Clinical Study Protocol
Sponsor:
GlaxoSmithKline Biologicals
Rue de l'Institut 89, 1330 Rixensart, Belgium

Primary Study vaccine	• GlaxoSmithKline (GSK) Biologicals' candidate *Plasmodium falciparum* malaria vaccine RTS,S/AS01$_E$ (SB257049).
Other Study vaccine	• GlaxoSmithKline (GSK) Biologicals' candidate *Plasmodium falciparum* malaria vaccine RTS,S/AS01$_B$ (SB257049).
eTrack study number and Abbreviated Title	205081 (MALARIA-092)
Investigational New Drug (IND) number	***17337***
Date of protocol	Final Version 1: 09 March 2016
Date of protocol amendment/administrative change	Amendment 1 Final: 23 June 2016 ***Administrative change 1 Final: 19 June 2017***
Title	Efficacy, immunogenicity and safety study of GSK Biologicals' candidate malaria vaccine (SB257049) evaluating various dose schedules in a sporozoite challenge model in healthy malaria-naïve adults.
Detailed Title	A Phase IIa, open-label, controlled, mono-center study to evaluate the efficacy, immunogenicity and safety of GSK Biologicals' candidate malaria vaccines RTS,S/AS01$_E$ and RTS,S/AS01$_B$ administered as various dose schedules according to a 0, 1, 7-month or a 0, 7-month schedule in healthy malaria-naïve subjects aged 18-55 years.
Co-ordinating author	• PPD (Scientific Writer - Keyrus Biopharma contractor for GSK Biologicals)
Contributing authors	• PPD (Clinical Research and Development Lead [CRDL]) • PPD, PPD (Project Statisticians) • PPD, PPD (Study Delivery Leads) • PPD, PPD (Vaccine Supply Coordinator)

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Contributing authors (continued)	• PPD, PPD (Clinical Laboratory Sciences Leads) • PPD, PPD (Clinical Safety representatives) • PPD, PPD (Study Data Managers) • PPD (Clinical Regulatory Affairs representative) • PPD, PPD (Global Patent Representatives)

GSK Biologicals' Protocol DS v 14.1.1

(Amended 19 June 2017)

Protocol Administrative Change 1 Sponsor Signatory Approval

eTrack study number and Abbreviated Title	205081 (MALARIA-092)
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Sponsor signatory	François Roman, Clinical & Epidemiology Project Lead, DDW Vaccines
Signature	_____
Date	_____

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Protocol Administrative Change 1 Rationale

Administrative change:	Administrative change 1

Rationale/background for changes:

The Investigational New Drug (IND) number, 17337, has been added to the title page.

Q^2 Solutions, as a new partner, will manage logistical aspects of serum samples sent from site to GSK for testing. Their details have been added to APPENDIX B 'CLINICAL LABORATORIES'.

Minor errors and inconsistencies have been corrected and contributing authors updated.

Intensity grades for aspartate aminotransferase were missing in Table 29 'Toxicity grading scales for blood testing'. These have been added.

Protocol Administrative Change 1 Investigator Agreement

I agree:

- To conduct the study in compliance with this protocol, any future protocol amendments or protocol administrative changes, with the terms of the clinical trial agreement and with any other study conduct procedures and/or study conduct documents provided by GlaxoSmithKline (GSK) Biologicals.
- To assume responsibility for the proper conduct of the study at this site.
- That I am aware of, and will comply with, 'Good Clinical Practice' (GCP) and all applicable regulatory requirements.
- To ensure that all persons assisting me with the study are adequately informed about the GSK Biologicals' investigational vaccines and other study-related duties and functions as described in the protocol.
- To acquire the reference ranges for laboratory tests performed locally and, if required by local regulations, obtain the laboratory's current certification or Quality Assurance procedure manual.
- To ensure that no clinical samples (including serum samples) are retained onsite or elsewhere without the approval of GSK Biologicals and the express written informed consent of the subject and/or the subject's legally acceptable representative.
- To perform no other biological assays on the clinical samples except those described in the protocol or its amendment(s).
- To co-operate with a representative of GSK Biologicals in the monitoring process of the study and in resolution of queries about the data.
- That I have been informed that certain regulatory authorities require the sponsor to obtain and supply, as necessary, details about the investigator's ownership interest in the sponsor or the investigational vaccines, and more generally about his/her financial ties with the sponsor. GSK Biologicals will use and disclose the information solely for the purpose of complying with regulatory requirements.

Hence I:

- Agree to supply GSK Biologicals with any necessary information regarding ownership interest and financial ties (including those of my spouse and dependent children).
- Agree to promptly update this information if any relevant changes occur during the course of the study and for one year following completion of the study.
- Agree that GSK Biologicals may disclose any information it has about such ownership interests and financial ties to regulatory authorities.
- Agree to provide GSK Biologicals with an updated Curriculum Vitae and other documents required by regulatory agencies for this study.

eTrack study number and Abbreviated Title	205081 (MALARIA-092)
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Investigator name	_____
Signature	_____
Date	_____

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Sponsor Information

1. Sponsor

GlaxoSmithKline Biologicals
Rue de l'Institut 89, 1330 Rixensart, Belgium

2. Sponsor Medical Expert for the Study

Refer to the local study contact information document.

3. Sponsor Study Monitor

Refer to the local study contact information document.

4. Sponsor Study Contact for Reporting of a Serious Adverse Event

GSK Biologicals Central Back-up Study Contact for Reporting SAEs: refer to protocol Section 9.4.2.

SYNOPSIS

Detailed Title

A Phase IIa, open-label, controlled, mono-center study to evaluate the efficacy, immunogenicity and safety of GSK Biologicals' candidate malaria vaccines RTS,S/AS01$_E$ and RTS,S/AS01$_B$ administered as various dose schedules according to a 0, 1, 7-month or a 0, 7-month schedule in healthy malaria-naïve subjects aged 18-55 years.

Indication

Primary immunization against malaria disease caused by *Plasmodium falciparum (P. falciparum)*.

Rationale for the study and study design

Rationale for the study

Results from controlled human malaria infection (CHMI) studies showed a simple way to improve the efficacy of the RTS,S/AS01 vaccine to the point where it might become useful either for improved malaria control in the pediatric population or as a tool to reduce malaria transmission. Combining several interventions such as vector control, prophylactic vaccination and mass drug administration, thus targeting different stages of the parasite cycle, may prove to offer the best control of malaria transmission.

This study aims to investigate whether changes in dosing schedule are associated with increased or equivalent protection, and to evaluate the immune mechanisms associated with vaccine efficacy under varying dosing schedules.

The study aims are the following:

a. Evaluation of the role of a fractional second and third dose.

b. Evaluation of a two-dose schedule where the second dose is fractional.

c. Evaluation of the use of the pediatric formulation to vaccinate adults, when a pediatric dose is delivered to adults.

d. Evaluation of the use of the pediatric formulation to vaccinate adults, when what has been considered so far as an adult dose is delivered using the pediatric formulation (increasing the volume of administration).

e. Evaluate the impact of varying dosing schedules on immune effectors and immune correlates of protection.

The ability to use the pediatric formulation (RTS,S/AS01$_E$) for adults would be a very positive step when considering product availability and implementation feasibility of a mass vaccination program in the context of an elimination campaign, therefore study aims c) and d) are being investigated. The pediatric formulation of RTS,S/AS01 has never been used in adults, but there are indications from other programs that the AS01$_E$ Adjuvant System may not be substantially inferior to AS01$_B$ for immunogenicity [EARLY-CLINRES-002, data on file; Montoya, 2013].

Evaluation of a two-dose schedule, separated by seven months, will contribute to explore the possibility that the close repetition of doses leads to some excessive priming that may be detrimental to the immune responses. If leading to sufficient protection, a two-dose strategy may be a great advantage when considering cost and logistics aspects in the contribution to malaria control and elimination efforts.

Rationale for the study design

This study is designed to evaluate efficacy, immunogenicity and safety of various dose schedules of GSK Biologicals' candidate malaria vaccines RTS,S/AS01$_B$ (adult formulation containing 50 µg RTS,S antigen and AS01$_B$ formulation with 50 µg of MPL and 50 µg of QS-21 per 0.5 ml) and RTS,S/AS01$_E$ (pediatric formulation containing 25 µg RTS,S antigen and AS01$_E$ formulation with 25 µg of MPL and 25 µg of QS-21 per 0.5 ml) in healthy malaria-naïve subjects aged 18-55 years. The study proposed herein will include six study groups. Four of these study groups will be vaccinated according to a 0, 1, 7-month schedule and one according to a 0, 7-month schedule. The immunization schedule of reference will be the same as the one used in study MALARIA-071 (117014), i.e. RTS,S/AS01$_B$ full dose at Month 0 and Month 1 and RTS,S/AS01$_B$ 1/5th dose at Month 7. Three months after the last dose of study vaccines, all subjects will undergo a sporozoite challenge and will be compared to an infectivity control group:

- **Group AduFx** will receive RTS,S/AS01$_B$ full dose at Month 0 and Month 1 + RTS,S/AS01$_B$ fractional dose (1/5th dose) at Month 7 (immunization schedule of reference).
- **Group 2PedFx** will receive double dose of RTS,S/AS01$_E$ at Month 0 and Month 1 + double dose of RTS,S/AS01$_E$ fractional dose (1/5th dose) at Month 7.
- **Group PedFx** will receive RTS,S/AS01$_E$ full dose at Month 0 and Month 1 + RTS,S/AS01$_E$ fractional dose (1/5th dose) at Month 7.

- **Group Adu2Fx** will receive RTS,S/AS01$_B$ full dose at Month 0 + RTS,S/AS01$_B$ fractional dose (1/5th dose) at Month 1 and Month 7.
- **Group Adu1Fx** will receive RTS,S/AS01$_B$ full dose at Month 0 + RTS,S/AS01$_B$ fractional dose (1/5th dose) at Month 7.
- **Group infectivity control** will not receive any immunization but will undergo the sporozoite challenge.

The sporozoite challenge model, in the RTS,S/AS candidate vaccine development program, has demonstrated a high relevance in its ability to predict efficacy under conditions of natural exposure in malaria-endemic countries. The study design is similar to that of other past CHMI studies successfully conducted. To better discriminate levels of protection across study groups and assess durability of protection, CHMI will occur three months after the last vaccine dose rather than three weeks as in past RTS,S studies. About 150 healthy adults will be enrolled and followed up closely under controlled conditions by investigators experienced in *P. falciparum* challenge studies. The proportion of immunized participants who remain free of *P. falciparum* infection following sporozoite challenge and the delay in the pre-patent period leading to infection will be evaluated as indicators of vaccine-induced protection. Immunological investigations are planned with the intent to characterize qualitatively and quantitatively the immune response induced by the immunization regimen. This design takes into account logistical restrictions on the size of human challenge procedures.

Immunological assays will be selected for their ability to demonstrate whether the administration of a fractional vaccine dose can affect quantitatively or qualitatively the vaccine-induced immune response, especially when considering the generation of antibodies against the circumsporozoite protein of *P. falciparum* (CS) antigen, but also the hepatitis B surface antigen (HBsAg) which is present in the vaccine construct. Qualitative aspect of the immune response will be characterized as much as possible (targeting different parts of the antigen and avidity). Samples from this study may be used in future assay development or testing to better understand the immune responses underlying vaccine induced protection, responses to the vaccine components and the disease under investigation.

Objectives

Primary

Efficacy

- To assess the vaccine efficacy against the occurrence of *P. falciparum* parasitemia (defined by a positive blood slide) for each vaccination schedule (AduFx, 2PedFx, PedFx, Adu2Fx, and Adu1Fx) versus the infectivity controls.

Secondary

Efficacy

- To assess the time to onset of *P. falciparum* parasitemia (defined by a positive blood slide) for each vaccination schedule (AduFx, 2PedFx, PedFx, Adu2Fx, and Adu1Fx) versus the infectivity controls.
- To assess the occurrence of *P. falciparum* parasitemia (defined by a positive blood slide) after vaccine administration for alternative vaccination schedules (2PedFx, PedFx, Adu2Fx, and Adu1Fx) versus the immunization schedule of reference (AduFx).
- To assess the time to onset of *P. falciparum* parasitemia (defined by a positive blood slide) after vaccine administration for alternative vaccination schedules (2PedFx, PedFx, Adu2Fx, and Adu1Fx) versus the immunization schedule of reference (AduFx).

Immunogenicity

For each vaccination schedule:

- To evaluate anti-CS repeat region antibody response at specified timepoints.
- To evaluate anti-HBs immunoglobulin G (IgG) antibody response at specified timepoints.

Safety

For each vaccination schedule:

- To assess the safety (unsolicited AEs/AEs of specific interest/serious adverse events [SAEs]) and reactogenicity (solicited AEs).

Tertiary

Immunogenicity

For each vaccination schedule:

- To evaluate anti-CS repeat region IgG avidity index at specified timepoints.
- To evaluate anti-full length CS protein IgG concentrations and anti-C terminal portion of the protein (C-term) IgG concentrations at specified timepoints.
- To evaluate anti-full length CS protein and anti-C-term IgG avidity at specified timepoints.

Note: other immuno-assays evaluating the immune response targeting the CS and HBsAg might be performed.

Study design

- Experimental design: Phase IIA, open, randomized, controlled, mono-centric, single-country study with five parallel groups and one infectivity control group.
- Duration of the study: Approximately 16 months for each vaccinated subject and three months for the infectivity control subjects.
  - Epoch 001: Primary starting at Visit 1 (Screening) (Screening for vaccinated subjects) and ending at Visit 10 (Day 226).
  - Epoch 002: Challenge starting at Visit 11 (Day 286) or Visit 1b (Screening) (Screening for infectivity controls), as applicable, and ending at Visit 31 (Day 376).
- Study groups:

Synopsis Table 1 Study groups and epochs foreseen in the study

Study groups	Vaccination schedule	Number of subjects	Age (Min/Max)	Epochs	
				Epoch 001	Epoch 002
AduFx	RTS,S/AS01$_B$ full dose at Month 0, Month 1 + RTS,S/AS01$_B$ 1/5th dose at Month 7	26	18 years - 55 years	X	X
2PedFx	RTS,S/AS01$_E$ double dose at Month 0, Month 1 + double dose RTS,S/AS01$_E$ 1/5th dose at Month 7	26	18 years - 55 years	X	X
PedFx	RTS,S/AS01$_E$ full dose at Month 0, Month 1 + RTS,S/AS01$_E$ 1/5th dose at Month 7	26	18 years - 55 years	X	X
Adu2Fx	RTS,S/AS01$_B$ full dose at Month 0 + RTS,S/AS01$_B$ 1/5th dose at Month 1, Month 7	26	18 years - 55 years	X	X
Adu1Fx	RTS,S/AS01$_B$ full dose at Month 0 + RTS,S/AS01$_B$ 1/5th dose at Month 7	26	18 years - 55 years	X	X
Infectivity control	Subjects will not receive any immunization but will undergo sporozoite challenge	30*	18 years - 55 years		X

* between 20 and 30 infectivity controls will be enrolled.

Synopsis Table 2 Study groups and treatment foreseen in the study

Treatment name	Vaccine/Product name	Injectable volume	Study Groups					
			AduFx	2PedFx	PedFx	Adu2Fx	Adu1Fx	Infectivity control
RTS,S/AS01$_B$ (Full dose)	RTS,S (50 µg)	0.5 ml	X			X	X	
	AS01B		X			X	X	
RTS,S/AS01$_B$ (1/5th dose)	RTS,S (50 µg)	0.1 ml	X			X	X	
	AS01B		X			X	X	
RTS,S/AS01$_E$ (Full dose)	RTS,S (25 µg)	0.5 ml		X*	X			
	AS01E			X*	X			
RTS,S/AS01$_E$ (1/5th dose)	RTS,S (25 µg)	0.1 ml		X**	X			
	AS01E			X**	X			

* Injected volume will be 1.0 ml
** Injected volume will be 0.2 ml

- Control: active control (Group AduFx [RTS,S/AS01$_B$ full dose at Month 0 and Month 1 + RTS,S/AS01$_B$ fractional dose at Month 7] is the immunization schedule of reference) and infectivity controls for the challenge.
- Vaccination schedule are provided in Synopsis Table 1.
- Treatment allocation: randomized
- Blinding:

Synopsis Table 3 Blinding of study epochs

Study Epochs	Blinding
Epoch 001	open
Epoch 002	open

- Sampling schedule:
  - Blood samples for assessment of anti-CS and anti-HBs immune response and for serum repository will be collected before vaccine administration (Day 0), one month post-Dose 2 (Day 58), six months post-Dose 2 (Day 196), one month post-Dose 3 (Day 226), on the day of challenge (Day 286), 28 days post-challenge (Day 314), and at study end (Day 376) for subjects from **AduFx, 2PedFx, PedFx, and Adu2Fx groups** and before vaccine administration (Day 0), before Dose 2 (Day 196), one month post-Dose 2 (Day 226), on the day of challenge (Day 286), 28 days post-challenge (Day 314), and at study end (Day 376) for subjects from **Adu1Fx group**.
  - Blood samples for peripheral blood mononuclear cells (PBMC) and plasma repository will be collected before vaccine administration (Day 0), one month post-Dose 1 (Day 28), seven days post-Dose 2 (Day

35), one month post-Dose 2 (Day 58), six months post-Dose 2 (Day 196), seven days post-Dose 3 (Day 203), one month post-Dose 3 (Day 226), on the day of challenge (Day 286), and at study end (Day 376) for subjects from **AduFx, 2PedFx, PedFx, and Adu2Fx groups** and before vaccine administration (Day 0), one month post-Dose 1 (Day 28), before Dose 2 (Day 196), seven days post-Dose 2 (Day 203), one month post-Dose 2 (Day 226), on the day of challenge (Day 286), and at study end (Day 376) for subjects from **Adu1Fx group**.

- Blood samples for the evaluation of biochemistry (alanine aminotransferase [ALT], aspartate aminotransferase [AST] and creatinine) and hematology (hemoglobin, leukocytes [white blood cells; WBC] and platelets) parameters will be collected before vaccine administration (screening), seven days post-Dose 2 (Day 35), one month post-Dose 2 (Day 58), seven days post-Dose 3 (Day 203), one month post-Dose 3 (Day 226), the day of first parasitemia, and 28 days post-challenge (Day 314) for subjects from **AduFx, 2PedFx, PedFx, and Adu2Fx groups**, before vaccine administration (screening), seven days post-Dose 2 (Day 203), one month post-Dose 2 (Day 226), the day of first parasitemia, and 28 days post-challenge (Day 314) for subjects from **Adu1Fx group** and at screening, the day of first parasitemia and 28 days post-challenge (Day 314) for the **infectivity control subjects**.
- Blood samples for RNA sequencing (messenger ribonucleic acid [mRNA] sequencing analysis) will be collected before vaccine administration (Day 0), at Dose 2 (Day 28), one day post-Dose 2 (Day 29), at Dose 3 (Day 196), and one day post-Dose 3 (Day 197) for subjects from **AduFx, 2PedFx, PedFx, and Adu2Fx groups** and before vaccine administration (Day 0), one month post-Dose 1 (Day 28), at Dose 2 (Day 196) and one day post-Dose 2 (Day 197) for subjects from **Adu1Fx group**.
- Blood sample for assessment of parasitemia (PCR testing) will be collected on the day of subject entry into the hotel phase (Day 295 [Visit 16]) to provide an estimate of the number of subjects likely to develop malaria within the first few days of the hotel phase.

  - Blood sample for assessment of parasitemia (blood smear) will be collected daily for 14 days (from Day 291 [Visit 12] to Day 304 [Visit 25]) and then every two days for nine days (Day 306 [Visit 26], Day 308 [Visit 27], Day 310 [Visit 28], Day 312 [Visit 29], and Day 314 [Visit 30]). For subjects who develop malaria, blood smear may be discontinued once the subject has three consecutive negative smears (separated by more than 12 hours) following the initial treatment.
  - Blood samples for testing of human immunodeficiency virus (HIV), hepatitis C virus (HCV) and HBsAg will be collected from all subjects at screening.
  - Urinary pregnancy test (urine beta-human chorionic gonadotropin [β-HCG]) will be performed on all women at screening, before each vaccine dose for vaccinated subjects and on the day of challenge.
- Type of study: self-contained.
- Data collection: electronic Case Report Form (eCRF).
- Safety monitoring:
  - Each subject will be observed for at least 30 minutes after vaccination to evaluate and treat any acute AEs.
  - Each subject will be observed for at least 30 minutes following completion of the sporozoite challenge to evaluate and treat any acute allergic reactions.
  - All SAEs (all, fatal, related to the investigational vaccine) and pregnancies will be reported until study end (Day 376).
  - All AEs and SAEs leading to withdrawal from further vaccination will be reported until study end (Day 376) for all vaccinated subjects (AduFx, 2PedFx, PedFx, Adu2Fx, and Adu1Fx groups).
  - Meningitis and potential immune-mediated diseases (pIMDs) are AEs of specific interest to be reported until study end (Day 376) for all vaccinated subjects (AduFx, 2PedFx, PedFx, Adu2Fx, and Adu1Fx groups).
  - Solicited local and general AEs will be collected from all vaccinated subjects (AduFx, 2PedFx, PedFx, Adu2Fx, and Adu1Fx groups) during seven days (day of vaccination and six subsequent days) after each dose of study vaccine.

- Unsolicited AEs will be collected from all vaccinated subjects (AduFx, 2PedFx, PedFx, Adu2Fx, and Adu1Fx groups) during 30 days (day of vaccination and 29 subsequent days) after each dose of study vaccine.
- AEs post-challenge will be collected from all subjects (AduFx, 2PedFx, PedFx, Adu2Fx, Adu1Fx, and infectivity control groups) during 30 days (day of challenge and 29 subsequent days) after the sporozoite challenge.
- Biochemistry (ALT, AST and creatinine) and hematology (hemoglobin, WBC and platelets) parameters will be assessed as described in 'Sampling schedule'.

Case definition

Case definition of *P. falciparum* infection: asexual blood stage *P. falciparum* parasite density > 0 detected by blood slide reading.

Number of subjects

The target is to enroll approximately 150 subjects (26 subjects per vaccine group and between 20 and 30 subjects in the infectivity control group) aged 18 to 55 years at the time of first vaccination.

Endpoints

Primary

Efficacy

- Occurrence of *P. falciparum* parasitemia (defined by a positive blood slide) following sporozoite challenge, comparing RTS,S/AS01$_B$ administered as full doses at Month 0 and Month 1 and 1/5th dose at Month 7 (AduFx group) versus infectivity controls.
- Occurrence of *P. falciparum* parasitemia (defined by a positive blood slide) following sporozoite challenge, comparing RTS,S/AS01$_E$ administered as double dose at Month 0 and Month 1 and double 1/5th dose at Month 7 (2PedFx group) versus infectivity controls.
- Occurrence of *P. falciparum* parasitemia (defined by a positive blood slide) following sporozoite challenge, comparing RTS,S/AS01$_E$ administered as full doses at Month 0 and Month 1 and 1/5th dose at Month 7 (PedFx group) versus infectivity controls.
- Occurrence of *P. falciparum* parasitemia (defined by a positive blood slide) following sporozoite challenge, comparing RTS,S/AS01$_B$ administered as one full dose at Month 0 and 1/5th doses at Month 1 and Month 7 (Adu2Fx group) versus infectivity controls.

- Occurrence of *P. falciparum* parasitemia (defined by a positive blood slide) following sporozoite challenge, comparing RTS,S/AS01$_B$ administered as one full dose at Month 0 and 1/5th doses at Month 7 (Adu1Fx group) versus infectivity controls.

Secondary

Efficacy

- Occurrence of *P. falciparum* parasitemia (defined by a positive blood slide) following sporozoite challenge, comparing RTS,S/AS01$_E$ administered as double dose at Month 0 and Month 1 and double 1/5th dose at Month 7 (2PedFx group) versus immunization schedule of reference (AduFx group).
- Occurrence of *P. falciparum* parasitemia (defined by a positive blood slide) following sporozoite challenge, comparing RTS,S/AS01$_E$ administered as full doses at Month 0 and Month 1 and 1/5th dose at Month 7 (PedFx) versus immunization schedule of reference (AduFx group).
- Occurrence of *P. falciparum* parasitemia (defined by a positive blood slide) following sporozoite challenge, comparing RTS,S/AS01$_B$ administered as one full dose at Month 0 and 1/5th full dose at Month 1 and Month 7 (Adu2Fx) versus immunization schedule of reference (AduFx group).
- Occurrence of *P. falciparum* parasitemia (defined by a positive blood slide) following sporozoite challenge, comparing RTS,S/AS01$_B$ administered as one full dose at Month 0 and 1/5th full doses at Month 7 (Adu1Fx) versus immunization schedule of reference (AduFx group).
- Time to onset of *P. falciparum* parasitemia (defined by a positive blood slide) following sporozoite challenge in each vaccination schedule versus immunization schedule of reference (AduFx group).
- Time to onset of *P. falciparum* parasitemia (defined by a positive blood slide) following sporozoite challenge in each vaccination schedule versus infectivity controls.

Immunogenicity

- For each vaccination schedule, the immune response to the CS antigen of the investigational vaccine:
  - Anti-CS repeat region antibody concentrations before vaccine administration (Day 0), one month post-Dose

2 (Day 58), six months post-Dose 2 (Day 196), one month post-Dose 3 (Day 226), on the day of challenge (Day 286), 28 days post-challenge (Day 314), and at study end (Day 376) for subjects from AduFx, 2PedFx, PedFx, and Adu2Fx groups and before vaccine administration (Day 0), before Dose 2 (Day 196), one month post-Dose 2 (Day 226), on the day of challenge (Day 286), 28 days post-challenge (Day 314), and at study end (Day 376) for subjects from Adu1Fx group.

- For each vaccination schedule, the immune response to the hepatitis B antigen of the investigational vaccine:
  - Anti-HBs IgG antibody concentrations before vaccine administration (Day 0), one month post-Dose 2 (Day 58), six months post-Dose 2 (Day 196), one month post-Dose 3 (Day 226), on the day of challenge (Day 286), 28 days post-challenge (Day 314), and at study end (Day 376) for subjects from AduFx, 2PedFx, PedFx, and Adu2Fx groups and before vaccine administration (Day 0), before Dose 2 (Day 196), one month post-Dose 2 (Day 226), on the day of challenge (Day 286), 28 days post-challenge (Day 314), and at study end (Day 376) for subjects from Adu1Fx group.

Safety

- 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 within 30 days (day of vaccination and 29 subsequent days) after each vaccination, according to the Medical Dictionary for Regulatory Activities (MedDRA) classification.
  - Occurrence of AEs within 30 days (day of challenge and 29 subsequent days) after challenge, according to the 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 (from Dose 1 [Day 0] up to study conclusion [Day 376]), 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 376), according to the MedDRA classification, for each vaccinated subject.
  - Occurrence of pIMDs from Dose 1 (Day 0) up to study conclusion (Day 376), according to the MedDRA classification, for each vaccinated subject.
  - Occurrence of meningitis from Dose 1 (Day 0) up to study conclusion (Day 376), according to the MedDRA classification, for each vaccinated subject.
  - Occurrence of abnormal laboratory values at screening, Day 35, Day 58, Day 203, Day 226, the day of first parasitemia, and Day 314 for each vaccinated subject and at screening, the day of first parasitemia and Day 314 for the infectivity control subjects.
- Safety after sporozoite challenge in the infectivity control group.
  - Occurrence of AEs within 30 days (day of challenge and 29 subsequent days) after challenge, according to the MedDRA classification.
  - Occurrence of SAEs (all, fatal, related) from day of challenge (Day 286) to the end of the challenge phase (Day 314), according to the MedDRA classification.

Tertiary

Immunogenicity

- For each vaccination schedule, the immune response to the CS antigen of the investigational vaccine:
  - Anti-CS repeat region IgG avidity index before vaccine administration (Day 0), one month post-Dose 2 (Day 58), six months post-Dose 2 (Day 196), one month post-Dose 3 (Day 226), on the day of challenge (Day 286), 28 days post-challenge (Day 314), and at study end (Day 376) for subjects from AduFx, 2PedFx, PedFx, and Adu2Fx groups and before vaccine administration (Day 0), before Dose 2 (Day 196), one month post-Dose 2 (Day 226), on the day of challenge (Day 286), 28 days post-challenge (Day 314), and at study end (Day 376) for subjects from Adu1Fx group.
  - Anti-full length CS protein IgG concentrations and anti-C-term IgG concentrations before vaccine administration (Day 0), one month post-Dose 2 (Day 58), six months post-Dose 2 (Day 196), one month

post-Dose 3 (Day 226), on the day of challenge (Day 286), 28 days post-challenge (Day 314), and at study end (Day 376) for subjects from AduFx, 2PedFx, PedFx, and Adu2Fx groups and before vaccine administration (Day 0), before Dose 2 (Day 196), one month post-Dose 2 (Day 226), on the day of challenge (Day 286), 28 days post-challenge (Day 314), and at study end (Day 376) for subjects from Adu1Fx group.

– Anti-full length CS protein and anti-C-term IgG avidity before vaccine administration (Day 0), one month post-Dose 2 (Day 58), six months post-Dose 2 (Day 196), one month post-Dose 3 (Day 226), on the day of challenge (Day 286), 28 days post-challenge (Day 314), and at study end (Day 376) for subjects from AduFx, 2PedFx, PedFx, and Adu2Fx groups and before vaccine administration (Day 0), before Dose 2 (Day 196), one month post-Dose 2 (Day 226), on the day of challenge (Day 286), 28 days post-challenge (Day 314), and at study end (Day 376) for subjects from Adu1Fx group.

Note: other immuno-assays evaluating the immune response targeting the CS and HBsAg might be performed.

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LIST OF ABBREVIATIONS

012M:	Group of subjects enrolled in study MALARIA-071, who were randomized to receive three full doses of RTS,S/AS01$_B$ at Month 0, Month 1 and Month 2.
AduFx:	Subjects receiving RTS,S/AS01$_B$ full dose at Month 0, Month 1 + RTS,S/AS01$_B$ 1/5th dose at Month 7
Adu1Fx:	Subjects receiving RTS,S/AS01$_B$ full dose at Month 0 + RTS,S/AS01$_B$ 1/5th dose at Month 7
Adu2Fx:	Subjects receiving RTS,S/AS01$_B$ full dose at Month 0 + RTS,S/AS01$_B$ 1/5th dose at Month 1, Month 7
AE:	Adverse event
ALT:	Alanine aminotransferase
Anti-CS:	Antibody to the *Plasmodium falciparum* circumsporozoite (CS) repeat domain
Anti-HBs:	Antibody to the hepatitis B surface antigen
AS01$_B$:	GSK's proprietary Adjuvant System containing MPL, QS-21 and liposome (50 µg MPL and 50 µg QS-21)
AS01$_E$:	GSK's proprietary Adjuvant System containing MPL, QS-21 and liposome (25 µg MPL and 25 µg QS-21)
AST:	Aspartate aminotransferase
ATP:	According-to-protocol
β-HCG:	Beta-human chorionic gonadotropin
CEVAC:	Center for vaccinology, Ghent University, Belgium
CHMI:	Controlled human malaria infection
CI:	Confidence interval
CLIA:	Chemiluminescence enzyme immunoassay
CLS:	Clinical laboratory sciences
CS:	Circumsporozoite protein of *Plasmodium falciparum*
C-term:	CS terminal portion of the protein

DoC:	Day of challenge
eCRF:	Electronic case report form
EDD:	Estimated date of delivery
EGA:	Estimated gestational age
ELISA:	Enzyme-linked immunosorbent assay
EPT:	Endpoint titer
eTDF:	Electronic temperature excursion decision form
FDA:	Food and Drug Administration, United States of America
Fx:	Fractional
Fx017M:	Group of subjects enrolled in study MALARIA-071, who were randomized to receive two full doses of RTS,S/AS01$_B$ at Month 0 and Month 1 and a third fractional dose (1/5th dose) at Month 7.
GCP:	Good clinical practice
GMC:	Geometric mean concentration
GSK:	GlaxoSmithKline
HBsAg:	Hepatitis B surface antigen
HCV:	Hepatitis C virus
HIV:	Human immunodeficiency virus
IB:	Investigator brochure
ICF:	Informed consent form
ICH:	International conference on harmonization
IEC:	Independent ethics committee
IgG:	Immunoglobulin G
IM:	Intramuscular
IMP:	Investigational medicinal product
IND:	Investigational new drug

IRB:	Institutional review board
LMP:	Last menstrual period
MedDRA:	Medical dictionary for regulatory activities
MPL:	3-O-desacyl-4'-monophosphoryl lipid A (produced by GSK)
mRNA:	messenger ribonucleic acid
NHANES I:	National health and nutrition examination survey I
PBMC:	Peripheral blood mononuclear cells
PCR:	Polymerase chain reaction
PedFx:	Subjects receiving RTS,S/AS01$_E$ full dose at Month 0, Month 1 + RTS,S/AS01$_E$ 1/5th dose at Month 7
2PedFx:	Subjects receiving RTS,S/AS01$_E$ double dose at Month 0, Month 1 + double dose RTS,S/AS01$_E$ 1/5th dose at Month 7
***P. falciparum*:**	*Plasmodium falciparum*
pIMD:	Potential immune-mediated disease
QS-21:	(*Quillaja saponaria* Molina, fraction 21) (Licensed by GSK from Antigenics Inc, a wholly owned subsidiary of Agenus Inc., a Delaware, USA corporation)
RTS,S:	Particulate antigen, containing both RTS and S (hepatitis B surface antigen) proteins
RTS,S/AS01$_B$:	GSK Biologicals' candidate *Plasmodium falciparum* malaria vaccine adjuvanted with GSK Biologicals' proprietary Adjuvant System AS01$_B$
RTS,S/AS01$_E$:	GSK Biologicals' candidate *Plasmodium falciparum* malaria vaccine adjuvanted with GSK Biologicals' proprietary Adjuvant System AS01$_E$
SAE:	Serious adverse event
SBIR:	Randomization system on internet
SD:	Standard deviation

SDV:	Source document verification
SOP:	Standard operating procedure
SPM:	Study procedures manual
TVC:	Total vaccinated cohort
ULN:	Upper limit of normal range
WBC:	White blood cells
WHB:	Whole blood
WRAIR:	Walter Reed Army Institute of Research

GLOSSARY OF TERMS

Adequate contraception: Adequate contraception is defined as a contraceptive method with failure rate of less than 1% per year when used consistently and correctly and when applicable, in accordance with the product label for example:

- abstinence from penile-vaginal intercourse, when this is their preferred and usual lifestyle,
- Oral contraceptives , either combined or progestogen alone,
- injectable progestogen,
- implants of etenogestrel or levonorgestrel,
- estrogenic vaginal ring,
- percutaneous contraceptive patches,
- intrauterine device or intrauterine system,
- male partner sterilization prior to the female subject's entry into the study, and this male is the sole partner for that subject,

  The information on the male sterility can come from the site personnel's review of the subject's medical records; or interview with the subject on her medical history.
- male condom combined with a vaginal spermicide (foam, gel, film, cream or suppository
- male condom combined with a female diaphragm, either with or without a vaginal spermicide (foam, gel, film, cream, or suppository).

Adequate contraception does not apply to subjects of child bearing potential with same sex partners, when this is their preferred and usual lifestyle.

Adverse event: Any untoward medical occurrence in a patient or clinical investigation subject, temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

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

Blinding:	A procedure in which one or more parties to the trial are kept unaware of the treatment assignment in order to reduce the risk of biased study outcomes. The level of blinding is maintained throughout the conduct of the trial, and only when the data are cleaned to an acceptable level of quality will appropriate personnel be unblinded or when required in case of a serious adverse event. In an open-label study, no blind is used. Both the investigator and the subject know the identity of the treatment assigned.
Eligible:	Qualified for enrolment into the study based upon strict adherence to inclusion/exclusion criteria.
Electronic case report form:	Auditable electronic record designed to capture information required by the clinical trial protocol to be reported to the sponsor on each trial subject.
Epoch:	An epoch is a self-contained set of consecutive timepoints or a single timepoint from a single protocol. Self-contained means that data collected for all subjects at all timepoints within that epoch allows to draw a complete conclusion to define or precise the targeted label of the product (e.g. primary, booster, yearly immunogenicity follow-ups, and surveillance periods for efficacy or safety).
eTrack:	GSK's tracking tool for clinical trials.
Evaluable:	Meeting all eligibility criteria, complying with the procedures defined in the protocol, and, therefore, included in the according-to-protocol (ATP) analysis.
Immunological correlate of protection:	The defined immune response above which there is a high likelihood of protection in the absence of any host factors that might increase susceptibility to the infectious agent.
Investigational vaccine: **(Synonym of Investigational Medicinal Product)**	A pharmaceutical form of an active ingredient or placebo being tested or used as a reference in a clinical trial, including a product with a marketing authorization when used in a way different from the approved form, or when used for an unapproved indication, or when used to gain further information about an approved use.
Menarche:	Menarche is the onset of menses for the first time in a young female and is preceded by several changes associated with puberty including breast development and pubic hair growth. Menarche usually occurs within 1-2 years of breast development, thelarche. However, a young

	female can become pregnant before her first menses. Thus, a conservative definition of non-childbearing potential in a pre-menarcheal female is a young female who has not yet entered puberty as evidenced by lack of breast development (palpable glandular breast tissue).
Menopause:	Menopause is the age associated with complete cessation of menstrual cycles, menses, and implies the loss of reproductive potential by ovarian failure. A practical definition accepts menopause after 1 year without menses with an appropriate clinical profile at the appropriate age e.g. > 45 years.
Potential Immune-Mediated Disease:	Potential immune-mediated diseases (pIMDs) are a subset of AEs that include autoimmune diseases and other inflammatory and/or neurologic disorders of interest which may or may not have an autoimmune etiology.
Primary completion date:	The date that the final subject was examined or received an intervention for the purpose of final collection of data for the primary outcome, whether the clinical trial concluded according to the pre-specified protocol or was terminated.
Protocol amendment:	The International Conference on Harmonization (ICH) defines a protocol amendment as: 'A written description of a change(s) to or formal clarification of a protocol.' GSK Biologicals further details this to include a change to an approved protocol that affects the safety of subjects, scope of the investigation, study design, or scientific integrity of the study.
Protocol administrative change:	A protocol administrative change addresses changes to only logistical or administrative aspects of the study.
Randomization:	Process of random attribution of treatment to subjects in order to reduce bias of selection.
Self-contained study:	Study with objectives not linked to the data of another study.
Serious adverse event:	A serious adverse event (SAE) is any untoward medical occurrence that: • results in death; • is life-threatening; • requires hospitalization or prolongation of existing hospitalization; • results in disability/incapacity;

	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 or blood dyscrasias or convulsions that do not result in hospitalization. In this study, the following AEs of specific interest: pIMDs and meningitis will be reported as SAEs.
Site Monitor:	An individual assigned by the sponsor who is responsible for assuring proper conduct of clinical studies at one or more investigational sites.
Solicited adverse event:	Adverse events to be recorded as endpoints in the clinical study. The presence/occurrence/intensity of these events is actively solicited from the subject or an observer during a specified post-vaccination follow-up period.
Subject:	Term used throughout the protocol to denote an individual who has been contacted in order to participate or participates in the clinical study, either as a recipient of the vaccine(s)/product(s) or as a control.
Subject number:	A unique number identifying a subject, assigned to each subject consenting to participate in the study.
Treatment:	Term used throughout the clinical study to denote a set of investigational product(s) or marketed product(s) or placebo intended to be administered to a subject, identified by a unique number, according to the study randomization or treatment allocation.
Treatment number:	A number identifying a treatment to a subject, according to the study randomization or treatment allocation.
Unsolicited adverse event:	Any AE reported in addition to those solicited during the clinical study. Also any 'solicited' symptom with onset outside the specified period of follow-up for solicited symptoms will be reported as an unsolicited adverse event.

TRADEMARKS

The following trademarks are used in the present protocol.

Note: In the body of the protocol (including the synopsis), the names of the products will be written without the superscript symbol ™ or ® and in *italics*.

Trademarks of the GlaxoSmithKline group of companies	Generic description
Malarone®	Atovaquone and proguanil hydrochloride

Trademarks not owned by the GlaxoSmithKline group of companies	Generic description
QS-21 (*Quillaja saponaria* Molina, fraction 21; licensed by GSK from Antigenics Inc, a wholly owned subsidiary of Agenus Inc., a Delaware, USA corporation)	Triterpene glycoside immune enhancer
Imodium® (Johnson & Johnson Limited)	Loperamide
Coartem® (Novartis Pharmaceuticals UK Ltd.)	Artemether/lumefantrine

1. INTRODUCTION

1.1. Background

GlaxoSmithKline (GSK) Biologicals in partnership with PATH is developing a first generation *Plasmodium falciparum* (*P. falciparum)* malaria vaccine for routine immunization of infants and children living in malaria-endemic areas with the objective of reducing the risk of malaria and severe malaria during the first years of life.

GSK Biologicals has developed several formulations of the candidate pre-erythrocytic *P. falciparum* malaria vaccine, RTS,S/AS01, which consists of sequences of the circumsporozoite (CS) protein of *P. falciparum* and hepatitis B surface antigen (HBsAg) adjuvanted with the adjuvant system AS01 containing two immune enhancers MPL (3'-O-desacyl-4'-monophosphoryl-lipid A) and QS-21 (*Quillaja saponaria* Molina, fraction 21), in a liposomes suspension.

A large Phase III study (MALARIA-055 PRI [110021]), conducted in infants and children at 11 study sites in seven countries across sub-Saharan Africa, showed the potential for the current pediatric formulation of the candidate vaccine to play a role in future malaria control strategies. The vaccine efficacy of this pediatric formulation RTS,S/AS01$_E$, (25 µg of RTS,S antigen with AS01$_E$ containing 25 µg MPL, 25 µg QS-21 and liposomes) against all episodes of clinical malaria, when delivered according to a 0, 1, 2-month schedule, in children aged 5-17 months was estimated to be 55.1% (95% confidence interval [CI]: 50.5, 59.3; [The RTS,S Clinical Trials Partnership, 2011]) and 31.4% (95% CI: 24.2, 37.9) in infants aged 6-12 weeks [The RTS,S Clinical Trials Partnership, 2012] over 12 months post-Dose 3. Vaccine efficacy for clinical malaria waned over time [The RTS,S Clinical Trials Partnership, 2014; The RTS,S Clinical Trials Partnership, 2015]. The administration of a RTS,S/AS01$_E$ fourth dose enhanced protection.

While the Phase III evaluation of the pediatric candidate malaria vaccine RTS,S/AS01$_E$ was ongoing, GSK Biologicals, in collaboration with PATH, continued to investigate ways to further improve vaccine efficacy levels. Higher vaccine efficacy levels may lead to improved malaria control and contribute to the malaria elimination goal set as a long-term target by the global health community [Malaria Vaccine Technology Roadmap, 2013].

In a previous controlled human malaria infection (CHMI) study (WRMAL-003) evaluating, in malaria-naïve adults, vaccine formulations based on the RTS,S antigen, one study group showed high levels of vaccine efficacy against sporozoite challenge, with six out of seven subjects protected [Stoute, 1997]. Subjects in this study group had received an adjuvanted formulation of RTS,S antigen, referred to as RTS,S/AS02. The immunization schedule under evaluation was a 0, 1, 7-month schedule. After having observed high general reactogenicity in two subjects after the second immunization, and in view of limited experience of adjuvant safety at the time, there was a decision to reduce the third immunization dose to a fifth of the full 0.5 ml dose (fractional dose).

Later in the RTS,S clinical development program, immunization schedules with three subsequent identical doses given one month apart were used. In a further Phase II study, MALARIA-050, the spacing of the third (full) dose at Month 7 (i.e., a 0, 1, 7-month schedule) showed no increased benefit over the 0, 1, 2-month schedule [Asante, 2011]. In view of the supportive safety, immunogenicity and efficacy data, as well as considerations related to vaccine implementation in sub-Saharan Africa, the 0, 1, 2-month schedule was selected for further vaccine development.

The 0, 1, 7-month schedule, with a fractional dose delivered as the third immunization (Fx017M), has been further investigated recently in a CHMI study in malaria-naïve adults (MALARIA-071). The biological hypothesis was that the delayed fractional dose allowed a better qualitative immune maturation in the germinal center, with antigen selection of the B-cells harboring surface immunoglobulin with highest antigen affinity. Preliminary analyses showed high efficacy in the Fx017M group compared to standard doses given at 0, 1, 2-months (012M group): 4/30 subjects in the Fx017M group developed parasitemia (vaccine efficacy: 86.7% [95% CI: 66.8, 94.6]); 6/16 subjects in the 012M group developed parasitemia (vaccine efficacy: 62.5% [95% CI: 29.4, 80.1]); all 12 control subjects (no vaccine) developed parasitemia, confirming the validity of the malaria challenge. MALARIA-071 was not powered to detect superiority of the Fx017M group over the 012M group, but the study did show some evidence of a difference in vaccine efficacy comparing the two groups (increase in proportion of protected subjects = 64.4% [95% CI: -7.9, 88.3], p=0.0741, Fisher's exact; difference in time to parasitemia p=0.0455, logrank). Immunological investigations showed that the delayed fractional dose regimen did not generate higher absolute anti-CS antibody titers as compared to the standard regimen, but increased anti-CS antibody avidity. The mean (standard deviation) avidity index on day of challenge (using CS full length as target antigen, was 0.55 (0.08) in the 012M group vs 0.68 (0.1) in the Fx017M group ($p<0.0001$). Further immunological investigations are ongoing. In the follow-up phase of the study, subjects who were protected in the initial challenge were randomized to receive a fractional fourth dose or no fourth dose, before being exposed to a second sporozoite challenge approximately six months after the initial challenge. Amongst subjects initially in the Fx017M group, three out of seven subjects who did not receive a fourth dose were protected in this second challenge, while nine out of 10 subjects who were initially protected and received a fractional fourth dose were protected. The results from the study follow-up phase and the second challenge suggest that there is waning immunity with the fractional schedule too but that the protection can be extended with an additional fractional dose.

Please refer to the current Investigator Brochure (IB) for information regarding the pre-clinical and clinical studies of RTS,S/AS01.

1.2. Rationale for the study and study design

1.2.1. Rationale for the study

Results from CHMI studies showed a simple way to improve the efficacy of the RTS,S/AS01 vaccine to the point where it might become useful either for improved malaria control in the pediatric population or as a tool to reduce malaria transmission. Combining several interventions such as vector control, prophylactic vaccination and mass drug administration, thus targeting different stages of the parasite cycle, may prove to offer the best control of malaria transmission.

This study aims to investigate whether changes in dosing schedule are associated with increased or equivalent protection, and to evaluate the immune mechanisms associated with vaccine efficacy under varying dosing schedules.

The study aims are the following:

a. Evaluation of the role of a fractional second and third dose.

b. Evaluation of a two-dose schedule where the second dose is fractional.

c. Evaluation of the use of the pediatric formulation to vaccinate adults, when a pediatric dose is delivered to adults.

d. Evaluation of the use of the pediatric formulation to vaccinate adults, when what has been considered so far as an adult dose is delivered using the pediatric formulation (increasing the volume of administration).

e. Evaluate the impact of varying dosing schedules on immune effectors and immune correlates of protection.

The ability to use the pediatric formulation (RTS,S/$AS01_E$) for adults would be a very positive step when considering product availability and implementation feasibility of a mass vaccination program in the context of an elimination campaign, therefore study aims c) and d) are being investigated. The pediatric formulation of RTS,S/AS01 has never been used in adults, but there are indications from other programs that the $AS01_E$ Adjuvant System may not be substantially inferior to $AS01_B$ for immunogenicity [EARLY-CLINRES-002, data on file; Montoya, 2013].

Evaluation of a two-dose schedule, separated by seven months, will contribute to explore the possibility that the close repetition of doses leads to some excessive priming that may be detrimental to the immune responses. If leading to sufficient protection, a two-dose strategy may be a great advantage when considering cost and logistics aspects in the contribution to malaria control and elimination efforts.

1.2.2. Rationale for the study design

This study is designed to evaluate efficacy, immunogenicity and safety of various dose schedules of GSK Biologicals' candidate malaria vaccines RTS,S/AS01$_B$ (adult formulation containing 50 µg RTS,S antigen and AS01$_B$ formulation with 50 µg of MPL and 50 µg of QS-21 per 0.5 ml) and RTS,S/AS01$_E$ (pediatric formulation containing 25 µg RTS,S antigen and AS01$_E$ formulation with 25 µg of MPL and 25 µg of QS-21 per 0.5 ml) in healthy malaria-naïve subjects aged 18-55 years. The study proposed herein will include six study groups. Four of these study groups will be vaccinated according to a 0, 1, 7-month schedule and one according to a 0, 7-month schedule. The immunization schedule of reference will be the same as the one used in study MALARIA-071 (117014), i.e. RTS,S/AS01$_B$ full dose at Month 0 and Month 1 and RTS,S/AS01$_B$ 1/5th dose at Month 7. Three months after the last dose of study vaccines, all subjects will undergo a sporozoite challenge and will be compared to an infectivity control group:

- **Group AduFx** will receive RTS,S/AS01$_B$ full dose at Month 0 and Month 1 + RTS,S/AS01$_B$ fractional dose (1/5th dose) at Month 7 (immunization schedule of reference).
- **Group 2PedFx** will receive double dose of RTS,S/AS01$_E$ at Month 0 and Month 1 + double dose of RTS,S/AS01$_E$ fractional dose (1/5th dose) at Month 7.
- **Group PedFx** will receive RTS,S/AS01$_E$ full dose at Month 0 and Month 1 + RTS,S/AS01$_E$ fractional dose (1/5th dose) at Month 7.
- **Group Adu2Fx** will receive RTS,S/AS01$_B$ full dose at Month 0 + RTS,S/AS01$_B$ fractional dose (1/5th dose) at Month 1 and Month 7.
- **Group Adu1Fx** will receive RTS,S/AS01$_B$ full dose at Month 0 + RTS,S/AS01$_B$ fractional dose (1/5th dose) at Month 7.
- **Group infectivity control** will not receive any immunization but will undergo the sporozoite challenge.

The sporozoite challenge model, in the RTS,S/AS candidate vaccine development program, has demonstrated a high relevance in its ability to predict efficacy under conditions of natural exposure in malaria-endemic countries. The study design is similar to that of other past CHMI studies successfully conducted. To better discriminate levels of protection across study groups and assess durability of protection, CHMI will occur three months after the last vaccine dose rather than three weeks as in past RTS,S studies. About 150 healthy adults will be enrolled and followed up closely under controlled conditions by investigators experienced in *P. falciparum* challenge studies. The proportion of immunized participants who remain free of *P. falciparum* infection following sporozoite challenge and the delay in the pre-patent period leading to infection will be evaluated as indicators of vaccine-induced protection. Immunological investigations are planned with the intent to characterize qualitatively and quantitatively the immune response induced by the immunization regimen. This design takes into account logistical restrictions on the size of human challenge procedures.

Immunological assays will be selected for their ability to demonstrate whether the administration of a fractional vaccine dose can affect quantitatively or qualitatively the vaccine-induced immune response, especially when considering the generation of antibodies against the CS antigen, but also the HBsAg which is present in the vaccine construct. Qualitative aspect of the immune response will be characterized as much as possible (targeting different parts of the antigen and avidity). Samples from this study may be used in future assay development or testing to better understand the immune responses underlying vaccine induced protection, responses to the vaccine components and the disease under investigation.

1.3. Benefit : Risk assessment

Please refer to the current IB for the summary of potential risks and benefits of RTS,S/AS01.

The following section outlines the risk assessment and mitigation strategy for this study protocol:

1.3.1. Risk assessment

Important Potential/Identified Risk	Data/Rationale for Risk	Mitigation Strategy
Investigational study vaccine (RTS,S/AS01)		
Important potential risk: **Meningitis**	In the large Phase III study, MALARIA-055 PRI, an imbalance of meningitis cases of any etiology (i.e. including cases with confirmed etiology and cases with no etiology found), with no cluster in time-to-onset, has been observed in children 5-17 months of age at first dose. Meningitis has not been a safety concern in RTS,S studies in adults.	Subjects will be instructed on the need to attend the clinic if they are unwell. A high level of medical supervision is in place to detect and treat meningitis if it occurs. Meningitis is an AE of specific interest (see Section 9.1.5). Clinical details of each case will be captured through the expedited AE report and in a specific eCRF screen.
Important potential risk: **Hypersensitivity (including anaphylaxis)**	As with other vaccines, hypersensitivity and anaphylaxis to one or several components of the vaccine can rarely occur. One case of erythema multiforme and two cases of bronchospasm within 30 days following RTS,S/AS01 vaccination were reported as hypersensitivity reactions in past pediatric studies. No case of anaphylaxis has been reported following RTS,S/AS01 vaccination to date.	Subjects will be observed closely for at least 30 minutes following administration of the vaccine with appropriate medical treatment readily available in case of anaphylaxis. History of anaphylaxis post-vaccination and history of any reaction or hypersensitivity likely to be exacerbated by any component of the vaccine are exclusion criteria in this study (see Section 5.3). Previous anaphylactic reaction to a vaccine is a contraindication to further experimental vaccinations in this study (see Section 7.5).

Important Potential/Identified Risk	Data/Rationale for Risk	Mitigation Strategy
Important potential risk: **potential immune-mediated disease (pIMD)**	pIMD is a theoretical concern with adjuvanted vaccines as no evidence of autoimmune disease caused by RTS,S/AS01 has been observed.	Subjects will be informed of this theoretical risk and the need to attend the clinic if they are unwell. pIMD is an AE of specific interest (see Section 9.1.5). The occurrence of pIMD cases will be described.
Study procedures		
Pain when taking blood samples	When taking the blood samples, the subject may feel faint; or experience mild pain, bruising, irritation or redness.	Subjects will be advised to inform or call the study doctor immediately if they have any side effects that they perceive as serious.
Risks associated with malaria challenge	The risks associated with the sporozoïte challenge include local inflammatory reactions or potential allergic reactions to mosquito bites as well as the development of malaria infection. Transient abnormalities, such as fever, headache, mild anemia, leukopenia, splenomegaly, hepatic tenderness and fatigue, are expected consequences of malaria. The complications of malaria which can lead to kidney, liver or brain damage, and death, are seen during naturally acquired malaria when diagnosis and treatment are delayed and high levels of parasitemia develop.	Anti-malaria treatment will be administered to malaria infected subjects. Under the carefully controlled conditions of this study, the chance of serious illness or death from malaria infection is very small.

1.3.2. Benefit assessment

No direct benefit is foreseen from the study participation. 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.

1.3.3. Overall Benefit:Risk conclusion

The potential risks in association with RTS,S/AS01 (SB257049) are considered acceptable and the potential risks associated with malaria challenges are minimized by the carefully controlled condition of this study.

2. OBJECTIVES

2.1. Primary objective

2.1.1. Efficacy

- To assess the vaccine efficacy against the occurrence of *P. falciparum* parasitemia (defined by a positive blood slide) for each vaccination schedule (AduFx, 2PedFx, PedFx, Adu2Fx, and Adu1Fx) versus the infectivity controls.

Refer to Section 11.1 for the definition of the primary endpoint.

2.2. Secondary objectives

2.2.1. Efficacy

- To assess the time to onset of *P. falciparum* parasitemia (defined by a positive blood slide) for each vaccination schedule (AduFx, 2PedFx, PedFx, Adu2Fx, and Adu1Fx) versus the infectivity controls.
- To assess the occurrence of *P. falciparum* parasitemia (defined by a positive blood slide) after vaccine administration for alternative vaccination schedules (2PedFx, PedFx, Adu2Fx, and Adu1Fx) versus the immunization schedule of reference (AduFx).
- To assess the time to onset of *P. falciparum* parasitemia (defined by a positive blood slide) after vaccine administration for alternative vaccination schedules (2PedFx, PedFx, Adu2Fx, and Adu1Fx) versus the immunization schedule of reference (AduFx).

2.2.2. Immunogenicity

For each vaccination schedule:

- To evaluate anti-CS repeat region antibody response at specified timepoints.
- To evaluate anti-HBs immunoglobulin G (IgG) antibody response at specified timepoints.

2.2.3. Safety

For each vaccination schedule:

- To assess the safety (unsolicited AEs/AEs of specific interest/serious adverse events [SAEs]) and reactogenicity (solicited AEs).

Refer to Section 11.2 for the definition of the secondary endpoints.

2.3. Tertiary objectives

2.3.1. Immunogenicity

For each vaccination schedule:

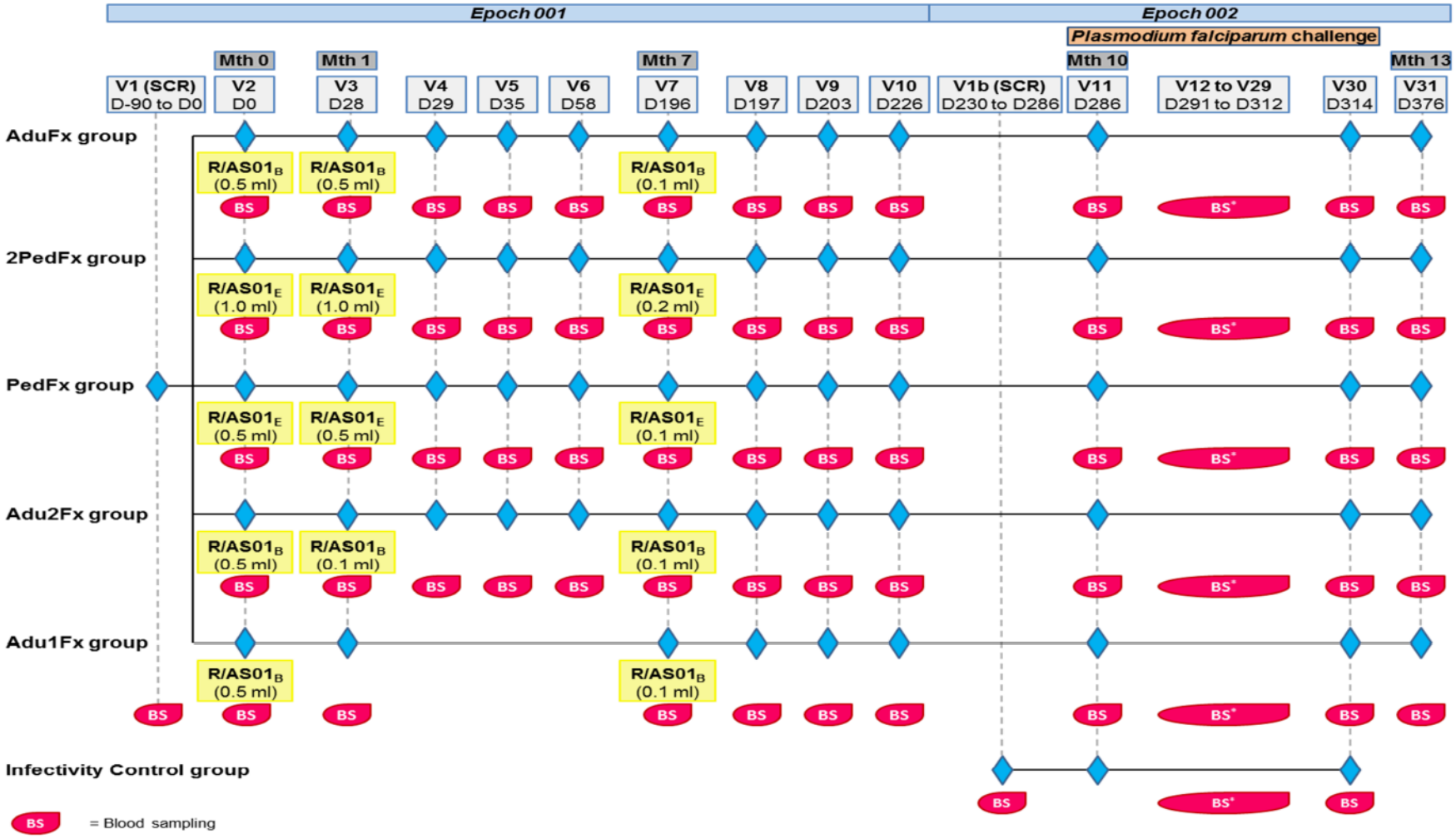
- To evaluate anti-CS repeat region IgG avidity index at specified timepoints.
- To evaluate anti-full length CS protein IgG concentrations and anti-C terminal portion of the protein (C-term) IgG concentrations at specified timepoints.
- To evaluate anti-full length CS protein and anti-C-term IgG avidity at specified timepoints.

Note: other immuno-assays evaluating the immune response targeting the CS and HBsAg might be performed.

Refer to Section 11.3 for the definition of the tertiary endpoints.

3. STUDY DESIGN OVERVIEW

Figure 1 Study design



Footnotes to Figure 1:

D: Day; Mth: month; R: RTS,S; SCR: Screening; V: visit. * Blood sample for estimation of subjects with parasitemia (PCR testing) will be collected on the day of subject entry into the hotel phase (Day 295 [Visit 16]); blood sample for biochemistry and hematology parameters will be collected the day of first parasitemia and blood sample for assessment of parasitemia (blood smear) will be collected daily for 14 days (from Day 291 [Visit 12] to Day 304 [Visit 25]) and then every two days for nine days (Day 306 [Visit 26], Day 308 [Visit 27], Day 310 [Visit 28], Day 312 [Visit 29], and Day 314 [Visit 30]).

(Amended 19 June 2017)

Protocol waivers or exemptions are not allowed with the exception of immediate safety concerns. Therefore, adherence to the study design requirements, including those specified in the outline of study procedures (Section 6.5), are essential and required for study conduct.

- Experimental design: Phase IIA, open, randomized, controlled, mono-centric, single-country study with five parallel groups and one infectivity control group.
- Duration of the study: Approximately 16 months for each vaccinated subject and three months for the infectivity control subjects.
  - Epoch 001: Primary starting at Visit 1 (Screening) (Screening for vaccinated subjects) and ending at Visit 10 (Day 226).
  - Epoch 002: Challenge starting at Visit 11 (Day 286) or Visit 1b (Screening) (Screening for infectivity controls), as applicable, and ending at Visit 31 (Day 376).
- Study groups:

Table 1 Study groups and epochs foreseen in the study

Study groups	Vaccination schedule	Number of subjects	Age (Min/Max)	Epochs	
				Epoch 001	Epoch 002
AduFx	RTS,S/AS01$_B$ full dose at Month 0, Month 1 + RTS,S/AS01$_B$ 1/5th dose at Month 7	26	18 years - 55 years	X	X
2PedFx	RTS,S/AS01$_E$ double dose at Month 0, Month 1 + double dose RTS,S/AS01$_E$ 1/5th dose at Month 7	26	18 years - 55 years	X	X
PedFx	RTS,S/AS01$_E$ full dose at Month 0, Month 1 + RTS,S/AS01$_E$ 1/5th dose at Month 7	26	18 years - 55 years	X	X
Adu2Fx	RTS,S/AS01$_B$ full dose at Month 0 + RTS,S/AS01$_B$ 1/5th dose at Month 1, Month 7	26	18 years - 55 years	X	X
Adu1Fx	RTS,S/AS01$_B$ full dose at Month 0 + RTS,S/AS01$_B$ 1/5th dose at Month 7	26	18 years - 55 years	X	X
Infectivity control	Subjects will not receive any immunization but will undergo sporozoite challenge	30*	18 years - 55 years		X

* between 20 and 30 infectivity controls will be enrolled.

Table 2 Study groups and treatment foreseen in the study

Treatment name	Vaccine/Product name	Injectable volume	Study Groups					
			AduFx	2PedFx	PedFx	Adu2Fx	Adu1Fx	Infectivity control
RTS,S/AS01$_B$ (Full dose)	RTS,S (50 µg)	0.5 ml	X			X	X	
	AS01B		X			X	X	
RTS,S/AS01$_B$ (1/5th dose)	RTS,S (50 µg)	0.1 ml	X			X	X	
	AS01B		X			X	X	
RTS,S/AS01$_E$ (Full dose)	RTS,S (25 µg)	0.5 ml		X*	X			
	AS01E			X*	X			
RTS,S/AS01$_E$ (1/5th dose)	RTS,S (25 µg)	0.1 ml		X**	X			
	AS01E			X**	X			

* Injected volume will be 1.0 ml
** Injected volume will be 0.2 ml

Table 3 Overview of the vaccine administration per study groups

Groups	Visit 2 (Day 0)	Visit 3 (Day 28)	Visit 7 (Day 196)
AduFx	RTS,S/AS01$_B$ (Full dose) (0.5 ml)	RTS,S/AS01$_B$ (Full dose) (0.5 ml)	RTS,S/AS01$_B$ (1/5th dose) (0.1 ml)
2PedFx	RTS,S/AS01$_E$ (Full dose) (1.0 ml)	RTS,S/AS01$_E$ (Full dose) (1.0 ml)	RTS,S/AS01$_E$ (1/5th dose) (0.2 ml)
PedFx	RTS,S/AS01$_E$ (Full dose) (0.5 ml)	RTS,S/AS01$_E$ (Full dose) (0.5 ml)	RTS,S/AS01$_E$ (1/5th dose) (0.1 ml)
Adu1Fx	RTS,S/AS01$_B$ (Full dose) (0.5 ml)	-	RTS,S/AS01$_B$ (1/5th dose) (0.1 ml)
Adu2Fx	RTS,S/AS01$_B$ (Full dose) (0.5 ml)	RTS,S/AS01$_B$ (1/5th dose) (0.1 ml)	RTS,S/AS01$_B$ (1/5th dose) (0.1 ml)

- Control: active control (Group AduFx [RTS,S/AS01$_B$ full dose at Month 0 and Month 1 + RTS,S/AS01$_B$ fractional dose at Month 7] is the immunization schedule of reference) and infectivity controls for the challenge.
- Vaccination schedule are provided in Table 1.
- Treatment allocation: randomized

Table 4 Blinding of study epochs

Study Epochs	Blinding
Epoch 001	open
Epoch 002	open

- Sampling schedule:
  - Blood samples for assessment of anti-CS and anti-HBs immune response and for serum repository will be collected before vaccine administration (Day 0), one month post-Dose 2 (Day 58), six months post-Dose 2 (Day 196), one month post-Dose 3 (Day 226), on the day of challenge (Day 286), 28 days post-challenge (Day 314), and at study end (Day 376) for subjects from **AduFx, 2PedFx, PedFx, and Adu2Fx groups** and before vaccine administration (Day 0), before Dose 2 (Day 196), one month post-Dose 2 (Day 226), on the day of challenge (Day 286), 28 days post-challenge (Day 314), and at study end (Day 376) for subjects from **Adu1Fx group**.
  - Blood samples for peripheral blood mononuclear cells (PBMC) and plasma repository will be collected before vaccine administration (Day 0), one month post-Dose 1 (Day 28), seven days post-Dose 2 (Day 35), one month post-Dose 2 (Day 58), six months post-Dose 2 (Day 196), seven days post-Dose 3 (Day 203), one month post-Dose 3 (Day 226), on the day of challenge (Day 286), and at study end (Day 376) for subjects from **AduFx, 2PedFx, PedFx, and Adu2Fx groups** and before vaccine administration (Day 0), one month post-Dose 1 (Day 28), before Dose 2 (Day 196), seven days post-Dose 2 (Day 203), one month post-Dose 2 (Day 226), on the day of challenge (Day 286), and at study end (Day 376) for subjects from **Adu1Fx group**.

- Blood samples for the evaluation of biochemistry (alanine aminotransferase [ALT], aspartate aminotransferase [AST] and creatinine) and hematology (hemoglobin, leukocytes [white blood cells; WBC] and platelets) parameters will be collected before vaccine administration (screening), seven days post-Dose 2 (Day 35), one month post-Dose 2 (Day 58), seven days post-Dose 3 (Day 203), one month post-Dose 3 (Day 226), the day of first parasitemia, and 28 days post-challenge (Day 314) for subjects from **AduFx, 2PedFx, PedFx, and Adu2Fx groups**, before vaccine administration (screening), seven days post-Dose 2 (Day 203), one month post-Dose 2 (Day 226), the day of first parasitemia, and 28 days post-challenge (Day 314) for subjects from **Adu1Fx group** and at screening, the day of first parasitemia and 28 days post-challenge (Day 314) for the **infectivity control subjects.** Following a bleeding procedure failure or laboratory failure, repeat bleeds can be considered upon investigator discretion no more than three times, or if medically indicated for full investigation of a potential adverse event.
- Blood samples for RNA sequencing (messenger ribonucleic acid [mRNA] sequencing analysis) will be collected before vaccine administration (Day 0), at Dose 2 (Day 28), one day post-Dose 2 (Day 29), at Dose 3 (Day 196), and one day post-Dose 3 (Day 197) for subjects from **AduFx, 2PedFx, PedFx, and Adu2Fx groups** and before vaccine administration (Day 0), one month post-Dose 1 (Day 28), at Dose 2 (Day 196) and one day post-Dose 2 (Day 197) for subjects from **Adu1Fx group**.
- Blood sample for assessment of parasitemia (PCR testing) will be collected on the day of subject entry into the hotel phase (Day 295 [Visit 16]) to provide an estimate of the number of subjects likely to develop malaria within the first few days of the hotel phase.
- Blood sample for assessment of parasitemia (blood smear) will be collected daily for 14 days (from Day 291 [Visit 12] to Day 304 [Visit 25]) and then every two days for nine days (Day 306 [Visit 26], Day 308 [Visit 27], Day 310 [Visit 28], Day 312 [Visit 29], and Day 314 [Visit 30]). For subjects who develop malaria, blood smear may be discontinued once the subject has three consecutive negative smears (separated by more than 12 hours) following the initial treatment (see Section 6.4.1).
- Blood samples for testing of HIV, hepatitis C virus (HCV) and HBsAg will be collected from all subjects at screening.
- Urinary pregnancy test (urine beta-human chorionic gonadotropin [β-HCG]) will be performed on all women at screening, before each vaccine dose for vaccinated subjects and on the day of challenge.

- Type of study: self-contained.
- Data collection: electronic Case Report Form (eCRF).

- Safety monitoring:
  - Each subject will be observed for at least 30 minutes after vaccination to evaluate and treat any acute AEs.
  - Each subject will be observed for at least 30 minutes following completion of the sporozoite challenge to evaluate and treat any acute allergic reactions.
  - All SAEs (all, fatal, related to the investigational vaccine) and pregnancies will be reported until study end (Day 376).
  - All AEs and SAEs leading to withdrawal from further vaccination will be reported until study end (Day 376) for all vaccinated subjects (AduFx, 2PedFx, PedFx, Adu2Fx, and Adu1Fx groups).
  - Meningitis and pIMDs are AEs of specific interest to be reported until study end (Day 376; see Section 9.1.5) for all vaccinated subjects (AduFx, 2PedFx, PedFx, Adu2Fx, and Adu1Fx groups).
  - Solicited local and general AEs will be collected from all vaccinated subjects (AduFx, 2PedFx, PedFx, Adu2Fx, and Adu1Fx groups) during seven days (day of vaccination and six subsequent days) after each dose of study vaccine.
  - Unsolicited AEs will be collected from all vaccinated subjects (AduFx, 2PedFx, PedFx, Adu2Fx, and Adu1Fx groups) during 30 days (day of vaccination and 29 subsequent days) after each dose of study vaccine.
  - AEs post-challenge will be collected from all subjects (AduFx, 2PedFx, PedFx, Adu2Fx, Adu1Fx, and infectivity control groups) during 30 days (day of challenge and 29 subsequent days) after the sporozoite challenge.
  - Biochemistry (ALT, AST and creatinine) and hematology (hemoglobin, WBC and platelets) parameters will be assessed as described in 'Sampling schedule'.

4. CASE DEFINITION

Table 5 Case definition of *P. falciparum* infection

Case definition	Asexual blood stage *P. falciparum* parasite density > 0 detected by blood slide reading.

5. STUDY COHORT

5.1. Number of subjects/centers

The target is to enroll approximately 150 subjects (26 subjects per vaccine group and between 20 and 30 subjects in the infectivity control group) aged 18 to 55 years at the time of first vaccination. Refer to Section 11.4 for the determination of sample size.

Overview of the recruitment plan

Recruitment will be conducted using multiple methods such as advertisements, flyers, posters, word of mouth and other institutional review board (IRB) approved methods. There will be an opportunity for subjects who enroll in the study to receive compensation for recruitment of additional subjects.

Recruitment for the vaccine groups will be terminated when 26 subjects per vaccine group have been enrolled (i.e. informed consent form signed), eligible per screening and inclusion/exclusion criteria and available on the day of first vaccination.

Recruitment for infectivity control group will be terminated when a maximum of 30 subjects have been enrolled (i.e. informed consent form signed), eligible per screening and inclusion/exclusion criteria, and available on the day of challenge. For infectivity control group **only**, in the rare event that malaria-naïve volunteers cannot be recruited, subjects may be enrolled if they have not been diagnosed with malaria (clinical disease or parasitemia) within the last five years (inclusive).

5.2. Inclusion criteria for enrolment

Deviations from inclusion criteria are not allowed because they can potentially jeopardize the scientific integrity of the study, regulatory acceptability or subject safety. Therefore, adherence to the criteria as specified in the protocol is essential.

All subjects must satisfy ALL the following criteria at study entry:

- Subjects who, in the opinion of the investigator, can and will comply with the requirements of the protocol (e.g. completion of the diary cards, return for follow-up visits).
- Written informed consent obtained from the subject prior to performing of any study specific procedure.
- A male or female between, and including, 18 and 55 years of age at the time of enrolment.
- Healthy subjects as established by medical history and clinical examination before entering into the study.

- Available to participate for the duration of the study (approximately 16 months per vaccinated subject and approximately three months per subject in the infectivity control group).
- Female subjects of non-childbearing potential may be enrolled in the study.
  - Non-childbearing potential is defined as pre-menarche, current tubal ligation, hysterectomy, ovariectomy or post-menopause.

Please refer to the glossary of terms for the definition of menarche and menopause.

- Female subjects of childbearing potential may be enrolled in the study, if the subject:
  - has practiced adequate contraception for 30 days prior to vaccination, and
  - has a negative pregnancy test at enrolment, and
  - has agreed to continue adequate contraception during the entire treatment period and for two months after completion of the vaccination series and/or malaria challenge.

Please refer to the glossary of terms for the definition of adequate contraception.

5.3. Exclusion criteria for enrolment

Deviations from exclusion criteria are not allowed because they can potentially jeopardize the scientific integrity of the study, regulatory acceptability or subject safety. Therefore, adherence to the criteria as specified in the protocol is essential.

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:

- Use of any investigational or non-registered product (drug or vaccine) other than the study vaccines during the period starting 30 days before the first dose of study vaccines (Day -29 to Day 0), or planned use during the study period.
- Any medical condition that in the judgment of the investigator would make intramuscular (IM) injection unsafe.
- 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 $\geq$ 20 mg/day (for adult subjects), or equivalent. Inhaled and topical steroids are allowed.
- Administration of long-acting immune-modifying drugs at any time during the study period (e.g. infliximab).
- Chronic use of antibiotics with antimalarial effects (e.g. tetracyclines for dermatologic patients, sulfa for recurrent urinary tract infections, etc.).
- 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 (pharmaceutical product or device).
- Seropositive for HBsAg or HCV.
- Documented HIV-positive subject.
- Previous vaccination against malaria.
- History of malaria chemoprophylaxis within 60 days prior to vaccination.
- Any history of malaria (for the vaccine groups).
- Planned travel to malaria endemic areas during the study period.
- History of splenectomy.
- Any confirmed or suspected immunosuppressive or immunodeficient condition, based on medical history and physical examination.
- Family history of congenital or hereditary immunodeficiency.
- History of any reaction or hypersensitivity likely to be exacerbated by any component of the vaccine.
- History of anaphylaxis post-vaccination.
- Hypersensitivity to latex.
- History of any reaction or hypersensitivity likely to be exacerbated by chloroquine.
- History of psoriasis and porphyria, which may be exacerbated after chloroquine treatment.
- Current use of medications known to cause drug reactions to chloroquine.
- History of severe reactions to mosquito bites.
- Major congenital defects.
- Serious chronic illness.
- History of any neurological disorders or seizures (except for a single episode of simple febrile seizure in childhood).
- Acute disease and/or fever at the time of enrolment.
  - Fever is defined as temperature $\geq$ 37.5°C/99.5°F for oral, axillary or tympanic route, or $\geq$ 38.0°C/100.4°F for rectal route.
  - Subjects with a minor illness (such as mild diarrhea, mild upper respiratory infection) without fever may be enrolled at the discretion of the investigator.
- Acute or chronic, clinically significant pulmonary, cardiovascular, hepatic or renal functional abnormality, as determined by physical examination or laboratory screening tests.

- Any abnormal baseline laboratory screening tests: ALT, AST, creatinine, hemoglobin, platelet count, total WBC, out of normal range as defined in the protocol.
- Evidence of increased cardiovascular disease risk, "moderate" or "high", according to the National health and nutrition examination survey I (NHANES I) criteria.

  Note: NHANES I criteria will be applied for all subjects including subjects aged 18-35 years old (see APPENDIX C).

  – An abnormal baseline screening electrocardiogram, defined as one showing pathologic Q waves and significant ST-T wave changes; left ventricular hypertrophy; any non-sinus rhythm excluding isolated premature atrial contractions; right or left bundle branch block; or advanced (secondary or tertiary) A-V heart block.
- 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.
- Pregnant or lactating female.
- History of chronic alcohol consumption and/or drug abuse.
- Female planning to become pregnant or planning to discontinue contraceptive precautions.
- History of blood donation within 56 days preceding enrolment.
- Any other significant finding that in the opinion of the investigator would increase the risk of having an adverse outcome from participating in this study.

6. CONDUCT OF THE STUDY

6.1. Regulatory and ethical considerations, including the informed consent process

The study will be conducted in accordance with all applicable regulatory requirements.

The study will be conducted in accordance with the ICH Guideline for Good Clinical Practice (GCP), all applicable subject privacy requirements and the guiding principles of the Declaration of Helsinki.

GSK will obtain favorable opinion/approval to conduct the study from the appropriate regulatory agency, in accordance with applicable regulatory requirements, prior to a site initiating the study in that country.

Conduct of the study includes, but is not limited to, the following:

- IRB/Independent Ethics Committee (IEC) review and favorable opinion/approval of study protocol and any subsequent amendments.
- Subject informed consent.
- Investigator reporting requirements as stated in the protocol.

GSK will provide full details of the above procedures to the investigator, either verbally, in writing, or both.

Freely given and written or witnessed/ thumb printed informed consent must be obtained from each subject, as appropriate, prior to participation in the study.

GSK Biologicals will prepare a model Informed Consent Form (ICF) which will embody the ICH GCP and GSK Biologicals required elements. While it is strongly recommended that this model ICF is to be followed as closely as possible, the informed consent requirements given in this document are not intended to pre-empt any local regulations which require additional information to be disclosed for informed consent to be legally effective. Clinical judgment, local regulations and requirements should guide the final structure and content of the local version of the ICF.

The investigator has the final responsibility for the final presentation of the ICF, respecting the mandatory requirements of local regulations. The ICF generated by the investigator with the assistance of the sponsor's representative must be acceptable to GSK Biologicals and be approved (along with the protocol, and any other necessary documentation) by the IRB/IEC.

6.2. Subject identification and randomization of treatment

6.2.1. Subject identification

Subject identification numbers will be assigned sequentially to the subjects who have consented to participate in the study, according to the range of subject identification numbers allocated to the center.

6.2.2. Randomization of treatment

6.2.2.1. Randomization of supplies

The list of treatment numbers for the supplies is generated at GSK Biologicals, using MATerial EXcellence (MATEX), a program developed for use in Statistical Analysis System (SAS) (Cary, NC, USA) by GSK Biologicals.

6.2.2.2. Treatment allocation to the subject

The treatment numbers will be allocated by dose.

6.2.2.2.1. Study group and treatment number allocation

The target is to enroll approximately 150 subjects (26 subjects per vaccine group and between 20 and 30 subjects in the infectivity control group). Randomization will be performed on the investigational vaccine groups (AduFx, 2PedFx, PedFx, Adu2Fx, and Adu1Fx) and not for the infectivity controls. The first enrolled 130 subjects will be allocated to the vaccine groups (AduFx, 2PedFx, PedFx, Adu2Fx, and Adu1Fx) and the subsequent subjects will be allocated to the infectivity controls group.

Allocation of the subject to a study group at the investigator site will be performed using a randomization system on internet (SBIR). The randomization algorithm will use a minimization procedure accounting for center.

After obtaining the signed and dated ICF from the subject and having checked the eligibility of the subject, the site staff in charge of the vaccine administration will access SBIR. Upon providing the subject identification number, the randomization system will determine the study group and will provide the treatment number to be used for the first dose.

The number of each administered treatment must be recorded in the eCRF on the Vaccine Administration screen.

When SBIR is not available, please refer to the SBIR user guide or the Study Procedures Manual (SPM) for specific instructions.

6.2.2.2.2. Treatment number allocation for subsequent doses

For each dose subsequent to the first dose, the study staff in charge of the vaccine administration will access SBIR, provide the subject identification number, and the system will provide a treatment number consistent with the allocated study group.

The number of each administered treatment must be recorded in the eCRF on the Vaccine Administration screen.

6.3. Method of blinding

This study is open label.

The laboratory in charge of the laboratory testing will be blinded to the treatment, and codes will be used to link the subject and study (without any link to the treatment attributed to the subject) to each sample.

6.4. General study aspects

Supplementary study conduct information not mandated to be present in this protocol is provided in the accompanying SPM. The SPM provides the investigator and the site personnel with administrative and detailed technical information that does not impact the safety of the subjects.

6.4.1. Mosquito challenge

Subjects from the five vaccine groups (AduFx, 2PedFx, PedFx, Adu2Fx, and Adu1Fx) completing their immunization course will be challenged with sporozoite-infected mosquitoes to determine whether the expected protective response has developed. Such challenges are mandated by the absence of any laboratory tests that will unequivocally predict protection. The challenge is scheduled to occur approximately 90 days (three months) after the last vaccination (Day 286 [Visit 11]).

Unimmunized infectivity control subjects will also be challenged to verify the adequacy of the challenge.

Subjects undergoing malaria challenge will receive an emergency notification card that outlines their participation in the study with details on the exposure to malaria, as well as the appropriate investigator contact telephone numbers. Details of this information may vary, depending on the venue selected for the hotel phase of the study.

6.4.1.1. Contraindications to malaria challenge

The only absolute contraindication to mosquito challenge is pregnancy. If this occurs after immunization but before undergoing challenge, the subject must not be challenged and she will be followed for the duration of the study or pregnancy whichever is longer.

A subject with a minor illness but who does not have fever during the pre-challenge assessment may be challenged at the discretion of the investigator. If the subject is moderately to severely ill with fever on the day of the challenge, he/she will not be challenged. Challenge can be performed within the time window defined in Table 9, Table 10 and Table 11, at the discretion of the investigator if the subject becomes well. Subjects who will not be challenged will be followed for the duration of the study.

Any conditions which according to the judgment of the investigator would make the challenge unsafe.

6.4.1.2. Parasite and mosquito strains

The 3D7 clone of *P. falciparum* is a human malaria isolate that has never been passed through monkeys, is well adapted to culture, is a good producer of gametocytes that can infect mosquitoes, is susceptible to currently available, approved, anti-malarial compounds, and has previously been used in the Walter Reed Army Institute of Research (WRAIR) challenge model to successfully infect human subjects under a Biologics Master File submitted to the Food and Drug Administration (FDA). Master seed lots of these parasites have been developed and stored at WRAIR. All blood products used for malaria and mosquito culturing will be commercially tested for HIV, HBsAg, HCV, and syphilis. The mosquitoes used will be laboratory-born and reared *Anopheles stephensi*.

6.4.1.3. Infection of human subjects

Mosquitoes infected approximately 2-3 weeks earlier that are likely to contain sporozoites in their salivary glands will be allowed to feed on the subjects. For each subject, five mosquitoes will be allowed to feed over five minutes, after which they will be dissected to confirm how many were infected, and the salivary glands scored. If required, additional mosquitoes will be allowed to feed until a total of five infected mosquitoes with a minimum 2+ salivary glands score have fed [Wirtz, 1987]. The mosquito feedings will be performed in a secure insectary of the Department of entomology at WRAIR. Subjects will be observed for at least 30 minutes following completion of the sporozoite challenge in order to assess them for any evidence of acute allergic reactions related to mosquito exposure. To date, no severe allergic reactions related to mosquito bites have been documented in the context of the WRAIR malaria challenge model. Routinely, transient local allergic reactions (itching, rash) typical of mosquito bites occur at sites of the bites.

6.4.1.4. Determining parasitemia

Post-challenge, parasitemia will be determined by microscopy of Giemsa-stained thick blood films (smear). Microscopy will be performed on thick smears using a validated standard operation procedure.

All blood films (positive and negative) will be archived at the study centers for later re-examination and confirmation, if required.

6.4.1.5. Management of infected human subjects

The pre-patent period for *P. falciparum* in man normally averages 9-12 days. In previous studies, the pre-patent period in controls varied from 7-18 days. The shortest reported pre-patent period in man is five days, and the longest is 25 days [Ballou, 1987]. An immunized individual who does not have complete protection may have a prolongation of the parasite pre-patent period.

Beginning on Day 5 (Day 291 [Visit 12]) after their challenge, subjects will be seen and evaluated daily by a study investigator and blood will be drawn for blood smears to check for the presence of parasites as described in Section 6.4.1.4. If fever or symptoms develop at any time, blood smears will be done more frequently (every 6 to 12 hours), and a study investigator will evaluate the subject. A confirmed positive result will be relayed immediately to the on-call investigator/study personnel by the microscopists. The infection will be treated early (i.e. as soon as parasites can be identified on thick smear) according to the treatment regimen outlined in Section 6.4.1.6.

Beginning on Day 9 post-challenge (Day 295 [Visit 16]), a group of hotel rooms in the local area of WRAIR, will be reserved for malaria-challenged subjects. The subjects will be required to spend their nights there to allow for more rapid assessment of any potential symptoms of malaria during the hours that the study centers are closed. There will be an investigator present on-site and available for subject assessment. There will also be qualified study personnel on site 24 hours per day during the hotel phase of the study.

A blood sample for assessment of parasitemia (PCR testing) will be collected on the day of subject entry into the hotel phase (Day 295 [Visit 16]) to provide the study team an estimate of the number of subjects likely to develop malaria within the first few days of the hotel phase. This will allow the principal investigator and the study team to maximize subject safety by properly allocating resources and medical personnel to meet any high demand periods (i.e. periods when a large number of subjects are/will become ill). These samples will be processed by and assessed at WRAIR.

During the hotel phase, all challenged subjects will be assessed on a daily basis in an identical manner. An evaluation will be done each morning (headache, muscle aches, etc.) and blood will be drawn for smear. All challenged subjects will be instructed to check in with clinical staff by telephone call or in-person each evening during the hotel stay until they are positive for malaria. They will be asked if they feel any differently since they were seen in the morning. At any time required, the on-duty investigator will arrange for the timely production of blood smears, along with their examination and interpretation, in order to treat rapidly those subjects in whom therapy for malaria is indicated. Once a positive smear is identified, daily blood films will continue to be obtained until three consecutive films are negative (separated by more than 12 hours). A complete blood count and serum chemistry tests will be done when parasites are initially found in the blood (this could be done on the day of parasitemia detection or the day after).

The maximum hotel stay for malaria-challenged subjects should be approximately 10 days (Day 295 [Visit 16] to Day 304 [Visit 25]). A subject who develops malaria, is treated, and has three consecutive negative malaria smears (separated by more than 12 hours), will not need to remain in the hotel. The investigators will be responsible for accounting for any subjects who do not arrive in the hotel during the challenge phase. If required, the investigators will physically locate and treat any malaria-infected subject who is unable to maintain the follow-up dictated by this study.

If infection does not develop within 18 days, the subject will be released from staying nightly at the hotel. Subjects who do not develop malaria will be required to come to the clinical center for evaluation and blood drawing for smears every two days up to 28 days post-challenge (Days 306 [Visit 26], 308 [Visit 27], 310 [Visit 28], 312 [Visit 29], and 314 [Visit 30]). A subject who develops malaria and has three consecutive (separated by more than 12 hours) negative smears following initial treatment may be excused from the remaining hotel visits and the late post-challenge clinic visits at Days 306, 308, 310, and 312 (20, 22, 24 and 26 days post-challenge), but will be required to come to the clinic center at Day 314 (28 days post-challenge [Visit 30]). Telephone contact will be made if the subject does not keep a scheduled follow-up appointment. Symptom screening via telephone may suffice in lieu of clinical visits for Days 306, 308, 310, or 312 which fall upon a weekend.

6.4.1.6. Malaria treatment

During the evaluation of protective efficacy, as soon as a malaria infection is documented in a subject, he/she will be treated with standard doses of oral chloroquine (a total of 1500 mg chloroquine base: 600 mg base initially, followed by 300 mg base given approximately 6, 24, and 48 hours later) under direct observation. This regimen has been

100% effective in previous WRAIR malaria vaccine studies using the same malaria strain as will be used in this challenge model. Such early treatment minimizes the risk of developing a complicated malaria infection. Alternatively, atovaquone/proguanil (*Malarone*) standard oral dosage of 1 g/400 mg (four adult tablets per day for three consecutive days) or artemether/lumefantrine (*Coartem*) standard oral dosage of 80 mg/480 mg (four tablets initial dose, four tablets 8 hours later, and then four tablets twice daily for the following two days) can be used to treat subjects.

The malaria strain used for challenge (*P. falciparum* strain NF54/clone 3D7) is sensitive to several currently available, licensed, anti-malarial drugs that are safe, effective and have a low incidence of side effects. The investigators will have available approved antipyretics, such as acetaminophen and ibuprofen, for subjects experiencing fever and myalgias. In addition, other approved medications will be available to the investigators, which may include, but are not limited to: ondansetron and loperamide (*Imodium*) to treat other signs/symptoms as necessary. Investigators will always assure that subjects do not have underlying allergies to any of these medications prior to their use. An alternative antipyretic will be provided if the subject is allergic to a prescribed drug.

It is anticipated that treatment of malaria will be curative, since relapses do not occur after adequate treatment of *P. falciparum* infections. No previous *P. falciparum* subject infected and treated by WRAIR has had a malaria relapse. Subjects will be advised to contact the study physician, or to advise their personal physician of their participation in this malaria study, if fever, headache, or other symptoms possibly related to malaria develop at any time within one year after completion of the study. In the unlikely event that malaria recurs, the subject will be retreated with chloroquine, atovaquone/proguanil (*Malarone*) standard oral dosage of 1 g/400 mg (four adult tablets per day for three consecutive days), or artemether/lumefantrine (*Coartem*) standard oral dosage of 80 mg/480 mg (four tablets initial dose, four tablets 8 hours later, and then four tablets twice daily for the following two days).

No human viruses are known to be transmitted by colonized *Anopheles* mosquitoes. Blood purchased for malaria feedings has been commercially tested using FDA-approved test methods for antibodies to HIV, HCV, and syphilis, as well as for the presence of HBsAg; all blood has tested negatively for these tests. No documented cases of HIV or viral hepatitis transmission from mosquitoes to humans have occurred. The risk of accidentally transmitting malaria to a person in the community will be negligible because:

- The infected mosquitoes will be raised in secure insectary at WRAIR.
- All malaria challenges occur in a secure insectary.
- The infected mosquitoes never leave the secured insectary area at any time.
- Malaria infections in subjects will be treated promptly before gametocytes can develop (generally ten days after the development of patent malaria), thus the risk of transmission to local mosquitoes is reduced.

6.5. Outline of study procedures

Table 6 List of study procedures for the investigational vaccine groups: AduFx, 2PedFx, PedFx, and Adu2Fx

Epoch	Epoch 001										Epoch 002				
Type of contact	Visit 1 (Screening)	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9	Visit 10	Visit 11	Visit 12 to Visit 29*	Visit Par**	Visit 30	Visit 31
Study Month		0	1				7				10				13
Study Day	-90 to 0	0	28	29	35	58	196	197	203	226	286	291 to 312		314	376
Vaccination timepoint		Vacc 1	Vacc 2				Vacc 3				CHMI phase				
Informed consent	●														
Check inclusion/exclusion criteria[a]	●	O													
Check screening laboratory results[b]	●														
Collect demographic data	●														
Medical history	●														
Record information on past hepatitis B immunization[c]	●														
Physical examination	●	O	O	O	O	O	O	O	O	O	O	O	O	O	O
Urine pregnancy test (β-HCG)	●	●	●				●				●				
Check contraindications to vaccination[d]		●	●				●								
Record pre-vaccination body temperature		●	●				●								
Randomization	●														
Treatment number allocation		O	O				O								
Recording of administered treatment number		●	●				●								
Vaccines administration		●	●				●								
Check contraindications to challenge[d]											●				
Sporozoite challenge											●				
Distribution of emergency notification card											O				
Blood sampling for HIV, HCV and HBsAg (17 ml)	●														
Blood sampling for assessment of parasitemia (blood smear; 2.0 ml)												●***		●	
Blood sampling for assessment of parasitemia at start of hotel phase (PCR; 2.0 ml)												O****			

Epoch	Epoch 001										Epoch 002				
Type of contact	Visit 1 (Screening)	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9	Visit 10	Visit 11	Visit 12 to Visit 29*	Visit Par**	Visit 30	Visit 31
Study Month		0	1				7				10				13
Study Day	-90 to 0	0	28	29	35	58	196	197	203	226	286	291 to 312		314	376
Vaccination timepoint		Vacc 1	Vacc 2				Vacc 3				CHMI phase				
Blood sampling for assessment of antibody determination and serum repository (20.0 ml)		●				●	●			●	●			●	●
Blood sampling for RNA sequencing (12 ml)		●	●	●			●	●							
Blood sampling for PBMC and plasma repository (30.0 ml)		●	●		●	●	●		●	●	●				●
Blood sampling for hematology and biochemistry analysis (7.5 ml)[e]	●				●	●			●	●			●	●	
Record any concomitant medications/vaccinations	●	●	●	●	●	●	●	●	●	●	●	●		●	●
Record any intercurrent medical conditions	●	●	●	●	●	●	●	●	●	●	●	●		●	●
Distribution of diary cards		O	O				O								
Return of diary cards			O			O				O					
Diary card transcription by investigator			●			●				●					
Recording of solicited local and general AEs (Days 0–6) post-vaccination		●	●				●								
Recording of unsolicited AEs (Day 0-29) post-vaccination		●	●	●	●	●	●	●	●						
Recording of AEs (Day 0-29) post-challenge											●	●	●	●	
Recording of SAEs (all, fatal, related) and pregnancies		●	●	●	●	●	●	●	●	●	●	●	●	●	●
Recording of SAEs related to study participation, or to a concurrent GSK medication/vaccine	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Recording of all AEs and SAEs leading to withdrawal from further vaccination		●	●	●	●	●	●	●	●	●	●	●	●	●	●
Recording of AEs of specific interest[f]		●	●	●	●	●	●	●	●	●	●	●	●	●	●
Study Conclusion															●

β-HCG: Beta-human chorionic gonadotropin; PBMC: peripheral blood mononuclear cells; Vacc: vaccination.

* Visit 12 = 291, Visit 13 = Day 292, Visit 14 = Day 293, Visit 15 = Day 294, Visit 16 = Day 295, Visit 17 = Day 296, Visit 18 = Day 297, Visit 19 = Day 298, Visit 20 = Day 299, Visit 21 = Day 300, Visit 22 = Day 301, Visit 23 = Day 302, Visit 24 = Day 303, Visit 25 = Day 304, Visit 26 = Day 306, Visit 27 = Day 308, Visit 28 = Day 310, Visit 29 = Day 312.

** A blood sample for biochemistry and hematology parameters will be collected the day of first parasitemia (between Visit 12 [Day 291] and Visit 29 [Day 312]).

*** Blood sample for assessment of parasitemia (blood smear) will be collected daily for 14 days (from Day 291 [Visit 12] to Day 304 [Visit 25]) and then every two days for nine days (Day 306 [Visit 26], Day 308 [Visit 27], Day 310 [Visit 28], Day 312 [Visit 29], and Day 314 [Visit 30]). For subjects who develop malaria, blood smear may be discontinued once the subject has three consecutive negative smears (separated by more than 12 hours) following initial treatment.

**** Blood sample for assessment of parasitemia (PCR testing) will be collected on the day of subject entry into the hotel (Day 295).

a Including a check of NHANES I criteria and electrocardiogram (see APPENDIX C).

[b] The screening laboratory results (HIV, HCV, HBsAg, ALT, AST, creatinine, hemoglobin, leukocytes [WBC], platelets, and urine β-HCG) must be checked during the screening activities and before randomization.

[c] Based on best evidences available.

[d] There is no specific section in the eCRF to record the contraindications, warnings and precautions. The absolute contraindications to further administration of study vaccines or to challenge have to be recorded in the AE or SAE section of the eCRF.

[e] Blood sampling for hematology and biochemistry analysis includes ALT, AST, creatinine, hemoglobin, leukocytes (WBC), and platelets.

[f] AEs of specific interest include meningitis and pIMDs (see Section 9.1.5).

● is used to indicate a study procedure that requires documentation in the individual eCRF.

○ is used to indicate a study procedure that does not require documentation in the individual eCRF.

Table 7 List of study procedures for the investigational vaccine group: Adu1Fx

Epoch	Epoch 001							Epoch 002				
Type of contact	Visit 1 (Screening)	Visit 2	Visit 3	Visit 7	Visit 8	Visit 9	Visit 10	Visit 11	Visit 12 to Visit 29*	Visit Par**	Visit 30	Visit 31
Study Month		0	1	7				10				13
Study Day	-90 to 0	0	28	196	197	203	226	286	291 to 312		314	376
Vaccination timepoint		Vacc 1		Vacc 2				CHMI phase				
Informed consent	●											
Check inclusion/exclusion criteria[a]	●	O										
Check screening laboratory results[b]	●											
Collect demographic data	●											
Medical history	●											
Record information on past hepatitis B immunization[c]	●											
Physical examination	●	O	O	O	O	O	O	O	O	O	O	O
Urine pregnancy test (β-HCG)	●	●		●				●				
Check contraindications to vaccination[d]		●		●								
Record pre-vaccination body temperature		●		●								
Randomization	●											
Treatment number allocation		O		O								
Recording of administered treatment number		●		●								
Vaccines administration		●		●								
Check contraindications to challenge[d]								●				
Sporozoite challenge								●				
Distribution of emergency notification card								O				
Blood sampling for HIV, HCV and HBsAg (17 ml)	●											
Blood sampling for assessment of parasitemia (blood smear; 2.0 ml)									●***		●	
Blood sampling for assessment of parasitemia at start of hotel phase (PCR; 2.0 ml)									O****			
Blood sampling for assessment of antibody determination and serum repository (20.0 ml)		●		●			●	●			●	●
Blood sampling for RNA sequencing (12 ml)		●	●	●	●							

Epoch	Epoch 001							Epoch 002				
Type of contact	Visit 1 (Screening)	Visit 2	Visit 3	Visit 7	Visit 8	Visit 9	Visit 10	Visit 11	Visit 12 to Visit 29*	Visit Par**	Visit 30	Visit 31
Study Month		0	1	7				10				13
Study Day	-90 to 0	0	28	196	197	203	226	286	291 to 312		314	376
Vaccination timepoint		Vacc 1		Vacc 2				CHMI phase				
Blood sampling for PBMC and plasma repository (30.0 ml)		●	●	●		●	●	●				●
Blood sampling for hematology and biochemistry analysis (7.5 ml)[e]	●					●	●			●	●	
Record any concomitant medications/vaccinations	●	●	●	●	●	●	●	●	●		●	●
Record any intercurrent medical conditions	●	●	●	●	●	●	●	●	●		●	●
Distribution of diary cards		O		O								
Return of diary cards			O				O					
Diary card transcription by investigator			●				●					
Recording of solicited local and general AEs (Days 0–6) post-vaccination		●		●								
Recording of unsolicited AEs (Day 0-29) post-vaccination		●	●	●	●	●						
Recording of AEs (Day 0-29) post-challenge								●	●	●	●	
Recording of SAEs (all, fatal, related) and pregnancies		●	●	●	●	●	●	●	●	●	●	●
Recording of SAEs related to study participation, or to a concurrent GSK medication/vaccine	●	●	●	●	●	●	●	●	●	●	●	●
Recording of all AEs and SAEs leading to withdrawal from further vaccination		●	●	●	●	●	●	●	●	●	●	●
Recording of AEs of specific interest[f]		●	●	●	●	●	●	●	●	●	●	●
Study Conclusion												●

β-HCG: Beta-human chorionic gonadotropin; PBMC: peripheral blood mononuclear cells; Vacc: vaccination.
*Visit 12 = Day 291, Visit 13 = Day 292, Visit 14 = Day 293, Visit 15 = Day 294, Visit 16 = Day 295, Visit 17 = Day 296, Visit 18 = Day 297, Visit 19 = Day 298, Visit 20 = Day 299, Visit 21 = Day 300, Visit 22 = Day 301, Visit 23 = Day 302, Visit 24 = Day 303, Visit 25 = Day 304, Visit 26 = Day 306, Visit 27 = Day 308, Visit 28 = Day 310, Visit 29 = Day 312.
** A blood sample for biochemistry and hematology parameters will be collected the day of first parasitemia (between Visit 12 [Day 291] and Visit 29 [Day 312]).
*** Blood sample for assessment of parasitemia (blood smear) will be collected daily for 14 days (from Day 291 [Visit 12] to Day 304 [Visit 25]) and then every two days for nine days (Day 306 [Visit 26], Day 308 [Visit 27], Day 310 [Visit 28], Day 312 [Visit 29], and Day 314 [Visit 30]). For subjects who develop malaria, blood smear may be discontinued once the subject has three consecutive negative smears (separated by more than 12 hours) following initial treatment.
**** Blood sample for assessment of parasitemia (PCR testing) will be collected on the day of subject entry into the hotel (Day 295).
[a] Including a check of NHANES I criteria and electrocardiogram (see APPENDIX C).

[b] The screening laboratory results (HIV, HCV, HBsAg, ALT, AST, creatinine, hemoglobin, leukocytes [WBC], platelets, and urine β-HCG) must be checked during the screening activities and before randomization.
[c] Based on best evidences available.
[d] There is no specific section in the eCRF to record the contraindications, warnings and precautions. The absolute contraindications to further administration of study vaccines or to challenge have to be recorded in the AE or SAE section of the eCRF.
[e] Blood sampling for hematology and biochemistry analysis includes ALT, AST, creatinine, hemoglobin, leukocytes (WBC), and platelets.
[f] AEs of specific interest include meningitis and pIMDs (see Section 9.1.5).
● is used to indicate a study procedure that requires documentation in the individual eCRF.
○ is used to indicate a study procedure that does not require documentation in the individual eCRF.

Table 8 List of study procedures for the infectivity control group

Epoch	Epoch 002				
Type of contact	Visit 1b (Screening)*	Visit 11	Visit 12 to Visit 29**	Visit Par***	Visit 30
Study Day	230 to 286	286	291 to 312		314
Informed consent	●				
Check inclusion/exclusion criteria[a]	●	O			
Check screening laboratory results[b]	●				
Collect demographic data	●				
Medical history	●				
Record information on past hepatitis B immunization[c]	●				
Physical examination	●	O	O	O	O
Urine pregnancy test (β-HCG)	●	●			
Check contraindications to challenge[d]		●			
Sporozoite challenge		●			
Distribution of emergency notification card		O			
Blood sampling for HIV, HCV and HBsAg (17 ml)	●				
Blood sampling for assessment of parasitemia (blood smear; 2.0 ml)			●****		●
Blood sampling for assessment of parasitemia at start of hotel phase (PCR; 2.0 ml)			O*****		
Blood sampling for hematology and biochemistry analysis (7.5 ml)[e]	●			●	●
Record any concomitant medications/vaccinations	●	●	●		●
Record any intercurrent medical conditions	●	●	●		●
Recording of AEs (Day 0-29) post-challenge		●	●	●	●
Recording of SAEs (all, fatal, related) and pregnancies		●	●	●	●
Recording of SAEs related to study participation, or to a concurrent GSK medication/vaccine	●	●	●	●	●
Study Conclusion					●

β-HCG: Beta-human chorionic gonadotropin; PBMC: peripheral blood mononuclear cells.
* This visit is only applicable to the infectivity control group
**Visit 12 = Day 291, Visit 13 = Day 292, Visit 14 = Day 293, Visit 15 = Day 294, Visit 16 = Day 295, Visit 17 = Day 296, Visit 18 = Day 297, Visit 19 = Day 298, Visit 20 = Day 299, Visit 21 = Day 300, Visit 22 = Day 301, Visit 23 = Day 302, Visit 24 = Day 303, Visit 25 = Day 304, Visit 26 = Day 306, Visit 27 = Day 308, Visit 28 = Day 310, Visit 29 = Day 312.
*** A blood sample for biochemistry and hematology parameters will be collected the day of first parasitemia (between Visit 12 [Day 291] and Visit 29 [Day 312]).
**** Blood sample for assessment of parasitemia (blood smear) will be collected daily for 14 days (from Day 291 [Visit 12] to Day 304 [Visit 25]) and then every two days for nine days (Day 306 [Visit 26], Day 308 [Visit 27], Day 310 [Visit 28], Day 312 [Visit 29], and Day 314 [Visit 30]). For subjects who develop malaria, blood smear may be discontinued once the subject has three consecutive negative smears (separated by more than 12 hours) following initial treatment.
***** Blood sample for assessment of parasitemia (PCR testing) will be collected on the day of subject entry into the hotel (Day 295)

[a] Including a check of NHANES I criteria and electrocardiogram (see APPENDIX C).

[b] The screening laboratory results (HIV, HCV, HBsAg, ALT, AST, creatinine, hemoglobin, leukocytes [WBC], platelets, and urine β-HCG) must be checked during the screening activities and before randomization.

[c] Based on best evidences available.

[d] There is no specific section in the eCRF to record the contraindications, warnings and precautions. The absolute contraindications to challenge have to be recorded in the AE or SAE section of the eCRF.

[e] Blood sampling for hematology and biochemistry analysis includes ALT, AST, creatinine, hemoglobin, leukocytes (WBC), and platelets.

● is used to indicate a study procedure that requires documentation in the individual eCRF.

○ is used to indicate a study procedure that does not require documentation in the individual eCRF.

Table 9 Intervals between study visits for the investigational vaccine groups: AduFx, 2PedFx, PedFx, and Adu2Fx

Interval	Optimal length of interval [1]	Allowed interval[2]
Visit 1 (Screening) → Visit 2 (1st vaccination)	0 to 90 days	-
Visit 2 → Visit 3 (2nd vaccination)	28 days	± 7 days
Visit 3 → Visit 5	7 days	± 1 day
Visit 3 → Visit 6	30 days	± 7 days
Visit 3 → Visit 7 (3rd vaccination)	168 days	± 14 days
Visit 7 → Visit 9	7 days	± 1 day
Visit 7 → Visit 10	30 days	± 7 days
Visit 7 → Visit 11 (Challenge)	90 days	+ 14 days
Visit 11 → Visit 30	28 days	± 7 days
Visit 30 → Visit 31	62 days	± 14 days

[1] Whenever possible the investigator should arrange study visits within this interval.
[2] Subjects may not be eligible for inclusion in the according-to-protocol (ATP) cohort for analysis of immunogenicity and efficacy if they make the study visit outside this interval.

Table 10 Intervals between study visits for the investigational vaccine groups: Adu1Fx

Interval	Optimal length of interval [1]	Allowed interval[2]
Visit 1 (Screening) → Visit 2 (1st vaccination)	0 to 90 days	-
Visit 2 → Visit 7 (2nd vaccination)	196 days	± 14 days
Visit 7 → Visit 9	7 days	± 1 day
Visit 7 → Visit 10	30 days	± 7 days
Visit 7 → Visit 11 (Challenge)	90 days	+ 14 days
Visit 11 → Visit 30	28 days	± 7 days
Visit 30 → Visit 31	62 days	± 14 days

[1] Whenever possible the investigator should arrange study visits within this interval.
[2] Subjects may not be eligible for inclusion in the ATP cohort for analysis of immunogenicity and efficacy if they make the study visit outside this interval.

Table 11 Intervals between study visits for the infectivity control group

Interval	Optimal length of interval [1]	Allowed interval[2]
Visit 1b (Screening) → Visit 11 (Challenge)	0 to 56 days	-
Visit 11 → Visit 30	28 days	± 7 days

[1] Whenever possible the investigator should arrange study visits within this interval.
[2] Subjects may not be eligible for inclusion in the ATP cohort for analysis of immunogenicity and efficacy if they make the study visit outside this interval.

6.6. Detailed description of study procedures

6.6.1. Informed consent

The signed/witnessed/thumb printed informed consent of the subject must be obtained before study participation. Refer to Section 6.1 for the requirements on how to obtain informed consent.

6.6.2. Check inclusion and exclusion criteria

Check all inclusion and exclusion criteria as described in Sections 5.2 and 5.3 before enrolment.

The check of inclusion and exclusion criteria will include an electrocardiogram and the check of NHANES I criteria (see APPENDIX C).

6.6.3. Check screening laboratory results

Check the results of the laboratory tests for HIV, HCV, HBsAg, ALT, AST, creatinine, hemoglobin, leukocytes (WBC), platelets, and urine β-HCG to assess if the subject meets the related exclusion criteria as described in Section 5.3.

6.6.4. Collect demographic data

Record demographic data such as date of birth, gender, and geographic ancestry in the subject's eCRF.

6.6.5. Medical history

Obtain the subject's medical history by interview and/or review of the subject's medical records and record any pre-existing conditions or signs and/or symptoms present in a subject prior to the first study vaccination in the eCRF.

6.6.6. Record information on past hepatitis B immunization

Record information on past hepatitis B immunization in the subject's eCRF, based on best evidences available.

6.6.7. Physical examination

Perform a physical examination of the subject, including assessment of body temperature and recording of height and body weight. Collected information needs to be recorded in the eCRF.

Physical examination at each study visit subsequent to the first visit, will be performed only if the subject indicates during questioning that there might be some underlying pathology(ies) or if deemed necessary by the investigator or delegate.

Treatment of any abnormality observed during physical examination has to be performed according to local medical practice outside this study or by referral to an appropriate health care provider.

6.6.8. Urine pregnancy test

Female subjects are to have a urine pregnancy test at the screening visit and prior to any study vaccine administration. The study vaccines may only be administered if the pregnancy test is negative. Note: The urine pregnancy test must be performed even if the subject is menstruating at the time of the study visit.

Female subjects are also to have a urine pregnancy test prior to the sporozoite challenge. The sporozoite challenge may only be performed if the pregnancy test is negative.

6.6.9. Check contraindications to vaccination

Contraindications to vaccination must be checked at the beginning of each vaccination visit. Refer to Section 7.5 for more details.

This procedure is only applicable to subjects of the investigational vaccine groups, i.e. AduFx, 2PedFx, PedFx, Adu2Fx, and Adu1Fx.

6.6.10. Assess pre-vaccination body temperature

The axillary, rectal, oral or tympanic body temperature of all subjects needs to be measured prior to any study vaccines administration. The preferred route for recording temperature in this study will be oral. If the subject has fever (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) on the day of vaccination, the vaccination visit will be rescheduled within the allowed interval for this visit (see Table 9 and Table 10).

This procedure is only applicable to subjects of the investigational vaccine groups, i.e. AduFx, 2PedFx, PedFx, Adu2Fx, and Adu1Fx.

6.6.11. Randomization and treatment number allocation

Study group and treatment number allocation will be performed as described in Section 6.2.2. The number of each administered treatment must be recorded in the eCRF.

This procedure is only applicable to subjects of the investigational vaccine groups, i.e. AduFx, 2PedFx, PedFx, Adu2Fx, and Adu1Fx.

6.6.12. Study vaccines administration

- After completing all prerequisite procedures prior to vaccination, one dose of study vaccine will be administered intramuscularly in the deltoid of the non-dominant arm (refer to Section 7.3 for detailed description of the vaccines administration procedure). If the investigator or delegate determines that the subject's health on the day of administration temporarily precludes vaccine administration, the visit will be rescheduled within the allowed interval for this visit (see Table 9 and Table 10).
- The subjects will be observed closely for at least 30 minutes following the administration of the vaccine, with appropriate medical treatment readily available in case of anaphylaxis.
- This procedure is only applicable to subjects of the investigational vaccine groups, i.e. AduFx, 2PedFx, PedFx, Adu2Fx, and Adu1Fx.

6.6.13. Check contraindications to challenge

Contraindications to sporozoite challenge are to be checked for all subjects at the beginning of the challenge visit as described in Section 6.4.1.1.

6.6.14. Sporozoite challenge

After completing the prerequisite procedures prior to challenge, all subjects will be challenged with malaria as outlined in Section 6.4.1.3.

6.6.15. Distribution of emergency notification card

Upon entry into the challenge phase of the study, all subjects will be issued an emergency notification card containing the subject's name, details on the exposure to malaria and a 24-hour emergency telephone contact numbers for study investigators.

6.6.16. Sampling

Refer to the Module on Biospecimen Management in the SPM for detailed instructions for the collection, handling and processing of the samples.

- **Blood sampling for HIV, HCV and HBsAg**

  A volume of approximately 17.0 ml of whole blood should be drawn from all subjects screened to assess HIV, HCV and HBsAg status (see Table 6, Table 7 and Table 8).

- **Blood sampling for initial assessment of parasitemia (PCR)**

  A volume of approximately 2.0 ml should be drawn from all subjects on the day of subject entry to the hotel phase (Day 295 [Visit 16]) for the assessment of parasitemia by PCR testing to provide the study team an estimate of the number of subjects likely to develop malaria within the first few days of the hotel phase (see Table 6, Table 7 and Table 8).

- **Blood sampling for assessment of parasitemia (blood smear)**

  A volume of approximately 2.0 ml should be drawn from all subjects at each pre-defined timepoint for the assessment of parasitemia by blood smear (see Table 6, Table 7 and Table 8). For subjects who develop malaria, blood samples for smears may be discontinued once the subject has three consecutive negative smears (separated by more than 12 hours) following initial treatment.

- **Blood sampling for assessment of antibody determination and serum repository**

  For subjects of the investigational vaccine groups (AduFx, 2PedFx, PedFx, Adu2Fx, and Adu1Fx), a volume of at least 20.0 ml of whole blood (to provide at least 10 ml of serum for anti-CS enzyme-linked immunosorbent assay [ELISA], anti-CS avidity, anti-HBs, and serum repository) should be drawn at each pre-defined timepoint (see Table 6 and Table 7). After centrifugation, serum samples should be kept at –20°C/ –4°F or below until shipment.

- **Blood sampling for RNA sequencing**

  For subjects of the investigational vaccine groups (AduFx, 2PedFx, PedFx, Adu2Fx, and Adu1Fx), a volume of approximately 12.0 ml of whole blood should be drawn at each pre-defined timepoint for RNA sequencing (mRNA sequencing analysis; see Table 6 and Table 7). The blood should be stored at room temperature (20 to 25°C) until they are transferred to the designated laboratory for further processing.

- **Blood sampling for PBMC and plasma repository**

  For subjects of the investigational vaccine groups (AduFx, 2PedFx, PedFx, Adu2Fx, and Adu1Fx), a volume of approximately 30.0 ml of whole blood should be drawn at each pre-defined timepoint for the purpose of the PBMC and plasma repository (see Table 6 and Table 7). The blood should be stored at room temperature until it is transferred to the designated laboratory for further processing. The purified PBMC should be stored in liquid nitrogen and plasma at -20°C or colder until further processing.

- **Blood sampling for hematology and biochemistry analysis**

  A volume of approximately 7.5 ml of whole blood should be drawn from all subjects at each pre-defined timepoint for the assessment of safety parameters (ALT, AST, creatinine, hemoglobin, leukocytes [WBC], and platelets; see Table 6, Table 7 and Table 8).

6.6.17. Check and record concomitant medication/vaccination and intercurrent medical conditions

Concomitant medication/vaccination must be checked and recorded in the eCRF as described in Section 7.6.

Intercurrent medical conditions must be checked and recorded in the eCRF as described in Section 7.7.

6.6.18. Recording of AEs, SAEs, pregnancies and AEs of specific interest

- For subjects of the investigational vaccine groups (AduFx, 2PedFx, PedFx, Adu2Fx, and Adu1Fx)
  - Refer to Section 9.3 for procedures for the investigator to record AEs, SAEs, pregnancies and AEs of specific interest (meningitis and pIMDs). Refer to Section 9.4 for guidelines and how to report SAE, pregnancy and AEs of specific interest reports to GSK Biologicals.
  - The subjects will be instructed to contact the investigator immediately should they manifest any signs or symptoms they perceive as serious.
  - At each vaccination visit, diary cards will be provided to the subject. The subject will record body (oral) temperature and any solicited local/general AEs (i.e. on the day of vaccination and during the next six days) or any unsolicited AEs (i.e. on the day of vaccination and during the next 29 days) occurring after vaccination. The subject will be instructed to return the completed diary card to the investigator at the next study visit.
  - Collect and verify completed diary cards during discussion with the subject on Visit 3, Visit 6 and Visit 10 for subjects of AduFx, 2PedFx, PedFx, and Adu2Fx groups and on Visit 3 and Visit 10 for subjects of Adu1Fx groups.
  - Any unreturned diary cards will be sought from the subject through telephone call(s) or any other convenient procedure. The investigator will transcribe the collected information into the eCRF in English.
- For subjects of the infectivity controls group
  - Refer to Section 9.3 for procedures for the investigator to record AEs, SAEs and pregnancies. Refer to Section 9.4 for guidelines and how to report SAE and pregnancy reports to GSK Biologicals.
  - The subjects will be instructed to contact the investigator immediately should they manifest any signs or symptoms they perceive as serious.

6.6.19. Study conclusion

The investigator will:

- review data collected to ensure accuracy and completeness
- complete the Study Conclusion screen in the eCRF.

6.7. Biological sample handling and analysis

Please refer to the SPM for details on biospecimen management (handling, storage and shipment).

Samples will not be labeled with information that directly identifies the subject but will be coded with the identification number for the subject (subject number).

- Collected samples will be used for protocol mandated research and purposes related to the improvement, development and quality assurance of the laboratory tests described in this protocol. This may include the management of the quality of these tests, the maintenance or improvement of these tests, the development of new test methods, as well as making sure that new tests are comparable to previous methods and work reliably.
- It is also possible that future findings may make it desirable to use the samples acquired in this study for future research, not described in this protocol. Therefore, all subjects in countries where this is allowed, will be asked to give a specific consent to allow GSK or a contracted partner to use the samples for future research. Future research will be subject to the laws and regulations in the respective countries and will only be performed once an independent Ethics Committee or Review Board has approved this research.

Information on further investigations and their rationale can be obtained from GSK Biologicals.

Any sample testing will be done in line with the consent of the individual subject.

Refer also to the Investigator Agreement, where it is noted that the investigator cannot perform any other biological assays except those described in the protocol or its amendment(s).

If additional testing is performed, the marker priority ranking given in Section 6.7.4 may be changed.

Collected samples will be stored for a maximum of 20 years (counting from when the last subject performed the last study visit), unless local rules, regulations or guidelines require different timeframes or different procedures, which will then be in line with the subject consent. These extra requirements need to be communicated formally to and discussed and agreed with GSK Biologicals.

6.7.1. Use of specified study materials

When materials are provided by GSK Biologicals, it is MANDATORY that all clinical samples (including serum samples) be collected and stored exclusively using those materials in the appropriate manner. The use of other materials could result in the exclusion of the subject from the ATP analysis (See Section 11.5 for the definition of cohorts to be analyzed). The investigator must ensure that his/her personnel and the laboratory(ies) under his/her supervision comply with this requirement. However, when GSK Biologicals does not provide material for collecting and storing clinical samples, appropriate materials from the investigator's site must be used. Refer to the Module on Clinical Trial Supplies in the SPM.

6.7.2. Biological samples

Details of the quantity of biological sample to be taken at each timepoint during the study are provided in Table 12.

Table 12 Biological samples (whole blood)

Study Phase	Timepoint	Sample type	Total volume of blood per visit (ml)					
			AduFx	2PedFx	PedFx	Adu2Fx	Adu1Fx	Infectivity control
Pre-vaccination	Visit 1 (Screening)	Blood sampling for HIV, HCV and HBsAg	17.0 ml	17.0 ml	17.0 ml	17.0 ml	17.0 ml	-
		Blood sampling for hematology and biochemistry analysis	7.5 ml	7.5 ml	7.5 ml	7.5 ml	7.5 ml	-
		Total:	**24.5 ml**	**24.5 ml**	**24.5 ml**	**24.5 ml**	**24.5 ml**	-
	Visit 2 (Day 0)	Blood sampling for antibody determination and serum repository	20.0 ml	20.0 ml	20.0 ml	20.0 ml	20.0 ml	-
		Blood sampling for RNA sequencing	12.0 ml	12.0 ml	12.0 ml	12.0 ml	12.0 ml	-
		Blood sampling for PBMC and plasma repository	30.0 ml	30.0 ml	30.0 ml	30.0 ml	30.0 ml	-
		Total:	**62.0 ml**	**62.0 ml**	**62.0 ml**	**62.0 ml**	**62.0 ml**	-
Post-Dose 1	Visit 3 (Day 28)	Blood sampling for RNA sequencing	12.0 ml	12.0 ml	12.0 ml	12.0 ml	12.0 ml	-
		Blood sampling for PBMC and plasma repository	30.0 ml	30.0 ml	30.0 ml	30.0 ml	30.0 ml	-
		Total:	**42.0 ml**	**42.0 ml**	**42.0 ml**	**42.0 ml**	**42.0 ml**	-
Post-Dose 2* or Post-Dose 1**	Visit 4 (Day 29)	Blood sampling for RNA sequencing	12.0 ml	12.0 ml	12.0 ml	12.0 ml	-	-
		Total:	**12.0 ml**	**12.0 ml**	**12.0 ml**	**12.0 ml**	-	-
	Visit 5 (Day 35)	Blood sampling for PBMC and plasma repository	30.0 ml	30.0 ml	30.0 ml	30.0 ml	-	-
		Blood sampling for hematology and biochemistry analysis	7.5 ml	7.5 ml	7.5 ml	7.5 ml	-	-
		Total:	**37.5 ml**	**37.5 ml**	**37.5 ml**	**37.5 ml**	-	-
	Visit 6 (Day 58)	Blood sampling for antibody determination and serum repository	20.0 ml	20.0 ml	20.0 ml	20.0 ml	-	-
		Blood sampling for PBMC and plasma repository	30.0 ml	30.0 ml	30.0 ml	30.0 ml	-	-
		Blood sampling for hematology and biochemistry analysis	7.5 ml	7.5 ml	7.5 ml	7.5 ml	-	-
		Total:	**57.5 ml**	**57.5 ml**	**57.5 ml**	**57.5 ml**	-	-
	Visit 7 (Day 196)	Blood sampling for antibody determination and serum repository	20.0 ml	20.0 ml	20.0 ml	20.0 ml	20.0 ml	-
		Blood sampling for RNA sequencing	12.0 ml	12.0 ml	12.0 ml	12.0 ml	12.0 ml	-
		Blood sampling for PBMC and plasma repository	30.0 ml	30.0 ml	30.0 ml	30.0 ml	30.0 ml	-
		Total:	**62.0 ml**	**62.0 ml**	**62.0 ml**	**62.0 ml**	**62.0 ml**	-

Study Phase	Timepoint	Sample type	Total volume of blood per visit (ml)					
			AduFx	2PedFx	PedFx	Adu2Fx	Adu1Fx	Infectivity control
Post-Dose 3* or Post-Dose 2**	Visit 8 (Day 197)	Blood sampling for RNA sequencing	12.0 ml	12.0 ml	12.0 ml	12.0 ml	12.0 ml	-
		Total:	**12.0 ml**	**12.0 ml**	**12.0 ml**	**12.0 ml**	**12.0 ml**	-
	Visit 9 (Day 203)	Blood sampling for PBMC and plasma repository	30.0 ml	30.0 ml	30.0 ml	30.0 ml	30.0 ml	-
		Blood sampling for hematology and biochemistry analysis	7.5 ml	7.5 ml	7.5 ml	7.5 ml	7.5 ml	-
		Total:	**37.5 ml**	**37.5 ml**	**37.5 ml**	**37.5 ml**	**37.5 ml**	-
	Visit 10 (Day 226)	Blood sampling for antibody determination and serum repository	20.0 ml	20.0 ml	20.0 ml	20.0 ml	20.0 ml	-
		Blood sampling for PBMC and plasma repository	30.0 ml	30.0 ml	30.0 ml	30.0 ml	30.0 ml	-
		Blood sampling for hematology and biochemistry analysis	7.5 ml	7.5 ml	7.5 ml	7.5 ml	7.5 ml	-
		Total:	**57.5 ml**	**57.5 ml**	**57.5 ml**	**57.5 ml**	**57.5 ml**	-
Pre-challenge	Visit 1b (Screening)	Blood sampling for HIV, HCV and HBsAg	-	-	-	-	-	17.0 ml
		Blood sampling for hematology and biochemistry analysis	-	-	-	-	-	7.5 ml
		Total:	-	-	-	-	-	**24.5 ml**
	Visit 11 (Day 286)	Blood sampling for antibody determination and serum repository	20.0 ml	20.0 ml	20.0 ml	20.0 ml	20.0 ml	-
		Blood sampling for PBMC and plasma repository	30.0 ml	30.0 ml	30.0 ml	30.0 ml	30.0 ml	-
		Total:	**50.0 ml**	**50.0 ml**	**50.0 ml**	**50.0 ml**	**50.0 ml**	-
Post-challenge	Visit 12 (Day 291)	Blood sampling for assessment of parasitemia (blood smear)	2.0 ml	2.0 ml	2.0 ml	2.0 ml	2.0 ml	2.0 ml
	Visit 13 (Day 292)	Blood sampling for assessment of parasitemia (blood smear)	2.0 ml	2.0 ml	2.0 ml	2.0 ml	2.0 ml	2.0 ml
	Visit 14 (Day 293)	Blood sampling for assessment of parasitemia (blood smear)	2.0 ml	2.0 ml	2.0 ml	2.0 ml	2.0 ml	2.0 ml
	Visit 15 (Day 294)	Blood sampling for assessment of parasitemia (blood smear)	2.0 ml	2.0 ml	2.0 ml	2.0 ml	2.0 ml	2.0 ml
	Visit 16 (Day 295)	Blood sampling for assessment of parasitemia (blood smear)	2.0 ml	2.0 ml	2.0 ml	2.0 ml	2.0 ml	2.0 ml
		Blood sampling for assessment of parasitemia at start of hotel phase (PCR)****	2.0 ml	2.0 ml	2.0 ml	2.0 ml	2.0 ml	2.0 ml
	Visit 17 (Day 296)	Blood sampling for assessment of parasitemia (blood smear)	2.0 ml	2.0 ml	2.0 ml	2.0 ml	2.0 ml	2.0 ml
	Visit 18 (Day 297)	Blood sampling for assessment of parasitemia (blood smear)	2.0 ml	2.0 ml	2.0 ml	2.0 ml	2.0 ml	2.0 ml
	Visit 19 (Day 298)	Blood sampling for assessment of parasitemia (blood smear)	2.0 ml	2.0 ml	2.0 ml	2.0 ml	2.0 ml	2.0 ml
	Visit 20 (Day 299)	Blood sampling for assessment of parasitemia (blood smear)	2.0 ml	2.0 ml	2.0 ml	2.0 ml	2.0 ml	2.0 ml
	Visit 21 (Day 300)	Blood sampling for assessment of parasitemia (blood smear)	2.0 ml	2.0 ml	2.0 ml	2.0 ml	2.0 ml	2.0 ml
	Visit 22 (Day 301)	Blood sampling for assessment of parasitemia (blood smear)	2.0 ml	2.0 ml	2.0 ml	2.0 ml	2.0 ml	2.0 ml
	Visit 23 (Day 302)	Blood sampling for assessment of parasitemia (blood smear)	2.0 ml	2.0 ml	2.0 ml	2.0 ml	2.0 ml	2.0 ml
	Visit 24 (Day 303)	Blood sampling for assessment of parasitemia (blood smear)	2.0 ml	2.0 ml	2.0 ml	2.0 ml	2.0 ml	2.0 ml

Study Phase	Timepoint	Sample type	Total volume of blood per visit (ml)					
			AduFx	2PedFx	PedFx	Adu2Fx	Adu1Fx	Infectivity control
	Visit 25 (Day 304)	Blood sampling for assessment of parasitemia (blood smear)v	2.0 ml	2.0 ml	2.0 ml	2.0 ml	2.0 ml	2.0 ml
	Visit 26 (Day 306)	Blood sampling for assessment of parasitemia (blood smear)	2.0 ml	2.0 ml	2.0 ml	2.0 ml	2.0 ml	2.0 ml
	Visit 27 (Day 308)	Blood sampling for assessment of parasitemia (blood smear)	2.0 ml	2.0 ml	2.0 ml	2.0 ml	2.0 ml	2.0 ml
	Visit 28 (Day 310)	Blood sampling for assessment of parasitemia (blood smear)	2.0 ml	2.0 ml	2.0 ml	2.0 ml	2.0 ml	2.0 ml
	Visit 29 (Day 312)	Blood sampling for assessment of parasitemia (blood smear)	2.0 ml	2.0 ml	2.0 ml	2.0 ml	2.0 ml	2.0 ml
	Visit 30 (Day 314)	Blood sampling for assessment of parasitemia (blood smear)	2.0 ml	2.0 ml	2.0 ml	2.0 ml	2.0 ml	2.0 ml
		Blood sampling for antibody determination and serum repository	20.0 ml	20.0 ml	20.0 ml	20.0 ml	20.0 ml	-
		Blood sampling for hematology and biochemistry analysis	7.5 ml	7.5 ml	7.5 ml	7.5 ml	7.5 ml	7.5 ml
		Total:	**29.5 ml**	**31.5 ml**	**31.5 ml**	**31.5 ml**	**31.5 ml**	**9.5 ml**
	Visit Par***	Blood sampling for hematology and biochemistry analysis	7.5 ml	7.5 ml	7.5 ml	7.5 ml	7.5 ml	7.5 ml
		Total:	**7.5 ml**	**7.5 ml**	**7.5 ml**	**7.5 ml**	**7.5 ml**	**7.5 ml**
Study end	Visit 31 (Day 376)	Blood sampling for antibody determination and serum repository	20.0 ml	20.0 ml	20.0 ml	20.0 ml	20.0 ml	-
		Blood sampling for PBMC and plasma repository	30.0 ml	30.0 ml	30.0 ml	30.0 ml	30.0 ml	-
		Total:	**50.0 ml**	**50.0 ml**	**50.0 ml**	**50.0 ml**	**50.0 ml**	-
		Total blood collected over the entire study:	**579.5 ml**	**579.5 ml**	**579.5 ml**	**579.5 ml**	**472.5 ml**	**79.5 ml**

* For groups AduFx, 2PedFx, PedFx and Adu2Fx.
** For group Adu1Fx.
*** Biochemistry and hematology parameters will be collected the day of first parasitemia (between Visit 12 [Day 291] and Visit 29 [Day 312]).
**** Blood sample for assessment of parasitemia by PCR will be collected on the day of subject entry into the hotel (Day 295 [Visit 16]).

6.7.3. Laboratory assays

Please refer to APPENDIX A for a detailed description of the assays performed in the study. Please refer to APPENDIX B for the address of the clinical laboratories used for sample analysis.

Serological assays for the determination of anti-CS antibodies will be performed by ELISA at laboratories designated by GSK Biologicals using standardized and validated procedures (refer to Table 13).

Serological assays for the determination of anti-HBs antibodies will be performed by chemiluminescence enzyme immunoassay (CLIA) at a GSK Biologicals' laboratory using standardized and validated procedures (refer to Table 13).

Table 13 Assays for humoral immunity (antibody determination)

System	Component	Method	Kit / Manufacturer	Unit	Cut-off	Laboratory
SERUM	Plasmodium falciparum.Circumsporozoite Protein.R32LR Ab.IgG	ELISA	In house	EU/ml	0.5	CEVAC
SERUM	Plasmodium falciparum.Circumsporozoide Protein.R32LR Ab.IgG Avidity	ELISA	In house	%	Not applicable	CEVAC
SERUM	Plasmodium falciparum.anti-C-Term Circumsporozoide Ab.IgG	ELISA	In house	EPT	100	WRAIR
SERUM	Plasmodium falciparum.anti-C-Term Circumsporozoide Ab.IgG avidity	ELISA	In house	Avidity Index	Not applicable	WRAIR
SERUM	Plasmodium falciparum.Circumsporozoite Full length(N+C-Terminal) Recombinant+NANP Protein+NVDP Protein Ab.IgG	ELISA	In house	1/DIL	Not applicable	WRAIR
SERUM	Plasmodium falciparum.anti-full length Circumsporozoide Ab.IgG avidity	ELISA	In house	Avidity Index	Not applicable	WRAIR
SERUM	Hepatitis B Virus.Surface Ab	CLIA	ADVIA Centaur anti-HBs2 (Siemens Healthcare)	mIU/ml	6.2	GSK Biologicals*

CEVAC: Center for Vaccinology; CLIA: chemiluminescence enzyme immunoassay; ELISA: Enzyme-linked immunosorbent assay; EPT: endpoint titer; IgG: Immunoglobulin G; WRAIR: Walter Reed Army Institute of Research

* GSK Biologicals laboratory refers to Clinical Laboratory Sciences (CLS) in Rixensart, Belgium; Wavre, Belgium.

Other assays on stored serum, PBMC and plasma samples may be performed based on results of ongoing studies to investigate the safety and/or vaccine induced anti-malaria and hepatitis B immune responses.

Table 14 Assessment of *P. falciparum* parasitemia

System	Component	Method	Unit	Laboratory
WHOLE BLOOD	Plasmodium falciparum parasites*	Not applicable**	parasite/μl	WRAIR
WHOLE BLOOD	Plasmodium falciparum parasites*	Real-time PCR	Positive/negative***	WRAIR

WRAIR: Walter Reed Army Institute of Research
* *P. falciparum* parasite count includes blood-stage parasites.
** Method used will be blood slide microscope reading.
*** For assessment and processing by WRAIR of parasitemia on the day of subject entry into the hotel (Day 295 [Visit 16])

Table 15 Hematology, biochemistry and screening tests

System	Component	Method	Scale	Laboratory
SERUM	HIV-IgG + Ag (SER,GLR)	Immunoassay	Qualitative	Quest Diagnostics, Inc.
SERUM	Hepatitis B Virus.Surface Ab	CLIA	Qualitative	Quest Diagnostics, Inc.
SERUM	HCV Ab	Immunoassay	Qualitative	Quest Diagnostics, Inc.
URINE	β-HCG*	Immunoassay	Qualitative	WRAIR
WHOLE BLOOD	Hemoglobin Leukocytes (White Blood Cells) Platelets	Not applicable	Quantitative	Quest Diagnostics, Inc.
SERUM	Alanine Aminotransferase Aspartate Aminotransferase Creatinine	Not applicable	Quantitative	Quest Diagnostics, Inc.

β-HCG: Beta-human chorionic gonadotropin; CLIA: chemiluminescence enzyme immunoassay, HCV: hepatitis C virus, HIV: human immunodeficiency virus, WRAIR: Walter Reed Army Institute of Research
*Urinary pregnancy test

PBMC for mRNA expression profiling will be collected and stored in a repository to allow mRNA sequencing or future next generation technologies, during the current study or in future research, to assess transcriptome signals. Scope of this assay will be based on ongoing investigations.

The repository laboratory for serum samples will be GSK Biologicals' clinical laboratories and for plasma and PBMC samples will be Precision Bioservices Inc.

The GSK Biologicals' clinical laboratories have established a Quality System supported by procedures. The activities of GSK Biologicals' clinical laboratories are audited regularly for quality assessment by an internal (sponsor-dependent) but laboratory-independent Quality Department.

6.7.4. Biological samples evaluation

6.7.4.1. Immunological read-outs

Table 16 Immunological read-outs

Blood sampling timepoint		Study groups	No. subjects	Sample	Component	Components priority rank
Type of contact and timepoint	Sampling timepoint					
Visit 2 (Day 0)	Pre-vaccination	AduFx, 2PedFx, PedFx, Adu2Fx, Adu1Fx	130	Serum	Plasmodium falciparum.Circumsporozoite Protein.R32LR Ab.IgG	1
					Plasmodium falciparum.Circumsporozoide Protein.R32LR Ab.IgG Avidity	2
					Plasmodium falciparum.anti-C-Term Circumsporozoide Ab.IgG	3
					Plasmodium falciparum.anti-C-Term Circumsporozoide Ab.IgG avidity	4
					Hepatitis B Virus.Surface Ab	5
				Serum repository*	To be determined	Not applicable
				PBMC and plasma repository*	To be determined	Not applicable
Visit 3 (Day 28)	One month post-Dose 1	AduFx, 2PedFx, PedFx, Adu2Fx, Adu1Fx	130	PBMC and plasma repository*	To be determined	Not applicable
Visit 5 (Day 35)	Seven days post-Dose 2	AduFx, 2PedFx, PedFx, Adu2Fx	104	PBMC and plasma repository*	To be determined	Not applicable

Blood sampling timepoint		Study groups	No. subjects	Sample	Component	Components priority rank
Type of contact and timepoint	Sampling timepoint					
Visit 6 (Day 58)	One month post-Dose 2	AduFx, 2PedFx, PedFx, Adu2Fx	104	Serum	Plasmodium falciparum.Circumsporozoite Protein.R32LR Ab.IgG	1
					Plasmodium falciparum.Circumsporozoide Protein.R32LR Ab.IgG Avidity	2
					Plasmodium falciparum.anti-C-Term Circumsporozoide Ab.IgG	3
					Plasmodium falciparum.anti-C-Term Circumsporozoide Ab.IgG avidity	4
					Hepatitis B Virus.Surface Ab	5
				Serum repository*	To be determined	Not applicable
				PBMC and plasma repository*	To be determined	Not applicable
Visit 7 (Day 196)	Six months post-Dose 2** or seven month post-Dose 1***	AduFx, 2PedFx, PedFx, Adu2Fx, Adu1Fx	130	Serum	Plasmodium falciparum.Circumsporozoite Protein.R32LR Ab.IgG	1
					Plasmodium falciparum.Circumsporozoide Protein.R32LR Ab.IgG Avidity	2
					Plasmodium falciparum.anti-C-Term Circumsporozoide Ab.IgG	3
					Plasmodium falciparum.anti-C-Term Circumsporozoide Ab.IgG avidity	4
					Hepatitis B Virus.Surface Ab	5
				Serum repository*	To be determined	Not applicable
				PBMC and plasma repository*	To be determined	Not applicable
Visit 9 (Day 203)	Seven days post-Dose 3** or post-Dose 2***	AduFx, 2PedFx, PedFx, Adu1Fx, Adu2Fx	130	PBMC and plasma repository*	To be determined	Not applicable

Blood sampling timepoint		Study groups	No. subjects	Sample	Component	Components priority rank
Type of contact and timepoint	Sampling timepoint					
Visit 10 (Day 226)	One month post-Dose 3** or post-Dose 2***	AduFx, 2PedFx, PedFx, Adu2Fx, Adu1Fx	130	Serum	Plasmodium falciparum.Circumsporozoite Protein.R32LR Ab.IgG	1
					Plasmodium falciparum.Circumsporozoide Protein.R32LR Ab.IgG Avidity	2
					Plasmodium falciparum.anti-C-Term Circumsporozoide Ab.IgG	3
					Plasmodium falciparum.anti-C-Term Circumsporozoide Ab.IgG avidity	4
					Hepatitis B Virus.Surface Ab	5
				Serum repository*	To be determined	Not applicable
				PBMC and plasma repository*	To be determined	Not applicable
Visit 11 (Day 286)	Day of challenge	AduFx, 2PedFx, PedFx, Adu2Fx, Adu1Fx	130	Serum	Plasmodium falciparum.Circumsporozoite Protein.R32LR Ab.IgG	1
					Plasmodium falciparum.Circumsporozoide Protein.R32LR Ab.IgG Avidity	2
					Plasmodium falciparum.anti-C-Term Circumsporozoide Ab.IgG	3
					Plasmodium falciparum.anti-C-Term Circumsporozoide Ab.IgG avidity	4
					Hepatitis B Virus.Surface Ab	5
				Serum repository*	To be determined	Not applicable
				PBMC and plasma repository*	To be determined	Not applicable
Visit 30 (Day 314)	28 days post-challenge	AduFx, 2PedFx, PedFx, Adu2Fx, Adu1Fx	130	Serum	Plasmodium falciparum.Circumsporozoite Protein.R32LR Ab.IgG	1
					Plasmodium falciparum.Circumsporozoide Protein.R32LR Ab.IgG Avidity	2
					Plasmodium falciparum.anti-C-Term Circumsporozoide Ab.IgG	3
					Plasmodium falciparum.anti-C-Term Circumsporozoide Ab.IgG avidity	4
					Hepatitis B Virus.Surface Ab	5
				Serum repository*	To be determined	Not applicable

Blood sampling timepoint		Study groups	No. subjects	Sample	Component	Components priority rank
Type of contact and timepoint	Sampling timepoint					
Visit 31 (Day 376)	Study end	AduFx, 2PedFx, PedFx, Adu2Fx, Adu1Fx	130	Serum	Plasmodium falciparum.Circumsporozoite Protein.R32LR Ab.IgG	1
					Plasmodium falciparum.Circumsporozoide Protein.R32LR Ab.IgG Avidity	2
					Plasmodium falciparum.anti-C-Term Circumsporozoide Ab.IgG	3
					Plasmodium falciparum.anti-C-Term Circumsporozoide Ab.IgG avidity	4
					Hepatitis B Virus.Surface Ab	5
				Serum repository*	To be determined	Not applicable
				PBMC and plasma repository*	To be determined	Not applicable

* Assays on stored serum, PBMC and plasma samples may be performed based on results of ongoing studies to investigate the safety and/or vaccine induced anti-malaria and hepatitis B immune responses.
** For groups AduFx, 2PedFx, PedFx and Adu2Fx.
*** For group Adu1Fx.

In case of insufficient blood sample volume to perform assays for all antibodies, the samples will be analysed according to priority ranking provided in Table 16.

6.7.4.2. Hematology/Blood Chemistry

Table 17 Hematology and biochemistry read-outs

Blood sampling timepoint		Study groups	No. subjects	Component
Type of contact and timepoint	Sampling timepoint			
Visit 1 (Screening)	Pre-vaccination	AduFx, 2PedFx, PedFx, Adu2Fx, Adu1Fx	≥ 130	HIV-IgG + Ag (SER,GLR)
				Hepatitis B Virus.Surface Ab
				HCV Ab
				Alanine Aminotransferase
				Aspartate Aminotransferase
				Creatinine
				Hemoglobin
				Leukocytes (White Blood Cells)
				Platelets
Visit 5 (Day 35)	Seven days post-Dose 2	AduFx, 2PedFx, PedFx, Adu2Fx	104	Alanine Aminotransferase
				Aspartate Aminotransferase
				Creatinine
				Hemoglobin
				Leukocytes (White Blood Cells)
				Platelets
Visit 6 (Day 58)	One month post-Dose 2	AduFx, 2PedFx, PedFx, Adu2Fx	104	Alanine Aminotransferase
				Aspartate Aminotransferase
				Creatinine
				Hemoglobin
				Leukocytes (White Blood Cells)
				Platelets
Visit 9 (Day 203)	Seven days post-Dose 3* or post-Dose 2**	AduFx, 2PedFx, PedFx, Adu2Fx, Adu1Fx	130	Alanine Aminotransferase
				Aspartate Aminotransferase
				Creatinine
				Hemoglobin
				Leukocytes (White Blood Cells)
				Platelets
Visit 10 (Day 226)	One month post-Dose 3* or post-Dose 2**	AduFx, 2PedFx, PedFx, Adu2Fx, Adu1Fx	130	Alanine Aminotransferase
				Aspartate Aminotransferase
				Creatinine
				Hemoglobin
				Leukocytes (White Blood Cells)
				Platelets
Visit 1b (Screening)	Pre-challenge	Infectivity control	≥ 20***	HIV-IgG + Ag (SER,GLR)
				Hepatitis B Virus.Surface Ab
				HCV Ab
				Alanine Aminotransferase
				Aspartate Aminotransferase
				Creatinine
				Hemoglobin
				Leukocytes (White Blood Cells)
				Platelets
Visit Par	Event-driven (first parasitemia)	AduFx, 2PedFx, PedFx, Adu2Fx, Adu1Fx, Infectivity control	~ 150	Alanine Aminotransferase
				Aspartate Aminotransferase
				Creatinine
				Hemoglobin
				Leukocytes (White Blood Cells)
				Platelets

Blood sampling timepoint		Study groups	No. subjects	Component
Type of contact and timepoint	Sampling timepoint			
Visit 30 (Day 314)	28 days post-challenge	AduFx, 2PedFx, PedFx, Adu2Fx, Adu1Fx, Infectivity control	~ 150	Alanine Aminotransferase
				Aspartate Aminotransferase
				Creatinine
				Hemoglobin
				Leukocytes (White Blood Cells)
				Platelets

* For groups AduFx, 2PedFx, PedFx and Adu2Fx.
** For group Adu1Fx.
*** between 20 and 30 infectivity controls will be enrolled.

6.7.4.3. Molecular biology

Table 18 RNA sequencing (mRNA sequencing analysis)

Blood sampling timepoint		Study groups	No. subjects	Component	Components priority rank
Type of contact and timepoint	Sampling timepoint				
Visit 2 (Day 0)	Pre-vaccination	AduFx, 2PedFx, PedFx, Adu1Fx, Adu2Fx	130	mRNA	Not applicable
Visit 3 (Day 28)	Day of Dose 2* or one month post-Dose 1**	AduFx, 2PedFx, PedFx, Adu1Fx Adu2Fx	130	mRNA	Not applicable
Visit 4 (Day 29)	One day post-Dose 2	AduFx, 2PedFx, PedFx, Adu2Fx	104	mRNA	Not applicable
Visit 7 (Day 196)	Day of Dose 3* or Dose 2**	AduFx, 2PedFx, PedFx, Adu1Fx, Adu2Fx	130	mRNA	Not applicable
Visit 8 (Day 197)	One day post-Dose 3* or Dose 2**	AduFx, 2PedFx, PedFx, Adu1Fx, Adu2Fx	130	mRNA	Not applicable

mRNA: messenger ribonucleic acid
* For groups AduFx, 2PedFx, PedFx and Adu2Fx.
** For group Adu1Fx.

6.7.4.4. Parasitemia

Table 19 Parasitemia (blood smear)

Blood sampling timepoint	Study groups	No. subjects	Component	Components priority rank
Visit 12 (Day 291)	AduFx, 2PedFx, PedFx, Adu2Fx, Adu1Fx, Infectivity control	~ 150	Blood smear	1
Visit 13 (Day 292)	AduFx, 2PedFx, PedFx, Adu2Fx, Adu1Fx, Infectivity control	~ 150	Blood smear	1
Visit 14 (Day 293)	AduFx, 2PedFx, PedFx, Adu2Fx, Adu1Fx, Infectivity control	~ 150	Blood smear	1
Visit 15 (Day 294)	AduFx, 2PedFx, PedFx, Adu2Fx, Adu1Fx, Infectivity control	~ 150	Blood smear	1
Visit 16 (Day 295)	AduFx, 2PedFx, PedFx, Adu2Fx, Adu1Fx, Infectivity control	~ 150	Blood smear	1
	AduFx, 2PedFx, PedFx, Adu2Fx, Adu1Fx, Infectivity control	~ 150	PCR*	2
Visit 17 (Day 296)	AduFx, 2PedFx, PedFx, Adu2Fx, Adu1Fx, Infectivity control	~ 150	Blood smear	1
Visit 18 (Day 297)	AduFx, 2PedFx, PedFx, Adu2Fx, Adu1Fx, Infectivity control	~ 150	Blood smear	1
Visit 19 (Day 298)	AduFx, 2PedFx, PedFx, Adu2Fx, Adu1Fx, Infectivity control	~ 150	Blood smear	1
Visit 20 (Day 299)	AduFx, 2PedFx, PedFx, Adu2Fx, Adu1Fx, Infectivity control	~ 150	Blood smear	1
Visit 21 (Day 300)	AduFx, 2PedFx, PedFx, Adu2Fx, Adu1Fx, Infectivity control	~ 150	Blood smear	1
Visit 22 (Day 301)	AduFx, 2PedFx, PedFx, Adu2Fx, Adu1Fx, Infectivity control	~ 150	Blood smear	1
Visit 23 (Day 302)	AduFx, 2PedFx, PedFx, Adu2Fx, Adu1Fx, Infectivity control	~ 150	Blood smear	1
Visit 24 (Day 303)	AduFx, 2PedFx, PedFx, Adu2Fx, Adu1Fx, Infectivity control	~ 150	Blood smear	1
Visit 25 (Day 304)	AduFx, 2PedFx, PedFx, Adu2Fx, Adu1Fx, Infectivity control	~ 150	Blood smear	1
Visit 26 (Day 306)	AduFx, 2PedFx, PedFx, Adu2Fx, Adu1Fx, Infectivity control	~ 150	Blood smear	1
Visit 27 (Day 308)	AduFx, 2PedFx, PedFx, Adu2Fx, Adu1Fx, Infectivity control	~ 150	Blood smear	1
Visit 28 (Day 310)	AduFx, 2PedFx, PedFx, Adu2Fx, Adu1Fx, Infectivity control	~ 150	Blood smear	1
Visit 29 (Day 312)	AduFx, 2PedFx, PedFx, Adu2Fx, Adu1Fx, Infectivity control	~ 150	Blood smear	1
Visit 30 (Day 314)	AduFx, 2PedFx, PedFx, Adu2Fx, Adu1Fx, Infectivity control	~ 150	Blood smear	1

* For assessment and processing by WRAIR of parasitemia on the day of subject entry into the hotel (Day 295 [Visit 16])

6.7.5. Immunological correlates of protection

No correlate of protection has been demonstrated so far for the CS antigen.

For the HBsAg, the conventional correlate of protection is anti-HBs antibody concentrations above 10 mIU/ml [European Consensus Group on Hepatitis B Immunity, 2000].

The investigator is encouraged to share the immunological assay results for non-responders with the study subjects.

For the subjects identified as non-responders, it remains the responsibility of the investigator in charge of the subject's clinical management to determine the medical need for re-vaccination and to re-vaccinate the subjects as per local/regional practices.

7. STUDY VACCINES AND ADMINISTRATION

7.1. Description of study vaccines

The candidate RTS,S/AS01 vaccine to be used has been developed and manufactured by GSK Biologicals.

The Quality Control Standards and Requirements for the 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 labeled and packed according to applicable regulatory requirements.

Table 20 Study vaccines

Treatment name	Vaccine name	Formulation	Presentation	Volume to be administered	Number of doses
RTS,S/AS01$_E$ (Full dose)	RTS,S	RTS,S=25µg	Lyophilized pellet in a glass vial	0.5 ml	2*
	AS01E	MPL=25µg; QS21=25µg; Liposomes	Liquid solution in a glass vial		
RTS,S/AS01$_E$ (1/5th dose)	RTS,S	RTS,S=25µg	Lyophilized pellet in a glass vial	0.1 ml	1**
	AS01E	MPL=25µg; QS21=25µg; Liposomes	Liquid solution in a glass vial		
RTS,S/AS01$_B$ (Full dose)	RTS,S	RTS,S=50µg	Lyophilized pellet in a glass vial	0.5 ml	1 or 2***
	AS01B	MPL=50µg; QS21=50µg; Liposomes	Liquid solution in a glass vial		
RTS,S/AS01$_B$ (1/5th dose)	RTS,S	RTS,S=50µg	Lyophilized pellet in a glass vial	0.1 ml	1 or 2****
	AS01B	MPL=50µg; QS21=50µg; Liposomes	Liquid solution in a glass vial		

* RTS,S/AS01$_E$ full dose (0.5 ml): Group 2PedFx will receive two times two doses; Group PedFx will receive two doses.
** RTS,S/AS01$_E$ 1/5th dose (0.1 ml): Group 2PedFx will receive one time two doses; Group PedFx will receive one dose.
*** RTS,S/AS01$_B$ full dose (0.5 ml): Group AduFx will receive two doses; Group Adu1Fx and Adu2Fx will receive one dose.
**** RTS,S/AS01$_B$ 1/5th dose (0.1 ml): Group AduFx and Adu1Fx will receive one dose; Group Adu2Fx will receive two doses.

7.2. Storage and handling of study vaccines

The study vaccines must be stored at the respective label storage temperature conditions in a safe and locked place. Access to the storage space should be limited to authorized study personnel. The storage conditions will be assessed during pre-study activities under the responsibility of the sponsor study contact. The storage temperature should be continuously monitored with calibrated (if not validated) temperature monitoring device(s) and recorded. Refer to the Module on Clinical Trial Supplies in the SPM for more details on storage of the study vaccines.

Temperature excursions must be reported in degree Celsius.

Any temperature excursion outside the range of 0.0 to +8.0°C (for +2 to +8°C/+36 to +46°F label storage condition) impacting investigational medicinal products (IMPs) must be reported in the appropriate (electronic) temperature excursion decision form ([e]TDF). The impacted IMPs must not be used and must be stored in quarantine at label temperature conditions until usage approval has been obtained from the sponsor.

In case of temperature excursion below +2.0°C down to 0.0°C impacting IMP(s) there is no need to report in (e)TDF, but adequate actions must be taken to restore the +2 to +8°C/+36 to +46°F label storage temperature conditions. The impacted IMP(s) may still be administered, but the site should avoid re-occurrence of such temperature excursion. Refer to the Module on Clinical Trial Supplies in the SPM for more details on actions to take.

Refer to the Module on Clinical Trial Supplies in the SPM for details and instructions on the temperature excursion reporting and usage decision process, packaging and accountability of the study vaccines.

7.3. Dosage and administration of study vaccines

7.3.1. Dosage of RTS,S/AS01

7.3.1.1. RTS,S/AS01$_E$

In this study, the commercial presentation of RTS,S/AS01$_E$ will be used, i.e. a two-doses glass vial of lyophilized RTS,S antigen (50 µg) to be reconstituted with a two-doses glass vial of AS01$_E$ Adjuvant System (1.0 ml). The final product for administration will be prepared by reconstitution of the lyophilized antigen with the liquid adjuvant. From the reconstituted vaccine vial:

- 0.5 ml will be withdrawn to administer RTS,S/AS01$_E$ full doses, or
- 0.1 ml will be withdrawn to administered RTS,S/AS01$_E$ fractional doses (1/5th dose), or
- 1.0 ml will be withdrawn to administer RTS,S/AS01$_E$ double doses, or
- 0.2 ml will be withdrawn to administered double dose of RTS,S/AS01$_E$ fractional doses (1/5th dose).

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

7.3.1.2. RTS,S/AS01$_B$

In this study, the clinical presentation of RTS,S/AS01$_B$ will be used, i.e. a two-dose glass vial of lyophilized RTS,S antigen (50 µg) to be reconstituted with a mono-dose glass vial of AS01$_B$ 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/AS01$_B$ full doses, or
- 0.1 ml will be withdrawn to administered RTS,S/AS01$_B$ fractional doses (1/5th dose).

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

7.3.2. Administration of RTS,S/AS01

Disinfect the top of the vaccine vial (pellet) and adjuvant vial with alcohol swabs and let dry. Withdraw the content of the adjuvant vial in a syringe and inject the adjuvant into the vial of lyophilized antigen. The pellet is then dissolved by gently shaking the vial. Wait for one minute to ensure the complete dissolution of the vial content before withdrawing the correct dose (see Section 7.3.1.1 and Section 7.3.1.2). The full dose of RTS,S/AS01$_E$ or RTS,S/AS01$_B$ should be administered using a fresh 25 gauge needle with a length of one inch (25 mm). For the fractional dose of RTS,S/AS01$_E$ or RTS,S/AS01$_B$, a 1 ml syringe and fresh 25 gauge needle with a length of one inch (25 mm) should be used and the volume administered should be as shown in Table 21. The reconstituted vaccine should be administered by slow IM injection into the non-dominant deltoid. The vaccine should be injected within four hours of reconstitution (storage at +2°C to +8°C).

Table 21 Dosage and administration

Type of contact and timepoint	Volume to be administered	Study group	Treatment name	Route	Site	Side
Visit 2 (Day 0)	0.5 ml	AduFx	RTS,S/AS01$_B$ (Full dose)	IM	Deltoid	Non-dominant
	1.0 ml	2PedFx	RTS,S/AS01$_E$ (Full dose)	IM	Deltoid	Non-dominant
	0.5 ml	PedFx	RTS,S/AS01$_E$ (Full dose)	IM	Deltoid	Non-dominant
	0.5 ml	Adu2Fx	RTS,S/AS01$_B$ (Full dose)	IM	Deltoid	Non-dominant
	0.5 ml	Adu1Fx	RTS,S/AS01$_B$ (Full dose)	IM	Deltoid	Non-dominant
Visit 3 (Day 28)	0.5 ml	AduFx	RTS,S/AS01$_B$ (Full dose)	IM	Deltoid	Non-dominant
	1.0 ml	2PedFx	RTS,S/AS01$_E$ (Full dose)	IM	Deltoid	Non-dominant
	0.5 ml	PedFx	RTS,S/AS01$_E$ (Full dose)	IM	Deltoid	Non-dominant
	0.1 ml	Adu2Fx	RTS,S/AS01$_B$ (1/5th dose)	IM	Deltoid	Non-dominant
Visit 7 (Day 196)	0.1 ml	AduFx	RTS,S/AS01$_B$ (1/5th dose)	IM	Deltoid	Non-dominant
	0.2 ml	2PedFx	RTS,S/AS01$_E$ (1/5th dose)	IM	Deltoid	Non-dominant
	0.1 ml	PedFx	RTS,S/AS01$_E$ (1/5th dose)	IM	Deltoid	Non-dominant
	0.1 ml	Adu2Fx	RTS,S/AS01$_B$ (1/5th dose)	IM	Deltoid	Non-dominant
	0.1 ml	Adu1Fx	RTS,S/AS01$_B$ (1/5th dose)	IM	Deltoid	Non-dominant

IM: intramuscular

7.4. Replacement of unusable vaccine doses

In addition to the vaccine doses provided for the planned number of subjects (including over-randomization when applicable), at least 15% additional vaccine doses will be supplied to replace those that are unusable.

7.5. Contraindications to vaccination

The following events constitute absolute contraindications to administration of RTS,S/AS01$_B$ and RTS,S/AS01$_E$. If any of these events occur during the study, the subject must not receive doses of vaccine but may continue other study procedures at the discretion of the investigator (see Section 9.5).

- Anaphylaxis following the administration of vaccine(s).
- Pregnancy (see Section 9.2.1).
- Any confirmed or suspected immunosuppressive or immunodeficient condition, including HIV infection.
- RTS,S/AS01$_B$ and RTS,S/AS01$_E$ malaria vaccine should not be administered to subjects with known hypersensitivity to any component of the vaccine or to a previous dose of RTS,S/AS01$_B$ or RTS,S/AS01$_E$ malaria vaccine or hepatitis B vaccines. Any subject who shows signs of hypersensitivity to the vaccine should not be given further doses.
- Any condition that in the judgment of the investigator would make intramuscular injection unsafe.
- Occurrence of a new pIMD or the exacerbation of an existing pIMD that, in the opinion of the investigator, expose the subject to unacceptable risk from subsequent vaccination. In such cases, the investigator should use his/her clinical judgment prior to administering the next dose of the vaccine(s)/product(s). Refer to Section 9.1.5.1 for the definition of pIMDs.

The following events constitute contraindications to administration of RTS,S/AS01$_B$ and RTS,S/AS01$_E$ at that point in time; if any of these events occur at the time scheduled for vaccination, the subject may be vaccinated at a later date, within the time window specified in the protocol (see Section 6.5), or the subject may be withdrawn at the discretion of the investigator (see Section 9.5).

- Acute disease and/or fever at the time of vaccination.
  - 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.
  - Subjects with a minor illness (such as mild diarrhea, mild upper respiratory infection) without fever can be administered all vaccines.

7.6. Concomitant medications/products and concomitant vaccinations

At each study visit, the investigator should question the subject about any medications/products taken and vaccinations received by the subject.

7.6.1. Recording of concomitant medications/products and concomitant vaccinations

The following concomitant medication(s)/product(s)/vaccine(s) must be recorded in the eCRF.

- All concomitant medications/products, except vitamins and dietary supplements, administered during the period starting within seven days following each dose of study vaccines (Day 0 to Day 6) and during the entire sporozoite challenge (Day 286 to Day 314).
- Any concomitant vaccination administered in the period starting seven days before the first dose of study vaccines and ending at the last study visit (Day -7 to Day 376) and during the entire sporozoite challenge (Day 286 to Day 314).
- Prophylactic medication (i.e. medication administered in the absence of ANY symptom and in anticipation of a reaction to the vaccination).

  E.g. an anti-pyretic is considered to be prophylactic when it is given in the absence of fever and any other symptom, to prevent fever from occurring (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).
- Any concomitant medications/products/vaccines listed in Section 7.6.2.
- Any concomitant medications/products/vaccines relevant to a SAE/pIMD to be reported as per protocol or administered at any time during the study period for the treatment of a SAE /pIMD. In addition, concomitant medications relevant to SAEs and pIMD need to be recorded on the expedited Adverse Event report.

7.6.2. Concomitant medications/products/vaccines that may lead to the elimination of a subject from ATP analyses

The use of the following concomitant medications/products/vaccines will not require withdrawal of the subject from the study but may determine a subject's evaluability in the ATP analysis. See Section 11.5 for cohorts to be analyzed.

- Any investigational or non-registered product (drug or vaccine) other than the study vaccines used during the study period.
- Immunosuppressants or other immune-modifying drugs administered chronically (i.e. more than 14 days in total) during the study period. For corticosteroids, this will mean prednisone ≥ 20 mg/day (for adult subjects), or equivalent. Inhaled and topical steroids are allowed.

- Long-acting immune-modifying drugs administered at any time during the study period (e.g. infliximab).
- A vaccine not foreseen by the study protocol administered during the period starting seven days before each dose and ending seven days after each dose of vaccines administration*.

  * In case an emergency mass vaccination for an unforeseen public health threat (e.g.: a pandemic) is organized by the public health authorities, outside the routine immunization program, the time period described above can be reduced if necessary for that vaccine provided it is licensed and used according to its Summary of Product Characteristics or Prescribing Information and according to the local governmental recommendations and provided a written approval of the Sponsor is obtained.
- Immunoglobulins and/or any blood products administered during the study period.
- Drugs known to have anti-*Plasmodium* properties, used during the challenge period before identification of parasitemia.

7.7. Intercurrent medical conditions that may lead to elimination of a subject from ATP analyses

For vaccinated subjects, at each study visit subsequent to the first vaccination/the vaccination visit, it must be verified if the subject has experienced or is experiencing any intercurrent medical condition. If it is the case, the condition(s) must be recorded in the eCRF.

For the infectivity controls group, at each study visit subsequent to the sporozoite challenge visit, it must be verified if the subject has experienced or is experiencing any intercurrent medical condition. If it is the case, the condition(s) must be recorded in the eCRF.

Subjects may be eliminated from the ATP cohort for analysis of immunogenicity and efficacy if, during the study, they incur a condition that has the capability of altering their immune response or are confirmed to have an alteration of their initial immune status.

8. HEALTH ECONOMICS

Not applicable

9. 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.

9.1. Safety definitions

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

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 or a concurrent medication (overdose per se should not be reported as an AE/SAE).
- Signs, symptoms temporally associated with vaccines 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).

AEs to be recorded as endpoints (solicited AEs) are described in Section 9.1.3. All other AEs will be recorded as UNSOLICITED AEs.

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 eCRF.

9.1.2. 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 fulfills 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, diarrhea, 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.

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.

9.1.3. Solicited adverse events

9.1.3.1. Solicited local (injection-site) adverse events

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

Table 22 Solicited local adverse events

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

9.1.3.2. Solicited general adverse events

The following general AEs will be solicited:

Table 23 Solicited general adverse events

Adult
Fatigue
Fever
Gastrointestinal symptoms †
Headache

† Gastrointestinal symptoms include nausea, vomiting, diarrhea 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 eCRF.

9.1.4. 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, hematology, 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 (refer to Sections 9.1.1 and 9.1.2). 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.

9.1.5. Adverse events of specific interest

AEs of specific interest for safety monitoring include meningitis and pIMDs.

9.1.5.1. Potential immune-mediated diseases

pIMDs are a subset of AEs that include autoimmune diseases and other inflammatory and/or neurologic disorders of interest which may or may not have an autoimmune etiology. AEs that need to be recorded and reported as pIMDs include those listed in Table 24.

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.

Table 24 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 disease, microscopic polyangiitis, Wegener's granulomatosis, Churg–Strauss syndrome (allergic granulomatous angiitis), Buerger's disease (thromboangiitis obliterans), necrotizing vasculitis and anti-neutrophil cytoplasmic antibody (ANCA) positive vasculitis (type unspecified), Henoch-Schonlein purpura, Behcet's syndrome, leukocytoclastic vasculitis.	• Autoimmune hemolytic anemia • Autoimmune thrombocytopenia • Antiphospholipid syndrome • Pernicious anemia • Autoimmune aplastic anaemia • Autoimmune neutropenia • Autoimmune pancytopenia	• Autoimmune glomerulonephritis (including IgA nephropathy, glomerulonephritis rapidly progressive, membranous glomerulonephritis, membranoproliferative glomerulonephritis, and mesangioproliferative glomerulonephritis) • Ocular autoimmune diseases (including autoimmune uveitis and autoimmune retinopathy) • Autoimmune myocarditis/cardiomyopathySarcoidosis • Stevens-Johnson syndrome • Sjögren's syndrome • Idiopathic pulmonary fibrosis • Goodpasture syndrome • Raynaud's phenomenon
Liver disorders	**Gastrointestinal disorders**	**Endocrine disorders**
• Autoimmune hepatitis • Primary biliary cirrhosis • Primary sclerosing cholangitis • Autoimmune cholangitis	• Inflammatory Bowel disease, including Crohn's disease, ulcerative colitis, microscopic colitis, ulcerative proctitis • Celiac disease • Autoimmune pancreatitis	• Autoimmune thyroiditis (including Hashimoto thyroiditis) • Grave's or Basedow's disease • Diabetes mellitus type I • Addison's disease • Polyglandular autoimmune syndrome • Autoimmune hypophysitis

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.

In order to facilitate the documentation of pIMDs in the eCRF, a pIMD standard questionnaire and a list of preferred terms and preferred terms codes corresponding to the above diagnoses will be available to investigators at study start.

9.1.5.2. Meningitis

For the further evaluation of the safety signal of meningitis in the investigational vaccine groups (AduFx, 2PedFx, PedFx, Adu2Fx, and Adu1Fx), all cases of meningitis occurring during the study will be reported as a SAE and clinical details of each case will be reported in a specific eCRF screen.

9.2. Events or outcomes not qualifying as adverse events or serious adverse events

9.2.1. Pregnancy

Female subjects who are pregnant or lactating at the time of vaccination 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.

Note: The pregnancy itself should always be recorded on an electronic pregnancy report.

The following should always be considered as SAE and will be reported as described in Sections 9.4.1 and 9.4.3:

- Spontaneous pregnancy loss, including:
  - spontaneous abortion, (spontaneous pregnancy loss before/at 22 weeks of gestation)
  - ectopic and molar pregnancy
  - stillbirth (intrauterine death of fetus after 22 weeks of gestation).

  Note: the 22 weeks cut-off in gestational age is based on WHO-ICD 10 noted in the EMA Guideline on pregnancy exposure [EMA, 2006]. It is recognized that national regulations might be different.
- 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 (as per [CDC MACDP] guidelines) identified in the offspring of a study subject (either during pregnancy, at birth or later) regardless of whether the fetus 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 to GSK Biologicals as described in Section 9.4.3. 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.

9.3. Detecting and recording adverse events, serious adverse events and pregnancies

9.3.1. Time period for detecting and recording adverse events, serious adverse events and pregnancies

All AEs starting within 30 days following administration of each dose of study vaccines (Day 0 to Day 29) must be recorded into the appropriate section of the eCRF, irrespective of intensity or whether or not they are considered vaccination-related.

For vaccinated subjects, the time period for collecting and recording SAEs will begin at the first receipt of study vaccine (Day 0) and will end at the last study visit (Day 376). For the infectivity controls group, the time period for collecting and recording SAEs will begin on the first day of the sporozoite challenge (Day 286) and will end at the last study visit (Day 314). See Section 9.4 for instructions on reporting of SAEs.

All AEs/SAEs leading to withdrawal from the study will be collected and recorded from the time of the first receipt of study vaccines (Day 0) for AduFx, 2PedFx, PedFx, Adu2Fx, and Adu1Fx groups.

In addition to the above-mentioned reporting requirements and in order to fulfill international reporting obligations, SAEs that are related to study participation (i.e. protocol-mandated procedures, invasive tests, a change from existing therapy) or are related to a concurrent GSK medication/vaccine will be collected and recorded from the time the subject consents to participate (i.e. Screening) in the study until she/he is discharged from the study.

For vaccinated subjects, the time period for collecting and recording pregnancies will begin at the first receipt of study vaccines (Day 0) and will end at the last study visit (Day 376). For the infectivity controls group, the time period for collecting and recording pregnancies will begin on the first day of the sporozoite challenge (Day 286) and will end at the last study visit (Day 314). See section 9.4 for instructions on reporting of pregnancies.

For vaccinated subjects, the time period for collecting and recording of AEs of specific interest (meningitis and pIMDs) will begin at the first receipt of study vaccines (Day 0) and will end at the last study visit (Day 376). See section 9.4 for instructions on reporting of pIMDs.

An overview of the protocol-required reporting periods for AEs, SAEs and pregnancies is given in Table 25, Table 26 and Table 27.

Table 25 Reporting periods for collecting safety information for the investigational vaccine groups: AduFx, 2PedFx, PedFx, and Adu2Fx)

Study visits	1	2		3		6	7		10	11 to 30			31
Study days	-90 to 0	0	6	28	34	58	196	202	226	286	314	315	376
Solicited local and general AEs post-vaccination													
Unsolicited AEs post-vaccination													
AEs post-challenge													
AEs and SAEs leading to withdrawal from further vaccination													
SAEs (all, fatal, related)													
SAEs related to study participation, or to a concurrent GSK medication/vaccine													
AEs of specific interest (meningitis and pIMDs)													
Pregnancies													

Table 26 Reporting periods for collecting safety information for the investigational vaccine groups: Adu1Fx

Study visits	1	2		3	7		10	11 to 30			31
Study days	-90 to 0	0	6	28	196	202	226	286	314	315	376
Solicited local and general AEs post-vaccination											
Unsolicited AEs post-vaccination											
AEs post-challenge											
AEs and SAEs leading to withdrawal from further vaccination											
SAEs (all, fatal, related)											
SAEs related to study participation, or to a concurrent GSK medication/vaccine											
AEs of specific interest (meningitis and pIMDs)											
Pregnancies											

Table 27 Reporting periods for collecting safety information for the infectivity control group

Study visits	1b	11 to 30		
Study days	258 to 286	286	314	315
AEs post-challenge				
SAEs (all, fatal, related)				
SAEs related to study participation, or to a concurrent GSK medication/vaccine				
Pregnancies				

9.3.2. Post-Study adverse events and serious adverse events

A post-study AE/SAE is defined as any event that occurs outside of the AE/SAE reporting period defined in Table 25, Table 26 and Table 27. 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.

9.3.3. Evaluation of adverse events and serious adverse events

9.3.3.1. 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 then record all relevant information regarding an AE/SAE in the eCRF. The investigator is not allowed to send photocopies of the subject's medical records to GSK Biologicals instead of appropriately completing the eCRF. However, there may be instances when copies of medical records for certain cases are requested by GSK Biologicals. In this instance, all subject identifiers will be blinded on the copies of the medical records prior to submission to GSK Biologicals.

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.

9.3.3.2. Assessment of adverse events

9.3.3.2.1. Assessment of intensity

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

Table 28 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, diarrhea 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 at GSK Biologicals 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 at GSK Biologicals 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. An event is defined as 'serious' when it meets one of the pre-defined outcomes as described in Section 9.1.2.

The normal ranges and toxicity grading for laboratory safety parameters used in this study are presented in Table 29.

Table 29 Toxicity grading scales for blood testing

Adverse event	Intensity grade	Intensity*
Hemoglobin (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
Hemoglobin (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	1.1 - 2.5 x ULN
	2	2.6 – 5 x ULN
	3	> 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
*Grading scale adapted from [FDA guidance for industry: toxicity grading scale for healthy adult and adolescent volunteers enrolled in preventive vaccine clinical trials (September 2007)].

(Amended 19 June 2017)

9.3.3.2.2. Assessment of causality

The investigator is obligated to assess the relationship between investigational vaccines 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 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 to include in the initial report to GSK Biologicals. However, it is very important that the investigator always makes an assessment of causality for every event prior to submission of the Expedited Adverse Events Report to GSK Biologicals. 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 multiple vaccines, it may not be possible to determine the causal relationship of general AEs to the individual vaccine administered. The investigator should, therefore, assess whether the AE could be causally related to vaccination rather than to the individual vaccine.

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?

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

If an event meets the criteria to be determined as 'serious' (see Section 9.1.2), 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).

9.3.3.3. Assessment of outcomes

The investigator will assess the outcome of all unsolicited 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).

9.4. Reporting of serious adverse events, pregnancies, and other events

9.4.1. Prompt reporting of serious adverse events, pregnancies, and other events to GSK Biologicals

SAEs that occur in the time period defined in Section 9.3 will be reported promptly to GSK within the timeframes described in Table 30, once the investigator determines that the event meets the protocol definition of a SAE.

Pregnancies that occur in the time period defined in Section 9.3 will be reported promptly to GSK within the timeframes described in Table 30, once the investigator becomes aware of the pregnancy.

AEs of specific interest (meningitis and pIMDs) that occur in the time period defined in Section 9.3 will be reported promptly to GSK within the timeframes described in Table 30, once the investigator determines that the event meets the protocol definition of an AEs of specific interest.

Table 30 Timeframes for submitting serious adverse event, pregnancy and other events reports to GSK Biologicals

Type of Event	Initial Reports		Follow-up of Relevant Information on a Previous Report	
	Timeframe	Documents	Timeframe	Documents
SAEs	24 hours*‡	electronic Expedited Adverse Events Report	24 hours*	electronic Expedited Adverse Events Report
Pregnancies	2 weeks*	electronic pregnancy report	2 weeks*	electronic pregnancy report
AEs of specific interest (meningitis and pIMDs)	24 hours**‡	electronic Expedited Adverse Events Report	24 hours*	electronic Expedited Adverse Events Report

* Timeframe allowed after receipt or awareness of the information.
**Timeframe allowed once the investigator determines that the event meets the protocol definition of an AEs of specific interest.
‡ The investigator will be required to confirm review of the SAE/AEs of specific interest causality by ticking the 'reviewed' box in the electronic Expedited Adverse Events Report within 72 hours of submission of the SAE/AEs of specific interest.

9.4.2. Contact information for reporting serious adverse events, pregnancies and adverse events of specific interest

Study Contact for Reporting SAEs, AEs of specific interest and pregnancies
Refer to the local study contact information document.
Back-up Study Contact for Reporting SAEs, AEs of specific interest and pregnancies
24/24 hour and 7/7 day availability: GSK Biologicals Clinical Safety & Pharmacovigilance US sites only: Fax: PPD

9.4.3. Completion and transmission of serious adverse event reports to GSK Biologicals

Once an investigator becomes aware that a SAE has occurred in a study subject, the investigator (or designate) must complete the information in the electronic Expedited Adverse Events Report WITHIN 24 HOURS. The report will always be completed as thoroughly as possible with all available details of the event. Even if the investigator does not have all information regarding a SAE, the report should still be completed within 24 hours. Once additional relevant information is received, the report should be updated WITHIN 24 HOURS.

The investigator will always provide an assessment of causality at the time of the initial report. The investigator will be required to confirm the review of the SAE causality by ticking the 'reviewed' box in the electronic Expedited Adverse Events Report within 72 hours of submission of the SAE.

9.4.3.1. Back-up system in case the electronic reporting system does not work

If the electronic reporting system does not work, the investigator (or designate) must complete, then date and sign a paper Expedited Adverse Events Report and fax it to the Study Contact for Reporting SAEs (refer to the Sponsor Information) or to GSK Biologicals Clinical Safety and Pharmacovigilance department within 24 hours.

This back-up system should only be used if the electronic reporting system is not working and NOT if the system is slow. As soon as the electronic reporting system is working again, the investigator (or designate) must complete the electronic Expedited Adverse Events Report within 24 hours. The final valid information for regulatory reporting will be the information reported through the electronic SAE reporting system.

9.4.4. Completion and transmission of pregnancy reports to GSK Biologicals

Once the investigator becomes aware that a subject is pregnant, the investigator (or designate) must complete the required information onto the electronic pregnancy report WITHIN 2 WEEKS.

Note: Conventionally, the estimated gestational age (EGA) of a pregnancy is dated from the first day of the last menstrual period (LMP) of the cycle in which a woman conceives. If the LMP is uncertain or unknown, dating of EGA and the estimated date of delivery (EDD) should be estimated by ultrasound examination and recorded in the pregnancy report.

9.4.5. Reporting of adverse events of specific interest to GSK Biologicals

Once an AE of specific interest (meningitis and pIMDs) is diagnosed (serious or non-serious) in a study subject, the investigator (or designate) must complete the information in the electronic Expedited Adverse Events Report WITHIN 24 HOURS after he/she becomes aware of the diagnosis. The report allows to specify that the event is a pIMD and whether it is serious or non serious. For pIMDs the report will always be completed as thoroughly as possible with all available details of the event, in accordance with the pIMD standard questionnaire provided. Even if the investigator does not have all information regarding an AE of specific interest, the report should still be completed within 24 hours. Once additional relevant information is received, the report should be updated WITHIN 24 HOURS.

The investigator will always provide an assessment of causality at the time of the initial report. The investigator will be required to confirm the review of the causality of an AE of specific interest by ticking the 'reviewed' box in the electronic Expedited Adverse Events Report within 72 hours of submission of the AE of specific interest.

Refer to Section 9.4.3.1 for back-up system in case the electronic reporting system does not work.

9.4.6. Updating of SAE, pregnancy, and adverse events of specific interest information after removal of write access to the subject's eCRF

When additional SAE, pregnancy, or AEs of specific interest information is received after removal of the write access to the subject's eCRF, new or updated information should be recorded on the appropriate paper report, with all changes signed and dated by the investigator. The updated report should be faxed to the Study Contact for Reporting SAEs (refer to the Sponsor Information) or to GSK Biologicals Clinical Safety and Pharmacovigilance department within the designated reporting time frames specified in Table 30.

9.4.7. Regulatory reporting requirements for serious adverse events

The investigator will promptly report all SAEs to GSK in accordance with the procedures detailed in Section 9.4.1. GSK Biologicals has a legal responsibility to promptly notify, as appropriate, both the local regulatory authority and other regulatory agencies about the safety of a product under clinical investigation. Prompt notification of SAEs by the investigator to the Study Contact for Reporting SAEs is essential so that legal obligations and ethical responsibilities towards the safety of other subjects are met.

Investigator safety reports are prepared according to the current GSK policy and are forwarded to investigators as necessary. An investigator safety report is prepared for a SAE(s) that is both attributable to the investigational vaccines and unexpected. The purpose of the report is to fulfill specific regulatory and GCP requirements, regarding the product under investigation.

9.5. Follow-up of adverse events, serious adverse events, and pregnancies

9.5.1. Follow-up of adverse events and serious adverse events

9.5.1.1. Follow-up during the study

After the initial AE/SAE report, the investigator is required to proactively follow each subject and provide additional relevant information on the subject's condition to GSK Biologicals (within 24 hours for SAEs; refer to Table 30).

All SAEs and AEs of specific interest (meningitis and pIMDs) (serious or non-serious) documented at a previous visit/contact and designated as not recovered/not resolved or recovering/resolving will be reviewed at subsequent visits/contacts until the end of the study.

All AEs documented at a previous visit/contact and designated as not recovered/not resolved or recovering/resolving will be reviewed at subsequent visits/contacts until 30 days after the last vaccination.

9.5.1.2. Follow-up after the subject is discharged from the study

The investigator will follow subjects:

- with SAEs, AEs of specific interest (meningitis and pIMDs) (serious or non-serious), or subjects withdrawn from the study as a result of an AE, until the event has resolved, subsided, stabilized, disappeared, or until the event is otherwise explained, or the subject is lost to follow-up.

If the investigator receives additional relevant information on a previously reported SAE, he/she will provide this information to GSK Biologicals using a paper/ electronic Expedited Adverse Events Report and/or pregnancy report as applicable.

GSK Biologicals may request that the investigator performs or arranges the conduct of additional clinical examinations/tests and/or evaluations to elucidate as fully as possible the nature and/or causality of the AE or SAE. The investigator is obliged to assist. If a subject dies during participation in the study or during a recognized follow-up period, GSK Biologicals will be provided with any available post-mortem findings, including histopathology.

9.5.2. Follow-up of pregnancies

Pregnant subjects will be followed to determine the outcome of the pregnancy. At the end of the pregnancy, whether full-term or premature, information on the status of the mother and child will be forwarded to GSK Biologicals using the electronic pregnancy report and the Expedited Adverse Events Report if applicable. Generally, the follow-up period doesn't need to be longer than six to eight weeks after the estimated date of delivery.

Regardless of the reporting period for SAEs for this study, if the pregnancy outcome is a SAE, it should always be reported as SAE.

9.6. Treatment of adverse events

Treatment of any AE is at the sole discretion of the investigator and according to current good medical practice. Any medication administered for the treatment of an AE should be recorded in the subject's eCRF (refer to Section 7.6).

9.7. Subject card

Study subjects must be provided with the address and telephone number of the main contact for information about the clinical study.

The investigator (or designate) must therefore provide a "subject card" to each subject. In an emergency situation this card serves to inform the responsible attending physician that the subject is in a clinical study and that relevant information may be obtained by contacting the investigator.

Subjects must be instructed to keep subject cards in their possession at all times.

10. SUBJECT COMPLETION AND WITHDRAWAL

10.1. Subject completion

A subject who returns for the concluding visit foreseen in the protocol is considered to have completed the study.

10.2. Subject withdrawal

Withdrawals will not be replaced.

10.2.1. Subject withdrawal from the study

From an analysis perspective, a 'withdrawal' from the study refers to any subject who did not come back for the concluding visit foreseen in the protocol.

All data collected until the date of withdrawal/last contact of the subject will be used for the analysis.

A subject is considered 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 subject from the date of withdrawal/last contact.

Investigators will make an attempt to contact those subjects who do not return for scheduled visits or follow-up.

Information relative to the withdrawal will be documented in the eCRF. The investigator will document whether the decision to withdraw a subject from the study was made by the subject himself/herself, or by the investigator, as well as which of the following possible reasons was responsible for withdrawal:

- SAE.
- Non-SAE.
- Protocol violation (specify).
- Consent withdrawal, not due to an AE*.
- Moved from the study area.
- Lost to follow-up.
- Other (specify).

*In case a subject is withdrawn from the study because he/she has withdrawn consent, the investigator will document the reason for withdrawal of consent, if specified by the subject, in the eCRF.

Subjects who are withdrawn from the study because of SAEs/AEs must be clearly distinguished from subjects who are withdrawn for other reasons. Investigators will follow subjects who are withdrawn from the study as result of a SAE/AE until resolution of the event (see Section 9.5.1.2).

10.2.2. Subject withdrawal from investigational vaccines

A 'withdrawal' from the investigational vaccines refers to any subject who does not receive the complete treatment, i.e. when no further planned dose is administered from the date of withdrawal. A subject withdrawn from the investigational vaccines may not necessarily be withdrawn from the study as further study procedures or follow-up may be performed (safety or immunogenicity) if planned in the study protocol.

Information relative to premature discontinuation of the investigational vaccines will be documented on the Vaccine Administration screen of the eCRF. The investigator will document whether the decision to discontinue further vaccination/treatment was made by the subject himself/herself, or by the investigator, as well as which of the following possible reasons was responsible for withdrawal:

- SAE.
- Non-SAE.
- Other (specify).

10.3. Extension study

At the end of the study (study conclusion visit), the investigator will ask each subject if they are interested to participate in a booster study/long-term study. If a subject is not interested in participating in the booster study/long-term study the reason for refusal will be documented in the subject's eCRF.

10.4. Screen and baseline failures

Screening failures are defined as subjects who are withdrawn from the study after giving informed consent, but who do not meet the inclusion and exclusion criteria. Reason for screening failure will be collected.

11. STATISTICAL METHODS

11.1. Primary endpoint

11.1.1. Efficacy

- Occurrence of *P. falciparum* parasitemia (defined by a positive blood slide) following sporozoite challenge, comparing RTS,S/AS01$_B$ administered as full doses at Month 0 and Month 1 and 1/5th dose at Month 7 (AduFx group) versus infectivity controls.
- Occurrence of *P. falciparum* parasitemia (defined by a positive blood slide) following sporozoite challenge, comparing RTS,S/AS01$_E$ administered as double dose at Month 0 and Month 1 and double 1/5th dose at Month 7 (2PedFx group) versus infectivity controls.
- Occurrence of *P. falciparum* parasitemia (defined by a positive blood slide) following sporozoite challenge, comparing RTS,S/AS01$_E$ administered as full doses at Month 0 and Month 1 and 1/5th dose at Month 7 (PedFx group) versus infectivity controls.
- Occurrence of *P. falciparum* parasitemia (defined by a positive blood slide) following sporozoite challenge, comparing RTS,S/AS01$_B$ administered as one full dose at Month 0 and 1/5th doses at Month 1 and Month 7 (Adu2Fx group) versus infectivity controls.
- Occurrence of *P. falciparum* parasitemia (defined by a positive blood slide) following sporozoite challenge, comparing RTS,S/AS01$_B$ administered as one full dose at Month 0 and 1/5th doses at Month 7 (Adu1Fx group) versus infectivity controls.

11.2. Secondary endpoints

11.2.1. Efficacy

- Occurrence of *P. falciparum* parasitemia (defined by a positive blood slide) following sporozoite challenge, comparing RTS,S/AS01$_E$ administered as double dose at Month 0 and Month 1 and double 1/5th dose at Month 7 (2PedFx group) versus immunization schedule of reference (AduFx group).
- Occurrence of *P. falciparum* parasitemia (defined by a positive blood slide) following sporozoite challenge, comparing RTS,S/AS01$_E$ administered as full doses at Month 0 and Month 1 and 1/5th dose at Month 7 (PedFx) versus immunization schedule of reference (AduFx group).
- Occurrence of *P. falciparum* parasitemia (defined by a positive blood slide) following sporozoite challenge, comparing RTS,S/AS01$_B$ administered as one full dose at Month 0 and 1/5th full dose at Month 1 and Month 7 (Adu2Fx) versus immunization schedule of reference (AduFx group).

- Occurrence of *P. falciparum* parasitemia (defined by a positive blood slide) following sporozoite challenge, comparing RTS,S/AS01$_B$ administered as one full dose at Month 0 and 1/5th full doses at Month 7 (Adu1Fx) versus immunization schedule of reference (AduFx group).
- Time to onset of *P. falciparum* parasitemia (defined by a positive blood slide) following sporozoite challenge in each vaccination schedule versus immunization schedule of reference (AduFx group).
- Time to onset of *P. falciparum* parasitemia (defined by a positive blood slide) following sporozoite challenge in each vaccination schedule versus infectivity controls.

11.2.2. Immunogenicity

- For each vaccination schedule, the immune response to the CS antigen of the investigational vaccine:
  - Anti-CS repeat region antibody concentrations before vaccine administration (Day 0), one month post-Dose 2 (Day 58), six months post-Dose 2 (Day 196), one month post-Dose 3 (Day 226), on the day of challenge (Day 286), 28 days post-challenge (Day 314), and at study end (Day 376) for subjects from AduFx, 2PedFx, PedFx, and Adu2Fx groups and before vaccine administration (Day 0), before Dose 2 (Day 196), one month post-Dose 2 (Day 226), on the day of challenge (Day 286), 28 days post-challenge (Day 314), and at study end (Day 376) for subjects from Adu1Fx group.
- For each vaccination schedule, the immune response to the hepatitis B antigen of the investigational vaccine:
  - Anti-HBs IgG antibody concentrations before vaccine administration (Day 0), one month post-Dose 2 (Day 58), six months post-Dose 2 (Day 196), one month post-Dose 3 (Day 226), on the day of challenge (Day 286), 28 days post-challenge (Day 314), and at study end (Day 376) for subjects from AduFx, 2PedFx, PedFx, and Adu2Fx groups and before vaccine administration (Day 0), before Dose 2 (Day 196), one month post-Dose 2 (Day 226), on the day of challenge (Day 286), 28 days post-challenge (Day 314), and at study end (Day 376) for subjects from Adu1Fx group.

11.2.3. Safety

- 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 within 30 days (day of vaccination and 29 subsequent days) after each vaccination, according to the Medical Dictionary for Regulatory Activities (MedDRA) classification.

  - Occurrence of AEs within 30 days (day of challenge and 29 subsequent days) after challenge, according to the 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 (from Dose 1 [Day 0] up to study conclusion [Day 376]), 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 376), according to the MedDRA classification, for each vaccinated subject.
  - Occurrence of pIMDs from Dose 1 (Day 0) up to study conclusion (Day 376), according to the MedDRA classification, for each vaccinated subject.
  - Occurrence of meningitis from Dose 1 (Day 0) up to study conclusion (Day 376), according to the MedDRA classification, for each vaccinated subject.
  - Occurrence of abnormal laboratory values at screening, Day 35, Day 58, Day 203, Day 226, the day of first parasitemia, and Day 314 for each vaccinated subject and at screening, the day of first parasitemia and Day 314 for the infectivity control subjects.
- Safety after sporozoite challenge in the infectivity control group.
  - Occurrence of AEs within 30 days (day of challenge and 29 subsequent days) after challenge, according to the MedDRA classification.
  - Occurrence of SAEs (all, fatal, related) from day of challenge (Day 286) to the end of the challenge phase (Day 314), according to the MedDRA classification.

11.3. Tertiary endpoints

11.3.1. Immunogenicity

- For each vaccination schedule, the immune response to the CS antigen of the investigational vaccine:
  - Anti-CS repeat region IgG avidity index before vaccine administration (Day 0), one month post-Dose 2 (Day 58), six months post-Dose 2 (Day 196), one month post-Dose 3 (Day 226), on the day of challenge (Day 286), 28 days post-challenge (Day 314), and at study end (Day 376) for subjects from AduFx, 2PedFx, PedFx, and Adu2Fx groups and before vaccine administration (Day 0), before Dose 2 (Day 196), one month post-Dose 2 (Day 226), on the day of challenge (Day 286), 28 days post-challenge (Day 314), and at study end (Day 376) for subjects from Adu1Fx group.

- Anti-full length CS protein IgG concentrations and anti-C-term IgG concentrations before vaccine administration (Day 0), one month post-Dose 2 (Day 58), six months post-Dose 2 (Day 196), one month post-Dose 3 (Day 226), on the day of challenge (Day 286), 28 days post-challenge (Day 314), and at study end (Day 376) for subjects from AduFx, 2PedFx, PedFx, and Adu2Fx groups and before vaccine administration (Day 0), before Dose 2 (Day 196), one month post-Dose 2 (Day 226), on the day of challenge (Day 286), 28 days post-challenge (Day 314), and at study end (Day 376) for subjects from Adu1Fx group.
- Anti-full length CS protein and anti-C-term IgG avidity before vaccine administration (Day 0), one month post-Dose 2 (Day 58), six months post-Dose 2 (Day 196), one month post-Dose 3 (Day 226), on the day of challenge (Day 286), 28 days post-challenge (Day 314), and at study end (Day 376) for subjects from AduFx, 2PedFx, PedFx, and Adu2Fx groups and before vaccine administration (Day 0), before Dose 2 (Day 196), one month post-Dose 2 (Day 226), on the day of challenge (Day 286), 28 days post-challenge (Day 314), and at study end (Day 376) for subjects from Adu1Fx group.

Note: other immuno-assays evaluating the immune response targeting the CS and HBsAg might be performed.

11.4. Determination of sample size

The target enrollment for this study will be approximately 150 subjects (26 subjects per vaccine group and between 20 and 30 subjects in the infectivity control group) to ensure at least 20 subjects in each group will undergo sporozoite challenge, given an approximate 20% estimated drop-out rate in vaccinees, based on past experience.

Assuming at least 19/20 controls and 6/20 vaccinees become positive (vaccine efficacy approximately 68%) the trial has 99% power to detect statistically significant vaccine efficacy of each of the vaccination groups over the infectivity controls for an overall power of 96% for the primary endpoint (p-value < 0.05).

For secondary efficacy endpoints the power of the trial to detect incremental vaccine efficacy of one schedule over any other is presented below:

Vaccine efficacy group x over controls	Vaccine efficacy group y over controls	Incremental Vaccine efficacy of group y over x	Power
50%	75%	50%	27%
50%	80%	60%	41%
50%	85%	70%	57%
50%	90%	80%	74%

11.5. Cohorts for analyses

11.5.1. Total vaccinated cohort

The total vaccinated cohort (TVC) will include all subjects who received at least one dose of study vaccine. The TVC analysis will be performed per treatment actually administered. All challenged infectivity control will also be included and will be presented as a separate study group.

11.5.2. According-to-protocol cohort for analysis of immunogenicity and efficacy

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

11.6. Derived and transformed data

- Immunogenicity
  - A subject seropositive for anti-CS antibody will be a subject whose antibody concentration will be greater than or equal to the cut-off value (anti-CS $\geq$ 0.5 EU/ml).
  - Seroprotection rate for anti-HBs antibody is defined as the percentage of subjects with antibody concentration greater than or equal to an established cut-off (anti-HBs $\geq$ 10 mIU/ml).
  - The geometric mean concentrations (GMC) calculations will be performed by taking the anti-log of the mean of the log transformations (base 10). Antibody concentrations below the cut-off of the assay will be given an arbitrary value of half the cut-off for the purpose of GMC calculation.
  - Handling of missing data: for a given subject and a given immunogenicity measurement, missing or non-evaluable measurements will not be replaced. Therefore, an analysis will exclude subjects with missing or non-evaluable measurements.
- Reactogenicity and safety
  - Handling of missing data: subjects who missed reporting symptoms (solicited/unsolicited or concomitant medications) will be treated as subjects without symptoms (solicited/unsolicited or concomitant medications, respectively). In case of significant non-compliance of study procedures for reporting symptoms, the analysis plan will be reassessed to ensure more accurate reporting of study data by further analysis.

– For the analysis of solicited symptom, missing or non-evaluable measurements will not be replaced. Therefore the analysis of the solicited symptoms based on the TVC will include only subjects/doses with documented safety data (i.e. symptom screen/sheet completed).

11.7. Analysis of demographics

A study flow diagram (consort) will be generated to present the number of subjects screened, randomized, receiving doses and included in the ATP analyses.

The analysis of demographics will be performed on the TVC and on the ATP cohort for immunogenicity and efficacy.

Demographic characteristics (age at vaccination in years, gender, and race) will be summarized by group using descriptive statistics:

- Frequency tables will be generated for categorical variable such as race.
- Mean, median, standard deviation (SD) and range will be provided for continuous data such as age.

Withdrawal status will be summarized by group using descriptive statistics:

- The number of subjects enrolled into the study as well as the number of subjects excluded from ATP analyses will be tabulated.
- The numbers of withdrawn subjects will be tabulated according to the reason for withdrawal.

11.8. Analysis of efficacy

The analysis of efficacy will be performed on the ATP cohort for immunogenicity and efficacy.

Efficacy will be assessed by comparison of *P. falciparum* parasitemia and time to onset of parasitemia after sporozoite challenge. Vaccine efficacy is defined as 100*(1-Relative Risk). Relative risk of infection and 95% CI will be calculated. Fisher's Exact test will be used for the comparison of *P. falciparum* parasitemia between each one of the vaccine groups (AduFx, 2PedFx, PedFx, Adu2Fx, and Adu1Fx) and the infectivity control group. Kaplan-Meier analysis will be performed on time to onset of parasitemia, for comparisons between each one of the vaccine groups (AduFx, 2PedFx, PedFx, Adu2Fx, and Adu1Fx) and the infectivity control group, using the log-rank statistic. The same methodology will be applied for the evaluation of the vaccinated groups versus the immunization schedule of reference (AduFx group). All statistical tests will be two-tailed at 5% significance level.

11.9. Analysis of immunogenicity

The analysis of immunogenicity will be performed on the ATP cohort for immunogenicity and efficacy.

For each study group, at each timepoint that a blood sample result is available:

- The percentage of subjects with seropositive levels of anti-CS (proportion of subjects with anti-CS antibody concentrations ≥ 0.5 EU/ml) with 95% CI will be determined at specified blood sampling timepoints in each groups (AduFx, 2PedFx, PedFx, Adu2Fx, and Adu1Fx; see Table 6 and Table 7). Anti-CS antibody concentrations will be summarized by GMC. Anti-CS antibody concentrations will be displayed using reverse cumulative curves. Similar analysis will be performed for anti-CS repeat region, anti-full length CS protein and anti-C-term antibodies.
- The percentage of subjects with seroprotection levels of anti-HBs (anti-HBs antibody concentrations ≥ 10 mIU/ml) with 95% CI will be determined at specified blood sampling timepoints in each groups (AduFx, 2PedFx, PedFx, Adu2Fx, and Adu1Fx; see Table 6 and Table 7). Anti-HBs antibody concentrations will be summarized by GMC. Anti-HBs antibody concentrations will be displayed using reverse cumulative curves.

For the analysis of secondary and tertiary objectives, avidity index will be summarized by mean, SD, median and quartile.

11.10. Analysis of safety

The analysis of safety will be performed on the TVC.

The percentage of subjects with at least one local AE (solicited and unsolicited), with at least one general AE (solicited and unsolicited) and with any AE during the 7-day or 30-day follow-up period after each 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 30-day follow-up period 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 within 30 days (Day 0-29) after each vaccine dose (overall doses) and percentage of subjects reporting AEs 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 hematological (hemoglobin, WBC and platelets) laboratory values will be presented according to toxicity grading scales and tabulated by group.

The percentage of subjects using concomitant medication (any medication, any antipyretic and any antipyretic taken prophylactically, respectively) during the 7-day follow-up period (Day 0-6) after vaccination will be summarized by group after each dose and overall. The percentage of subjects using concomitant medication during the entire sporozoite challenge will be summarized by group.

11.11. Interpretation of analyses

For the primary efficacy evaluations the aim will be to establish significant vaccine efficacy over infectivity controls whereas secondary efficacy evaluations between vaccination schedules will be descriptive with the aim to characterize the difference in immunogenicity, efficacy and safety between groups.

11.12. Conduct of analyses

Any deviation(s) or change(s) from the original statistical plan outlined in this protocol will be described and justified in the final study report.

11.12.1. Sequence of analyses

The analysis will be performed when all data up to Day 376 are available. An integrated study report presenting all results until Day 376 will be written.

11.12.2. Statistical considerations for interim analyses

No interim analyses are planned.

12. ADMINISTRATIVE MATTERS

To comply with ICH GCP administrative obligations relating to data collection, monitoring, archiving data, audits, confidentiality and publications must be fulfilled.

12.1. Electronic Case Report Form instructions

A validated GSK defined electronic data collection tool will be used as the method for data collection.

In all cases, subject initials will not be collected nor transmitted to GSK. Subject data necessary for analysis and reporting will be entered/transmitted into a validated database or data system. Clinical data management will be performed in accordance with applicable GSK standards and data cleaning procedures.

While completed eCRFs are reviewed by a GSK Biologicals' Site Monitor at the study site, omissions or inconsistencies detected by subsequent eCRF review may necessitate clarification or correction of omissions or inconsistencies with documentation and approval by the investigator or appropriately qualified designee. In all cases, the investigator remains accountable for the study data.

The investigator will be provided with a CD-ROM of the final version of the data generated at the investigational site once the database is archived and the study report is complete and approved by all parties.

12.2. Study Monitoring by GSK Biologicals

GSK will monitor the study to verify that, amongst others, the:

- Data are authentic, accurate, and complete.
- Safety and rights of subjects are being protected.
- Study is conducted in accordance with the currently approved protocol, any other study agreements, GCP and all applicable regulatory requirements.

The investigator and the head of the medical institution (where applicable) agrees to allow the monitor direct access to all relevant documents.

The investigator must ensure provision of reasonable time, space and qualified personnel for monitoring visits.

Direct access to all study-site related and source data is mandatory for the purpose of monitoring review. The monitor will perform an eCRF review and a Source Document Verification (SDV). By SDV we understand verifying eCRF entries by comparing them with the source data that will be made available by the investigator for this purpose.

The Source Documentation Agreement Form describes the source data for the different data in the eCRF. This document should be completed and signed by the site monitor and investigator and should be filed in the monitor's and investigator's study file. Any data item for which the eCRF will serve as the source must be identified, agreed and documented in the source documentation agreement form.

For eCRF, the monitor freezes completed and approved screens at each visit.

Upon completion or premature discontinuation of the study, the monitor will conduct site closure activities with the investigator or site staff, as appropriate, in accordance with applicable regulations, GCP, and GSK procedures.

12.3. Record retention

Following closure of the study, the investigator must maintain all site study records (except for those required by local regulations to be maintained elsewhere) in a safe and secure location. The records must be easily accessible, when needed (e.g. audit or inspection), and must be available for review in conjunction with assessment of the facility, supporting systems, and staff. Where permitted by applicable laws/regulations or institutional policy, some or all of these records can be maintained in a validated format other than hard copy (e.g. microfiche, scanned, electronic); however, caution needs to be exercised before such action is taken. The investigator must ensure that all reproductions are legible and are a true and accurate copy of the original and meet accessibility and retrieval standards, including re-generating a hard copy, if required. Furthermore, the investigator must ensure that an acceptable back-up of the reproductions exists and that there is an acceptable quality control procedure in place for making these reproductions.

GSK will inform the investigator/institution of the time period for retaining these records to comply with all applicable regulatory requirements. However, the investigator/institution should seek the written approval of the sponsor before proceeding with the disposal of these records. The minimum retention time will meet the strictest standard applicable to a particular site, as dictated by ICH GCP, any institutional requirements, applicable laws or regulations, or GSK standards/procedures.

The investigator/institution must notify GSK of any changes in the archival arrangements, including, but not limited to archival at an off-site facility, transfer of ownership of the records in the event the investigator leaves the site.

12.4. Quality assurance

To ensure compliance with GCP and all applicable regulatory requirements, GSK may conduct a quality assurance audit. Regulatory agencies may also conduct a regulatory inspection of this study. Such audits/inspections can occur at any time during or after completion of the study. If an audit or inspection occurs, the investigator and institution agree to allow the auditor/inspector direct access to all relevant documents and to allocate his/her time and the time of his/her staff to the auditor/inspector to discuss findings and any relevant issues.

12.5. Posting of information on publicly available clinical trial registers and publication policy

Study information from this protocol will be posted on publicly available clinical trial registers before enrollment of subjects begins.

Summaries of the results of GSK interventional studies (phase I-IV) are posted on publicly available results registers within 12 months of the primary completion date.

GSK also aims to publish the results of these studies in the searchable, peer reviewed scientific literature. Manuscripts are submitted for publication within 24 months of the last subject's last visit. At the time of publication, this protocol will be fully disclosed.

12.6. Provision of study results to investigators

Where required by applicable regulatory requirements, an investigator signatory will be identified for the approval of the study report. The investigator will be provided reasonable access to statistical tables, figures, and relevant reports and will have the opportunity to review the complete study results at a GSK site or other mutually-agreeable location.

GSK Biologicals will also provide the investigator with the full summary of the study results. The investigator is encouraged to share the summary results with the study subjects, as appropriate.

13. COUNTRY SPECIFIC REQUIREMENTS

Not applicable.

14. REFERENCES

Asante KP, Abdulla S, Agnandji S, et al. Safety and efficacy of the RTS,S/AS01$_E$ candidate malaria vaccine given with expanded-programme-on-immunisation vaccines: 19 month follow-up of a randomised, open-label, phase 2 trial. *Lancet Infect Dis.* 2011; 11:741-49.

Ballou WR, Sherwood JA, Neva FA, et al. Safety and efficacy of a recombinant DNA *Plasmodium falciparum* sporozoite vaccine. *Lancet* 1987;8545:1277-1281.

Centers for Disease Control and Prevention Metropolitan Atlanta Congenital Defects Program (CDC MACDP) guidelines. Birth defects and genetic diseases branch 6-digit code for reportable congenital anomalies; http://www.cdc.gov/ncbddd/birthdefects/documents/MACDPcode0807.pdf (last access: 09-MAR-2016)

Clement F, Dewar V, Van Braeckel E et al. Validation of an enzyme-linked immunosorbent assay for the quantification of human IgG directed against the repeat region of the circumsporozoite protein of the parasite *Plasmodium falciparum*. *Malar J.* 2012; 11:384.

EMA Guideline on the exposure to medicinal products during pregnancy: need for post-authorization data (Doc. Ref. EMEA/CHMP/313666/2005) 'adopted at Community level in May 2006); *http://www.ema.europa.eu/docs/en_GB/document_library/Regulatory_and_procedural_guideline/2009/11/WC500011303.pdf* (last access: 09-MAR-2016)

European Consensus Group on Hepatitis B Immunity. Are booster immunisations needed for lifelong hepatitis B immunity? *Lancet.* 2000; 355(9203):561-565.

FDA guidance for industry: toxicity grading scale for healthy adult and adolescent volunteers enrolled in preventive vaccine clinical trials (September 2007). http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Vaccines/ucm074775.htm (last access: 09-MAR-2016).

Malaria Vaccine Funders Group. Malaria Vaccine Technology Roadmap. 2013. http://www.who.int/immunization/topics/malaria/vaccine_roadmap/TRM_update_nov13.pdf?ua=1 (last access: 09-MAR-2016).

Montoya J, Solon JA, Cunanan SR, et al. A randomized, controlled dose-finding phase II study of the M72/AS01 candidate tuberculosis vaccine in healthy PPD-positive adults. *J Clin Immunol* 2013; 33(8): 1360-75.

Stoute JA, Slaoui M, Heppner DG et al. A preliminary evaluation of a recombinant circumsporozoite protein vaccine against *Plasmodium falciparum* malaria. *N Engl J Med.* 1997; 336:86-91.

The RTS,S Clinical Trials Partnership. First results of a phase 3 trial of RTS,S/AS01 malaria vaccine in African children. *N Engl J Med.* 2011; 365:1863-75.

The RTS,S Clinical Trials Partnership. A Phase 3 Trial of RTS,S/AS01 Malaria Vaccine in African Infants. *N Engl J Med*. 2012; 367:2284-2295.

The RTS,S Clinical Trials Partnership. The RTS,S/AS01 Malaria Vaccine Phase 3 Trial: 18 Month Efficacy and Safety in Children and Young Infants at 11 African Sites. *PLoS Med.* 2014; 11(7):e1001685.

The RTS,S Clinical Trials Partnership. Efficacy and safety of RTS,S/AS01 malaria vaccine with or without a booster dose in infants and children in Africa: final results of a phase 3, individually randomised, controlled trial. *Lancet.* 2015;386(9988):31-45.

Wirtz RA, Ballou WR, Schneider I et al. *Plasmodium falciparum*: Immunogenicity of circumsporozoite protein constructs produced in Escherichia coli. *Exp Parasitol* 1987;63:166-72.

APPENDIX A LABORATORY ASSAYS

Anti-CS antibody

Antibody concentrations against *P. falciparum* CS-repeat region will be measured at CEVAC by a standard ELISA methodology using plate adsorbed recombinant R32LR antigen, as described by Clement *et al*. [Clement, 2012]. Anti-CS antibody concentrations will be determined relative to a standard reference antibody as a control according to standard operating procedures from the laboratory. The cut-off for the assay is 0.5 EU/ml. Results will be reported in EU/ml.

Anti-HBs antibody

Anti-HBs antibody concentrations will be determined using commercially available CLIA kits ADVIA® Centaur anti-HBs2 manufactured by Siemens Healthcare. The cut-off for the assay is 6.2 mIU/ml. Results will be reported in mIU/ml.

Avidity assays against CS repeat region, CS full length and C-term antigens

The anti-CS repeat region avidity assay uses NH_4SCN to demonstrate the avidity of the antibodies in the assay, according to standard operating procedures (SOPs) from the laboratory. Briefly, one extra step is introduced in the classic anti-CS quantification assay. After addition and incubation of the serum sample an extra incubation with the chaotropic reagent (NH_4SCN at 1M) is inserted in order to introduce a detaching force to the antigen-antibody complex. The remaining antibodies, demonstrating a higher binding force to the antigen, are further quantified and the avidity index % (anti-CS repeat region concentration under chaotropic reagent/anti-CS repeat region concentration without chaotropic reagent) is calculated and reported.

The avidity assay against full length CS protein and C-term uses 4M urea as chaotropic reagent and the result is also reported as avidity index % (anti-CSP full length concentration under chaotropic reagent/anti-CS protein full length concentration without chaotropic reagent and anti-C-term concentration under chaotropic reagent/anti-C-term titer without chaotropic reagent, respectively).

Blood smear and PCR testing for assessment of *P. falciparum* parasitemia

Blood slide preparation and reading and PCR testing will be performed according to laboratory SOPs.

Hematological and biochemical testing

Hematological and biochemical testing will be performed at Quest Diagnostics, Inc. using laboratory SOPs.

RNA sequencing (mRNA expression analysis)

mRNA sequencing is a technique using next generation sequencing technology to sequence and quantify mRNA samples. It concerns analysis of mRNA transcription, not genetic testing as the data obtained is used to assess relative abundance of mRNA molecules. The Illumina Genome Analyzer II uses clonal array formation and reversible terminator technology to generate 20-30 million short sequence reads per sample lane.

These short sequences are aligned with the genome and counted to provide a relative frequency of sequences in the library population. The experiments will be instructed by the results obtained from systems biology results in previous CHMI studies.

APPENDIX B CLINICAL LABORATORIES

Table 31 GSK Biologicals' laboratories

Laboratory	Address
GSK Biologicals Clinical Laboratory Sciences, Rixensart	Biospecimen Reception - B7/44 Rue de l'Institut, 89 - B-1330 Rixensart - Belgium
GSK Biologicals Clinical Laboratory Sciences, Wavre-Nord Noir Epine	Avenue Fleming, 20 - B-1300 Wavre - Belgium

Table 32 Outsourced laboratories

Laboratory	Address
CEVAC - University of Gent	De Pintelaan, 185 Gent Belgium
Division of Malaria Vaccine Development - WRAIR	Walter Reed Army Institute of Research Silver Spring, MD 20910, United States
Precision Bioservices, Inc.	8425 Progress Drive Frederick, MD 21701, United States
Quest Diagnostics, Inc.	1901 Sulphur Spring Road Baltimore, MD 21227, United States
Q² Solutions	***27027 Tourney Road, Suite 2E*** ***Valencia, CA 91355*** ***United States***

(Amended 19 June 2017)

APPENDIX C NHANES I CARDIOVASCULAR RISK CRITERIA

Volunteers will be screened for cardiac risk factors and be given a screening electrocardiogram. The information will be recorded on a source document with the following noted:

Study ID # _____

Risk factors

Weight___ kg

Blood pressure _____

Height_____

Smoker Y / N

Calculated BMI (kg/m^2)_____

Diabetes Y / N

Using Table A (males) or Table B (females), 5-year cardiovascular risk:

□ Low

□ Moderate

□ High

Electrocardiogram (EKG)

12-lead EKG taken? Y / N

If not,
reason_____

Electrocardiogram interpreted by

_____

Electrocardiogram interpretation

_____

Only volunteers with NHANES I low risk criteria as well as non-significant EKG, as determined by expert consultant cardiologist, are accepted in the study.

Table A (for Males)

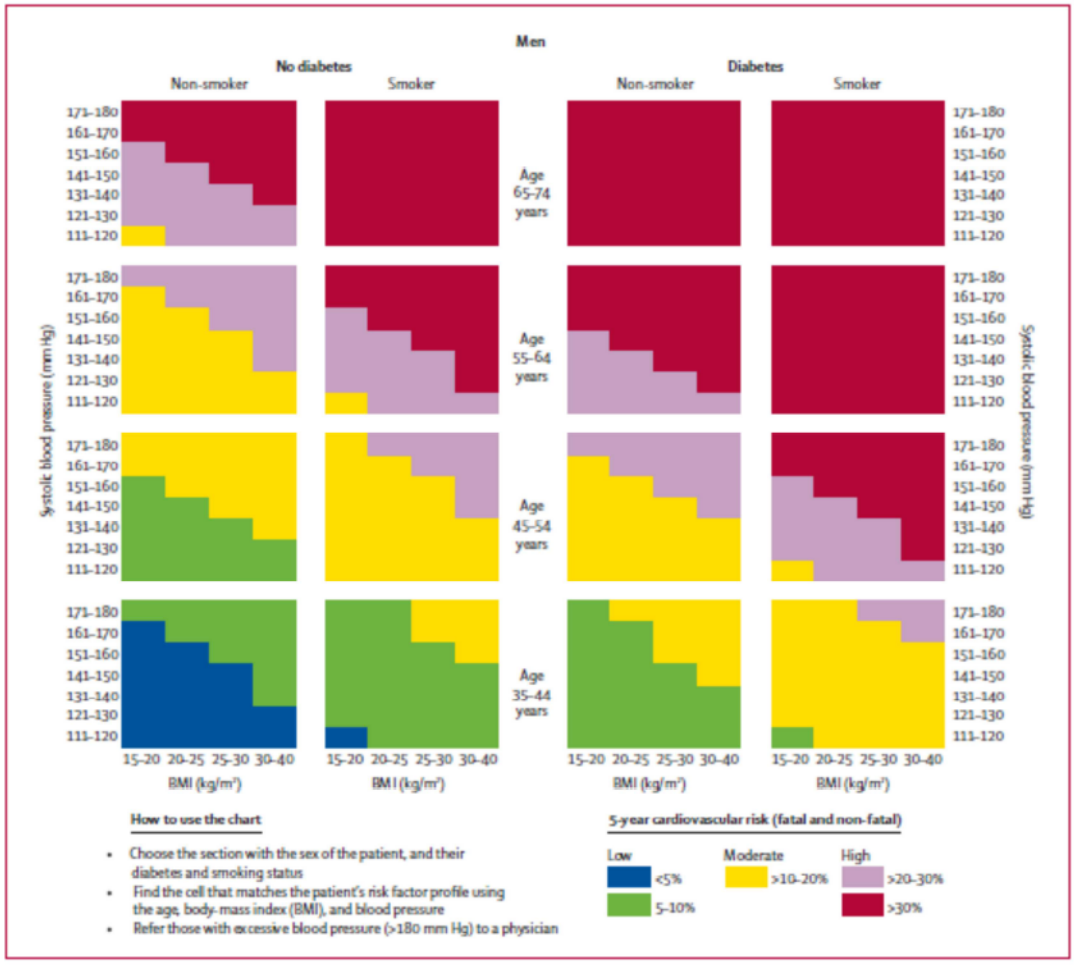
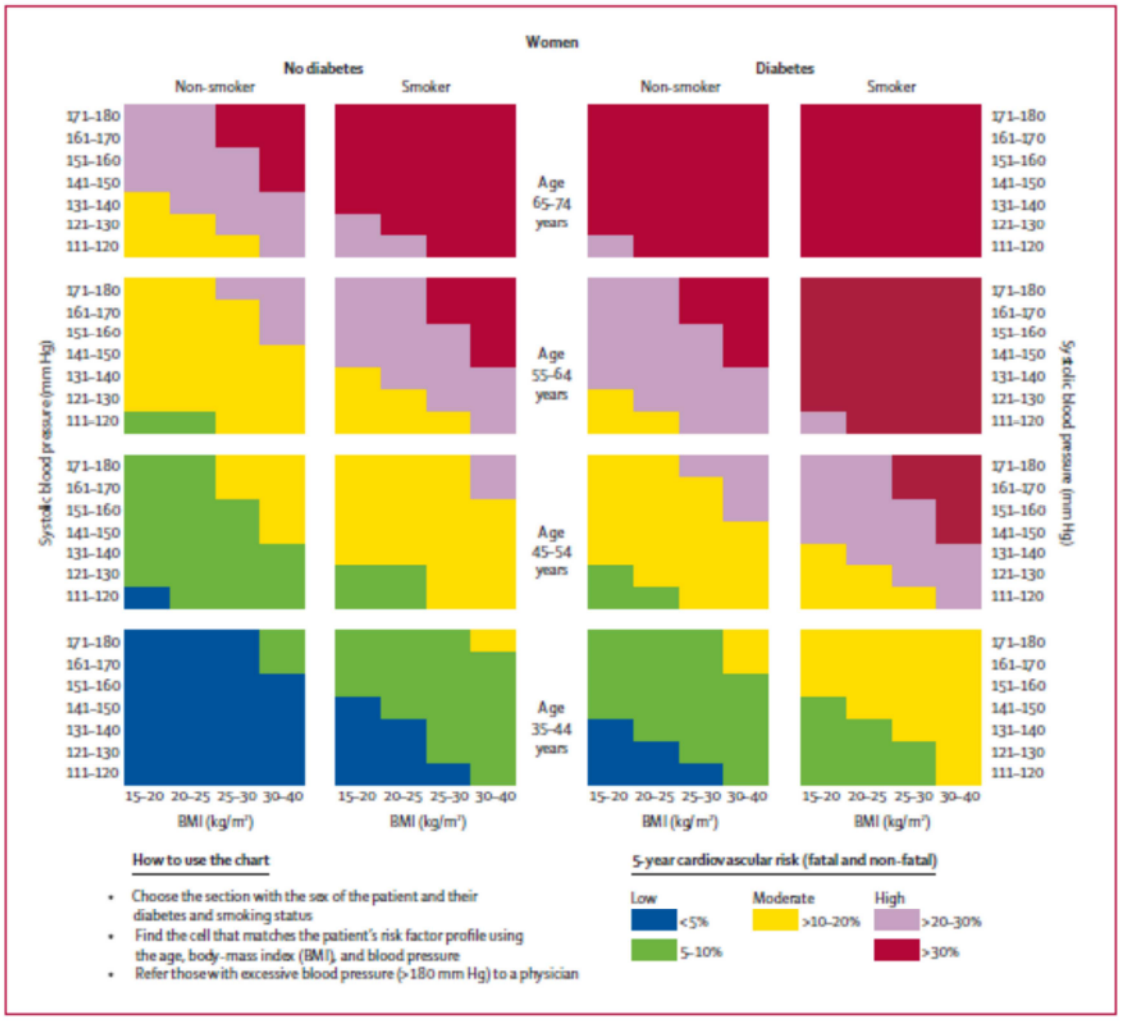


Table B (for Females)



APPENDIX D AMENDMENTS AND ADMINISTRATIVE CHANGES TO THE PROTOCOL

GlaxoSmithKline Biologicals Vaccine Value & Health Science (VVHS) **Protocol Amendment 1**	
eTrack study number and Abbreviated Title	205081 (MALARIA-092)
Amendment number:	Amendment 1
Amendment date:	23 June 2016
Co-ordinating author:	PPD Scientific Writer, Freelance Contractor for GSK Biologicals

Rationale/background for changes:

An additional blood sample of approximately 2 ml for assessment of parasitemia (polymerase chain reaction [PCR] testing) will be collected on the day of subject entry into the hotel phase (Day 295 [Visit 16]) to provide the study team an estimate of the number of subjects likely to develop malaria within the first few days of the hotel phase. This will allow the principal investigator and the study team to maximize subject safety by properly allocating resources and medical personnel to meet any high demand periods (i.e. periods when a large number of subjects are/will become ill). These samples will be processed by and assessed at WRAIR.

The text has been revised to provide clarification that blood samples for both PBMC **and plasma** will be collected for repository storage.

The final protocol dated 09 March 2016 details that 1 ml whole blood samples will be taken for parasitemia assessment. EDTA tubes of 1 ml volume are unavailable in the US hence 2 ml EDTA tubes will be provided. Whole blood samples of 2 ml will be drawn and the required sample volumes will be extracted for the blood slide smear.

In Section 6.6.8, the text has been revised to clarify that urine pregnancy tests will be conducted for all females and not just those of childbearing potential.

On Day 1 post day of challenge no blood samples are scheduled. As there is no safety concern at this timepoint and no specific assessments are scheduled, this visit has been deleted from the protocol.

The proposed genetic testing will investigate the immune response to the vaccine or to malaria through RNA sequencing, which looks at the signature for immune molecules being produced by the body's cells. Deep sequencing refers to sequencing a genomic region multiple times in order to detect rare events in the subject's genome, identifying rare genetic mutations and specific hereditary characteristics. Deep sequencing is not proposed in this study, reference to which has been replaced by RNA sequencing.

Amended text has been included in *bold italics* and deleted text in ~~strikethrough~~ in the following sections:

SYNOPSIS

Study design

- Experimental design: Phase IIA, open, randomized, controlled, mono-centric, single-country study with five parallel groups and one infectivity control group.
- Duration of the study: Approximately 16 months for each vaccinated subject and three months for the infectivity control subjects.
  - Epoch 001: Primary starting at Visit 1 (Screening) (Screening for vaccinated subjects) and ending at Visit 10 (Day 226).
- Epoch 002: Challenge starting at Visit 11 (Day 286) or Visit 1b (Screening) (Screening for infectivity controls), as applicable, and ending at Visit 3***1***~~2~~ (Day 376).
- Sampling schedule:
  - Blood samples for peripheral blood mononuclear cells (PBMC) ***and plasma*** repository will be collected before vaccine administration (Day 0), one month post-Dose 1 (Day 28), seven days post-Dose 2 (Day 35), one month post-Dose 2 (Day 58), six months post-Dose 2 (Day 196), seven days post-Dose 3 (Day 203), one month post-Dose 3 (Day 226), on the day of challenge (Day 286), and at study end (Day 376) for subjects from **AduFx, 2PedFx, PedFx, and Adu2Fx groups** and before vaccine administration (Day 0), one month post-Dose 1 (Day 28), before Dose 2 (Day 196), seven days post-Dose 2 (Day 203), one month post-Dose 2 (Day 226), on the day of challenge (Day 286), and at study end (Day 376) for subjects from **Adu1Fx group**.
  - Blood samples for ~~deep~~***RNA*** sequencing (messenger ribonucleic acid [mRNA] sequencing analysis) will be collected before vaccine administration (Day 0), at Dose 2 (Day 28), one day post-Dose 2 (Day 29), at Dose 3 (Day 196), and one day post-Dose 3 (Day 197) for subjects from **AduFx, 2PedFx, PedFx, and Adu2Fx groups** and before vaccine administration (Day 0), one month post-Dose 1 (Day 28), at Dose 2 (Day 196) and one day post-Dose 2 (Day 197) for subjects from **Adu1Fx group**.

  - ***Blood sample for assessment of parasitemia (PCR testing) will be collected on the day of subject entry into the hotel phase (Day 295 [Visit 16]) to provide an estimate of the number of subjects likely to develop malaria within the first few days of the hotel phase.***
  - Blood sample for assessment of parasitemia (blood smear) will be collected daily for 14 days (from Day 291 [Visit 1~~2~~***3***] to Day 304 [Visit 2~~5~~***6***]) and then every two days for nine days (Day 306 [Visit 2~~6~~***7***], Day 308 [Visit 2~~7~~***8***], Day 310 [Visit 2~~8~~***9***], Day 312 [Visit ***29***~~30~~], and Day 314 [Visit 3~~0~~***1***]). For subjects who develop malaria, blood smear may be discontinued once the subject has three consecutive negative smears (separated by more than 12 hours) following the initial treatment.

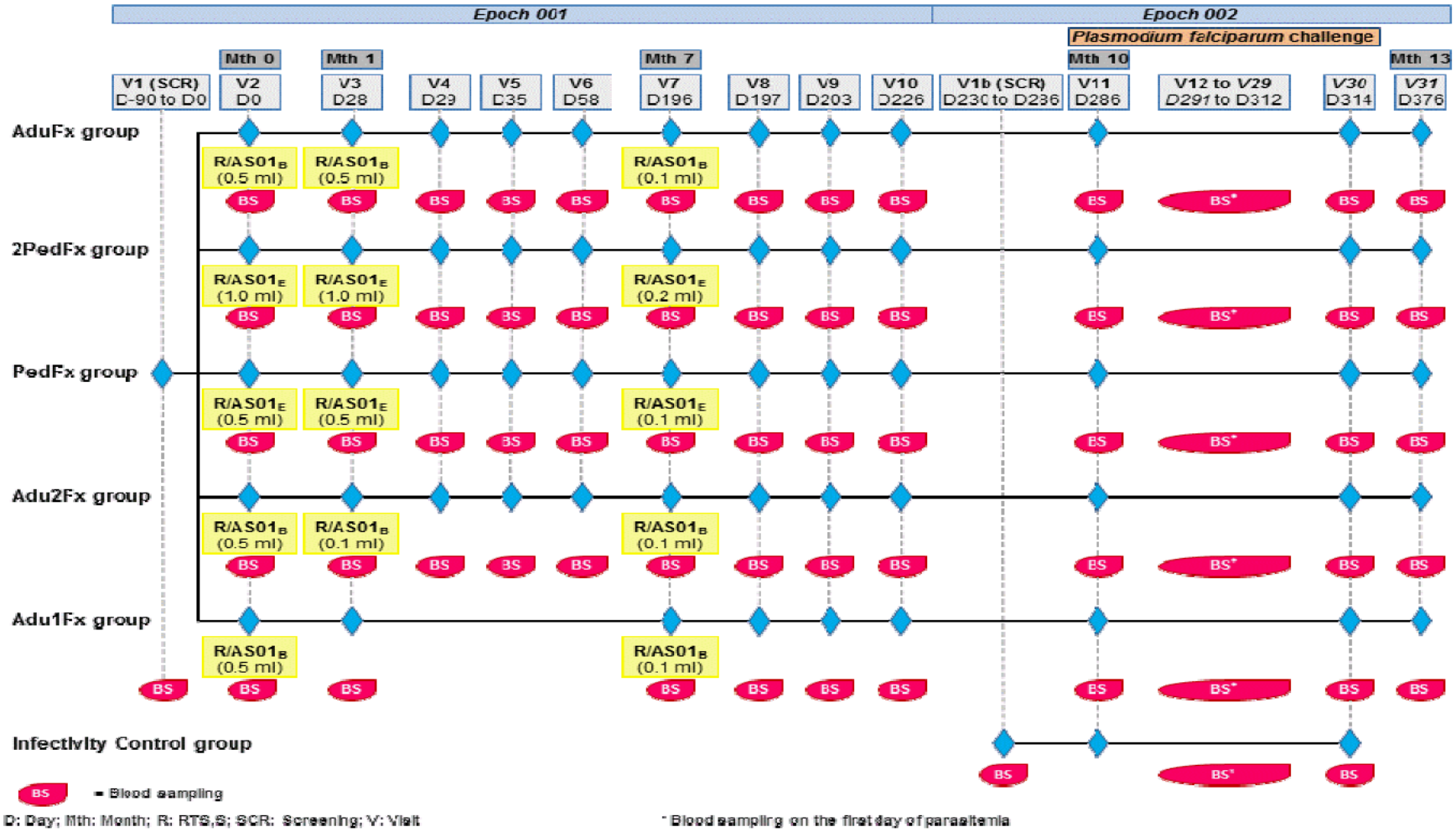
- Safety monitoring:
  - Biochemistry (ALT, AST and creatinine) and hematology (hemoglobin, WBC and platelets) parameters will be assessed ~~before vaccine administration (screening), seven days post-Dose 2 (Day 35), one month post-Dose 2 (Day 58), seven days post-Dose 3 (Day 203), one month post-Dose 3 (Day 226), the day of first parasitemia, and 28 days post-challenge (Day 314) for subjects from AduFx, 2PedFx, PedFx, and Adu2Fx groups, before vaccine administration (screening), seven days post-Dose 2 (Day 203), one month post-Dose 2 (Day 226), the day of first parasitemia, and 28 days post-challenge (Day 314) for subjects from Adu1Fx group and at screening, the day of first parasitemia and 28 days post-challenge (Day 314) for the infectivity control subjects~~***as described in 'Sampling schedule'***.
-

LIST OF ABBREVIATIONS

PCR:	***Polymerase chain reaction***

3. STUDY DESIGN OVERVIEW

Figure 1 Study design



Footnotes to Figure 1:
D: Day; Mth: month; R: RTS,S; SCR: Screening; V: visit. * ***Blood sample for estimation of subjects with parasitemia (PCR testing) will be collected on the day of subject entry into the hotel phase (Day 295 [Visit 16]); b****~~B~~*lood sample for biochemistry and hematology parameters will be collected the day of first parasitemia and blood sample for assessment of parasitemia (blood smear) will be collected daily for 14 days (from Day 291 [Visit 1***2~~3~~***] to Day 304 [Visit 2***5~~4~~***]) and then every two days for nine days (Day 306 [Visit 2***6~~7~~***], Day 308 [Visit 2***7~~8~~***], Day 310 [Visit 2***8~~9~~***], Day 312 [Visit ***29~~30~~***], and Day 314 [Visit 3***0~~1~~***]).

- Experimental design: Phase IIA, open, randomized, controlled, mono-centric, single-country study with five parallel groups and one infectivity control group.
- Duration of the study: Approximately 16 months for each vaccinated subject and three months for the infectivity control subjects.
  - Epoch 001: Primary starting at Visit 1 (Screening) (Screening for vaccinated subjects) and ending at Visit 10 (Day 226).
  - Epoch 002: Challenge starting at Visit 11 (Day 286) or Visit 1b (Screening) (Screening for infectivity controls), as applicable, and ending at Visit 3*1*~~2~~ (Day 376).

- Sampling schedule:
  - Blood samples for peripheral blood mononuclear cells (PBMC) ***and plasma*** repository will be collected before vaccine administration (Day 0), one month post-Dose 1 (Day 28), seven days post-Dose 2 (Day 35), one month post-Dose 2 (Day 58), six months post-Dose 2 (Day 196), seven days post-Dose 3 (Day 203), one month post-Dose 3 (Day 226), on the day of challenge (Day 286), and at study end (Day 376) for subjects from **AduFx, 2PedFx, PedFx, and Adu2Fx groups** and before vaccine administration (Day 0), one month post-Dose 1 (Day 28), before Dose 2 (Day 196), seven days post-Dose 2 (Day 203), one month post-Dose 2 (Day 226), on the day of challenge (Day 286), and at study end (Day 376) for subjects from **Adu1Fx group**.
  - Blood samples for ~~deep~~***RNA*** sequencing (messenger ribonucleic acid [mRNA] sequencing analysis) will be collected before vaccine administration (Day 0), at Dose 2 (Day 28), one day post-Dose 2 (Day 29), at Dose 3 (Day 196), and one day post-Dose 3 (Day 197) for subjects from **AduFx, 2PedFx, PedFx, and Adu2Fx groups** and before vaccine administration (Day 0), one month post-Dose 1 (Day 28), at Dose 2 (Day 196) and one day post-Dose 2 (Day 197) for subjects from **Adu1Fx group**.
  - ***Blood sample for assessment of parasitemia (PCR testing) will be collected on the day of subject entry into the hotel phase (Day 295 [Visit 16]) to provide an estimate of the number of subjects likely to develop malaria within the first few days of the hotel phase.***
  - Blood sample for assessment of parasitemia (blood smear) will be collected daily for 14 days (from Day 291 [Visit 1*2*~~3~~] to Day 304 [Visit 2*5*~~6~~]) and then every two days for nine days (Day 306 [Visit 2*6*~~7~~], Day 308 [Visit 2*7*~~8~~], Day 310 [Visit 2*8*~~9~~], Day 312 [Visit *29*~~30~~], and Day 314 [Visit 3*0*~~1~~]). For subjects who develop malaria, blood smear may be discontinued once the subject has three consecutive negative smears (separated by more than 12 hours) following the initial treatment.
- Safety monitoring:
  - Biochemistry (ALT, AST and creatinine) and hematology (hemoglobin, WBC and platelets) parameters will be assessed ~~before vaccine administration (screening), seven days post-Dose 2 (Day 35), one month post-Dose 2 (Day 58),~~

~~seven days post Dose 3 (Day 203), one month post Dose 3 (Day 226), the day of first parasitemia, and 28 days post-challenge (Day 314) for subjects from AduFx, 2PedFx, PedFx, and Adu2Fx groups, before vaccine administration (screening), seven days post Dose 2 (Day 203), one month post Dose 2 (Day 226), the day of first parasitemia, and 28 days post-challenge (Day 314) for subjects from Adu1Fx group and at screening, the day of first parasitemia and 28 days post-challenge (Day 314) for the infectivity control subjects~~***as described in 'Sampling schedule'.***

5.2. Inclusion criteria for enrolment

Deviations from inclusion criteria are not allowed because they can potentially jeopardize the scientific integrity of the study, regulatory acceptability or subject safety. Therefore, adherence to the criteria as specified in the protocol is essential.

All subjects must satisfy ALL the following criteria at study entry:

- Female subjects of childbearing potential may be enrolled in the study, if the subject:
  - has practiced adequate contraception for 30 days prior to vaccination, and
  - has a negative pregnancy test at enrolment, and
  - has agreed to continue adequate contraception during the entire treatment period and for two months after completion of the vaccination series ***and/or malaria challenge***.

6.4.1.5. Management of infected human subjects

The pre-patent period for *P. falciparum* in man normally averages 9-12 days. In previous studies, the pre-patent period in controls varied from 7-18 days. The shortest reported pre-patent period in man is five days, and the longest is 25 days [Ballou, 1987]. An immunized individual who does not have complete protection may have a prolongation of the parasite pre-patent period.

~~Subjects will return at the study clinic on Day 1 (Day 287 [Visit 12]) after their primary phase challenge for evaluation by a study investigator. If for any reason subjects do not come at the study clinic, they can be contacted by phone to collect information on their well-being.~~ Beginning on Day 5 (Day 291 [Visit 1~~2~~***3***]) after their challenge, subjects will be seen and evaluated daily by a study investigator and blood will be drawn for blood smears to check for the presence of parasites as described in Section 6.4.1.4. If fever or symptoms develop at any time, blood smears will be done more frequently (every 6 to 12 hours), and a study investigator will evaluate the subject. A confirmed positive result will be relayed immediately to the on-call investigator/study personnel by the microscopists. The infection will be treated early (i.e. as soon as parasites can be identified on thick smear) according to the treatment regimen outlined in Section 6.4.1.6.

Beginning on Day 9 post-challenge (Day 295 [Visit 1~~6~~***7***]), a group of hotel rooms in the local area of WRAIR, will be reserved for malaria-challenged subjects. The subjects will be required to spend their nights there to allow for more rapid assessment of any potential

symptoms of malaria during the hours that the study centers are closed. There will be an investigator present on-site and available for subject assessment. There will also be qualified study personnel on site 24 hours per day during the hotel phase of the study.

A blood sample for assessment of parasitemia (PCR testing) will be collected on the day of subject entry into the hotel phase (Day 295 [Visit 16]) to provide the study team an estimate of the number of subjects likely to develop malaria within the first few days of the hotel phase. This will allow the principal investigator and the study team to maximize subject safety by properly allocating resources and medical personnel to meet any high demand periods (i.e. periods when a large number of subjects are/will become ill). These samples will be processed by and assessed at WRAIR.

During the hotel phase, all challenged subjects will be assessed on a daily basis in an identical manner. An evaluation will be done each morning (headache, muscle aches, etc.) and blood will be drawn for smear. All challenged subjects will be instructed to check in with clinical staff by telephone call or in-person each evening during the hotel stay until they are positive for malaria~~ or post day of challenge Day 28 whichever comes first~~. They will be asked if they feel any differently since they were seen in the morning. At any time required, the on-duty investigator will arrange for the timely production of blood smears, along with their examination and interpretation, in order to treat rapidly those subjects in whom therapy for malaria is indicated. Once a positive smear is identified, daily blood films will continue to be obtained until three consecutive films are negative (separated by more than 12 hours). A complete blood count and serum chemistry tests will be done when parasites are initially found in the blood (this could be done on the day of parasitemia detection or the day after).

The maximum hotel stay for malaria-challenged subjects should be approximately 10 days (Day 295 [Visit 1***6***~~7~~] to Day 304 [Visit 2***5***~~6~~]). A subject who develops malaria, is treated, and has three consecutive negative malaria smears (separated by more than 12 hours), will not need to remain in the hotel. The investigators will be responsible for accounting for any subjects who do not arrive in the hotel during the challenge phase. If required, the investigators will physically locate and treat any malaria-infected subject who is unable to maintain the follow-up dictated by this study.

If infection does not develop within 18 days, the subject will be released from staying nightly at the hotel. Subjects who do not develop malaria will be required to come to the clinical center for evaluation and blood drawing for smears every two days up to 28 days post-challenge (Days 306 [Visit 2***6***~~7~~], 308 [Visit 2***7***~~8~~], 310 [Visit 2***8***~~9~~], 312 [Visit ***29***~~30~~], and 314 [Visit 3***0***~~1~~]). A subject who develops malaria and has three consecutive (separated by more than 12 hours) negative smears following initial treatment may be excused from the remaining hotel visits and the late post-challenge clinic visits at Days 306, 308, 310, and 312 (20, 22, 24 and 26 days post-challenge), but will be required to come to the clinic center at Day 314 (28 days post-challenge [Visit 3***0***~~1~~]). Telephone contact will be made if the subject does not keep a scheduled follow-up appointment. Symptom screening via telephone may suffice in lieu of clinical visits for Days 306, 308, 310, or 312 which fall upon a weekend.

6.5. Outline of study procedures

Table 6 List of study procedures for the investigational vaccine groups: AduFx, 2PedFx, PedFx, and Adu2Fx

Epoch	Epoch 001										Epoch 002				
Type of contact	Visit 1 (Screening)	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9	Visit 10	Visit 11	Visit 12 to Visit ~~30~~29*	Visit Par**	Visit ~~31~~30	Visit ~~32~~31
Study Month		0	1				7				10				13
Study Day	-90 to 0	0	28	29	35	58	196	197	203	226	286	~~28~~791 to 312		314	376
Vaccination timepoint		Vacc 1	Vacc 2				Vacc 3				CHMI phase				
Informed consent	●														
Check inclusion/exclusion criteria[a]	●	O													
Check screening laboratory results[b]	●														
Collect demographic data	●														
Medical history	●														
Record information on past hepatitis B immunization[c]	●														
Physical examination	●	O	O	O	O	O	O	O	O	O	O	O	O	O	O
Urine pregnancy test (β-HCG)	●	●	●				●				●				
Check contraindications to vaccination[d]		●	●				●								
Record pre-vaccination body temperature		●	●				●								
Randomization	●														
Treatment number allocation		O	O				O								
Recording of administered treatment number		●	●				●								
Vaccines administration		●	●				●								
Check contraindications to challenge[d]											●				
Sporozoite challenge											●				
Distribution of emergency notification card											O				
Blood sampling for HIV, HCV and HBsAg (17 ml)	●														
Blood sampling for assessment of parasitemia (***blood smear;*** ~~2~~4.0 ml)												●***		●	
Blood sampling for assessment of parasitemia at start of hotel phase (PCR; 2.0 ml)												***O*******			

Epoch	Epoch 001										Epoch 002				
Type of contact	Visit 1 (Screening)	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9	Visit 10	Visit 11	Visit 12 to Visit ~~30~~29*	Visit Par**	Visit ~~31~~30	Visit ~~32~~31
Study Month		0	1				7				10				13
Study Day	-90 to 0	0	28	29	35	58	196	197	203	226	286	~~28~~791 to 312		314	376
Vaccination timepoint		Vacc 1	Vacc 2				Vacc 3				CHMI phase				
Blood sampling for assessment of antibody determination and serum repository (20.0 ml)		●				●	●			●	●			●	●
Blood sampling for ~~deep~~*RNA* sequencing (12 ml)		●	●	●			●	●							
Blood sampling for PBMC ***and plasma*** repository (30.0 ml)		●	●		●	●	●		●	●	●				●
Blood sampling for hematology and biochemistry analysis (7.5 ml)[e]	●				●	●			●	●			●	●	
Record any concomitant medications/vaccinations	●	●	●	●	●	●	●	●	●	●	●	●		●	●
Record any intercurrent medical conditions	●	●	●	●	●	●	●	●	●	●	●	●		●	●
Distribution of diary cards		O	O				O								
Return of diary cards			O			O				O					
Diary card transcription by investigator			●			●				●					
Recording of solicited local and general AEs (Days 0–6) post-vaccination		●	●				●								
Recording of unsolicited AEs (Day 0-29) post-vaccination		●	●	●	●	●	●	●	●						
Recording of AEs (Day 0-29) post-challenge											●	●	●	●	
Recording of SAEs (all, fatal, related) and pregnancies		●	●	●	●	●	●	●	●	●	●	●	●	●	●
Recording of SAEs related to study participation, or to a concurrent GSK medication/vaccine	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Recording of all AEs and SAEs leading to withdrawal from further vaccination		●	●	●	●	●	●	●	●	●	●	●	●	●	●
Recording of AEs of specific interest[f]		●	●	●	●	●	●	●	●	●	●	●	●	●	●
Study Conclusion															●

β-HCG: Beta-human chorionic gonadotropin; PBMC: peripheral blood mononuclear cells; Vacc: vaccination.

* ~~Visit 12 = Day 287,~~ Visit 1~~2~~3 = Day 291, Visit 1~~3~~4 = Day 292, Visit 1~~4~~5 = Day 293, Visit 1~~5~~6 = Day 294, Visit 1~~6~~7 = Day 295, Visit 1~~7~~8 = Day 296, Visit 1~~8~~9 = Day 297, Visit 1~~9~~20 = Day 298, Visit 2~~0~~1 = Day 299, Visit 2~~1~~2 = Day 300, Visit 2~~2~~3 = Day 301, Visit 2~~3~~4 = Day 302, Visit 2~~4~~5 = Day 303, Visit 2~~5~~6 = Day 304, Visit 2~~6~~7 = Day 306, Visit 2~~7~~8 = Day 308, Visit 2~~8~~9 = Day 310, Visit ***2***~~9~~30 = Day 312.

** A blood sample for biochemistry and hematology parameters will be collected the day of first parasitemia (between Visit 12 [Day ~~28~~***791***] and Visit ***2***~~9~~30 [Day 312]).

*** Blood sample for assessment of parasitemia (blood smear) will be collected daily for 14 days (from Day 291 [Visit 123] to Day 304 [Visit 256]) and then every two days for nine days (Day 306 [Visit 267], Day 308 [Visit 278], Day 310 [Visit 289], Day 312 [Visit 2930], and Day 314 [Visit 301]). For subjects who develop malaria, blood smear may be discontinued once the subject has three consecutive negative smears (separated by more than 12 hours) following initial treatment.

**** ***Blood sample for assessment of parasitemia (PCR testing) will be collected on the day of subject entry into the hotel (Day 295).***

[a] Including a check of NHANES I criteria and electrocardiogram (see Appendix C).

[b] The screening laboratory results (HIV, HCV, HBsAg, ALT, AST, creatinine, hemoglobin, leukocytes [WBC], platelets, and urine β-HCG) must be checked during the screening activities and before randomization.

[c] Based on best evidences available.

[d] There is no specific section in the eCRF to record the contraindications, warnings and precautions. The absolute contraindications to further administration of study vaccines or to challenge have to be recorded in the AE or SAE section of the eCRF.

[e] Blood sampling for hematology and biochemistry analysis includes ALT, AST, creatinine, hemoglobin, leukocytes (WBC), and platelets.

[f] AEs of specific interest include meningitis and pIMDs (see Section 9.1.5).

● is used to indicate a study procedure that requires documentation in the individual eCRF.

○ is used to indicate a study procedure that does not require documentation in the individual eCRF.

Table 7 List of study procedures for the investigational vaccine group: Adu1Fx

Epoch	Epoch 001							Epoch 002				
Type of contact	Visit 1 (Screening)	Visit 2	Visit 3	Visit 7	Visit 8	Visit 9	Visit 10	Visit 11	Visit 12 to Visit 2930*	Visit Par**	Visit 304	Visit 312
Study Month		0	1	7				10				13
Study Day	-90 to 0	0	28	196	197	203	226	286	29187 to 312		314	376
Vaccination timepoint		Vacc 1		Vacc 2				CHMI phase				
Informed consent	●											
Check inclusion/exclusion criteria[a]	●	O										
Check screening laboratory results[b]	●											
Collect demographic data	●											
Medical history	●											
Record information on past hepatitis B immunization[c]	●											
Physical examination	●	O	O	O	O	O	O	O	O	O	O	O
Urine pregnancy test (β-HCG)	●	●		●				●				
Check contraindications to vaccination[d]		●		●								
Record pre-vaccination body temperature		●		●								
Randomization	●											

Epoch	Epoch 001							Epoch 002				
Type of contact	**Visit 1 (Screening)**	**Visit 2**	**Visit 3**	**Visit 7**	**Visit 8**	**Visit 9**	**Visit 10**	**Visit 11**	**Visit 12 to Visit ~~29~~30***	**Visit Par****	**Visit 3~~0~~4**	**Visit 3~~1~~2**
Study Month		**0**	**1**	**7**				**10**				**13**
Study Day	**-90 to 0**	**0**	**28**	**196**	**197**	**203**	**226**	**286**	**29~~187~~ to 312**		**314**	**376**
Vaccination timepoint		**Vacc 1**		**Vacc 2**				**CHMI phase**				
Treatment number allocation		O		O								
Recording of administered treatment number		●		●								
Vaccines administration		●		●								
Check contraindications to challenge[d]								●				
Sporozoite challenge								●				
Distribution of emergency notification card								O				
Blood sampling for HIV, HCV and HBsAg (17 ml)	●											
Blood sampling for assessment of parasitemia (***blood smear;*** 2~~4~~.0 ml)									●***		●	
Blood sampling for assessment of parasitemia at start of hotel phase (PCR; 2.0 ml)									O****			
Blood sampling for assessment of antibody determination and serum repository (20.0 ml)		●		●			●	●			●	●
Blood sampling for ~~deep~~***RNA*** sequencing (12 ml)		●	●	●	●							
Blood sampling for PBMC ***and plasma*** repository (30.0 ml)		●	●	●		●	●	●				●
Blood sampling for hematology and biochemistry analysis (7.5 ml)[e]	●					●	●			●	●	
Record any concomitant medications/vaccinations	●	●	●	●	●	●	●	●	●		●	●
Record any intercurrent medical conditions	●	●	●	●	●	●	●	●	●		●	●
Distribution of diary cards		O		O								
Return of diary cards			O				O					
Diary card transcription by investigator			●				●					
Recording of solicited local and general AEs (Days 0–6) post-vaccination		●		●								
Recording of unsolicited AEs (Day 0-29) post-vaccination		●	●	●	●	●						
Recording of AEs (Day 0-29) post-challenge								●	●	●	●	
Recording of SAEs (all, fatal, related) and pregnancies		●	●	●	●	●	●	●	●	●	●	●
Recording of SAEs related to study participation, or to a concurrent GSK medication/vaccine	●	●	●	●	●	●	●	●	●	●	●	●

Epoch	Epoch 001							Epoch 002				
Type of contact	Visit 1 (Screening)	Visit 2	Visit 3	Visit 7	Visit 8	Visit 9	Visit 10	Visit 11	Visit 12 to Visit ~~29~~30*	Visit Par**	Visit 3~~0~~1	Visit 3~~1~~2
Study Month		0	1	7				10				13
Study Day	-90 to 0	0	28	196	197	203	226	286	29~~187~~ to 312		314	376
Vaccination timepoint		Vacc 1		Vacc 2				CHMI phase				
Recording of all AEs and SAEs leading to withdrawal from further vaccination		●	●	●	●	●	●	●	●	●	●	●
Recording of AEs of specific interest[f]		●	●	●	●	●	●	●	●	●	●	●
Study Conclusion												●

β-HCG: Beta-human chorionic gonadotropin; PBMC: peripheral blood mononuclear cells; Vacc: vaccination.

* ~~Visit 12 = Day 287,~~ Visit 1~~2~~3 = Day 291, Visit 1~~3~~4 = Day 292, Visit 1~~4~~5 = Day 293, Visit 1~~5~~6 = Day 294, Visit 1~~6~~7 = Day 295, Visit 1~~7~~8 = Day 296, Visit 1~~8~~9 = Day 297, Visit 1~~9~~20 = Day 298, Visit 2~~0~~1 = Day 299, Visit 2~~1~~2 = Day 300, Visit 2~~2~~3 = Day 301, Visit 2~~3~~4 = Day 302, Visit 2~~4~~5 = Day 303, Visit 2~~5~~6 = Day 304, Visit 2~~6~~7 = Day 306, Visit 2~~7~~8 = Day 308, Visit 2~~8~~9 = Day 310, Visit ***29***~~30~~ = Day 312.

** A blood sample for biochemistry and hematology parameters will be collected the day of first parasitemia (between Visit 12 [Day 2~~87~~***91***] and Visit ***29***~~30~~ [Day 312]).

*** Blood sample for assessment of parasitemia (blood smear) will be collected daily for 14 days (from Day 291 [Visit 1~~2~~3] to Day 304 [Visit 2~~5~~6]) and then every two days for nine days (Day 306 [Visit 2~~6~~7], Day 308 [Visit 2~~7~~8], Day 310 [Visit 2~~8~~9], Day 312 [Visit ***29***~~30~~], and Day 314 [Visit 3~~0~~1]). For subjects who develop malaria, blood smear may be discontinued once the subject has three consecutive negative smears (separated by more than 12 hours) following initial treatment.

**** ***Blood sample for assessment of parasitemia (PCR testing) will be collected on the day of subject entry into the hotel (Day 295).***

[a] Including a check of NHANES I criteria and electrocardiogram (see Appendix C).

[b] The screening laboratory results (HIV, HCV, HBsAg, ALT, AST, creatinine, hemoglobin, leukocytes [WBC], platelets, and urine β-HCG) must be checked during the screening activities and before randomization.

[c] Based on best evidences available.

[d] There is no specific section in the eCRF to record the contraindications, warnings and precautions. The absolute contraindications to further administration of study vaccines or to challenge have to be recorded in the AE or SAE section of the eCRF.

[e] Blood sampling for hematology and biochemistry analysis includes ALT, AST, creatinine, hemoglobin, leukocytes (WBC), and platelets.

[f] AEs of specific interest include meningitis and pIMDs (see Section 9.1.5).

● is used to indicate a study procedure that requires documentation in the individual eCRF.

○ is used to indicate a study procedure that does not require documentation in the individual eCRF.

Table 8 List of study procedures for the infectivity control group

Epoch	Epoch 002				
Type of contact	Visit 1b (Screening)*	Visit 11	Visit 12 to Visit ~~29~~30**	Visit Par***	Visit 3~~0~~4
Study Day	230 to 286	286	29~~18~~7 to 312		314
Informed consent	●				
Check inclusion/exclusion criteria[a]	●	O			
Check screening laboratory results[b]	●				
Collect demographic data	●				
Medical history	●				
Record information on past hepatitis B immunization[c]	●				
Physical examination	●	O	O	O	O
Urine pregnancy test (β-HCG)	●	●			
Check contraindications to challenge[d]		●			
Sporozoite challenge		●			
Distribution of emergency notification card		O			
Blood sampling for HIV, HCV and HBsAg (17 ml)	●				
Blood sampling for assessment of parasitemia (***blood smear;*** ***2~~1~~***.0 ml)			●****		●
Blood sampling for assessment of parasitemia at start of hotel phase (PCR; 2.0 ml)			***O********		
Blood sampling for hematology and biochemistry analysis (7.5 ml)[e]	●			●	●
Record any concomitant medications/vaccinations	●	●	●		●
Record any intercurrent medical conditions	●	●	●		●
Recording of AEs (Day 0-29) post-challenge		●	●	●	●
Recording of SAEs (all, fatal, related) and pregnancies		●	●	●	●
Recording of SAEs related to study participation, or to a concurrent GSK medication/vaccine	●	●	●	●	●
Study Conclusion					●

β-HCG: Beta-human chorionic gonadotropin; PBMC: peripheral blood mononuclear cells.

* This visit is only applicable to the infectivity control group

** ~~Visit 12 = Day 287,~~ Visit 1~~2~~***3*** = Day 291, Visit 1~~3~~***4*** = Day 292, Visit 1~~4~~***5*** = Day 293, Visit 1~~5~~***6*** = Day 294, Visit 1~~6~~***7*** = Day 295, Visit 1~~7~~***8*** = Day 296, Visit 1~~8~~***9*** = Day 297, Visit 1~~9~~***20*** = Day 298, Visit 2~~0~~***1*** = Day 299, Visit 2~~1~~***2*** = Day 300, Visit 2~~2~~***3*** = Day 301, Visit 2~~3~~***4*** = Day 302, Visit 2~~4~~***5*** = Day 303, Visit 2~~5~~***6*** = Day 304, Visit 2~~6~~***7*** = Day 306, Visit 2~~7~~***8*** = Day 308, Visit 2~~8~~***9*** = Day 310, Visit ***29~~3~~30*** = Day 312.

*** A blood sample for biochemistry and hematology parameters will be collected the day of first parasitemia (between Visit 12 [Day 2~~87~~***91***] and Visit ***29~~3~~30*** [Day 312]).

**** Blood sample for assessment of parasitemia (blood smear) will be collected daily for 14 days (from Day 291 [Visit 1~~2~~***3***] to Day 304 [Visit 2~~5~~***6***]) and then every two days for nine days (Day 306 [Visit 2~~6~~***7***], Day 308 [Visit 2~~7~~***8***], Day 310 [Visit 2~~8~~***9***], Day 312 [Visit ***29~~3~~30***], and Day 314 [Visit 3~~0~~***1***]). For subjects who develop malaria, blood smear may be discontinued once the subject has three consecutive negative smears (separated by more than 12 hours) following initial treatment.

***** ***Blood sample for assessment of parasitemia (PCR testing) will be collected on the day of subject entry into the hotel (Day 295).***

[a] Including a check of NHANES I criteria and electrocardiogram (see Appendix C).

[b] The screening laboratory results (HIV, HCV, HBsAg, ALT, AST, creatinine, hemoglobin, leukocytes [WBC], platelets, and urine β-HCG) must be checked during the screening activities and before randomization.

[c] Based on best evidences available.

[d] There is no specific section in the eCRF to record the contraindications, warnings and precautions. The absolute contraindications to challenge have to be recorded in the AE or SAE section of the eCRF.

[e] Blood sampling for hematology and biochemistry analysis includes ALT, AST, creatinine, hemoglobin, leukocytes (WBC), and platelets.

● is used to indicate a study procedure that requires documentation in the individual eCRF.

○ is used to indicate a study procedure that does not require documentation in the individual eCRF.

Table 9 Intervals between study visits for the investigational vaccine groups: AduFx, 2PedFx, PedFx, and Adu2Fx

Interval	Optimal length of interval [1]	Allowed interval[2]
Visit 1 (Screening) → Visit 2 (1st vaccination)	0 to 90 days	-
Visit 2 → Visit 3 (2nd vaccination)	28 days	± 7 days
Visit 3 → Visit 5	7 days	± 1 day
Visit 3 → Visit 6	30 days	± 7 days
Visit 3 → Visit 7 (3rd vaccination)	168 days	± 14 days
Visit 7 → Visit 9	7 days	± 1 day
Visit 7 → Visit 10	30 days	± 7 days
Visit 7 → Visit 11 (Challenge)	90 days	+ 14 days
Visit 11 → Visit 30~~4~~	28 days	± 7 days
Visit 30~~4~~ → Visit 3~~12~~	62 days	± 14 days

[1] Whenever possible the investigator should arrange study visits within this interval.
[2] Subjects ~~will~~*may* not be eligible for inclusion in the according-to-protocol (ATP) cohort for analysis of immunogenicity and efficacy if they make the study visit outside this interval.

Table 10 Intervals between study visits for the investigational vaccine groups: Adu1Fx

Interval	Optimal length of interval [1]	Allowed interval[2]
Visit 1 (Screening) → Visit 2 (1st vaccination)	0 to 90 days	-
Visit 2 → Visit 7 (2nd vaccination)	196 days	± 14 days
Visit 7 → Visit 9	7 days	± 1 day
Visit 7 → Visit 10	30 days	± 7 days
Visit 7 → Visit 11 (Challenge)	90 days	+ 14 days
Visit 11 → Visit 30~~4~~	28 days	± 7 days
Visit 30~~4~~ → Visit 3~~12~~	62 days	± 14 days

[1] Whenever possible the investigator should arrange study visits within this interval.
[2] Subjects ~~will~~*may* not be eligible for inclusion in the ATP cohort for analysis of immunogenicity and efficacy if they make the study visit outside this interval.

Table 11 Intervals between study visits for the infectivity control group

Interval	Optimal length of interval [1]	Allowed interval[2]
Visit 1b (Screening) → Visit 11 (Challenge)	0 to 56 days	-
Visit 11 → Visit 30~~4~~	28 days	± 7 days

[1] Whenever possible the investigator should arrange study visits within this interval.
[2] Subjects ~~will~~*may* not be eligible for inclusion in the ATP cohort for analysis of immunogenicity and efficacy if they make the study visit outside this interval.

6.6.8. Urine pregnancy test

Female subjects ~~of childbearing potential~~are to have a urine pregnancy test at the screening visit and prior to any study vaccine administration. The study vaccines may only be administered if the pregnancy test is negative. Note: The urine pregnancy test must be performed even if the subject is menstruating at the time of the study visit.

Female subjects are also to have a urine pregnancy test prior to the sporozoite challenge. The sporozoite challenge may only be performed if the pregnancy test is negative.

6.6.16. Sampling

Refer to the Module on Biospecimen Management in the SPM for detailed instructions for the collection, handling and processing of the samples.

- **Blood sampling for HIV, HCV and HBsAg**

  A volume of ***approximately*** 17.0 ml of whole blood should be drawn from all subjects screened to assess HIV, HCV and HBsAg status (see Table 6, Table 7 and Table 8).

- ***Blood sampling for initial assessment of parasitemia (PCR)***

  A volume of approximately 2.0 ml should be drawn from all subjects on the day of entry to the hotel phase (Day 295 [Visit 16]) for the assessment of parasitemia by PCR testing to provide the study team an estimate of the number of subjects likely to develop malaria within the first few days of the hotel phase (see Table 6, Table 7 and Table 8).

- **Blood sampling for assessment of parasitemia *(blood smear)***

  A volume of ***approximately 2***~~1~~.0 ml should be drawn from all subjects at each pre-defined timepoint for the assessment of parasitemia ***by blood smear*** (see Table 6, Table 7 and Table 8). For subjects who develop malaria, blood samples for smears may be discontinued once the subject has three consecutive negative smears (separated by more than 12 hours) following initial treatment.

- **Blood sampling for assessment of antibody determination and serum repository**

  For subjects of the investigational vaccine groups (AduFx, 2PedFx, PedFx, Adu2Fx, and Adu1Fx), a volume of at least 20.0 ml of whole blood (to provide at least 10 ml of serum for anti-CS enzyme-linked immunosorbent assay [ELISA], anti-CS avidity, anti-HBs, and serum repository) should be drawn at each pre-defined timepoint (see Table 6 and Table 7). After centrifugation, serum samples should be kept at –20°C/ –4°F or below until shipment.

- **Blood sampling for ~~deep~~*RNA* sequencing**

  For subjects of the investigational vaccine groups (AduFx, 2PedFx, PedFx, Adu2Fx, and Adu1Fx), a volume of ***approximately*** 12.0 ml of whole blood should be drawn at each pre-defined timepoint for ~~deep~~***RNA*** sequencing (mRNA sequencing analysis; see Table 6 and Table 7). The blood should be stored at room temperature (20 to 25°C) until they are transferred to the designated laboratory for further processing.

- **Blood sampling for PBMC *and plasma* repository**

  For subjects of the investigational vaccine groups (AduFx, 2PedFx, PedFx, Adu2Fx, and Adu1Fx), a volume of ***approximately*** 30.0 ml of whole blood should be drawn at each pre-defined timepoint for the purpose of the PBMC ***and plasma*** repository (see Table 6 and Table 7). The blood should be stored at room temperature until it is transferred to the designated laboratory for further processing. The purified PBMC

should be stored in liquid nitrogen ***and plasma at -20°C or colder*** until further processing.

- **Blood sampling for hematology and biochemistry analysis**

  A volume of ***approximately*** 7.5 ml of whole blood should be drawn from all subjects at each pre-defined timepoint for the assessment of safety parameters (ALT, AST, creatinine, hemoglobin, leukocytes [WBC], and platelets; see Table 6, Table 7 and Table 8).

6.7.2. Biological samples

Details of the quantity of biological sample to be taken at each timepoint during the study are provided in Table 12.

Table 12 Biological samples (whole blood)

Study Phase	Timepoint	Sample type	Total volume of blood per visit (ml)					
			AduFx	2PedFx	PedFx	Adu2Fx	Adu1Fx	Infectivity control
Pre-vaccination	Visit 1 (Screening)	Blood sampling for HIV, HCV and HBsAg	17.0 ml	17.0 ml	17.0 ml	17.0 ml	17.0 ml	-
		Blood sampling for hematology and biochemistry analysis	7.5 ml	7.5 ml	7.5 ml	7.5 ml	7.5 ml	-
		Total:	**24.5 ml**	**24.5 ml**	**24.5 ml**	**24.5 ml**	**24.5 ml**	-
	Visit 2 (Day 0)	Blood sampling for antibody determination and serum repository	20.0 ml	20.0 ml	20.0 ml	20.0 ml	20.0 ml	-
		Blood sampling for ~~deep~~***RNA*** sequencing	12.0 ml	12.0 ml	12.0 ml	12.0 ml	12.0 ml	-
		Blood sampling for PBMC ***and plasma*** repository	30.0 ml	30.0 ml	30.0 ml	30.0 ml	30.0 ml	-
		Total:	**62.0 ml**	**62.0 ml**	**62.0 ml**	**62.0 ml**	**62.0 ml**	-
Post-Dose 1	Visit 3 (Day 28)	Blood sampling for ~~deep~~***RNA*** sequencing	12.0 ml	12.0 ml	12.0 ml	12.0 ml	12.0 ml	-
		Blood sampling for PBMC ***and plasma*** repository	30.0 ml	30.0 ml	30.0 ml	30.0 ml	30.0 ml	-
		Total:	**42.0 ml**	**42.0 ml**	**42.0 ml**	**42.0 ml**	**42.0 ml**	-
Post-Dose 2* or Post-Dose 1**	Visit 4 (Day 29)	Blood sampling for ~~deep~~***RNA*** sequencing	12.0 ml	12.0 ml	12.0 ml	12.0 ml	-	-
		Total:	***12.0 ml***	***12.0 ml***	***12.0 ml***	***12.0 ml***	-	-
	Visit 5 (Day 35)	Blood sampling for PBMC ***and plasma*** repository	30.0 ml	30.0 ml	30.0 ml	30.0 ml	-	-
		Blood sampling for hematology and biochemistry analysis	7.5 ml	7.5 ml	7.5 ml	7.5 ml	-	-
		Total:	**37.5 ml**	**37.5 ml**	**37.5 ml**	**37.5 ml**	-	-
	Visit 6 (Day 58)	Blood sampling for antibody determination and serum repository	20.0 ml	20.0 ml	20.0 ml	20.0 ml	-	-
		Blood sampling for PBMC ***and plasma*** repository	30.0 ml	30.0 ml	30.0 ml	30.0 ml	-	-
		Blood sampling for hematology and biochemistry analysis	7.5 ml	7.5 ml	7.5 ml	7.5 ml	-	-
		Total:	**57.5 ml**	**57.5 ml**	**57.5 ml**	**57.5 ml**	-	-
	Visit 7 (Day 196)	Blood sampling for antibody determination and serum repository	20.0 ml	20.0 ml	20.0 ml	20.0 ml	20.0 ml	-
		Blood sampling for ~~deep~~***RNA*** sequencing	12.0 ml	12.0 ml	12.0 ml	12.0 ml	12.0 ml	-
		Blood sampling for PBMC ***and plasma*** repository	30.0 ml	30.0 ml	30.0 ml	30.0 ml	30.0 ml	-
		Total:	**62.0 ml**	**62.0 ml**	**62.0 ml**	**62.0 ml**	**62.0 ml**	-
Post-Dose 3* or Post-Dose 2**	Visit 8 (Day 197)	Blood sampling for ~~deep~~***RNA*** sequencing	12.0 ml	12.0 ml	12.0 ml	12.0 ml	12.0 ml	-
		Total:	***12.0 ml***	***12.0 ml***	***12.0 ml***	***12.0 ml***	***12.0 ml***	-
	Visit 9 (Day 203)	Blood sampling for PBMC ***and plasma*** repository	30.0 ml	30.0 ml	30.0 ml	30.0 ml	30.0 ml	-
		Blood sampling for hematology and biochemistry analysis	7.5 ml	7.5 ml	7.5 ml	7.5 ml	7.5 ml	-

Study Phase	Timepoint	Sample type	Total volume of blood per visit (ml)					
			AduFx	2PedFx	PedFx	Adu2Fx	Adu1Fx	Infectivity control
		Total:	**37.5 ml**	**37.5 ml**	**37.5 ml**	**37.5 ml**	**37.5 ml**	-
	Visit 10 (Day 226)	Blood sampling for antibody determination and serum repository	20.0 ml	20.0 ml	20.0 ml	20.0 ml	20.0 ml	-
		Blood sampling for PBMC ***and plasma*** repository	30.0 ml	30.0 ml	30.0 ml	30.0 ml	30.0 ml	-
		Blood sampling for hematology and biochemistry analysis	7.5 ml	7.5 ml	7.5 ml	7.5 ml	7.5 ml	-
		Total:	**57.5 ml**	**57.5 ml**	**57.5 ml**	**57.5 ml**	**57.5 ml**	-
Pre-challenge	Visit 1b (Screening)	Blood sampling for HIV, HCV and HBsAg	-	-	-	-	-	17.0 ml
		Blood sampling for hematology and biochemistry analysis	-	-	-	-	-	7.5 ml
		Total:	-	-	-	-	-	**24.5 ml**
	Visit 11 (Day 286)	Blood sampling for antibody determination and serum repository	20.0 ml	20.0 ml	20.0 ml	20.0 ml	20.0 ml	-
		Blood sampling for PBMC ***and plasma*** repository	30.0 ml	30.0 ml	30.0 ml	30.0 ml	30.0 ml	-
		Total:	**50.0 ml**	**50.0 ml**	**50.0 ml**	**50.0 ml**	**50.0 ml**	-
Post-challenge	Visit 1~~23~~ (Day 291)	Blood sampling for assessment of parasitemia ***(blood smear)***	2~~4~~.0 ml	2~~4~~.0 ml	2~~4~~.0 ml	2~~4~~.0 ml	2~~4~~.0 ml	2~~4~~.0 ml
	Visit 1~~34~~ (Day 292)	Blood sampling for assessment of parasitemia ***(blood smear)***	2~~4~~.0 ml	2~~4~~.0 ml	2~~4~~.0 ml	2~~4~~.0 ml	2~~4~~.0 ml	2~~4~~.0 ml
	Visit 1~~45~~ (Day 293)	Blood sampling for assessment of parasitemia ***(blood smear)***	2~~4~~.0 ml	2~~4~~.0 ml	2~~4~~.0 ml	2~~4~~.0 ml	2~~4~~.0 ml	2~~4~~.0 ml
	Visit 1~~56~~ (Day 294)	Blood sampling for assessment of parasitemia ***(blood smear)***	2~~4~~.0 ml	2~~4~~.0 ml	2~~4~~.0 ml	2~~4~~.0 ml	2~~4~~.0 ml	2~~4~~.0 ml
	Visit 1~~67~~ (Day 295)	Blood sampling for assessment of parasitemia ***(blood smear)***	2~~4~~.0 ml	2~~4~~.0 ml	2~~4~~.0 ml	2~~4~~.0 ml	2~~4~~.0 ml	2~~4~~.0 ml
		Blood sampling for assessment of parasitemia at start of hotel phase (PCR)*	***2.0 ml***	***2.0 ml***	***2.0 ml***	***2.0 ml***	***2.0 ml***	***2.0 ml***
	Visit 1~~78~~ (Day 296)	Blood sampling for assessment of parasitemia ***(blood smear)***	2~~4~~.0 ml	2~~4~~.0 ml	2~~4~~.0 ml	2~~4~~.0 ml	2~~4~~.0 ml	2~~4~~.0 ml
	Visit 1~~89~~ (Day 297)	Blood sampling for assessment of parasitemia ***(blood smear)***	2~~4~~.0 ml	2~~4~~.0 ml	2~~4~~.0 ml	2~~4~~.0 ml	2~~4~~.0 ml	2~~4~~.0 ml
	Visit ~~1920~~ (Day 298)	Blood sampling for assessment of parasitemia ***(blood smear)***	2~~4~~.0 ml	2~~4~~.0 ml	2~~4~~.0 ml	2~~4~~.0 ml	2~~4~~.0 ml	2~~4~~.0 ml
	Visit 2~~01~~ (Day 299)	Blood sampling for assessment of parasitemia ***(blood smear)***	2~~4~~.0 ml	2~~4~~.0 ml	2~~4~~.0 ml	2~~4~~.0 ml	2~~4~~.0 ml	2~~4~~.0 ml
	Visit 2~~12~~ (Day	Blood sampling for assessment of parasitemia ***(blood smear)***	2~~4~~.0 ml	2~~4~~.0 ml	2~~4~~.0 ml	2~~4~~.0 ml	2~~4~~.0 ml	2~~4~~.0 ml

Study Phase	Timepoint	Sample type	Total volume of blood per visit (ml)					
			AduFx	2PedFx	PedFx	Adu2Fx	Adu1Fx	Infectivity control
	300)							
	Visit 223 (Day 301)	Blood sampling for assessment of parasitemia ***(blood smear)***	24.0 ml	24.0 ml	24.0 ml	24.0 ml	24.0 ml	24.0 ml
	Visit 234 (Day 302)	Blood sampling for assessment of parasitemia ***(blood smear)***	24.0 ml	24.0 ml	24.0 ml	24.0 ml	24.0 ml	24.0 ml
	Visit 245 (Day 303)	Blood sampling for assessment of parasitemia ***(blood smear)***	24.0 ml	24.0 ml	24.0 ml	24.0 ml	24.0 ml	24.0 ml
	Visit 256 (Day 304)	Blood sampling for assessment of parasitemia ***(blood smear)v***	24.0 ml	24.0 ml	24.0 ml	24.0 ml	24.0 ml	24.0 ml
	Visit 267 (Day 306)	Blood sampling for assessment of parasitemia ***(blood smear)***	24.0 ml	24.0 ml	24.0 ml	24.0 ml	24.0 ml	24.0 ml
	Visit 278 (Day 308)	Blood sampling for assessment of parasitemia ***(blood smear)***	24.0 ml	24.0 ml	24.0 ml	24.0 ml	24.0 ml	24.0 ml
	Visit 289 (Day 310)	Blood sampling for assessment of parasitemia ***(blood smear)***	24.0 ml	24.0 ml	24.0 ml	24.0 ml	24.0 ml	24.0 ml
	Visit 2930 (Day 312)	Blood sampling for assessment of parasitemia ***(blood smear)***	24.0 ml	24.0 ml	24.0 ml	24.0 ml	24.0 ml	24.0 ml
	Visit 301 (Day 314)	Blood sampling for assessment of parasitemia ***(blood smear)***	24.0 ml	24.0 ml	24.0 ml	24.0 ml	24.0 ml	24.0 ml
		Blood sampling for antibody determination and serum repository	20.0 ml	20.0 ml	20.0 ml	20.0 ml	20.0 ml	-
		Blood sampling for hematology and biochemistry analysis	7.5 ml	7.5 ml	7.5 ml	7.5 ml	7.5 ml	7.5 ml
		Total:	**298.5 ml**	**298.5 ml**	**298.5 ml**	**298.5 ml**	**298.5 ml**	**98.5 ml**
	Visit Par***	Blood sampling for hematology and biochemistry analysis	7.5 ml	7.5 ml	7.5 ml	7.5 ml	7.5 ml	7.5 ml
		Total:	***7.5 ml***	***7.5 ml***	***7.5 ml***	***7.5 ml***	***7.5 ml***	***7.5 ml***
Study end	Visit 312 (Day 376)	Blood sampling for antibody determination and serum repository	20.0 ml	20.0 ml	20.0 ml	20.0 ml	20.0 ml	-
		Blood sampling for PBMC ***and plasma*** repository	30.0 ml	30.0 ml	30.0 ml	30.0 ml	30.0 ml	-
		Total:	**50.0 ml**	**50.0 ml**	**50.0 ml**	**50.0 ml**	**50.0 ml**	-
		Total blood collected over the entire study:	**57958.5 ml**	**57958.5 m**	**57958.5 m**	**57958.5 m**	**57958.5 m**	**7958.5 ml**

6.7.3. Laboratory assays

Other assays on stored serum***, PBMC and plasma*** samples~~and PBMC~~ may be performed based on results of ongoing studies to investigate the safety and/or vaccine induced anti-malaria and hepatitis B immune responses.

Table 14 Assessment of *P. falciparum* parasitemia

System	Component	Method	Unit	Laboratory
WHOLE BLOOD	Plasmodium falciparum parasites*	Not applicable**	parasite/μl	WRAIR
WHOLE BLOOD	***Plasmodium falciparum parasites****	***Real-time PCR***	***Positive/negative*****	***WRAIR***

WRAIR: Walter Reed Army Institute of Research
* *P. falciparum* parasite count includes blood-stage parasites.
** Method used will be blood slide microscope reading.
***** For assessment and processing by WRAIR of parasitemia on the day of subject entry into the hotel (Day 295 [Visit 16])***

PBMC for ~~gene~~***RNA*** expression profiling will be collected and stored in a repository to allow mRNA sequencing or future next generation technologies***, during the current study or in future research,*** to assess transcriptome signals. Scope of this assay will be based on ongoing investigations.

The repository laboratory for serum samples will be GSK Biologicals' clinical laboratories and for plasma and PBMC samples will be Precision Bioservices Inc.

6.7.4. Biological samples evaluation

6.7.4.1. Immunological read-outs

Table 16 Immunological read-outs

Blood sampling timepoint		Study groups	No. subjects	Sample	Component	Components priority rank
Type of contact and timepoint	Sampling timepoint					
Visit 2 (Day 0)	Pre-vaccination	AduFx, 2PedFx, PedFx, Adu2Fx, Adu1Fx	130	Serum	Plasmodium falciparum.Circumsporozoite Protein.R32LR Ab.IgG	1
					Plasmodium falciparum.Circumsporozoide Protein.R32LR Ab.IgG Avidity	2
					Plasmodium falciparum.anti-C-Term Circumsporozoide Ab.IgG	3
					Plasmodium falciparum.anti-C-Term Circumsporozoide Ab.IgG avidity	4
					Hepatitis B Virus.Surface Ab	5
				Serum repository*	To be determined	Not applicable
				PBMC ***and***	To be determined	Not applicable

Blood sampling timepoint		Study groups	No. subjects	Sample	Component	Components priority rank
Type of contact and timepoint	Sampling timepoint					
				plasma repository*		
Visit 3 (Day 28)	One month post-Dose 1	AduFx, 2PedFx, PedFx, Adu2Fx, Adu1Fx	130	PBMC ***and plasma*** repository*	To be determined	Not applicable
Visit 5 (Day 35)	Seven days post-Dose 2	AduFx, 2PedFx, PedFx, Adu2Fx	104	PBMC ***and plasma*** repository*	To be determined	Not applicable
Visit 6 (Day 58)	One month post-Dose 2	AduFx, 2PedFx, PedFx, Adu2Fx	104	Serum	Plasmodium falciparum.Circumsporozoite Protein.R32LR Ab.IgG	1
					Plasmodium falciparum.Circumsporozoide Protein.R32LR Ab.IgG Avidity	2
					Plasmodium falciparum.anti-C-Term Circumsporozoide Ab.IgG	3
					Plasmodium falciparum.anti-C-Term Circumsporozoide Ab.IgG avidity	4
					Hepatitis B Virus.Surface Ab	5
				Serum repository*	To be determined	Not applicable
				PBMC ***and plasma*** repository*	To be determined	Not applicable
Visit 7 (Day 196)	Six months post-Dose 2** or seven month post-Dose 1***	AduFx, 2PedFx, PedFx, Adu2Fx, Adu1Fx	130	Serum	Plasmodium falciparum.Circumsporozoite Protein.R32LR Ab.IgG	1
					Plasmodium falciparum.Circumsporozoide Protein.R32LR Ab.IgG Avidity	2
					Plasmodium falciparum.anti-C-Term Circumsporozoide Ab.IgG	3
					Plasmodium falciparum.anti-C-Term Circumsporozoide Ab.IgG avidity	4
					Hepatitis B Virus.Surface Ab	5
				Serum repository*	To be determined	Not applicable
				PBMC ***and plasma*** repository*	To be determined	Not applicable

Blood sampling timepoint		Study groups	No. subjects	Sample	Component	Components priority rank
Type of contact and timepoint	Sampling timepoint					
Visit 9 (Day 203)	Seven days post-Dose 3** or post-Dose 2***	AduFx, 2PedFx, PedFx, Adu1Fx, Adu2Fx	130	PBMC ***and plasma*** repository*	To be determined	Not applicable
Visit 10 (Day 226)	One month post-Dose 3** or post-Dose 2***	AduFx, 2PedFx, PedFx, Adu2Fx, Adu1Fx	130	Serum	Plasmodium falciparum.Circumsporozoite Protein.R32LR Ab.IgG	1
					Plasmodium falciparum.Circumsporozoide Protein.R32LR Ab.IgG Avidity	2
					Plasmodium falciparum.anti-C-Term Circumsporozoide Ab.IgG	3
					Plasmodium falciparum.anti-C-Term Circumsporozoide Ab.IgG avidity	4
					Hepatitis B Virus.Surface Ab	5
				Serum repository*	To be determined	Not applicable
				PBMC ***and plasma*** repository*	To be determined	Not applicable
Visit 11 (Day 286)	Day of challenge	AduFx, 2PedFx, PedFx, Adu2Fx, Adu1Fx	130	Serum	Plasmodium falciparum.Circumsporozoite Protein.R32LR Ab.IgG	1
					Plasmodium falciparum.Circumsporozoide Protein.R32LR Ab.IgG Avidity	2
					Plasmodium falciparum.anti-C-Term Circumsporozoide Ab.IgG	3
					Plasmodium falciparum.anti-C-Term Circumsporozoide Ab.IgG avidity	4
					Hepatitis B Virus.Surface Ab	5
				Serum repository*	To be determined	Not applicable
				PBMC ***and plasma*** repository*	To be determined	Not applicable

Blood sampling timepoint		Study groups	No. subjects	Sample	Component	Components priority rank
Type of contact and timepoint	Sampling timepoint					
Visit 3~~0~~1 (Day 314)	28 days post-challenge	AduFx, 2PedFx, PedFx, Adu2Fx, Adu1Fx	130	Serum	Plasmodium falciparum.Circumsporozoite Protein.R32LR Ab.IgG	1
					Plasmodium falciparum.Circumsporozoide Protein.R32LR Ab.IgG Avidity	2
					Plasmodium falciparum.anti-C-Term Circumsporozoide Ab.IgG	3
					Plasmodium falciparum.anti-C-Term Circumsporozoide Ab.IgG avidity	4
					Hepatitis B Virus.Surface Ab	5
				Serum repository*	To be determined	Not applicable
Visit 3~~1~~2 (Day 376)	Study end	AduFx, 2PedFx, PedFx, Adu2Fx, Adu1Fx	130	Serum	Plasmodium falciparum.Circumsporozoite Protein.R32LR Ab.IgG	1
					Plasmodium falciparum.Circumsporozoide Protein.R32LR Ab.IgG Avidity	2
					Plasmodium falciparum.anti-C-Term Circumsporozoide Ab.IgG	3
					Plasmodium falciparum.anti-C-Term Circumsporozoide Ab.IgG avidity	4
					Hepatitis B Virus.Surface Ab	5
				Serum repository*	To be determined	Not applicable
				PBMC ***and plasma*** repository*	To be determined	Not applicable

* Assays on stored serum***, PBMC and plasma*** samples ~~or stored PBMC samples~~ may be performed based on results of ongoing studies to investigate the safety and/or vaccine induced anti-malaria and hepatitis B immune responses.
** For groups AduFx, 2PedFx, PedFx and Adu2Fx.
*** For group Adu1Fx.

6.7.4.2. Hematology/Blood Chemistry

Table 17 Hematology and biochemistry read-outs

Blood sampling timepoint		Study groups	No. subjects	Component
Type of contact and timepoint	Sampling timepoint			
Visit 1 (Screening)	Pre-vaccination	AduFx, 2PedFx, PedFx, Adu2Fx, Adu1Fx	≥ 130	HIV-IgG + Ag (SER,GLR)
				Hepatitis B Virus.Surface Ab
				HCV Ab
				Alanine Aminotransferase
				Aspartate Aminotransferase
				Creatinine
				Hemoglobin
				Leukocytes (White Blood Cells)
				Platelets
Visit 5 (Day 35)	Seven days post-Dose 2	AduFx, 2PedFx, PedFx, Adu2Fx	104	Alanine Aminotransferase
				Aspartate Aminotransferase
				Creatinine
				Hemoglobin
				Leukocytes (White Blood Cells)
				Platelets
Visit 6 (Day 58)	One month post-Dose 2	AduFx, 2PedFx, PedFx, Adu2Fx	104	Alanine Aminotransferase
				Aspartate Aminotransferase
				Creatinine
				Hemoglobin
				Leukocytes (White Blood Cells)
				Platelets
Visit 9 (Day 203)	Seven days post-Dose 3* or post-Dose 2**	AduFx, 2PedFx, PedFx, Adu2Fx, Adu1Fx	130	Alanine Aminotransferase
				Aspartate Aminotransferase
				Creatinine
				Hemoglobin
				Leukocytes (White Blood Cells)
				Platelets
Visit 10 (Day 226)	One month post-Dose 3* or post-Dose 2**	AduFx, 2PedFx, PedFx, Adu2Fx, Adu1Fx	130	Alanine Aminotransferase
				Aspartate Aminotransferase
				Creatinine
				Hemoglobin
				Leukocytes (White Blood Cells)
				Platelets
Visit 1b (Screening)	Pre-challenge	Infectivity control	≥ 20***	HIV-IgG + Ag (SER,GLR)
				Hepatitis B Virus.Surface Ab
				HCV Ab
				Alanine Aminotransferase
				Aspartate Aminotransferase
				Creatinine
				Hemoglobin
				Leukocytes (White Blood Cells)
				Platelets
Visit Par	Event-driven (first parasitemia)	AduFx, 2PedFx, PedFx, Adu2Fx, Adu1Fx, Infectivity control	~ 150	Alanine Aminotransferase
				Aspartate Aminotransferase
				Creatinine
				Hemoglobin
				Leukocytes (White Blood Cells)
				Platelets

Blood sampling timepoint		Study groups	No. subjects	Component
Type of contact and timepoint	Sampling timepoint			
Visit 30~~4~~ (Day 314)	28 days post-challenge	AduFx, 2PedFx, PedFx, Adu2Fx, Adu1Fx, Infectivity control	~ 150	Alanine Aminotransferase
				Aspartate Aminotransferase
				Creatinine
				Hemoglobin
				Leukocytes (White Blood Cells)
				Platelets

* For groups AduFx, 2PedFx, PedFx and Adu2Fx.
** For group Adu1Fx.
*** between 20 and 30 infectivity controls will be enrolled.

6.7.4.3. Molecular biology

Table 18 ~~Deep~~*RNA* sequencing (mRNA sequencing analysis)

Blood sampling timepoint		Study groups	No. subjects	Component	Components priority rank
Type of contact and timepoint	Sampling timepoint				
Visit 2 (Day 0)	Pre-vaccination	AduFx, 2PedFx, PedFx, Adu1Fx, Adu2Fx	130	mRNA	Not applicable
Visit 3 (Day 28)	Day of Dose 2* or one month post-Dose 1**	AduFx, 2PedFx, PedFx, Adu1Fx Adu2Fx	130	mRNA	Not applicable
Visit 4 (Day 29)	One day post-Dose 2	AduFx, 2PedFx, PedFx, Adu2Fx	104	mRNA	Not applicable
Visit 7 (Day 196)	Day of Dose 3* or Dose 2**	AduFx, 2PedFx, PedFx, Adu1Fx, Adu2Fx	130	mRNA	Not applicable
Visit 8 (Day 197)	One day post-Dose 3* or Dose 2**	AduFx, 2PedFx, PedFx, Adu1Fx, Adu2Fx	130	mRNA	Not applicable

mRNA: messenger ribonucleic acid
* For groups AduFx, 2PedFx, PedFx and Adu2Fx.
** For group Adu1Fx.

6.7.4.4. Parasitemia

Table 19 Parasitemia (blood smear)

Blood sampling timepoint	Study groups	No. subjects	Component	Components priority rank
Visit 1~~23~~ (Day 291)	AduFx, 2PedFx, PedFx, Adu2Fx, Adu1Fx, Infectivity control	~ 150	Blood smear	1
Visit 1~~34~~ (Day 292)	AduFx, 2PedFx, PedFx, Adu2Fx, Adu1Fx, Infectivity control	~ 150	Blood smear	1
Visit 1~~45~~ (Day 293)	AduFx, 2PedFx, PedFx, Adu2Fx, Adu1Fx, Infectivity control	~ 150	Blood smear	1
Visit 1~~56~~ (Day 294)	AduFx, 2PedFx, PedFx, Adu2Fx, Adu1Fx, Infectivity control	~ 150	Blood smear	1
Visit 1~~67~~ (Day 295)	AduFx, 2PedFx, PedFx, Adu2Fx, Adu1Fx, Infectivity control	~ 150	Blood smear	1
	AduFx, 2PedFx, PedFx, Adu2Fx, Adu1Fx, Infectivity control	***~ 150***	***PCR****	***2***
Visit 1~~78~~ (Day 296)	AduFx, 2PedFx, PedFx, Adu2Fx, Adu1Fx, Infectivity control	~ 150	Blood smear	1
Visit 1~~89~~ (Day 297)	AduFx, 2PedFx, PedFx, Adu2Fx, Adu1Fx, Infectivity control	~ 150	Blood smear	1
Visit ***19***~~20~~ (Day 298)	AduFx, 2PedFx, PedFx, Adu2Fx, Adu1Fx, Infectivity control	~ 150	Blood smear	1
Visit 2~~01~~ (Day 299)	AduFx, 2PedFx, PedFx, Adu2Fx, Adu1Fx, Infectivity control	~ 150	Blood smear	1
Visit 2~~12~~ (Day 300)	AduFx, 2PedFx, PedFx, Adu2Fx, Adu1Fx, Infectivity control	~ 150	Blood smear	1
Visit 2~~23~~ (Day 301)	AduFx, 2PedFx, PedFx, Adu2Fx, Adu1Fx, Infectivity control	~ 150	Blood smear	1
Visit 2~~34~~ (Day 302)	AduFx, 2PedFx, PedFx, Adu2Fx, Adu1Fx, Infectivity control	~ 150	Blood smear	1
Visit 2~~45~~ (Day 303)	AduFx, 2PedFx, PedFx, Adu2Fx, Adu1Fx, Infectivity control	~ 150	Blood smear	1
Visit 2~~56~~ (Day 304)	AduFx, 2PedFx, PedFx, Adu2Fx, Adu1Fx, Infectivity control	~ 150	Blood smear	1
Visit 2~~67~~ (Day 306)	AduFx, 2PedFx, PedFx, Adu2Fx, Adu1Fx, Infectivity control	~ 150	Blood smear	1
Visit 2~~78~~ (Day 308)	AduFx, 2PedFx, PedFx, Adu2Fx, Adu1Fx, Infectivity control	~ 150	Blood smear	1
Visit 2~~89~~ (Day 310)	AduFx, 2PedFx, PedFx, Adu2Fx, Adu1Fx, Infectivity control	~ 150	Blood smear	1
Visit ***29***~~30~~ (Day 312)	AduFx, 2PedFx, PedFx, Adu2Fx, Adu1Fx, Infectivity control	~ 150	Blood smear	1
Visit 3~~01~~ (Day 314)	AduFx, 2PedFx, PedFx, Adu2Fx, Adu1Fx, Infectivity control	~ 150	Blood smear	1

**** For assessment and processing by WRAIR of parasitemia on the day of subject entry into the hotel (Day 295 [Visit 16])***

6.7.5. Immunological correlates of protection

No correlate of protection has been demonstrated so far for the CS antigen.

For the HBsAg, the conventional correlate of protection is anti-HBs antibody concentrations above 10 mIU/ml [European Consensus Group on Hepatitis B Immunity, 2000].

The investigator is encouraged to share the immunological assay results for non-responders with the study subjects~~/subjects' parent(s)/LAR(s).~~

For the subjects identified as non-responders, it remains the responsibility of the investigator in charge of the subject's clinical management to determine the medical need for re-vaccination and to re-vaccinate the subjects as per local/regional practices.

9.3.1. Time period for detecting and recording adverse events, serious adverse events and pregnancies

Table 25 Reporting periods for collecting safety information for the investigational vaccine groups: AduFx, 2PedFx, PedFx, and Adu2Fx)

Study visits	1	2		3		6	7		10	11 to 304			312
Study days	-90 to 0	0	6	28	34	58	196	202	226	286	314	315	376
Solicited local and general AEs post-vaccination													
Unsolicited AEs post-vaccination													
AEs post-challenge													
AEs and SAEs leading to withdrawal from further vaccination													
SAEs (all, fatal, related)													
SAEs related to study participation, or to a concurrent GSK medication/vaccine													
AEs of specific interest (meningitis and pIMDs)													
Pregnancies													

Table 26 Reporting periods for collecting safety information for the investigational vaccine groups: Adu1Fx

Study visits	1	2		3	7		10	11 to 304			312
Study days	-90 to 0	0	6	28	196	202	226	286	314	315	376
Solicited local and general AEs post-vaccination											
Unsolicited AEs post-vaccination											
AEs post-challenge											
AEs and SAEs leading to withdrawal from further vaccination											
SAEs (all, fatal, related)											
SAEs related to study participation, or to a concurrent GSK medication/vaccine											
AEs of specific interest (meningitis and pIMDs)											
Pregnancies											

Table 27 Reporting periods for collecting safety information for the infectivity control group

Study visits	1b	11 to 30~~4~~		
Study days	258 to 286	286	314	315
AEs post-challenge				
SAEs (all, fatal, related)				
SAEs related to study participation, or to a concurrent GSK medication/vaccine				
Pregnancies				

APPENDIX A LABORATORY ASSAYS

Blood smear *and PCR testing* for assessment of *P. falciparum* parasitemia

Blood slide preparation and~~slide~~ reading ***and PCR testing*** will be performed according to laboratory SOPs.

~~Deep~~*RNA* sequencing (mRNA expression analysis)

mRNA sequencing is a technique using next generation sequencing technology to sequence and quantify mRNA samples. It concerns analysis of mRNA transcription, not genetic testing as the data obtained is used to assess relative abundance of mRNA molecules. The Illumina Genome Analyzer II uses clonal array formation and reversible terminator technology to generate 20-30 million short sequence reads per sample lane. These short sequences are aligned with the genome and counted to provide a relative frequency of sequences in the library population. The experiments will be instructed by the results obtained from systems biology results in previous CHMI studies.

GlaxoSmithKline Biologicals SA

Vaccines R &D
Protocol Administrative Change 1

eTrack study number and Abbreviated Title	205081 (MALARIA-092)
IND number	17337
EudraCT number	205081 (MALARIA-092)
Administrative change number:	Administrative change 1
Administrative change date:	19 June 2017
Co-ordinating author:	PPD, Scientific Writer, Freelance Contractor for GSK Biologicals

Rationale/background for changes:

The Investigational New Drug (IND) number, 17337, has been added to the title page.

Q^2 Solutions, as a new partner, will manage logistical aspects of serum samples sent from site to GSK for testing. Their details have been added to APPENDIX B 'CLINICAL LABORATORIES'.

Minor errors and inconsistencies have been corrected and contributing authors updated.

Intensity grades for aspartate aminotransferase were missing in Table 29 'Toxicity grading scales for blood testing'. These have been added.

Amended text has been included in *bold italics* and deleted text in ~~strikethrough~~ in the following sections:

Clinical Study Protocol
Sponsor:
GlaxoSmithKline Biologicals
Rue de l'Institut 89, 1330 Rixensart, Belgium

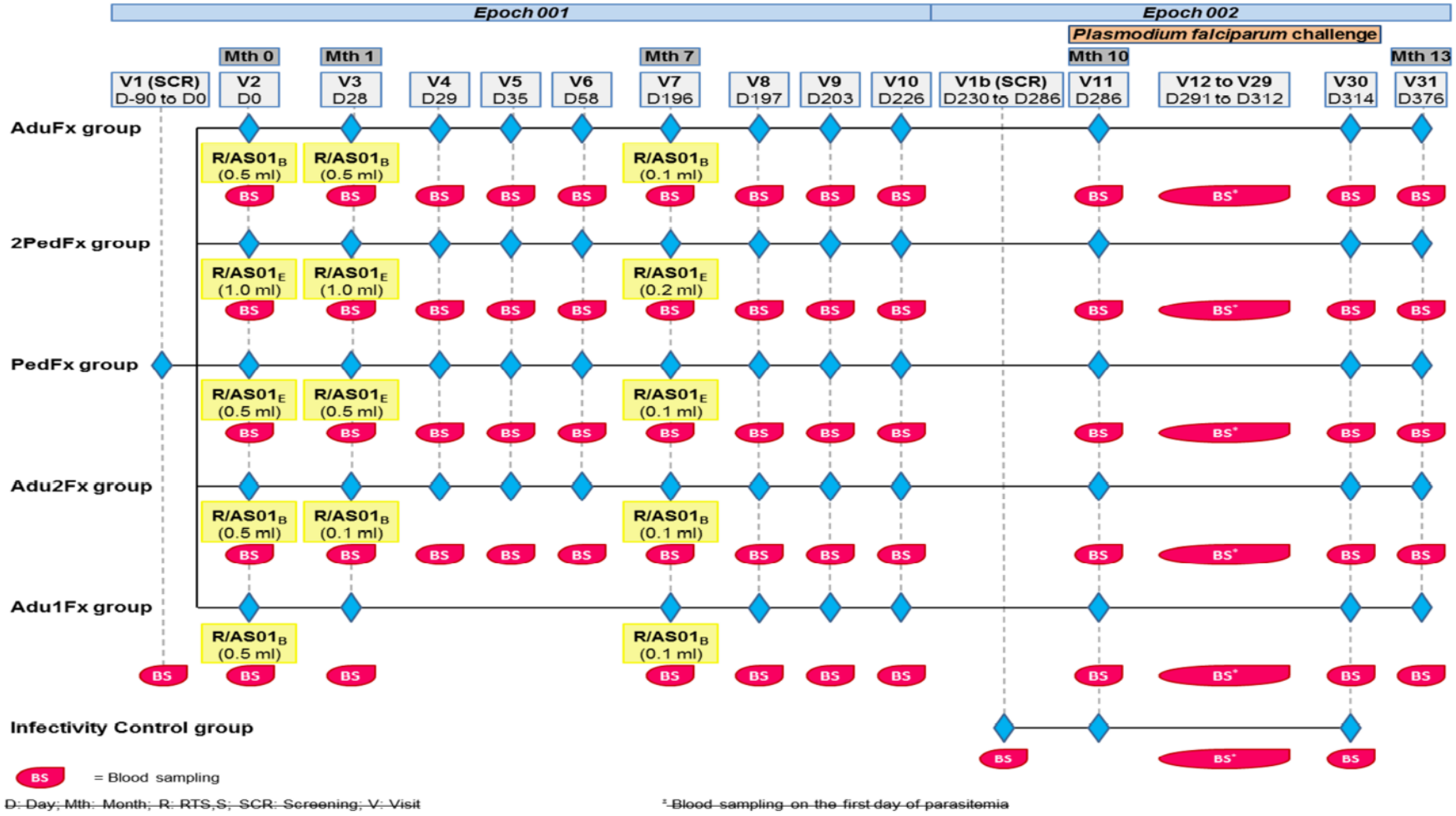
Primary Study vaccine	• GlaxoSmithKline (GSK) Biologicals' candidate *Plasmodium falciparum* malaria vaccine RTS,S/AS01$_E$ (SB257049).
Other Study vaccine	• GlaxoSmithKline (GSK) Biologicals' candidate *Plasmodium falciparum* malaria vaccine RTS,S/AS01$_B$ (SB257049).
eTrack study number and Abbreviated Title	205081 (MALARIA-092)
Investigational New Drug (IND) number	***17337***
Date of protocol	Final Version 1: 09 March 2016
Date of protocol amendment/administrative change	Amendment 1 Final: 23 June 2016 ***Administrative change 1 Final: 19 June 2017***
Title	Efficacy, immunogenicity and safety study of GSK Biologicals' candidate malaria vaccine (SB257049) evaluating various dose schedules in a sporozoite challenge model in healthy malaria-naïve adults.
Detailed Title	A Phase IIa, open-label, controlled, mono-center study to evaluate the efficacy, immunogenicity and safety of GSK Biologicals' candidate malaria vaccines RTS,S/AS01$_E$ and RTS,S/AS01$_B$ administered as various dose schedules according to a 0, 1, 7-month or a 0, 7-month schedule in healthy malaria-naïve subjects aged 18-55 years.
Co-ordinating author	• PPD (Scientific Writer - Keyrus Biopharma contractor for GSK Biologicals)
Contributing authors	• PPD (Clinical Research and Development Lead [CRDL]) • PPD, PPD (Project Statisticians) • PPD, PPD (Study Delivery Leads) • PPD, PPD (Vaccine Supply Coordinator)

eTrack study number and Abbreviated Title	205081 (MALARIA-092)
Investigational New Drug (IND) number	***17337***
Detailed Title	A Phase IIa, open-label, controlled, mono-center study to evaluate the efficacy, immunogenicity and safety of GSK Biologicals' candidate malaria vaccines RTS,S/AS01$_E$ and RTS,S/AS01$_B$ administered as various dose schedules according to a 0, 1, 7-month or a 0, 7-month schedule in healthy malaria-naïve subjects aged 18-55 years.
Contributing authors (continued)	• PPD, PPD (Clinical Laboratory Sciences Lead*s*) • PPD, PPD (Clinical Safety representative*s*) • PPD, PPD (Study Data Manager*s*) • PPD (Clinical Regulatory Affairs representative) • PPD, PPD (Global Patent Representative*s*)

GSK Biologicals' Protocol DS v 14.1.1

3. Study Design Overview

Figure 1 Study design



D: Day; Mth: Month; R: RTS,S; SCR: Screening; V: Visit

[a] Blood sampling on the first day of parasitemia

Footnotes to Figure 1:

D: Day; Mth: month; R: RTS,S; SCR: Screening; V: visit. * Blood sample for estimation of subjects with parasitemia (PCR testing) will be collected on the day of subject entry into the hotel phase (Day 295 [Visit 16]); blood sample for biochemistry and hematology parameters will be collected the day of first parasitemia and blood sample for assessment of parasitemia (blood smear) will be collected daily for 14 days (from Day 291 [Visit 12] to Day 304 [Visit 25]) and then every two days for nine days (Day 306 [Visit 26], Day 308 [Visit 27], Day 310 [Visit 28], Day 312 [Visit 29], and Day 314 [Visit 30]).

9.3. Detecting and recording adverse events, serious adverse events and pregnancies

9.3.3.2. Assessment of adverse events

9.3.3.2.1. Assessment of intensity

Table 29 Toxicity grading scales for blood testing

Adverse event	Intensity grade	Intensity*
Hemoglobin (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
Hemoglobin (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	1.1 - 2.5 x ULN
	2	2.6 – 5 x ULN
	3	> 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
*Grading scale adapted from [FDA guidance for industry: toxicity grading scale for healthy adult and adolescent volunteers enrolled in preventive vaccine clinical trials (September 2007)].

APPENDIX B CLINICAL LABORATORIES

Table 32 Outsourced laboratories

Laboratory	Address
CEVAC - University of Gent	De Pintelaan, 185 Gent Belgium
Division of Malaria Vaccine Development - WRAIR	Walter Reed Army Institute of Research Silver Spring, MD 20910, United States
Precision Bioservices, Inc.	8425 Progress Drive Frederick, MD 21701, United States
Quest Diagnostics, Inc.	1901 Sulphur Spring Road Baltimore, MD 21227, United States
Q² Solutions	***27027 Tourney Road, Suite 2E*** ***Valencia, CA 91355*** ***United States***

Protocol Administrative Change 1 Sponsor Signatory Approval

eTrack study number and Abbreviated Title	205081 (MALARIA-092)
Investigational New Drug (IND) number	17337
Date of protocol administrative change	Administrative change 1 Final: 19 June 2017
Detailed Title	A Phase IIa, open-label, controlled, mono-center study to evaluate the efficacy, immunogenicity and safety of GSK Biologicals' candidate malaria vaccines RTS,S/AS01$_E$ and RTS,S/AS01$_B$ administered as various dose schedules according to a 0, 1, 7-month or a 0, 7-month schedule in healthy malaria-naïve subjects aged 18-55 years.
Sponsor signatory	François Roman, Clinical & Epidemiology Project Lead, DDW Vaccines
Signature	PPD
Date	12 JUL 2017

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