Clinical Study Protocol

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

Primary study vaccine and number	• GlaxoSmithKline (GSK) Biologicals' candidate *Plasmodium falciparum* (*P. falciparum*) malaria vaccine RTS,S/AS01$_E$ (SB257049)
eTrack study number and Abbreviated Title	209003 (MALARIA-102 BST:092)
Investigational New Drug (IND) number	17337
Date of Protocol	Final Version 2: 26 September 2018
Title	Efficacy, immunogenicity and safety study evaluating a fractional (Fx) booster dose of GSK Biologicals' candidate malaria vaccine (SB257049) in a sporozoite challenge model in healthy malaria-naïve adults.
Detailed Title	A Phase IIa, open-label, non-randomized, controlled, mono-center study to evaluate the efficacy, immunogenicity and safety of a fractional (Fx) booster dose of GlaxoSmithKline Biologicals' malaria candidate vaccine RTS,S/AS01$_E$ when given to healthy adults subjects previously receiving various primary dose schedules in a sporozoite challenge model.
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GSK Biologicals' Protocol DS v 15.0

Protocol Sponsor Signatory Approval

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Sponsor signatory	François Roman, Clinical and Epidemiology Project Lead, Diseases of the Developing World project
Signature	_____
Date	_____

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Protocol 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 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' study vaccine 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 vaccine, 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.

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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 (SAE)

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, non-randomized, controlled, mono-center study to evaluate the efficacy, immunogenicity and safety of a fractional (Fx) booster dose of GlaxoSmithKline Biologicals' malaria candidate vaccine RTS,S/AS01$_E$ when given to healthy adults subjects previously receiving various primary dose schedules in a sporozoite challenge model.

Indication

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

Rationale for the study and study design

- **Rationale for the study**

This follow-up study (MALARIA-102) to the MALARIA-092 study will be conducted at the Walter Reed Army Institute of Research (WRAIR) and is designed to evaluate the waning efficacy with the fractional schedules and to confirm if protection can be extended with an additional Fx dose.

In this booster study, vaccinated subjects from the MALARIA-092 study [previously protected or unprotected following controlled human malaria infection (CHMI)] will receive a booster and undergo a second CHMI three to four weeks after vaccination.

- **Rationale for the study design**

The MALARIA-092 study was designed to evaluate varying vaccination schedules, a Fx dose and the use of the pediatric formulation, RTS,S/AS01$_E$, in the adult CHMI model. The current study will assess if a Fx dose booster will maintain protection in those previously protected and convert previously unprotected individuals to protected status.

Given the positive efficacy derived in MALARIA-092 study in the group that received RTS,S/AS01$_E$ (PedFx), a decision has been taken to boost subjects only with a Fx dose from the RTS,S/AS01$_E$ formulation (0.1 mL). This will facilitate deployment in Africa where the same dosage and formulation will be used across all age groups. Interim analysis from the MALARIA-092 CHMI study showed that vaccine efficacy (VE) with the RTS,S/AS01$_B$ (comparator group) or RTS,S/AS01$_E$ were comparable [51% (95% CI: 19%, 70%; p-value = 0.0010) and 60% (95% CI: 30%, 77%; p-value = 0.0001), respectively]. Thus for this MALARIA-102 study, subjects will receive a booster dose with a Fx dose of a RTS,S/AS01$_E$ vaccine.

This study will be sponsored by GSK Biologicals and funded by PATH.

Objectives

Primary

- To assess vaccine efficacy against the occurrence of *P. falciparum* parasitemia (defined by a positive blood slide):
  - In subjects who were protected following challenge in the MALARIA-092 study and who receive a Fx booster dose versus infectivity controls.
  - In subjects who were not protected following challenge in the MALARIA-092 study and who receive a Fx booster dose versus infectivity controls.

Secondary

Efficacy

- To assess the time-to-onset of *P. falciparum* parasitemia (defined by a positive blood slide):
  - In subjects who were protected following challenge in the MALARIA-092 study and who receive a Fx booster dose versus the infectivity controls.
  - In subjects who were not protected following challenge in the MALARIA-092 study and who receive a Fx booster dose versus the infectivity controls.

Immunogenicity

- To evaluate anti-circumsporozoite protein (CS) repeat region antibody response at specified timepoints.
- To evaluate anti-hepatitis B antigen (HBs) Igtot antibody response at specified timepoints.

Safety

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

Tertiary

Efficacy

- To assess vaccine efficacy against the occurrence of *P. falciparum* parasitemia (defined by a positive blood slide):
  - In subjects who were protected versus subjects who were not protected following challenge in the MALARIA-092 study and who receive a Fx booster dose.

- To assess the time-to-onset of *P. falciparum* parasitemia (defined by a positive blood slide):
  - In subjects who were protected versus subjects who were not protected following challenge in the MALARIA-092 study and who receive a Fx booster dose.
- To assess the occurrence of *P. falciparum* parasitemia, defined by a positive PCR.
- To assess the time-to-onset of *P. falciparum* parasitemia, defined by a positive PCR.

Immunogenicity

- To evaluate the anti-CS repeat region IgG avidity index at specified timepoints.
- To evaluate the anti-full length CS protein IgG concentrations and anti-C terminal portion of the protein (C-term) IgG concentrations at specified timepoints.
- To evaluate the 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-label, non-randomized, controlled, mono-centric, single-country study with two parallel groups (protected and non-protected) and one infectivity control group.
- **Duration of the study:** Approximately seven months for subjects from the MALARIA-092 study (excluding screening) and approximately one month for infectivity control subjects (excluding screening).
  - Epoch 001: Screening period for subjects from the MALARIA-092 study (Day -90 to Day -1).
  - Epoch 002: Vaccination starting at Visit 1 (Day 1) and ending at Visit 3 (Day 22).
  - Epoch 003: Screening period for infectivity control subjects (Day -68 to Day 21).
  - Epoch 004: Challenge starting at Visit 3 (Day 22) and ending at Visit 23 (Day 190) or Visit 22 (Day 50) for the infectivity control group.

- **Primary completion Date (PCD):** Visit 23 (Day 190).
- **End of Study (EoS):** Last testing results released of samples collected at Visit 23 (Day 190).
- **Study groups:** Refer to Synopsis Table 1 and Synopsis Table 2.

Synopsis Table 1 Study groups and epochs foreseen in the study

Study groups	Approximate number of subjects*	Age (Min - Max)	Epochs			
			Epoch 001	Epoch 002	Epoch 003	Epoch 004
P-Fx	20	18-55 years**	x	x		x
NP-Fx	20	18-55 years**	x	x		x
InfectivityCtrl	6-24***	18-55 years			x	x

P-Fx = Pooled subjects from the MALARIA-092 study vaccinated with RTS,S/AS01 (different doses/formulations) and protected following the first challenge who will receive a Fx booster dose of RTS,S/AS01$_E$
NP-Fx = Pooled subjects from the MALARIA-092 study vaccinated with RTS,S/AS01 (different doses/formulations) and not protected following the first challenge who will receive a Fx booster dose of RTS,S/AS01$_E$
InfectivityCtrl = Subjects who will not receive any vaccination but will undergo sporozoite challenge
*The actual number of subjects will be known after screening. A minimum of 20 subjects per group (P-Fx and NP-Fx) is anticipated
**Age at the time of enrollment in the primary study (MALARIA-092)
***Up to 24 subjects, or 4-6 per day, depending on the expected number of days of challenge

Synopsis Table 2 Study groups and treatment foreseen in the study

Treatment name	Vaccine/Product name	Volume to be administered	Study groups		
			P-Fx	NP-Fx	InfectivityCtrl
RTS,S/AS01$_E$	RTS,S	0.1 mL	x	x	-
	AS01E		x	x	-

- **Control:** Non-interventional control. For the challenge, this will be the InfectivityCtrl group.
- **Vaccination schedule:** All previously vaccinated and challenged subjects in MALARIA-092 study will receive one Fx dose of RTS,S/AS01$_E$ at Visit 1 (Day 1).
- **Treatment allocation:** Non-randomized.
- **Blinding:** Open (refer to Synopsis Table 3).

Synopsis Table 3 Blinding of study epochs

Study Epochs	Blinding
Epoch 001	Open
Epoch 002	Open
Epoch 003	Open
Epoch 004	Open

- **Sampling schedule:**

  For subjects from the P-Fx and NP-Fx groups:

  – Blood samples for assessment of anti-CS and anti-HBs immune response and for serum repository will be collected at screening, Day 1*, on the day of challenge (Day 22), 28 days post-challenge (Day 50) and at study end (Day 190).

  – Blood samples for peripheral blood mononuclear cells (PBMCs) and plasma repository will be collected at screening, Day 1*, 7 days post-booster (Day 8), on the day of challenge (Day 22), 28 days post-challenge (Day 50) and at study end (Day 190).

    *Before vaccine administration. These samples might not be drawn if Visit 1 occurs less than one week after screening, at the discretion of the investigator.

  For all subjects:

  – Blood samples for the evaluation of biochemistry (alanine aminotransferase [ALT], aspartate aminotransferase [AST], creatinine) and hematology (hemoglobin, leukocytes [white blood cells; WBC], platelets) parameters will be collected at screening, Day 1*, 7 days post-booster* (Day 8), Day 22, the day of first parasitemia and 28 days post-challenge (Day 50). 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 or clarification of subject eligibility.

    *Not applicable for the infectivity controls.

  – Blood sample for assessment of parasitemia (blood smear and PCR) will be collected daily for 14 days (from Day 27 [Visit 4] to Day 40 [Visit 17]) and then every two days for nine days (Day 42 [Visit 18], Day 44 [Visit 19], Day 46 [Visit 20], Day 48 [Visit 21] and Day 50 [Visit 22]). Exceptionally this can occur within 1 or 3 days if the subject cannot make the visit or for schedule conflicts. For subjects who develop malaria, blood smear and PCR 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 HIV, hepatitis C virus (HCV) and hepatitis B surface antigen (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, at Day 1* and on the day of challenge (Day 22).

    *Before vaccine administration. Not applicable for the infectivity controls.

- **Type of study:** extension of other protocol(s) (MALARIA-092).
- **Data collection**: electronic Case Report Form (eCRF).

Case definition

The following will be used as 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 all subjects who were vaccinated and have undergone challenge in MALARIA-092 study and who are willing to take part in this MALARIA-102 booster study. A minimum of 40 subjects (20/group) are expected to be enrolled in this study. However, there may be more or fewer subjects who consent to join the study. In addition, up to 24 subjects in the infectivity control group will be newly enrolled.

Endpoints

Primary

- Occurrence of *P. falciparum* parasitemia (defined by a positive blood slide) following sporozoite challenge (in all study groups versus infectivity controls).

Secondary

Efficacy

- Time-to-onset of *P. falciparum* parasitemia (defined by a positive blood slide) following sporozoite challenge.

Immunogenicity

- Anti-CS repeat region antibody concentrations at screening, Day 1, prior to challenge (Day 22), 28 days post-challenge (Day 50) and at study end (Day 190).
- Anti-HBs Igtot antibody concentrations at screening, Day 1, prior to challenge (Day 22), 28 days post-challenge (Day 50) and at study end (Day 190).

Safety

- Occurrence of solicited local and general AEs within 7 days after vaccination (day of vaccination and 6 subsequent days) in the booster vaccination groups.
- Occurrence of unsolicited AEs within 21 days after vaccination (day of vaccination and 20 subsequent days), according to the Medical Dictionary for Regulatory Activities (MedDRA) classification, in the booster vaccination groups.
- Occurrence of AEs within 29 days after challenge (day of challenge and 28 subsequent days), according to the MedDRA classification, in all study groups.
- Occurrence of AEs of specific interest (potential immune-mediated diseases [pIMDs] and meningitis) from Day 1 up to study conclusion (Day 190), according to the MedDRA classification, in all study groups.
- Occurrence of SAEs (all, fatal, related to investigational vaccine) during the whole study period (from screening up to study conclusion [Day 190]), according to the MedDRA classification, in all study groups.
- Occurrence of abnormal laboratory values at screening, Day 1, Day 8, Day 22, the day of first parasitemia and 28 days post-challenge (Day 50) for the booster vaccination groups; and at screening, Day 22, the day of first parasitemia and 28 days post-challenge (Day 50) for the infectivity control subjects.

Tertiary

Efficacy

- Occurrence of *P. falciparum* parasitemia (defined by a positive blood slide) following sporozoite challenge (between study groups).
- Time-to-onset of *P. falciparum* parasitemia (defined by a positive blood slide) following sporozoite challenge (between study groups).
- Occurrence of *P. falciparum* parasitemia (defined by a positive PCR) following sporozoite challenge (between study groups).
- Time-to-onset of *P. falciparum* parasitemia (defined by a positive PCR) following sporozoite challenge (between study groups).

Immunogenicity

- Anti-CS repeat region IgG avidity index at Day 1, prior to challenge (Day 22), 28 days post-challenge (Day 50) and at study end (Day 190).
- Anti-full length CS protein IgG concentrations and anti-C-term IgG concentrations at Day 1, prior to challenge (Day 22), 28 days post-challenge (Day 50) and at study end (Day 190).
- Anti-full length CS protein and anti-C-term IgG avidity at Day 1, prior to challenge (Day 22), 28 days post-challenge (Day 50) and at study end (Day 190).

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LIST OF ABBREVIATIONS

AE:	Adverse Event
ALT:	Alanine Aminotransferase
AS01E:	GSK's proprietary Adjuvant System containing MPL, QS-21 (25 µg MPL and 25 µg QS-21) and liposome
AST:	Aspartate Aminotransferase
β-HCG:	Beta-Human Chorionic Gonadotropin
CHMI:	Controlled Human Malaria Infection
CI:	Confidence Interval
CLIA:	Chemiluminescence Enzyme Immunoassay
CS:	Circumsporozoite protein of *Plasmodium falciparum*
eCRF:	electronic Case Report Form
ELISA:	Enzyme-Linked Immunosorbent Assay
EoS:	End of Study
ES:	Exposed Set
eTDF:	Electronic Temperature excursion Decision Form
FDA:	Food and Drug Administration, United States of America
Fx:	Fractional
GCP:	Good Clinical Practice
GSK:	GlaxoSmithKline
GMC:	Geometric Mean Concentration
HBsAg:	Hepatitis B surface antigen
HCV:	Hepatitis C Virus
HIV:	Human Immunodeficiency Virus
ICF:	Informed Consent Form
ICH:	International Conference on Harmonization

IEC:	Independent Ethics Committee
IgG:	Immunoglobulin G
IMP:	Investigational Medicinal Product
IRB:	Institutional Review Board
MedDRA:	Medical Dictionary for Regulatory Activities
MPL:	3-O-desacyl-4'-monophosphoryl lipid A (produced by GSK)
NHANES I:	National Health And Nutrition Examination Survey I
***P. falciparum*:**	*Plasmodium falciparum*
PBMC:	Peripheral blood mononuclear cells
PCD:	Primary Completion Date
PCR:	Polymerase Chain Reaction
pIMD:	Potential Immune-Mediated Disease
PPS:	Per-Protocol Set
QS-21:	*Quillaja saponaria* Molina, fraction 21 (Licensed by GSK from Antigenics Inc., a wholly owned subsidiary of Agenus Inc., a Delaware, USA corporation)
RCC:	Reverse Cumulative Curve
SAE:	Serious Adverse Event
SBIR:	Randomization System on Internet
SD:	Standard Deviation
SDV:	Source Document Verification
SPM:	Study Procedures Manual
VE:	Vaccine Efficacy
WBC:	White Blood Cell
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,
- Combined estrogen and progesterone oral contraceptives,
- Injectable progestogen,
- Implants of etenogestrel or levonorgestrel,
- Contraceptive 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), and/or progesterone alone oral contraceptive.

Adequate contraception does not apply to subjects of child bearing potential with same sex partners, or for subjects who are and will continue to be abstinent from penile-vaginal intercourse on a long term and persistent basis, when this is their preferred and usual lifestyle.

Adverse event (AE): 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 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 enrollment into the study based upon strict adherence to inclusion/exclusion criteria.
End of Study (EoS): (Synonym of End of Trial)	For studies with collection of Human Biologicals Samples or imaging data, EoS is defined as the date of the last testing/reading released of the Human Biological Samples or imaging data, related to primary and secondary endpoints. EoS must be achieved no later than 8 months after Last Subject Last Visit.
Epoch:	An epoch is a set of consecutive timepoints or a single timepoint from a single protocol. Epochs are defined to support a main purpose which is either to draw conclusions on subject participation or to draw a complete conclusion to define or precise the targeted label of the product. Supporting means that data collected at the timepoints included in an epoch must be sufficient to fulfil the purpose of the epoch. Typical examples of epochs are screening, primary vaccinations, boosters, 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 Per-Protocol analysis (see Sections 7.6.2 and 11.5 for details on criteria for evaluability).
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 being tested 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 all primary outcomes, whether the clinical trial was concluded according to the pre-specified protocol or was terminated.
Randomization:	Process of random attribution of treatment to subjects in order to reduce bias of selection.
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:	AEs 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.

Study vaccine/product:	Any investigational vaccine/product being tested and/or any authorized use of a vaccine/product/placebo as a reference or administered concomitantly, in a clinical trial that evaluates the use of an investigational vaccine/product.
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 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.
Treatment number:	A number identifying a treatment to a subject, according to 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 AE.

TRADEMARKS

The following trademarks are used in the present protocol.

Note: In the body of the protocol (including the synopsis), the names of the vaccines/products and/or medications 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
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 *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, 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].

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. A previous controlled human malaria infection (CHMI) study in malaria-naïve adults (MALARIA-071) where a 0, 1, 7-month schedule, with a fractional (Fx) dose delivered as the third immunization (Fx017M) was evaluated showed high efficacy in the Fx017M group compared to standard doses given at 0, 1, 2-months (012M group). 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). While all volunteers in the infectivity control group developed parasitemia after CHMI, 26 of 30 subjects in the Fx017M group (VE = 86.7% [95% CI; 66.8, 94.6]; p-value < 0.0001 by the Fisher exact test) and 10 of 16 subjects in the 012M group (VE = 62.5% [95% CI: 29.4, 80.1]; p-value = 0.0009) were protected. In the follow-up phase of the study, subjects who were protected in the initial challenge were randomized to receive a Fx 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 ten subjects who were initially protected and received a Fx 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 Fx dose [Regules, 2016].

The MALARIA-092 CHMI in healthy malaria-naïve subjects aged 18-55 years was designed to evaluate the efficacy, immunogenicity and safety of various dose schedules and formulations of GSK Biologicals' candidate malaria vaccines. The study, conducted at the Walter Reed Army Institute of Research (WRAIR), aimed to evaluate: (a) the role of a Fx second and third dose, (b) a two-dose schedule where the second dose is fractional, (c) the use of the pediatric formulation to vaccinate adults, when a pediatric dose is delivered to adults, (d) 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), and (e) the impact of varying dosing schedules on immune effectors and immune correlates of protection. Group AduFx receiving RTS,S/AS01$_B$ full dose at Month 0 and Month 1 + RTS,S/AS01$_B$ fractional (Fx) dose (1/5th dose) at Month 7.

The groups evaluated were:

- Group 2PedFx receiving double dose of RTS,S/AS01$_E$ at Month 0 and Month 1 + double dose of RTS,S/AS01$_E$ Fx dose (1/5th dose) at Month 7.
- Group PedFx receiving RTS,S/AS01$_E$ full dose at Month 0 and Month 1 + RTS,S/AS01$_E$ Fx dose (1/5th dose) at Month 7.
- Group Adu2Fx receiving RTS,S/AS01$_B$ full dose at Month 0 + RTS,S/AS01$_B$ Fx dose (1/5th dose) at Month 1 and Month 7.
- Group Adu1Fx receiving RTS,S/AS01$_B$ full dose at Month 0 + RTS,S/AS01$_B$ Fx dose (1/5th dose) at Month 7.

Please refer to the current Investigator's Brochure 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

This follow-up study (MALARIA-102) to the MALARIA-092 study will be conducted at WRAIR and is designed to evaluate the waning efficacy with the fractional schedules and to confirm if protection can be extended with an additional Fx dose.

In this booster study, vaccinated subjects from the MALARIA-092 study (previously protected or unprotected following CHMI) will receive a booster and undergo a second CHMI three to four weeks after vaccination.

1.2.2. Rationale for the study design

The MALARIA-092 study was designed to evaluate varying vaccination schedules, a Fx dose and the use of the pediatric formulation, RTS,S/AS01$_E$, in the adult CHMI model. The current study will assess if a Fx dose booster will maintain protection in those previously protected and convert previously unprotected individuals to protected status.

Given the positive efficacy derived in MALARIA-092 study in the group that received RTS,S/AS01$_E$ (PedFx), a decision has been taken to boost subjects only with a Fx dose from the RTS,S/AS01$_E$ formulation (0.1 mL). This will facilitate deployment in Africa where the same dosage and formulation will be used across all age groups. Interim analysis from the MALARIA-092 CHMI study showed that vaccine efficacy (VE) with the RTS,S/AS01$_B$ (comparator group) or RTS,S/AS01$_E$ were comparable [51% (95% CI: 19%, 70%; p-value = 0.0010) and 60% (95% CI: 30%, 77%; p-value = 0.0001), respectively]. Thus for this MALARIA-102 study, subjects will receive a booster dose with a Fx dose of a RTS,S/AS01$_E$ vaccine.

This study will be sponsored by GSK Biologicals and funded by PATH.

1.3. Benefit : Risk assessment

Please refer to the current Investigator's Brochure 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 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 adverse event (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/Identified Risk	Data/Rationale for Risk	Mitigation Strategy
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 multiform 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 risk: **potential immune-mediated disease** (pIMD)	pIMD is a theoretical concern with adjuvanted vaccines as no evidence of auto-immune 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.

Important Potential/Identified Risk	Data/Rationale for Risk	Mitigation Strategy
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 sporozoite 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 study participation. Indirect benefits may be possible since subjects will be screened for 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 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

- To assess vaccine efficacy against the occurrence of *P. falciparum* parasitemia (defined by a positive blood slide):
  - In subjects who were protected following challenge in the MALARIA-092 study and who receive a Fx booster dose versus infectivity controls.
  - In subjects who were not protected following challenge in the MALARIA-092 study and who receive a Fx booster dose versus infectivity controls.

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

2.2. Secondary objectives

Efficacy

- To assess the time-to-onset of *P. falciparum* parasitemia (defined by a positive blood slide):
  - In subjects who were protected following challenge in the MALARIA-092 study and who receive a Fx booster dose versus the infectivity controls.
  - In subjects who were not protected following challenge in the MALARIA-092 study and who receive a Fx booster dose versus the infectivity controls.

Immunogenicity

- To evaluate anti-circumsporozoite protein (CS) repeat region antibody response at specified timepoints.
- To evaluate anti-hepatitis B antigen (HBs) Igtot antibody response at specified timepoints.

Safety

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

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

2.3. Tertiary objectives

Efficacy

- To assess vaccine efficacy against the occurrence of *P. falciparum* parasitemia (defined by a positive blood slide):
  - In subjects who were protected versus subjects who were not protected following challenge in the MALARIA-092 study and who receive a Fx booster dose.
- To assess the time-to-onset of *P. falciparum* parasitemia (defined by a positive blood slide):
  - In subjects who were protected versus subjects who were not protected following challenge in the MALARIA-092 study and who receive a Fx booster dose.
- To assess the occurrence of *P. falciparum* parasitemia, defined by a positive PCR.
- To assess the time-to-onset of *P. falciparum* parasitemia, defined by a positive PCR.

Immunogenicity

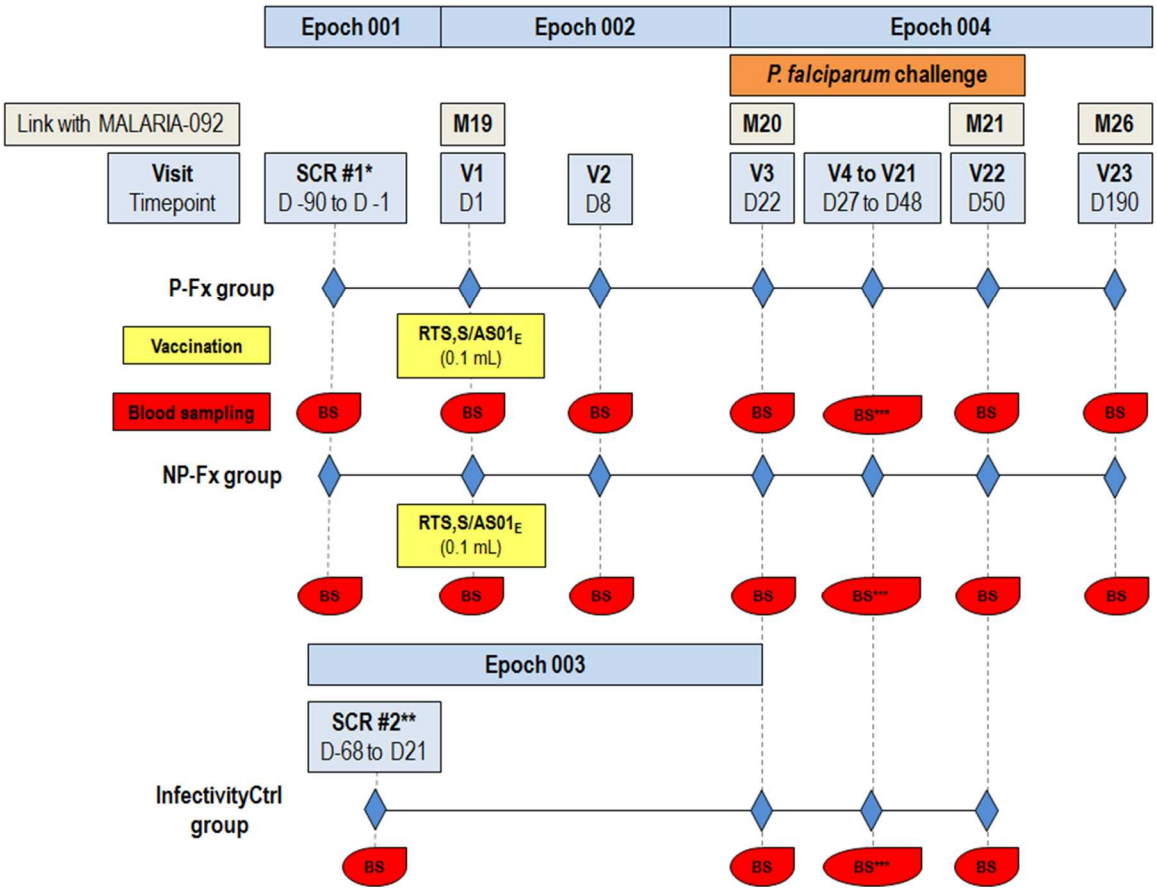
- To evaluate the anti-CS repeat region IgG avidity index at specified timepoints.
- To evaluate the anti-full length CS protein IgG concentrations and anti-C terminal portion of the protein (C-term) IgG concentrations at specified timepoints.
- To evaluate the anti-full length CS protein and anti-C-term 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 and to section 11.13.1 for the reporting of tertiary endpoint results.

3. STUDY DESIGN OVERVIEW

Figure 1 Study design overview



SCR = screening; **V** = visit; **D** = day; **M** = month; **BS** = blood sampling
*Screening visit applicable for the groups from the MALARIA-092 study receiving the fractional booster dose
**Screening visit applicable for subjects in the infectivity control group
***Blood sample for biochemistry and hematology parameters will be collected the day of first parasitemia and blood sample for assessment of parasitemia (blood smear and PCR) will be collected daily for 14 days (from Day 27 [Visit 4] to Day 40 [Visit 17]) and then every two days for nine days (Day 42 [Visit 18], Day 44 [Visit 19], Day 46 [Visit 20], Day 48 [Visit 21] and Day 50 [Visit 22]). Exceptionally this can occur within 1 or 3 days if the subject cannot make the visit or for schedule conflicts.

Protocol waivers or exemptions are not allowed unless necessary for the management 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-label, non-randomized, controlled, mono-centric, single-country study with two parallel groups (protected and non-protected) and one infectivity control group.
- **Duration of the study:** Approximately seven months for subjects from the MALARIA-092 study (excluding screening) and approximately one month for infectivity control subjects (excluding screening).
  - Epoch 001: Screening period for subjects from the MALARIA-092 study (Day -90 to Day -1).
  - Epoch 002: Vaccination starting at Visit 1 (Day 1) and ending at Visit 3 (Day 22).
  - Epoch 003: Screening period for infectivity control subjects (Day -68 to Day 21).
  - Epoch 004: Challenge starting at Visit 3 (Day 22) and ending at Visit 23 (Day 190) or Visit 22 (Day 50) for the infectivity control group.
- **Primary Completion Date (PCD):** Visit 23 (Day 190).

  Refer to glossary of terms for the definition of PCD.
- **End of Study (EoS):** Last testing results released of samples collected at Visit 23 (Day 190).

  Refer to glossary of terms for the definition of EoS.
- **Study groups:** Refer to Table 1 and Table 2.

Table 1 Study groups and epochs foreseen in the study

Study groups	Approximate number of subjects*	Age (min - max)	Epochs			
			Epoch 001	Epoch 002	Epoch 003	Epoch 004
P-Fx	20	18-55 years**	x	x		x
NP-Fx	20	18-55 years**	x	x		x
InfectivityCtrl	6-24***	18-55 years			x	x

P-Fx = Pooled subjects from the MALARIA-092 study vaccinated with RTS,S/AS01 (different doses/formulations) and protected following the first challenge who will receive a Fx booster dose of RTS,S/AS01$_E$
NP-Fx = Pooled subjects from the MALARIA-092 study vaccinated with RTS,S/AS01 (different doses/formulations) and not protected following the first challenge who will receive a Fx booster dose of RTS,S/AS01$_E$
InfectivityCtrl = Subjects who will not receive any vaccination but will undergo sporozoite challenge
*The actual number of subjects will be known after screening. A minimum of 20 subjects per group (P-Fx and NP-Fx) is anticipated
**Age at the time of enrollment in the primary study (MALARIA-092)
***Up to 24 subjects, or 4-6 per day, depending on the expected number of days of challenge

Table 2 Study groups and treatment foreseen in the study

Treatment name	Vaccine/Product name	Volume to be administered	Study groups		
			P-Fx	NP-Fx	InfectivityCtrl
RTS,S/AS01$_E$	RTS,S	0.1 mL	x	x	-
	AS01E		x	x	-

- **Control:** Non-interventional control. For the challenge, this will be the InfectivityCtrl group.
- **Vaccination schedule:** All previously vaccinated and challenged subjects in MALARIA-092 study will receive one Fx dose of RTS,S/AS01$_E$ at Visit 1 (Day 1).
- **Treatment allocation:** Non-randomized.
- **Blinding:** Open (refer to Table 3).

Table 3 Blinding of study epochs

Study Epochs	Blinding
Epoch 001	Open
Epoch 002	Open
Epoch 003	Open
Epoch 004	Open

- **Sampling schedule:**

  For subjects from the P-Fx and NP-Fx groups:

  – Blood samples for assessment of anti-CS and anti-HBs immune response and for serum repository will be collected at screening, Day 1*, on the day of challenge (Day 22), 28 days post-challenge (Day 50) and at study end (Day 190).

  – Blood samples for peripheral blood mononuclear cells (PBMCs) and plasma repository will be collected at screening, Day 1*, 7 days post-booster (Day 8), on the day of challenge (Day 22), 28 days post-challenge (Day 50) and at study end (Day 190).

  *Before vaccine administration. These samples might not be drawn if Visit 1 occurs less than one week after screening, at the discretion of the investigator.

  For all subjects:

  – Blood samples for the evaluation of biochemistry (alanine aminotransferase [ALT], aspartate aminotransferase [AST], creatinine) and hematology (hemoglobin, leukocytes [white blood cells; WBC], platelets) parameters will be collected at screening, Day 1*, 7 days post-booster* (Day 8), Day 22, the day of first parasitemia and 28 days post-challenge (Day 50). 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 or clarification of subject eligibility.

  *Not applicable for the infectivity controls.

  – Blood sample for assessment of parasitemia (blood smear and PCR) will be collected daily for 14 days (from Day 27 [Visit 4] to Day 40 [Visit 17]) and then every two days for nine days (Day 42 [Visit 18], Day 44 [Visit 19], Day 46 [Visit 20], Day 48 [Visit 21] and Day 50 [Visit 22]). Exceptionally this can occur within 1 or 3 days if the subject cannot make the visit or for schedule conflicts. For subjects who develop malaria, blood smear and PCR 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 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, at Day 1* and on the day of challenge (Day 22).

  *Before vaccine administration. Not applicable for the infectivity controls.

- **Type of study:** extension of other protocol(s) (MALARIA-092).
- **Data collection:** electronic Case Report Form (eCRF).

4. CASE DEFINITION

The following will be used as case definition of *P. falciparum* infection:

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

5. STUDY COHORT

5.1. Number of subjects/centres

Subjects who were vaccinated (different formulations/schedules at 18-55 years of age at the time of first vaccination) and challenged in the MALARIA-092 study will be enrolled and allocated to the groups as follows:

- **P-Fx group***: Subjects who were protected following the first challenge and who will receive a Fx booster dose of RTS,S/AS01$_E$ 12 months after completion of the vaccination course in the MALARIA-092 study.
- **NP-Fx group***: Subjects who were not protected following the first challenge and who will receive a Fx booster dose of RTS,S/AS01$_E$ 12 months after completion of the vaccination course in the MALARIA-092 study.

*The actual number of subjects in these groups will be determined by subjects from the MALARIA-092 study who agree to participate in this booster study and fulfil the eligibility criteria for each of the groups. A minimum of 20 subjects per group (P-Fx and NP-Fx) is anticipated.

In addition, the following group will be enrolled for this study:

- **InfectivityCtrl group**: Subjects who will be newly enrolled in the MALARIA-102 study and will only undergo sporozoite challenge.

Refer to the table below for a description of the MALARIA-092 study vaccination groups:

Study groups	Vaccination schedule	Age (Min/Max)
AduFx	RTS,S/AS01$_B$ full dose at M0, M1 + RTS,S/AS01$_B$ Fx dose (1/5th) at M7	18 - 55 years
2PedFx	RTS,S/AS01$_E$ double dose at M0, M1 + double dose RTS,S/AS01$_E$ Fx dose (1/5th) at M7	18 - 55 years
PedFx	RTS,S/AS01$_E$ full dose at M0, M1 + RTS,S/AS01$_E$ Fx dose (1/5th) at M7	18 - 55 years
Adu2Fx	RTS,S/AS01$_B$ full dose at M0 + RTS,S/AS01$_B$ Fx dose (1/5th) at M1, M7	18 - 55 years
Adu1Fx	RTS,S/AS01$_B$ full dose at M0 + RTS,S/AS01$_B$ Fx dose (1/5th) at M7	18 - 55 years

M = month

The target is to enroll all subjects who were vaccinated and have undergone challenge in MALARIA-092 study and who are willing to take part in this MALARIA-102 booster study. A minimum of 40 subjects (20/group) who were vaccinated in the MALARIA-092 study are expected to be enrolled in this study. However there may be more or fewer subjects who consent to join the study. In addition, up to 24 subjects in the infectivity control group will be newly enrolled. Refer to Section 11.4 for a detailed description of the criteria used in the determination of sample size.

Overview of the recruitment plan

On or about the last study visit of the MALARIA-092 study, participants will be asked about their willingness to be contacted to take part in a booster study, which will also be conducted at WRAIR. The investigator and his team will explain the rationale for MALARIA-102 and explain in general terms what the study entails. Only subjects who have completed all vaccinations and the primary challenge will be eligible for screening for the MALARIA-102 study. Such subjects who are interested in continuing on to participation in the MALARIA-102 study will be contacted and scheduled for a screening visit during the approved study recruitment window. Screened subjects who fulfill all inclusion criteria (and none of the exclusion criteria) for the study will be considered eligible to participate in the study.

For the infectivity control group, subjects will be recruited and screened in order to provide approximately 6 control subjects for each malaria challenge day required for the trial (the number of which will depend on the final number of experimental subjects). Up to 24 subjects may eventually be enrolled (defined as undergoing malaria challenge) as infectivity controls.

To be deemed eligible to participate, control subjects must fulfill all inclusion criteria (and none of the exclusion criteria) for the study. For the 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).

WRAIR has an established process whereby an IRB-approved generic screening protocol is used to screen potential subjects managed by the WRAIR Clinical Trials Center. If subjects from this pre-screened group agree to be infectivity controls in the MALARIA-102 study, the required biochemistry, hematology, HIV, HCV and HBsAg and ECG test results can be rolled over to the study provided they occur within the screening period, at the discretion of the Principal Investigator.

5.2. Inclusion criteria for enrollment

Deviations from inclusion criteria are not allowed because they can potentially jeopardize the scientific integrity, regulatory acceptability of the study 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:

Only for subjects from the MALARIA-092 study:

- Subjects vaccinated and having undergone sporozoite challenge during the primary study (MALARIA-092).

For all subjects:

- 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 any study-specific procedure.
- 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 seven months for all subjects from MALARIA-092 and one month for the infectivity control subjects; excluding the screening period).
- Female subjects of non-childbearing potential may be enrolled in the study.
  - Non-childbearing potential is defined as pre-menarche, current bilateral tubal ligation or occlusion, hysterectomy, bilateral 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 Day 1, and has agreed to continue adequate contraception during the entire treatment period and for two months after malaria challenge (only for subjects from the MALARIA-092 study).
  - Has practiced adequate contraception for 30 days prior to malaria challenge, and has agreed to continue adequate contraception up to two months after malaria challenge (only for the infectivity control subjects).
  - Has a negative pregnancy test at enrollment.

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

For the infectivity control subjects:

- Male or female subjects between, and including, 18 and 55 years of age.

5.3. Exclusion criteria for enrollment

Deviations from exclusion criteria are not allowed because they can potentially jeopardize the scientific integrity, regulatory acceptability of the study 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:

For all subjects except the infectivity control subjects:

- Any medical condition that in the judgment of the investigator would make intramuscular injection unsafe.
- History of any reaction or hypersensitivity likely to be exacerbated by any component of the vaccine.
- History of anaphylaxis post-vaccination.

For all subjects:

- Use of any investigational or non-registered product (drug or vaccine) other than the study vaccine during the period starting 30 days before Day 1 (Day -29 to Day 1) (for P-Fx and NP-Fx groups)/before the malaria challenge (for infectivity control subjects), or planned use during the study period.
- 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 Day 1 (for P-Fx and NP-Fx groups) or malaria challenge (for infectivity control subjects). For corticosteroids, this will mean prednisone ≥ 20 mg/day, 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 anti-malarial 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 within seven days of Day 1 (for P-Fx and NP-Fx groups) or the malaria challenge (for infectivity control subjects).
- 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 HIV, HBsAg or HCV.
- Planned travel to malaria endemic areas during the study period.
- Any confirmed or suspected immunosuppressive or immunodeficient condition, based on medical history and physical examination.

- History of any reaction or hypersensitivity that would prevent the subject from utilizing all of the following: chloroquine, atovaquone/proguanil, artemether/lumefantrine.
- Current use of medications known to cause drug reactions that would prevent the subject from utilizing any of the following: chloroquine, atovaquone/proguanil, artemether/lumefantrine.
- History of severe reactions to mosquito bites.
- Acute disease and/or fever at the time of enrollment.
  - Fever is defined as temperature ≥ 37.5°C/99.5°F for oral, axillary or tympanic 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.
- Hepatomegaly, right upper quadrant abdominal pain or tenderness.
- Any abnormal (clinically significant) baseline laboratory screening tests: ALT, AST, creatinine, hemoglobin, platelet count, total WBC, out of normal range.
- Personal history of auto-immune disease.
- Administration of immunoglobulins and/or any blood products during the period starting three months before Day 1 (for P-Fx and NP-Fx groups)/the malaria challenge (for infectivity control subjects), or planned administration during the study period.
- Pregnant or lactating female.
- Female planning to become pregnant or planning to discontinue contraceptive precautions.
- History of chronic alcohol consumption and/or drug abuse.
- History of blood donation within 56 days preceding enrollment.
- 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.
- Acute or chronic, clinically significant pulmonary, cardiovascular, hepatic or renal functional abnormality, as determined by physical examination or laboratory screening tests.
- 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 20-35 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.

Only for infectivity control subjects:

- Previous vaccination against malaria.
- History of splenectomy.
- Family history of congenital or hereditary immunodeficiency.
- Major congenital defects.
- Serious chronic illness.
- History of any neurological disorders or seizures (except for a single episode of simple febrile seizure in childhood).
- Diagnosed with malaria within the last 5 years (inclusive).

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 International Conference on Harmonization (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:

- Institutional Review Board (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 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 judgement, 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 randomisation

6.2.1. Subject identification

Subject identification numbers will be the same as the ones assigned in the primary study MALARIA-092, with the exception that the identifying protocol number will be changed to reflect the new study, as applicable. New identification numbers will be generated for infectivity controls.

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. The allocation will be sequential in the order in which the subjects are vaccinated.

6.2.2.2.1. Study group and treatment number allocation

The target is to enroll all subjects who were vaccinated and have undergone challenge in the MALARIA-092 study and who are willing to take part in this MALARIA-102 booster study. A minimum of 40 subjects (20/vaccine group) are expected to be enrolled in this study. However there may be more or fewer subjects who consent to join the study.

Subjects from the MALARIA-092 study will not be randomized but the treatment allocation will be done via a randomization system on internet (SBIR) to allow monitoring of vaccine storage and expiry dates.

Subjects from the infectivity control group will not be randomized and will not appear in SBIR; they will simply be registered in the eCRF database.

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

6.3. 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 two vaccine groups (P-Fx and NP-Fx) 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 three weeks after vaccination (Day 22 [Visit 3]).

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 6 and Table 7, 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 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) and PCR. 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 (period between infection with a parasite and the demonstration of the parasite in the body) 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 5 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 the fifth day after their challenge (Day 27 [Visit 4]), subjects will be seen and evaluated daily by a study investigator and blood will be drawn for blood smears and PCR 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. Additional PCR tests may also be performed at investigator discretion, but may not necessarily be run in real time.

Beginning on the ninth day post-challenge (Day 31 [Visit 8]), a group of hotel rooms in the local area of WRAIR will be reserved for malaria-challenged subjects. 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.

During the hotel phase, all challenged subjects will be assessed on a daily basis in an identical manner. An evaluation will be done ideally each morning (headache, muscle aches, etc.) and blood will be drawn for smear and PCR. There may be the rare occasion where subject assessment is done later in the day but every effort must be made for the assessment to be done in the morning. All challenged subjects will be instructed to check in with clinical staff by telephone call or texting or in-person each afternoon or evening during the hotel stay until they are positive for malaria. A member of the WRAIR staff will contact him/her and if they are not available during the time of contact. A telephone script will be used to guide the site staff during these contacts. 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 and PCR, 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 nights (between Day 31 [Visit 8] to Day 40 [Visit 17], inclusive). 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 and PCR every two days up to 28 days post-challenge (Days 42 [Visit 18], 44 [Visit 19], 46 [Visit 20], 48 [Visit 21] and 50 [Visit 22]). Exceptionally this can occur within 1 or 3 days if the subject cannot make the visit or for schedule conflicts. 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 42, 44, 46 and 48 (20, 22, 24 and 26 days post-challenge), but will be required to come to the clinic center at Day 50 (28 days post-challenge [Visit 22]). Telephone contact (spoken or written) 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 42, 44, 46 and 48 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 with a positive blood smear 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 (once a day for three consecutive days) or artemether/lumefantrine (*Coartem*) standard oral dosage of 80 mg/480 mg (initial dose, additional dose 8 hours later, and then 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 (once a day for three consecutive days), or artemether/lumefantrine (*Coartem*) standard oral dosage of 80 mg/480 mg (initial dose, additional dose 8 hours later, and then 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 4 Study procedures for the P-Fx and NP-Fx groups

Epoch	Epoch 001	Epoch 002		Epoch 004				
Equivalent timepoints based on MALARIA-092 study		M19		M20			M21	M26
Type of contact (Visit #)	SCR #1	Visit 1	Visit 2	Visit 3	Visit 4 to Visit 21	Visit Par*	Visit 22	Visit 23
Timepoints	Day -90 to -1	Day 1	Day 8	Day 22	Day 27 to Day 48[⇑]		Day 50	Day 190
Sampling timepoints	SCR1	Vacc	Post-vacc	DoC	Post-challenge			
Informed consent	●							
Check inclusion/exclusion criteria [a]	●	O						
Check screening laboratory results [b]	●							
Collect demographic data	●							
Medical history	●							
Physical examination	●	O[#]	O[#]	O[#]	O[#]	O[#]	O[#]	O[#]
Urine pregnancy test	●	●		●				
Check contraindications to vaccination [c]		O						
Pre-vaccination body temperature		●						
Treatment number allocation		O						
Recording of administered treatment number		●						
Vaccine administration		●						
Check contraindications to challenge [c]				O				
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 mL)					●**		●	
Blood sampling for assessment of parasitemia (PCR) (~2 mL)					●**		●	
Blood sampling for antibody determination (~20 mL)	●	●[e]		●			●	●
Blood sampling for PBMCs and plasma repository (~40 mL)	●	●[e]	●	●			●	●
Blood sampling for hematology/biochemical analysis (~7.5 mL) [d]	●	●	●	●		●	●	
Record any concomitant medications/vaccinations	●	●	●	●	●		●	●
Record any intercurrent medical conditions	●	●	●	●	●		●	●
Distribution of diary cards		O						

Epoch	Epoch 001	Epoch 002		Epoch 004				
Equivalent timepoints based on MALARIA-092 study		**M19**		**M20**			**M21**	**M26**
Type of contact (Visit #)	**SCR #1**	**Visit 1**	**Visit 2**	**Visit 3**	**Visit 4 to Visit 21**	**Visit Par***	**Visit 22**	**Visit 23**
Timepoints	**Day -90 to -1**	**Day 1**	**Day 8**	**Day 22**	**Day 27 to Day 48⇑**		**Day 50**	**Day 190**
Sampling timepoints	**SCR1**	**Vacc**	**Post-vacc**	**DoC**	**Post-challenge**			
Return of diary cards			O[f]	O[g]				
Diary card transcription by investigator			●	●				
Recording of solicited AEs within 7 days post-vaccination		●	●					
Recording of unsolicited AEs within 21 days post-vaccination		●	●					
Recording of AEs post-challenge				●	●	●	●	
Recording of SAEs and pregnancies		●	●	●	●	●	●	●
Recording of pIMDs and meningitis		●	●	●	●	●	●	●
Recording of SAEs related to study participation, or to a concurrent GSK medication/vaccine	●	●	●	●	●	●	●	●
Study conclusion								●

M = month; **SCR #1** = screening for subjects from the MALARIA-092 study; **Vacc** = vaccination; **DoC** = day of challenge; **HIV** = human immunodeficiency virus; **HCV** = hepatitis C virus, **HBsAg** = hepatitis B surface antigen; **PBMCs** = peripheral blood mononuclear cells; **AEs** = adverse events; **SAEs** = serious adverse events; **pIMDs** = potential immune-mediated diseases

⇑**Visit 5** = Day 28 / **Visit 6** = Day 29 / **Visit 7** = Day 30 / **Visit 8** = Day 31 / **Visit 9** = Day 32 / **Visit 10** = Day 33 / **Visit 11** = Day 34 / **Visit 12** = Day 35 / **Visit 13** = Day 36 / **Visit 14** = Day 37 / **Visit 15** = Day 38 / **Visit 16** = Day 39 / **Visit 17** = Day 40 / **Visit 18** = Day 42 / **Visit 19** = Day 44 / **Visit 20** = Day 46

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

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

#History-directed physical examination

*A blood sample for biochemistry and hematology parameters will be collected the day of first parasitemia (between Visit 4 [Day 27] and Visit 21 [Day 48]).

**A blood sample for assessment of parasitemia (blood smear and PCR) will be collected daily for 14 days (from Day 27 [Visit 4] to Day 40 [Visit 17]) and then every two days for 9 days (Day 42 [Visit 18], Day 44 [Visit 19], Day 46 [Visit 20], Day 48 [Visit 21] and Day 50 [Visit 22]). Exceptionally this can occur within 1 or 3 days if the subject cannot make the visit or for schedule conflicts. For subjects who develop malaria (positive blood smear), blood smear and PCR may be discontinued once the subject has three consecutive negative smears (separated by more than 12 hours) following initial treatment.

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 vaccination.

c There is no specific section in the eCRF to record the contraindications. The absolute contraindications to administration of study vaccine or challenge have to be recorded in the AE or SAE section of the eCRF.

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

e Blood sample might not be drawn at Visit 1 if this visit occurs less than one week after the screening visit, at the discretion of the investigator
f Return of diary cards for the collection of solicited AEs
g Return of diary cards for the collection of unsolicited AEs

Table 5 Study procedures for the InfectivityCtrl group

Epoch	Epoch 003	Epoch 004			
Equivalent timepoints based on MALARIA-092 study		M20			M21
Type of contact (Visit #)	SCR #2	Visit 3	Visit 4 to Visit 21	Visit Par*	Visit 22
Timepoints	Day -68 to 21	Day 22	Day 27 to Day 48⇑		Day 50
Sampling timepoints	SCR2	DoC	Post-challenge		
Informed consent	●				
Check inclusion/exclusion criteria [a]	●	O			
Check screening laboratory results [b]	●				
Collect demographic data	●				
Medical history	●				
Physical examination	●	O#	O#	O#	O#
Urine pregnancy test	●	●			
Check contraindications to challenge [c]		O			
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 mL)			●**		●
Blood sampling for assessment of parasitemia (PCR) (~2 mL)			●**		●
Blood sampling for hematology/biochemical analysis (~7.5 mL) [d]	●	●		●	●
Record any concomitant medications/vaccinations	●	●	●		●
Record any intercurrent medical conditions	●	●	●		●
Recording of AEs post-challenge		●	●	●	●
Recording of SAEs and pregnancies		●	●	●	●
Recording of SAEs related to study participation, or to a concurrent GSK medication/vaccine	●	●	●	●	●
Study conclusion					●

SCR #2 = screening for infectivity control subjects; **DoC** = Day of challenge; **HIV** = human immunodeficiency virus; **HCV** = hepatitis C virus, **HBsAg** = hepatitis B surface antigen; **AEs** = adverse events; **SAEs** = serious adverse events

⇧**Visit 5** = Day 28 / **Visit 6** = Day 29 / **Visit 7** = Day 30 / **Visit 8** = Day 31 / **Visit 9** = Day 32 / **Visit 10** = Day 33 / **Visit 11** = Day 34 / **Visit 12** = Day 35 / **Visit 13** = Day 36 / **Visit 14** = Day 37 / **Visit 15** = Day 38 / **Visit 16** = Day 39 / **Visit 17** = Day 40 / **Visit 18** = Day 42 / **Visit 19** = Day 44 / **Visit 20** = Day 46

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

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

#History-directed physical examination

*A blood sample for biochemistry and hematology parameters will be collected the day of first parasitemia (between Visit 4 [Day 27] and Visit 21 [Day 48]).

**A blood sample for assessment of parasitemia (blood smear and PCR) will be collected daily for 14 days (from Day 27 [Visit 4] to Day 40 [Visit 17]) and then every two days for 9 days (Day 42 [Visit 18], Day 44 [Visit 19], Day 46 [Visit 20], Day 48 [Visit 21] and Day 50 [Visit 22]). Exceptionally this can occur within 1 or 3 days if the subject cannot make the visit or for schedule conflicts. For subjects who develop malaria (positive blood smear), blood smear and PCR may be discontinued once the subject has three consecutive negative smears (separated by more than 12 hours) following initial treatment.

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.

c There is no specific section in the eCRF to record the contraindications. The absolute contraindications to administration of study vaccine or challenge have to be recorded in the AE or SAE section of the eCRF.

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

Whenever possible, the investigator should arrange study visits within the interval described in Table 6 and Table 7

Table 6 Intervals between study visits for the P-Fx and NP-Fx groups

Interval	Optimal length of interval*	Allowed interval**
Screening #1 → Visit 1	1 to 90 days	-
Visit 1 → Visit 2	7 days	6-8 days
Visit 1 → Visit 3 (challenge)	21 days	21-28 days
Visit 3 → Visit 22	28 days	21-35 days
Visit 22 → Visit 23	140 days	126-154 days

*Whenever possible the investigator should arrange study visits within this interval.
**Subjects may not be eligible for inclusion in the Per-Protocol Set (PPS) for analysis of immunogenicity and efficacy if they make the study visit outside this interval.

Table 7 Intervals between study visits for the infectivity control group

Interval	Optimal length of interval*	Allowed interval**
Screening #2 → Visit 3 (challenge)	1 to 90 days	-
Visit 3 → Visit 22	28 days	21-35 days

*Whenever possible the investigator should arrange study visits within this interval.
**Subjects may not be eligible for inclusion in the PPS 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 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 enrollment and prior to Day 1 (for P-Fx and NP-Fx groups)/to the malaria challenge (for infectivity control group).

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.

WRAIR has an established process whereby an IRB-approved generic screening protocol is used to screen potential subjects managed by the WRAIR Clinical Trials Center. For infectivity controls, please refer to Section 5.1 (overview of the recruitment plan) for further information.

6.6.4. Collect demographic data

Record demographic data such as date of birth, sex, race and ethnicity 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 Day 1 (for P-Fx and NP-Fx groups)/to the malaria challenge (for infectivity control group) in the eCRF.

6.6.6. Physical examination

Perform a physical examination of the subject, including assessment of oral 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 (screening) 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.

6.6.7. Urine pregnancy test

Female subjects of childbearing potential are to have a urine pregnancy test at screening and at Day 1 (prior to any study vaccine administration). The study vaccine may only be administered if the pregnancy test is negative.

Note: Pregnancy test must be performed even if the subject is menstruating at the time of the study visit.

All 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.

6.6.8. Check contraindications to vaccination

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

6.6.9. Assess pre-vaccination body temperature

The oral body temperature of each subject needs to be measured prior to the study vaccine administration. If the subject has fever (fever is defined as temperature ≥ 37.5°C/99.5°F regardless the location of measurement) on the day of vaccination, the vaccination visit will be rescheduled within the allowed interval for this visit (Table 6).

6.6.10. Study group and treatment number allocation

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.

6.6.11. Study vaccine administration

- After completing all prerequisite procedures prior to vaccination, one dose of study vaccine will be administered intramuscularly in the deltoid muscle of the non-dominant arm (preferably), except, at the discretion of the investigator, if there are tattoos, rashes, burns or other skin disorders which make the evaluation of AE(s) at the injection site impossible (refer to Section 7.3 for detailed description of the vaccine 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 (refer to Table 6).
- 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.

6.6.12. 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.

6.6.13. 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.

6.6.14. Distribution of emergency notification card

In addition to the subject card (refer to Section 9.7), 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.

6.6.15. Sampling

Blood samples will be taken during certain study visits as specified in Section 6.5 List of Study Procedures.

For P-Fx and NP-Fx groups:

- Blood sampling for assessment of antibody determination and serum repository

  A volume of approximately 20 mL of whole blood (to provide at least 10 mL of serum) should be drawn from all subjects for each analysis of humoral immune response (anti-CS ELISA, anti-CS avidity, anti-HBs and serum repository) at each pre-defined timepoint. After centrifugation, serum samples should be kept at –20°C/–4°F or below until shipment. Refer to the SPM for more details on sample storage conditions.

- Blood sampling for PBMC and plasma repository

  A volume of approximately 40 mL of whole blood should be drawn at each pre-defined timepoint for the purpose of the PBMC and plasma repository. 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.

For all subjects:

- Blood sampling for HIV, HCV and HBsAg

  A volume of approximately 17 mL of whole blood should be drawn from all screened subjects to assess HIV, HCV and HBsAg status.

- Blood sampling for assessment of parasitemia (blood smear and PCR)

  A volume of approximately 4 mL should be drawn from all subjects at each pre-defined timepoint for the assessment of parasitemia by blood smear and PCR. For subjects who develop malaria (positive blood smear), blood samples for smears and PCR may be discontinued once the subject has three consecutive negative smears (separated by more than 12 hours) following initial treatment.

- 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).

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

6.6.16. 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.

6.6.17. Recording of AEs, SAEs, pregnancies and pIMDs

- Refer to Section 9.3 for procedures for the investigator to record AEs, SAEs, pregnancies and AEs of specific interest (pIMDs and meningitis). Refer to Section 9.4 for guidelines and how to report SAE, pregnancy and AE 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.

For the P-Fx and NP-Fx groups:

- At the vaccination visit, diary cards will be provided to the subject. The subject will be instructed to measure and record the oral body temperature and any solicited local/general AEs (i.e., on the day of vaccination and during the next 6 days) or any unsolicited AEs (i.e., on the day of vaccination and during the next 20 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 2 and Visit 3.
- Any unreturned diary cards will be sought from the subject through telephone call(s) or any other convenient procedure.
- The investigator or delegate will transcribe the collected information into the eCRF in English.

6.6.18. 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 labelled 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 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 United States 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 Per-Protocol 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

Table 8 Biological samples

Sample type	Sample type	Quantity	Timepoint	Study groups
Whole blood	Blood sampling for HIV, HCV and HBsAg	~17 mL	Screening #1	P-Fx NP-Fx
			Screening #2	Infectivity control
	Blood sampling for assessment of parasitemia (blood smear and PCR)	~4 mL	Visit 4 (Day 27) Visit 5 (Day 28) Visit 6 (Day 29) Visit 7 (Day 30) Visit 8 (Day 31) Visit 9 (Day 32) Visit 10 (Day 33) Visit 11 (Day 34) Visit 12 (Day 35) Visit 13 (Day 36) Visit 14 (Day 37) Visit 15 (Day 38) Visit 16 (Day 39) Visit 17 (Day 40) Visit 18 (Day 42) Visit 19 (Day 44) Visit 20 (Day 46) Visit 21 (Day 48) Visit 22 (Day 50)	All subjects
	Blood sampling for antibody determination and serum repository	~20 mL	Screening #1 Visit 1 (Day 1)** Visit 3 (Day 22) Visit 22 (Day 50) Visit 23 (Day 190)	P-Fx NP-Fx
	Blood sampling for PBMC and plasma repository	~40 mL	Screening #1 Visit 1 (Day 1)** Visit 2 (Day 8) Visit 3 (Day 22) Visit 22 (Day 50) Visit 23 (Day 190)	P-Fx NP-Fx
	Blood sampling for hematology and biochemistry analysis	~7.5 mL	Screening #1 Visit 1 (Day 1) Visit 2 (Day 8)	P-Fx NP-Fx
			Screening #2	Infectivity control
			Visit 3 (Day 22) Visit 22 (Day 50) Visit Par*	All subjects

*Biochemistry and hematology parameters will be collected the day of first parasitemia (between Visit 4 [Day 27] and Visit 21 [Day 48]).
**Blood sample might not be drawn at Visit 1 if this visit occurs less than one week after the screening visit, at the discretion of the investigator

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 enzyme-linked immunosorbent assay (ELISA) at laboratories designated by GSK Biologicals using standardized and validated procedures.

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

Table 9 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	1.9	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 (Total Ig)	CLIA	ADVIA Centaur anti-HBs2 (Siemens Healthcare)	mIU/mL	6.2	GSK Biologicals*

Ab = antibody; **Ig** = immunoglobulin; **ELISA** = enzyme-linked immunosorbent assay; **CEVAC** = Center For Vaccinology; **EPT** = endpoint titer; **WRAIR** = Walter Reed Army Institute of Research; **CLIA** = chemiluminescence enzyme immunoassay
*GSK Biologicals laboratory refers to Clinical Laboratory Sciences (CLS) In Rixensart, Belgium; Wavre, Belgium.

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

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

Assessment of *P. falciparum* parasitemia will be performed by blood slide microscopy reading and real-time PCR at WRAIR, as applicable.

Table 10 Assessment of *P. falciparum* parasitemia

System	Component	Method	Unit	Laboratory
Whole blood	Plasmodium falciparum parasites*	Blood slide microscopy reading	Parasite/μL	WRAIR
Whole blood	Plasmodium falciparum parasites*	Real-time PCR	Positive/negative	WRAIR

WRAIR = Walter Reed Army Institute of Research; **PCR** = polymerase chain reaction
**P. falciparum* parasite count includes blood-stage parasites.

Hematology, biochemistry and screening tests (e.g., HIV, leukocytes) will be performed by different methods/assays at Quest Diagnostics, Inc., (all except pregnancy testing) and at the WRAIR (pregnancy testing).

Table 11 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	Hepatitis C virus 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.

HIV = human immunodeficiency virus; **IgG** = immunoglobulin G; **Ag** = antigen; **Ab** = antibody; **CLIA** = chemiluminescence enzyme immunoassay; **HCV** = hepatitis C virus; **β-HCG** = beta-human chorionic gonadotropin; **WRAIR** = Walter Reed Army Institute of Research
*Urinary pregnancy test

Additional exploratory testing on the vaccine and/or on the disease under study may be performed within the framework of the study if deemed necessary for accurate interpretation of the data or should such assay(s) become available at GSK. These assays may not be represented in the objectives/endpoints of the study protocol.

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 12 Immunological read-outs

Blood sampling timepoint		Study groups	Number of subjects	Component	Components priority rank
Type of contact and timepoint	Sampling timepoint				
Screening #1 Visit 1 (Day 1) Visit 3 (Day 22) Visit 22 (Day 50) Visit 23 (Day 190)	Screening Vaccination day Challenge day Post-challenge Post-challenge	P-Fx NP-Fx	~40*	Anti-CS Ab	1
				Anti-CS avidity index	2
				Anti-C term Ab	3
				Anti-C term avidity index	4
				HBsAg Ab	5

CS = circumsporozoite; **Ab** = antibody; **HBsAg** = hepatitis B surface antigen
*A minimum of 20 subjects per group (P-Fx and NP-Fx) is anticipated

In case of insufficient blood sample volume to perform assays for all antibodies, the samples will be analyzed according to priority ranking provided in Table 12.

Additional serological assays for the determination of anti-full length CS antibodies titers and avidity will be performed by ELISA at WRAIR following the same order as in the table above (i.e., determination of antibody titers before avidity).

6.7.4.2. Hematology/Blood chemistry

Table 13 Hematology/biochemistry read-outs

Blood sampling timepoint		Study groups	Number of subjects	Component
Type of contact and timepoint	Sampling timepoint			
Screening #1	SCR#1	P-Fx NP-Fx	~40**	HIV IgG + Ag HBsAg Ab HCV Ab Hemoglobin Leukocytes (WBC) Platelets ALT AST Creatinine
Screening #2	SCR#2	Infectivity control	~24	
Visit 1 (Day 1) Visit 2 (Day 8)	Vaccination day Post-vaccination	P-Fx NP-Fx	~40**	Hemoglobin Leukocytes (WBC) Platelets ALT AST Creatinine
Visit 3 (Day 22) Visit 22 (Day 50) Visit Par*	Post-challenge Post-challenge	All subjects	~64	

HIV = human immunodeficiency virus; **IgG** = immunoglobulin G; **Ag** = antigen; **HBsAg** = hepatitis B surface antigen; **Ab** = antibody; **HCV** = hepatitis C virus; **WBC** = white blood cell; **ALT** = alanine aminotransferase; **AST** = aspartate aminotransferase
*Biochemistry and hematology parameters will be collected the day of first parasitemia (between Visit 4 [Day 27] and Visit 21 [Day 48]).
**A minimum of 20 subjects per group (P-Fx and NP-Fx) is anticipated

6.7.4.3. Parasitemia

Table 14 Parasitemia read-outs

Blood sampling timepoint	Study groups	Number of subjects	Component	Components priority rank
Visit 4 (Day 27) Visit 5 (Day 28) Visit 6 (Day 29) Visit 7 (Day 30) Visit 9 (Day 32) Visit 8 (Day 31) Visit 10 (Day 33) Visit 11 (Day 34) Visit 12 (Day 35) Visit 13 (Day 36)	All subjects	~64	Blood smear	1
Visit 14 (Day 37) Visit 15 (Day 38) Visit 16 (Day 39) Visit 17 (Day 40) Visit 18 (Day 42) Visit 19 (Day 44) Visit 20 (Day 46) Visit 21 (Day 48) Visit 22 (Day 50)			PCR	2

PCR = polymerase chain reaction

In case of insufficient blood sample volume to perform assays for parasitemia assessment, samples will be analyzed according to priority ranking provided in Table 14.

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 VACCINE AND ADMINISTRATION

7.1. Description of study vaccine

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 vaccine is labelled and packed according to applicable regulatory requirements.

Table 15 Study vaccine

Treatment name	Vaccine name	Formulation	Presentation	Volume to be administered*	Number of doses
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		

MPL = Monophosphoryl lipid; **QS-21** = *Quillaja saponaria* Molina, fraction 21 (Licensed by GSK from Antigenics Inc., a wholly owned subsidiary of Agenus Inc., a Delaware, USA corporation)
*After reconstitution

7.2. Storage and handling of study vaccine

The study vaccine 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 vaccine.

Temperature excursions must be reported in degree Celsius.

Any temperature excursion outside the range of 0.0°C to +8.0°C/+32°F to +46°F (for +2°C to +8°C/+36°F to +46°F label storage condition) impacting investigational medicinal products (IMPs) must be reported in the appropriate (electronic) temperature excursion decision form. 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.

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 vaccine.

7.3. Dosage and administration of study vaccine

In this study, the commercial presentation of RTS,S/AS01$_E$ will be used, i.e., a glass vial of lyophilized RTS,S antigen to be reconstituted with a glass vial of AS01$_E$ Adjuvant System. A full dose of RTS,S/AS01$_E$ (0.5 mL) contains 25 μg of the RTS,S antigen and 25 μg of each immunostimulant MPL and QS-21.

From the reconstituted vaccine vial, 0.1 mL will be withdrawn to administer a Fx booster dose of RTS,S/AS01$_E$.

Table 16 Dosage and administration

Type of contact and timepoint	Study group	Treatment name	Volume to be administered	Route	Site	
					Location	Laterality*
Visit 1 (Day 1)	P-Fx NP-Fx	RTS,S/AS01$_E$ (1/5th dose)	0.1 mL	IM	Deltoid	Non-Dominant

IM: intramuscular

*The non-dominant arm is the preferred arm of injection. In case it is not possible to administer the vaccine in the non-dominant arm (e.g., in case of skin alterations due to tattoos, rashes, burns or other skin disorders subject to the discretion of the investigator), an injection in the dominant arm may be performed.

7.4. Replacement of unusable vaccine

In addition to the vaccine doses provided for the planned number of subjects (including over-randomization when applicable), at least 10% additional vaccine doses will be supplied to replace those that are unusable.

7.5. Contraindications to vaccination

The following event constitutes contraindications to administration of RTS,S/AS01$_E$ at that point in time; if this event occurs 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. The preferred location for measuring temperature in this study will be the oral cavity.
  - Subjects with a minor illness (such as mild diarrhea, mild upper respiratory infection) without fever can be administered the vaccine.

7.6. Concomitant medications/products and concomitant vaccinations

At each study visit, the investigator or delegate 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 within seven days following Day 1 (Day 1 to Day 8) and during the entire sporozoite challenge (Day 22 to Day 50).
- Any concomitant vaccination administered in the period starting seven days before Day 1 and ending at the last study visit (Day -7 to Day 190).
- Prophylactic medication (i.e., medication administered in the absence of ANY symptom and in anticipation of a reaction to the vaccination) (for P-Fx and NP-Fx groups).

  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 regardless the location of measurement). The preferred location for measuring temperature in this study will be the oral cavity.
- 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 SAE(s) and pIMD(s) 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 Per-Protocol 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 Per-Protocol analysis. See Section 11.5 for cohorts to be analyzed:

- Any investigational or non-registered product (drug or vaccine) other than the study vaccine 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, 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 dose and ending seven days after the dose of vaccine 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.

  Although vaccination with routine recommended national vaccination such as with the influenza or pneumococcal vaccines will not lead to elimination, every effort should be made to have the vaccinations occurring either more than seven days before vaccination or more than seven days after vaccination.

- 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 Per-Protocol analyses

For vaccinated subjects, at each study visit subsequent to 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 Per-Protocol set (PPS) 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 they/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 study vaccine 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 study vaccine or a concurrent medication (overdose per se should not be reported as an AE/SAE).
- Signs, symptoms temporally associated with study vaccine 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 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 fulfils any other serious criteria, the event will also be considered serious. When in doubt as to whether 'hospitalization' occurred or was necessary, the AE should be considered serious.

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

d. Results in disability/incapacity, OR

   Note: The term disability means a substantial disruption of a person's ability to conduct normal life functions. This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, 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 judgement should be exercised in deciding whether reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the 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 17 Solicited local adverse events

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 18 Solicited general adverse events

Fatigue
Fever
Gastrointestinal symptoms*
Headache

*Gastrointestinal symptoms include nausea, vomiting, diarrhea and/or abdominal pain.

Note: It is recommended that subjects measure and record the oral body temperature in the evening. Should temperature (including additional) measurements be performed at other times of day, subjects will be instructed to record the highest temperature in the diary card.

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 judgement in deciding whether an abnormal laboratory finding or other abnormal assessment is clinically significant.

9.1.5. Adverse events of specific interest

9.1.5.1. Potential immune-mediated diseases

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 aetiology. AEs that need to be recorded and reported as pIMDs include those listed in Table 19.

However, the investigator will exercise his/her medical and scientific judgement 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 19 List of potential immune-mediated diseases

Neuro-inflammatory disorders	Musculoskeletal disorders	Skin disorders
• Cranial nerve neuropathy, including paralysis and 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. • Demyelinating peripheral neuropathies including: - Chronic inflammatory demyelinating polyneuropathy, - Multifocal motor neuropathy, - Polyneuropathies associated with monoclonal gammopathy. • Narcolepsy.	• Systemic lupus erythematosus and associated conditions. • Systemic scleroderma (systemic sclerosis), including: - Diffuse scleroderma, - CREST syndrome. • Idiopathic inflammatory myopathies, including: - Dermatomyositis, - Polymyositis. • Anti-synthetase syndrome. • Rheumatoid arthritis and associated conditions including: - Juvenile idiopathic arthritis, - Still's disease. • Polymyalgia rheumatica. • Spondyloarthropathies, including: - Ankylosing spondylitis, - Reactive arthritis (Reiter's syndrome), - Undifferentiated spondyloarthritis, - Psoriatic arthritis, - Enteropathic arthritis. • Relapsing polychondritis. • Mixed Connective Tissue disorder. • Gout.	• Psoriasis. • Vitiligo. • Erythema nodosum. • Autoimmune bullous skin diseases (including pemphigus, pemphigoid and dermatitis herpetiformis). • Lichen planus. • Sweet's syndrome. • Localized scleroderma (Morphoea).

Vasculitis	Blood disorders	Others
• Large vessels vasculitis including: - Giant Cell Arteritis (Temporal Arteritis), - Takayasu's Arteritis. • Medium sized and/or small vessels vasculitis including: - Polyarteritis nodosa, - Kawasaki's disease, - Microscopic Polyangiitis, - Wegener's Granulomatosis (granulomatosis with polyangiitis), - Churg–Strauss syndrome (allergic granulomatous angiitis or eosinophilic granulomatosis with polyangiitis), - Buerger's disease (thromboangiitis obliterans), - Necrotizing vasculitis (cutaneous or systemic), - Anti-neutrophil cytoplasmic antibody (ANCA) positive vasculitis (type unspecified), - Henoch-Schonlein purpura (IgA vasculitis), - Behcet's syndrome, - Leukocytoclastic vasculitis.	• Autoimmune hemolytic anemia. • Autoimmune thrombocytopenia. • Antiphospholipid syndrome. • Pernicious anemia. • Autoimmune aplastic anemia. • Autoimmune neutropenia. • Autoimmune pancytopenia.	• Autoimmune glomerulonephritis including: - IgA nephropathy, - Glomerulonephritis rapidly progressive, - Membranous glomerulonephritis, - Membrano-proliferative glomerulonephritis, - Mesangioproliferative glomerulonephritis, - Tubulointerstitial nephritis and uveitis syndrome. • Ocular autoimmune diseases including: - Autoimmune uveitis, - Autoimmune retinitis. • Autoimmune myocarditis. • Sarcoidosis. • Stevens-Johnson syndrome. • Sjögren's syndrome. • Alopecia areata. • 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 (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 (PTs) and PT 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, all cases of meningitis occurring during the study will be reported as a SAE.

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

9.2.1. Pregnancy

Female subjects who become pregnant after the vaccination will not receive the sporozoite challenge may continue the study for safety follow-up only, 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 study vaccine 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

Only for the P-Fx and NP-Fx groups:

All AEs starting within 21 days following administration of the dose of study vaccine (Day 1 to Day 21) must be recorded into the appropriate section of the eCRF, irrespective of intensity or whether or not they are considered vaccination-related.

SAEs that are related to the study vaccine will be collected and recorded from the time of the receipt of study vaccine until the subject is discharged from the study.

For all subjects:

The time period for collecting and recording AEs post-challenge will begin at the first day of challenge (Day 22) up to 29 days post-challenge (Day 50).

The time period for collecting and recording AEs of specific interest (pIMDs and meningitis) will begin at Day 1 (for P-Fx and NP-Fx groups)/the first day of sporozoite challenge (Day 22) (for infectivity control group) and will end at the last study visit (Day 190 for the P-Fx and NP-Fx groups/Day 50 for the infectivity control group). See Section 9.4 for instructions on reporting of pIMDs.

The time period for collecting and recording SAEs will begin at Day 1 (for P-Fx and NP-Fx groups)/the first day of sporozoite challenge (Day 22) (for infectivity control group) and will end at the last study visit (Day 190 for the P-Fx and NP-Fx groups/Day 50 for the infectivity control group) for each subject. 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 Day 1 (for P-Fx and NP-Fx groups)/the first day of sporozoite challenge (Day 22) (for infectivity control group).

In addition to the above-mentioned reporting requirements and in order to fulfil 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 in the study (screening) until she/he is discharged from the study.

The time period for collecting and recording pregnancies will begin at Day 1 (for P-Fx and NP-Fx groups)/the first day of sporozoite challenge (Day 22) (for infectivity control group) and will end at the last study visit (Day 190 for the P-Fx and NP-Fx groups/Day 50 for the infectivity control group). See Section 9.4 for instructions on reporting of pregnancies.

An overview of the protocol-required reporting periods for AEs, SAEs and pregnancies is given in Table 20.

Table 20 Reporting periods for collecting safety information

Event	SCR*	V1 D1	D7	D21	V3 D22	V7 D30	V22 D50	Study Conclusion** V23 D190
Solicited local and general AEs post-vaccination								
Unsolicited AEs post-vaccination								
AEs post-challenge								
SAEs (all, fatal, related) Pregnancies AEs of specific interest#								
SAEs related to study participation or concurrent GSK medication/vaccine								

SCR = screening; **D** = Day; **V** = Visit
*I.e., consent obtained.
**Visit applicable for all groups except for the infectivity control subjects (note that Day 50 is the study end for infectivity controls)
#pIMDs and meningitis

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 20. 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 study vaccine, 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 vaccine 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 21 Intensity scales for solicited symptoms

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. The preferred location for measuring temperature in this study will be the oral cavity.

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 normal ranges and toxicity grading for laboratory safety parameters used in this study are presented in Table 22.

Table 22 Toxicity grading scales for blood testing

Adverse event	Intensity grade	Intensity*
Hemoglobin (males)	Normal range	≥ 12.5 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 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	≤ 10 799 cells/mm^3
	1	10 800 – 15 000 cells/mm^3
	2	15 001 – 20 000 cells/mm^3
	3	> 20 001 cells/mm^3
Decrease in leukocytes (WBC)	Normal range	≥ 3200 cells/mm^3
	1	2500 - 3199 cells/mm^3
	2	1500 - 2499 cells/mm^3
	3	< 1500 cells/mm^3
Decrease in platelets	Normal	≥ 140 000 cells/mm^3
	1	125 000 – 139 000 cells/mm^3
	2	100 000 – 124 000 cells/mm^3
	3	< 100 000 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	≤ 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	≤ 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)].

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 judgement.

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.

9.3.3.2.2. Assessment of causality

The investigator is obligated to assess the relationship between study vaccine and the occurrence of each AE/SAE using clinical judgement. In case of concomitant administration of multiple vaccines/products, if possible, the investigator should specify if the AE could be causally related to a specific vaccine/product administered (i.e., investigational, control/placebo or co-administered vaccine). When causal relationship to a specific vaccine(s)/product(s) cannot be determined the investigator should indicate the AE to be related to all products.

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 study vaccine will be considered and investigated. The investigator will also consult the Investigator's Brochure 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.

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 study vaccine?"*

YES: There is a reasonable possibility that the study vaccine contributed to the AE.

NO: There is no reasonable possibility that the AE is causally related to the administration of the study vaccine. There are other, more likely causes and administration of the study vaccine 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 vaccine, 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 23, 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 23, once the investigator becomes aware of the pregnancy.

AEs of specific interest (pIMDs and meningitis) that occur in the time period defined in Section 9.3 will be reported promptly to GSK within the timeframes described in Table 23, once the investigator determines that the event meets the protocol definition of a AEs of specific interest.

Table 23 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 (pIMDs and meningitis)	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 a AE of specific interest.
#The investigator will be required to confirm review of the SAE/AE of specific interest causality by ticking the 'reviewed' box in the electronic Expedited Adverse Events Report within 72 hours of submission of the SAE/ AE of specific interest.

9.4.2. Contact information for reporting serious adverse events, pregnancies and pIMDs

Study Contact for Reporting SAEs, pIMDs and pregnancies
Refer to the local study contact information document.
Back-up Study Contact for Reporting SAEs, pIMDs 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 SAE 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 should be estimated by ultrasound examination and recorded in the pregnancy report.

9.4.5. Reporting of AEs of specific interest to GSK Biologicals

Once a AE of specific interest (pIMD and meningitis) 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. 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 a 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 AE of specific interest causality 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 AE of specific interest information after removal of write access to the subject's eCRF

When additional SAE, pregnancy or AE 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 timeframes specified in Table 23.

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 study vaccine and unexpected. The purpose of the report is to fulfil 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 23).

All SAEs and AE of specific interest (pIMDs and meningitis) (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 last visit of the subject.

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 vaccination/challenge, as applicable.

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

The investigator will follow subjects:

- With SAEs, AE of specific interest (pIMDs or meningitis) (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 does not 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 a SAE/AE of specific interest (pIMDs and meningitis) should be recorded in Expedited Adverse Event Report of 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 during the study duration.

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.
- Unsolicited non-serious AE.
- Solicited AE.
- 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).

10.3. 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 criteria. Reason for screening failure will be collected.

11. STATISTICAL METHODS

11.1. Primary endpoint

- Occurrence of *P. falciparum* parasitemia (defined by a positive blood slide) following sporozoite challenge (in all study groups versus infectivity controls).

11.2. Secondary endpoints

Efficacy

- Time-to-onset of *P. falciparum* parasitemia (defined by a positive blood slide) following sporozoite challenge.

Immunogenicity

- Anti-CS repeat region antibody concentrations at screening, Day 1, prior to challenge (Day 22), 28 days post-challenge (Day 50) and at study end (Day 190).
- Anti-HBs Igtot antibody concentrations at screening, Day 1, prior to challenge (Day 22), 28 days post-challenge (Day 50) and at study end (Day 190).

Safety

- Occurrence of solicited local and general AEs within 7 days after vaccination (day of vaccination and 6 subsequent days) in the booster vaccination groups.
- Occurrence of unsolicited AEs up to 21 days after vaccination (day of vaccination and 20 subsequent days), according to the Medical Dictionary for Regulatory Activities (MedDRA) classification, in the booster vaccination groups.
- Occurrence of AEs up to 29 days after challenge (day of challenge and 28 subsequent days), according to the MedDRA classification, in all study groups.
- Occurrence of AEs of specific interest (potential immune-mediated diseases [pIMDs] and meningitis) from Day 1 up to study conclusion (Day 190), according to the MedDRA classification, in all study groups.
- Occurrence of SAEs (all, fatal, related to investigational vaccine) during the whole study period (from screening up to study conclusion [Day 190]), according to the MedDRA classification, in all study groups.

- Occurrence of abnormal laboratory values at screening, Day 1, Day 8, Day 22, the day of first parasitemia and 28 days post-challenge (Day 50) for the booster vaccination groups; and at screening, Day 22, the day of first parasitemia and 28 days post-challenge (Day 50) for the infectivity control subjects.

11.3. Tertiary endpoints

Efficacy

- Occurrence of *P. falciparum* parasitemia (defined by a positive blood slide) following sporozoite challenge (between study groups).
- Time-to-onset of *P. falciparum* parasitemia (defined by a positive blood slide) following sporozoite challenge (between study groups).
- Occurrence of *P. falciparum* parasitemia (defined by a positive PCR) following sporozoite challenge (between study groups).
- Time-to-onset of *P. falciparum* parasitemia (defined by a positive PCR) following sporozoite challenge (between study groups).

Immunogenicity

- Anti-CS repeat region IgG avidity index at Day 1, prior to challenge (Day 22), 28 days post-challenge (Day 50) and at study end (Day 190).
- Anti-full length CS protein IgG concentrations and anti-C-term IgG concentrations at Day 1, prior to challenge (Day 22), 28 days post-challenge (Day 50) and at study end (Day 190).
- Anti-full length CS protein and anti-C-term IgG avidity at Day 1, prior to challenge (Day 22), 28 days post-challenge (Day 50) and at study end (Day 190).

11.4. Determination of sample size

The target enrollment for the MALARIA-092 study was ~130 vaccinated subjects (~26/vaccine group) and up to 30 subjects (or 4-6 per day, depending on the expected number of days of challenge) in the infectivity control group. Approximately 20 subjects in each group were expected to undergo sporozoite challenge (~100 vaccinated subjects overall). Based on past experience, an approximate 20% will refuse to participate in the booster study and there will be an approximate 20% drop-out rate in vaccinees for the booster study. With an assumed vaccine efficacy (VE) of ~68% in the primary study, there will be a minimum of ~20 subjects per group in this MALARIA-102 study. In addition, up to 24 infectivity control subjects will be newly enrolled for this study.

Assuming at least 19/20 controls and 6/20 vaccinees become positive (VE of ~68%), the trial has 99% power to detect statistically significant vaccine efficacy of each of the vaccination groups over the infectivity controls (p-value < 0.05).

The power to detect a statistically significant VE of each of the vaccination groups over the infectivity controls (p-value < 0.05) is presented in the table below:

Controls (N)	Vaccines (N)	Vaccine efficacy (%)	Protected controls (N)	Protected vaccinees (N)	Power* (%)
20	20	89	1	18	100
20	20	78	1	16	100
20	20	68	1	14	99.1
20	20	58	1	12	94.6
20	20	47	1	10	81.8

*Two independent proportions power analysis - Test statistic: Fisher exact test

11.5. Cohorts for Analyses

11.5.1. Enrolled Set

The Enrolled Set will include all screened subjects who provide informed consent and provide demographic and/or other baseline screening measurements, regardless of the subject's randomization and vaccination status in the trial, and receive a subject ID (for infectivity control subjects).

11.5.2. Intent-to-treat set

The Intent-To-Treat Set (ITTS) will include all subjects from the P-Fx and NP-Fx groups who received the Fx dose of study vaccine. All challenged infectivity controls will also be included and will be presented as a separate study group.

11.5.3. Per-protocol set for analysis of immunogenicity and efficacy

The Per-Protocol Set (PPS) for analysis of immunogenicity and efficacy will include all subjects included in the ITTS who fulfilled all eligibility criteria and received the Fx dose according to protocol procedures, did not use any medication, vaccine or blood products forbidden by the protocol, did not report any underlying medical condition influencing the efficacy response, had available data concerning immunogenicity endpoint measures, and underwent *P. falciparum* challenge. This will include subjects for whom assay results are available for antibodies against at least one study vaccine antigen after Day 1.

11.5.4. Adapted Per-Protocol Set for analysis of efficacy

The Adapted PPS for analysis of efficacy will include all subjects included in the ITTS who fulfilled all eligibility criteria and received the Fx dose according to protocol procedures and underwent *P. falciparum* 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 ≥ 1.9 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 ≥ 10 mIU/mL).
- The percentage of subjects seropositive/seroprotected and associated two-sided 95% Clopper-Pearson confidence intervals (CIs) will be computed by vaccine group for each available immunogenicity monitoring.
- The geometric mean concentrations (GMC) calculations will be performed by taking the anti-log of the mean of the log transformations. Antibody concentrations below the cut-off of the assay will be given an arbitrary value of half the cut-off of the assay 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 (P-Fx and NP-Fx groups) and safety (all subjects):

- Handling of missing data:
  - Subjects who missed reporting symptoms (unsolicited or concomitant medications) will be treated as subjects without symptoms (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 a given subject and the analysis of solicited symptoms within 7 days post-vaccination, missing or non-evaluable measurements will not be replaced. Therefore the analysis of the solicited symptoms based on the ITTS will include only vaccinated subjects for doses with documented safety data (i.e., symptom screen completed). More specifically the following rules will be used:
  - Subjects who documented the absence of a solicited symptom after the Fx dose will be considered not having that symptom after that dose.
  - Subjects who documented the presence of a solicited symptom after the Fx booster dose and fully or partially recorded daily measurement over the solicited period will be included in the summaries at the Fx dose and classified according to their maximum observed daily recording over the solicited period.

  - Subjects who documented the presence of a solicited symptom after the Fx dose without having recorded any daily measurement will be assigned to the lowest intensity category at that dose (i.e., 37.5°C for fever or grade 1 for other symptoms).
  - Fx dose without symptom sheets documented will be excluded.

- For analysis of unsolicited AEs, such as SAEs or AEs by primary MedDRA term, and for the analysis of concomitant medications, all subjects, as applicable, will be considered.

11.7. Analysis of demographics

A study flow table (consort) will be generated to present the number of subjects screened, randomized, receiving the Fx dose and included in the PPS for analyses of immunogenicity and efficacy.

The analysis of demographics will be performed on the ITTS and on the PPS for analysis of immunogenicity and efficacy.

Demographic characteristics (age at booster vaccination in years for the vaccinated groups and at the time of the screening for the infectivity control group, gender, and race) will be summarized by group and overall using descriptive statistics:

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

Previous study group (i.e., in the MALARIA-092 study) will be tabulated.

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 PPS for analyses of immunogenicity and efficacy will be tabulated.
- The number of withdrawn subjects will be tabulated according to the reason for withdrawal.

11.8. Analysis of exposure

The number and percentage of subjects with vaccination will be summarized overall and by group for all study groups where a vaccination is part of the study procedure. The analysis of exposure will be performed on the ITTS.

11.9. Analysis of efficacy

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

Efficacy will be assessed by comparison of *P. falciparum* parasitemia incidence and time-to-onset of *P. falciparum* parasitemia after sporozoite challenge. The analysis of the primary objective will based on blood smears results; additional exploratory analyses will be performed based PCR results (tertiary objectives).

Vaccine efficacy (VE) 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 incidence after challenge between each one of the study groups (P-Fx and NP-Fx) and the infectivity control group.

Kaplan-Meier analysis will be performed on time-to-onset of *P. falciparum* parasitemia, for comparisons between each one of the study groups (P-Fx and NP-Fx) and the infectivity control group, using the log-rank statistic.

The same methodology will be applied for the evaluation of the study groups (P-Fx vs. NP-Fx) versus each other. If there are sufficient evaluable subjects to draw some conclusions, descriptive analyses based on initial group assignment versus control will be performed.

All statistical tests will be two-tailed at 5% significance level.

Risk periods

For the analyses of time-to-onset of parasitemia (Kaplan-Meier and log-rank), time-at-risk will start on first day of challenge. Time-at-risk will be censored on Visit 22 (Day 50; 28 days post-challenge), drop-out date, start date of anti-malarial treatment or date meeting an endpoint, whichever occurs first. Time-at-risk will be calculated as: censor date – date challenge + 1.

For the analysis of proportion affected (relative risk), all subjects included in the PPS for analysis of immunogenicity and efficacy will be considered at risk of infection and no censoring or elimination will be applied for subjects not completing the entire protocol defined post-challenge follow-up (Day 50; 28 days post challenge).

11.10. Analysis of immunogenicity

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

For each group, at each timepoint where a blood sample is collected:

- The percentage of subjects with seropositive levels of anti-CS (proportion of subjects with anti-CS antibody concentrations ≥ 1.9 EU/mL) with 95% CI will be determined at specified blood sampling timepoints. Anti-CS antibody concentrations will be summarized by GMC. Anti-CS antibody concentrations will be displayed using reverse cumulative curves (RCCs). Similar analysis will be performed for the anti-full length CS protein (proportion of subjects with anti-CS antibody titers ≥ 200 1/DIL) and anti-C-term antibodies (proportion of subjects with anti-CS antibody concentrations ≥ 100 Endpoint Titer).
- 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. Anti-HBs antibody concentrations will be summarized by GMC. Anti-HBs antibody concentrations will be displayed using RCCs.
- Anti-CS, anti-full length CS protein and anti-CS avidity index will be summarized by mean, SD, median and quartile and box and whiskers plots will be generated.

11.11. Analysis of safety

The analysis of safety will be performed on the ITTS.

For the P-Fx and NP-Fx groups:

- 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 21-day follow-up period after the booster Fx dose will be tabulated with exact 95% CI. 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 each individual solicited general AE (any grade, grade 3, any related, grade 3 related, resulting in medically attended visit) during the 7-day follow-up period (Days 1-7) after the booster Fx dose will be tabulated for each group, with exact 95% CI.
- For fever (defined as temperature ≥ 37.5°C/99.5°F for oral, axillary or tympanic route), the number and percentage of subjects reporting fever by half degree (°C) cumulative increments during the first seven days (Days 1-7) after the booster Fx dose will be tabulated. Similar tabulations will be performed for any fever with a causal relationship to vaccination and grade 3 (> 39.0°C) causally related fever. The maximum temperature reported over the 7-day follow-up period will be tabulated.

- The percentage of subjects reporting unsolicited AEs within 21 days (Days 1-21) after the booster Fx dose 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 using concomitant medication (any medication, any antipyretic and any prophylactic medication, respectively) during the 7-day follow-up period after vaccination will be summarized by group.

For all subjects:

- The percentage of subjects reporting AEs during 29 days after the sporozoite challenge (Day 22-Day 50) 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 AEs of specific interest (meningitis and pIMDs) from Day 1 until last study visit (Day 190) (for P-Fx and NP-Fx groups)/from the day of challenge until last study visit (Day 50) (for infectivity control group), classified by MedDRA preferred term level, will be tabulated with exact 95% CI.
- The percentage of subjects reporting SAEs (all, fatal, related) occurring from Day 1 until last study visit (Day 190) (for P-Fx and NP-Fx groups)/from the day of challenge until last study visit (Day 50) (for infectivity control group), classified by the MedDRA preferred term level will be tabulated with exact 95% CI.
- Subjects reporting pregnancies will be described.
- The percentage of subjects reporting an AE or SAE leading to withdrawal from study from Day 1 until last study visit (Day 190) (for P-Fx and NP-Fx groups)/from the day of challenge (for infectivity control group) until last study visit (Day 50), classified by MedDRA preferred term level, will be tabulated with exact 95% CI.
- The percentage of subjects using concomitant medication during the entire sporozoite challenge (Days 22-50) will be summarized by group.
- Biochemistry (ALT, AST and creatinine) and hematological (hemoglobin, WBC and platelets) laboratory values will be presented by visit according to toxicity grading scales and tabulated by group. The normal ranges and toxicity grading for laboratory safety parameters used in this study are presented in Table 22.

11.12. Interpretation of analyses

All analyses performed during this study will be descriptive. The inferential analyses will not be considered confirmatory but rather used as an estimation of the probability that an effect is true.

11.13. 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.

11.13.1. Sequence of analyses

The analyses will be performed stepwise:

- An interim analysis will be performed as soon as the results of the challenge phase will be available on data as clean as possible, including safety and immunogenicity results, if available. This analysis will be descriptive and performed on the Adapted PPS for analysis of efficacy. Results will be used to make a decision on future field trials.
- When all the remaining data up to Day 190 will be available, the main analysis will be performed. An integrated clinical study report containing all data will be written and made available to the investigators.

If the data for exploratory assessments become available at a later stage, (an) additional analysis/analyses will be performed and results will be reported in a publication, if applicable.

11.13.2. Statistical considerations for interim analyses

A descriptive analysis to evaluate vaccine efficacy against infection of *P. falciparum* will be performed on the Adapted PPS for analysis of efficacy at the time of the interim analysis. Note that the main objective of the study linked to this endpoint will be analysed at the end of the study, on the PPS for analysis of immunogenicity and efficacy and can output different results.

12. ADMINISTRATIVE MATTERS

To comply with ICH GCP administrative obligations relating to data collection, monitoring, archiving data, audits, confidentiality, public disclosure requirements 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 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 other items, 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 a 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 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.

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. 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, otherwise, the minimum retention period will default to 25 years after completion of the study report.

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

GSK assures that the key design elements of this protocol will be posted on the GSK website and in publicly accessible database(s) such as clinicaltrials.gov, in compliance with the current regulations.

GSK also assures that results of this study will be posted on the GSK website and in publicly accessible regulatory registry(ies) within the required timeframe, in compliance with the current regulations. The minimal requirement is to have primary endpoint summary results disclosed at latest 12 months post-PCD and to have secondary endpoint disclosed at latest 12 months after the last subject last visit (LSLV) as described in the protocol.

GSK also aims to publish the results of these studies in searchable, peer-reviewed scientific literature and follows the guidance from the International Committee of Medical Journal Editors.

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.

12.7. Data Sharing

Under the framework of the SHARE initiative, results of GSK studies may be combined with non-GSK studies, to investigate further about the study product(s) and other product(s), and/or the disease/condition under investigation and related diseases and conditions.

13. COUNTRY SPECIFIC REQUIREMENTS

Not applicable.

14. REFERENCES

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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: 25 April 2018)

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FDA guidance for industry: toxicity grading scale for healthy adult and adolescent volunteers enrolled in preventive vaccine clinical trials (September 2007). *https://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Vaccines/UCM091977.pdf* (last access: 25 April 2018).

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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, 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 1.9 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.

Antibody titers and subclasses against full-length CS and the C-term of CS

Standardized procedures established in the Clinical Immunology Laboratory at WRAIR to measure IgG antibodies against the full-length recombinant CS protein and the recombinant C-term of CS protein will be performed according to Standard Operating Procedures (SOPs) from the laboratory. Results are expressed in endpoint titer and the cut-off is set at 100.

Antibody IgG1, IgG2, IgG3 and IgG4 subclasses against the full-length recombinant CS protein and the recombinant C-term of CS protein will be performed using a Luminex assay according to SOPs from the laboratory. Results are expressed in Mean Fluorescent Intensity and the cut-off is set at 50.

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.

APPENDIX B CLINICAL LABORATORIES

Table 24 GSK Biologicals' laboratories

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

Table 25 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
Q^2 Solutions Clinical Trials (US)	27027 Tourney Road, Suite 2E Valencia, CA 91355 United States

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 (ECG)

12-lead ECG taken? Y / N

If not, reason_____

Electrocardiogram interpreted by:

_____

Electrocardiogram interpretation:

_____

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

Table A (for Males)

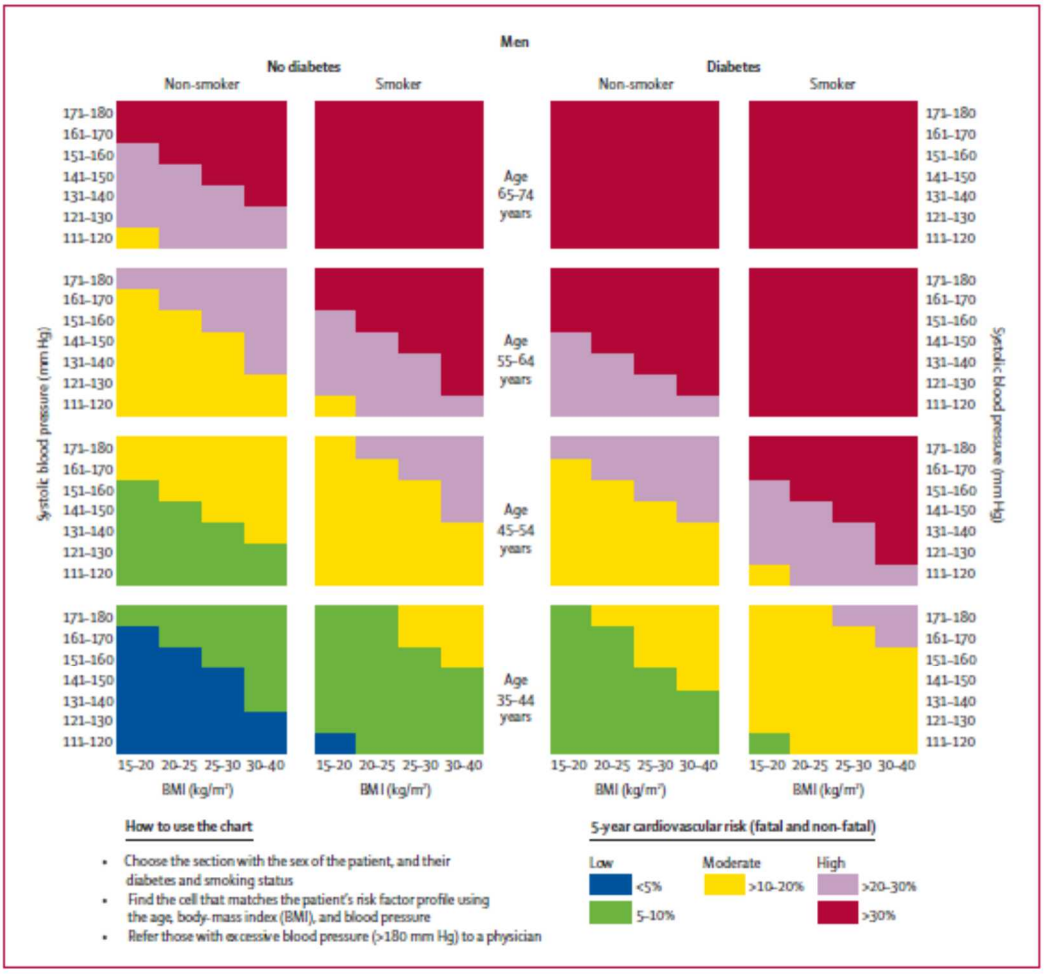

Figure 5: Risk prediction chart for cardiovascular disease using non-laboratory-based measures (men)

Note: Subjects aged between 18 and 35 years will be assessed as if they are 35 (the minimum age covered by the NHANES criteria/tables). While this process likely over-estimates (minimally) the cardiac risk of these young subjects, the only associated effect would be eliminating a healthy volunteer than including a cardiac risk.

Table B (for Females)

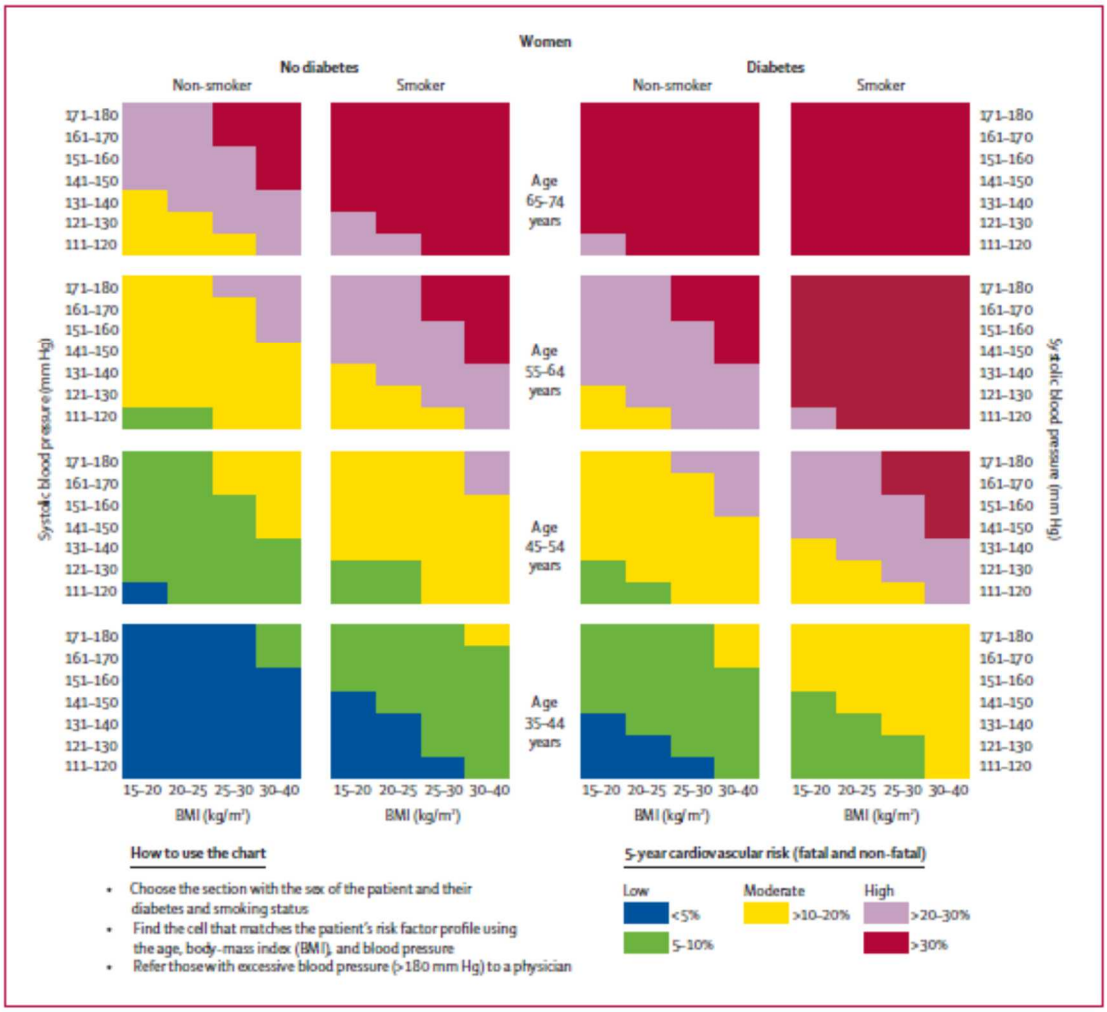

Figure 4: Risk prediction chart for cardiovascular disease using non-laboratory-based measures (women)

Note: Subjects aged between 18 and 35 years will be assessed as if they are 35 (the minimum age covered by the NHANES criteria/tables). While this process likely over-estimates (minimally) the cardiac risk of these young subjects, the only associated effect would be eliminating a healthy volunteer than including a cardiac risk.