Statistical Analysis Plan Amendment 2

Study ID: 204889

Official Title of Study: Efficacy, Safety and Immunogenicity Study of GSK Biologicals' Candidate Malaria Vaccine (SB257049) Evaluating Schedules With or With-out Fractional Doses, Early Dose 4 and Yearly Doses, in Children 5-17 Months of Age

NCTID: NCT03276962

Date of Document: 1 October 2020

GlaxoSmithKline	**Statistical Analysis Plan**
Detailed Title:	Phase IIb randomized, open-label, controlled, multi-center study of the efficacy, safety and immunogenicity of GSK Biologicals' candidate malaria vaccine RTS,S/AS01$_E$ evaluating schedules with or without fractional doses, early Dose 4 and yearly doses, in children 5-17 months of age living in sub-Saharan Africa
eTrack study number and Abbreviated Title	204889 (MALARIA-094)
Scope:	All data pertaining to the above study.
Date of Statistical Analysis Plan	Final: 24 September 2020

APP 9000058193 Statistical Analysis Plan Template V4 (Effective date: 3 June 2019)

TABLE OF CONTENTS

PAGE

LIST OF TABLES

PAGE

LIST OF FIGURES

PAGE

LIST OF ABBREVIATIONS

ADI	Active detection of infection
AE	Adverse event
ALT	Alanine aminotransferase
Anti-CS	Antibody to the *Plasmodium falciparum* circumsporozoite (CS) repeat domain
Anti-HBs	Antibody to the hepatitis B surface antigen
$AS01_E$	GSK's proprietary Adjuvant System containing MPL, QS-21 Stimulon® and liposome (25 μg MPL and 25 μg QS-21 Stimulon®)
BMI	Body mass index
CDISC	Clinical Data Interchange Standards
CI	Confidence Interval
CRF/eCRF	Case Report Form/electronic Case Report Form
CS	Circumsporozoite protein of *Plasmodium falciparum*
CTRS	Clinical Trial Registry Summary
EU/ml	ELISA unit per milliliter
ELISA	Enzyme-linked immunosorbent assay
ES	Exposed Set
Fx	Fractional
eTMF	Electronic Trial Master File
GMC	Geometric mean concentration
GMT	Geometric mean titer
GSK	GlaxoSmithKline
HR	Hazard ratio
ICH	International Committee on Harmonization
IDMC	Independent Data Monitoring Committee
ITN	Insecticide-treated bednet
IU/ml	International units per milliliter
LAR	Legally acceptable representative
MedDRA	Medical Dictionary for Regulatory Activities

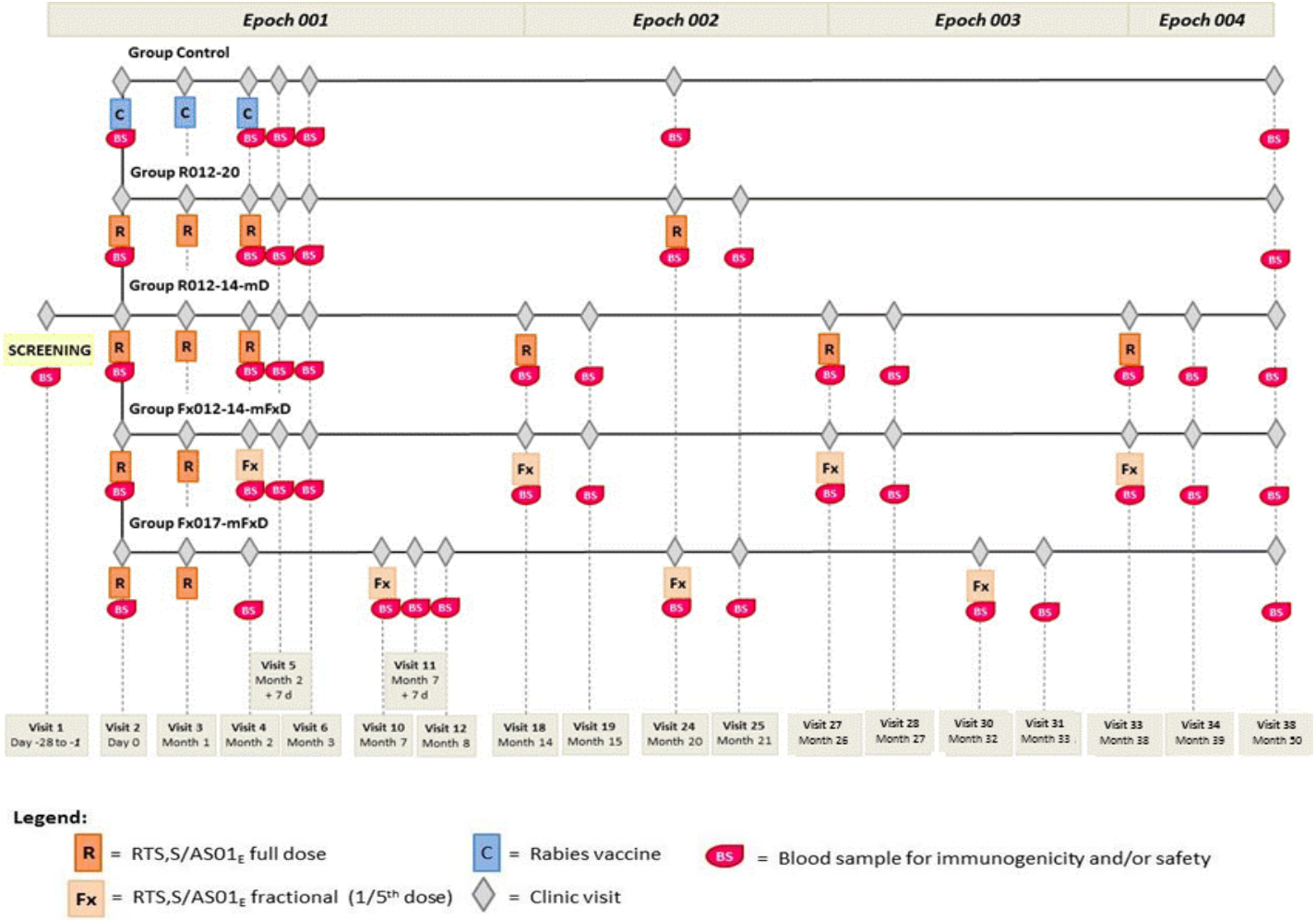
PCD	Passive case detection
PCR	Polymerase chain reaction
pIMD	Potential immune-mediated disease
PPS	Per Protocol Set
RCD	Reverse cumulative distribution
RTS,S	Particulate antigen, containing both RTS and S (hepatitis B surface antigen) proteins
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SAS	Statistical analysis system
SBIR	GSK Biological's Internet Randomization System
SD	Standard Deviation
SHS	Study Headline Summary
SR	Study Report
SDTM	Study Data Tabulation Model
TFL	Tables Figures and Listings
TOC	Table of Content
ULN	Upper limit of normal
WBC	White blood cells

1. DOCUMENT HISTORY

Date	Description	Protocol Version
04 SEP 2019	First Version	Amendment 2 Final : 06 APR 2017
24 SEPT 2020	second Version: - update of the follow-up period up to M21 and not M20, up to M33 and not M32 - for the elimination codes: when the same elimination code is given for immunogenicity and efficacy. For some of these elimination codes which are for immunogeniticty Per Protocol (PP) set and efficacy PP set, for the immunogenicity PP, all the doses have to be considered but for efficacy PP set, only the three first doses have to be considered. Therefor for efficacy PP set in these cases, a new elimination code has been given	Amendment 2 Final : 06 APR 2017
01 OCT 2020	Third Version: - for the elimination codes for immunogencity: for the dynamic allocation: o the elimination code 2100 (which is all serological result not available post-vaccination) is eliminated from the list of elimination codes involved in the dynamic allocation o the elimination code 2080 (which is a vaccination out of schedule) goes from visit-specific elimination to elimination of all the visits posterior to the dose affected.	Amendment 2 Final : 06 APR 2017

2. STUDY DESIGN

Figure 1 Study design: clinic visits



In addition to the clinic visits presented in the figure above, parasite prevalence will be assessed at the following visits (these will be fieldworker visits or clinic visits depending on the timepoint and the group):

Figure 2 Study design: visits for assessment of parasite prevalence

Visits for parasite prevalence													
Year 1	Visit 2 Day 0	Visit 3 Month 1	Visit 4 Month 2	Visit 6 Month 3	Visit 7 Month 4	Visit 8 Month 5	Visit 9 Month 6	Visit 10 Month 7	Visit 12 Month 8	Visit 13 Month 9	Visit 14 Month 10	Visit 15 Month 11	Visit 16 Month 12
Year 2		Visit 17 Month 13	Visit 18 Month 14	Visit 19 Month 15	Visit 20 Month 16	Visit 21 Month 17	Visit 22 Month 18	Visit 23 Month 19	Visit 24 Month 20			Visit 26 Month 23	
Year 3			Visit 27 Month 26			Visit 29 Month 29			Visit 30 Month 32			Visit 32 Month 35	
Year 4			Visit 33 Month 38			Visit 35 Month 41			Visit 36 Month 44			Visit 37 Month 47	
Year 5			Visit 38 Month 50										

- **Experimental design:** Phase IIB, randomized, open-label, controlled, multi-centric, study with five parallel groups.
- **Randomization:** minimization method accounting for center was used.
- **Study groups:** 5 study groups are defined for the study, each consisting of 300 subjects (aged 5-17 months).
- **Treatment allocation:** randomized.
- **Blinding:** open.
- **Efficacy surveillance:**
  - Capture of malaria cases: malaria cases will be captured by passive case detection (PCD). Parents/LARs will be advised to present their child to the health center if the child is unwell. Care will be administered according to local recommendations. All children with fever and/or history of fever will have a blood sample taken for the evaluation of vaccine efficacy (blood slide and filter paper for polymerase chain reaction [PCR]). All anti-malaria treatments administered during the study should be recorded in the subject's eCRF.
  - Cross-sectional surveys: to evaluate the vaccine efficacy against incident and prevalent *P. falciparum* infections, monthly cross-sectional surveys will be performed from Day 0 until Month 20 to measure *P. falciparum* parasitemia. Thereafter until study end (Month 50) parasitemia will be measured every three months. Blood slides from cross-sectional surveys will not be read in real time.
  - The research team at each study center will provide all screened subjects with an insecticide-treated bednet (ITN), and malaria diagnosis and treatment will be ensured.

- **Sampling schedule:**

Blood sampling timepoints

		Visit 1	Visit 2	Visit 4	Visit 5	Visit 6	Visit 10	Visit 11	Visit 12	Visit 18	Visit 19	Visit 24	Visit 25	Visit 27	Visit 28	Visit 30	Visit 31	Visit 33	Visit 34	Visit 38
Study group	**Type of blood sampling**	**Scr**	**D0**	**M2**	**M2 + 7d**	**M3**	**M7**	**M7 + 7d**	**M8**	**M14**	**M15**	**M20**	**M21**	**M26**	**M27**	**M32**	**M33**	**M38**	**M39**	**M50**
R012-20	Safety	x*		x**	x**	x**														
	Immunogenicity§		x	x		x						x	x							x
R012-14	Safety	x*		x**	x**	x**														
	Immunogenicity§		x	x		x				x	x			x	x			x	x	x
Fx012-14	Safety	x*		x**	x**	x**														
	Immunogenicity§		x	x		x				x	x			x	x			x	x	x
Fx017-20	Safety	x*					x**	x**	x**											
	Immunogenicity§		x	x			x		x			x	x			x	x			x
Control	Safety	x*		x**	x**	x**														
	Immunogenicity§		x	x		x						x								x

* At screening, hemoglobin will be assessed in all subjects screened.
** Blood samples for hematology and biochemistry will be taken in a sub-cohort consisting of the first 50 subjects enrolled in each group.
§ Blood samples for immunogenicity will be taken in all subjects enrolled.
Scr: Screening
M: Month
D: Day

- **Safety monitoring:**

Study visits		1	2			3			4			10			18			24			27			30			33			38
Event		Scr*	Vac 1			Vac 2			Vac 3			Vac 4			Vac 5			Vac 6			Vac 7			Vac 8			Vac 9			Study end
Study timepoint:		D -28 to -1	D0	D0 + 3d	D0 + 29d	M1	M1 + 3d	M1 + 29d	M2	M2 + 3d	M2 + 29d	M7	M7 + 3d	M7 + 29d	M14	M14 + 3d	M14 + 29d	M20	M20 + 3d	M20 + 29d	M26	M26 + 3d	M26 + 29d	M32	M32 + 3d	M32 + 29d	M38	M38 + 3d	M38 + 29d	M50
For ALL groups																														
AEs/SAEs leading to withdrawal from further vaccination			■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
SAEs related to study participation or concurrent GSK medication/vaccine	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
SAEs (All, fatal, related to the investigational vaccine)			■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
AEs of specific interest**			■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Group R012-20																														
Solicited local and general AEs***									■	■								■	■											
Unsolicited AEs			■	■	■	■	■	■	■	■	■							■	■	■										
Group R012-14 and Fx012-14																														
Solicited local and general AEs***									■	■					■	■					■	■					■	■		

Study visits		1	2			3			4			10			18			24			27			30			33			38
Event		Scr*	Vac 1			Vac 2			Vac 3			Vac 4			Vac 5			Vac 6			Vac 7			Vac 8			Vac 9			Study end
Study timepoint:		D -28 to -1	D0	D0 + 3d	D0 + 29d	M1	M1 + 3d	M1 + 29d	M2	M2 + 3d	M2 + 29d	M7	M7 + 3d	M7 + 29d	M14	M14 + 3d	M14 + 29d	M20	M20 + 3d	M20 + 29d	M26	M26 + 3d	M26 + 29d	M32	M32 + 3d	M32 + 29d	M38	M38 + 3d	M38 + 29d	M50
Unsolicited AEs																														
Group Fx017-20																														
Solicited local and general AEs***																														
Unsolicited AEs																														
Control group																														
Solicited local and general AEs***																														
Unsolicited AEs																														

* i.e. consent obtained.
** AEs of specific interest include seizures occurring within 30 days post-vaccination, meningitis and pIMDs.
*** Solicited local and general AEs will be collected in the first 50 subjects enrolled in each group (reactogenicity sub-cohort).
Scr: Screening; Vac: Vaccination; D: Day; M: Month; 3d: 3 days; 29d: 29 days.

- Intervals between study visits

Interval	Optimal length of interval [1]	Allowed interval[2]
Visit 1→Visit 2 (Vacc)	0 to 28 days	0 - 28 days
Visit 2→Visit 3 (Vacc)	1 month	Minimum 21 days (3 weeks) - Maximum 35 days (5 weeks)
Visit 3→Visit 4 (Vacc)	1 month	Minimum 21 days (3 weeks) - Maximum 35 days (5 weeks)
Visit 4→Visit 5	7 days	Minimum 6 days - Maximum 8 days
Visit 4→Visit 6	1 month	Minimum 21 days (3 weeks) - Maximum 35 days (5 weeks)
Visit 6→Visit 7	1 month	Minimum 21 days (3 weeks) - Maximum 35 days (5 weeks)
Visit 7→Visit 8	1 month	Minimum 21 days (3 weeks) - Maximum 35 days (5 weeks)
Visit 8→Visit 9	1 month	Minimum 21 days (3 weeks) - Maximum 35 days (5 weeks)
Visit 3→Visit 10 (Vacc)	6 months	Minimum 161 days (23 weeks) - Maximum 203 days (29 weeks)
Visit 10→Visit 11	7 days	Minimum 6 days - Maximum 8 days
Visit 10→Visit 12	1 month	Minimum 21 days (3 weeks) - Maximum 35 days (5 weeks)
Visit 12→Visit 13	1 month	Minimum 21 days (3 weeks) - Maximum 35 days (5 weeks)
Visit 13→Visit 14	1 month	Minimum 21 days (3 weeks) - Maximum 35 days (5 weeks)
Visit 14→Visit 15	1 month	Minimum 21 days (3 weeks) - Maximum 35 days (5 weeks)
Visit 15→Visit 16	1 month	Minimum 21 days (3 weeks) - Maximum 35 days (5 weeks)
Visit 16→Visit 17	1 month	Minimum 21 days (3 weeks) - Maximum 35 days (5 weeks)
Visit 4→Visit 18 (Vacc)	12 months	Minimum 336 days (48 weeks) - Maximum 392 days (56 weeks)
Visit 18→Visit 19	1 month	Minimum 21 days (3 weeks) - Maximum 35 days (5 weeks)
Visit 19→Visit 20	1 month	Minimum 21 days (3 weeks) - Maximum 35 days (5 weeks)
Visit 20→Visit 21	1 month	Minimum 21 days (3 weeks) - Maximum 35 days (5 weeks)
Visit 21→Visit 22	1 month	Minimum 21 days (3 weeks) - Maximum 35 days (5 weeks)
Visit 22→Visit 23	1 month	Minimum 21 days (3 weeks) - Maximum 35 days (5 weeks)
Visit 4→Visit 24 (Vacc)*	18 months	Minimum 518 days (74 weeks) - Maximum 574 days (82 weeks)
Visit 10→Visit 24 (Vacc)**	13 months	Minimum 364 days (52 weeks) - Maximum420 days (60 weeks)
Visit 24→Visit 25	1 month	Minimum 21 days (3 weeks) - Maximum 35 days (5 weeks)
Visit 2***4*** → Visit 26	***3*** months	Minimum ***77*** days (***11*** weeks) **-** Maximum ***105*** days (***15*** weeks)
Visit 18→Visit 2***7*** (Vacc)	12 months	Minimum 336 days (48 weeks) - Maximum 392 days (56 weeks)
Visit 2***7***→Visit 2***8***	1 month	Minimum 21 days (3 weeks) - Maximum 35 days (5 weeks)
Visit 2***7***→Visit ***29***	3 months	Minimum 77 days (11 weeks) - Maximum 105 days (15 weeks)
Visit 24→Visit 3***0*** (Vacc)	12 months	Minimum 336 days (48 weeks) - Maximum 392 days (56 weeks)
Visit 3***0***→Visit 3***1***	1 month	Minimum 21 days (3 weeks) - Maximum 35 days (5 weeks)
Visit 3***0***→Visit 3***2***	3 months	Minimum 77 days (11 weeks) - Maximum 105 days (15 weeks)
Visit 2***7***→Visit 3***3*** (Vacc)	12 months	Minimum 336 days (48 weeks) - Maximum 392 days (56 weeks)
Visit 3***3***→Visit 3***4***	1 month	Minimum 21 days (3 weeks) - Maximum 35 days (5 weeks)
Visit 3***3***→Visit 3***5***	3 months	Minimum 77 days (11 weeks) - Maximum 105 days (15 weeks)
Visit 3***5***→Visit 3***6***	3 months	Minimum 77 days (11 weeks) - Maximum 105 days (15 weeks)
Visit 3***6***→Visit 3***7***	3 months	Minimum 77 days (11 weeks) - Maximum 105 days (15 weeks)
Visit 3***7***→Visit 3***8***	3 months	Minimum 77 days (11 weeks) - Maximum 105 days (15 weeks)

[1].Whenever possible the investigator should arrange study visits within this interval.

[2].Subjects will not be eligible for inclusion in the according-to-protocol (ATP) cohorts if they make the study visit outside this interval: to be included in the ATP cohort for efficacy, subjects should receive all vaccinations within the protocol specified intervals; to be included in the ATP cohort for immunogenicity, subjects should receive all vaccinations within the protocol specified intervals and perform all blood samplings for immunogenicity within the protocol specified intervals.

* Interval between Visit 4 and Visit 24 is for groups Control and R012-20.

** Interval between Visit 10 and Visit 24 is for group Fx017-20.

Vacc: vaccination in at least one group.

3. OBJECTIVES

3.1. Primary objective

- **Efficacy: Incremental efficacy of a schedule with a fractional third dose at Month 2 over the standard schedule**
  - To demonstrate the superiority of a 3-dose schedule of GSK Biologicals' malaria vaccine RTS,S/AS01$_E$ with a fractional third dose at Month 2 compared to a standard schedule of RTS,S/AS01$_E$ with three full doses in terms of vaccine efficacy against clinical malaria (primary case definition) over 12 months post-Dose 3.

Criterion:

The lower limit of the 95% confidence interval (CI) of the incremental vaccine efficacy estimate is above 0.

Refer to Section 5.1 for the definition of the primary endpoint.

3.2. Secondary objectives

- **Efficacy**
  - To assess the incremental vaccine efficacy against clinical malaria of a schedule with a fractional third dose at Month 2 versus a schedule with 3 full doses (primary and secondary case definitions) over 12 months post-Dose 3.
  - To assess the incremental vaccine efficacy against clinical malaria, over 7 and 12 months post-Dose 3, of a schedule with a fractional third dose at Month 7 versus a schedule with a fractional third dose at Month 2.
  - To assess the incremental vaccine efficacy against clinical malaria, over 7 and 12 months post-Dose 3, of a schedule with a fractional third dose at Month 7 versus a schedule with 3 full doses.
  - To assess the incremental vaccine efficacy against clinical malaria, over 12 months post-Dose 4, post-Dose 5 and post-Dose 6 of a schedule with a fractional third dose at Month 2 and yearly fractional doses versus a schedule with full doses at Month 0, Month 1, Month 2 and yearly full doses.
  - To assess the incremental vaccine efficacy against clinical malaria, over 12 months post-Dose 4, of a schedule with a fractional third dose at Month 7 versus a standard schedule with 4 full doses at 0,1,2,20 months.
  - To assess the vaccine efficacy and impact of each RTS,S/AS01$_E$ schedule over the entire study period by measuring the efficacy against clinical malaria at Months 14, 20, 26, 32, 38 and 50.
  - To assess the prevalence of *P. falciparum* infections of each RTS,S/AS01$_E$ schedule at cross-sectional visits (monthly from Month 0-20 and every three months thereafter till study end).

- To assess the vaccine efficacy against incident *P. falciparum* infections defined by positive blood slide over the entire study period.

- **Immunogenicity: Immune response to the CS and HBs antigens**
  - To describe the antibody response to the anti-circumsporozoite protein of *P. falciparum* (anti-CS) for each schedule.
  - To describe the antibody response to the hepatitis B surface antigen (anti-HBs) for each schedule.

- **Safety and reactogenicity**
  - To assess the safety of RTS,S/AS01$_E$ for each schedule in terms of serious adverse events (SAEs), unsolicited adverse events (AEs) and AEs of specific interest.
  - To assess the reactogenicity of RTS,S/AS01$_E$ in terms of solicited local and general AEs.
  - To assess the safety of RTS,S/AS01$_E$ in terms of biochemistry (alanine aminotransferase [ALT], creatinine) and hematology (hemoglobin, white blood cells [WBC], platelets) parameters.

Refer to Section 5.2 for the definition of the secondary endpoints.

3.3. Tertiary objectives

- **Immunogenicity**
  - To describe the anti-CS antibody response in terms of avidity.

Of note, blood samples taken in this study may also be used in ancillary studies. More details on ancillary studies are provided in the study design section of the protocol.

Refer to Section 5.3 for the definition of the tertiary endpoints**.**

4. CASE DEFINITIONS

Case definitions of Clinical Malaria

Primary case definition	*P. falciparum* asexual parasitemia > 5000 parasites/µl **AND** presence of fever (axillary temperature ≥ 37.5°C) at the time of presentation **AND** occurring in a child who is unwell and brought for treatment to a healthcare facility
Secondary case definition	*P. falciparum* asexual parasitemia > 0 **AND** presence of fever (axillary temperature ≥ 37.5°C) at the time of presentation or history of fever within 24 hours of presentation **AND** occurring in a child who is unwell and brought for treatment to a healthcare facility

Case definition of incident *P. falciparum* infection

Case definition 1	A documented *P. falciparum* asexual parasite density > 0 detected by blood slide reading at a cross-sectional survey or as captured by the secondary case definition of clinical malaria (active ***detection of infection*** and passive ***case*** detection).

Case definition of prevalent *P. falciparum* infection

Case definition 1	A documented *P. falciparum* asexual parasite density > 0 detected by blood slide reading at a cross-sectional survey (active detection of infection [ADI]).

Table 1 Case definition of severe *P. falciparum* malaria

Adapted from [WHO, 2015].

P. falciparum parasitemia > 0 detected by microscopy and/or rapid diagnostic test (RDT) **AND** one or more of the following, occurring in the absence of an identified alternative cause:
• Impaired consciousness: a Glasgow coma score < 11 in children two years of age or older (≥ 2 years) or a Blantyre coma score < 3 in children less than two years of age (< 2 years); • Prostration: generalized weakness so that the person is unable to sit, stand or walk without assistance; • Multiple convulsions: more than two episodes within 24h; • Acidosis: a base deficit of > 8 mEq/l or, if not available, a plasma bicarbonate level of < 15 mmol/l or venous plasma lactate ≥ 5 mmol/l. Severe acidosis manifests clinically as respiratory distress (rapid, deep, labored breathing). • Hypoglycemia: blood or plasma glucose < 2.2 mmol/l (< 40 mg/dl); • Severe malarial anemia: hemoglobin concentration ≤ 5 g/dl or a hematocrit of ≤ 15% in children < 12 years of age with a parasite count > 10 000/µl; • Renal impairment: plasma or serum creatinine > 265 µmol/l (3 mg/dl) or blood urea > 20 mmol/l; • Jaundice: plasma or serum bilirubin > 50 µmol/l (3 mg/dl) with a parasite count > 100 000/µl; • Pulmonary edema: radiologically confirmed or oxygen saturation < 92% on room air with a respiratory rate > 30/min, often with chest indrawing and crepitations on auscultation; • Significant bleeding: including recurrent or prolonged bleeding from the nose, gums or venipuncture sites; hematemesis or melaena; • Shock: compensated shock is defined as capillary refill ≥ 3 s or temperature gradient on leg (mid to proximal limb), but no hypotension. Decompensated shock is defined as systolic blood pressure < 70 mm Hg in children, with evidence of impaired perfusion (cool peripheries or prolonged capillary refill); • Hyperparasitemia: *P. falciparum* parasitemia > 10% (i.e. percentage of infected red blood cells > 10%; corresponding to > 500 000/µl).

Table 2 Case definition of cerebral *P. falciparum* malaria

Adapted from [WHO, 2015].

Severe *P. falciparum* malaria with coma (Glasgow coma score < 11 in children two years of age or older [≥ 2 years] or Blantyre coma score < 3 in children less than two years of age [(< 2 years]); **AND** If malaria with seizure: coma persisting for > 30 min after the seizure. Other treatable causes of coma should be excluded before diagnosing cerebral malaria (e.g. hypoglycaemia, bacterial meningitis).

5. ENDPOINTS

5.1. Primary endpoint

- **Efficacy:**
  - The occurrence of clinical malaria meeting the primary case definition from Month 2.5 up to Month 14.

5.2. Secondary endpoints

- **Efficacy:**
  - The occurrence of clinical malaria meeting the primary and secondary case definitions from Day 0 up to Month 50.
  - The occurrence of incident *P. falciparum* infections from Day 0 to Month 50.
  - The prevalence of *P. falciparum* infections defined by positive blood slide at each cross-sectional survey.
- **Immunogenicity**: Immune response to the CS and HBs antigens (immunogenicity subset)
  - Anti-HBs antibody concentrations, seropositivity and seroprotection measured before Dose 1, one month post-Dose 2 (group Fx017-20 only), before and one month post-Dose 3, before and one month after Dose 4, before and one month after each yearly dose and at study end (Month 50).
  - Anti-CS antibody concentrations and seropositivity measured before Dose 1, one month post-Dose 2 (group Fx017-20 only), before and one month post-Dose 3, before and one month after Dose 4, before and one month after each yearly dose and at study end (Month 50).
- **Safety**
  - The occurrence of SAEs (all, fatal and related) during the whole study period according to the Medical Dictionary for Regulatory Activities (MedDRA) classification.

- The occurrence of AEs and SAEs leading to withdrawal from further vaccination from Dose 1 (Day 0) up to Month 50, according to the MedDRA classification.
- The occurrence of severe malaria cases and cerebral malaria cases during the whole study period.
- The occurrence of potential immune-mediated diseases (pIMDs) from Day 0 up to Month 50, according to the MedDRA classification.
- The occurrence of meningitis from Day 0 up to Month 50, according to the MedDRA classification.
- The occurrence of seizures within 30 days (day of vaccination and 29 subsequent days) after each dose of study vaccine, according to the MedDRA classification.
- The occurrence of generalized convulsive seizure within seven days (day of vaccination and six subsequent days) after each dose of study vaccine, according to the Brighton collaboration guidelines [Bonhoeffer, 2004].
- The occurrence of unsolicited AEs within 30 days (day of vaccination and 29 subsequent days) after each dose of study vaccine, according to the MedDRA classification.
- The concentration of biochemistry (ALT, creatinine) and hematology (hemoglobin, WBC, platelets) parameters in the first 50 subjects (25 subjects per site) enrolled in each group (reactogenicity sub-cohort) before Dose 3, seven days post-Dose 3 and 30 days post-Dose 3.

- **Reactogenicity** (reactogenicity sub-cohort)
  - The occurrence of solicited local and general AEs in a the first 50 subjects enrolled in each group within four days (day of vaccination and three subsequent days) after Dose 3 of study vaccine (all groups including controls), after Dose 4 (groups R012-20, R012-14, Fx012-14 and Fx017-20), after Dose 5 (groups R012-14, Fx012-14 and Fx017-20) and after Dose 6 (groups R012-14 and Fx012-14).

5.3. Tertiary endpoints

- **Immunogenicity**
  - Anti-CS antibody avidity at same time points specified in secondary endpoints

6. ANALYSIS SETS

6.1. Definition

Note that in order to align to International Committee on Harmonization (ICH) and Clinical Data Interchange Standards (CDISC) terminology the Total Vaccinated Cohort and the According To Protocol cohort have been renamed Exposed Set (ES) and Per-Protocol Set (PPS) respectively. A solicited safety set is an added set for tables including solicited data only.

6.1.1. All Enrolled Set

The enrolled set will include all subjects who signed informed consent.

6.1.2. Exposed Set (ES)

The ES will include all subjects who received at least one dose of study vaccine. The ES analysis will be performed per treatment actually administered.

6.1.3. Per-Protocol Set for analysis of efficacy (PPS for efficacy)

For the primary analysis up to month 14, the PPS for efficacy will include all subjects from the ES who fulfilled eligibility criteria and who received all vaccinations according to protocol procedures within the protocol specified intervals that contribute to the time at risk in the follow-up period starting 14 days post-Dose 3.

For the final analysis up to month 50, all subjects who have not received Dose 4 of study vaccine or subsequent yearly doses (Dose 5 and Dose 6) according to protocol will be censored at the last contact date prior to the first missed dose recorded and will contribute to the PPS analysis up to censoring.

6.1.4. Per-Protocol Set for analysis of immunogenicity (PPS for immunogenicity)

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

6.1.5. Solicited Safety Set

The solicited safety set- including the solicited safety set within the reactogenicity sub cohort- will include all subjects who received at least one dose of study vaccine and had solicited safety data.

6.2. Criteria for eliminating data from Analysis Sets

Elimination codes are used to identify subjects to be eliminated from analysis. Detail is provided below for each set.

6.2.1. Elimination from Exposed Set (ES)

Code 1030 (study vaccine not administered at all), code 800 (fraudulent data) and code 900 (invalid informed consent) will be used for identifying subjects eliminated from the ES and the reactogenicity sub-cohort.

6.2.2. Elimination from the PPS for analysis of efficacy and immunogenicity

A subject will be excluded from the PPS under the following conditions:

Code	Condition under which the code is used	Visit (timepoints) where the code is applicable	Applicable analysis set
800	Fraudulent data	All visits	immunology, efficacy
900	Invalid informed consent	All visits	immunology, efficacy
1030	Study vaccine not administered at all but subject number allocated Subject randomized but not vaccinated.	Visit 2, and onwards	immunology, efficacy
1040*	Administration of concomitant vaccine(s) forbidden in the protocol to be determined by CRDL from review of individual data listings	For efficacy set (3035 elimination code): a vaccine not foreseen by the study protocol administered during the period starting seven days before each dose and ending seven days after each dose of vaccine. It applies only for the three first doses.	immunology, efficacy
1050	Randomisation failure First vaccination not equal to the group the subject was randomized to. To be attributed by Statistician and CDR: check SBIR, replacement, vaccine administration	Time of randomization, and onwards	immunology, efficacy

Code	Condition under which the code is used	Visit (timepoints) where the code is applicable	Applicable analysis set
1070*	Vaccination not according to protocol: • Incomplete vaccination course before treatment withdrawal • Wrong replacement or study vaccine administered (not compatible with the vaccine regimen associated to the treatment number).	All visits were vaccinations are planned: • Control→ Visit 2, 3 and 4 • R012-20→Visit 2, 3, 4 and 24 • R012-14 → Visit 2, 3, 4, 18, 27 and 33 • Fx012-14 → Visit 2, 3, 4, 18, 27, and 33 • Fx017-20 → Visit 2, 3, 10, 24 and 30	immunology
1080*	Vaccine temperature deviation vaccine administered despite a Good Manufacturing Practices (GMP) no-go temperature deviation	For efficacy set (3075 elimination code): applies at the time of the first 3 doses. For control, R012-20, R012-14 and Fx012-14 at visit 2,3 and 4. For Fx017-20 at visit 2,3 and 7.	Immunology, efficacy
1090*	Expired vaccine administered	For efficacy set (3090 elimination code): applies at the time of the first 3 doses. For control, R012-20, R012-14 and Fx012-14 at visit 2,3 and 4. For Fx017-20 at visit 2,3 and 7.	Immunology, efficacy
2010	Protocol violation (inclusion/exclusion criteria) including age Subject's age should be 5-17 months at first study visit.	Visit 2 • Subjects for whom date of birth → Visit 2 is outside [5-17 months] • All other criteria that are not met	Immunology, efficacy
2040*	Administration of any medication forbidden by the protocol To be determined by CRDL from review of individual data listings	For the efficacy set (3040 elimination code): A vaccine not foreseen by the study protocol administered during the period starting seven days before each dose and ending seven days after each dose of vaccine. It applies only for the three first doses	Immunology, efficacy

Code	Condition under which the code is used	Visit (timepoints) where the code is applicable	Applicable analysis set
2080*	Subjects did not comply with vaccination schedule vac1=first vaccination, vac2 = second vaccination etc.	For the immuno set it applies under following conditions: • All groups: vac1→vac2 is outside [21-35 days] • Control, R012-20, R012-14, Fx012-14: vac2→vac3 is outside [21-35 days] • R012-20: vac3→vac4 is outside [518-574 days] • R012-14, Fx012-14: vac3→vac4 is outside [336-392 days] vac4→vac5 is outside [336-392 days] vac5→vac6 is outside [336-392 days] • Fx017: vac2→vac3 is outside [161-203 days] vac3→vac4 is outside [364-420 days] vac4→vac5 is outside [336-392 days] For efficacy set (3080 elimination code) it applies to the time of the first 3 doses. For control, R012-20, R012-14 and Fx012-14 at visit 2,3 and 4. For Fx017-20 at visit 2,3 and 7.	Immunology, efficacy
2090*	Subjects did not comply with blood sample schedule Ser1=first serum collection, ser2 = second serum collection etc. +For these intervals, calculations were done by adding up both minimum and maximum days of the	Applies under following conditions: • Control, R012-20, R012-14, Fx012-14: vac3→ser3 (M3) is outside [21-35 days] • Control:	Immunology

Code	Condition under which the code is used	Visit (timepoints) where the code is applicable	Applicable analysis set
	visit intervals specified in table 14 of the protocol	vac3→ser4(M20) is outside [518-574 days] vac3→ser5 (M50) is outside [+1316-1596 days] • R012-20: vac4→ser5 (M21) is outside [21-35 days] vac4→ser6 (M50) is outside [+812-1008 days] • R012-14, Fx012-14: vac4→ser5 (M15) is outside [21-35 days] vac5→ser7 (M27) is outside [21-35 days] vac6→ser9 (M34) is outside [21-35 days] vac6→ser10 (m50) is outside [+308-420 days] • Fx017- 20: vac2→ser2(M2) is outside [21-35 days] vac3→ser4 (M8) is outside [21-35 days] vac4→ser6 (M21) is outside [21-35 days] vac5→ser7 (M33) is outside [21-35 days] vac5→ser9 (M50) is outside [+476-616 days]	
2100	Serological results not available post-vaccination if ALL are missing and subject in Immunogenicity Subset	All visits where serum samples are planned (see Table 1– time points for immunogenicity)	Immunology
2120	Incomplete assessment and mislabelling (sample tested/not tested) of	All visits where serum samples are planned (see Table 1– time points for immunog enicity)	Immunology

Code	Condition under which the code is used	Visit (timepoints) where the code is applicable	Applicable analysis set
	blood sample, for specific assays		

Code	Condition under which the code is used	Visit (timepoints) where the code is applicable	Applicable analysis set
3100	Subjects without follow up data starting 14 days post dose 3 Note that subjects not receiving dose 4, 5 and 6 will be censored at the last contact prior to whichever first missed dose recorded	Applies under following conditions: • First 3 vaccinations are administered • No data available 14 days after visit 4 for Control, R012-20, R012-14, Fx012-14 • No data available 14 days after visit 10 for Fx017-20	Efficacy
3200	Subjects not receiving first 3 doses	Applies under following conditions • For control, R012-20, R012-14, Fx012-14 → no vaccination administered at visit 2,3 and 4. • For Fx017-20 → no vaccination administered at visit 2,3 and 10	Efficacy

*a Dynamic elimination approach will be used for the definition of the immunogenicity PPS (Analysis M21 E1_02, Analysis M33 E1_03, Final analysis). This means that for codes 1040, 1070, 1080,1090, 2080, only the data relevant/posterior to the deviation will be eliminated. For codes 2040, 2090, 2120, the data elimination will be visit specific. For the efficacy PPS subjects will be fully excluded based on the elimination codes. However, subjects included in the efficacy analysis could be censored at last contact date.

6.2.3. Elimination from solicited safety set

Code 1030 (study vaccine not administered at all), code 800 (fraudulent data) and code 900 (invalid informed consent) and code 1160 (no post-vaccination solicited safety data) will be used for identifying subjects eliminated from the solicited safety set and subjects of the solicited safety set who are part of the reactogenicity sub-cohort.

6.3. Protocol deviation not leading to elimination from per-protocol analysis set

Protocol deviations not leading to elimination from PPS analyses are defined in the MALARIA-094 Protocol Deviation Management Plan.

https://biodocumentum.bio.corpnet1.com/webtoppr/drl/objectId/090f45f6898bbf02 (MALARIA-094 (204889) Protocol Deviation Management Plan V3 corrected on 22 Aug 2019 (09-Jul-2019))

7. STATISTICAL ANALYSES

Note that standard data derivation rules and stat methods are described in the annex and will not be repeated below. The study specific data derivation rules and stat methods will be described in section 11.1.

7.1. Demography

7.1.1. Analysis of demographics/baseline characteristics planned in the protocol

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

Demographic characteristics (age and gender) and the baseline hemoglobin level will be tabulated per study group overall, and by site, for each cohort (ES, PPS cohort for efficacy and PPS cohort for immunogenicity).

The mean age at first vaccination (in months) (plus range and standard deviation [SD]) of the vaccinated subjects as a whole, and per group, will be calculated.

7.1.2. Additional considerations

Demographic characteristics, cohort description, withdrawal status will be summarized by group using descriptive statistics:

- Frequency tables will be generated for categorical variables such as gender.
- Mean, median, SD, minimum and maximum will be provided for continuous data such as age.
- The 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 analyses will be tabulated.
- The numbers of withdrawn subjects will be tabulated according to the reason for withdrawal.

7.2. Exposure

The number and percentage of subjects who received study vaccine doses will be tabulated overall for each study group separately (ES).

7.3. Efficacy/Effectiveness

7.3.1. Analysis of efficacy planned in the protocol

The primary analysis of efficacy will be based on the PPS for efficacy. The effect of the vaccine will be characterized using different complementary standard methodologies.

7.3.1.1. Strategy of analysis and rationale

The strategy of analysis for the present study has been developed considering the following points:

- Prevalent infections are only captured by ADI during the cross-sectional surveys performed at pre-defined timepoints throughout the study. Incident infections are captured via both ADI at cross-sectional surveys and via passive detection upon spontaneous presentation at health centers (see Section 4.2 of the protocol for case definitions of *P. falciparum* infections). Episodes of clinical malaria will be treated when diagnosed. Samples collected at cross-sectional visits in asymptomatic children will not be processed in real time and will not lead to systematic treatment.
- Vaccine efficacy against prevalent *P. falciparum* infections at the successive cross-sectional surveys is an important measure as it is not influenced by care seeking behavior and possibly less influenced by blood-stage immunity. Reducing the prevalence of blood-stage infections at successive timepoints will be a good indicator of the potential to reduce transmission as it is directly associated with the potential to infect mosquitoes.
- Vaccine efficacy against first or only episode of incident *P. falciparum* infection or against first or only episode of clinical malaria minimizes the bias related to differential acquisition of blood-stage immunity in the vaccine and Control groups, and is assumed to be the best way to assess the biological effect of the third dose fractioning and spacing.
- Vaccine efficacy against all episodes of clinical malaria is more relevant to public health but over longer time periods. There is a possibility of bias related to the fact that it will compare a Control group with frequent exposure and an intervention group with reduced exposure and a different build-up of blood-stage immunity over time, possibly influencing health seeking behaviors (hence the importance of also evaluating vaccine efficacy against prevalent infection and first or only episode).
- Considering the measures of incidence:
  - For group comparisons with identical calendar time follow-up periods, the incidence rates will be directly compared. The incremental vaccine efficacy estimate is defined as 1-Incidence Rate Ratio in each group.
  - When different calendar time follow up periods are compared, as is done for comparison Fx017 versus both Fx012 and R012 during the period 7 and 12 months post dose 3, the following analysis will be performed:

1. An analysis comparing the treatment schedules Fx017-20 versus Fx012-14 and R012-14 with respect to clinical malaria and incident infection taking into account potential confounders. The following covariates are considered to be prognostic for clinical malaria: monthly prevalence of parasitemia for control group and study site. An example will be described for comparison Fx017-20 versus both Fx012-14 7 months post dose 3. For the analysis with outcome first or only episode of clinical malaria, a cox regression with outcome time to clinical malaria will be modeled, with treatment as exposure (comparing schedule Fx012-14 from month 2-9 versus Fx017-

20 from month 7-14), monthly prevalence of parasitemia for control group as covariate (continuous) and study site (Ghana versus Kenya). For the analysis with all episodes of clinical malaria, a negative binomial regression (method described in section 7.3.1.2) will be performed. The same exposures and covariates as used for the cox regression will be entered in this model. The adjusted incremental efficacy will be defined as 1 minus the hazard ratio/incidence ratio, during the corresponding time frame for first or only episode/all episodes.

– The estimation of the number of cases of clinical malaria averted will inform on how the vaccine efficacy translates into impact, in a way that is dependent on exposure and hence transmission intensity.

7.3.1.2. Measures of incidence (*P. falciparum* infection and clinical malaria)

- **First or only episode**

Vaccine efficacy against first or only episode of incident *P. falciparum* infection or clinical malaria will be analyzed by Cox regression stratified by study site, and overall. The 95% CI and p-values of the vaccine efficacy estimates (1- Hazard Ratio) will be calculated from this model. Cumulative incidence graphs will be produced.

- **All episodes**

Vaccine efficacy against all episodes of clinical malaria will be analyzed by negative binomial regression allowing for interdependence between episodes within the same subject (non-linear mixed model with over dispersion parameter estimated from the random effect). The 95% CI and p-values of the vaccine efficacy estimates (1- Incidence Rate Ratio) will be calculated from this model. In case models do not converge or showed poor fit, alternative negative binomial models without random effects will be applied. fourteen days following an episode meeting the case definition under evaluation will be subtracted from the follow-up time. The distribution of the total number of events per subject will be tabulated by group. Results per site and overall (adjusted for site as a fixed effect) will be summarized in tables and forest plots.

7.3.1.3. Measure of prevalence (*P. falciparum* infection)

The prevalence of *P. falciparum* infection will be evaluated on the cross-sectional ADI samples only. At each pre-defined timepoint (see protocol section 6.5) the prevalence (number of subjects with *P.falciparium* infection/total number of subjects) of *P. falciparum* infection will be calculated by group together with 95% CIs. Graphical presentations of the prevalence over time will be produced. Results per site and overall will be summarized in tables. In addition the number of positive prevalent infections will be analyzed by a negative binomial regression to compare the groups (Fx012-14, Fx017-20, R012) against the control to obtain vaccine efficacy estimates and corresponding 95% CI (applying the same strategy as for all episodes in section 7.3.1.2).

The prevalence of gametocytemia will be tabulated for each group (Fx017-20, Fx012-14, R012-20, R012-14 and control) at each monthly cross-sectional visit (from M0 until M20) and thereafter at each three-monthly cross-sectional visit (from M23 until M50).

Within gametocytes positive slides the mean male/female ratio will be tabulated by group and time point and by site. Within parasitemia positive subjects the geometric mean will be tabulated by group and time point and by site.

7.3.1.4. Measure of impact (clinical malaria)

The vaccine impact will be estimated by the number of cases of clinical malaria averted over the relevant time frame per 1000 subjects vaccinated. The number of cases averted will be calculated as the difference of the estimated cases between the Control group and the vaccine group. Estimated cases in each group will be calculated as the area under the curve of the incidence of clinical malaria over time and expressed as cumulative cases averted per 1000 subjects followed-up during the relevant period. Results per site and overall will be summarized in tables.

7.3.1.5. Primary analysis of efficacy (primary objective)

A) Clinical malaria:

Analysis performed at Month 20:

– Incremental efficacy of Fx012-14 versus (R012-20 + R012-14) over M2.5-M14 (first or only episode, PPS).

7.3.1.6. Secondary analyses of efficacy

A) Clinical malaria:

Analysis performed at Month 20:

– Incremental efficacy of Fx012-14 versus (R012-20 + R012-14) over M2.5-M14 (all episodes, PPS).

– Incremental efficacy of Fx012-14 versus (R012-20 + R012-14) over D0-M14 (first or only and all episode, ES).

– Vaccine efficacy of Fx012-14 versus Control group over M2.5-M9 (first or only and all episodes, PPS).

– Vaccine efficacy of Fx017-20 versus Control group over M7.5-M14 (first or only and all episodes, PPS)

– Vaccine efficacy of R012-20 + R012-14 versus Control group over M2.5-M9 (first or only and all episodes, PPS).

– Vaccine efficacy of Fx012-14 versus Control group over M2.5-M14 (first or only and all episodes, PPS).

– Vaccine efficacy of Fx017-20 versus Control group over M7.5-M19 (all episodes, PPS).

– Vaccine efficacy of R012-20 + R012-14 versus Control group over M2.5-M14 (first or only and all episodes, PPS).

- Incremental efficacy of Fx017-20 over M7.5-M14 versus Fx012-14 over M2.5-M9 (first or only and all episodes, PPS).
- Incremental efficacy of Fx017-20 over M7.5-M14 versus groups R012-20 + R012-14 over M2.5-M9 (first or only and all episodes, PPS).
- Incremental efficacy of Fx017-20 over M7.5-M19 versus Fx012-14 over M2.5-M14 (first or only and all episodes, PPS).
- Incremental efficacy of Fx017-20 over M7.5-M19 versus groups R012-20 + R012-14 over M2.5-M14 (first or only and all episodes, PPS).
- Vaccine efficacy and impact of R012-20 + R012-14 versus Control group over D0-M14 (first or only for efficacy and all episodes for efficacy and impact, ES).
- Vaccine efficacy and impact of Fx012-14 versus Control group over D0-M14 (first or only for efficacy and all episodes for efficacy and impact, ES).
- Vaccine efficacy and impact of Fx017-20 versus Control group over D0-M14 (first or only and all episodes, ES).
- Vaccine efficacy and impact of R012-20 versus Control group over D0-M20 (all episodes, ES).
- Vaccine efficacy and impact of R012-14 versus Control group over D0-M20 (all episodes, ES).
- Vaccine efficacy and impact of Fx012-14 versus Control group over D0-M20 (all episodes, ES).
- Vaccine efficacy and impact of Fx017-20 versus Control group over D0-M20 (all episodes, ES).

Analysis performed at Month 32:

- Vaccine efficacy and impact of R012-20 versus Control group over D0-M26 (all episodes, ES).
- Vaccine efficacy and impact of R012-14 versus Control group over D0-M26 (all episodes, ES).
- Vaccine efficacy and impact of Fx012-14 versus Control group over D0-M26 (all episodes, ES).
- Vaccine efficacy and impact of Fx017-20 versus Control group over D0-M26 (all episodes, ES).
- Incremental efficacy of Fx017-20 versus R012-20 over M20-M32 (all episodes, PPS).
- Incremental efficacy of Fx012-14 versus R012-14 over M14-M26 (all episodes, PPS).
- Vaccine efficacy and impact of R012-20 versus Control group over D0-M32 (all episodes, ES).

- Vaccine efficacy and impact of R012-14 versus Control group over D0-M32 (all episodes, ES).
- Vaccine efficacy and impact of Fx012-14 versus Control group over D0-M32 (all episodes, ES).
- Vaccine efficacy and impact of Fx017-20 versus Control group over D0-M32 (all episodes, ES).

Analysis performed at Month 50:

- Vaccine efficacy and impact of R012-20 versus Control group over D0-M38 (all episodes, ES).
- Vaccine efficacy and impact of R012-20 versus Control group over D0-M50 (all episodes, ES).
- Vaccine efficacy and impact of R012-14 versus Control group over D0-M38 (all episodes, ES).
- Vaccine efficacy and impact of R012-14 versus Control group over D0-M50 (all episodes, ES).
- Vaccine efficacy and impact of Fx012-14 versus Control group over D0-M38 (all episodes, ES).
- Vaccine efficacy and impact of Fx012-14 versus Control group over D0-M50 (all episodes, ES).
- Vaccine efficacy and impact of Fx017-20 versus Control group over D0-M38 (all episodes, ES).
- Vaccine efficacy and impact of Fx017-20 versus Control group over D0-M50 (all episodes, ES).
- Incremental efficacy of Fx012-14 versus R012-14 over M26-M38 (all episodes, PPS).
- Incremental efficacy of Fx012-14 versus R012-14 over M38-M50 (all episodes, PPS).

B) Incident *P. falciparum* infections

Analysis performed at Month 20:

- Incremental efficacy of Fx012-14 versus (R012-20 + R012-14) over M2.5-M14 (first or only episode, PPS).
- Incremental efficacy of Fx012-14 versus (R012-20 + R012-14) over D0-M14 (first or only episodes, ES).
- Vaccine efficacy of Fx012-14 versus Control group over M2.5-M9 (first or only episodes, PPS).
- Vaccine efficacy of Fx017-20 versus Control group over M7.5-M14 (first or only episodes, PPS).
- Vaccine efficacy of R012-20 + R012-14 versus Control group over M2.5-M9 (first or only episodes, PPS).
- Vaccine efficacy of Fx012-14 versus Control group over M2.5-M14 (first or only episodes, PPS).
- Vaccine efficacy of Fx017-20 versus Control group over M7.5-M19 (first or only episodes, PPS).
- Vaccine efficacy of R012-20 + R012-14 versus Control group over M2.5-M14 (first or only episodes, PPS).
- Incremental efficacy of Fx017-20 over M7.5-M14 versus Fx012-14 over M2.5-M9 (first or only episodes, PPS).
- Incremental efficacy of Fx017-20 over M7.5-M14 versus groups R012-20 + R012-14) over M2.5-M9 (first or only episodes, PPS).
- Incremental efficacy of Fx017-20 over M7.5-M19 versus Fx012-14 over M2.5-M14 (first or only episodes, PPS).
- Incremental efficacy of Fx017-20 over M7.5-M19 versus groups R012-20 + R012- over M2.5-M14 (first or only episodes, PPS).
- Vaccine efficacy of R012-20 + R012-14 versus Control group over D0-M14 (first or only and all episodes, ES).
- Vaccine efficacy of Fx012-14 versus Control group over D0-M14 (first or only and all episodes, ES).
- Vaccine efficacy of Fx017-20 versus Control group over D0-M14 (first or only and all episodes, ES).
- Vaccine efficacy of R012-20 versus Control group over D0-M20 (all episodes, ES).
- Vaccine efficacy of R012-14 versus Control group over D0-M20 (all episodes, ES).
- Vaccine efficacy of Fx012-14 versus Control group over D0-M20 (all episodes, ES).

– Vaccine efficacy of Fx017-20 versus Control group over D0-M20 (all episodes, ES).

C) Prevalent *P. falciparum* infections

Prevalent *P. falciparum* infections will be assessed in the ES.

Analysis performed at Month 20:

– Prevalent *P. falciparum* infections at each cross-sectional survey

Analysis performed at Month 32:

– Prevalent *P. falciparum* infections at each cross-sectional survey

Analysis performed at Month 50:

– Prevalent *P. falciparum* infections at each cross-sectional survey

7.3.2. Additional considerations

7.3.2.1. All planned VE Analyses and corresponding risk periods

This section describes all VE analyses (planned in protocol and additional analyses).

For each endpoint, the time at risk will be calculated separately. The time at risk will be counted in days, and expressed as person years at risk (days/365.25). In order to avoid mathematical problems because of time equals zero when an event occurred the same day that the time at risk started, the first day counts as 1 thus the duration is calculated as (date of event or censoring – date of start follow up +1). Time at risk will end at the date of next visit minus 1 to avoid including post safety and efficacy data that may have occurred on the same day.

For endpoints evaluating all episodes where time at risk does not end when the episode meets the case definition, 14 days following the episode will be subtracted from the time at risk (day of episode + 14). If an episode is detected during a period of time not counting for the time at risk it will not be included in the analysis however a table will be presented showing the number of such episodes not included in the analysis.

Table 3 Risk periods for clinical malaria & incident infection for the exposed set

Risk Period (months)	Start of risk period (at subject level)	End of risk period (at subject level)*	Group comparisons	Clinical malaria	Incident Infection
M20 analyses					
[0-14]	Day of first vaccination of RTS,S or control vaccine	Date of Month 14 visit – 1 or drop out date from study conclusion, whichever occurs first	Fx017-20 vs control	First or only- and all episodes	First or only episode
			Fx012-14 vs control	First or only- and all episodes	First or only episode
			R012-20 +R012-14 vs control	First or only- and all episodes	First or only episode
			Fx012-14 Vs R012-20 +R012-14	First or only- and all episodes	First or only episode
[0-20]	Day of first vaccination of RTS,S or control vaccine	Date of Month 20 visit – 1 or drop out date from study conclusion, whichever occurs first	Fx017-20 vs control	All episodes	First or only episode
			Fx012-14 vs control	All episodes	First or only episode
			R012-20 vs control	All episodes	First or only episode
			R012-14 vs control	All episodes	First or only episode
			Fx012-14 Vs R012-20	All episodes	First or only episode
			Fx012-14 Vs R012-14	All episodes	First or only episode
M32 analyses					
[0-26] [0-32]	Day of first vaccination of RTS,S or control vaccine	Date of Month 26/32 visit – 1 or drop out date from study conclusion, whichever occurs first	Fx017-20 vs control	All episodes	NA
			Fx012-14 vs control	All episodes	
			R012-20 vs control	All episodes	
			R012-14 vs control	All episodes	
			Fx012-14 Vs R012-20	All episodes	
			Fx012-14 Vs R012-14	All episodes	
M50 analyses					
[0-38]	Day of first vaccination of RTS,S or control vaccine	Date of Month 38 visit – 1 or drop out date from study conclusion, whichever occurs first	Fx017-20 vs control	All episodes	NA
			Fx012-14 vs control	All episodes	
			R012-20 vs control	All episodes	
			R012-14 vs control	All episodes	
			Fx012-14 Vs R012-20	All episodes	
			Fx012-14 Vs R012-14	All episodes	
[0-50]	Day of first vaccination of RTS,S or control vaccine	Date of Month 50 visit – 1 or drop out date from study conclusion, whichever occurs first	Fx017-20 vs control	All episodes	NA
			Fx012-14 vs control	All episodes	
			R012-20 vs control	All episodes	
			R012-14 vs control	All episodes	
			Fx012-14 Vs R012-20	All episodes	
			Fx012-14 Vs R012-14	All episodes	
			Fx017-20 vs R012-14	All episodes	
			Fx017-20 vs Fx012-14	All episodes	

* if visit date and last contact date are both missing then subjects will be censored at month [x] times 30.5 days post dose 1.

Clinical malaria

For endpoints evaluating the first or only clinical malaria, subjects who withdraw from the study before the study end will contribute to the time at risk computation. This will be explained with the help of the following figure. In these illustrative examples, for the sake of simplicity, it has been considered that visits were effectively conducted at exact monthly intervals.

	M1 30.5d	M2 30.5d	M3 30.5d	M4 30.5d	M5 30.5d	M6 30.5 d	M7 30.5 d	M8 30.5 d	M9 30.5 d	M10 30.5d
Subject A	-	-	-	.	.	-	-	+		
Subject B	-	-	-	+	-	+	-	-	-	-

-negative
+positive
. missing

Example: Subject A has two missed visits at month 4 and month 5, and an episode of clinical malaria at start of month 8. This subject will be considered to have an event at month 8, and therefore the time at risk will be (30.5d*7months) 214 days.

For endpoints evaluating all episodes 14 days following each episode will be subtracted from the time at risk (day of episode + 14). For subject B the time at risk will be (30.5*10 months) – (2*14 days) 277 days.

Incident infection

Only subjects with a confirmed negative blood slide at the beginning of the follow-up period (defined in Table 3) will be included in the analysis. The subjects will be followed-up until either having a case, or being censored (having a missing cross-sectional visit or being a drop-out whichever occurs first).

Example: the subject A described in the previous example would have been censored at the start of month 4 and contributes (3*30.5) 92 days to the time at risk.

Risk periods for prevalent parasitemia & Gametocytemia

At month 20 there are two types of analysis that will be presented:

- First, the prevalence of parasitemia/gametocytemia will be tabulated for each group (Fx017-20, Fx012-14, R012-20, R012-14 and control) at each cross sectional visit (from M0 until M20).
  - Within gametocytes positive slides the mean male/female ratio will be tabulated by group and time point and by site.
  - Within parasitemia positives the geometric mean with will be tabulated by group and time point and by site.
- Second, an analysis with endpoint all episodes of prevalent infections within risk period [M1-M20] will be performed comparing the groups (Fx017-20, Fx012-14, R012-20, R012-14) with the control. In this analysis subjects with missing visits before the last contact date will only contribute to the time at risk at the time periods

they are not missing. See example below. The subject contributes (3*30.5) +(2*30.5)=153 days to the time at risk.

	M1	M2	M3	M4	M5	M6	M7	M8	M9	M20
Subject	-	-	-	.	-	-	+	.	.	.
	→			→						

-negative
+positive
. missing

- In both analyses described above subjects with clinical malaria (case definition 1 or 2) at start day of detection of parasitemia + subsequent 14 days will be excluded.

At month 32 and month 50 the following analyses will be performed for each group, at each cross sectional visit and by site:

- the prevalence of parasitemia/gametocytemia
- male/female ratio for gametocytes positive slides
- and geometric means for parasitemia positives

Table 4 Risk periods for clinical Malaria & incident infection for the per protocol set: Comparisons with equal calendar times

Risk Period (months)	Start of risk period (at subject level)	End of risk period (at subject level)*	Group comparisons	Clinical Malaria	Incident infection
Analyses 12 months post dose 3: • **Primary objective: incremental efficacy of fractional dose versus full dose** • **Secondary objective: vaccine efficacy of all groups versus controls**					
[2½-14]	14 days post dose 3 of RTS,S or control vaccine (day of dose 3 vaccination + 14)	Date of Month 14 visit – 1 or drop out date from study conclusion, whichever occurs first	**Primary objective: Fx012-14 vs R012-20 +R012-14**	First or only- and all episodes	First or only episode
			Fx012-14 vs control	First or only- and all episodes	First or only episode
			R012-20 +R012-14 vs control	First or only- and all episodes	First or only episode
Analyses 7 months post dose 2 and 3: • **vaccine efficacy of all groups versus controls**					
[2½-9]	14 days post dose 3 of RTS,S or control vaccine (day of dose 3 vaccination + 14)	Date of Month 9 visit – 1 or drop out date from study conclusion, whichever occurs first	R012-20+ R012-14 vs Control	First or only- and all episodes	First or only episode
			Fx012-14 vs control	First or only- and all episodes	First or only episode

Risk Period (months)	Start of risk period (at subject level)	End of risk period (at subject level)*	Group comparisons	Clinical Malaria	Incident infection
[7½-14]	14 days post dose 3 of RTS,S or control vaccine (day of dose 3 vaccination + 14)	Date of Month 14 visit – 1 or drop out date from study conclusion, whichever occurs first	Fx017-20 vs control	First or only- and all episodes	First or only episode
[1-7]	Day of second vaccination of RTS,S or control vaccine	Date of Month 14 visit – 1 or drop out date from study conclusion, whichever occurs first	Fx017-20 vs control	First or only- and all episodes	First or only episode
Analyses up to 38 months after dose 3: • **incremental of fractional dose versus full dose** • **vaccine efficacy of all groups versus controls**					
[2½-20]	14 days post dose 3 of RTS,S or control vaccine (day of dose 3 vaccination + 14)	Date of Month 20 visit – 1 or drop out date from study conclusion, whichever occurs first	Fx012-14 vs control	All episodes	First or only episode
			R012-14 vs control	All episodes	First or only episode
			R012-20 vs control	All episodes	First or only episode
			Fx012-14 vs R012-20	All episodes	First or only episode
			Fx012-14 vs R012-14	All episodes	First or only episode
			R012-20 vs R012-14	All episodes	First or only episode
[7½-19]	14 days post dose 3 of RTS,S or control vaccine (day of dose 3 vaccination + 14)	Date of Month 19 visit – 1 or drop out date from study conclusion, whichever occurs first	Fx017-20 vs control	All episodes	First or only episode
[2½-26] [2½-32] [2½-38] [2½-50]	14 days post dose 3 of RTS,S or control vaccine (day of dose 3 vaccination + 14)	Date of Month 26/ 32/38/50 visit – 1 or drop out date from study conclusion, whichever occurs first	R012-14 vs control	All episodes	NA
			Fx012-14 vs control	All episodes	
			R012-20 vs control	All episodes	
			Fx012-14 vs R012-20	All episodes	
			Fx012-14 vs R012-14	All episodes	
			R012-20 vs R012-14	All episodes	
[7½-26] [7½-32] [7½-38] [7½-50]	14 days post dose 3 of RTS,S or control vaccine (day of dose 3 vaccination + 14)	Date of Month 26/32/38/50 visit – 1 or drop out date from study conclusion, whichever occurs first	Fx017-20 vs control	All episodes	NA

Risk Period (months)	Start of risk period (at subject level)	End of risk period (at subject level)*	Group comparisons	Clinical Malaria	Incident infection
Analyses post booster dose (post dose 4, 5 and 6):					
[M14-M20]	Day of fourth RTS,S dose or month 14 visit for control	Date of Month 20 visit – 1 or drop out date from study conclusion, whichever occurs first	Fx012-14 vs R012-14	All episodes	First or only episode
			R012-14 vs control	All episodes	First or only episode
			Fx012-14 vs control	All episodes	First or only episode
[M14-M26] [M26-M32] [M26-M38] [M38-M50]	Day of fourth/fifth/sixth RTS,S dose or month 14/26/38 visit for control	Date of Month 26/32/38/50 visit – 1 or drop out date from study conclusion, whichever occurs first	Fx012-14 vs R012-14	All episodes	NA
			R012-14 vs control	All episodes	
			Fx012-14 vs control	All episodes	
[M20-M32]	Day of fourth RTS,S dose or month 20 visit for control	Date of Month 32 visit – 1 or drop out date from study conclusion, whichever occurs first	Fx017-20 vs R012-20	All episodes	NA
			Fx017-20 vs Control	All episodes	
			R012-20 vs Control	All episodes	
[M32-M50]	Day of fifth RTS,S dose or M32 visit for control	Date of Month 50 visit – 1 or drop out date from study conclusion, whichever occurs first	Fx017-20 vs Control	All episodes	NA

* if visit date and last contact date are both missing then subjects will be censored at month [x] times 30.5 days post dose 2/3/4/5/6.

Table 5 Risk periods for clinical Malaria & incident infection for the per protocol set: Comparisons with unequal calendar time

Risk Period (months)	Start of risk period (at subject level)	Stop (maximum)*	Group comparisons	Clinical Malaria	Incident infection
Analyses 6 months post dose 3: fractional dose versus full dose					
[6.5 months post dose 3] [M7.5-M14] [2.5-9]	14 days post dose 3 of RTS,S (day of dose 3 vaccination + 14)	Fx017-14: Date of Month 14-1 or drop out date from study conclusion, whichever occurs first All other groups: Date of Month 9 or drop out date from study conclusion, whichever occurs first	Fx017-20 vs Fx012-14	First or only- and all episodes	First or only episode
			Fx017-20 vs R012-20 +R012-14	First or only- and all episodes	First or only episode

Risk Period (months)	Start of risk period (at subject level)	Stop (maximum)*	Group comparisons	Clinical Malaria	Incident infection
Analyses 12 months post dose 3: fractional dose versus full dose					
[1 year post dose 3] [M7.5-M19] [M2.5-M14]	14 days post dose 3 of RTS,S (day of dose 3 vaccination + 14)	R012-20: Date of Month 14-1 or drop out date from study conclusion, whichever occurs first All other groups: Date of the visit of fourth dose -1 or drop out date from study conclusion, whichever occurs first	Fx017-20 vs Fx012-14	First or only- and all episodes	First or only episode
			Fx017-20 vs R012-20 +R012-14	First or only- and all episodes	First or only episode

* if visit date and last contact date are both missing then subjects will be censored at month [x] times 30.5 days post dose 3.

Clinical Malaria

The rules for calculating the risk periods are the same as for the exposed set.

Incident infection

Only subjects with a confirmed negative blood slide at the beginning of the follow-up period (defined in Table 2 and Table 3) will be included in the analysis. The subjects will be followed-up until being censored (either having a case, having a missing cross-sectional visit or being a drop-out whichever occurs first). For follow-up periods starting from M2.5 and M7.5, only subjects with a confirmed negative blood slide at M2 and M7 will be included in the analysis.

7.3.2.2. Additional analyses for efficacy

This section summarizes the additional analyses as compared to the protocol defined analyses (described in section 7.3.1.6).

Table 6 Incremental efficacy analyses against all episodes of clinical malaria.

Risk Period (months)	Full dose groups, control	Fractional dose groups, control			Objectives
		Fx012-14	Fx017-20	Control	
Exposed set					
D0-M20 D0-M26 D0-M32 D0-M38	R012-20	x			• Prolonged follow-up period for primary objective
	R012-14	x			
D0-M50	R012-20	x			• Prolonged follow-up period for primary objective
	R012-14	x	x		• Prolonged follow-up period for primary objective • To compare a delayed factional dose schedule with early fractional- and early full dose schedule
	-	x	x		
Per protocol set					
M1-M7	Control		x		• VE of delayed fractional third dose
M2.5-M20	R012-20	x		x	• Prolonged follow-up period for primary objective • Vaccine efficacy of early fractional/full dose schedule • Incremental efficacy of full dose (standard schedule versus early booster) schedule
	R012-14			x	
	Control	x			
	R012-20 vs R012-14				
M2.5-M26 M2.5-M32 M2.5-M38 M2.5- M50	R012-20	x		x	• Prolonged follow-up period for primary objective • Vaccine efficacy of early fractional/full dose schedule • Incremental efficacy of full dose (standard schedule versus early booster) schedule
	R012-14	x		x	
	Control	x			
	R012-20 vs R012-14				
M7.5-M26 M7.5-M32 M7.5-M38 M7.5-M50	Control		x		• VE of delayed fractional third dose at prolonged follow-up periods
M14-M20 M26-M32	R0-12-14	x		x	• Incremental efficacy 6 months post dose 4 and 5 (fractional vs full dose) • VE 6 months post dose 4 and 5 of early fractional- and full dose schedule
	Control	x			
M14-M26 M26-M38 M38-M50	R0-12-14			X	• VE 12 months post dose 4, 5 and 6 of the early fractional- and full dose schedule
	Control	x			
M20-M32	R012-20			x	• VE 12 months post dose 4 of standard full dose schedule
M32-M50	Control		x		• VE 18 months post dose 5 of fractional delayed schedule

X=group comparisons

Table 7 Incremental efficacy analyses against first or only episodes of incident infection

Risk Period (months)	Full dose groups, control	Fractional dose groups, control			Objectives
		Fx012-14	Fx017-20	Control	
Exposed set					
D0-M20	R012-20	x			• Prolonged follow-up period for primary objective
	R012-14	x			
Per protocol set					
M1-M7	Control		x		• VE of delayed fractional third dose
M2.5-M20	R012-20	x		x	• Prolonged follow-up period for primary objective • Vaccine efficacy of early fractional/full dose schedule • Incremental efficacy of full dose (standard schedule versus early booster) schedule
	R012-14			x	
	Control	x			
	R012-14 vs R012-20				
M14-M20	R012-14	x		x	• Incremental efficacy 6 months post dose 4 (fractional vs full dose) • VE 6 months post dose 4 of early fractional- and full dose schedule
	Control	x			

X=group comparisons

7.3.2.3. Excluded analyses for efficacy

The following protocol defined analyses will not be performed anymore. These will be addressed in the ancillary MALARIA-095 study.

Incident infection:

Analysis performed at Month 33:

- Vaccine efficacy of R012-20 versus Control group over D0-M26 (all episodes, ES).
- Vaccine efficacy of R012-14 versus Control group over D0-M26 (all episodes, ES).
- Vaccine efficacy of Fx012-14 versus Control group over D0-M26 (all episodes, ES).
- Vaccine efficacy of Fx017-20 versus Control group over D0-M26 (all episodes, ES).
- Incremental efficacy of Fx017-20 versus R012-20 over M20-M32 (all episodes, PPS).
- Incremental efficacy of Fx012-14 versus R012-14-mD over M14-M26 (all episodes, ES).

Analysis performed at Month 50:

- Vaccine efficacy of R012-20 versus Control group over D0-M38 (all episodes, ES).
- Vaccine efficacy of R012-20 versus Control group over D0-M50 (all episodes, ES).
- Vaccine efficacy of R012-14 versus Control group over D0-M38 (all episodes, ES).
- Vaccine efficacy of R012-14 versus Control group over D0-M50 (all episodes, ES).
- Vaccine efficacy of Fx012-14 versus Control group over D0-M38 (all episodes, ES).
- Vaccine efficacy of Fx012-14 versus Control group over D0-M50 (all episodes, ES).
- Vaccine efficacy of Fx017-20 versus Control group over D0-M38 (all episodes, ES).
- Vaccine efficacy of Fx017-20 versus Control group over D0-M50 (all episodes, ES).
- Incremental efficacy of Fx012-14 versus R012-14-mD over M26-M38 (all episodes, PPS).
- Incremental efficacy of Fx012-14 versus R012-14-mD over M38-M50 (all episodes, PPS).

7.3.2.4. Additional considerations for efficacy analyses

Schoenfeld residual test will be used to test the independence between residuals and time. This test will be performed on the primary objective and on the secondary objective of incremental efficacy over the 12-month period post-dose 3 of a schedule with a fractional dose at month 7 versus 3 full doses

7.3.2.5. Additional considerations for analyses for cases averted

For each site and overall, the number of cases of clinical malaria will be calculated (difference between incidences of control group and vaccine groups, expressed per 1000 population vaccinated) for each 3-monthly time period and totaled over the follow-up periods month [0-14], [0-26], [0-32], [0-38] and [0-50].

7.3.2.6. Additional considerations for exploratory analyses

Malaria species
A listing of cases with *P. ovale* parasite, *P. malariae* malaria and *P. vivax* parasites by group will be provided.

Waning efficacy
Waning efficacy will be explored by plots of hazard ratios of study groups versus the control over time. Piece wise cox models will be performed comparing Fx017-20, Fx012-14, R012-20, R012-14 to the control over periods of 6 months to estimate hazard ratios. The Hazard ratios obtained from the models will be plotted in graphs.

7.4. Immunogenicity

7.4.1. Analysis of immunogenicity planned in the protocol

The primary analysis of immunogenicity will be based on the PPS for immunogenicity; however, analyses on the ES will also be performed.

In the immunogenicity subset (first 50 subjects enrolled in each group), the percentage of subjects with seropositive levels of anti-CS (proportion of subjects with anti-CS antibody concentrations greater than or equal to 1.9 EU/ml) with 95% CI will be determined at specified blood sampling timepoints in each group (see Section 2 – Sampling schedule). Anti-CS antibody concentrations will be summarized by geometric mean concentrations (GMCs) with 95% CI. Anti-CS antibody concentrations one month after the third dose and each subsequent dose of study vaccine will also be investigated using reverse cumulative distribution (RCD) curves.

The seroprotective level for anti-HBs is ≥ 10 mIU/ml. In the immunogenicity subset, the percentage of subjects with protective levels of anti-HBs (≥ 10 mIU/ml) with 95% CI will be determined at specified blood sampling timepoints in each group (see Section 2 – Sampling schedule). Anti-HBs antibody concentrations will be summarized by GMCs with 95% CI. Anti-HBs antibody concentrations one month after the third dose and each subsequent dose of study vaccine will also be investigated using RCD curves.

For the analysis of the tertiary objective, avidity/affinity index and avidity maturation will be summarized by mean, SD, median and quartile. CCI [redacted]

7.4.2. Additional considerations

Statistical methods for Geometric Mean Concentrations

GMCs and associated two-sided 95% CIs will be computed for each group at each available immunogenicity monitoring timepoint. The 95% CI for the mean of log-transformed concentration will be obtained assuming that log-transformed concentrations are normally distributed with unknown variance. Logarithmic transformations use base 10. The 95% CI for the GMC will then be obtained by exponential transformation (base 10) of the 95% CI for the mean of the log-transformed concentrations.

Reverse cumulative distribution curves

RCD curves for antibody concentrations will be plotted: the x-axis represents the antibody concentrations value (log-10 scale), while the y-axis represents the percentage of subjects having a log-transformed antibody value greater or equal to the corresponding x-value.

Statistical methods for seropositive/seroprotection rates

The percentage of subjects seropositive/seroprotected and associated two-sided 95% Clopper-Pearson CIs will be computed by vaccine group at each available immunogenicity monitoring.

CCI

[redacted]

7.5. Analysis of safety and reactogenicity

7.5.1. Analysis of safety and reactogenicity planned in the protocol

The primary analysis of safety will be based on the ES. Analyses containing only solicited data will be based on the solicited safety set.

Among the reactogenicity sub-cohort (first 50 subjects enrolled in each group) 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 solicited follow-up period (i.e. four days post-Dose 3, Dose 4, Dose 5 and Dose 6) will be tabulated with exact 95% CI after each vaccine dose and overall. The percentage of doses followed by at least one local AE (solicited and unsolicited), by at least one general AE (solicited and unsolicited) and by any AE will be tabulated for the whole solicited follow-up period (i.e. four days post-Dose 3, Dose 4, Dose 5 and Dose 6), with exact 95% CI. Similar tables will be generated for Grade 3 AEs and AEs considered as causally related to vaccination.

Among the reactogenicity sub-cohort (first 50 subjects enrolled in each group), the percentage of subjects reporting each individual solicited local and general AE during the solicited follow-up period (i.e. four days post-Dose 3, Dose 4, Dose 5 and Dose 6) will be tabulated with exact 95% CI. The percentage of doses followed by each individual solicited local and general AE will be tabulated for each dose and for the whole solicited follow-up period (i.e. four days post-Dose 3, Dose 4, Dose 5 and Dose 6), with exact 95% CI. Similar tables will be generated for Grade 3 AEs, causal events and for fever, temperature in 0.5°C increments.

The verbatim reports of unsolicited symptoms will be reviewed by a physician and the signs and symptoms will be coded according to the MedDRA. The percentage of subjects with at least one report of an unsolicited AE classified by the MedDRA preferred term level, occurring within 30 days (day of vaccination and 29 subsequent days) after each dose of study vaccine will be tabulated with exact 95% CI.

The percentage of subjects reporting an SAE (all, fatal, related) occurring within 30 days (day of vaccination and 29 subsequent days) after each dose of study vaccine, classified by the MedDRA preferred term level will be tabulated with exact 95% CI.

The percentage of subjects reporting an SAE (all, fatal, related) from Dose 1 (Day 0) until Month 14, from Day 0 until Month 21, from Day 0 until Month 26, from Day 0 until Month 33 and over the whole study duration (Dose 1 [Day 0] until Month 50), classified by the MedDRA preferred term level, will be tabulated with exact 95% CI.

The percentage of subjects reporting an AE or SAE leading to withdrawal from further vaccination from Dose 1 (Day 0) until Month 50, classified by the MedDRA preferred term level, will be tabulated with exact 95% CI.

The percentage of subjects reporting severe malaria cases and cerebral malaria cases from Day 0 up to Month 14, Day 0 up to Month 21, Day 0 up to Month 26, Day 0 up to Month 33 and Day 0 up to Month 50 and over successive years within the study will be tabulated with exact 95% CI.

The percentage of subjects reporting pIMDs from Day 0 up to Month 14, Day 0 up to Month 21, Day 0 up to Month 26, Day 0 up to Month 33 and Day 0 up to Month 50, classified by the MedDRA preferred term level will be tabulated with exact 95% CI.

The percentage of subjects reporting meningitis from Day 0 up to Month 14, Day 0 up to Month 20, Day 0 up to Month 26, Day 0 up to Month 32 and Day 0 up to Month 50 classified by the MedDRA preferred term level will be tabulated with exact 95% CI.

The percentage of subjects reporting seizures occurring within 30 days (day of vaccination and 29 subsequent days) after each dose of study vaccine, classified by the MedDRA preferred term level will be tabulated with exact 95% CI.

For generalized convulsive seizures occurring within seven days following a dose of study vaccine, an analysis will be performed based on the Brighton Collaboration guidelines [Bonhoeffer, 2004]. This includes descriptive tables of the time relationship of seizures to vaccination, the duration of seizures and the level of diagnostic certainty.

Biochemistry (ALT, creatinine) and hematology (hemoglobin, WBC, platelets) values that are outside of the reference ranges (see protocol section 7.5.2.1) will be described for the reactogenicity sub-cohort (first 50 subjects enrolled in each group) before Dose 3, seven days post-Dose 3 and 30 days post-Dose 3. Frequency distribution of results by toxicity grades will be tabulated by group.

7.5.2. Additional considerations

Note that the study will be converted in CDISC. Accordingly Day 0-Day N will be replaced by Day 1-Day N+1 for the statistical analysis.

7.5.2.1. Clinical Safety Laboratory Investigations

For the analysis of hematology and biochemistry parameters, the following grading scale will be used:

Test	Acceptable limit/Normal range	Grade 1	Grade 2	Grade 3	Grade 4
Hemoglobin	≥ 8.0 g/dl	> 8.0 to 6.0 g/dl	> 6.0 to 5.0 g/dl	< 5.0 g/dl	< 5.0 g/dl and clinical signs of heart failure
WBC	≥ 4.0 x10^3/µl < 17 x10^3/µl	2.5 to 4.0 x 10^3/µl	1.5 to 2.4 x 10^3/µl	1.0 to 1.4 x 10^3/µl	< 1.0 x 10^3/µl
Platelets	≥ 100 x10^3/µl	50 to 99 x 10^3/µl	25 to 49 x 10^3/µl	< 25 x 10^3/µl	< 25 x 10^3/µl and clinical signs of bleeding
ALT	≤ 60 IU/L	1.1 to 2.5 x ULN	2.6 to 5.0 x ULN	5.1 to 10.0 x ULN	> 10.0 x ULN
Creatinine	≤ 60 µmol/L (or 0.6 mg/dl)	1.1 to 1.5 x ULN	1.6 to 3.0 x ULN	3.1 to 6.0 x ULN	> 6.0 x ULN or requires dialysis

ALT = alanine aminotransferase; ULN = upper limit of the normal range; WBC = white blood cells
Grading scale adapted from: [NIAID, 2004] Division of AIDS table for grading the severity of adult and pediatric adverse events, December 2004.

7.5.2.2. Concomitant Medication and Concomitant Vaccination

Medications will be coded using the GSKDRUG dictionary.

The frequencies and percentages of subjects reporting antipyretic medications (administered during seven days following each dose of study vaccine, Day 0 to Day 6) will be tabulated by vaccine group for each study dose and across doses. All concomitant medication will be provided in individual listings.

Antipyretics will be further considered prophylactic when administered in the absence of ANY symptom and in anticipation of a reaction to the vaccination.

Concomitant vaccinations administered in the period starting seven days before the first dose of study vaccines and ending at the last study visit (Day -7 to Month 50) will be listed.

7.5.2.3. Additional analysis for severe malaria

A listing for severe malaria cases with associated sign and symptoms will be provided. The same listing will be provided for cerebral malaria cases.

8. ANALYSIS INTERPRETATION

Vaccine efficacy estimates with a lower limit of the 95% CI above 0 are to be considered statistically significant.

If the standard schedule of 3 full doses at month 0, 1 and 2 is not statistically significantly superior to the control group in terms of efficacy, the other secondary objectives should be interpreted in the light of this finding.

In order to control alpha not only for the primary objective but also for the secondary objective of incremental efficacy over the 12-month period post-dose 3 of a schedule with a fractional dose at month 7 versus 3 full doses, a sequential approach will be implemented (hierarchical testing). The endpoints will be analyzed sequentially and any conclusion on the secondary endpoint will be conditional to reaching the primary endpoint. All other secondary endpoints should be interpreted descriptively.

9. CONDUCT OF ANALYSES

9.1. Sequence of analyses

All analyses (including interim analyses) will be conducted on data as clean as possible.

- Sequence of analyses and conditional continuation:
  - A first analysis will be performed on data collected up to Month 21 (Visit 25) to evaluate the primary objective (cleaned data) and relevant secondary objectives.
  - A second analysis will be performed on data collected up to Month 33 (Visit 31) to evaluate relevant secondary objectives.
  - A final analysis will be performed at study end (Month 50) on data collected up to Month 50. An integrated study report presenting all results until Month 50 will be produced.
- After the interim analyses, the Independent Data Monitoring Committee (IDMC) will have the possibility to recommend the suspension of further vaccinations and termination of specific study group(s) upon consideration of the study results. In the event that the IDMC recommend the suspension of further vaccinations, the group(s) impacted will be followed up for 12 months after their last study vaccination.

Description	**Disclosure Purpose (CTRS=public posting, SR=study report, internal)**
Final analysis E1_01	SR, CTRS
Analysis M21 E1_02	SR, CTRS
*Analysis M33 E1_03	CTRS

*The need for a SR will be evaluated at a later time point depending on regulatory need.

9.2. Statistical considerations for interim analyses

The analysis of the primary endpoint will be performed after Month 21 (Visit 25) on cleaned data collected up to Month 14. No alpha adjustment is foreseen because final analysis of the primary endpoint will occur at the time of the first interim analysis after Month 21 (Visit 25) on cleaned data collected up to Month 14. Data up to Month 21 (Visit 25) will also be as clean as possible.

10. CHANGES FROM PLANNED ANALYSES

The Total Vaccinated Cohort and the According To Protocol cohort have been renamed Exposed Set (ES) and Per-Protocol Set (PPS) respectively.

The seropositivity threshold for anti-CS has been updated from 0.5 EU/ml to 1.9 EU/ml.

A Solicited Safety Set has been added in the analysis set section to fit with the new statistical rules and layouts applicable from the 12th of November 2018.

For purposes of analyses and to simplify the naming convention of the groups in Figure 1 haven been simplified. The label names of the study groups have been renamed according to the following table:

Vaccination schedule	**Group label in protocol**	**Group label in SAP**
RTS,S/AS01$_E$ full dose given at Month 0, Month 1, Month 2 and a fourth dose at Month 20	R012-20	R012-20
RTS,S/AS01$_E$ full dose at Month 0, Month 1, Month 2, an early fourth dose at Month 14 and yearly full doses at Month 26 and Month 38	R012-14-mD	R012-14
RTS,S/AS01$_E$ full dose at Month 0 and Month 1, RTS,S/AS01$_E$ fractional dose (1/5th dose) at Month 2 and yearly fractional doses at Month 14, Month 26, Month 38	Fx012-14-mFxD	Fx012-14
RTS,S/AS01$_E$ full dose at Month 0 and Month 1, RTS,S/AS01$_E$ delayed fractional third dose (1/5th dose) at Month 7 and yearly fractional doses at Month 20 and Month 32	Fx017-mFxD	Fx017-20
Rabies vaccine at Month 0, Month 1 and Month 2	Control	Control

A sequential approach to the analysis of incremental efficacy over the 12-month period post-dose 3 of a schedule with a fractional dose at month 7 versus 3 full doses was implemented (see section 8).

The following analyses were added or modified:

- Unsolicited adverse events within 7 days after each vaccination will be described in addition to the 30 days analysis
  - Analyses on the occurrence of PIMDs (described in section Secondary endpoints) were added in section Analysis of safety and reactogenicity planned in the protocol, as they were missing in the protocol
- Secondary endpoint for efficacy
  - Analyses considering different calendar follow-up time was updated. In the protocol an indirect comparison was suggested taking into account incidence in controls, while for the SAP another proposal is suggested as described in section 7.3.1.1
  - Analysis of incident infection will only be performed up to M20 since infection with *P. falciparum* was regularly (monthly) evaluated until this time point.
  - Analysis of prevalent infection will include a model of time to all episodes to have a summary VE measure up to M20 (described in section 7.3.2.1)
  - Additional analysis for endpoints of clinical malaria and incident infection were added mainly to investigate shorter periods (6-monthly post booster dose efficacy7.3.2 (described in section 7.3.2.2).
  - The wording of the impact analyses as planned per protocol has been corrected from “first or only and all episodes” to “all episodes” only (see section 7.3.1.6).
  - The wording of the prevalent *P. falciparum* infections analyses as planned per protocol has been changed to “Prevalent *P. falciparum* infections at each cross-sectional survey” removing “up to Month 14”, “up to Month 20 “, “up to Month 26”, “up to Month 32 “, “up to Month 38” and “up to Month 50 “, to improve readability (see section 7.3.1.6).
  - For all episodes analyses fourteen (instead of seven) days following an episode meeting the case definition will be subtracted from the follow-up time, to be consistent with analyses performed in MALARIA-055

11. ANNEXES

11.1. Business rules for standard data derivations and statistical methods

This section contains GSK Vaccines' standard rules for data display and derivation for clinical and epidemiological studies. These rules will be applied along with those detailed in section 10 (additional study-specific rules)

11.1.1. Handling of missing data

11.1.1.1. Dates

When partially completed dates (i.e. with missing day or month) are used in calculations, the following standard rules will be applied:

- A missing day will be replaced by 15
- A missing day and month will be replaced by June 30th.

The following exceptions apply:

- Adverse event start dates with missing day:
  - If the event starts in the same month as at least one of the study doses, the contents of AE.AESTRTPT (the flag indicating if the event occurred before or after vaccination) will be used to complete the date. If 'after vaccination' is selected, the imputed start date will match the first (or only) study dose given during that month. If 'before vaccination' is selected, the imputed date will be one day before the first (or only) study dose given during that month.
- Adverse event start dates with missing day and month:
  - If the event starts in the same year as at least one of the study doses, the contents of AE.AESTRTPT (the flag indicating if the event occurred before or after vaccination) will be used to complete the date. If 'after vaccination' is selected, the imputed start date will match the first (or only) study dose given during that year. If 'before vaccination' is selected, the imputed date will be one day before the first (or only) study dose given during that year.

All other cases of incomplete AE or concomitant medication/vaccination start date will follow the standard rules above.

11.1.1.2. Laboratory data

Missing laboratory results (including immunological data) will not be replaced.

11.1.1.3. Daily recording of solicited symptoms

11.1.1.3.1. Studies with electronic diaries

For studies using electronic diaries for the collection of solicited symptoms, symptoms will be considered present only when a daily recording of grade 1 or more is present.

11.1.1.3.2. Studies with paper diaries

For studies using paper diaries which have questions in the CRF indicating the presence or absence of solicited symptoms, the following rules are applicable.

Denominators for the summary of local (or general) solicited symptoms will be calculated using the number of subjects who respond "Yes" or "No" to the question concerning the occurrence of local (or general) symptoms.

When a specific symptom is marked as having not occurred following a specific vaccination (i.e. SDTM CE.CEOCCUR=N for the specified post-vaccination period for the symptom in question), all daily measurements will be imputed as Grade 0.

When a specific symptom is marked as having occurred following a specific vaccination (i.e. SDTM CE.CEOCCUR=Y for the specified post-vaccination period for the symptom in question), any missing daily recordings will be given imputed values to allow them to contribute to the 'Any' rows but not to specific grade rows of the symptom summary tables.

When the occurrence of a specific symptom is not present (i.e. SDTM CE.CEOCCUR is neither Y nor N for the specified post-vaccination period for the symptom in question) but the group of symptoms (local or general) is marked as having occurred (i.e. SDTM CE.CEOCCUR=Y), all missing daily recordings will be given imputed values to allow them to contribute to the 'Any' rows but not to specific grade rows of the symptom summary tables.

The following table shows how subjects contribute to each category for a specific solicited symptom over the Day X to Day Y post-vaccination period:

Solicited symptom category	Subjects included in the calculation of the numerator
Any	All subjects with at least one occurrence of the symptom at grade 1, grade 2, or grade 3 between Day X and Day Y or with the symptom marked as present and at least one missing daily recording between Day X and Day Y
At least grade 1	All subjects with at least one occurrence of the symptom at grade 1, grade 2, or grade 3 between Day X and Day Y
At least grade 2	All subjects with at least one occurrence of the symptom at grade 2 or grade 3 between Day X and Day Y
At least grade 3	All subjects with at least one occurrence of the symptom at grade 3 between Day X and Day Y

11.1.1.4. Unsolicited adverse events

Missing severity, relationship with study vaccine, and outcome of unsolicited adverse events will not be replaced and will appear as 'UNKNOWN' in all statistical output.

11.1.2. Data derivation

11.1.2.1. Age at vaccination in days

When age at vaccination is to be displayed in days, it will be calculated as:

Age = date of vaccination minus date of birth

11.1.2.2. Age at vaccination in months

When age at vaccination is to be displayed in months, it will be calculated as the number of complete calendar months between the date of birth (DOB) and the date of vaccination. For example:

DOB = 10JUN2017, Date of vaccination = 09JUL2018 -> Age = 12 months

DOB = 10JUN2017, Date of vaccination = 10JUL2018 -> Age = 13 months

11.1.2.3. Age at vaccination in years

When age at vaccination is to be displayed in years, it will be calculated as the number of complete calendar years between the date of birth and the date of vaccination. For example:

DOB = 10SEP1983, Date of vaccination = 09SEP2018 -> Age = 34 years

DOB = 10SEP1983, Date of vaccination = 10SEP2018 -> Age = 35 years

11.1.2.4. Weight

Weight will be presented in kilograms. Weights reported in pounds will be converted as follows:

Weight in kilograms = Weight in pounds / 2.2

11.1.2.5. Height

Height will be presented in centimeters. Heights reported in feet and inches will be converted as follows:

Height in centimeters = Height in inches x 2.54

11.1.2.6. Body mass index (BMI)

BMI will be calculated as follows:

$$BMI = (\text{Weight in kilograms}) / (\text{Height in meters})^2$$

11.1.2.7. Temperature

Temperatures will be presented in degrees Celsius (°C). Temperatures reported in degrees Fahrenheit (°F) will be converted as follows:

$$\text{Temperature (Celsius)} = ((\text{Temperature (Fahrenheit)} - 32) \times 5)/9$$

11.1.2.8. Numerical serology results

Numerical serology results will be derived from the content of IS.ISORRES in the SDTM dataset. For assays with a specific cut-off, the following derivation rules apply:

IS.ISORRES	Derived value
"NEG", "-", or "(-)"	cut-off/2
"POS", "+", or "(+)"	cut-off
"< value" and value is <= assay cut-off	cut-off/2
"< value" and value is > assay cut-off	value
"> value" and value is < assay cut-off	cut-off/2
"> value" and value is >= assay cut-off	value
"value" and value is < cut-off	cut-off/2
"value" and value is >= cut-off	value
All other cases	missing

11.1.2.9. Geometric mean titers (GMTs) and concentrations (GMCs)

Geometric Mean Titre (GMT) or Concentration (GMC) calculations are performed by taking the inverse logarithm of the mean of the log titre or concentration transformations. Antibody titres or 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 GMT/GMC calculation. The cut-off value is defined by the laboratory before the analysis.

11.1.2.10. Onset day

The onset day for an event (e.g. AE, medication, vaccination) is the number of days between the last study vaccination and the start date of the event. This is 1 for an event occurring on the same day as a vaccination (and reported as starting after vaccination).

11.1.2.11. Duration of events

The duration of an event with a start and end date will be the number of days between the start and end dates plus one day, i.e. an event that starts on 03MAR2018 and ends on 12MAR2018 has a duration of 10 days.

The duration of solicited events will be calculated as the sum of the individual days with the symptom reported at grade 1 or higher.

11.1.2.12. Counting rules for combining solicited and unsolicited adverse events

For output combining solicited and unsolicited adverse events, all serious adverse events will be considered general events since the administration site flag is not included in the expedited adverse event CRF pages.

Multiple events with the same preferred term which start on the same day are counted as only one occurrence.

11.1.2.13. Counting rules for occurrences of solicited adverse events

When the occurrences of solicited adverse events are summarized, each event recorded as having occurred during a specific period will be counted as only one occurrence regardless of the number of days on which it occurs. Also, in the case of co-administered study vaccines, an injection site reaction recorded for a subject following multiple vaccines will be counted as only one occurrence.

11.1.3. Display of decimals

11.1.3.1. Percentages

Percentages and their corresponding confidence limits will be displayed with:

- no decimals when there are fewer than 50 subjects in each tabulated group
- one decimal when there are at least 50 subjects in at least one tabulated group
  - Exceptions will be made for percentages that are not 0% or 100% but appear as 0% or 100% due to rounding. For these specific cases the number of decimals will be increased until the displayed value is no longer 0% or 100%. Examples are given in the following table.

n/N	Displayed percentage
10/45	22%
1/45	2%
10/55	18.2%
1/55	1.8%
1/300	0.3%
1/3000	0.03%
1/30000	0.003%
299/300	99.7%
2999/3000	99.97%
29999/30000	99.997%

- The display of additional decimals for values close to 0% or 100% will be applied only to point estimates and not confidence limits, which can be rounded and displayed as 0% or 100%.
- Values of exactly 0% or 100% will be presented with no decimals regardless of the number of subjects per tabulated group.

11.1.3.2. Differences in percentages

Differences in percentages and their corresponding confidence limits will be displayed with one more decimal than the maximum number used to display the individual percentages, for example the difference between two percentages displayed with one decimal will be displayed with two decimals.

11.1.3.3. Demographic/baseline characteristics statistics

The mean, median, and standard deviation for continuous baseline characteristics (height, weight, BMI, pre-vaccination body temperature) will be presented with one decimal.

The minimum and maximum values and quartile values (if required) will be presented with the same number of decimals as the observed values.

The maxima and minima of transformed height variables will be displayed with no decimals.

The maxima and minima of transformed weight variables will be displayed with no decimals with the exception of values are below 10kg where one decimal will be displayed.

The maximum and minima of transformed body temperatures will be displayed with one decimal.

11.1.3.4. Serological summary statistics

The number of decimals used when displaying GMTs or GMCs and their confidence limits is shown in the following table:

GMT or GMC value	Number of decimals to display
<0.1	3
>=0.1 and <10	2
>=10 and <1000	1
>=1000	0

When multiple categories of GMT or GMC values are present in the same table, the number of decimals displayed should match that of the smallest category (i.e. the one with the higher number of decimals). For example, if GMT or GMC values of <0.1 appear in the same table as values of >=0.1 and <10, 3 decimals should be displayed for both.

GMT or GMC ratios and their confidence limits will be displayed with 2 decimals regardless of the actual values.

11.1.4. Statistical methodology

11.1.4.1. Exact confidence intervals around proportions

The exact CIs around within-group proportions are derived using the method of Clopper and Pearson [Bonhoeffer, 2004; Clopper, 1934].

11.1.4.2. Standardized asymptotic confidence intervals around differences in proportions

The standardized asymptotic CIs around differences in proportions are derived using the method of Mietinnen and Nurminen [Miettinen, 1985].

11.1.4.3. Adjusted GMT or GMC ratios

When between-group GMT or GMC ratios are computed and adjusted for two-level categorical co-variables, these co-variables should be included as dummy continuous variables in the Statistical Analysis System (SAS) procedure.

11.1.4.4. Vaccine efficacy

Vaccine efficacy with adjustment for time-to-first-event is calculated using a Cox's Proportional Hazards regression model [Cox, 1972]. All covariates to be included in the regression model should be detailed in the main body of the Statistical Analysis Plan (SAP). The between-group hazard ratio (HR) and its confidence limits are computed using the model, and vaccine efficacy is derived as 100 x (1-HR). The corresponding vaccine efficacy confidence limits are 1 minus each of the HR confidence limits. When

applying a Cox's Proportional Hazards regression model, the proportional hazards assumption should be verified by means of a Schoenfeld residual plot (Schoenfeld residuals versus time) and a log cumulative hazard plot (the log of the cumulative hazard versus the log of the survival time).

If either of these methods is expanded upon (e.g. to take multiple events into account), additional methodological details and references should be included in the main body of the SAP.

11.2. TFL and/or TFL ToC

The Tables Figures and Listings (TFL) Table of Contents (ToC) provides the list of tables/listings and figures needed for the study report. It also identifies the tables eligible for each analysis and their role (synopsis, in-text, post-text, Study Headline Summary [SHS], Clinical Trial Registry Summary [CTRS], Annex). The TFL and/or the TFL ToC can be found in eTMF folder section 11.01.01.

12. REFERENCES

Bonhoeffer J, Menkes J, Gold MS et al. Generalized convulsive seizure as an adverse event following immunization: case definition and guidelines for data collection, analysis and presentation. *Vaccine*. 2004; 22:557-562.

Clopper CJ, Pearson E. The Use of Confidence or Fiducial Limits Illustrated in the case of the Binomial. *Biometrika.* 1934;26:404-13.

Cox, D. R. Regression Models and Life-Tables. Journal of the Royal Statistical Society. Series B (Methodological), 1972;34:187–220.

Miettinen, O. S. and Nurminen, M. Comparative analysis of two rates. Statistics in Medicine, 1985;4,213-226.

National Institute of Allergy and Infectious Diseases (NIAID). National Institutes of Health (NIH). Division of AIDS table for grading the severity of adult and pediatric adverse events, December 2004. http://www.niaid.nih.gov/labsandresources/resources/daidsclinrsrch/documents/daidsaegradingtable.pdf. Accessed: 25 January 2016.

WHO. Guidelines for the treatment of malaria. 3rd Edition. 2015. http://www.who.int/malaria/publications/atoz/9789241549127/en/. Accessed: 25 January 2016.