include those listed below. However, the investigator will exercise his/her medical and scientific judgment in deciding whether other diseases have an autoimmune origin (i.e. pathophysiology involving systemic or organ-specific pathogenic autoantibodies) and should also be recorded as a pIMD.

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). • Narcolepsy	• 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 • Mixed connective tissue disorder	• Psoriasis • Vitiligo • Erythema nodosum • Autoimmune bullous skin diseases (including pemphigus, pemphigoid and dermatitis herpetiformis) • Alopecia areata • Lichen planus • Sweet's syndrome • Localised Scleroderma (Morphoea)

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	• Autoimmune hemolytic anemia • Autoimmune thrombocytopenia • Antiphospholipid syndrome • Pernicious anemia	• Autoimmune glomerulonephritis (including IgA nephropathy, glomerulonephritis rapidly progressive, membranous glomerulonephritis, membranoproliferative glomerulonephritis, and

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 aplastic anaemia • Autoimmune neutropenia • Autoimmune pancytopenia	mesangioproliferative glomerulonephritis) • Ocular autoimmune diseases (including autoimmune uveitis and autoimmune retinopathy) • Autoimmune myocarditis/cardiomyopathyS arcoidosis • 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

When there is enough evidence to make any of the above diagnoses, the AE must be reported as a pIMD. Symptoms, signs or conditions which might (or might not) represent the above diagnoses, should be recorded and reported as AEs but not as pIMDs until the final or definitive diagnosis has been determined, and alternative diagnoses have been eliminated or shown to be less likely.

6.11 Meningitis

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.

6.12 Events or outcomes not qualifying as adverse events or serious adverse events

6.13 Pregnancy

Female volunteers who are pregnant or intend to become pregnant will not be enrolled. Female subjects who become unexpectedly pregnant during of vaccination period (M0 to M2) must not receive additional doses of study vaccines but may continue other study procedures at the discretion of the investigator. While pregnancy itself is not considered an AE or SAE, any adverse pregnancy outcome or complication or elective termination of a pregnancy for medical reasons will be recorded and reported as an AE or a SAE.

The following should always be considered as SAE

- Spontaneous pregnancy loss, including:
  - spontaneous abortion, (spontaneous pregnancy loss before/at 22 weeks of gestation)
  - ectopic and molar pregnancy

- stillbirth (intrauterine death of foetus after 22 weeks of gestation).

- Any early neonatal death (i.e. death of a live born infant occurring within the first 7 days of life).
- Any congenital anomaly or birth defect identified in the offspring of a study subject (either during pregnancy, at birth or later) regardless of whether the foetus is delivered dead or alive. This includes anomalies identified by prenatal ultrasound, amniocentesis or examination of the products of conception after elective or spontaneous abortion.

Furthermore, any SAE occurring as a result of a post-study pregnancy AND considered by the investigator to be reasonably related to the investigational vaccines will be reported. While the investigator is not obligated to actively seek this information from former study participants, he/she may learn of a pregnancy through spontaneous reporting.

6.14 Detecting and recording adverse events and serious adverse events

6.15 Recording adverse events, serious adverse events and pregnancies

All AEs must be recorded into the appropriate section of the CRF, irrespective of intensity or whether or not they are considered vaccination-related.

6.16 Post-Study adverse events and serious adverse events

Investigators are not obligated to actively seek AEs or SAEs in former study participants. However, if the investigator learns of any SAE at any time after a subject has been discharged from the study, and he/she considers the event reasonably related to the investigational vaccines, the investigator will promptly notify the Study Contact for Reporting SAEs.

6.17 Active questioning to detect adverse events and serious adverse events

As a consistent method of collecting AEs, the subject should be asked a non-leading question such as:

'*Have you felt different in any way since receiving the vaccines or since the previous visit?*'

When an AE/SAE occurs, it is the responsibility of the investigator to review all documentation (e.g. hospital progress notes, laboratory and diagnostics reports) relative to the event. The investigator will attempt to establish a diagnosis pertaining to 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.

6.18 Assessment of intensity

The intensity of the following solicited AEs will be assessed as described:

Intensity scales for solicited symptoms in adults

Adults		
Adverse Event	Intensity grade	Parameter
Pain at injection site	0	None
	1	Mild: Any pain neither interfering with nor preventing normal every day activities.
	2	Moderate: Painful when limb is moved and interferes with every day activities.
	3	Severe: Significant pain at rest. Prevents normal every day activities.
Redness at injection site		Record greatest surface diameter in mm
Swelling at injection site		Record greatest surface diameter in mm
Fever*		Record temperature in °C/°F
Headache	0	Normal
	1	Mild: Headache that is easily tolerated
	2	Moderate: Headache that interferes with normal activity
	3	Severe: Headache that prevents normal activity
Fatigue	0	Normal
	1	Mild: Fatigue that is easily tolerated
	2	Moderate: Fatigue that interferes with normal activity
	3	Severe: Fatigue that prevents normal activity
Gastrointestinal symptoms (nausea, vomiting, diarrhoea and/or abdominal pain)	0	Normal
	1	Mild: Gastrointestinal symptoms that are easily tolerated
	2	Moderate: Gastrointestinal symptoms that interfere with normal activity
	3	Severe: Gastrointestinal symptoms that prevent normal activity

*Fever is defined as temperature ≥ 37.5°C / 99.5°F for oral, axillary or tympanic route, or ≥ 38.0°C / 100.4°F for rectal route. The preferred route for recording temperature in this study will be oral.

The maximum intensity of local injection site redness and swelling will be scored as follows:

0 : 0 mm

1 : > 0 to ≤ 50 mm

2 : > 50 mm to ≤ 100 mm

3 : > 100 mm

The maximum intensity of fever will be scored as follows:

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)

The investigator will assess the maximum intensity that occurred over the duration of the event for all unsolicited AEs (including SAEs) recorded during the study. The assessment will be based on the investigator's clinical judgment.

The intensity should be assigned to one of the following categories:

1 (mild) = An AE which is easily tolerated by the subject, causing minimal discomfort and not interfering with everyday activities.

2 (moderate) = An AE which is sufficiently discomforting to interfere with normal everyday activities.

3 (severe) = An AE which prevents normal, everyday activities. In adults, such an AE would, for example, prevent attendance at work/school and would necessitate the administration of corrective therapy.

An AE that is assessed as Grade 3 (severe) should not be confused with a SAE. Grade 3 is a category used for rating the intensity of an event; and both AEs and SAEs can be assessed as Grade 3.

The normal ranges and toxicity grading for laboratory safety parameters used in this study: Adverse event	Intensity grade	Intensity*
Haemoglobin (males)	Normal range	12.5 - 17.0 g/dl
	1	< 12.5 but ≥ 11.0 g/dl
	2	< 11.0 but ≥ 10.0 g/dl
	3	< 10.0 g/dl
Haemoglobin (females)	Normal range	11.5 - 15.0 g/dl
	1	< 11.5 but ≥ 10.5 g/dl
	2	< 10.5 but ≥ 9.5 g/dl
	3	< 9.5 g/dl
Increase in leukocytes (WBC)	Normal range	3200 - 10799 cells/mm^3
	1	10800 - 15000 cells/mm^3
	2	15001 - 20000 cells/mm^3
	3	> 20001 cells/mm^3
Decrease in leukocytes (WBC)	Normal range	3200 - 10800 cells/mm^3
	1	2500 - 3199 cells/mm^3
	2	1500 - 2499 cells/mm^3
	3	< 1500 cells/mm^3
Decrease in platelets	Normal	140000 - 400000 cells/mm^3
	1	125000 - 139000 cells/mm^3
	2	100000 - 124000 cells/mm^3
	3	< 100000 cells/mm^3
Alanine Aminotransferase	Normal range	Below ULN (60 U/l for males; 40 U/l for females
	1	1.1 - 2.5 x ULN
	2	2.6 – 5 x ULN
	3	> 5 x ULN
Aspartate Aminotransferase	Normal range	Below ULN (40 U/l for males; 35 U/l for females
		1.1 - 2.5 x ULN
		2.6 – 5 x ULN
		> 5 x ULN
Creatinine (males)	Normal range	0.5 - 1.39 mg/dl
	1	1.4 - 1.79 mg/dl
	2	1.8 - 2.0 mg/dl
	3	> 2.0 mg/dl
Creatinine (females)	Normal range	0.5 - 1.29 mg/dl
	1	1.3 – 1.69 mg/dl
	2	1.7 – 1.9 mg/dl
	3	>1.9 mg/dl

ULN: upper limit of normal range

The normal ranges and toxicity grading for laboratory safety parameters used in this study: Adverse event	Intensity grade	Intensity*

6.19 Assessment of causality

The investigator is obligated to assess the relationship between investigational vaccines and study antimalarial medications and the occurrence of each AE/SAE. The investigator will use clinical judgment to determine the relationship. Alternative plausible causes, such as natural history of the underlying diseases, concomitant therapy, other risk factors, and the temporal relationship of the event to the investigational vaccines and study antimalarial medications will be considered and investigated. The investigator will also consult the IB and/or Summary of Product Characteristics to determine his/her assessment.

There may be situations when a SAE has occurred and the investigator has minimal information. However, it is very important that the investigator always makes an assessment of causality. The investigator may change his/her opinion of causality in light of follow-up information and update the SAE information accordingly. The causality assessment is one of the criteria used when determining regulatory reporting requirements.

In case of concomitant administration of vaccine and study antimalarial medication, it may not be possible to determine the causal relationship of general AEs to the individual vaccine or medication administered. All solicited local (injection site) reactions will be considered causally related to vaccination. Causality of all other AEs should be assessed by the investigator using the following question:

Is there a reasonable possibility that the AE may have been caused by the investigational vaccine or study antimalarial medications?

YES : There is a reasonable possibility that the vaccines or study antimalarial medications contributed to the AE.

NO : There is no reasonable possibility that the AE is causally related to the administration of the study vaccines or study antimalarial medications. There are other, more likely causes and administration of the study vaccines and study antimalarial medications is not suspected to have contributed to the AE.

If an event meets the criteria to be determined as ‘serious’, additional examinations/tests will be performed by the investigator in order to determine ALL possible contributing factors for each SAE.

Possible contributing factors include:

- Medical history.
- Other medication.
- Protocol required procedure.
- Other procedure not required by the protocol.
- Lack of efficacy of the vaccines, if applicable.
- Erroneous administration.
- Other cause (specify).

6.20 Assessment of outcomes

The investigator will assess the outcome of all AEs (including SAEs) recorded during the study as:

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

6.21 Safety (ANALYSIS)

- Safety and reactogenicity of the investigational vaccine for each vaccination schedule:
  - Occurrence of solicited local and general AEs within seven days (day of vaccination and six subsequent days) after each vaccination.
  - Occurrence of unsolicited AEs from the date of the first vaccination to 29 days after the last vaccination, according to the Medical Dictionary for Regulatory Activities (MedDRA) classification.
  - Occurrence of SAEs (all, fatal, related to investigational vaccine) within 30 days (day of vaccination and 29 subsequent days) after each vaccination, according to the MedDRA classification.
  - Occurrence of SAEs (all, fatal, related to investigational vaccine) during the whole study period according to the MedDRA classification.
  - Occurrence of AEs and SAEs leading to withdrawal from further vaccination from Dose 1 (Day 0) up to study conclusion (Day 180), according to the MedDRA classification, for each vaccinated subjects.
  - Occurrence of pIMDs from Dose 1 (Day 0) up to study conclusion (Day 180), according to the MedDRA classification, for each vaccinated subjects.
  - Occurrence of meningitis from Dose 1 (Day 0) up to study conclusion (Day 180), according to the MedDRA classification, for each vaccinated subjects.

6.22 Analysis of safety

The percentage of subjects with at least one local AEs with at least one general AE (solicited and unsolicited) and with any AE during the 7-day or total follow-up period up to 29 days after the last vaccine dose and overall will be tabulated with exact 95% CI. The percentage of doses followed by at least one local AE (solicited and unsolicited), by at least one general AE (solicited and unsolicited) and by any AE during the 7-day or the total follow up period up to 29days after the last vaccine will be tabulated with exact 95% CI. The same computations will be done for Grade 3 AEs, for any AEs considered related to vaccination and for any Grade 3 AEs considered related to vaccination.

The percentage of subjects reporting each individual solicited local AE (any grade and Grade 3) and solicited general AE (any grade, Grade 3, any related, Grade 3 related, resulting in medically attended visit) during the 7-day follow-up period (Day 0-6) after each vaccine dose and overall will be tabulated for each group. Similarly, the percentage of doses followed by each individual solicited local and general AE will be tabulated, overall vaccination course, with exact 95% CI.

For fever, the number and percentage of subjects reporting fever by half degree (°C) cumulative increments during the first seven days (Day 0-6) after each vaccine dose and overall will be tabulated. Similar tabulations will be performed for any fever with a causal relationship to vaccination and Grade 3 (> 39.5°C) causally related fever.

The percentage of subjects reporting unsolicited AEs from the date of the first vaccine dose up to 29 days after the last vaccine dose and after the sporozoite challenge will be tabulated by group and by MedDRA preferred term with exact 95% CI. Similar tabulation will be done for Grade 3 unsolicited AEs, for any causally related unsolicited AEs and for Grade 3 causally related unsolicited AEs.

The percentage of subjects reporting SAEs and pregnancies will be described in detail.

The percentage of vaccinated subjects reporting AEs of specific interest (meningitis and pIMDs) will be described in detail.

Biochemistry (ALT, AST and creatinine) and haematological (haemoglobin, WBC and platelets) laboratory values will be presented according to toxicity grading scales and tabulated by group.

7 HOLDING RULES AND SAFETY MONITORING

7.1. No group holding rules will be pre-defined considering the evidence for a favourable safety profile of this candidate vaccine in Phase III infant evaluation.

7.2. Individual subject holding rules. Individual study participants who present at least one of the following stopping rules for individual subjects will be withdrawn from further vaccination:

a) Local reactions: upon investigator discretion.
b) Systemic solicited adverse events: The subject develops a Grade 3 systemic solicited adverse event beginning within 2 days after vaccination (day of vaccination and one subsequent day) and persisting at Grade 3 for >2 consecutive days.
c) Unsolicited adverse events: The subject has any Grade 3 adverse event considered related to vaccination, persisting at Grade 3 for >2 consecutive days.

d) The subject has a serious adverse event considered related to vaccination.
e) The subject has an acute allergic reaction or anaphylactic shock following the administration of vaccine investigational product.
f) Laboratory adverse events: The subject has any Grade 3 laboratory adverse event considered related to vaccination.

7.3. Safety Oversight

7.3.1. Medical Monitor

A Medical Monitor, representing the Sponsor will be appointed for oversight of safety in this clinical study. The Medical Monitor will be responsible for safety assessments as outlined below. The monitor will review the study prior to initiation and will be available to advise the Investigators on study-related medical issues and to act as a representative for the welfare of the subjects. The monitor does not have direct involvement in the conduct of the study and does not have other interests with any collaborating pharmaceutical firms or their competitors. All serious adverse events and any AEs that fulfil the criteria for pausing or halting will be reported to the medical monitor within 24 hours of becoming aware of the event. The monitor is responsible for the review of the safety data and communicate with the PI and/or the DSMB, as appropriate.. The Medical Monitor may also provide recommendation for continuation, modification, or termination of the study, if there is urgent need.

7.3.2. Data and Safety Monitoring Board

The DSMB will review the study prior to initiation; review the interim safety data reports; and review all SAEs at the end of the study. The Board may convene additional reviews if deemed necessary, on review of the safety data, as sent, periodically by the Medical monitor. All SAEs will be reported by the PI to the DSMB at the same time they are submitted to the IRB. The PI will notify the Board at the time pausing or halting criteria are met and obtain a recommendation concerning continuation, modification, or termination of the study. The PI will submit the written DSMB summary reports with recommendations to the IRB(s).

8 STATISTICAL ANALYSIS

8.1 Endpoints

We will assess the RTS,S recipients with regards to:

- Safety: the occurrence of AEs and SAEs (please see safety section)
- Immunogenicity: serum anti-CS antibody titre responses and anti-CS antibody avidity.

8.2 The Number of Participants

The sample size calculations are based on the objective of comparing the serologic response to RTS,S/AS01E vaccine co-administered with DHA-PIP + SLD-PQ with RTS,S/AS01E or RTS,S/AS01B vaccine alone in adults. This is a phase II trial. Sample sizes for phase II trials are usually small, as safety is still of paramount importance. Valid sample sizes for comparing sample sizes between two groups are usually obtained using exact test methods such as the Fisher's exact test power calculations. This is done using simulations as sample size formulas are not well developed. To estimate the sample size we use a four-fold increase in anti –CS antibody titre as an assumed correlate of protection. For the sample size calculation, we assume that the background rate of response in the reference groups will be 5% and the true rate of anti-CS antibody response in the vaccine groups is 80%. We wish to exclude, for the vaccine, a one-tailed lower 95% CI for the reference-vaccine group difference of lower than 30%. The study aims to detect this difference with 90% power (because the detectable difference is big) and testing at 5% significance level. With these assumptions, and using the method of Blackwelder[37] for precision-based sample size calculations, a total of 20 participants per group would be needed for arms 1 and 2 because the anticipated difference is too big. However, for arms 3 to 7 (Table 3 below), we will need more participants per group because a smaller detectable effect is anticipated. Using the Fisher's exact test power simulations, a difference in serologic response of 30% (say 65% vs 95%) gives approximately 80% power with a sample size of 30 participants in each group testing at 5% significance level. Therefore the total sample size for all the 7 arms will be 190 participants.

The size of study groups is relatively small hence it is imperative to minimise potential losses. Whenever possible an attempt will be made to replace study participates should a participant be withdrawn or choose to withdraw. The stata command for obtaining the Fishers exact test power simulations is: power two proportions 0.65 0.95, test(fisher) n1(30) n2(30). This has been performed in Stata 14.

The required sample sizes are summarized as follows:

Table 3: Sample size and vaccine vials required for each arm

Group	# participants	Vaccine	Dose M0	Dose M1	Dose M2	Antimalarial	vaccine vials required
1	20	RTS,S/AS01B	Single standard dose (0.5mL)	Single standard dose (0.5mL)	Fx dose (0.1mL)		60
2	20	RTS,S /AS01E	Double standard dose (1.0mL)	Double standard dose (1.0mL)	Double Fx dose (0.2mL)		60
3	30	RTS,S/AS01E	Single standard dose (0.5mL)	Single standard dose (0.5mL)	Single standard dose (0.5mL)		90
4	30	RTS,S/AS01E	Single standard dose (0.5mL)	Single standard dose (0.5mL)	Single standard dose (0.5mL)	DHA-PIP+PQ	90
5	30	RTS,S/AS01E	Single standard dose (0.5mL)	Single standard dose (0.5mL)	Fx dose (0.1mL)		90
6	30	RTS,S/AS01E	Single standard dose (0.5mL)	Single standard dose (0.5mL)	Fx dose (0.1mL)	DHA-PIP+PQ	90
7	30	RTS,S/AS01E	Single standard dose (0.5mL)		Fx dose (0.1mL)	DHA-PIP+PQ	60
Total	190						540

Fx dose= fractional dose

RTS,S/AS01B = adult formulation containing 50µg of RTS,S and 50µg MPL and 50µg QS-21 in the standard dose of 0.5mL.

RTS,S/AS01E = standard formulation containing 25µg of RTS,S and 25µg MPL and 25µg QS-21 in the standard dose of 0.5mL.

DHA-PIP = dihydroartemisinin-piperaquine

PQ = single low dose primaquine

Fx dose = fractional dose i.e. $1/5^{th}$ of standard dose of RTS,S/AS01B or RTS,S/AS01E

8.3 Inclusion in Analysis

All patients who received at least one dose of vaccine will be included in the safety analyses. Patients lost to follow-up before the completion of the follow-up period assessments will be censored at the last day seen.

Immunology analyses
Given the incidence of malaria in the study area, it is highly unlikely that any volunteer will become infected with any *Plasmodium* species during the trial. However, to exclude the remote possibility that a participant becomes infected we will screen participants for malaria at screening, Month 0, Month 1, Month 2, Month 3, and Month 6. The malaria screen consists of a blood film which is read immediately in the hospital laboratory. The result will be available in <30 min. Subjects positive for malaria at screening or Month 0 are excluded from participation in the study and will not be enrolled or vaccinated. Subjects positive for malaria at Month 1 or Month 2 will be withdrawn from further vaccinations. In the remote scenario that a volunteer becomes parasitaemic during the study results obtained from that point in time onward will be confounded by the immune response to the natural infection. Immunology results obtained during and after an episode of parasitaemia will be excluded from the immunology analysis. The results can be included in the safety analysis.

8.4 Overall plan

Both intention-to-treat and per-protocol analyses will be carried out.

- In the intention-to-treat analysis, every participant randomized in the study (who receive the correct or incorrect study agent, one or more doses, and complete or incomplete doses) will be analysed, except if he/she did not receive any dose of the study vaccine or if no post-randomization data was collected for this participant.

- The per-protocol analysis will compare participants according to the study agent actually received and will include only those participants who satisfied the inclusion/exclusion criteria, followed the protocol, and received three complete, correct doses. The following non-compliant participants will be excluded:
    - Participants included without meeting at least one inclusion criterion
    - Participants included despite meeting at least one exclusion criterion
    - Participants found non-compliant with the blood sampling schedule
    - Participants vaccinated with the wrong study agent (non-compliance with the randomization code)

- Participants excluded from the intention-to-treat analysis.

No interim analysis is planned.

8.5 Analysis of baseline characteristics

The demographic characteristics of enrolled participants will be compared by study group.

8.6 Analysis of immunogenicity

The serum anti-CS antibody titre and avidity responses for each treatment arm will be compared to the titre before vaccine administration as follows:

For group 1 to 6:

- Number (%) in each study group with >4-fold rise in titre from baseline to:
  - One month after first dose
  - One month after second dose
  - One month after the third dose
  - Six months after the first dose
- The geometric mean-fold rise in each study group from baseline to:
  - One month after first dose
  - One month after second dose
  - One month after the third dose
  - Six months after the first dose.

For group 7:

- Number (%) in study group with >4-fold rise in titre from baseline to:
  - One month after first dose
  - Two months after first dose
  - One month after the second dose
  - Six months after the first dose
- The geometric mean-fold rise in study group from baseline to:
  - One month after first dose
  - Two months after first dose
  - One month after the second dose
  - Six months after the first dose.

9 DIRECT ACCESS TO SOURCE DATA/DOCUMENTS

Direct access to all trial related source data/documents will be granted to authorized representatives from the sponsor, PATH Malaria Vaccine Initiative, GSK, Research Ethics Committees (RECs), and the regulatory authorities to permit trial-related monitoring, audits and inspections.

10 QUALITY CONTROL AND QUALITY ASSURANCE PROCEDURES

The study will be conducted in accordance with the current approved protocol, ICH GCP, relevant regulations and standard operating procedures. Data will be evaluated for compliance with the protocol and accuracy in relation to source documents. Following written standard operating procedures, the monitors will verify that the clinical trial is conducted and data are generated, documented and reported in compliance with the protocol.

11 ETHICS

Ethical approval will be sought prior to commencing the study through the relevant Research Ethics Committees. Indemnity for the trial will be provided by the University of Oxford. SAEs will be reported to the medical monitor, DSMB, ethics committees and the Sponsor. GCP training will be provided to all staff/investigators who have no valid training certificate prior to commencing the studies.

11.1 Declaration of Helsinki

The Investigators at each site will ensure that this study is conducted in accordance with the principles of the Declaration of Helsinki.

11.2 ICH Guidelines for Good Clinical Practice

The trial will adhere to the Research Governance policies of the University of Oxford and the ICH GCP.

11.3 Approvals

The protocol, informed consent form, participant information sheet, and other written participant information / materials will be submitted to appropriate Research Ethics Committees (RECs), and regulatory authorities for written approval. The Principal Investigator will submit and, where necessary, obtain approval from the above parties for all amendments to the original approved documents.

11.4 Participant Confidentiality

The trial staff will ensure that the participants' anonymity is maintained. The participants will be identified only by participants ID number on the CRF and in any electronic databases. All documents will be stored securely and only accessible to trial staff and authorized personnel. Only the sponsor representative, investigators, the clinical monitor, authorized individuals from the PATH Malaria Vaccine Initiative, GSK, the ethical committee(s) and the regulatory authorities will have access to the records. Data will be anonymized as soon as it is practical to do so.
With subject's consent, subject's clinical data and results from blood analyses stored in our database may be shared with PATH Malaria Vaccine Initiative, GSK, other researchers to use in the future. However, the other researchers will not be given any information that could identify the subject.

12 DATA HANDLING AND RECORD KEEPING

All study data will be stored under lock and key. The investigators will maintain and retain appropriate medical and research records and essential documents for this trial in compliance with ICH E6 GCP and regulatory and institutional requirements for the protection of confidentiality of volunteers. Only authorized, trained study staff will have access to study records. The participants will be identified by a study specific participants ID number and/or code in any database. The participants' names and any other identifying details will NOT be included in any study data electronic file.

The principal Investigator will be responsible for data management and for delegating the receiving, entering, cleaning, querying, analysing and storing all data that accrues from the study. The study staff will enter the data into the volunteers' CRFs, which will be in a paper and/or electronic format. This includes safety data, laboratory data (both clinical and immunological) and outcome data. Data will be managed and stored in MACRO® database, a GCP-compliant electronic data capture system.

The investigators will permit authorized representatives of the sponsor, ethical committee(s), regulatory agencies, authorized individuals from PATH Malaria Vaccine Initiative, GSK and the monitors to examine (and when required by applicable law, to copy) clinical records for the purposes of quality assurance reviews, audits and evaluation of the study safety and progress.

13 INSURANCE

The University of Oxford has a specialist insurance policy in place: - Newline Underwriting Management Ltd, at Lloyd's of London – which would operate in the event of any Participant suffering harm as a result of their involvement in the research.

14 PUBLICATION POLICY

All Investigators will be involved in reviewing drafts of the manuscripts, abstracts, press releases and any other publications arising from the study. Authorship will be determined in accordance with the ICMJE guidelines and other contributors will be acknowledged.

15 APPENDIX A: SUMMARY OF STUDY ARMS AND PROCEDURES

Month	Group 1 (n=20) $R_BR_Br_B$	Group 2 (n=20) $2xR_E2xR_E2xr_E$	Group 3 (n=30) $R_ER_ER_E$	Group 4 (n=30) $R_ER_ER_E$ + DHA-PIP + PQ	Group 5 (n=30) $R_ER_Er_E$	Group 6 (n=30) $R_ER_Er_E$ + DHA-PIP+ PQ	Group 7(n=30) R_Er_E + DHA-PIP + PQ
0	RTS,S/AS01B Standard dose (0.5mL)	RTS,S/AS01E Double Standard dose (1.0mL)	RTS,S/AS01E Standard dose (0.5mL)	RTS,S/AS01E Standard dose (0.5mL) + DHA-PIP+PQ	RTS,S/AS01E Standard dose (0.5mL)	RTS,S/AS01E Standard dose (0.5mL) + DHA-PIP+PQ	RTS,S/AS01E Standard dose (0.5mL) + DHA-PIP+PQ
1	RTS,S/AS01B Standard dose (0.5mL)	RTS,S/AS01E Double Standard dose (1.0mL)	RTS,S/AS01E Standard dose (0.5mL)	RTS,S/AS01E Standard dose (0.5mL) + DHA-PIP+PQ	RTS,S/AS01E Standard dose (0.5mL)	RTS,S/AS01E Standard dose (0.5mL) + DHA-PIP+PQ	No intervention in M1
2	RTS,S/AS01B 1/5 Standard dose (0.1mL)	RTS,S/AS01E Double 1/5 Standard dose (0.2mL)	RTS,S/AS01E Standard dose (0.5mL)	RTS,S/AS01E Standard dose (0.5mL) + DHA-PIP+PQ	RTS,S/AS01E 1/5 Standard dose (0.1mL)	RTS,S/AS01E 1/5 Standard dose (0.1mL) + DHA-PIP+PQ	RTS,S/AS01E 1/5 Standard dose (0.1mL) + DHA-PIP+PQ

R_B = Standard dose RTS,S/AS01B: 50µg RTS,S + standard dose AS01B (0.5ml containing 50µg MPL + 50µg QS21)

R_E = Standard dose RTS,S/AS01E: 25µg RTS,S + standard dose AS01E (0.5ml containing 25µg MPL + 25µg QS21)

r_B = Fractional dose RTS,S/AS01B ($1/5^{th}$ dose): 10µg RTS,S + 1/5 standard dose AS01B (0.1ml containing 10µg MPL + 10µg QS21)

r_E = Fractional dose RTS,S/AS01E ($1/5^{th}$ dose): 2.5µg RTS,S + 1/5 standard dose AS01E (0.1ml containing 2.5µg MPL + 2.5µg QS21)

2x = Double dose of **R_E** (i.e. 1.0ml) or **r_E** (i.e. 0.2ml), as indicated.

DHA-PIP = Dihydroartemisinin/piperaquine (for 3 days; day of vaccination and two subsequent days)

PQ = Primaquine (single low dose on day of vaccination)

Study procedures

Month		**Scr**	**M0**				**M1**				**M2**				**M3**	**M6[a]**
Day of month			**0[f]**	**1[b]**	**2[b]**	**7**	**0**	**1[b]**	**2[b]**	**7**	**0**	**1[b]**	**2[b]**	**7**	**0**	**0**
Informed Consent		x														
Inclusion / Exclusion criteria		x	x													
Vaccination			x				x*				x					
DHA/piperaquine**			x	x[b]	x[b]		x*	x[b]	x[b]		x	x[b]	x[b]			
PQ single low dose primaquine**			x				x*				x					
History of fever, current axillary temperature***		x	x	x[b]	x[b]	x	x	x[b]	x[b]	x	x	x[b]	x[b]	x	x	x
Full medical history, vital signs, physical examination		x	x				x				x					
Ask about any concomitant medication		x	x	x[b]	x[b]	x	x	x[b]	x[b]	x	x	x[b]	x[b]	x	x	x
Assessment of any adverse event(s)			x	x[b]	x[b]	x	x	x[b]	x[b]	x	x	x[b]	x[b]	x	x	x
Assess for any withdrawal criteria			x	x[b]	x[b]	x	x	x[b]	x[b]	x	x	x[b]	x[b]	x	x	x
Blood sample (mL)	Parasite microscopy****	2	2			2	0.5			2	0.5			2	2	0.5
	Haematology Hb, WBC, PLT															
	Biochemistry ALT, AST, CREA	3	3			3				3				3	3	
	HBsAg, HCV, HIV	1														
	Immunology panel [c]		10				10				10				10	10
	Piperaquine & Primaquine levels*****		1[g]	1[e]	1[d]	1[d]	1[d]	1[e]	1[d]	1[d]	1[d]	1[e]	1[d]	1[d]		
	Blood volume per visit (mL)	6	16	1	1	6	11.5	1	1	6	11.5	1	1	6	15	10.5
	Maximum cumulative blood volume (mL)															94.5
Electrocardiogram (ECG)		x														
Urine pregnancy test		x	x				X				x					

* Procedures are not applicable for study group 7 ** Only for study groups 4, 6 and 7, where applicable *** Axillary temperature will be record from D0-D7 after each vaccination	a) first day of M6 b) Visits on days 1 and 2 post-vaccination are only applicable for study groups 4, 6, and 7 in Months 0 and 2, and for study groups 4 and 6 in Month 1. c) the immunology panel consists of six assays to be processed at WRAIR:

**** Parasite microscopy for purposes of indicating whether volunteer has been exposed, and not as an assessment of protection. ***** Piperaquine and Primaquine levels for group 4 and 6 only. Additional drug level assessment for Primaquine will be done if additional data are required.	• *Plasmodium falciparum*. Circumsporozoite Protein. (NANP)6 Ab.IgG • *Plasmodium falciparum*. Circumsporozoide Protein. (NANP)6 Ab.IgG Avidity • *Plasmodium falciparum*. anti-C-Term Circumsporozoide Ab.IgG • *Plasmodium falciparum*. anti-C-Term Circumsporozoide Ab.IgG avidity • *Plasmodium falciparum*. Circumsporozoite Full length (N+C-Terminal) • *P falciparum*. anti-full length CSP Ab.IgG avidity d) Drug level only for Piperaquine e) Drug level for Piperaquine and Primaquine f) If interval between screening and M0D0 is 3 days or less, screening lab results (haematology, biochemistry, parasite microscopy, and urine pregnancy test) can be used for enrolment (M0D0) g) Drug level for Piperaquine and/or Primaquine will be assessed if additional data are required

Summary of participant visits

Group	**Number of visit**	**Visit timepoints**														
		Scr	**M0**				**M1**				**M2**				**M3**	**M6**
			D0	**D1**	**D2**	**D7**	**D0**	**D1**	**D2**	**D7**	**D0**	**D1**	**D2**	**D7**	**D0**	**D0**
1	9	x	x			x	x			x	x			x	x	x
2	9	x	x			x	x			x	x			x	x	x
3	9	x	x			x	x			x	x			x	x	x
4	15	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
5	9	x	x			x	x			x	x			x	x	x
6	15	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
7	13	x	x	x	x	x	x			x	x	x	x	x	x	x

All the study procedure will be performed by research team including research doctor, research nurse and research physician at healthy volunteer unit at Faculty of Tropical Medicine, Mahidol University.

Intervals between study visits

Interval	**Optimal length of interval**	**Allowed interval**
Screening to Month 0 / Day 0 (1st vaccination)	0 to 30 days	-
Month 0 / Day 0 (1st vaccination) to Month 1 / Day 0 (2nd vaccination)	28 days	+/ - 7 days
Month 1 / Day 0 (2nd vaccination) to Month 2 / Day 0 (3rd vaccination)	28 days	+/ - 7 days
Month 2 / Day 0 (3rd vaccination) to Month 3 / Day 0	28 days	+/ - 7 days
Month 3 / Day 0 to Month 6 / Day 0	84 days	+/ - 14 days

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