GlaxoSmithKline

Statistical Analysis Plan

Protocol Title:	An open extension to the phase III, multi-center study MALARIA-055 PRI (110021) to evaluate long-term efficacy, safety and immunogenicity of the RTS,S/AS01$_E$ candidate vaccine against malaria disease caused by *Plasmodium falciparum* in infants and children in Africa.
eTrack study number	200599
eTrack abbreviated title	MALARIA-076
Protocol version/date	Amendment 1 (18 February 2014)
Scope:	Safety analyses for Nanoro site year 1 follow up
Version:	Final
Date:	12 October 2015
Co-ordinating author:	PPD (GSK)
Other author(s):	

Approved by:

Clinical Development Manager	Name	Signature	dd-mmm-yyyy
Lead Statistician	Name	Signature	dd-mmm-yyyy
Project Statistician	Name	Signature	dd-mmm-yyyy

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The analysis plan is divided into 2 parts: the first part detailing the analyses to be performed (SAP) and a second part, annex (-es) (called TFL) describing the flow and format of tables, figures and listings to be annexed to the SR.

1. DOCUMENT HISTORY

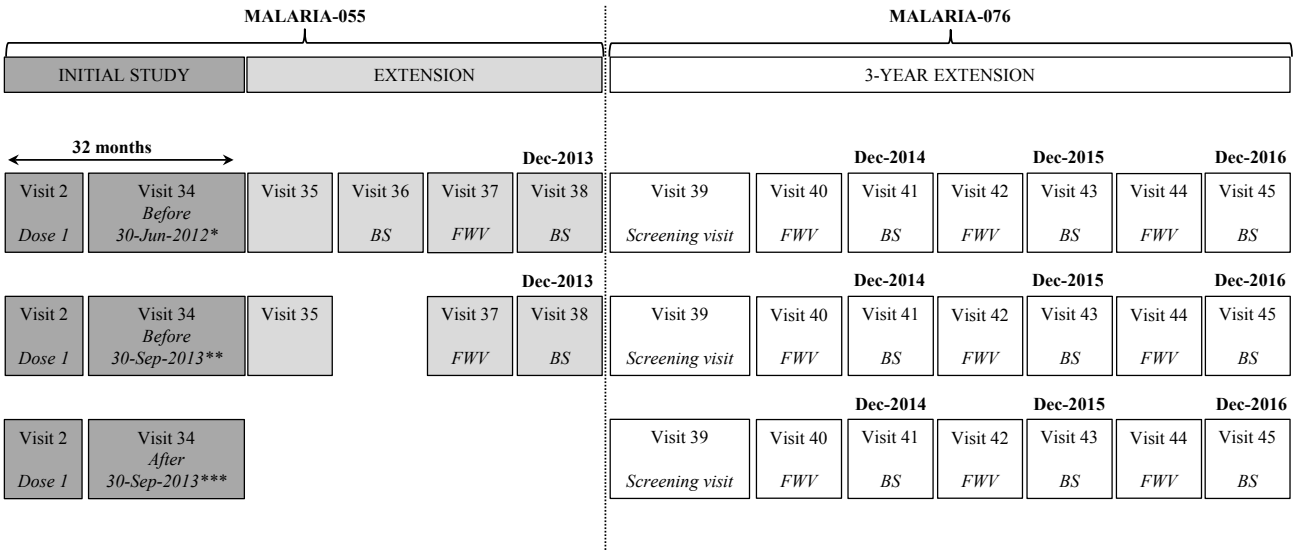
Date	Version	Description
28 Sep 2015	Draft	
12 October	Final	

2. STUDY DESIGN

2.1. Introduction

This Statistical Analysis Plan (SAP) provides detailed descriptions of the statistical analyses of safety endpoints on all subjects followed up until 31 December 2014. All analyses will be presented for each age category (6-12 weeks and 5-17 months at first vaccination) separately. The protocol specifies efficacy, safety and immunogenicity objectives in 3 sites. However, due to delays in protocol approvals only one of sites (Nanoro) has collected the first year of follow up at this point in time. Also, the protocol primary objective to describe the incidence of severe malaria according to a specific case definition will not be performed. Due to late protocol approvals and retrospective data collection the sites did not collect the data according to protocol and as such the case definition cannot be applied. Severe malaria will therefore be evaluated on malaria SAEs reported (preferred terms Malaria, Plasmodium falciparum infection and Cerebral malaria). The secondary objective to describe the incidence of clinical malaria will not be looked at now but will be analysed at a later time point. Lastly, due to the unavailability of the anti-CS assay, secondary immunogenicity objectives will also be reported at a later stage.

2.2. Design



* Subjects that had their last contact in the primary trial phase (Visit 34) BEFORE (and including) 30-Jun-2012, had 3 clinic visits and one field workers visit in the extension part of MALARIA-055 PRI.
** Subjects that had their last contact in the primary trial phase (Visit 34) BETWEEN 01-Jul-2012 and 30-Sep-2013 had 2 clinic visits and one field workers visit in the extension part of MALARIA-055 PRI.
*** Subjects that had their last contact in the primary trial phase (Visit 34) AFTER 30-Sep-2013 were not enrolled in the extension part of MALARIA-055 PRI.
BS: blood sampling; FWV: Field Worker Visit

- Experimental design: extension to the phase III, randomized, controlled, multi-centric study MALARIA-055 PRI (110021) that comprised 3 parallel groups
- Duration of the study: approximately 3 years
  - Epoch 001: starting at Visit 39, including and ending at Visit 41 (December 2014).
  - Epoch 002: starting at Visit 42, including and ending at Visit 43 (December 2015).
  - Epoch 003: starting at Visit 44, including and ending at Visit 45 (December 2016).
- Study groups: same study groups as in the primary study MALARIA-055 PRI (110021). Subjects enrolled in each of the 2 age categories (5-17 months and 6-12 weeks at first vaccination) were randomized in 3 study groups:
  - R3C: infants/children randomized to receive 3 doses of RTS,S/AS01$_E$ on a 0-, 1-, 2-month schedule with a dose of comparator vaccine at Month 20 during the primary study MALARIA-055 PRI (110021).
  - R3R: infants/children randomized to receive 3 doses of RTS,S/AS01$_E$ on a 0-, 1-, 2-month schedule with an RTS,S/AS01$_E$ booster dose at Month 20 during the primary study MALARIA-055 PRI (110021).

– C3C: infants/children randomized to receive 3 doses of a comparator vaccine on a 0-, 1-, 2-month schedule with a dose of comparator vaccine at Month 20 during the primary study MALARIA-055 PRI (110021).

3. OBJECTIVES

A complete list of protocol objectives and endpoints is available in the protocol. The current SAP describes analyses to be performed on SAEs collected from January 2014 (end of extension MAL055 PRI) and December 2014 (Year 1 follow up of MAL 076) in the Nanoro site. SAEs summaries pooling MAL 055 and MAL 076 over the complete follow up will also be generated.

3.1. Secondary objectives

3.1.1. Safety objective

- To describe the incidence of the following reported serious adverse events (SAEs): fatalities, related SAEs (related to vaccine administration in the primary study MALARIA-055 PRI [110021] and to study participation), malaria hospitalization, potential Immune-Mediated Disease (pIMDs), ***and*** meningitis from January 2014 to December 2016.

4. ENDPOINTS

4.1. Secondary endpoints

4.1.1. Safety endpoint

Protocol endpoint:

- The occurrence of the following reported SAEs: fatalities, related SAEs (related to vaccine administration in the primary study MALARIA-055 PRI [110021] and to study participation), malaria hospitalization, pIMDs, ***and*** meningitis over the time period starting January 2014 until the end of the 3-year follow-up period (Visit 45).

Analysis endpoints:

Analysis endpoints are restricted to the Nanoro Site:

Occurrence of malaria SAEs until the end of year 1 follow up (visit 41, December 2014).

Incidence of all episodes of malaria SAEs until the end of year 1 follow up (visit 41, December 2014).

Occurrence of fatal SAEs until the end of year 1 follow up (visit 41, December 2014).

Occurrence of related SAEs until the end of year 1 follow up (visit 41, December 2014).

Occurrence of pIMDs until the end of year 1 follow up (visit 41, December 2014).

Occurrence of meningitis SAEs until the end of year 1 follow up (visit 41, December 2014).

5. STUDY POPULATION

5.1. Modified Intention to treat population (ITT)

ITT classically includes all subjects randomized. However, for operational reasons randomized subjects who did not receive study vaccine are not followed-up further. Therefore, the modified ITT population will include all subjects that received at least 1 dose of study vaccine (RTS,S/AS01$_E$ or Control). The analyses on the ITT population will be performed per treatment assignment.

As there are different timeframes to be evaluated this translates in:

ITT [0-DEC14] and ITT [0-20]: N=number of subjects in Nanoro receiving dose 1 in MAL 055 PRI

ITT [21-DEC13]: N= number of subjects in Nanoro receiving dose 1 and have follow up time in the timeframe [21-DEC13]

ITT [JAN14-DEC14]: N=number of subjects in Nanoro consenting to MAL 076

5.2. Risk periods

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 (end date follow up – start date follow up +1).

Table 1 Time at risk definitions

Risk Period	Start	Stop
ITT [0-DEC14] (055 PRI + 076)	Day of first vaccination of RTS,S or control vaccine in MAL 055 PRI	For subjects not consenting to MAL 076: Date of visit 38 (MAL 055 PRI) or drop out date from end of extension, whichever occurs first For subjects consenting to MAL 076: 31 December 2014
ITT [0-20] (055)	Day of first vaccination of RTS,S or control vaccine in MAL 055 PRI	Date of month 20 visit (MAL 055 PRI) or drop out date from month 20 conclusion, whichever occurs first
ITT [21-DEC13] (055)	Day of 4th dose in MAL 055 PRI Subjects not receiving the booster dose will start FU at month 20 visit date+1 or last contact date+1 if visit not performed	31 December 2013
ITT [JAN14-DEC14] (076)	For subjects consenting to MAL 076: 1 January 2014	For subjects consenting to MAL 076: 31 December 2014

For subjects who consented to the extension phase after visit 34 (month 32 visit) in MAL 055 PRI, time at risk will be adjusted by removing the time gap between the month 32

visit and the start of the extension phase (defined as consent date). The MAL 076 protocol includes retrospective data collection and as a result consenting subjects are considered at risk as of the end of MAL 055 PRI.

6. STATISTICAL ANALYSES

6.1. Analysis on all infants (6-12w) and children (5-17m)

All analyses described below (demography and safety) will be performed on the ITT population. All analyses will be presented separately for both age categories.

6.1.1. Demography

A study flow diagram (consort) will be generated to present the number of subjects enrolled in MAL 055 PRI, completed visit 34 and consented to the extension and were seen at the last study contact in December 2013. Reasons for non-attendance will be summarized. The number of subjects approached and eligible for MAL-76 will be tabulated along with the number of subjects completing visit 41 (year 1 follow up, December 2014). Reasons for non-attendance will also be summarized.

Baseline characteristics at enrolment in MAL-076 (age, gender) will be tabulated by group.

Follow up time from dose 1 in MAL 055 PRI until December 2014 (Visit 41) will be tabulated by study group by age category. Follow-up time will be calculated in number of months between Dose 1 and the time at which study participation ended (i.e. visit 41 or censored). The number of months will be calculated as number of days divided by 30.4.

Categorical variables will be presented by percentages and numerical variables will be summarized by mean, standard deviation, median, minimum and maximum.

6.1.2. Safety

6.1.2.1. Occurrence of SAEs

Safety will be evaluated by examining SAEs for the 3 study groups from study start (Dose 1 in MAL 055 PRI) up to December 2014 (Visit 41 in MAL 076) over the risk periods defined in section 5.2 for the ITT population. All safety analyses will be performed separately for both age categories.

The proportion of subjects with SAEs (malaria PTs, fatal, related, meningitis/encephalitis, PTs and pIMDs), classified by the MedDRA preferred term level, will be tabulated by group with exact 95% CI.

6.1.2.2. Incidence of Malaria SAEs

Incidence rates of all episodes of malaria SAEs will be tabulated by group for risk periods defined in section 5.2

6.2. Individual data listings

All individual demography and safety data presented will be listed and appended to the study report.

6.3. Case definitions

6.3.1. Malaria SAEs

Malaria SAEs are defined as SAEs coded at Meddra preferred term level as 'Malaria', 'Plasmodium falciparum infection' or 'Cerebral malaria'.

6.3.2. Meningitis SAEs

Meningitis/encephalitis SAEs are defined as SAEs coded at LLT code, Meddra preferred term level as:

10027199 Meningitis

10027241 Meningitis haemophilus

10027249 Meningitis meningococcal

10027254 Meningitis salmonella

10027253 Meningitis pneumococcal

10027255 Meningitis staphylococcal

10027259 Meningitis tuberculous

10027242 Meningitis herpes

10027205 Meningitis candida

10027232 Meningitis enterococcal

10027233 Meningitis enteroviral

10058780 Meningitis neonatal

10048848 Meningitis toxoplasmal

10027250 Meningitis mumps

10027209 Meningitis cryptococcal

10027243 Meningitis histoplasma

10027258 Meningitis trypanosomal

10029339 Neurosyphilis

10027247 Meningitis leptospiral

10027248 Meningitis listeria

10008131 Meningitis in sarcoidosis code in PT "Cerebral sarcoidosis"

10027202 Meningitis bacterial

10027260 Meningitis viral

10027201 Meningitis aseptic

10027236 Meningitis fungal

7. CONDUCT OF ANALYSES

7.1. Sequence of analyses

For GSK internal use:

Description	Analysis ID (SDD sub-folder)
YEAR 1	E_02

7.2. Blinding

Open.

8. CHANGES FROM PLANNED ANALYSES

The analysis is restricted to year 1 follow up in the Nanoro site.

An analysis of incidence of malaria SAEs (all episodes over follow up time) was added.

Analyses of efficacy and immunogenicity will be performed at a subsequent timepoint.

9. ABBREVIATIONS

CI	Confidence Interval
GSK	GlaxoSmithKline
MedDRA	Medical Dictionary for Regulatory Activities
SAP	Statistical Analysis Plan
TFL	Tables Figures and Listing template annexed to SAP

Statistical Analysis Plan

Study alias & e-track number(s): MALARIA-076 (200599)

Detailed Title:	An open extension to the phase III, multi-center study MALARIA-055 PRI (110021) to evaluate long-term efficacy, safety and immunogenicity of the RTS,S/AS01E candidate vaccine against malaria disease caused by Plasmodium falciparum in infants and children in Africa.
SAP version	Version 3.1
SAP date	04-JUL-2017
Scope:	All data pertaining to the above study.
Co-ordinating author:	PPD
Other author(s):	PPD
Adhoc reviewers:	PPD (SERM Physician), PPD (SERM Scientist), PPD (RA DDW)
Approved by:	PPD (CRDL), PPD (Lead ScW), PPD (Acting Lead Stat), PPD (Director Early and Exploratory Clinical Statistics)

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The complete statistical analysis plan and results presentation is divided into 2 parts: the first part detailing the analyses to be performed (known as SAP, current document) and a second part, annex (-es) (called TFL) describing the flow and format of tables, figures and listings to be annexed to the SR.

LIST OF ABBREVIATIONS

$AS01_E$:	GSK's proprietary liposome-based Adjuvant System containing MPL and QS21
CI	Confidence Interval
CS	Circumsporozoite protein of Plasmodium falciparum
CTRS	Clinical Trial Registry
EL.U/ml	ELISA unit per milliliter
ELISA	Enzyme-linked immunosorbent assay
GMC	Geometric mean antibody concentration
GSK	GlaxoSmithKline
ITT	Intention-to-Treat
LAR	Legally Acceptable Representative
LL	Lower Limit of the confidence interval
M	Months
MAL-055	MALARIA-055 PRI (110021)
MAL-076	MALARIA-076 (200599)
MedDRA	Medical Dictionary for Regulatory Activities
pIMD	Potential Immune-Mediated Disease
RR	Risk Ratio
RTS,S	Particulate antigen, containing both RTS and S (hepatitis B surface antigen) proteins
RTS,S/AS	GSK Biologicals' candidate Plasmodium falciparum malaria vaccine adjuvanted with GSK Biologicals' proprietary Adjuvant Systems
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SBIR	GSK Biological's Internet Randomization System
SD	Standard Deviation

SR	Study Report
TFL	Tables Figures and Listing template annexed to SAP
UL	Upper Limit of the confidence interval
VE	Vaccine Efficacy
W	Weeks

1. DOCUMENT HISTORY

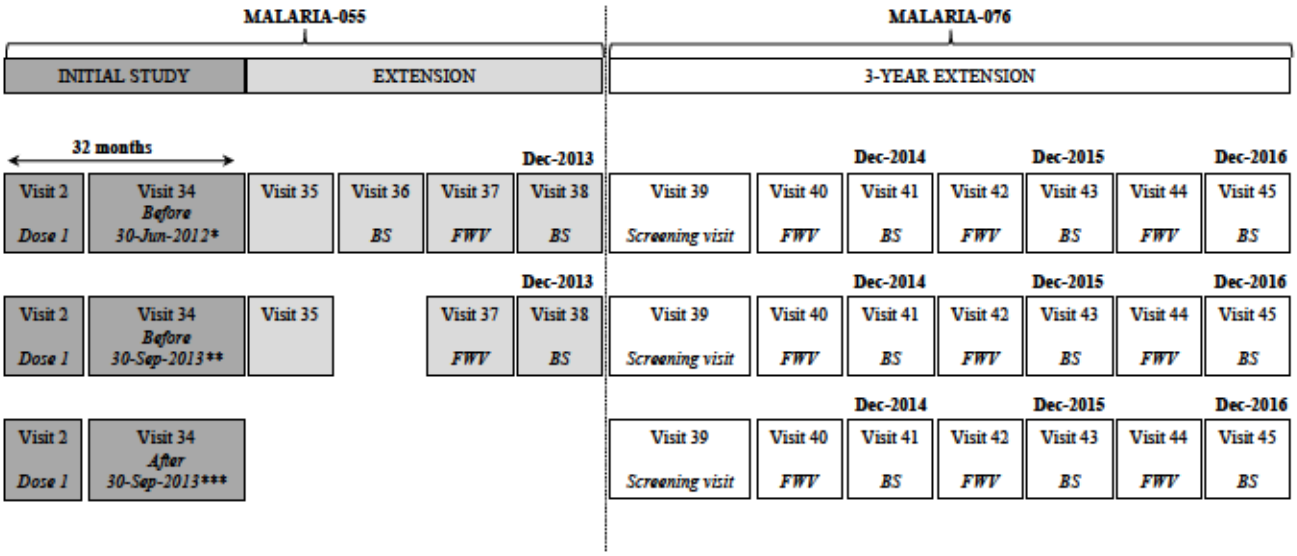
Date	Description	Protocol Version
12-OCT-2015	Version 1 : Safety analyses for Nanoro site year 1 follow up	Protocol Amendment 1 (18-FEB-2014)
09-AUG-2016	Version 2 : Year 2 follow-up Safety analysis	Protocol Amendment 2 (15-APR-2015)
18-MAY-2017	Version 3 final draft Final analysis for internal and IDMC review	Protocol Amendment 2 (15-APR-2015)
19-JUN-2017	Version 3 : Final analysis	Protocol Amendment 2 (15-APR-2015)
04-JUL-2017	Version 3.1 : updated risks periods B&C, Severe Malaria cases definition 1&2, some typo	Protocol Amendment 2 (15-APR-2015)

2. STUDY DESIGN

2.1. Introduction

This Statistical Analysis Plan (SAP) provides detailed descriptions of the statistical analyses of demography, efficacy, safety and immunogenicity endpoints for all subjects enrolled in this extension study and their corresponding data collected in the MAL-055 study. All analyses will be presented for each age category (6-12 weeks and 5-17 months at first vaccination in MAL-055) separately. Efficacy and immunogenicity analyses will be presented for each study site separately and overall. Safety analyses will be presented pooled across study sites.

2.2. Design



* Subjects that had their last contact in the primary trial phase (Visit 34) BEFORE (and including) 30-Jun-2012, had 3 clinic visits and one field workers visit in the extension part of MAL-055.
** Subjects that had their last contact in the primary trial phase (Visit 34) BETWEEN 01-Jul-2012 and 30-Sep-2013 had 2 clinic visits and one field workers visit in the extension part of MAL-055.
*** Subjects that had their last contact in the primary trial phase (Visit 34) AFTER 30-Sep-2013 were not enrolled in the extension part of MAL-055.
BS: blood sampling; FWV: Field Worker Visit

- Experimental design: extension to the phase III, randomized, controlled, multi-centric study MAL-055 that comprised 3 parallel groups
- Duration of the study: approximately 3 years
  - Epoch 001: starting at Visit 39, including and ending at Visit 41 (December 2014).
  - Epoch 002: starting at Visit 42, including and ending at Visit 43 (December 2015).
  - Epoch 003: starting at Visit 44, including and ending at Visit 45 (December 2016).
- Study groups: same study groups as in the primary study MAL-055 . Subjects enrolled in each of the 2 age categories (5-17 months or 6-12 weeks at time of first vaccination) were randomized in 3 study groups:
  - R3R: infants/children randomized to receive 3 doses of RTS,S/AS01$_E$ on a 0-, 1-, 2-month schedule with an RTS,S/AS01$_E$ 4th dose at Month 20 during the primary study MAL-055.

- R3C: infants/children randomized to receive 3 doses of RTS,S/AS01$_E$ on a 0-, 1-, 2-month schedule with a dose of comparator vaccine at Month 20 during the primary study MAL-055 .
- C3C: infants/children randomized to receive 3 doses of a comparator vaccine on a 0-, 1-, 2-month schedule with a dose of comparator vaccine at Month 20 during the primary study MAL-055.

The following group names will be used for the statistical analyses:

Group order in tables	Group label in tables	Group definition for footnote
1	R3R	RTS,S/AS01E primary schedule with 4th dose
2	R3C	RTS,S/AS01E primary schedule without 4th dose
3	C3C	Control

Sub-group order in tables	Sub-group label in tables	Sub-group definition for footnote
1	6-12 W	Infants
2	5-17 M	Children

3. OBJECTIVES

3.1. Primary objective

- To describe the incidence of severe malaria in the long-term over a 3-year period (from January 2014 to December 2016) of follow-up pooled across transmission settings, in both age categories.
  - In children enrolled in the 5-17 months age category, starting on average 4 years post primary vaccination.
  - In children enrolled in the 6-12 weeks age category, starting on average 3.5 years post primary vaccination.

3.2. Secondary objectives

3.2.1. Efficacy objectives

In each age category (i.e. 5-17 months and 6-12 weeks) over 3 years of follow-up (from January 2014 to December 2016):

- To describe the incidence of clinical malaria in recipients of the RTS,S/AS01$_E$ candidate malaria vaccine and in controls.
- To describe the incidence of hospitalization due to malaria in recipients of the RTS,S/AS01$_E$ candidate malaria vaccine and in controls.
- To describe the prevalence of malaria infection at annual cross sectional timepoints in recipients of the RTS,S/AS01$_E$ candidate malaria vaccine and in controls.
- To describe the hemoglobin level and the prevalence of anemia at annual cross sectional timepoints in recipients of the RTS,S/AS01$_E$ candidate malaria vaccine and in controls.

In each age category since the start of the primary study (MAL-055) until December 2016:

- To describe the incidence of severe malaria in recipients of the RTS,S/AS01$_E$ candidate malaria vaccine and in controls.
- To describe the incidence of clinical malaria in recipients of the RTS,S/AS01$_E$ candidate malaria vaccine and in controls.
- To describe the incidence of hospitalization due to malaria in recipients of the RTS,S/AS01$_E$ candidate malaria vaccine and in controls.

3.2.2. Safety objective

- To describe the incidence of the following reported serious adverse events (SAEs): fatalities, related SAEs (related to vaccine administration in the primary study MAL-055 and to study participation), malaria hospitalization, potential Immune-Mediated Diseases (pIMDs), and meningitis from January 2014 to December 2016.

3.2.3. Immunogenicity objective

- To describe anti-circumsporozoite protein of *Plasmodium falciparum* (anti-CS) antibodies response over the 3-year follow-up period, in each age category.

4. ENDPOINTS

4.1. Primary endpoint

- The occurrence of severe malaria meeting the ~~primary~~[1] case definition analyzed over the time period starting January 2014 until the end of the 3-year follow-up period (Visit 45).

[1] changed from protocol

4.2. Secondary endpoints

4.2.1. Efficacy endpoints

- The occurrence of clinical malaria meeting ~~the primary and secondary~~ case definition~~s~~ analyzed over the time period starting January 2014 until the end of the 3-year follow-up period (Visit 45).
- The occurrence of malaria hospitalization meeting each of the case definitions analyzed over the time period starting January 2014 until the end of the 3-year follow-up period (Visit 45).
- *The occurrence of fatal malaria meeting each of the case definitions analyzed over the time period starting January 2014 until the end of the 3-year follow-up period (Visit 45). (not in protocol, see section 9.2)*
- *The occurrence of cerebral malaria meeting each of the case definitions analyzed over the time period starting January 2014 until the end of the 3-year follow-up period (Visit 45). (not in protocol, see section 9.2)*
- The prevalence of parasitemia at 3 annual timepoints (Visit 41, 43 and 45), in both age categories (6-12 weeks and 5-17 months).
- The prevalence of anemia at 3 annual timepoints (Visit 41, 43 and 45), in both age categories (6-12 weeks and 5-17 months).

- The level of hemoglobin at 3 annual timepoints (Visit 41, 43 and 45), in both age categories (6-12 weeks and 5-17 months).
- The occurrence of severe malaria meeting the primary and secondary case definitions analyzed over the time period starting at the beginning of the primary study (MAL-055; Visit 2) until the end of the follow-up period (Visit 45), in both age categories (6-12 weeks and 5-17 months).
- The occurrence of clinical malaria meeting the primary and secondary case definitions analyzed over the time period starting at the beginning of the primary study (MAL-055; Visit 2) until the end of the follow-up period (Visit 45), in both age categories (6-12 weeks and 5-17 months).
- The occurrence of malaria hospitalization meeting all case definitions analyzed over the time period starting at the beginning of the primary study (MAL-055; Visit 2) until the end of the follow-up period (Visit 45), in both age categories (6-12 weeks and 5-17 months).
- *The occurrence of fatal malaria meeting all case definitions analyzed over the time period starting at the beginning of the primary study (MAL-055; Visit 2) until the end of the follow-up period (Visit 45), in both age categories (6-12 weeks and 5-17 months). (not in protocol, see section 9.2)*
- *The occurrence of cerebral malaria meeting all case definitions analyzed over the time period starting at the beginning of the primary study (MAL-055; Visit 2) until the end of the follow-up period (Visit 45), in both age categories (6-12 weeks and 5-17 months). (not in protocol, see section 9.2)*

4.2.2. Safety endpoint

- The occurrence of the following reported SAEs: fatalities, related SAEs (related to vaccine administration in the primary study MAL-055 and to study participation), malaria hospitalization, pIMDs, and meningitis over the time period starting January 2014 until the end of the 3-year follow-up period (Visit 45).

4.2.3. Immunogenicity endpoint

- The annual anti-CS antibody titers (Visit 41, 43 and 45) and corresponding timepoints from MAL-055, for children of both age categories (6-12 weeks and 5-17 months).

5. STUDY POPULATION

5.1. Modified Intention to treat population (ITT)

ITT classically includes all subjects randomized. However, for operational reasons randomized subjects who did not receive study vaccine are not followed-up further. Therefore, the modified ITT population will include all subjects from the 3 centers of MAL-076 that received at least 1 dose of study vaccine (RTS,S/AS01$_E$ or Control) in MAL-055. The analyses on the ITT population will be performed per treatment assignment.

As there are different timeframes to be evaluated this translates in:

ITT [M0-DEC16] and ITT [M0-M20]: N=number of subjects receiving dose 1 in MAL-055

ITT [M21-DEC13]: N= number of subjects receiving dose 1 and have follow up time in the timeframe [M21-DEC13]

ITT [M21-DEC16]: N= number of subjects receiving dose 1 and have follow up time in the timeframe [M21-DEC16]

ITT [JAN14-DEC16]: N=number of subjects consenting to MAL-076 or with dead reported during the retrospective data collection

ITT [JAN14-DEC14]: N=number of subjects consenting to MAL-076 or with dead reported during the retrospective data collection

ITT [JAN15-DEC15]: N=number of subjects consenting to MAL-076 and having follow up time in the timeframe [JAN15-DEC15]

ITT [JAN16-DEC16]: N=number of subjects consenting to MAL-076 and having follow up time in the timeframe [JAN16-DEC16]

5.2. According-to-Protocol population for efficacy

Not applicable.

5.3. According-to-Protocol population for immunogenicity

Not applicable.

5.4. Risk periods

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 (end date follow up – start date follow up +1).

Table 1 Time at risk definitions

	Risk Period	Start	Stop
A	ITT [M0-DEC16] (MAL-055 + MAL-076)	Day of first vaccination of RTS,S or control vaccine in MAL-055	For subjects not consenting to MAL-076: Date of visit 38 (MAL-055) or drop out date from end of extension, whichever occurs first. For subjects consenting to MAL-076: 31 December 2016 or drop out date from study conclusion, whichever occurs first
B	ITT [M0-M20] (MAL-055)	Day of first vaccination of RTS,S or control vaccine in MAL-055	Date of month 20 visit (MAL-055) or drop out date from month 20 conclusion, whichever occurs first
C	ITT [M21-DEC13] (MAL-055)	Day of 4th dose in MAL-055 Subjects not receiving the 4th dose will start FU at month 20 visit date + 1D or last contact date + 1 D if visit not performed	Date of visit 38 (MAL-055) or drop out date from end of extension, whichever occurs first.
D	ITT [M21-DEC16] (MAL-055 + MAL-076)	Day of 4th dose in MAL-055 Subjects not receiving the 4th dose will start FU at month 20 visit date + 1D or last contact date + 1 D if visit not performed	For subjects not consenting to MAL-076: Date of visit 38 (MAL-055) or drop out date from MAL-055 end of extension, whichever occurs first For subjects consenting to MAL-076: 31 December 2016 or drop out date from study conclusion, whichever occurs first.
E	ITT [JAN14-DEC16] (MAL-076)	For subjects consenting to MAL-076 or with dead reported during the retrospective data collection: 1 January 2014	For subjects consenting to MAL-076: subject completion date from Study conclusion page (= completion or withdrawal or death date)
F	ITT [JAN14-DEC14] (MAL-076)	For subjects consenting to MAL-076 or with dead reported during the retrospective data collection: 1 January 2014	For subjects consenting to MAL-076: 31 December 2014 or drop out date from study conclusion or death date, whichever occurs first.
G	ITT [JAN15-DEC15] (MAL-076)	For subjects consenting to MAL-076: 1 January 2015	For subjects consenting to MAL-076: 31 December 2015 or drop out date from study conclusion or death date, whichever occurs first.
H	ITT [JAN16-DEC16] (MAL-076)	For subjects consenting to MAL-076: 1 January 2016	For subjects consenting to MAL-076: subject completion date from Study conclusion page (= completion or withdrawal or death date)

Note: letter to be used for start/end variables names

For subjects who consented to the extension phase after visit 34 (month 32 visit) in MAL-055, time at risk will be adjusted by removing the time gap between the month 32 visit and the start of the extension phase (defined as consent date). The MAL-076 protocol includes retrospective data collection and as a result consenting subjects are considered at risk as of the end of MAL-055.

No elimination codes applicable for this study -> no study specific form FORM-BIO-CLIN-9004-05 Criteria for eliminating subjects from the analyses exist.

6. STATISTICAL METHODS

6.1. Analysis of demographics

Study flow diagrams (consort, Overall and by site/age group) will be generated to present the number of subjects from the 3 centers of MAL-076 enrolled in MAL-055, who completed the last study contact in December 2013. Reasons for visit not done will be summarized. The number of subjects approached and eligible for MAL-76 will be tabulated along with the number of subjects completing visit 41 (year 1 follow up, December 2014), visit 43 (year 2 follow up) and visit 45 (year 3 follow up). Reasons for non-attendance will also be summarized.

Baseline characteristics at enrolment in MAL-055 and MAL-076 (age (M0 MAL-055 and at 01JAN2014, gender) will be tabulated by group and age category, overall and by site.

Follow up time from dose 1 in MAL-055 until December 2016 (Visit 45 in MAL-076) will be tabulated by study group by age category, overall and by site. For consenting children, follow up time in the 076 trial will also be presented (total: between the end of MAL-055 and Visit 45/ year 3 visit in MAL-076, retrospective: between the end of MAL-055 and ICF date in MAL-076 and prospective: between ICF date in MAL-076 and date of Visit 45/ year 3 visit in MAL-076). Follow-up time will be calculated in number of months between dates (i.e. visit date or censored). The number of months will be calculated as number of days divided by 30.4.

Categorical variables will be presented by percentages and numerical variables will be summarized by mean, standard deviation, median and quartiles.

For both age categories, the distribution of subjects enrolled among the study sites will be tabulated as a whole and per study group.

6.2. Analysis of efficacy

All analyses will be performed only on the modified ITT populations.

VE will be calculated on the entire follow-up period. Incidence comparisons, using the same formula and analytic methodology will be calculated for time period breakdown.

Malaria disease incidence will be estimated over the defined risk periods (see 5.4) and by 6-monthly breakdown periods covering the 3-year period.

6.2.1. Case definitions

Plasmodium falciparum asexual parasitemia from study start in MAL-055 was defined as a positive blood slide by independent double (or triple in the case of pre-defined discrepancies between the first two readers) reading according to a protocol defined process. The same process was planned per protocol in MALARIA 076. However, given the time gap between the end of MAL-055 and consent of MALARIA 076, protocol amendment 2 specified that other parasitological parameters representing local practice could be collected in the absence of the protocol defined slide reading process. As a result, in the case definitions used in this study, positive *Plasmodium falciparum* parasitemia will be defined within each case definition as either:

- A positive blood slide from the protocol defined reading process (double/triple read density > 0 parasites/µl)
- A positive blood slide from single reading process to guide treatment
- A positive RDT (rapid diagnostic test)

It is acknowledged that using this definition of positive *Plasmodium falciparum* parasitemia, malaria case definitions would have lower specificity. On the other hand, sensitivity is increased, ensuring that all potential malaria cases are included in the analyses.

6.2.1.1. Clinical malaria

Positive *Plasmodium falciparum* asexual parasitemia

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

6.2.1.2. Severe malaria

Case definition 1: Severe malaria during MAL-055 and after consent in MAL-076 was diagnosed based on symptoms and signs occurring at presentation or developing during admission according to the case definition : Positive *Plasmodium falciparum* asexual parasitemia (within -1 to +3 days of admission)	
AND with one or more marker of disease severity[1]:	- Prostration. - Respiratory distress. - Blantyre score ≤ 2.

	- Seizures 2 or more. - Hypoglycemia < 2.2 mmol/L. - Acidosis BE ≤ -10.0 mmol/L. - Lactate ≥ 5.0 mmol/L. - Anemia < 5.0 g/dL

Prostration is defined as, in an acutely sick child, the inability to perform previously-acquired motor function: in a child previously able to stand, inability to stand; in a child previously able to sit, inability to sit and in a very young child, inability to suck.
Respiratory distress is defined as lower chest wall indrawing or abnormally deep breathing.
2 or more seizures occurring in the total time period including 24 h prior to admission to the emergency room and the hospitalization.

Case definition 2:

During the gap period, no systematic collection of severe malaria disease markers was operational at the level of the trial site and potential episodes of severe malaria were collected as SAEs (preferred terms 'malaria', '*Plasmodium falciparum* infection' and 'cerebral malaria'). In order to include all potential episodes, severe malaria disease will be defined as:

Positive *Plasmodium falciparum* asexual parasitemia (within -1 to +3 days of admission) AND	
one or more marker of disease severity [1]:	- Prostration. - Respiratory distress. - Blantyre score ≤ 2. - Seizures 2 or more. - Hypoglycemia < 2.2 mmol/L. - Acidosis BE ≤ -10.0 mmol/L. - Lactate ≥ 5.0 mmol/L. - Anemia < 5.0 g/dL
OR: **SAE report including preferred terms** (within -1 to +3 days of admission)	Malaria *Plasmodium falciparum* infection Cerebral malaria

[1] See variable sev21_ in sevmal dataset of MAL-055 (run with w_slide.a_pf_dens = 5001 for positive)

This means that all severe malaria episodes as previously (MAL-055) and prospectively (076) detected according to the protocol described algorithm as well as malaria SAE reports for which we have parasitological evidence (whole study period + gap period) will be included. SAE reports for which no parasitological evidence exists will be reported in the SAE safety section.

6.2.1.3. Malaria hospitalization

Definition 1	A medical hospitalization with confirmed positive *Plasmodium falciparum* asexual parasitemia (excludes planned admissions for medical investigation/care or elective surgery and trauma)
Definition 2	A hospitalization for which, in the judgment of the principal investigator, *Plasmodium falciparum* infection was the sole or a major contributing factor to the presentation

6.2.1.4. Fatal Malaria

Definition 1	An SAE report with preferred terms 'malaria', 'plasmodium falciparum infection', 'cerebral malaria' with confirmed positive *Plasmodium falciparum* asexual parasitemia associated with a fatal outcome (excludes planned admissions for medical investigation/care or elective surgery and trauma)
Definition 2	An SAE report with preferred terms 'malaria', 'plasmodium falciparum infection', 'cerebral malaria' associated with a fatal outcome

6.2.1.5. Cerebral malaria

Positive *Plasmodium falciparum* asexual parasitemia (within -1 to +3 days of admission) AND EITHER	
marker of disease severity:	Blantyre score ≤ 2.
SAE report including preferred terms	Cerebral malaria

Case definition for sensitivity analysis (see § 7.3)

Positive *Plasmodium falciparum* asexual parasitemia (within -1 to +3 days of admission) AND Blantyre score ≤ 2

6.2.1.6. Prevalent anemia

Prevalent severe anemia	A documented hemoglobin < 5.0 g/dL identified at an annual visit
Prevalent moderate anemia	A documented hemoglobin < 8.0 g/dL identified at an annual visit

6.2.1.7. Prevalent parasitemia

Prevalent parasitemia	A documented *Plasmodium falciparum* asexual parasite density > 0 parasites/µL identified at an annual visit

6.2.2. Efficacy against Severe malaria

For both age categories (6-12 weeks and 5-17 months), VE/ incidence comparison against all episodes of clinical malaria will be estimated as 1-incidence ratio (IR; total number of events/follow-up time in the RTS,S/AS01$_E$ groups [R3R/R3C] over the total number of events/follow-up time in the control group [C3C]) calculated by negative binomial regression allowing for interdependence between episodes within the same subject (mixed model with over-dispersion parameter estimated from the random effect [Lievens, 2011]) and will be presented together with 95% CI and p-values calculated from this model. Results will be analyzed per site and overall.

6.2.3. Efficacy against Clinical malaria

Same as Severe malaria (see § 6.2.2)

6.2.4. Malaria hospitalization

For both age categories (6-12 weeks and 5-17 months), malaria hospitalization will be analyzed by the proportion of children affected. VE will be estimated as 1-the risk ratio (RR; proportion of subjects reporting events in the RTS,S/AS01$_E$ groups [R3R/R3C] over the proportion in controls [C3C]) over the entire follow-up period, and will be presented together with 95% CIs and p-values. Results will be analyzed per site and overall.

6.2.5. Cerebral malaria

Same as Malaria hospitalization (see § 6.2.4)

6.2.6. Fatal malaria

Same as Malaria hospitalization (see § 6.2.4)

6.2.7. Vaccine efficacy against prevalent parasitemia and prevalent anemia

For both age categories (6-12 weeks and 5-17 months), VE against prevalent endpoints (parasitemia, moderate and severe anemia) assessed annually will be estimated as 1-RR where RR is the risk ratio (proportion of subjects reporting events in the RTS,S/AS01$_E$ groups [R3R/R3C] over the proportion in controls [C3C]) and will be presented together with 95% CIs and p-values. Results will be analyzed per site and overall. The geometric mean parasite density and arithmetic mean hemoglobin level will be calculated per site and overall. The effect of the group will be evaluated using the t-test.

6.2.8. Vaccine impact

For both age categories (6-12 weeks and 5-17 months), each site and overall, the number of cases of clinical malaria, severe malaria (definition 1), cerebral malaria and malaria hospitalizations (definition 1) averted will be calculated (difference between incidences, expressed per 1000 population vaccinated) for each 6-monthly time period and total over the entire follow-up time (from dose 1 of Malaria-055 to Dec 2016). Graphical presentations will be generated showing the cumulative number of averted cases over time (breakdown of follow-up period).

6.3. Analysis of immunogenicity

All analyses will be performed on the modified ITT population.

For both age categories (6-12 weeks and 5-17 months), for each site and overall, the percentage of subjects with seropositive levels of anti-CS (proportion of subjects with anti-CS antibody titers ≥ 0.5 EU/mL) with 95% CI will be determined at each blood sampling timepoint. Antibody titers will be summarized by GMT with 95% CI at all timepoints at which serological samples are taken (MAL-076 and MAL-055).

6.4. Analysis of safety

6.4.1. Occurrence of SAEs

Safety will be evaluated by examining SAEs for the 3 study groups from study start (Dose 1 in MAL-055) up to December 2016 (Visit 45 in MAL-076) for the ITT [M0-DEC16] population, from dose 1 to dose 4 for ITT [M0-M20], from dose 4 to study end for ITT [M21-DEC16] and by year of study 076 ITT [JAN14-DEC14], ITT [JAN15-DEC15] and ITT [JAN15-DEC15].

The proportion of subjects with SAEs, classified by the MedDRA preferred term level, will be tabulated by group with exact 95% CI. Analyses will be performed separately for both age categories by study site and pooled across study sites.

6.4.2. Occurrence of Malaria SAEs

The proportion of subjects with Malaria SAEs, classified by the MedDRA preferred term level, will be tabulated by group with exact 95% CI from study start (Dose 1 in MAL-055) up to December 2016 (Visit 45 in MAL-076) for the ITT [M0-DEC16] population, from dose 1 to dose 4 for ITT [M0-M20], from dose 4 to study end for ITT [M21-DEC16] and by year of study 076 ITT [JAN14-DEC14], ITT [JAN15-DEC15] and ITT [JAN15-DEC15]. Analyses will be performed separately for both age categories by study site and pooled across study sites.

6.4.3. Meningitis/encephalitis SAEs

Meningitis/encephalitis SAEs will be listed by age category and group, including risk period of report

6.4.4. pIMDs

pIMDs will be listed by age category and group, including risk period of report.

6.4.5. Fatal SAEs

The proportion of subjects with fatal SAEs, classified by the MedDRA preferred term level, will be tabulated by group with exact 95% CI from study start (Dose 1 in MAL-055) up to December 2016 (Visit 45 in MAL-076) for the ITT [M0-DEC16] population

and by year of study 076 ITT [JAN14-DEC14], ITT [JAN15-DEC15] and ITT [JAN15-DEC16]. Analyses will be performed separately for both age categories pooled across study sites.

Fatal SAEs will be listed by age category and group, including risk period of report.

7. STATISTICAL CALCULATIONS

7.1. Derived and transformed data

7.1.1. Coding and Grading of Adverse events

- Adverse events and serious adverse events are coded according to the MedDRA dictionary (at the level of System Organ Class and Preferred Term) based on the verbatim reported. This coding is made by a medically qualified person experienced in the company-specific coding conventions. The latest available dictionary version at the time of analysis will be used.

- Subject who did not report any particular event will be considered as subject not experiencing this event.

7.1.2. Humoral immune response

- anti-CS antibody ELISA (Plasmodium falciparum Circumsporozoite Protein.R32LR Ab.IgG) assay cut-off: 0.5 EL.U/mL.
- A seropositive subject is a subject whose anti-CS antibody titer is greater than or equal to the cut-off value.
- The geometric mean concentration (GMC) is calculated by taking the anti-logarithm of the mean of the log10 concentration transformations. Antibody concentration below the assay cut-off will be given an arbitrary value of half of the cut-off for the purpose of the calculation. Antibody concentration between the assay cut-off will be kept unchanged.
- For a given subject and given immunogenicity measurement, results of missing or non-evaluable measurements will not be imputed. Therefore, an analysis will exclude subjects with missing or non-evaluable measurements.

7.1.3. Time-to-event calculation

In instances where periods between two dates are to be calculated (time-to-event endpoints), the convention to be used is as follows:

[later date] – [earlier date] + 1 day.

Should the result of this calculation be lower than 1, the time-to-event value will be re-set to 1 (event on Day 1) for the purpose of including the patient in the analysis.

When converting a number of days to other units, the following conversion factors will be used:

1 year = 365.25 days
1 month = 30.4 days.

7.2. Programming Algorithm for case definitions

Risk periods defined in MAL-055 will be carried over without changes for subjects enrolled in MAL-076, but the same updated case definitions will be applied to both MAL-055 and MAL-076 data.

7.2.1. Severe malaria episodes

Identification of severe malaria episodes is done through a programmatic algorithm using data from hospital admission and deterioration forms. The following rules will be applied for severe malaria disease markers and co-morbidities.

	Parameter	Timing (days)	Included for case definition	Excluded for case definition	Patient contact document
Parasitaemia	Blood slide or RDT	-1 DOA +3	Positivity in this timeframe	Any case for which there is no result within time window	Outpatient form, inpatient form, deterioration form
Marker of disease severity	Prostration	DOA +3	Any event within timeframe	CSF results not available within timeframe in children < 3 years	Inpatient form, Deterioration form
	Respiratory distress	DOA +3	Any event within timeframe	CXR result not available within timeframe	Inpatient form, Deterioration form
	Blantyre score ≤ 2	DOA +3	Any event within timeframe	CSF results not available within timeframe	Inpatient form, Deterioration form
	Seizures 2 or more	DOA +3	Any event within timeframe	CSF results not available within timeframe	Inpatient form (history of signs and symptoms), Deterioration form

	Parameter	Timing (days)	Included for case definition	Excluded for case definition	Patient contact document
	Hypoglycemia < 2.2 mmol/L	DOA +3	Any event within timeframe		Inpatient form, Deterioration form
	Acidosis BE ≤ -10.0 mmol/L	DOA +3	Any event within timeframe		Inpatient form, Deterioration form
	Lactate ≥ 5.0 mmol/L	DOA +3	Any event within timeframe		Inpatient form, Deterioration form
	Anemia < 5.0 g/dL	DOA +3	Any event within timeframe		Inpatient form, Deterioration form

DOA: Day Of Admission

7.2.2. Malaria hospitalization

Identification of malaria hospitalization is done through a programmatic algorithm using data from hospital admission and discharge forms. The following rules will be applied for derivation of the malaria hospitalization case definitions. One admission can only lead to one case of malaria hospitalization. No outpatient data contributes to these case definitions.

Parameter	definition
Definition 1	Admission with positive parasitemia within the time window -1 DOA +3
Definition 2	Admission which in the judgment of the principal investigator, *P. falciparum* infection was the sole or a major contributing factor to the presentation (recorded at time of discharge)

DOA: Day Of Admission

7.3. Sensitivity Efficacy Analyses

The primary efficacy analysis will be based on direct likelihood (analysis of the data as-is), which means that the output of the statistical models applied will be without further considerations of missing data.

The incidence of retrospective vs prospective severe Malaria data in each site will be documented

For the sensitivity analysis, retrospective data collected between the end of MAL-055 and the first MAL-076 visit will be removed:

For subjects who consented to the MAL-076, time at risk will be adjusted to remove the time gap between Dec 2013 and the start of MAL-076 (defined as consent date). All

cases occurring during this gap period (retrospectively reported) will not be counted in this sensitivity analysis.

Sensitivity analyses will be performed for Severe Malaria definition 1, Clinical Malaria and Cerebral malaria cases over the whole period (ITT [JAN14-DEC16], ITT [M0-DEC16]).

Specific case definitions have been set for sensitivity analysis of Cerebral malaria cases (see §6.2.1.5)

8. CONDUCT OF ANALYSES

8.1. Sequence of analyses

Description	Analysis ID	Disclosure Purpose	Reference for TFL
Final Analysis	E1_01	study report, CTRS, publication	MALARIA-076 (200599) TFL E1_01.doc
Year 1, Nanoro site	E1_02	Study report	MALARIA-076 (200599) TFL (Year 1 Nanoro).doc
Year 2	E1_03	Study report	MALARIA-076 (200599) TFL (Year 2).doc
AAR	E1_04 Cancelled	Response to Year 2 EMA question	MALARIA-076 (200599) Additional Analysis Request E01_04 - Questions PRAC rapporteur Year 2 E1_03.docx
Final Immuno	E1_05	Annex to study report, CTRS	MALARIA-076 (200599) TFL E1_05.doc

8.2. Statistical considerations for interim analyses

Two interim analyses were performed. As all analyses are descriptive, p-values will be informatory and not confirmatory. No alpha-adjustment will be planned.

9. CHANGES FROM PLANNED ANALYSES

9.1. Interim analyses

Two interim analyses of efficacy, immunogenicity and safety data were planned: at the end of Year 1 (i.e. Visit 41) and at the end of Year 2 (i.e. Visit 43)

However, due to delays in protocol approvals only one of the sites (Nanoro) had collected the first year of follow up in time to be included in the Year 1 interim analysis.

Due to late protocol approvals and retrospective data collection the sites did not collect the data according to protocol and as such the case definition cannot be applied. Efficacy against severe malaria in Year 2 analysis was therefore evaluated based on the malaria SAEs reported up to that point in time (using the MedDRA preferred terms: Malaria, Plasmodium falciparum infection and Cerebral malaria). An analysis of incidence of malaria SAEs (all episodes over follow up time) was added, together with an analysis of relative risks.

For the final analysis, specific case definitions based on available data were developed.

Due to the unavailability of the anti-CS assay results, secondary immunogenicity objectives were not reported at interim analyses.

9.2. Endpoints

Four secondary efficacy endpoints were added:

- The occurrence of fatal malaria meeting each of the case definitions analysed over the time period starting January 2014 until the end of the 3-year follow-up period (Visit 45).
- The occurrence of cerebral malaria meeting each of the case definitions analysed over the time period starting January 2014 until the end of the 3-year follow-up period (Visit 45).
- The occurrence of fatal malaria meeting all case definitions analyzed over the time period starting at the beginning of the primary study (MAL-055; Visit 2) until the end of the follow-up period (Visit 45), in both age categories (6-12 weeks and 5-17 months).
- The occurrence of cerebral malaria meeting all case definitions analyzed over the time period starting at the beginning of the primary study (MAL-055; Visit 2) until the end of the follow-up period (Visit 45), in both age categories (6-12 weeks and 5-17 months).

9.3. Final analyses

VE against first or only episodes of clinical malaria was removed, as not applicable after years of follow-up (majority of subjects have already 1st episode in MAL-055).

Due to retrospective data collection, efficacy will only be analysed on ITT: According-to-Protocol population for efficacy has been removed, and case definitions adapted.

Cases definitions were adapted to data collection, replacing primary and secondary case definitions stated in the protocol.

Due to the gap period, height, weight and mid arm circumference were not recorded annually as planned: corresponding z-scores tables and t-test will not be generated.

As no related SAE have been reported, corresponding summary tables will not be generated

As very few pIMDs have been reported, corresponding summary tables will not be generated: only one table with details, including risk period of report

9.4. Immunogenicity analyses

Immunogenicity will also only be analysed on ITT: According-to-Protocol population for Immunogenicity has been removed. Due to late immunogenicity results release, these will be analysed and reported in an annex to the CSR.

10. REFERENCES

- Lievens M, Aponte J, Williamson J, Mmbando B, Mohammed A et al. Statistical methodology for the evaluation of vaccine efficacy in a phase III multi-centre trial of the RTS,S/AS01 malaria vaccine in African children. *Malar J.* 2011;10:222 doi:10.1186/1475-2875-10-222