This supplement contains the following items.

1. Final RTS,S + SMC Extension study protocol, dated 27 January 2022.

2. Statistical analysis plan, dated 23 June 2022.

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Seasonal vaccination with the RTS,S/AS01$_E$ malaria vaccine given with or without seasonal malaria chemoprevention: extension of a randomised, double-blind Phase 3 trial until children reach the age of five years.

Brief Title: A trial of RTS,S/AS01$_E$ and SMC: extension study

Protocol Identifying Number - PATH: CVIA 083

Protocol Identifying Number - GSK: MALARIA-106 EXT:099 Y4-5 SUPP

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LIST OF ABBREVIATIONS

ACT	Artemisinin combination therapy
AE	Adverse event
AESI	Adverse event of special interest
A/L	Artemether/lumefantrine
anti-CS	Antibody to *P. falciparum* circumsporozoite protein repeat region
anti-HBs	Antibody to hepatitis B surface antigen
AS01	GSK's proprietary adjuvant system containing QS21, MPL, and liposomes
AQ	Amodiaquine
CSP	Circumsporozoite protein
DBS	Dried blood spot
DSMB	Data Safety and Monitoring Board
EMA	European Medicines Agency
GCP	Good Clinical Practice
HBsAg	Hepatitis B surface antigen
IB	Investigator's Brochure
ICH-GCP	International Committee on Harmonisation – Good Clinical Practise
ID	Identification
IRB	Institutional Review Board
ICH	International Council for Harmonisation
IRSS	Institut de Recherche en Sciences de la Santé
ITN	Insecticide-treated bed net
LLIN	Long-lasting insecticde-treated bednets
LDH	Lactate dehydrogenase
LSHTM	London School of Hygiene and Tropical Medicine
MMV	Medicines for Malaria Venture
MPAC	Malaria Policy Advisory Committee
MRTC	Malaria Research and Training Centre
NMCP	National Malaria Control Programme
pIMD	Potential immune-mediated disease
RDT	Rapid diagnostic test for malaria
RTS,S	Protein comprising CS and hepatitis B surface antigen
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SOP	Standard Operating Procedure
SP	Sulphadoxine/pyrimethamine
SMC	Seasonal Malaria Chemoprevention
WHO	World Health Organization

STATEMENT OF COMPLIANCE

The signature below constitutes the approval of this protocol and the attachments and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and ICH-GCP (E6) guidelines.

Study Director in Mali	NAME (PRINTED)	DATE
	Alassane Dicko	18.3.22
Study Director in Burkina Faso	NAME (PRINTED)	DATE
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PROTOCOL SUMMARY

Title	**Seasonal vaccination with the RTS,S/AS01$_E$ malaria vaccine given with or without seasonal malaria chemoprevention: extension until children reach the age of five years.**
Design	A trial of seasonal vaccination with the RTS,S/AS01$_E$ malaria vaccine with or without Seasonal Malaria Chemoprevention (SMC) was initiated in 2017 in 5887 children aged 5-17 months in Burkina Faso and Mali. The children were enrolled in an individually randomised, double-blind controlled trial to investigate (a) whether the combination of seasonal vaccination with RTS,S/AS01$_E$ following priming combined with SMC (sulphadoxine-pyrimethamine (SP) plus amodiaquine (AQ)) would be superior in providing protection against clinical episodes of malaria in young children than either intervention given alone and (b) whether seasonal vaccination with RTS,S/AS01$_E$ following priming given alone would be non-inferior to SMC given alone in preventing clinical attacks of malaria in children under five years of age. Consequently, children enrolled in the study have been randomised individually to one of three groups: a. Group 1 (SMC alone). Children in this group received three doses of rabies vaccine in April-June 2017 and one dose of hepatitis A vaccine in June 2018 and in June 2019 together with four rounds of SMC (SP+AQ) during the period August to November of 2017, 2018 and 2019. b. Group 2 (RTS,S/AS01$_E$ alone). Children in this group received three doses of RTS,S/AS01$_E$ vaccine in April-June 2017 and one dose of RTS,S/AS01$_E$ vaccine in June 2018 and in June 2019 together with four rounds of SMC placebo during the period August to November of 2017, 2018 and 2019. c. Group 3 (SMC + RTS,S/AS01$_E$ vaccine). Children in this group received three doses of RTS,S/AS01$_E$ vaccine in April-June 2017 and one dose of RTS,S/AS01$_E$ vaccine in June 2018 and in June 2019 together with four rounds of SMC (SP+AQ) during the period August to November of 2017, 2018 and 2019. The current study design is for continuation of the trial for a further two years. SMC is recommended until the age of five years. Thus, if seasonal vaccination with RTS,S/AS01$_E$, or with another malaria vaccine with high initial efficacy but a relatively short duration of protection, is to be considered as a potential supplement, or possible replacement, for SMC, it is important to determine the efficacy of annual seasonal vaccination in primed children until they reach the age of five years. If a recommendation is made to extend SMC until the age of 10 years, as may shortly be the case in several countries, it might then be appropriate to continue seasonal vaccination until this age but only if it can be shown that repeated booster doses of RTS,S/AS01$_E$ are safe and retain their efficacy. If it can be shown during the first three years of the trial that adding seasonal vaccination with RTS,S/AS01$_E$ to SMC provides added benefit or it is shown and that vaccination with RTS,S/AS01$_E$ is equivalent or superior to SMC given alone it will be appropriate to continue the trial for a further two years but if neither of these outcomes is achieved it will not. However, waiting until the definitive results from the first three years of trial are obtained

	in April/May 2020 would make it impossible to obtain the vaccine and drugs, and make the logistic arrangements needed to deploy them, in time for the trial to continue without interruption during the 2020 malaria transmission season (July-October). Therefore, a preliminary analysis of both efficacy and safety data obtained up to the end of November 2019 was recommended by one of the trial's donors and this review was undertaken by an independent expert appointed by the Bill and Melinda Gates Foundation in January 2020 to provide advice as to whether the trial should be continued beyond its present phase and, if so, whether the three study arms should be retained. The same set of data was reviewed at two meetings held by the Data and Safety Management Board (DSMB) on January 16th and 17th 2020. Both the independent expert and members of the DSMB recommended continuation of the trial with retention of all three study groups as outline in this proposal. This will, therefore, be a controlled, individually randomised trial with three study groups. The study groups and the interventions that they will receive are indicated below- **Group1 (SMC)** — **Group 2 (RTS,S)** — **Group 3 (RTS,S + SMC)** **2020** June: Tetanus or Tetanus/diphtheria toxoids x 1* — RTS,S/AS01$_E$ x 1 — RTS,S/AS01$_E$ x 1 July -Oct: SMC x 4 — SMC placebo x 4 — SMC x 4 **2021** June: Tetanus or Tetanus/diphtheria toxoids x 1* — RTS,S/AS01$_E$ x 1 — RTS,S/AS01$_E$ x 1 July -Nov: SMC x 4 (Mali) SMC x 5 (Burkina Faso) — SMC placebo x 4 (Mali) SMC placebo x 5 (Burkina Faso) — SMC x 4 (Mali) SMC x 5 (Burkina Faso) All children will have received previously five doses of RTS,S/AS01$_E$ vaccine or comparator vaccines together with SMC or placebo. * Depending on the ability to ship tetanus toxoid vaccine from India as a result of the COVID-19 outbreak, children in group 1 will either receive tetanus toxoid vaccine procured from the Serum Institute of India, or tetanus/diphtheria toxoids vaccine provided by the national infant immunization programmes of Burkina Faso and Mali from the stock utilized routinely in their national immunization programmes.
Study Objective	This trial, undertaken in children aged three or four years old who have previously received five doses of the RTS,S/ASO1 malaria vaccine or comparator vaccines, together with seasonal malaria chemoprevention (SMC) with sulphadoxine-pyrimethamine (SP) plus amodiaquine (AQ) or placebo seeks to determine whether :

	a. Administration of further doses of RTS,S/AS01$_E$ at the beginning of the malaria transmission until children reach the age of five years provides additional protection against clinical episodes of malaria when given together with SMC. b. Vaccination with a booster dose of the RTS,S/AS01$_E$ malaria vaccine at the beginning of the malaria transmission until children reach the age of five years is non-inferior to SMC in preventing clinical attacks of malaria and would be easier to deliver than SMC.
Endpoints	The primary end-point for the trial is the incidence of clinical episodes of malaria, defined as an episode of fever (temperature >= 37.5^o C) or a history of fever within the past 48 hours, that is severe enough to require treatment at a health centre or by a community health worker and which is accompanied by a positive blood film with a parasite density of 5,000 parasites per µl or more. Secondary end-points for the trial include – a. Clinical episodes of an uncomplicated febrile illness (temperature >= 37.5^o C), or a history of fever within the past 48 hours, with a positive blood film (any level of asexual parasitaemia) or a positive rapid diagnostic test (RDT) for malaria. b. Hospital admissions with malaria, including cases of severe malaria who meet WHO criteria for a diagnosis of severe malaria. c. The prevalence of malaria infection not severe enough to warrant a clinic visit detected in a subset of randomly selected children during home visits. This activity is currently suspended due to COVID-19. d. The prevalence of malaria parasitaemia, including gametocytaemia, moderate and severe anaemia and malnutrition at the end of the malaria transmission season. Minor adverse events following repeated booster doses of RTS,S/AS01$_E$ and serious adverse events (SAEs), including any deaths, occurring at any time during the study with special reference to any cases of meningitis or cerebral malaria. Exploratory end-points for the trial include: a. Anti-CSP antibody concentrations obtained before and after each booster dose, determined in a sub-sample of children. b. The prevalence of malaria parasitaemia at the end of the malaria transmission season in school-age children resident in the study areas, as determined during the previous three years, to provide information on whether there have been any changes in the overall level of malaria transmission in the study area during the five years of the study. c. The prevalence of mutations in the Th2 or Th3 locus of the *Plasmodium falciparum csp* gene in children who have received repeated doses of RTS,S/AS01$_E$. d. The presence of molecular markers of resistance to SP and AQ in parasite positive samples collected during the final cross-sectional survey.

	e. The 28-day treatment outcome in children with asymptomatic malaria parasitaemia treated with SP+AQ identified during the final cross-sectional survey. f. Evidence of 'rebound' malaria in study children who have reached the age of five years at the time of the last year of the trial and who are no longer eligible to receive either of the trial interventions. g. The presence of antibodies to a broad range of malaria antigens in children who have received RTS,S/AS01$_E$ alone, SMC alone and RTS,S/AS01$_E$ + SMC collected after three years of the interventions.
Population	Children of either sex, aged three or four years old who have been enrolled in the previous trial of seasonal vaccination with the RTS,S/AS01$_E$ vaccine will be eligible to join this extension study, remaining in their original trial allocation group.
Sample Size	It is estimated that approximately 4,800 of the 5887 enrolled originally in the trial will be available for recruitment into the extension study.
Phase	Phase 3
Number of sites enrolling participants	Two sites: The trial will be conducted in Houndé district, Burkina Faso and in Bougouni district, Mali.
Study duration	July 2020 – June 2022

SCHEMATIC OF STUDY DESIGN

Table 1. Group Description

This will be a controlled, individually randomised trial with three study groups. The study groups and the interventions that they will receive are -

	Group1 (SMC)	Group 2 (RTS,S/AS01$_E$)	Group 3 (RTS,S/AS01$_E$ + SMC)
2020			
June	Tetanus or Tetanus/diphtheria toxoids x 1	RTS,S/AS01$_E$ x 1	RTS,S/AS01$_E$ x 1
July -Oct	SMC x 4	SMC placebo x 4	SMC x 4
2021			
June	Tetanus or Tetanus/diphtheria toxoids x 1	RTS,S/AS01$_E$ x 1	RTS,S/AS01$_E$ x 1
July -Nov	SMC x 4 (Mali) SMC x 5 (Burkina Faso)	SMC placebo x 4 (Mali) SMC placebo x 5 (Burkina Faso)	SMC x 4 (Mali) SMC placebo x 5 (Burkina Faso)

All children will have received previously five doses of RTS,S/AS01$_E$ vaccine or comparator vaccines (3 doses of rabies and 2 doses of hepatitis A vaccines) together with SMC or placebo.

1. KEY ROLES

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2. INTRODUCTION: BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 The RTS,S/AS01$_E$ Vaccine

The RTS,S/AS01$_E$ malaria vaccine is a recombinant protein vaccine in which the fusion protein RTS containing parts of the circumsporozoite protein (CSP) of *Plasmodium falciparum* fused to hepatitis B surface antigen (HBsAg) is co-expressed in yeast, together with free HBsAg (S), to form a virus like particle (RTS,S); it is given with the powerful adjuvant AS01 [1]. RTS,S/AS01$_E$ induces a strong antibody response to *P. falciparum* CSP and high titres of anti-CSP antibody are associated with protection [2]. Following a long process of development, a phase 3 trial of RTS,S/AS01$_E$ conducted in 15,439 children in seven countries in Africa showed that three doses of RTS,S/AS01$_E$ given with a one month interval between doses, followed by a fourth dose 18 months after dose 3, gave 36.5 % [95% CI 31,41%] protection against clinical attacks of malaria when given to young children aged 5-17 months who were followed for 48 months; efficacy was less when the vaccine was given to infants at the age of 6-12 weeks [3]. A long term follow- up in three of the phase 3 trial sites showed that when given to children aged 5-17 months, vaccine efficacy against severe malaria was 36.7% (95% (5% CI 14.6, 53.1) over a period of seven years [4]. RTS,S/AS01$_E$ provides a high level of protection during the first three months after vaccination, modelled to be about 70% in the phase 3 trial, a level of initial efficacy similar to that observed in an earlier phase 2 trial in Gambian adults [5]. However, efficacy wanes progressively over the following months. A subsequent dose given 18 months after the primary series restores some but not all of the efficacy seen immediately after the primary series [3, 5]. In July 2015, the European Medicines Agency (EMA) reviewed data on the efficacy and safety of RTS,S/AS01$_E$ and concluded that the risk benefit balance favoured the vaccine and gave a positive opinion on its use in children aged 6 weeks to 17 months. WHO's SAGE committee reviewed the vaccine's efficacy and safety in October 2015 and made a number of recommendations on its further evaluation [6]. These included the pilot implementation of RTS,S/AS01$_E$ in children aged 5-17 months in three to five settings with moderate-to-high malaria transmission intensity, with a preference for areas where SMC is not being delivered, and recommended evaluation of alternative approaches to deployment of the vaccine. Following the WHO recommendations three large RTS,S/AS01$_E$ pilot studies commenced in Ghana, Kenya and Malawi in 2019 [7].

2.2 Seasonal Malaria Chemoprevention (SMC)

Seasonal malaria chemoprevention (SMC) involves monthly administration of an antimalarial drug or drug combination in a full therapeutic course to children on three or four occasions during the period of highest risk of malaria infection. Studies undertaken in several countries in West Africa, including Burkina Faso and Mali, showed that SMC with sulphadoxine/pyrimethamine (SP) and amodiaquine (AQ) is highly effective in areas where the transmission of malaria is markedly seasonal, reducing the incidence of severe and uncomplicated malaria by up to 80% [8-10]. SMC with a combination of SP and AQ is safe, with no serious drug related adverse event being reported after administration of over 800,000 courses in Senegal [11]. Areas where SMC would be an appropriate intervention based on the seasonality and incidence of malaria include most of the Sahel and sub-Sahel, a population of approximately 200 million, and possibly other areas in southern and eastern Africa [12]. A Technical Expert Group of the WHO reviewed all the available evidence on the efficacy and safety of SMC in May 2011 and recommended SMC with SP+AQ in areas of the Sahel and sub-Sahel with highly seasonal transmission. This recommendation was endorsed by the WHO Malaria Policy Advisory Committee (MPAC) in February 2012 [13]. Most countries in the Sahel and sub-Sahel region have incorporated SMC, along with other malaria control interventions, in their strategic malaria control plan and the

implementation of SMC at scale is in progress in many countries in this region with financial support from local governments and several international donor organisations. It is estimated that SMC will be delivered to about 20 million children in 2019. Preliminary evaluation suggests that SMC is providing about 50% protection against clinical malaria when delivered through a national programme (http://www.malariaconsortium.org/pages/access-smc.htm).

Despite the efficacy of SMC and its deployment together with long-lasting insecticide-treated bednets (LLIN), malaria remains a major cause of mortality and hospital admissions among young children in many areas of the Sahel and sub-Sahel and new approaches to malaria control are needed in these areas. One potential, novel approach is combining seasonal malaria vaccination with SMC [14]. Although RTS,S/AS01$_E$ may be a little less effective than SMC during the malaria transmission season, this may be balanced by provision of protection during the dry season when some malaria transmission still occurs and when SMC is not given. In addition, a single seasonal dose of vaccine may be easier to administer than four rounds of SMC. For this reason, a trial was initiated in Burkina Faso and Mali in 2017 to test whether the combination of seasonal vaccination with RTS,S/AS01$_E$ combined with SMC would be more effective than either intervention given alone. In addition, this trial has set out to establish whether seasonal vaccination with RTS,S/AS01$_E$ could be used as an alternative to SMC if, for example, resistance emerged to the anti-malarials used for SMC. Currently, there are no other combinations of licensed antimalarials that could be used to replace SP and AQ for SMC and it is likely to be 5-10 years before novel antimalarials under development are available for this purpose.

2.3 A trial of seasonal vaccination with the RTS,S/AS01$_E$ malaria vaccine with or without SMC

2.3.1 Outline

For the reasons given above, in the first quarter of 2017, 5887 children aged 5-17 months (2777 in Burkina Faso and 3143 in Mali) were enrolled in an individually randomised, double-blind controlled trial to investigate (a) whether the combination of seasonal vaccination with RTS,S/AS01$_E$ following priming combined with SMC with SP+AQ would be superior in providing protection against clinical episodes of malaria in young children than either intervention given alone and (b) whether seasonal vaccination with RTS,S/AS01$_E$ following priming given alone would be non-inferior to SMC given alone in preventing clinical attacks of malaria in young children [15]. Consequently, children enrolled in the study have been randomised individually to one of three groups:

a. Group 1 (SMC alone). Children in this group received three doses of rabies vaccine in April-June 2017 and one dose of hepatitis A vaccine in June 2018 and in June 2019 together with four rounds of SMC (SP+AQ) during the period August to November of 2017, 2018 and 2019.
b. Group 2 (RTS,S/AS01$_E$ alone). Children in this group received three doses of RTS,S/AS01$_E$ vaccine in April-June 2017 and one dose of RTS,S/AS01$_E$ vaccine in June 2018 and in June 2019 together with four rounds of SMC placebo during the period August to November of 2017, 2018 and 2019.
c. Group 3 (SMC + RTS,S/AS01$_E$ vaccine). Children in this group received three doses of RTS,S/AS01$_E$ vaccine in April-June 2017 and one dose of RTS,S/AS01$_E$ vaccine in June 2018 and in June 2019 together with four rounds of SMC (SP+AQ) during the period August to November of 2017, 2018 and 2019.

The primary end-point for the trial is the incidence of clinical episodes of malaria, defined as an episode of illness characterised by fever (temperature >= 37.5^{o} C), or a history of fever within the past 48 hours, that is severe enough to require treatment at a health centre or by a community health worker and which is accompanied by a positive blood film with a parasite density of 5,000 per µl or more. Secondary end-points include deaths, hospital admissions with malaria, the prevalence of malaria infection in healthy children at weekly surveys during the transmission season and the prevalence of malaria in study children at the end of the malaria transmission season. The economic cost of adding RTS,S/AS01$_E$ to SMC, its feasibility and the acceptability of each intervention are also being evaluated.

The trial is scheduled to finish collection of data in March 2020 when study children will have been followed through three malaria transmission seasons and two and a half dry seasons. By this time, children in the RTS,S/AS01$_E$ groups will have received two seasonal booster doses of the RTS,S/AS01$_E$ vaccine following priming.

2.3.2 Progress with the trial

In Burkina Faso, 92% of enrolled children received all three priming doses of RTS,S/AS01$_E$ or control (rabies) vaccine in 2017, 90% received their first booster dose in June 2018 and 90% received their second booster dose in June 2019. In Mali, 93% of children received all three priming doses of RTS,S/AS01$_E$ or comparator vaccine in 2017, 88% received their first booster dose of vaccine in June 2018 and 85% their second booster dose of vaccine in June 2019. Coverage with SMC or placebo has been high throughout the trial with nearly 90% of children receiving all four monthly SMC courses during the first and second years of the study. Retention of children in the trial has also been high. At a census undertaken early in 2019, 2547 (92%) of the 2777 children enrolled in Burkina Faso and 2816 (90%) of the 3143 children initially enrolled in Mali were still contributing to the study.

The investigators remain blind to the randomisation code. However, in March 2019 members of the trial's Data and Safety Monitoring Board (DSMB) met to review safety outcomes during the first two years of the trial. They reviewed data on deaths, deaths attributed to malaria, hospital admissions and hospital admissions attributed to malaria broken down by study group. The board concluded that it was safe for the trial to continue for a third year retaining the three initial study groups. This recommendation implies that during 2018 a single booster dose of RTS,S/AS01$_E$ was not markedly inferior to SMC in protecting against the outcomes considered by the board and provided encouragement for continuation of the trial into a third year and, potentially, for longer.

2.3.3 Rationale for continuation of the trial for a further two years

SMC is recommended until the age of five years (10 years in Senegal). Thus, if seasonal vaccination with RTS,S/AS01$_E$, or with another malaria vaccine with high initial efficacy but a relatively short duration of protection, is to be considered as a potential supplement, or possible replacement for SMC, it is important to determine the efficacy of annual seasonal vaccination in primed children until they reach the age of five years. If a recommendation is made to extend SMC until the age of 10 years, as may shortly be the case in several countries, it might then be appropriate to continue seasonal vaccination until this age but only if it can be shown that repeated booster doses of RTS,S/AS01$_E$ are safe and retain their efficacy.

If it can be shown during the first three years of the trial that adding seasonal vaccination with RTS,S/AS01$_E$ to SMC provides added benefit or it is shown and that vaccination with RTS,S/AS01$_E$ is equivalent or superior to SMC given alone it will be appropriate to continue the trial for a further two years but if neither of these outcomes is achieved it will not. However, waiting until the definitive results from the first three years of trial are obtained in April/May 2020 would make it impossible to obtain the vaccine and drugs, and make the logistic arrangements needed to deploy them, in time for the trial to continue without interruption during the 2020 malaria transmission season (July-October). Therefore, it was proposed by one of the trial's funders that a preliminary analysis of safety and efficacy on data collected up to the end of November 2009 should be undertaken to determine whether it would be useful to continue the trial until children reached the age of five years and, if so, whether all the trial groups should be retained. To guide the decision-making progress during this preliminary analysis, a decision tree (appendix 1) was developed. Efficacy data covering three transmission seasons and two dry seasons were provided to an independent expert appointed by the Bill and Melinda Gates Foundation at the beginning of January 2020. The same set of data was also provided to the trial's Data and Safety Management Board (DSMB) which reviewed it at meetings held on January 16th and 17th 2020. Both the independent expert and members of the DSMB recommended continuation of the trial with retention of all three study groups, as outline in this proposal.

2.4 Potential risks and benefits

Details of potential risks are provided in the Investigator's Brochure for RTS,S/AS01. Potential risks for licensed products, tetanus or tetanus/diphtheria toxoid vaccine, SP, and AQ may be found in the package insert for the product.

2.4.1 Risks of receipt of RTS,S/AS01$_E$ or tetanus toxoid or tetanus/diphtheria vaccine

Recipients of the investigational vaccine (RTS,S/AS01$_E$) may experience pain and/or swelling at the injection site, fever, headache, fatigue, nausea, vomiting and/or abdominal pain, joint pain and/or muscle aches but these features are usually only mild. About 1:1,000 children have been reported to develop a febrile convulsion after receipt of RTS,S/AS01$_E$. In the first three years of RTS,S/AS01$_E$ plus SMC trial only five children have experienced a febrile convulsion and it is not known if all these children had received RTS,S/AS01$_E$. In the large Phase III study, MALARIA-055, an imbalance in meningitis cases of any etiology (i.e. including cases with confirmed etiology and cases with no etiology found), with no cluster in time-to-onset, was observed in children 5-17 months of age at first dose [3]. Potential immune-mediated disease (pIMD) is a theoretical concern with adjuvanted vaccines but no evidence of autoimmune disease caused by RTS,S/AS01 has been observed. Nonetheless, both meningitis and pIMDs will be monitored as AEs of specific interest for the duration of the study.

Vaccination with tetanus or tetanus/diphtheria toxoids may cause mild local and systemic reactions and anaphylaxis has been recorded as a very rare adverse event following administration of this vaccine. Some children in the extension study will receive five doses of tetanus or tetanus/diphtheria toxoids during their first five years of life. Administration of a fourth dose of tetanus or tetanus/diphtheria/pertussis vaccine at the age of approximately 18 months is recommended in some routine infant immunization programmes. There is less experience of administration of a fifth dose. However, tetanus toxoid is recommended at each pregnancy in low income countries and many women must have received at least five doses of tetanus toxoid without any reports of adverse events.

2.4.2 Risks of receipt of SP and AQ

SP can cause Stevens-Johnson syndrome, but this side effect has been seen very rarely when SP has been used for either intermittent preventive treatment of malaria in pregnancy or SMC. Amodiaquine is bitter and may cause vomiting but serious side effects, which include liver damage and neurological side effects, are very rare. SMC with SP+AQ has proved to be very safe. A study conducted in Senegal in which the consequence of administration of 800,000 doses of SMC with SP+AQ were followed carefully identified only three serious potentially drug related adverse effects (two cases of hepatic damage and one extra-pyramidal syndrome) [11]. Enhanced pharmaco- vigilance in seven countries participating in the ACCESS-SMC project funded by UNITAID, through which approximately 15 and 30 million treatment courses were administered in 2015 and 2016 respectively has confirmed that SMC with SP+AQ is very safe.

2.4.3 Risks of accidental disclosure of private medical information

In order to ensure that all information collected on study volunteers is kept confidential, the following safeguards will be applied: access to study files and personal information will be limited to study personnel, ethics committees, regulatory authorities, and the sponsor. Any link between individual study identification number and an individual's personal identifying information will be maintained in accordance with the sites SOPs to maintain each individual's confidentiality.

2.4.4 Risks of participation in the study

If a participant is hurt as a direct result of participating in this study, the medical care will be provided by the study team at the respective research centres and the study will pay for the expenses.

2.4.5 Risks of phlebotomy

Venipuncture is a routine clinical procedure which the medical community commonly uses to obtain blood samples. Immediate complications may be slight pain during the entry of the needle into the skin, very rarely dizziness and syncope. Additionally, a haematoma may result from the venipuncture, but this has minimal risk. Infection of the skin/soft tissue at the puncture site, vein, or blood stream can all occur, though these are very rare with both finger sticks and venous blood draws. Late complications might include thrombosis of the vein due to trauma or infection. These complications are extremely rare. Participant monitoring, aseptic technique, including sterile disposable blood collection apparatus and adherence to standard medical precautions, reduce any risk to a minimum.

2.4.6 Known potential benefits

SMC is a policy established by the WHO, and provides significant benefit against clinical and severe malaria in West Africa where *P. falciparum* transmission is highly seasonal (https://www.who.int/malaria/mpac/feb2012/smc_policy_recommendation.pdf).

A booster dose of tetanus toxoid or tetanus/diphtheria/pertussis vaccine at about the age of 18 months is recommended by WHO but is rarely given so that a majority of the children in the control group will receive some benefit that they would not have obtained had they not been enrolled in the trial.

RTS,S/AS01$_E$ has been shown in a large Phase 3 trial to provide significant protection against both uncomplicated and severe malaria across different transmission settings.

3. OBJECTIVES AND PURPOSE

Primary objective:

The primary objectives of the extension study are to determine, among children aged three or four years of age who have previously received RTS,S/AS01$_E$ alone, SMC alone or both interventions since the age of 5-17 months:

a. Whether the combination of seasonal vaccination with the malaria vaccine RTS,S/AS01$_E$ with SMC with SP+AQ is superior to the administration of either intervention given alone in preventing clinical attacks of malaria, defined as an episode of illness characterised by fever (temperature >= 37.5^o C), or a history of fever within the past 48 hours, that is severe enough to require treatment at a health centre or by a community health worker and which is accompanied by a positive blood film with a parasite density of 5,000 parasites per µl or more.
b. Whether seasonal vaccination with RTS,S/AS01$_E$ alone is non-inferior to SMC alone in preventing clinical attacks of malaria, defined as above.

Secondary objectives:

Secondary objectives of the extension study are to determine, among children aged three or four years of age who have previously received RTS,S/AS01$_E$ alone, SMC alone or both interventions since the age of 5-17 months:

a. The incidence of uncomplicated clinical episodes of febrile illness (temperature ≥ 37.5°C) or a history of fever within the past 48 hours with a positive blood film (any level of asexual parasitaemia) or a positive rapid diagnostic (RDT) for malaria.
b. The incidence of hospital admissions with malaria including cases of severe malaria meeting the WHO criteria for severe malaria.
c. The prevalence of malaria infection not severe enough to warrant a clinic visit detected in a subset of randomly selected children during home visits.
d. The prevalence of malaria parasitaemia including gametocytaemia, moderate and severe anaemia and malnutrition at the end of malaria transmission season.
e. The incidence of minor adverse events following repeated booster of RTS.S/AS01 or tetanus and of serious adverse events (SAEs), including any deaths occurring at any time during the study, with special reference to any cases of meningitis or cerebral malaria.

Exploratory 0bjectives:

Exploratory objectives include:

a. Anti-CSP antibody concentrations obtained before and after priming and before and after each booster dose, determined in a sub-sample of children.
b. The prevalence of malaria parasitaemia at the end of the malaria transmission season in school-age children resident in the study areas, as determined during the previous three years, to provide information on whether there have been any changes in the overall level of malaria transmission in the study area during the five years of the study.
c. The prevalence of mutations in the Th2 or Th3 locus of the *Plasmodium falciparum csp* gene in children who have received repeated doses of RTS,S/AS01$_E$.
d. The presence of molecular markers of resistance to SP and AQ in parasite positive samples collected during the final cross-sectional survey.

e. The 28-day treatment outcome in children with asymptomatic malaria parasitaemia detected during the final cross sectional survey following treatment with SP+AQ.
f. Evidence of 'rebound' malaria in study children who have reached the age of five years at the time of the last year of the trial and who are no longer eligible to receive either of the interventions.
g. The acceptability to parents and health staff involved in delivery of the two interventions and the cost of adding RTS,S/AS01$_E$ to SMC.
h. The presence of antibodies to a broad range of malaria antigens in children who have received RTS,S/AS01$_E$ alone, SMC alone and RTS,S/AS01$_E$ + SMC collected after three years of receipt of the interventions.

4. STUDY DESIGN AND ENDPOINTS

4.1 Study areas

The trial will be conducted in Houndé health district, Burkina Faso and in Bougouni district, Mali. The Houndé district is situated 300 km from Ouagadougou and 100 Km from Bobo-Dioulasso where the CHUSS, the 2nd National Reference Hospital (University Hospital), is located and which is the also a base of the Institut de Recherche en Sciences de la Santé, Direction Régionale (IRSS). The study site in Mali is the district of Bougouni in the region of Sikasso, Mali, 150 km south of Bamako where the Malaria Research and Training Centre (MRTC) is based.

Figure 1 : Map of Mali and Burkina Faso showing the two study sites Bougouni and Houndé.

The population of Houndé district belongs primarily to the Bwaba ethnic group and that of Bougouni primarily to the Bambara and Fula ethnic groups. Farming is the main occupation in each area. Each district has a district hospital.

Malaria, due predominantly to *P. falciparum,* is highly seasonal in both districts with over 80% of cases occurring during the rainy season (July – October) and during the following month. The prevalence of *P. falciparum* malaria in school age children in December 2018 was 24% in Bougouni and 61% in Houndé. The main malaria vector in each study area is *Anopheles gambiae* ss. A high proportion of children sleep under an LLIN. The first line treatment for malaria in the public health system is artemether /lumefantrine in each district. Cases of uncomplicated malaria are treated at one of the health centres in the district and in Bougouni some cases are treated in the community by trained community health workers. Cases of severe malaria are managed in the district hospital.

In Burkina Faso, drugs and vaccines are stored at IRSS, Bobo-Dioulasso where there is a dedicated storage room which has pharmaceutical refrigerators with dynamic cooling and an automatic defrosting system, power failure and open-door alarms. The room is air conditioned 24 hours a day and the temperature maintained at 22°C with temperature and humidity controllers. In Mali, vaccines are stored at MRTC, Bamako, Mali. Vaccines are stored in a cold room with continuous monitoring of the temperature devices with alarm and telephone SMS and an email alert system. The system is also equipped with two back-up generators. Only authorized personnel have access to the cold room. The cold room has a capacity of 14.28 m^3 (2.45m long x 2.45m wide x 2.38m high). Standard operating procedures are in place for vaccine reception, storage in the cold room and transfer to field sites on a daily basis. Field laboratories, which are equipped to undertake parasitological and haematological investigations, have been established at each district hospital. A trial in 16,000 children of adding azithromycin to the antimalarial drugs used for SMC was conducted successfully in these two study sites in 2014-2016 [16].

4.2 Study endpoints

4.2.1 Primary endpoint

The primary end-point for the trial is the incidence of clinical episodes of malaria, defined as an episode of fever (temperature >= 37.5^o C), or a history of fever within the past 48 hours, that is severe enough to require treatment at a health centre or by a community health worker and which is accompanied by a positive blood film with a parasite density of 5,000 parasites per µl or more.

4.2.2 Secondary endpoints

Secondary end-points for the trial include:

a. Clinical episodes of an uncomplicated febrile illness (temperature >= 37.5^o C), or a history of fever within the past 48 hours, with a positive blood film (any level of asexual parasitaemia) or a positive rapid diagnostic test (RDT) for malaria.
b. Hospital admissions with malaria, including cases of severe malaria who meet WHO criteria for a diagnosis of severe malaria.
c. The prevalence of malaria infection not severe enough to warrant a clinic visit detected in a subset of randomly selected children during home visits.

d. The prevalence of malaria parasitaemia, including gametocytaemia, moderate and severe anaemia and malnutrition at the end of the malaria transmission season.
e. The safety of administering repeated doses of RTS,S/AS01$_E$ and tetanus toxoid as assessed by the immediate response to vaccination and by the incidence of serious adverse events (SAEs), including any deaths, occurring at any time during the study with special reference to any cases of meningitis or cerebral malaria.

4.2.3 Exploratory endpoints

Exploratory end-points for the trial include:

a. Anti-CSP antibody concentrations obtained before and after each booster dose, determined in a sub-sample of children.
b. The prevalence of malaria parasitaemia at the end of the malaria transmission season in school-age children resident in the study areas, as determined during the previous three years, to provide information on whether there have been any changes in the overall level of malaria transmission in the study area during the five years of the study.
c. The prevalence of mutations in the Th2 or Th3 locus of the *Plasmodium falciparum csp* gene in children who have received multiple doses of RTS,S/AS01$_E$.
d. The presence of molecular markers of resistance to SP and AQ in parasite positive samples collected during the final cross-sectional survey.
e. The 28-day treatment outcome in children with asymptomatic malaria parasitaemia detected during the final cross-sectional survey and who are treated with SP+AQ.
f. Evidence of 'rebound' malaria in study children who have reached the age of five years at the time of the last year of the trial and who are no longer eligible to receive either of the interventions.
g. The presence of antibodies to a broad range of malaria antigens in children who have received RTS,S/AS01$_E$ alone, SMC alone and RTS,S/AS01$_E$ + SMC after three years of receipt of the interventions.

5. STUDY RECRUITMENT, ENROLLMENT AND WITHDRAWAL

5.1 Participant inclusion criteria

In order to be eligible to participate in this study, a child must have been enrolled in the initial phase of the trial of seasonal vaccination with the RTS,S/AS01$_E$ vaccine and their parents or guardian must have provided consent for their inclusion in the extension study.

A child who has reached the age of five years and who is currently enrolled in the trial but who is no longer eligible to receive either of the interventions will be followed until the end of the extension study on safety grounds and for evidence of "rebound" malaria.

5.2 Participant exclusion criteria

Any child who meets any of the following criteria will be excluded from participation in the extension study:

a. The child has had an allergic reaction to the study drugs or vaccines.

b. The child had febrile convulsions on more than one occasion following vaccination.
c. The child has developed a serious underlying illness such as severe malnutrition (weight for age or mid arm circumference Z scores < 3 SD) which in view of the investigators might impair the response to vaccination.
d. The child has been enrolled in another malaria vaccine or other experimental malaria intervention study.

5.3 National and community consent

Approval of the trial by the national malaria control programme (NMCP) and the expanded programme of immunisation (EPI) of each country was obtained prior to the start of the first phase of the study. These organisations have been kept informed of its progress; directors of each NMCP have regularly attended project meetings. These organisations will be informed of the results from the initial phase of the trial as soon as they are known and the nature of the extension study will be discussed with them. Results will also be presented to the local health authorities in the study areas.

Community consent was obtained prior to the start of the first phase of the trial through community meetings. As soon as the final results of the trial are known, community meetings will be held to present these findings and their implications for the children who have participated in the trial. However, parents will not be told the nature of the study group to which their child was allocated in order to retain blinding during the period of the extension period. At these meetings, the parents or guardians of study children will be asked whether they found the number of contact points required for vaccination and drug administration acceptable to them and whether they were happy to continue with this approach to preventing malaria in their children. Community liaison groups will be established at each study site.

5.4 Recruitment

The first step for the extension study will be a visit to the families of all children enrolled in the initial phase of the trial to determine how many remain in the study area and are eligible for recruitment to the extension study. It is anticipated that approximately 4,800 will be eligible to join the trial.

Once the families of study children have been identified (each child has an ID card with a unique number), project staff will explain the outcome of the first phase of the study and its implications for them in a local language that they understand. The family will then be asked if they are willing for their child to continue in the trial for a further two years and it will explained to them what this would involve. Families will not be told which group their child belonged to and this will not be known to the field or laboratory staff participating in the trial so as to obtain blinding. Provided that the family provided their consent, and the child meets the inclusion criteria and does not fall within any of the exclusion criteria listed above, the child will be formally enrolled in the extension study and a new identify card with an updated photograph will be provided.

5.5 Participant withdrawal or termination

The following reasons may lead to withdrawal of individual subjects during the course of the extension study:

- Withdrawal of informed consent by parent/guardian.

- Age >60 months on May 2020.
- Any adverse event that, according to clinical judgment of the investigator, is considered as a definite contraindication to proceeding with the study procedures.
- Complete loss to follow-up.
- Any other protocol deviation that results in a significant risk to the subject's safety.

6. STUDY VACCINE

6.1 Study vaccines and antimalarial medications

The Investigational Brochure (IB) for RTS,S/AS01$_E$ will be provided to the ethical and regulatory review committees. The candidate RTS,S/AS01 vaccine to be used has been developed and manufactured by GSK Biologicals. The vaccine is labelled and packed according to applicable regulatory requirements.

RTS,S antigen is a lyophilized pellet containing 25 µg of RTS,S per vial. The pellet is reconstituted with adjuvant in liquid form and 0.5 mL of reconstituted vaccine contains 25 µg RTS,S. The AS01$_E$ adjuvant contains 25 µg of MPL®, 25 µg QS21 (QS21 is a triterpene glycoside purified from the bark of *Quillaja saponaria*) in a suspension of liposomes in phosphate buffered saline per 0.5 mL and is presented in 3 mL monodose vials. The presentation of the reconstituted RTS,S/AS01E candidate malaria vaccine is an opalescent liquid. RTS,S/AS01$_E$ will be provided by GSK and sent directly to the study sites in Burkina Faso and Mali. A single dose consists of 0.5 ml of RTS,S/AS01$_E$ final preparation. After reconstitution, the vaccine is administered by slow intramuscular injection, using a fresh 25G needle with length of one inch (25 mm), into the left deltoid. Vaccine is injected within four hours of reconstitution (storage at +2°C to +8°C).

Tetanus toxoid vaccine will be procured by LSHTM from the Serum Institute of India and sent directly to the study sites in Burkina Faso and Mali. Tetanus vaccine is provided in a 0.5ml single dose vial containing tetanus toxoid ≥ 40 IU adsorbed on Aluminium Phosphate ≤ 1.25 mg. Due to the impact of the COVID-19 pandemic, it may not be possible to air freight the tetanus toxoid vaccine ordered from the Serum Institute of India to Burkina Faso and Mali in time for this to be administered in June 2020. In this situation, tetanus/diphtheria toxoids vaccine will be provided by the national infant immunization programmes of Burkina Faso and Mali from the stock utilized routinely in their national immunization programmes and obtained from recognized manufacturers through UNICEF. The vaccine contains an aluminum phosphate adjuvant. Both vaccines are administered by slow intramuscular injection into the deltoid muscle.

6.1.1 Product storage and handling

All vaccine vials/pre-filled syringes (RTS,S) and AS01$_E$ adjuvant and the tetanus or tetanus/diphtheria toxoids vaccine will be stored in a refrigerator (+2^{o}C to +8^{o}C) and not frozen. All vaccine/adjuvant/water for injection vials will be stored in a safe place with no access for unauthorized personnel. The storage temperature will be monitored continuously with calibrated temperature monitoring device(s) and recorded according to SOPs at the investigator's site. An alarm system and a back-up refrigerator will be available in case of power failure/breakdown. GSK and the study monitor will be contacted if the cold chain is broken, for example if vaccines become frozen or refrigeration fails.

GSK will manage temperature deviations during the primary shipment until the reception at the clinical trials site. Temperature excursions are the responsibility of the sponsor upon receipt of the RTS,S/AS01$_E$ study product. Any temperature excursion outside the range of 0.0 to +8.0°C (for +2 to +8°C/+36 to +46°F label storage condition) will be reported to the sponsor (LSTMH) and GSK. In case of temperature excursion below +2.0°C down to 0.0°C there will be no need to report this deviation but adequate actions will be taken to restore the +2 to +8°C/+36 to +46°F label storage temperature conditions.

Anti-malarial medications will be stored below 30°C in designated storage rooms.

6.1.2 Preparation

For this study, the commercial presentation of RTS,S/AS01$_E$ will be used, i.e. a two-dose glass vial of lyophilized RTS,S antigen (50 µg) to be reconstituted with a two-dose glass vial of AS01$_E$ Adjuvant System (1.0 ml). The final product for administration will be prepared by reconstitution of the lyophilized antigen with the liquid adjuvant. From the reconstituted vaccine vial, 0.5 mL will be administered for RTS,S/AS01$_E$ full doses. All vials of vaccine provided in this study are intended for single use only. Loading of syringes with RTS,S/AS01$_E$ or tetanus or tetanus/diphtheria toxoids, and masking syringes with tape to disguise their content, will be done by a person who takes no further part in the trial. Vaccines will be administered by a nurse or other category of health worker trained to give vaccines.

6.1.3 Route of administration

The route of administration for both RTS,S/AS01$_E$ and tetanus or tetanus/diphtheria toxoid will be by the intramuscular route to the deltoid muscle

SP and AQ will be administered orally according to the dosages recommended by the National Malaria Control Programme.

6.2 Study vaccine accountability procedures

The study vaccines will be distributed to and maintained by the pharmacies in Burkina Faso and Mali and will include accounts of the amount of product shipped, documentation of adequate and safe handling and use, temperature logs for vaccine product and plans for returning or destroying unused product with the exception of any tetanus toxoid which, with permission of the sponsor, may be donated to the respective national EPI programmes.

7. STUDY INTERVENTIONS

7.1 Insecticide-treated bednets (LLIN)

At the start of extension study, all child in the trial who can be identified will be issued with a new LLIN regardless of whether or not their family consents for them to remain in the extension study.

7.2 The RTS,S/AS01$_E$ vaccine

As described previously, the RTS,S/AS01$_E$ vaccine is a virus like particle (VLP) composed of the CSP protein of *P. falciparum* and HBsAg which is given with the adjuvant, AS01$_E$. The vaccine may cause local side effects such as pain and redness at the site of vaccination in approximately 20% of recipients and minor systemic effects such as drowsiness and irritability are sometimes observed following vaccination. Fever post vaccination occurs in about 10% of children and febrile convulsions occurred in about 1% of

5-17 month old recipients enrolled in the phase 3 trial [3] but no persistent neurological effects were recorded. The main safety issues related to administration of RTS,S/AS01$_E$ during the phase 3 trial were a statistically significant increase in meningitis in children given the vaccine at the age of 5-17 months and an apparent increase in mortality in female vaccine recipients. However, during the first three years of the current trial, only five cases of febrile convulsion have been reported among the approximately 4,000 children who have received three priming and two booster doses of RTS,AS01 or control vaccine and no proven case of meningitis has been detected. Following a fifth dose of RTS,S/AS01$_E$ given in 2019, no increase in reported local or systemic side effects compared to previous vaccine doses was noted. To ensure that minor local and systemic adverse events, such as a reaction at the vaccine site or fever, are not enhanced following repeated doses of RTS,S, adverse events during the first seven days after vaccination will be recorded in a sub-set of children. SAEs will be recorded in all children throughout the period of the trial, as described below in Section 8 and Appendix 2.

7.3 Comparator vaccines

A single dose of tetanus or tetanus/diphtheria toxoid will be used as the comparator vaccine for children in SMC alone group in the first and second years of the extension study. A booster dose of tetanus or tetanus/diphtheria/ pertussis vaccine at about the age of 18 months is recommended by WHO but is rarely given so that a majority of the children in the control group will receive some benefit that they would not have obtained had they not been enrolled in the trial. Tetanus or tetanus/diphtheria toxoid vaccine may produce some minor local and systemic effects but is very safe.

7.4 Vaccine administration

Vaccination will be undertaken during a mass campaign lasting one to two weeks during June 2021 and June 2022. Children with a minor illness, such as mild diarrhea or a mild upper respiratory infection without fever, at the time they present for vaccination will be vaccinated. Those with recorded fever and/or other features of a more serious acute illness will not be vaccinated but will be treated for their illness and vaccinated when this has resolved. Children who miss the mass vaccination programme but who attend any of the subsequent rounds of SMC administration will be offered vaccination at that time.

7.5 Seasonal Malaria Chemoprevention (SMC)

Four or five courses of SMC with SP+AQ will be given at monthly intervals during the malaria transmission season to children in the SMC alone and SMC +RTS,S/AS01$_E$ groups. A course of SMC for children aged over the age of one year comprises a single treatment of SP (500mg/25 mg) and AQ 150mg on day 1 and AQ 150mg on days 2 and 3. SP and AQ and matching placebo will be obtained from Guilin Pharamceuticals, Shanghai, Co, a GMP-certified supplier. All treatments will be given under observation. SP can cause Stevens-Johnson syndrome, but this side effect has been seen very rarely when SP has been used for either intermittent preventive treatment of malaria in pregnancy or SMC. Amodiaquine is bitter and may cause vomiting but serious side effects, which include liver damage and neurological side effects, are very rare. SMC with SP+AQ has proved to be very safe. A study conducted in Senegal in which the consequence of administration of 800,000 doses of SMC with SP+AQ were followed carefully identified only three serious potentially drug related adverse effects (two cases of hepatic damage and one extra-pyramidal syndrome) [11]. Enhanced pharmaco- vigilance in 7 countries participating in the ACCESS-SMC project funded by UNITAID, through which approximately 15 and 30

million treatment courses were administered in 2015 and 2016, respectively, has confirmed that SMC with SP+AQ is very safe.

SMC drugs will pre-packed by a pharmacist, who takes no further part in the trial, in re-sealable envelopes bearing the child's unique number and containing tablets for the four cycles of treatment required for one malaria transmission season. Treatment with each dose of SMC is given by trained, paid volunteers at a central point in each study community under observation. Study children will be given an updated identity card containing their photo, study identity number and date of birth. At the time of vaccination and/or SMC administration, a child's photo ID card is scanned to ensure that the child is given the allocated intervention. Home visits are made to children who miss treatment on the designated day and their parents/guardians are asked if they would still like their child to receive SMC. If they agree, treatment is given at home.

The interventions that children in each study group will receive during the extension period are summarised in Table 1.

Table.1 Summary of the interventions that will be given to study children in each study group during the extension study

	Group1 (SMC)	**Group2 (RTSS)**	**Group3 (RTSS+SMC)**
2020			
June	Tetanus or Tetanus/diphtheria toxoids x 1	RTSS/AS01 x 1	RTSS/AS01 x 1
July -Oct	SMC x 4	SMC placebo x 4	SMC x 4
2021			
June	Tetanus or Tetatnus/diphtheria toxoids x 1	RTSS/AS01 x 1	RTSS/AS01 x 1
July -Nov	SMC x 4 (Mali) SMC x 5 (Burkina Faso)	SMC placebo x 4 (Mali) SMC placebo x 5 (Burkina Faso)	SMC x 4 (Mali) SMC x 5 (Burkina Faso)

(SMC or placebo is given at monthly intervals on four or five (Burkina Faso 2021) occasions during the malaria transmission season.)

7.6 Follow-up and measurement of endpoints

The following follow-up procedures required to measure the study outcomes will be undertaken.

7.6.1 Passive surveillance for uncomplicated and severe malaria.

In each country, project staff are based in the district hospital and in the main dispensaries that serve the study communities and, working with health service staff, they will be responsible for identifying and documenting all cases of malaria who present to these facilities. In Mali, cases of uncomplicated malaria may be treated by community health workers who have been taught to diagnose malaria with a RDT and to treat RDT positive cases. Cases of suspected malaria (fever, history of fever within 48 hours

or any other symptom/sign suggestive of malaria) will be managed on the basis of their RDT result but blood films and filter paper strips will obtained from all these cases for subsequent confirmation of the malaria diagnosis.

7.6.2 Active surveillance for malaria

Each month throughout the study period, 96 randomly selected children (32 from each arm of the study) in each country will be visited at home, their temperature measured and a blood film collected for subsequent detection of asymptomatic parasitaemia. Any child who is febrile or who has other features suggestive of a diagnosis of malaria at the time of the home visit will have an RDT done and if this is positive s/he will be treated with artemether/lumefantrine. This activity is suspended due to COVID-19.

7.6.3 Prevalence of malaria parasitaemia and anaemia.

A survey of all study children will be undertaken approximately one month after the last round of SMC administration at the end of each malaria transmission season. Temperature will be measured and any child who is febrile or who has other features suggestive of a diagnosis of malaria will have an RDT done; those who are positive will be treated with artemether/lumefantine. Finger prick blood samples will be collected for preparing blood slides and filter paper blood spots. The prevalence of parasitaemia, including the presence of gametocytes, will be detected by microscopy.

7.6.4 Evaluation of mutations in the *P.falciparum* csp gene

Because the efficacy of RTS,S/AS01$_E$ has been shown to be influenced by the occurrence of mutations in the *csp* gene [17] this will be measured in samples obtained from children with clinical episodes of malaria and in children with parasitaemia at the cross-sectional survey.

7.6.5 Immune response to the RTS,S/AS01$_E$ vaccine

Blood samples (4ml) will be collected from approximately 160 children in each of the groups (80 per country) who received RTS,S/AS01E and from approximately 20 children in the SMC alone group prior to and one month after administration of the third and fourth booster doses of RTS,SD/AS01 for measurement of anti-CSP antibodies. Children will be selected randomly for each blood draw.

7.6.6 Malaria endemicity in the study area

A malaria survey of schoolchildren will be conducted at approximately the same time as the cross-sectional surveys are undertaken in study children (November 2020 and November 2021) to assess the malaria parasite prevalence at the end of the transmission in the study areas and to monitor the trend in the distribution of molecular SP resistance markers. Two hundred randomly selected school children per country (total 400) aged 6-12 years resident in the study area who are well and have not received SMC will be tested for malaria by microscopy to assess the prevalence of malaria parasitaemia at the end of each malaria transmissions season and to determine the overall level of malaria transmission and to assess the distribution of molecular markers of resistance to SP in the study area during the final period of the trial.

7.6.7 Drug resistance

Dried blood spots from children who have malaria parasitaemia detected by microscopy at the final cross-sectional survey in November 2021 will be used for analysis of molecular markers of resistance to SP and AQ at MRTC, Bamako.

7.6.8 In vivo measurement of drug sensitivity to SP+AQ

At the end of the 2021 malaria transmission season, an *in vivo* study will be done to determine whether the local strains of *P. falciparum* still remain sensitive to SP+AQ. At the time of the cross-sectional survey, a RDT for malaria will be done in addition to collection of blood slides and filter paper samples. Blood smears will be examined the same day, or as soon as possible afterwards, for any child who has a positive RDT test. Children who have microscopically-confirmed *P. falciparum* infection, but who are otherwise quite well, will receive a full treatment course of SP+AQ over three days, dosed according to age. Children will then be followed for 28 days according to a standard WHO treatment efficacy protocol, with parasitaemia and symptoms assessed at days 0, 1, 2, 4, 7, 14 and 28 after treatment. Children who have fever, a history of fever, or are unwell and who have a positive RDT will be treated with artemether-lumefantine. Infections occurring after day 7 (late parasitological failures) will be investigated to determine if the recurrent infection was due to a reinfection or a recrudescence using molecular markers.

7.6.9 Investigation of possible rebound malaria

Approximately 60% of trial children will, in line with national guidelines, not be eligible to receive SMC during the 2021 malaria transmission season as they will have reached the age of five years. These children may be at increased risk of malaria due to the fact that they have been partially protected from malaria during the first few years of their life. Employing the surveillance systems described in section 7.1, these children will be identified if admitted to hospital with severe malaria or when presenting to a clinic with proven uncomplicated malaria. Details of how this case control study will be undertaken will be provided in another proposal that will be submitted to the ethics committee in due course.

In order to investigate further the potential of four years of administration of SMC, seasonal vaccination with RTS,S/AS01E or both, antibodies against a range of malaria antigens will be measured in a finger-prick blood sample obtained from all study children at the start of the 2021 malaria transmission season and the patterns of response observed related to the risk of malaria in the subsequent malaria transmission season. At the same time a questionnaire will be administered which asks about risk factors for malaria that might confound analysis of the association between antibody pattern and risk. A second blood spot for antibody determination will be obtained at the time of the cross-sectional survey described in section 7.6.3 above.

Among children no longer eligible for SMC, the incidence rates of uncomplicated malaria and hospitalisation for malaria will be compared according to age, sex and prior adherence to study interventions (SMC history and vaccination history), to determine if children who have been well-protected during the study period have been placed at increased risk during the period after SMC and/or vaccination is stopped, a possibility that has been little investigated. To explore this issue further, we will develop case-control studies that will obtain information on additional relevant risk factors and potential confounders that are not routinely collected from all study children (e.g. measures of socio-economic status, housing construction, knowledge of malaria, use of LLIN, etc.) from cases of either uncomplicated or severe malaria and from controls sampled at random from the study cohort.

8. ASSESSMENT OF SAFETY

8.1 Safety assessments

Because there is no previous experience of the administration of sixth and seventh doses of RTS,S/AS01$_E$, and to ensure there are no adverse outcomes following administration of a fifth dose of tetanus toxoid approximately 150 children in each country (50 in each intervention group) will be visited daily for seven days after their third and fourth booster doses and local and systemic side effects recorded. Additionally, the DSMB will undertake a safety review at the end of the 2020 malaria transmission season before progressing to the final year of the study.

Surveillance will be maintained throughout the study period for any SAEs. Any SAEs that are (a) considered by the investigators to likely to be linked to the administration of a study vaccine or study drug or (b) are suspected cases of meningitis or cerebral malaria or (c) are fatal or life threatening will be thoroughly investigated and reported to GSK and to the DSMB within 72 hours of their detection. All SAEs, whether considered related to the study interventions or not will be tabulated in a blinded fashion and provided to the DSMB and to GSK at the times requested by the DSMB. SAEs will reported to the LSHTM ethics committee annually in a tabulated format. SUSARs will be reported to the sponsor and to LSHTM ethics committee within 72 hours of notification in a format similar to that outlined in LSHTM SOP 008. SAE's will be reported to the ethics committees in Burkina Faso and in Mali in line with their instructions following review of the protocol. Details of definitions of adverse events and SAEs and of reporting procedures are described in Appendix 2.

Experienced project staff based at the district hospitals are responsible for the identification of any child in the trial admitted to hospital and for ensuring their referral to a study physician. Hospital staff have been provided with additional training on the recognition of cases of meningitis, cerebral malaria or immune deficiency diseases and standard operating procedures have been developed for management of children suspected of having one of these conditions. The aetiology of cases of meningitis is determined by microscopical examination of cerebrospinal fluid samples for bacteria and white blood cells and by subsequent PCR testing at a reference laboratory. The death of any study child is investigated and, if this occurred in the community, a verbal autopsy will be done.

9. LABORATORY PROCEDURES

9.1 Detection of malaria.

A histidine rich protein (HRP2) based Rapid Diagnostic Test (RDT) will be used for the initial diagnosis of malaria and to guide treatment. All blood films will be read twice by two separate readers following the guidelines developed for the phase 3 RTS.S/AS01 trial [18]. Slides which are judged to be discordant for either positivity or parasite density will be read by a third reader. For slides with high or medium density parasitaemia (> 400 parasites/µl) readings are considered discordant if the higher count divided by the lower count is > 2. In the case of slides with low density parasitaemia (< 400/µL), readings are considered discordant if the highest reading density is more than one $\log_{10}$ higher than the lowest reading. In cases when one reader gives a count > 400/µL and the other < 400/µL, the second criterion will apply. For cases of discrepancy in definition of positivity/negativity, the majority decision is adopted. If the majority decision is positive, the final result is the geometrical mean of the two positive readings. In the case of discrepancies in parasite density, the final result is the geometric mean of the two geometrically closest readings.

9.2 Detection of markers of resistance to SP and AQ.

Parasite DNA will be extracted from dried blood spots and nested PCR reactions will used to detect the presence of mutations in the *dhfr* and *dhps* genes associated with resistance to pyrimethamine and sulphadoxine respectively, and the *pfcrt* and *pfmdr* mutations associated with resistance to amodiaquine [19-21]. PCR-RFLP will be used to detect the N511, C59R, S108N and I164L mutations in the *dhfr* gene, the A437G and K540E mutations in the *dhps* gene, the N86Y mutation in the *pfmdr1* gene and the K76T mutation in the *pfcrt* gene.

9.3 Differentiation of malaria re-infections and recrudescences

DNA from dried blood spots will be used for molecular characterisation of the parasite using polymorphisms in the *msp1, msp2* and CA1 polymerase genes [22]

9.4 Detection of mutations in the *csp* gene of *P. falciparum*

DNA will be extracted from selected blood spots collected from children with malaria and analysed for mutations at the Th2 or Th3 locus of the *csp* gene of *P. falciparum* associated with evidence of reduced efficacy following RTS,S/AS01$_E$ vaccination [17] These assays will be conducted at the Broad Institute, Boston.

9.5 Measurement of haemoglobin concentration

Haemoglobin concentration will measured colorimetrically using a Hemocue colorimeter (Hemocue AB, Angelholm, Sweden).

9.6 Measurement of anti-CSP concentration

Antibodies to CSP will be measured by a standardised ELISA used in many previous trials of RTS,S/AS01$_E$ at the University of Ghent [23]

9.7 Measurement of a broad range of malaria antibodies.

Antibodies to a broad range of malaria antigens will be measured using the Luminex assay at LSHTM.

9.8 Measurement of malaria parasitaemia using a novel 18S rRNA/rDNA biomarker

Testing for malaria parasite DNA will be undertaken in a sub-set of samples by Prof Murphy at the University of Washington, Seattle, USA.

10. STATISTICAL CONSIDERATIONS

10.1 Statistical analysis plan

All data will be collected and verified prior to analysis. Detailed statistical procedures, listings, table shells, and figures will be provided in a statistical analysis plan (SAP) prior to analysis. The SAP will be finalized before study close-out and database lock and will be submitted to the DSMB before the study code is broken.

The following key statistical components and a detailed description will be documented in the SAP:

- Primary and secondary endpoints and how they will be measured.
- Statistical methods and tests that will be used to analyze the endpoints.
- Indication of whether the comparisons will be using one-tailed or two-tailed tests (with justification of the choice) and the level of significance to be used.
- Identification of whether any adjustments to the significance level or the overall p value will be made to account for any planned or unplanned subgroup analyses or multiple testing
- Specification of potential adjusted analyses and a statement with which covariates or factors will be included.
- Planned exploratory analyses and justification of their importance.

The number of subjects enrolled, vaccinated, completed, or withdrawn will be summarized. Reasons for withdrawal, when known, will be provided. Demographic data will be summarized by descriptive statistics and will include total number of observations (n), mean, standard deviation (SD) and range for continuous variables, and number and percentages for dichotomous variables.

All the investigators involved in the collection of clinical or laboratory data will be blind as to which group the individual study children have been allocated. However, based on the likely final results from the first phase of the trial the investigators are likely to know that none of the study groups was significantly inferior to another and it could be argued that this could influence how data are collected and analysed (anti-conservative bias). However, the investigators consider that this risk is small. The trial statistician undertaking the analysis of the initial phase of the study will need to be able to link study findings to an individual child but measures will be undertaken to enable this to be done without the statistician being unblended for the period of the extension. This will be done by developing a second set of IDs for the trial participants with only the independent trial statistician knowing how the numbers link to the child's initial number and study group. In this way, during the course of the extension study, the trial statistician dealing with data related to an individual child will not know the identity of the trial group to which that child belongs.

10.2 Sample size and analysis of the primary efficacy endpoint

At a survey conducted in April 2019, 5363 of the 5887 (91%) children enrolled in the first phase of the trial were contactable, an annual loss of about 5%. Assuming that this rate of attrition continues for a further year and that a similar proportion of families do not consent for their child to join the extension study, it is likely that approximately 4,800 children will enter the extension study.

The primary analyses will cover the entire study period, ie. the initial three years of the study and the two year extension period. The entire study period is defined as the time from enrolment in June 2017 until the end of the study year in which the child reaches five years of age and is no longer eligible for SMC, i.e. between 5 and 20 months of age (depending upon the exact age at first vaccination) and 60-71 months of age (depending upon the timing of the child's fifth birthday in relation to the SMC schedule). On the basis of date of enrolment, the exit dates above, and losses to follow-up as described above, we estimate that there will be approximately 20,625 person-years of follow-up during the entire study period: 6875 person-years per study group (i.e. an average of 3.5 person-years per child originally recruited).

The primary end-point for the trial is the incidence of clinical episodes of malaria. Based on data from the first two years of the ongoing study, we assume that the incidence of malaria will be 200 cases per 1000 children per year over the entire study period. Based on this assumption, the study will have 90% power to detect a 12.0% or greater relative difference (absolute difference: 24 cases per 1000 child-years) between the RTS,S/AS01$_E$ + SMC and the RTS,S/AS01$_E$ alone or SMC alone groups, through a standard superiority comparison.

The study will also have 90% power using a two sided 95% confidence interval to exclude a 17.5% relative difference (absolute difference: 35 cases per 1000 child-years) in incidence of clinical malaria between children in the RTS,S/AS01$_E$ alone or SMC alone group, using a non-inferiority design, over the entire study period. To determine the level of confidence with which this margin can be excluded, we will also calculate two sided 90% and 99% confidence intervals.

There will be approximately 4,100 child-years of observation in the year following the third booster; 1367 child-years in each study group. Assuming the incidence of clinical episodes of malaria remains at about 200 episodes per 1,000 children per year during this period, the study will have 90% power, through a standard superiority comparison, to detect a 25.9% or greater relative difference (absolute difference: 52 cases per 1000 child-years) between the RTS,S/AS01$_E$ + SMC and the RTS,S/AS01$_E$ alone or SMC alone group. In the same period, the study will have 90% power, using a two sided 95% confidence interval, to exclude a 39.2% relative difference (absolute difference: 78 cases per 1000 child-years) in the incidence of clinical malaria between children in the RTS,S/AS01$_E$ alone or SMC alone group, using a non-inferiority design.

A similar analysis will be undertaken for the final year of the study following administration of a fourth booster dose of the RTS,S/AS01$_E$ or comparator vaccine but study power will reduced because slightly more than half of the children enrolled (3860/5920) will not be eligible for SMC in 2021-22, and thus will not contribute further events nor person-time at risk to the study period.

Both intention-to-treat and per-protocol analyses will be undertaken. Children who received any dose of SMC or vaccine will be included in the intention to treat analysis. Children who received all scheduled doses of SMC, or who were treatment for a clinical episode of malaria at a time when SMC would have been given, or all scheduled doses of vaccine will be included in the per protocol analysis for each year of the study. Results obtained in Burkina Faso and Mali will be analysed separately. Additional sub-analyses will include analysis by age, gender, and bed net use during the transmission season, as determined by the history obtained at the cross-sectional surveys.

10.3 Sample size and analysis of secondary endpoint(s)

10.3.1 Sample size for measurement of prevalence of parasitaemia in school children.

Approximately 200 randomly selected children will be included in each school-survey. This sample size is based on the following assumptions: (1) the prevalence of malaria parasitaemia will be 50% and intra-cluster correlation coefficient (ICC) will be 0.1 (this is based on the observations from the surveys undertaken previously in the same study areas; (2) the design effect will be 1.9 if the cluster size is 10 children; (3) refusal to take part in the survey will be <5%; (4) the acceptable 95% precision of the prevalence will be +/-10%.

10.3.2 Sample size for the serological assays

For the analysis of the serological response to RTS,S/AS01E, comparisons will be made between mean anti-CSP antibody titres pre and post the sixth and seventh RTS,S/AS01E doses. Based on the standard deviations in antibody titres observed in children enrolled in the RTS,S/AS01E phase 2 and phase 3 trials, inclusion of around 160 individuals in each group (pre and post vaccination at each of the two time points) will give a study with approximately 80% power to detect a difference of 25% - 30% in mean titre between groups.

10.3.3 Sample size for the *in vivo* study of anti-malarial drug resistance

Assuming a 90% adequate clinical and parasitological response (ACPR) in asymptomatic children, for the study to be able to estimate this proportion with a precision of 5% at the 95% confidence level, 139 children will need to be enrolled. Anticipating up to 10% loss to follow-up, the number of children to be enrolled will be increased to a target of 155. It should be possible to reach this target in Burkina Faso but this may not be possible in Mali where the prevalence of infection is now lower than in Burkina Faso.

10.3.4 Sample size for the rebound study

An important secondary analysis will be for potential 'rebound' malaria when children become too old to receive SMC and/or RTS,S/AS01E. As slightly more than half of the children in the trial will no longer be eligible to receive SMC or RTS,S/AS01E in the last year of the trial, the study provides an excellent opportunity to assess of the risk of 'rebound' malaria in children who have received the combination of RTS,S/AS01E and SMC during the previous four years with that of children who received just SMC (standard of care). Here, the concern is that incidence will be higher among children who have received both SMC and RTS,S than among children who have received the single interventions. Consequently, the design will be a non-inferiority comparison (to rule out excess incidence in the RTS,S/AS01E + SMC group). In our previous study in this area, incidence among children in the year they became too old to receive SMC was three-fold higher than in the last year they received SMC (2068 versus 677 cases per 1000 person-years at risk). Assuming that incidence among children who are no longer protected by SMC increases from 200 to 600 cases per 1000 person-years, and that there are 800 child-years at risk in each group during the rebound period, the study will have 90% power to exclude a 29.6% relative increase (absolute increase, 178 cases per 1000 child-years) in the incidence of clinical attacks of malaria among children who received RTS,S + SMC, compared with children who received SMC alone. With the same assumptions, there will be 80% power to exclude a 25.6% relative increase (absolute increase, 153 cases per 1000 child-years).

11. SOCIO-ECONOMIC STUDIES

The costs, both direct and indirect, of adding RTS,S/AS01$_E$ to SMC or substituting RTS,S for SMC will be undertaken and these studies will be described in a separate protocol.

12. ASSESSMENT OF THE IMPACT OF THE COVID-19 PANDEMIC ON CONSULTATIONS AND HOSPITALISATIONS IN THE STUDY SITES

The COVID-19 pandemic reached Mali and Burkina Faso in late March 2020 and, as in many countries, has impacted peoples' lives and the use of health services. As of 17 December 2021, 19,062 cases

including 637 deaths were reported in Mali. The corresponding figures in Burkina Faso were 16,672 and 296. Although, based on published statistics, African countries including Mali and Burkina Faso are among the least affected by the virus the impact of the pandemic on the health services may still be large and vary from one area to another. In a study carried out by the Global Fund, a 23% drop in consultations was observed in services for children under 5 years of age in African countries [24]. The survey also found a 17% drop in malaria diagnoses in 2020, compared to 2019, in the 24 African countries surveyed. To better describe the context of this trial we will examine the impact of the COVID-19 pandemic on trends in health care seeking including pediatric consultations and admissions to hospitals in the areas where the RTS,S + SMC trial is being conducted. To achieve this, we will extract and examine all the data from heath registers for outpatients and hospital admission in the paediatric services of the district hospitals in the study areas over the period from January 2018 to December 2021. Information to be collected will include the date of consultation or hospital admission, age, gender, place of residence (village/quartier), reason for consultation or admission, the diagnosis, the treatment, and outcomes of the hospitalisation. No personal identifiers will be collected. A descriptive analysis will be performed using the total number of consultations and hospitalisations and the number of consultations and admissions due to malaria over time by age categories before and during the COVID-19 pandemic, considering the malaria transmission season and various periods of the pandemic. The latter will be characterised as the beginning of the pandemic, the period of lifting of the restrictions by the government, the waves of the pandemic and the period between the waves as well as the introduction of the COVID-19 vaccine. Parametric and nonparametric tests will be used to compare the average number of consultations and hospitalisations. The average monthly changes in the number of consultations and hospitalisations including for malaria will be described graphically over time according to the characteristics of the patients (age, residence) and comparison between the trends before COVID-19 and during various time periods during the pandemic as described above will be made. Comparisons will be made between the calendar months of 2018, 2019, 2020 and 2021 to explore variation in the seasonal pattern of care-seeking over different years. These data will be used to describe the context of the trial and facilitate interpretation of the trial results.

13. DATA MANAGEMENT AND CLINICAL MONITORING

Data collection and management will be undertaken using the Open Data Kit (ODK) (https://opendatakit.org) which was set up for the ongoing RTSS plus SMC trial and which is working well. This data collection and management system uses electronic case record forms (eCRF) loaded in tablet PCs. Project staff are well trained and competent in using ODK based eCRF and regularly upload data into a secured, central server. Data collected using ODK will be uploaded to ODK Aggregate Server based at LSHTM. This is supported by the LSHTM IT department, with routine daily backups created and stored on separate servers. Data will be migrated from the ODK Aggregate server and loaded into MS Access databases in both Burkina Faso and Mali for generating queries and cleaning the databases. On a fortnightly basis, these separate country-based files will be merged into single tables (combining data from each country) for each of the eCRFs used in the study. These merged files will be exported from MS Access to CSV format and placed on a secure server at LSHTM where they will be accessible only to the trial statistician.

An experienced independent GCP monitor (Raouf Osseni or one of his delegates) will continue to monitor the study to ensure the quality of the data collected and that GCP standards are met. The

monitor will conduct two visit each year together including a final close out visit. The monitor ensures that the trial is conducted according to the study protocol, that appropriate ethical procedures are in place and examines a random selection of clinical and laboratory records during each visit to confirm their validity.

14. ETHICS/PROTECTION OF HUMAN SUBJECTS

Inclusion in the trial of an RTS,S/AS01$_E$ alone group even though SMC is recommended policy is justified on the grounds that RTS,S/AS01$_E$ should provide some added protection against the malaria episodes that occur outside the main transmission season when SMC is given and that it may be easier to administer than SMC, thus creating a situation of equipoise.

Individual, written, informed consent will be obtained from the family or legally recognised guardian of each child entered into the extensions study. Ethical approval for the extension study will be obtained from the Ethics Committee of LSHTM, the Health Research Ethics Committee of Burkina Faso, the Institutional Ethics Committee of IRSS in Burkina Faso and the Ethics Committee of the Faculty of Medicine, Pharmacy and Dentistry, University of Bamako. Approval for the extension study will also be sought from the regulatory authorities in Burkina Faso and Mali. Conduct of the trial will not impose any additional costs on the local health services. The project will contribute to the costs of routine clinical care of study subjects during the trial and to strengthening of the district hospitals in the study areas.

14.1 Future Use of Stored Specimens

After the study is complete, sera, and dried blood spots will be stored at the MRTC in Mali, the IRSS in Burkina Faso or at the London School of Hygiene & Tropical Medicine in a biorepository. The samples will be kept for a maximum of 10 years.

14.2 Compensation

Participants will be insured against injury caused by the study according to legal requirements and compensation for research related injury (to include costs of long term and future medical care needs) is available, should it occur. Appropriate treatment during the trial will be provided by the site personnel and paid for by the project**.**

The participants will receive compensation for the time and travel of the protocol specified visits including the inclusion or vaccination visits, the end of season visits, the visits for serology and for the in vivo study. The compensation for the time and travel to the health center has been estimated to 1000 FCFA (~ US $ 2) per visit.

15. TRIAL MANAGEMENT AND ROLES OF THE INVESTIGATORS AND COLLABORATORS

The London School of Hygiene & Tropical Medicine will be the main sponsor for the trial. Delegated responsibilities will be assigned locally. The London School of Hygiene & Tropical Medicine holds Public Liability (''negligent harm'') and Clinical Trial (non-negligent harm'') insurance policies which apply to this trial. The study may be subject to audit by the London School of Hygiene & Tropical Medicine under their remit as the sponsor, the Study Coordination Centre and other regulatory bodies to ensure adherence to GCP.

An independent trial steering committee, established at the start of the first phase on the trial provides scientific oversight and holds regular meetings either through teleconferencing or face-face meetings and will continue to do so during the extension study. Members of the steering committee are listed in appendix 3.

A DSMB, established prior to the initiation of the first phase of the trial, oversees the safety of subjects in the trial and will continue to do so during the extension period. Membership of the DSMB is shown in appendix 3.

The trial adheres to the principles outlined in the International Conference on Harmonisation Good Clinical Practice (ICH GCP) guidelines, protocol and all applicable local regulations. The extension study will be registered as a new trial on clinicaltrials.gov.

The team from the IRSS, Burkina Faso, which includes Jean Bosco Ouédraogo, Halidou Tinto and Issaka Zongo, is responsible for conducting the part of the trial undertaken in Burkina Faso and they will participate in the analysis of the trial results. The team from MRTC, which includes Alassane Dicko, and Issaka Sagara is responsible for conducting the part of the trial undertaken in Mali and will participate in the analysis of the trial results. The LSHTM team (Brian Greenwood, Daniel Chandramohan, Matthew Cairns, Paul Milligan, Jayne Webster and Karen Slater) provides epidemiological, statistical, administrative and financial management support.

16. FUNDING

Funding for the first phase of the trial has been provided by grants from the UK MRC/DFID/NIHR/WT Joint Global Health Trials scheme and PATH. Funding from the same donors is being sought to support the extension study.

17. DISSEMINATION PLANS

Results from the trial will be presented at national and international conferences, in peer reviewed journals, to the ethics committees and will be discussed with the study communities at the end of the study. Trial results will be shared with the WHO's technical expert groups and Malaria Policy Advisory Group (MPAC).

Strong links have been established already with the Ministries of Health, NMCPs and EPI programmes in Burkina Faso and Mali and these will facilitate the incorporation of RTS,S/AS01$_E$ vaccine into SMC/EPI programme if this is found to be a useful intervention. The study team has also established good links with many other organisations involved in the delivery of SMC trials, including the ACCESS-SMC programme coordinated by the Malaria Consortium and with the WHO staff responsible for conducting the RTS,S/AS01$_E$ implementation studies Thus, if it is found that RTS,S/AS01$_E$ vaccine is a useful replacement for or addition to SMC regimens, routes have already been established through which this knowledge could be disseminated rapidly.

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19. TIME AND EVENT TABLE

Activities	2020												2021												2022										
	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D
Protocol submission for ethics approval	x																																			
Ethics and regulatory approval		x																																		
Amend trial registration		x																																		
Order study drugs	x																																			
Vaccine shipment				x												x																				
Steering committee meeting		x							x												x												x			
DSMB meeting	x						x												X												x					
Census of study children				x												x															x					
Re-consenting children				x																																
Printing ID cards & labels					x												x																			
Vaccination						x												x																		
Administration of SMC								x	x	x	x								X	x	x	x														
Health facility based morbidity and mortality surveillance	x	x	x	x	x	x	x	x	x	x	x		x	x	x	x	x	x	X	X	x	x	x	x	x	x	x	x	x	x						
Active surveillance of malaria infection	x	x	x	x	x	x	x	x	x	x	x		x	X	x	x	x	x	X	X	x	x	x	x	x	x	x	x	x	x						
End of transmission surveys												x												x												
Blood slide reading	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	X	X	x	x	x	x	x	x	x	x	x	x						
PCR assays													x												x											
Serology								x												X																
GCP monitoring						x													X											x						
Data management	x	x	x	x	x	x	x	x	x	x	x		x	X	x	x	x	x	X	X	x	x	x	x	x	x	x	x	x	x	x	x	x			
Blinded analysis & report for committees																			x																	
Locking of data																																	x			
Data analysis																																	x	x		
Dissemination																																			x	x

20. APPENDICES

Appendix 1

A decision tree developed to aid in making the decision as to whether the trial should be extended.

INTRODUCTION

It is proposed that preliminary analysis should be conducted on data obtained up to the end of 2019, a few months before data collection has finished (March 2020), by an independent investigator in January or February 2020. This preliminary analysis will be undertaken to allow a decision to be taken early enough in 2020 for preparations to be made for continuation of the trial beyond June 2020 without a break in continuity of the administration of the trial interventions or of surveillance.

DATA AVAILABLE FOR THE PRELIMINARY ANALYSIS

The data available for the preliminary analysis will include –

a. Deaths.
b. Deaths attributed to malaria.
c. Hospital admissions.
d. Hospital admissions due to malaria
e. The incidence of clinical episodes of malaria (the primary trial end-point).
f. The prevalence of malaria at the end of each transmission season.
g. The presence of anaemia at the end of each transmission season.

ANALYSES TO BE UNDERTAKEN FOR THE PRELIMINARY REVIEW

Analyses of efficacy against clinical episodes of malaria, the primary trial end-point, will be undertaken for the overall period from the start of the trial in June 2017 until December 2019 and separately for the periods June 2017 to May 2018, June 208 to May 2019 and June 2019 to November 2019. Efficacy against severe malaria will be measured as a secondary analysis but the study is only powered to detect major differences between groups for this end-point. Safety evaluations will be based on the number and characteristics of serious adverse events recorded throughout the period of the trial. Antibody titres to the CSP antigen measured before and after priming with the RTS.S/AS01 vaccine and before and after each of the two booster immunisations will be analysed in the final analysis but these results will not be available at the time of the preliminary review.
The primary analysis will be based on data for both sites combined, with site as a stratification factor, but site-specific analyses will also be undertaken. A test of interaction will be used to determine if there is any evidence that efficacy of RTSS varies between the two trial sites. If a substantial difference is found, further analysis may be required.

DECISIONS MADE ON THE GROUNDS OF EFFICACY

Because of the inclusion of three study groups, a number of potential efficacy outcome are possible but analyses will focus on answering two main questions.

a. *Is efficacy against clinical episodes of malaria of the combination of RTS,S/AS01$_E$ plus SMC superior to that of SMC alone (the standard of care)?*

   Deciding on the enhanced level of efficacy that would be sufficient to warrant a policy decision to implement the combination of RTS,S/AS01$_E$ and SMC, and hence a justification for continuation of the trial, is difficult. However, based on the results of the RTS,S/AS01$_E$ phase 3 trial and the percentage used frequently in setting a potential target, and hence sample size, for trials of other malaria control interventions a figure of 20% or more has been chosen to define the superiority in efficacy against clinical episodes of malaria of the combination of RTS,S/AS01$_E$ and SMC over that achieved with SMC alone.

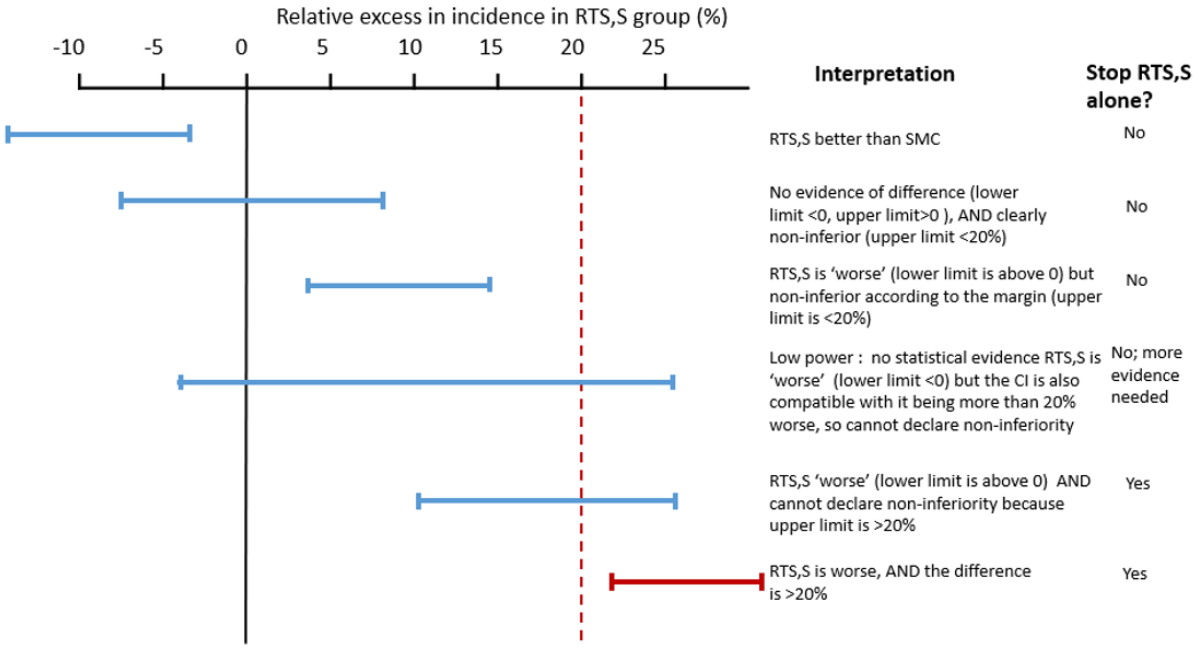
   Decision: If the trial data are consistent with an efficacy of the RTS,S +SMC combination over SMC alone of at least 20% (the upper 95% confidence limit on the efficacy is 20% or higher and the lower 95% confidence limit exceeds zero), the trial will continue with two or three groups depending on the outcome of the analysis described below. In the unlikely event that the combination of RTS,S/AS01$_E$ and SMC is not superior to either intervention used alone and RTS,S/AS01$_E$ given alone is not inferior to SMC given alone consideration could be given to continuation of the trial with the RTS,S/AS01$_E$ and SMC alone arms.

b. *Is RTS,S/AS01$_E$ given alone at least as effective in preventing clinical episodes of malaria as SMC given alone (the standard of care)?*

   SMC is the standard of care in both Burkina Faso and Mali. Consequently, if it is not possible to establish that RTS,S/AS01$_E$ given alone is as effective as SMC in preventing clinical episodes of malaria, it would not be justifiable to continue with the RTS,S/AS01$_E$ alone group in the trial. The efficacy of SMC (against placebo or no SMC) has been well established in clinical trials and, more recently, through case control studies in Mali and Burkina Faso. Efficacy of SMC against no SMC was about 50% over the whole year, although higher during the four months of the malaria transmission season. The non-inferiority margin is the largest reduction in this efficacy that would be considered acceptable if RTSS/AS01 was to replace SMC. A reduction in efficacy from 50% to 40%, which might be considered acceptable, translates to a 20% greater incidence in the RTSS/AS01-alone arm (the comparator) compared to the SMC-alone arm. This reduction in efficacy might be considered acceptable taking into account the potential advantages of RTS,S/AS01$_E$ over SMC in terms of ease of delivery and hence the likelihood of being able to sustain high levels of coverage. We have, therefore, chosen a margin of 20% in clinical incidence between the RTS,S/AS01$_E$ alone and SMC alone groups as the margin on which a decision will be based (this is slightly higher than the 16.1% margin used in the protocol to define the study's power to measure this end-point) .

   If the incidence rate ratio RTS,S/AS01$_E$ alone: SMC alone can be shown to be less than 1.20 then we can conclude that RTS,S alone is non-inferior to SMC alone. Two-sided 99%, 95% and 90% confidence intervals for the rate ratio will indicate the degree of confidence with which the margin of 1.20 (relative increase of 20%) can be excluded. Examples of how the confidence intervals might be interpreted are shown in Figure 1.

Figure 1. Interpretation of the possible findings in the comparison between RTS,S/AS01$_E$ and SMC.



A separate consideration is the performance of RTS,S/AS01$_E$ alone, compared to the combined group. It could be argued that if the RTS,S/AS01$_E$ alone group is inferior to the RTS,S/AS01$_E$ + SMC group, children in the RTS,S/AS01$_E$ alone group should be given SMC. However, RTS,S/AS01$_E$ is not standard of care and it is uncertain whether the combination would be sufficiently more effective to warrant its introduction. Therefore, it is justifiable to continue with the RTS,S/AS01$_E$ alone group, provided that it is not inferior to SMC, to investigate whether RTS,S/AS01$_E$ alone could be used as an alternative to SMC alone on grounds of increased feasibility or emergence of drug resistance.

Decision: If RTS,S/AS01$_E$ is demonstrated to be non-inferior to SMC alone, using a relative margin of 20%, it will be reasonable for this group to continue in the trial. If non-inferiority cannot be demonstrated, the children in the RTS,S alone group will not continue; these children will receive SMC as recommended in the national malaria programme and be followed until the end of the study.

The same questions will be considered in relation to the incidence of severe malaria (hospital admissions and deaths attributed to malaria) but the study is not powered to show differences between groups for these end-points Thus, although the findings from these analyses may help to support decisions based on the efficacy against clinical episodes of malaria, they will not be used alone to determine the decision on the future of the trial unless they raise issues of safety.

DECISIONS MADE ON THE GROUNDS OF SAFETY

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SMC alone is standard of care and so will act as the baseline for safety assessments (SMC has been shown in several large studies to be very safe). Thus, the trial's DSMB will be asked to consider the safety of children in the SMC plus RTS,S/AS01$_E$ and the RTS,S/AS01$_E$ alone groups in relation to findings in the SMC alone group based on an analysis of deaths overall, deaths attributed to malaria, SAEs overall and SAEs attributed to malaria. If there is a significant increase in the overall incidence of serious adverse events, primarily deaths or hospital admissions, not due to trauma or elective surgery or in the incidence of SAEs due to severe malaria in one of the RTS,S/AS01$_E$ groups compared with the SMC alone

group, the DSMB may recommend that the affected group should not continue in the trial. If there is a significant increase in both RTS,S/AS01$_E$ groups the DSMB may recommend that the trial should not be extended. Definition of what would considered to be sufficient evidence of risk in the groups receiving RTS,S/AS01$_E$ will be a decision for the DSMB but, in such circumstances, a difference in the incidence of serious adverse events between groups that reaches a statistical significance of P = 0.01 or less is usually considered to be one potential reason for dropping a trial group. These data will also be made available to GSK.

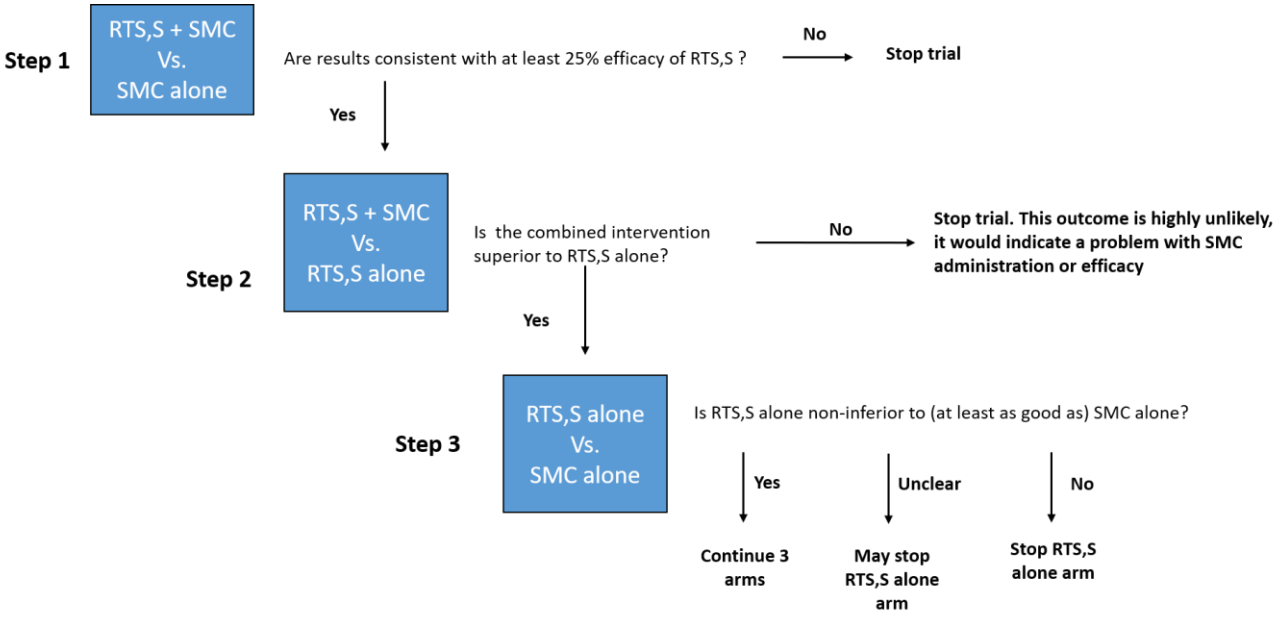
SEROLOGY

Serological findings will not be available in time for the preliminary evaluation.

CONCLUSIONS

Deciding on whether to continue the RTS,S/AS01$_E$ + SMC trial and, if so, whether this should be with all three arms will require some subjective decisions based on balancing the potential risks and benefits of doing so. However, this document should help to provide a framework that will aid in making these decisions and a potential pathway is shown in Figure 2.

Figure 2. Decision pathway



Appendix 2

Definitions of adverse and serious adverse events and reporting schedule

A. Definition of an adverse event, adverse events of special interest, and serious adverse event

A.1 Definition of adverse event (AE)

An adverse event (AE) is defined as any clinical symptom or sign that occurs in a study child after administration of the study vaccine or drugs that may or may not have a causal relationship with the study drugs. Examples of an AE include -

(1) Occurrence of symptom such as fever, vomiting or diarrhoea in a child who did not have these symptoms prior to the administration of drugs; (2) Unexpected worsening of an existing condition.

A.2 Definition of Adverse Events of Special Interest

AEs of specific interest for safety monitoring include all seizures occurring within 30 days post-vaccination, meningitis and pIMDs and will be recorded in the CRF.

Meningitis cases: For the further evaluation of the safety signal of meningitis in the investigational vaccine groups all cases of meningitis occurring during the study will be reported as a SAE.

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

Table A. List of Potential Immune-mediated Diseases:

Neuroinflammatory disorders	**Musculoskeletal disorders**	**Skin disorders**
• Cranial nerve disorders, including paralyses/paresis (e.g. Bell's palsy) • Optic neuritis • Multiple sclerosis • Transverse myelitis • Guillain-Barré syndrome, including Miller Fisher syndrome and other variants • Acute disseminated encephalomyelitis, including site specific variants: e.g. non-infectious encephalitis, encephalomyelitis, myelitis, myeloradiculoneuritis • Myasthenia gravis, including Lambert-Eaton myasthenic syndrome • Immune-mediated peripheral neuropathies and plexopathies,	• Systemic lupus erythematosus and associated conditions • Systemic scleroderma (Systemic sclerosis), including diffuse systemic form and CREST syndrome • Idiopathic inflammatory myopathies, including dermatomyositis • Polymyositis • Antisynthetase syndrome • Rheumatoid arthritis, and associated conditions including juvenile chronic arthritis and Still's disease • Polymyalgia rheumatica • Spondyloarthritis, including ankylosing spondylitis, reactive arthritis (Reiter's	• Psoriasis • Vitiligo • Erythema nodosum • Autoimmune bullous skin diseases (including pemphigus, pemphigoid and dermatitis herpetiformis) • Alopecia areata • Lichen planus • Sweet's syndrome • Localised Scleroderma (Morphoea)

(including chronic inflammatory demyelinating polyneuropathy, multifocal motor neuropathy and polyneuropathies associated with monoclonal gammopathy). • Narcolepsy	Syndrome) and undifferentiated spondyloarthritis • Psoriatic arthropathy • Relapsing polychondritis • Mixed connective tissue disorder	

Vasculitides	**Blood disorders**	**Others**
• Large vessels vasculitis including: giant cell arteritis such as Takayasu's arteritis and temporal arteritis. • Medium sized and/or small vessels vasculitis including: polyarteritis nodosa, Kawasaki's disease, microscopic polyangiitis, Wegener's granulomatosis, Churg–Strauss syndrome (allergic granulomatous angiitis), Buerger's disease (thromboangiitis obliterans), necrotizing vasculitis and anti-neutrophil cytoplasmic antibody (ANCA) positive vasculitis (type unspecified), Henoch-Schonlein purpura, Behcet's syndrome, leukocytoclastic vasculitis.	• Autoimmune hemolytic anemia • Autoimmune thrombocytopenia • Antiphospholipid syndrome • Pernicious anemia • Autoimmune aplastic anaemia • Autoimmune neutropenia • Autoimmune pancytopenia	• Autoimmune glomerulonephritis (including IgA nephropathy, glomerulonephritis rapidly progressive, membranous glomerulonephritis, membranoproliferative glomerulonephritis, and mesangioproliferative glomerulonephritis) • Ocular autoimmune diseases (including autoimmune uveitis and autoimmune retinopathy) • Autoimmune myocarditis/cardiomyopathySarcoidosis • Stevens-Johnson syndrome • Sjögren's syndrome • Idiopathic pulmonary fibrosis • Goodpasture syndrome • Raynaud's phenomenon

Liver disorders	**Gastrointestinal disorders**	**Endocrine disorders**
• Autoimmune hepatitis • Primary biliary cirrhosis • Primary sclerosing cholangitis • Autoimmune cholangitis	• Inflammatory Bowel disease, including Crohn's disease, ulcerative colitis, microscopic colitis, ulcerative proctitis • Celiac disease • Autoimmune pancreatitis	• Autoimmune thyroiditis (including Hashimoto thyroiditis) • Grave's or Basedow's disease • Diabetes mellitus type I • Addison's disease • Polyglandular autoimmune syndrome • Autoimmune hypophysitis

A3. Definition of a serious adverse event (SAE)

A serious adverse event is any clinical condition that fulfils at least one of the following criteria:

(1) Results in death.
(2) Results in admission to hospital.
(3) Is life-threatening (the child was at risk of death at the time of the adverse event).
(4) Results in disability/incapacity.

B. Severity, relationship of event to study drug or vaccine and outcome

The severity of a clinical adverse event is to be scored according to the following scale:

(1) Mild: Awareness of sign or symptom, but easily tolerated.
(2) Moderate: Discomfort enough to cause interference with usual activity
(3) Severe: Incapacitating with inability to work or perform usual activity.
(4) Life-threatening: Patient at risk of death at the time of the event.
(5) Death.

C. Assessment of Causality

The relationship between the study vaccines and drugs and the occurrence of each SAE will be determined by the project physician in consultation with the site PIs based on their clinical judgment. Alternative causes, such as the natural history of the underlying diseases, concomitant therapy, other risk factors, and the temporal relationship of the event to the study drug will be considered and investigated. The site PIs will consult the lead PIs and the DSMB if this is deemed to be necessary.

There may be situations where the PIs have very minimal information about a SAE to include in the initial report. However, every attempt will be made to make an assessment of causality for every SAE prior for reporting to the IDMC. The PIs may change their opinion of causality in light of follow-up information, and may amend the SAE case report form accordingly.

The relationship of an adverse event to study vaccine or drug will be assessed according to the following definitions:

(1) Definitely unrelated: events that had occurred prior to administration of the study drugs or events that are obviously unrelated to the study (e.g. accidental injury).

(2) Unlikely: There is no reasonable temporal association between the study drug and the suspected event and the event could have been produced by the child's clinical state or other concomitant medications.

(3) Possible: The suspected adverse event may or may not have a reasonable temporal association with the administration of study drug but the nature of the event is such that an association with the study drug cannot be ruled out. The event could be related to the child's clinical state or by concomitant medications.

(4) Probable: The suspected adverse event follows a reasonable temporal sequence after administration of study drugs, abates upon discontinuation of the drug, and cannot be reasonably explained by the known clinical state of the child.

(5) Definitely related: events that have no uncertainty in their association to the administration of study drugs.

The outcome of each AE must be assessed according to the following classification:

1) Completely recovered : The child has fully recovered with no observable residual effects.

2) Not yet completely recovered : The child's condition has improved, but still has some residual effects.

3) Deterioration : The child's overall condition has worsened

4) Permanent damage : The AE has resulted in a permanent impairment

5) Death : The child died due to the AE.

6) Ongoing : The AE remains the same as at onset.

7) Unknown : The outcome of the AE is not known because of lost to follow-up

D. Reporting of adverse events and SAEs

All serious adverse events will be reported using a SAE report form which will have a detailed narrative of the events including information on the date the event started, severity, possible relationship to study drugs, concomitant medications, action taken, and outcome of the event.

A list of all SAEs will be compiled monthly or three monthly as requested by the DSMB and provided to the DSMB and GSK. Any SAE potentially related to the vaccine or drug administration will be reported to the DSMB and institutional Ethics Committees within 72 hour.

SAEs will be reported promptly to GSK within the timeframes described in Table A, once the investigator determines that the event meets the protocol definition of a SAE.

Table B. Timeframes for submitting serious adverse event and other events reports to GSK Biologicals

Type of Event	Initial Reports		Follow-up of Relevant Information on a Previous Report	
	Timeframe	Documents	Timeframe	Documents
SAEs	72 hours*‡	electronic Expedited Adverse Events Report	72 hours***	electronic Expedited Adverse Events Report
AEs of specific interest**	72 hours*‡	electronic Expedited Adverse Events Report	72 hours***	electronic Expedited Adverse Events Report

* Timeframe allowed after receipt or awareness of the information.
** AEs of specific interest include meningitis and pIMDs.
*** Within receipt of information on the outcome of the SAE
‡ The investigator will be required to confirm review of the SAE/AEs of specific interest causality by ticking the 'reviewed' box in the GSK electronic Expedited Adverse Events Report within 72 hours of submission of the SAE/AEs of specific interest.

Reporting events of special interest

AEs of specific interest (pIMDs, and meningitis occurring within 30 or 42 days of vaccination) will be reported promptly to GSK within the timeframes described in Table B, once the investigator determines that the event meets the protocol definition of an AEs of specific interest (pIMDs and meningitis within 30 or 42 days of vaccination).

Contact information for reporting serious adverse events and AEs of specific interest

Study Contact for Reporting SAEs and AEs of specific interest*
Refer to the local study contact information document.
Back-up Study Contact for Reporting SAEs and AEs of specific interest*
24/24 hour and 7/7 day availability: **GSK Biologicals Clinical Safety & Pharmacovigilance** Outside US & Canada sites: Fax: +32 2 656 51 16 or +32 2 656 80 09 Email address: Rix.CT-safety-vac@gsk.com

Appendix 3

Membership of the trial committees.

Trial Steering Committee

Members of the trial steering committee are –

Daniel Chandramohan, LSHTM, London, UK (investigator).

Alassane Dicko, MRTC, Bamako, Mali (investigator).

Brian Greenwood, LSHTM, UK (investigator).

Jean Bosco Ouedraogo, IRSS Bobo-Dioulasso, Burkina Faso (investigator).

Opokua Ofori-Anyinam, GSK, Brussels, Belgium (independent member).

Kwadwo Koram, Noguchi Memorial Research Institute, Accra, Ghana (independent member).

Joaniter Nankabirwa, Makerere University, Kampala, Uganda (independent member).

Chris Ockenhouse, MVI Path, Washington, USA (independent member).

Caroline Harris (replacing Morven Roberts) MRC Head Office, London (donor representative).

Feiko ter Kuile, LSTM, Liverpool, UK (independent member) (chair).

Mahamdou Thera (MRTC, Bamako, Mali (independent member).

Data, Safety and Monitoring Board

Members of the Data Safety and Monitoring Board are -

Umberto D'Alessandro, MRC Unit, The Gambia.

Blaise Genton, Swiss Tropical and Public Health Institute, Basle, Switzerland (chair).

Mainga Hamaluba, KEMRI Wellcome Trust, Kilifi, Kenya

Francesca Little, University of Capetown, Capetown, South Africa.

Malcolm Molyneux, LSTM, Liverpool, UK

Jean-Louis Ndiaye, University Cheikh Anta Diop, Dakar, Senegal

23.06.22

Seasonal vaccination with the RTS,S/AS01$_E$ malaria vaccine given with or without seasonal malaria chemoprevention: extension of a randomised, double-blind Phase 3 trial until children reach the age of five years.

Brief Title: A trial of RTS,S/AS01$_E$ and SMC: extension study

(Protocol Identifying Number - PATH: CVIA 083)
(Protocol Identifying Number - GSK: MALARIA-106 EXT:099 Y4-5 SUPP)

Revisions to the Statistical Analysis Plan:

Revision Number	Date of Revision	Page Number(s)	Details

Summary Study Information

Study title

Seasonal vaccination with the RTS,S/AS01$_E$ malaria vaccine given with or without seasonal malaria chemoprevention: extension of a randomised, double-blind Phase 3 trial until children reach the age of five years.

Clinical trial registration

Clinicaltrials.gov Identifier: NCT04319380

This study is an extension of an earlier clinical trial, NCT03143218: 'A comparative trial of seasonal vaccination with the malaria vaccine RTS,S/AS01$_E$, seasonal malaria chemoprevention, and of the two interventions combined.'

Study duration

Original trial: April 2017 – March 2020;
Extension Study: April 2020 – March 2022.

Study site

The study is being conducted in Houndé district, Burkina Faso and in Bougouni district, Mali.

Study objectives

Original Trial

The original trial sought to determine

1. Whether seasonal vaccination with RTS,S/AS01$_E$ was non-inferior to four monthly courses of seasonal malaria chemoprevention (SMC) with sulphadoxine-pyrimethamine plus amodiaquine (SP+AQ) in preventing clinical malaria and other adverse outcomes.

2. Whether the combination of these two interventions (i.e. seasonal vaccination with RTS,S/AS01$_E$ and SMC with SP+AQ) was superior to RTS,S/AS01$_E$ alone or SMC alone in preventing clinical malaria and other adverse outcomes.

These aspects were considered to be of equal priority.

Extension Study

The extension study, undertaken in children aged three or four years old who had previously received five doses of the RTS,S/AS01$_E$ malaria vaccine or comparator vaccines, together with seasonal malaria chemoprevention (SMC) with sulphadoxine-pyrimethamine (SP) plus amodiaquine (AQ) or placebo set out to determine whether :

1. Vaccination with a booster dose of the RTS,S/AS01$_E$ malaria vaccine at the beginning of the malaria transmission until children reach the age of five years is non-inferior to SMC in preventing clinical attacks of malaria and would be easier to deliver than SMC.

2. Administration of further doses of RTS,S/AS01 $_E$ at the beginning of the malaria transmission until children reach the age of five years provides additional protection against clinical episodes of malaria when given together with SMC.

A key question for policy makers and malaria control programme managers is the impact of the two interventions and their combination in the first five years of life (the age range for which seasonal malaria chemoprevention is the current standard of care in most countries where it is policy). The primary focus of the overall study is, therefore, determination of the impact of the two interventions and their combination over the whole five-year period (the three-year initial study, plus the two-year extension), and its interpretation will, therefore, be based on the cumulative incidence of malaria until children reach the age of five years, and are no longer eligible for SMC.

The primary objective of the extension study is determination of potential added value of extending administration of the two interventions and their combination beyond the age of three years. This will be based on the results obtained in the final two years of the study, based on the objectives described above.

Important note on age groups within the study cohort, and eligibility for interventions

In most SMC programmes, children are eligible to receive the intervention if they are aged at least 3 months, and were under 60 months at the time of the first SMC administration each year (Senegal is an exception, with children aged 3-119 months eligible for SMC).

The original trial cohort was recruited in April 2017, when children were aged between 5-17 months. This means that some of the study cohort reached the age of five years prior to the 2021 rainy season and were, therefore, not eligible to receive the trial interventions in the final year of the extension study.

In the two-year extension phase of the trial, all children enrolled in the initial phase of the study received study interventions in 2020. In 2021, children enrolled in the initial phase of the study were eligible to receive study interventions if they were aged up to, and including, exactly 5 years (60 months) on the first of June 2021. Children above this age (i.e. with dates of birth on 31/05/2016, or earlier) remained under observation using identical surveillance measures as part of longer-term follow-up of the study cohort (to be reported separately) aimed at determining whether children in the trial are at any increased risk of malaria in the period after the interventions are no longer given., Other eligibility criteria are described below.

In the text below, the terms 'older' and 'younger' children are used as follows:

'**Older children**': children who were above the age of five years on 01 June 2021 (DOB 31/05/2016, or earlier), and who were not eligible for interventions in 2021.

'**Younger children**': children who were exactly five years of age, or below on 01 June 2021 (DOB 01/06/2016, or later), and who were eligible to receive interventions in 2021.

Study groups

This is a double-blind, individually-randomised trial with three study arms. For reference, the interventions given during the initial three-year trial are shown first, with the interventions given during the extension study shown separately.

Group 1 – SMC arm - 'SMC alone'

Original Trial:

In 2017, children in the SMC group were given three doses of rabies vaccine at monthly intervals and four rounds of SMC with SP+AQ at monthly intervals. In 2018 and 2019, these children received one dose of a control vaccine (Hepatitis A) and four rounds of SMC at monthly intervals.

Extension study:

All children in the SMC group received a single dose of a Tetanus Toxoid (in Burkina Faso) or Tetanus/Diphtheria toxoids (in Mali) vaccine in June 2020, and four rounds of SMC at monthly intervals between July and October 2020.

Children in the SMC group exactly five years of age, or below, on 1st June 2021 received an additional dose of Tetanus toxoid vaccine in June 2021 (in both Countries), and either four rounds of SMC (in Mali) or five rounds of SMC (in Burkina Faso) at monthly intervals between July and October 2021. This change in Burkina Faso for 2021 resulted from the National Malaria Control Programme choosing to deploy 5 courses of SMC in Houndé district.

Group 2 – RTSS/ $AS01_E$ arm – 'RTS,S alone'

Original Trial:

In 2017, children in the RTS,S/$AS01_E$ group received three doses of RTSS/$AS01_E$ vaccine and four rounds of placebo-SMC at monthly intervals. In 2018 and 2019, children received one dose of RTS,S/$AS01_E$ vaccine in June (prior to the malaria transmission season) and four rounds of placebo-SMC at monthly intervals.

Extension study:

All children in the RTS,S alone group received a single dose of RTS,S/$AS01_E$ in June 2020 (prior to the malaria transmission season), and four courses of placebo SMC at monthly intervals between July and October 2020.

Children in the RTS,S alone group exactly five years of age, or below, on 1st June 2021 received an additional dose of RTS,S/$AS01_E$ vaccine in June 2021 (prior to the malaria transmission season), and four courses of placebo SMC (in Mali) or five courses of placebo SMC (in Burkina Faso) at monthly intervals between July and October 2021.

Group 3 – RTS,S/AS01+SMC arm – 'Combined arm'

Original trial:

In 2017, children in the combined group were given three doses of RTS,S/$AS01_E$ vaccine and four rounds of SMC with SP+AQ at monthly intervals. In 2018 and 2019, children received one dose of RTS,S/$AS01_E$ vaccine in June (prior to the malaria transmission season) and four rounds of SMC with SP+AQ at monthly intervals.

Extension study:

All children in the Combined group received a single dose of RTS,S/AS01$_E$ in June 2020 (prior to the malaria transmission season), and four courses of SMC at monthly intervals between July and October 2020.
Children in the Combined group exactly five years of age, or below, on 1st June 2021 received an additional dose of RTS,S/AS01$_E$ vaccine in June 2021 (prior to the malaria transmission season), and either four rounds of SMC (in Mali) or five rounds of SMC (in Burkina Faso) at monthly intervals between July and October 2021.

The study groups, and abbreviated names used for ease of reference, are shown in the table below. The interventions given during the initial three-year trial, and the two-year extension are shown in separate sections.

Table 1. Study Interventions

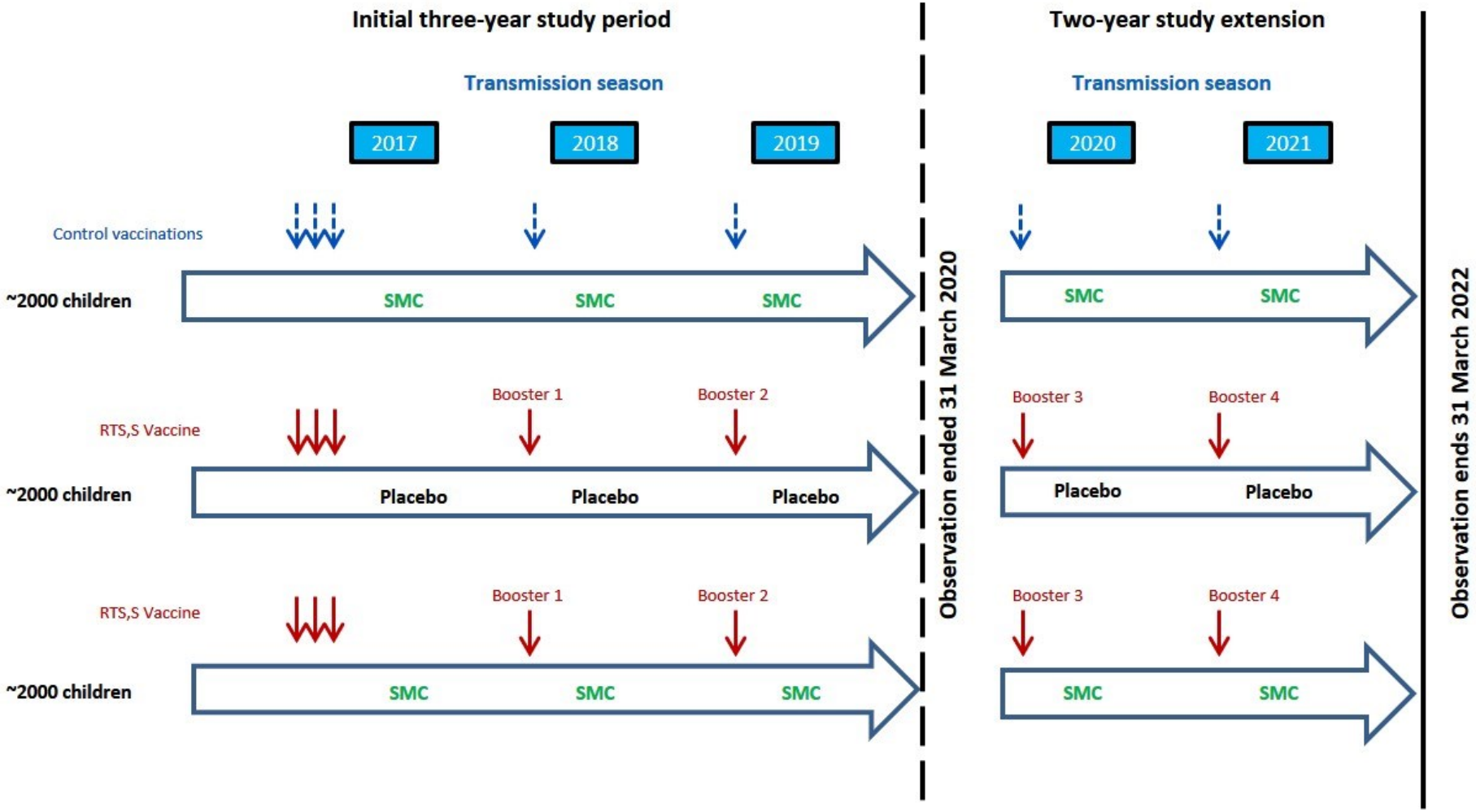
	Group		
	1) SMC 'SMC alone'	**2) RTS,S/AS01$_E$ 'RTS,S alone'**	**3) RTS,S/AS01$_E$ +SMC 'Combined group'**
Initial three-year trial			
April/May – June/July 2017	Rabies vaccine x 3	RTS,S/AS01$_E$ x 3	RTS,S/AS01$_E$ x 3
July/Aug – Oct/Nov 2017	SMC x 4	SMC placebo x 4	SMC x 4
June 2018	Hep A vaccine x 1	RTS,S/AS01$_E$ x 1	RTS,S/AS01$_E$ x 1
July-Oct 2018	SMC x 4	SMC placebo x 4	SMC x 4
June 2019	Hep A vaccine x 1	RTS,S/AS01$_E$ x 1	RTS,S/AS01$_E$ x 1
July-Oct 2019	SMC x 4	SMC placebo x 4	SMC x 4
Two-year Extension Study			
June 2020	Tetanus (BF) or Tetanus/Diphtheria toxoids (Mali) x 1[#]	RTS,S/AS01$_E$ x 1	RTS,S/AS01$_E$ x 1
July-Oct 2020	SMC x 4	SMC placebo x 4	SMC x 4
June 2021*	Tetanus toxoid x 1[#]	RTS,S/AS01$_E$ x 1	RTS,S/AS01$_E$ x 1
July-Nov 2021*[$]	SMC x 4 (Mali)	SMC placebo x 4 (Mali)	SMC x 4 (Mali)
	SMC x 5 (Burkina Faso)	SMC placebo x 5 (Burkina Faso)	SMC x 5 (Burkina Faso)

* Only children aged 5 years or below on June 1st 2021 were eligible to receive study intervention in 2021.

[#] In 2020, due to difficulties shipping tetanus toxoid vaccine from India as a result of the COVID-19 outbreak, children in group 1 in Burkina Faso received tetanus toxoid vaccine from stocks held by Burkina Faso's national infant immunization programme. Children in group 1 in Mali received tetanus/diphtheria toxoids vaccine from stock held by Mali's national infant immunization programme. In 2021, children in both countries received tetanus toxoid vaccine supplied by the Serum Institute of India.

[$] In Burkina Faso, in 2021, the National Malaria Control Programme began to deliver 5 monthly courses of SMC in the study area. To ensure that our intervention reflected the current standard of care, the study team also administered 5 SMC courses in 2021.

Figure 1. Schematic of Key Study Activities in the Initial Trial Period, and in the Two-Year Extension



Only children aged below 5 years on June 1st 2021 were eligible to receive study intervention in 2021.

Precise timing of interventions during the original trial, and the extension study

Administration of RTS,S/AS01$_E$ or control vaccine:
Original trial:
Administration of the first dose of study vaccines began in late April 2017 in all three study groups, and was completed by mid-May. The third dose was given between late June and early July 2017. In 2018 and 2019, single doses of RTS,S/AS01$_E$ or control vaccine were given in the first two weeks of June.

Extension study:
In June 2020, vaccinations were given to all children in the study. In June 2021, vaccinations were given to all children exactly five years of age, or below, on 1st June 2021.

Administration of SMC or placebo SMC:
Original trial:
In 2017, administration of the first SMC course began in late July and was completed by the end of the first week of August, with subsequent cycles on a monthly basis thereafter. In 2018 and 2019, administration of the first SMC course began in the second week of July, and was completed by mid-July. Subsequent courses were delivered monthly thereafter. Earlier delivery in 2018 and 2019 was necessary to ensure that drug administration was completed before SMC delivery through the national malaria control programme took place.

Extension study:
In 2020, SMC was administered to all children, as four monthly courses, starting in July. In 2021, all children exactly five years of age, or below, on 1st June 2021 received SMC, starting in July 2021. In Mali, children received four courses of SMC in 2021. In Burkina Faso, children received 5 cycles in 2021, because the national programme decided to deliver five cycles of SMC in Houndé district.

Study population in the original trial

Children of either sex were eligible for inclusion in the trial, provided that they were 5-17 months of age on the scheduled date of first vaccination in April 2017, they were living permanently in the study area, and the consent of a parent or legally acceptable representative was obtained.

Children with a history of an adverse reaction to SP or AQ, known to have a serious underlying illness including known HIV infection not well controlled by treatment, having severe malnutrition (z scores < 3 SD) or known to have previously received a malaria vaccine were excluded from the trial.

Children known to have received SMC during the year prior to enrolment (either through the previous study in these districts (AZ-SMC, NCT02211729), or from the national programme) were not excluded from this study.

The list of eligible children in each country was sorted by location (village), age in months, gender and prior receipt of SMC, before assigning randomisation codes in permuted blocks of 9, to give an implicit stratification on these factors.

Children who were randomized but who did not receive the first dose of study vaccines in 2017 were deemed to have withdrawn consent and were not followed up further. Consequently, analyses from baseline of all the children who remained under observation constitute a modified ITT population, rather than the full ITT population. Analyses during specific years of the study, or during the extension study period only, reflect the children remaining in follow-up at that point, rather than analysis by intention to treat.

Inclusion criteria for the Extension study

In order to be eligible to participate in the extension study, a child must have been enrolled in the initial phase of the trial of seasonal vaccination with the RTS,S/AS01$_E$ vaccine and their parents or guardian must have provided consent for their inclusion in the extension study.

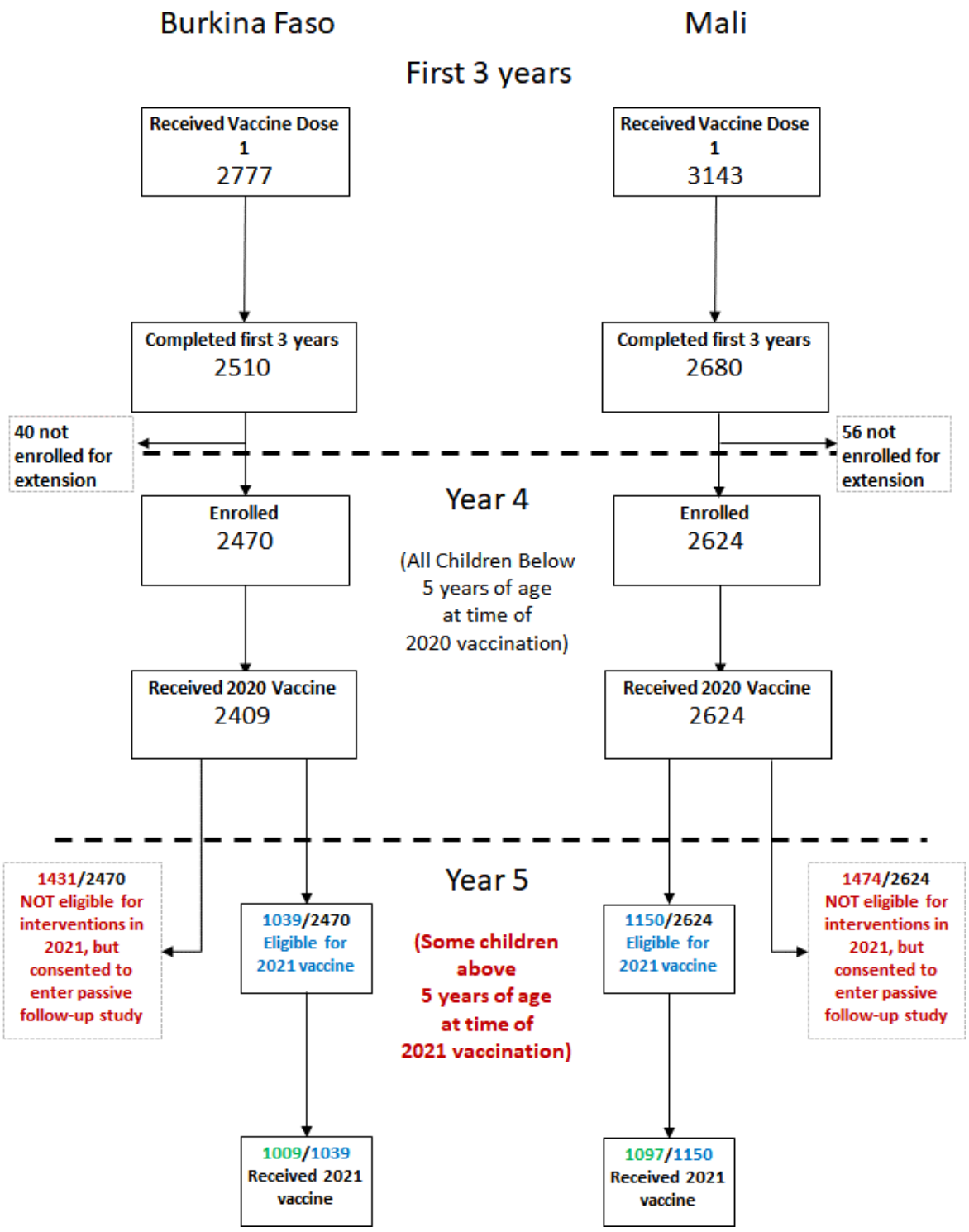
As described above, children above the age of 5 years on 1st June 2021 were no longer eligible to receive either of the interventions, but were followed until the end of the extension study on safety grounds and to begin longer-term monitoring for any increased risk of malaria in the period after the interventions were no longer given.

Exclusion criteria for the extension study

Any child who met any of the following criteria was excluded from participation in the extension study:

a. The child had had an allergic reaction to the study drugs or vaccines.
b. The child had febrile convulsions on more than one occasion following vaccination.
c. The child had a serious underlying illness, such as severe malnutrition (weight for age or mid arm circumference Z scores < 3 SD), which in view of the investigators might impair the response to vaccination.
d. The child had been enrolled in another malaria vaccine or other experimental malaria intervention study.

Figure 2. CONSORT chart for the initial phase of the trial and the extension study



* Some of the numbers in the figure above are provisional, and may change for the final analysis once all data cleaning is complete.

Illustrative sample size calculations for the extension study period

In Burkina Faso and Mali, 2777 and 3143 children respectively, received the first dose of study vaccine and were enrolled in the initial trial. In Burkina Faso 2410 out of 2510 children remaining in follow-up at the end of the three-year trial consented to join the extension study. In Mali, 2624 out of 2680 children remaining in follow-up after three years consented to join the extension phase of the trial (Figure 2).

Non-inferiority Comparison: SMC for four months of the year has an efficacy, assuming receipt of all four monthly cycles, of about 85% during this 4-month period. If, without intervention, the peak four months of malaria incidence would account for 60% of annual cases (with the other 40% falling during the other months) this equates to an efficacy over 12 months of at least 50%. The non-inferiority margin is the largest reduction in this efficacy that policy makers would be likely to accept if RTS,S/AS01 was to replace SMC, taking into account of the potential advantages of RTS,S over SMC in terms of ease of delivery and the potential to sustain a high level of coverage. A reduction in annual efficacy from 50% to 40% was considered the largest difference that would be likely to be acceptable. This lower efficacy would translate to a 20% greater incidence in the RTS,S/AS01-alone arm compared to the SMC-alone arm.

The study has greater than 90% power to exclude, at the 2.5% significance level, a relative difference in the incidence of clinical malaria of 20% over the five year study period between the RTS,S/AS01 and the SMC alone groups if these two interventions were equally effective. To determine the level of confidence with which this margin can be excluded, we will also calculate two sided 90% and 99% confidence intervals. With the anticipated incidence rates, there is adequate power to reject a smaller margin. However, 20% was considered the largest difference that would be considered unimportant.

Superiority Comparison: The primary end-point for the trial is the incidence of clinical episodes of malaria. Based on incidence during the first three years of the trial (305 cases per 1000 child-years in the SMC alone group), and taking account of the fact that the incidence of clinical episodes of malaria in children still protected by study interventions may decline with age, we assume that the incidence of malaria will be at least 200 cases per 1000 child-years at risk over the entire study period. Based on this assumption, the study will have 90% power to detect a 12.0% or greater relative difference (absolute difference: 24 cases per 1000 child-years) between the RTS,S/AS01$_E$ + SMC combined group and the RTS,S/AS01$_E$ alone or SMC alone groups, through a standard superiority comparison during the five years of the trial. In the first three years of the trial, the incidence was about 60% lower in the combined group (rather than 12%), so the extension study should have very high power for the superiority comparisons.

Analyses in individual years

There will be approximately 4,100 child-years of observation in the year following the third booster; 1367 child-years in each study group. Assuming the incidence of clinical episodes of malaria remains at about 200 episodes per 1,000 children per year during this period, the study will have 90% power, through a standard superiority comparison, to detect a 25.9% or greater

relative difference (absolute difference: 52 cases per 1000 child-years) between the Combined RTS,S/AS01$_E$ + SMC group and the RTS,S/AS01$_E$ alone or SMC alone group.

A similar analysis will be undertaken for the final year of the study following administration of a fourth booster dose of the RTS,S/AS01$_E$ or comparator vaccine but study power will reduced because slightly more than half of the children enrolled (3860/5920) will not be eligible for SMC in 2021-22, and thus will not contribute further events nor person-time at risk to the study period.

Study outcomes

Primary Endpoint

The primary end-point is the incidence of episodes of clinical malaria, as defined below, treated at a study health centre or hospital.

1.1 **Clinical malaria** is defined as an episode of fever (either measured axillary temperature ≥ 37.5°C, or a history of fever within the past 48 hours), and a positive blood film, with a *Plasmodium falciparum* parasite density of 5,000 per µl or more. This cut-off was used in the original three-year trial, as well as in previous studies of SMC in Burkina Faso and Mali, and in the phase 3 studies of the RTS,S/AS01 vaccine (1, 2, 5-7).

All passively-detected episodes of clinical malaria will be included in the analysis. Specifically, this includes visits to outpatient clinics and hospitals, as well as morbidity detected at the time of SMC or at the end of transmission season survey. These contacts can be considered 'passive' because the caregiver had to bring the child to the contact and because the SMC or survey was conducted at the health facility in many cases. As the focus is on passively detected cases, morbidity detected during home visits for serological sampling, and for the weekly parasitaemia survey (which was undertaken only in the initial phase of the study) are excluded. Morbidity detected at the time of vaccination is also excluded, because some children were visited at home and brought to the clinic in order to be vaccinated, so cases found at the time of vaccination cannot be regarded as passively detected.

To avoid double counting of disease episodes which result in more than one healthcare contact, episodes of the primary outcome documented within 7 days of a previous episode will not be counted. No adjustment is necessary to the person-time at risk (11).

Secondary Endpoints

Secondary end-points - not listed in order of priority - include the following:

2.1 Morbidity events detected passively at study health centres and hospitals

As for the primary outcome, episodes of the outcomes listed below which occur within 7 days of a previous event of the same type will be discounted. No adjustment is necessary to the person-time at risk (11).

2.1.1 **Clinical malaria with *P. falciparum* parasitaemia of any density.** Defined as an episode of fever (either measured temperature ≥ 37.5°C, or a history of fever within the past 48 hours), and a positive blood film for *P. falciparum* parasites. This includes hospitalisations for malaria meeting the above criteria (i.e. fever or history of fever, plus slide confirmed *P. falciparum* malaria of any density).

2.1.2 **Clinical malaria confirmed by rapid diagnostic test.** Defined as an episode of fever (either measured temperature ≥ 37.5°C, or a history of fever within the past 48 hours), and a positive rapid diagnostic test (RDT).

2.1.3 **Clinical malaria with non-falciparum parasitaemia of any density.** Defined as an episode of fever (either measured temperature ≥ 37.5°C, or a history of fever within the past 48 hours), and a positive blood film for non-falciparum *Plasmodium* parasites.

2.1.4 **Clinical malaria with *Plasmodium spp.* infection of any density.** Defined as an episode of fever (either measured temperature ≥ 37.5°C, or a history of fever within the past 48 hours), and a positive blood film for any *Plasmodium spp.* parasites (i.e. including *P. falciparum*).

2.2 **Severe outcomes detected passively at study health centres and hospitals, and through verbal autopsies**

For all hospital admissions and deaths, the primary diagnosis by a study physician was reviewed by a second independent clinician. A third clinician reviewed cases of disagreement to reach a consensus primary diagnosis. All verbal autopsies were also reviewed by the same process to obtain a consensus cause of death.

2.2.1 **Hospital admissions due to any cause**

2.2.2. **Hospital admissions excluding those due to external causes or surgical conditions**

2.2.3 **Hospital admissions due to malaria.** Defined as hospital admissions where malaria was the primary diagnosis, supported by a positive blood smear. Children who were admitted with a diagnosis of severe anaemia or neurological signs, with documented antimalarial treatment in the past 4 weeks, were also deemed to be due to malaria if their malaria RDT was positive (even if the blood smear result was negative or the blood smear result was not available). Additional analyses of children who meet the WHO criteria for a diagnosis of severe malaria including those with a) cerebral malaria, b) severe anaemia and c) other forms of severe malaria will be undertaken.

2.2.4 **The incidence of blood transfusions in study hospitals**

2.2.4 **Deaths due to any cause**

2.2.6 **Deaths due to any cause excluding external causes and surgical conditions**

2.2.7 **Deaths due to malaria**. Defined as hospital admissions resulting in death, where malaria was recorded as the primary cause of death supported by a positive blood smear. Children who were admitted with a diagnosis of severe anaemia or neurological signs, with documented antimalarial treatment in the past 4 weeks, were also deemed to be due to malaria

if their malaria RDT was positive (even if the blood smear result was negative or the blood smear result was not available). Deaths in the community will also be included when malaria is assigned by a panel of three independent physicians as the primary cause of death from verbal autopsy .

2.3 Outcomes measured at cross-sectional surveys at the end of the malaria transmission season

For each of the outcomes below, results will be analysed and presented separately for the two surveys conducted during the extension study (conducted in November/December 2020 and November/December 2021).

2.3.1 The prevalence of asexual stage *P. falciparum* infection of any density
2.3.2 The prevalence of asexual stage *P. falciparum* infection with density ≥ 5000 per ul
2.3.3 The prevalence of sexual stage *P. falciparum* infection (i.e. gametocytes)
2.3.4 The arithmetic mean *P. falciparum* parasite density, including samples which are parasite negatives as having a density of zero.
2.3.5 The prevalence of asexual stage infection of non-falciparum *Plasmodium* species.
2.3.6 The prevalence of sexual stage infection (i.e. gametocytes) of non-falciparum *Plasmodium* species.
2.3.7 The mean haemoglobin concentration in g/dL.
2.3.8 The prevalence of anaemia, defined as measured Hb < 10 g/dL.
2.3.9 The prevalence of moderate anaemia, defined as measured Hb < 7 g/dL.
2.3.10 The prevalence of severe anaemia, defined as measured Hb < 5 g/dL.

2.4 The antibody response to sixth and seventh doses of the RTS,S/AS01$_E$ vaccine

2.4.1 The *Plasmodium falciparum* anti-CSP antibody titre before and after the sixth and seventh doses (third and fourth 'booster' doses) of the RTS,S/AS01$_E$ malaria vaccine.
2.4.2 The association between the magnitude of the anti-CSP antibody response to the sixth and seventh doses of the RTS,S/AS01$_E$ vaccine and the risk of malaria during the subsequent malaria transmission season.

2.5 The treatment efficacy of the SP+AQ combination used for SMC

2.5.1 The percentage of children with asymptomatic *P. falciparum* parasitaemia at the cross-sectional survey in November/December 2021, who, when treated with SP+AQ, have an adequate clinical and parasitological response (ACPR) after 28 days.

Serious Adverse Events

In addition to the comparison of incidence rates described above, serious adverse events (SAEs) defined as hospitalisations or death, occurring at any time during the study will be tabulated by study group according to their cause.

Outcomes measured among school-age children

To help interpret results obtained in study children., end of season surveys have also been conducted among school-age children in the study areas at the same time as the end of transmission season surveys. The following outcomes will be calculated for school-age children.

3.1. The prevalence of asexual stage *P. falciparum* infection of any density
3.2 The prevalence of asexual stage *P. falciparum* infection with a density ≥ 5000 per ul

3.3 The arithmetic mean *P. falciparum* parasite density, including samples which are parasite negatives as having a density of zero.

Analysis periods and person-years at risk

Several analysis periods are of interest for the primary outcome of clinical malaria. These are detailed in the figures and text that follow.

Hypothesis testing and p-values will be presented for the whole intervention period (Period A characterised below) and for the two periods of interest in the extension study (periods B and C in the list below). Numbers of events and hazard ratios or other measures of effect (with confidence intervals) will be tabulated for other analysis populations.

A) Whole intervention period (all children).

The primary analysis population will be the whole intervention period. This is the period starting from enrolment in April 2017 until the end of the intervention year in which children reached 5 years of age (31 March 2021 for *older children*, 31 March 2022 for *younger children*).

B) Study Year 4, to assess effectiveness of the sixth dose of RTS,S/AS01$_E$

All study children still in follow-up at the start of the fourth year of the study will be included, starting from 1st April 2020 until 31 March 2021 (i.e., covering the 2020 rainy season and subsequent dry season).

C) Study Year 5, to assess effectiveness of the seventh dose of RTS,S/AS01$_E$ (younger children only)

Older children were not eligible for interventions in 2021. Consequently, this analysis will include only younger children, starting from 1st April 2021 until 31 March 2022 (i.e., covering the 2021 rainy season and subsequent dry season).

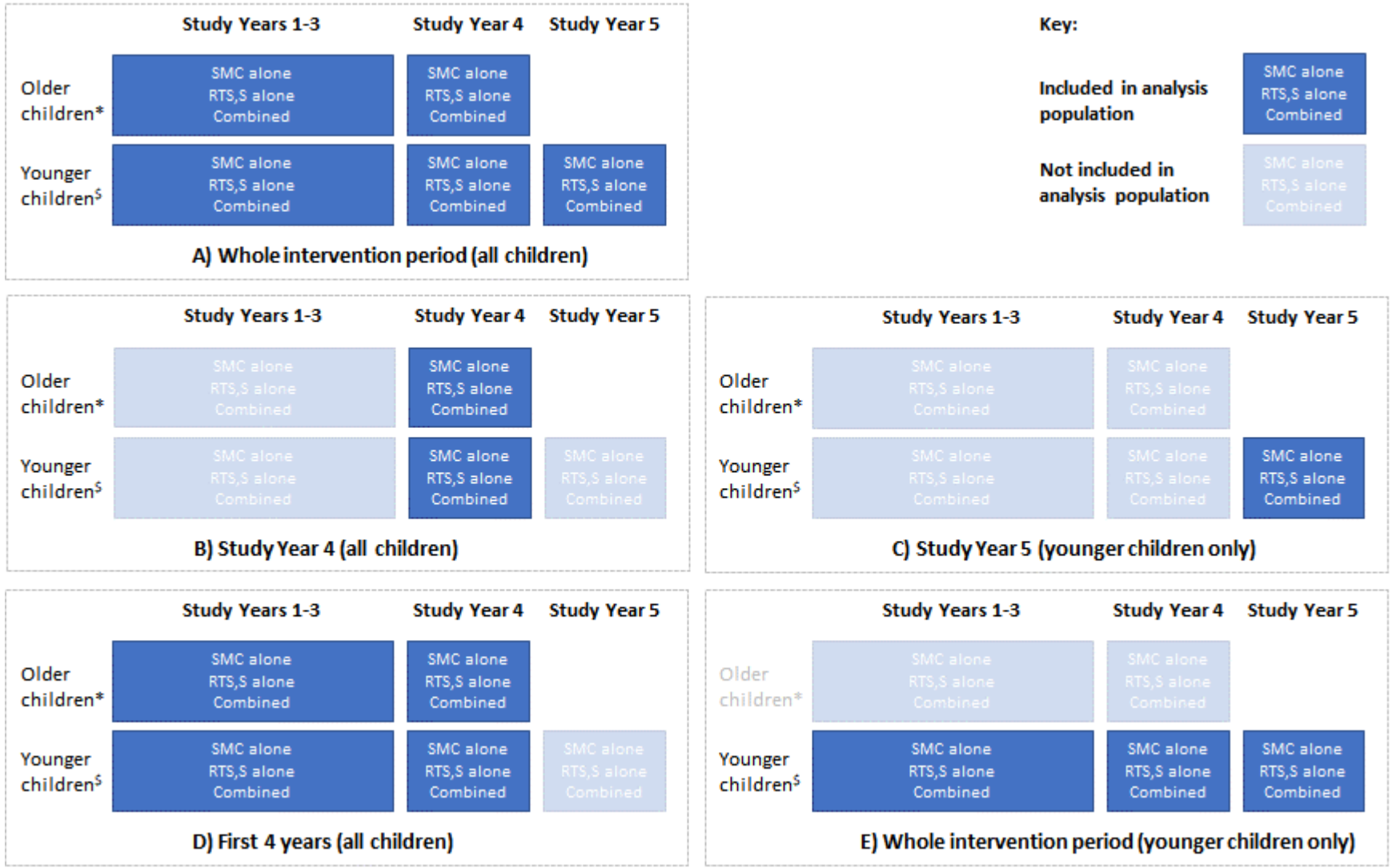
D) From study start until the end of the study year 4 (to assess the overall effectiveness of up to six doses)

All children originally enrolled in the trial were scheduled to receive study interventions during the first four years of the trial. This period will be analysed, starting from enrolment in April 2017 until 31 March 2021, after the end of the 2020 rainy season.

E) Whole intervention period, including only *younger children* (those eligible for interventions in 2021)

Starting from enrolment in April 2017 until 31 March 2022 (i.e. after the end of the 2021 rainy season, in which interventions were given to *younger children* for the last time). This period reflects the 'full' intervention over five years, with up to 7 doses of RTS,S and/or five complete seasons of SMC prior to 5 years of age.

Figure 3. Schematic of the analysis periods referred to in the text



Dark blue boxes show the periods that will be included in each analysis. *Older children are those aged 5 years or above on 1st June 2021, who were not eligible for interventions in Study Year 5 (2021). $Younger children were aged below 5 years on 1st June 2021, so were eligible for interventions in 2021.

Analysis strategy

Modified Intention to Treat

The primary analysis will be by modified intention to treat (mITT). The mITT population will include all children who were screened and who received the first dose of RTS,S/AS01$_E$ or control vaccine, irrespective of the number of doses of subsequent vaccines or SMC/SMC placebo received.

Children will contribute time at risk from the date of the first vaccination contact in 2017, until the end of the intervention period, which is either

i) 31st March 2021, if born prior to 1st June 2016 (and thus over the age of five years in June 2021), or
ii) 31st March, 2022, if born on or after 1st June 2016, and thus still under the age of five year in June 2021.

Prior to the end of the intervention period, children may be censored;
1) on the date last seen if lost-to-follow-up (LTFU),
2) on the date of permanent exit from the study area, or
3) on the date of death,

Children who temporarily left the study area with known exit and re-entry dates will have the corresponding person-time excluded from the analysis by intention to treat (and per protocol, if leaving the study area does not result in missed treatments).

Per Protocol Analysis

As a secondary analysis, the primary outcome of clinical malaria will also be analysed per protocol (PP). The PP population will be defined separately for each year of the study. Children who were vaccinated at all scheduled vaccination contacts in a particular year (3 in 2017, 1 in each subsequent year) and who, in the same year, were also seen at the first SMC/SMC placebo contact each month (4 per year) will be considered as 'per protocol' for that year.

Children who attended for SMC administration but who did not receive SMC because they had malaria and were referred for treatment will be included in the per protocol analysis.

'Per protocol' is defined differently for the two interventions (vaccination and SMC). For vaccination, a child must have *received* all vaccination doses that year; for SMC a child must only have *attended* all SMC contacts that year. This difference is necessary because the primary outcome of the trial (clinical malaria) can result in a specific SMC dose being missed permanently, whereas if a child had malaria at the time of vaccination, catch-up was attempted later in the season. The per protocol conditions will be applied equally to all three groups, i.e. to be considered as per protocol, a child must have received all doses of vaccine AND attended all SMC contacts, irrespective of which of these were active and placebo.

All secondary outcomes will be analysed by modified intention to treat, as described above.

Strategy to address multiplicity

Hypothesis testing for analyses of the primary outcome will follow the closed testing procedure, whereby there is initially a test of the null hypothesis that the incidence in the three groups is the same. If this is rejected at the 5% level, pairwise comparisons will be done also using a 5% significance level. Pairwise comparisons can be considered statistically significant only if the overall null hypothesis is rejected. P-values and 95% confidence intervals will be presented.

For the non-inferiority comparison of the primary outcome described above, we will calculate two-sided 95% confidence intervals, equivalent to the use of a one-sided significance level of 2.5%, as is recommended (4). To illustrate the level of confidence with which non-inferiority can be declared, we will plot the point estimate for the hazard ratio with two-sided 90, 95 and 99% confidence intervals, and the non-inferiority margin (a hazard ratio of 1.2, which would reflect 20% higher incidence of clinical malaria in children in the RTS,S/AS01$_E$ alone group, relative to the SMC alone group).

For secondary outcomes, we will present 95% confidence intervals, without adjustment for multiplicity, stating that no adjustment was made.

For safety outcomes, we will present p-values and 95% confidence intervals, without adjustment for multiplicity.

Trial profile – Original Study

The original trial profile showed the number of individual children enumerated at the initial census, the number of children eligible, and the number of children for whom consent was obtained. The profile also showed, for all the eligible children seen at the census who were randomised, the number of children seen at the first study contact who received the first dose of vaccine and who joined the study.

The number of children who exited the study population by the end of the first, second and third year of the original trial were shown, with reasons (where known) tabulated.

Uptake of the study interventions will be summarised, including:

- the number that received different combinations of vaccine doses;
- the distribution of the interval between doses;
- the number that received 0,1,2,3,4 SMC treatments each year;
- the actual timing of SMC cycle 1 in relation to the malaria transmission season;
- the mean and range of the intervals between the monthly SMC courses;
- the adherence to daily doses of SMC each month

Separate profiles were produced for each of the two trial centres.

Trial Profile - Extension Study

The number of individuals eligible to join the two-year extension study, and the number who gave consent to participate will be shown.

The number of children who exited the study population by the end of the fourth (2020 intervention year) will be tabulated with reasons (where known) indicated.

Children who reached the age of 5 years prior to the 2021 intervention year will be shown as a separate category (this does not count as 'loss to follow-up' as these children were excluded from further intervention by design, and continued to be monitored for longer-term for morbidity post-intervention).

Among children who remained below the age of five years in June 2021, the number of children who exited the study population by the end of the fifth (2021 intervention year) will be tabulated, with reasons (where known) indicated.

Uptake of the study interventions during the intervention period will be summarised, including:

- the number that received different combinations of vaccine doses;
- the distribution of the interval between doses;
- the number that received 0,1,2,3,4 (and 5 in Burkina Faso, in 2021) SMC treatments each year;
- the actual timing of SMC cycle 1 in relation to the malaria transmission season;
- the mean and range of the intervals between the monthly SMC courses;
- the adherence to daily doses of SMC each month

Statistical methods

Reference group

As SMC is the current standard of care, the SMC alone group will be considered as the reference group for comparisons with RTS,S/AS01$_E$ alone, and the combined group. Comparisons will also be made between the RTS,S/AS01$_E$ alone and the combined group.

Primary endpoint

Hazard Ratios

The hazard ratio for the primary outcome will be estimated using Cox regression models, with a robust standard error (i.e. the Andersen-Gill extension of the Cox model) to account for potential clustering of episodes within children. The Efron method will be used for tied event times.

The timescale will be calendar time. For analyses of the whole study period, calendar time will start from 1st April 2017, i.e. allowing delayed entry according to the precise timing of the first vaccination contact. This ensures that risk sets in the Cox models are comparable with respect to the timing of onset of transmission each year, and the timing of SMC. Due to variable timing in vaccine dose 1 in 2017, this would not be the case if the data were analysed on the time in study timescale. For the same reasons, analyses of the effectiveness of the sixth and seventh doses of RTS,S, calendar time will start from 1st April 2020 and 1st April 2021, respectively (i.e. the beginning of the study year in which the sixth and seventh doses were administered).

Nelson-Aalen Cumulative hazards will be plotted for each group to show the mean number of events per child during the study and the timing of events, and Kaplan Meier failure estimates will be plotted to show the risks during the study.

Incidence rate differences

In addition to the hazard ratios from Cox regression, as described above, the incidence rate differences (IRD) will also be calculated, i.e. the cases averted per 1000 child-years at risk. This is recommended in the updated CONSORT guidelines (8), as this gives an indication of the reduction in incidence attributable to the interventions, i.e. the absolute public health impact in similar contexts. This is an important question for the overall effect of the two interventions under study in this trial, which is a question of key interest for policy makers. This is also relevant to the interpretation of efficacy over successive years of the study. For example, if the hazard ratio between the intervention groups moves towards unity as children become older, but malaria incidence increases in older children, the absolute impact of the interventions could remain as important (or even more important), in older children. Alternatively, if the hazard ratio remains similar to that seen in the initial intervention period but incidence falls in older children, extension of vaccination into older children may not be warranted.

The IRD will be calculated using ordinary least squares regression of transformed variables for the intervention group, country and person-time at risk, as described by Xu et al. (9). This method uses a robust standard error and controls for unequal follow-up time, as well as

quantitative or multiple covariates. To aid interpretation, the risk of the primary outcome will also be estimated from the Kaplan-Meier estimate of the risk.

Proportion of children remaining free of malaria in individual years of the study

The proportion of children who remained free of malaria (1-risk), in individual study years will be calculated from the Kaplan-Meier estimates in each intervention group. The difference in this proportion between arms, with a 95% CI, and the relative difference (% increase in fraction remaining free of malaria) will also be calculated and presented.

Secondary endpoints

Secondary outcomes which are passively detected events, will be analysed in a similar way as for the primary outcome, i.e. estimating the hazard ratio using Cox regression with a robust standard error.

The prevalence ratio of secondary endpoints measured at the weekly survey (aggregated into three-month periods), and at end of season surveys (including *P. falciparum* parasitaemia, anaemia, etc) will be estimated using Poisson regression, with a robust standard error for the individual, as described in Zou, 2004 (10).

Linear regression models will be used to compare mean haemoglobin concentration between the groups.

Arithmetic mean parasite densities (including in the calculation samples which are parasite negatives, as having density of zero), will be compared between arms using Poisson regression with a robust standard error.

Covariates

All analyses (primary outcomes and secondary outcomes) will stratify on study country only (Burkina Faso or Mali).

For the primary outcome, we will also build a model adjusting for the following potential confounders:

- Age at enrolment
- Child's Sex
- Bednet use at baseline in 2017

Pre-specified subgroup analyses, interactions and exploratory analyses

As described above, the primary analysis will be pooled across the two study centres, stratified by (i.e. adjusted for) country. Efficacy (ratio and difference) measures will be presented for both sites combined. Investigation of any differences in intervention effects between the centres (formally, evidence for an interaction between intervention group and study centre) is pre-specified due to possible differences in performance of these interventions under different malaria transmission intensity. All outcomes will, therefore, also be tabulated by centre, and site-specific efficacy estimates will be presented.

Investigation of differences between study groups in each study year is also pre-specified, as described above, because it is possible that the efficacy of RTS,S/AS01 booster doses changes over time, or following successive doses. This will be assessed by exploring evidence for an interaction between intervention group and study year.

Evidence for effect modification by age at enrolment will be explored. It is possible that vaccination will perform differently according to the extent of prior exposure to malaria. As described above, analyses of the first four years of the study (analysis population D) will be conducted splitting children according to their age in June 2021 (children aged 5 years or more, vs. children who were still under 5 years; younger children subsequently received an extra year of intervention).

Finally, evidence for effect modification by child's sex (observed in the RTS,S Phase III trial) will be explored by inclusion of an interaction term in the Cox models for the primary outcome over the whole analysis period (period A). Severe malaria meeting the WHO definition and malaria deaths will also be included as exploratory analyses.

Pre-specified Secondary analyses

Safety signals from the RTS,S/AS01 phase 3 studies

The incidence of meningitis has been very low in the study cohort, with no events in the first three years of the trial. A 95% confidence interval for the incidence rate of meningitis among children vaccinated with RTS,S/AS01, over the whole intervention period, up to the age of five years, will be calculated.

The incidence of cerebral malaria among children vaccinated with RTS,S/AS01$_E$ will be investigated by comparing the RTS,S/AS01 groups with the SMC alone group, controlling for SMC status using an indicator variable. Cox regression will be used to obtain the hazard ratio and its 95% confidence interval over the whole intervention period, up to the age of five years.

The incidence of febrile convulsions not related to malaria or another obvious cause among children vaccinated with RTS,S/AS01 will be investigated as for cerebral malaria, i.e. by comparing the RTS,S groups with the SMC alone group, controlling for SMC status using an indicator variable. Cox regression will be used to obtain the hazard ratio and its 95% confidence interval over the whole intervention period, up to the age of five years. The excess cases per 1000 child-years at risk, based on the incidence rate differences will also be presented. The subset of febrile convulsions that occurred within 7 days of vaccination will also be analysed.

An exploratory analysis for data from the first three years of the trial found no evidence that RTS,S/AS01 increases mortality in girls. This will be repeated over the whole intervention period, up to the age of five years.

This was done by comparing the incidence of deaths using Cox regression, with an interaction between a dummy variable indicating receipt of RTS,S/AS01$_E$ and gender. The Wald test p-value for the interaction term will be used to assess evidence for effect modification. This model will also include a dummy variable for SMC to adjust for SMC receipt. This will enable the

female: male mortality ratio and its 95% confidence interval to be calculated separately for RTS,S/AS01$_E$ recipients, and non-recipients. We will use indicator variables to obtain the ratio of these ratios, with the 95% confidence interval. We will also present the mortality ratio for RTS,S recipients versus non-recipients separately for males and females. Since it is hypothesised that this effect may be age-dependent, we will also carry out these analyses restricted to the period after the first booster dose.

Ancillary analyses to be reported separately

1. Analysis of the prevalence and frequency of molecular markers of resistance to SP and AQ in children with *P. falciparum* infection, among samples collected at the end of season cross-sectional survey in 2019, and at the final cross-sectional survey in December 2021. The molecular markers of interest include the dhfr 51-59-108 triple mutation, dhps-A437G, and dhps-K540E mutations for resistance to SP, and the pfcrt K76T and pfmdr1 N86Y mutations for resistance to AQ.

2. Secondary analyses are planned to investigate the association between malaria event history, and completeness of adherence to study interventions, with malaria incidence over time.

3. Regression approaches applied to data from the first three years of the trial will be applied to data from the final two years of the study, to obtain estimate the duration and profile of protection over time from the sixth and seventh doses of RTS,S/AS01$_E$ and SMC. Further secondary analyses will explore the changing relative benefits of SMC and RTS,S/AS01$_E$ with age and transmission intensity (by comparing efficacy profiles with age between Burkina Faso, which has higher incidence rates, with Mali).

4. Analyses of the primary vaccine effect, through stratification on event number, to account for potential differences in the acquisition of immunity in the different study groups over the course of the trial, and to determine whether any appearance of diminishing effectiveness of the interventions over time, could be explained by differences in acquired immunity in the single intervention groups compared to the combined group.

5. Analyses of rebound morbidity. The study cohort is being followed up beyond the observation period of the extension study, to determine whether prior randomisation group, adherence to the allocated interventions, and disease history is associated with morbidity post-intervention. While older children in the study cohort have been observed during their first year post-interventions (during the 2021 rainy season), it would be premature to draw conclusions about delayed morbidity post-intervention, as these older children who received their final (sixth) dose of RTS,S vaccine in June 2020 may continue to be partially protected from vaccination in 2021. For this reason, analyses to investigate morbidity in the post-intervention period will be reported separately after a longer period of follow-up.

Database and randomisation codes

Data have been collected using electronic case record forms (eCRF) developed using Open Data Kit (ODK) software. Tablet PCs are used to document all intervention contacts and all active surveillance contacts such as the end of season cross-sectional surveys. For passive case detection, tablet PCs loaded with eCRFs are available at all study health centres that provide treatment. Data are transferred from the eCRFs held in the research sites to a central data management team. Automatic checks are performed on clinical and laboratory forms to ensure that they are complete and contain valid responses prior to transferring data. Further checking and cleaning of the data (including checks for duplicate entries, consistency and range checks of variables) is then carried out by the data management teams in Burkina Faso and Mali using MS Access.

The consistency of merges between different database tables will be undertaken blind to randomisation group. The analysis databases and analysis programmes (written as Stata do files) will also be prepared for the primary and key secondary analyses before the randomisation code is broken.

A final version of the database for analysis, following approval by the Data and Safety Monitoring Board (DSMB) will be archived on the LSHTM MyFiles system, and a copy sent to the chair of the DSMB for their records.

The study treatment group will only be released by the independent statistician when the final database is ready and authorisation is given by the DSMB.

All data used for analysis of the main trial report will be annotated and archived. Stata code used for the analyses will also be archived.

Planned main tables for published report

Dummy Table 1: Incidence of the primary outcome until the end of the intervention period: number of cases of clinical malaria; person-years at risk (PYAR); rates per 1000 person-years; and P-values from tests of homogeneity among all study children. Results will also be shown by country with results of the test of interaction by country.

	No. children	PYAR	No. events	Rate/1000 (95% CI)	Rate Ratio (95% CI)		Test of homogeneity[1]	Interaction by Country
All children								
SMC	2000	6000.0	1333	180.0 (120.0, 240.0)	(Reference)		P=0.0	
RTS,S	2000	6000.0	1333	180.0 (120.0, 240.0)	1.00 (0.90, 1.10)	Reference		
Combined	2000	6000.0	1333	180.0 (120.0, 240.0)	1.00 (0.90, 1.10)	1.00 (0.90, 1.10)		
Total	**6000**	**18000.0**	**4000**	**180.0 (120.0, 240.0)**	-	-	-	
Burkina Faso								P=0.0
SMC	2000	6000.0	1333	180.0 (120.0, 240.0)	(Reference)		P=0.0	
RTS,S	2000	6000.0	1333	180.0 (120.0, 240.0)	1.00 (0.90, 1.10)	Reference		
Combined	2000	6000.0	1333	180.0 (120.0, 240.0)	1.00 (0.90, 1.10)	1.00 (0.90, 1.10)		
Total	**6000**	**18000.0**	**4000**	**180.0 (120.0, 240.0)**	-	-	-	
Mali								
SMC	2000	6000.0	1333	180.0 (120.0, 240.0)	(Reference)		P=0.0	
RTS,S	2000	6000.0	1333	180.0 (120.0, 240.0)	1.00 (0.90, 1.10)	Reference		
Combined	2000	6000.0	1333	180.0 (120.0, 240.0)	1.00 (0.90, 1.10)	1.00 (0.90, 1.10)		
Total	**6000**	**18000.0**	**4000**	**180.0 (120.0, 240.0)**	-	-	-	

Numbers are included only to give an idea of layout / spacing.

Dummy Table 2: Incidence of the primary outcome by study year: number of cases of clinical malaria; person-years at risk (PYAR); rates per 1000 person-years; and P-values from tests of homogeneity among all study children. The results of the test of interaction by study year will also be shown.

	No. children	PYAR	No. events	Rate/1000 (95% CI)	Rate Ratio (95% CI)		Test of homogeneity[1]	Interaction by Year
Study Year 4								
SMC	2000	2000.0	1333	180.0 (120.0, 240.0)	(Reference)		P=0.0	
RTS,S	2000	2000.0	1333	180.0 (120.0, 240.0)	1.00 (0.90, 1.10)	Reference		
Combined	2000	2000.0	1333	180.0 (120.0, 240.0)	1.00 (0.90, 1.10)	1.00 (0.90, 1.10)		
Total	**6000**	**6000.0**	**4000**	**180.0 (120.0, 240.0)**	**-**	**-**	**-**	
Study Year 5								P=0.0
SMC	1000	1000.0	1333	180.0 (120.0, 240.0)	(Reference)		P=0.0	
RTS,S	1000	1000.0	1333	180.0 (120.0, 240.0)	1.00 (0.90, 1.10)	Reference		
Combined	1000	1000.0	1333	180.0 (120.0, 240.0)	1.00 (0.90, 1.10)	1.00 (0.90, 1.10)		
Total	**3000**	**3000.0**	**4000**	**180.0 (120.0, 240.0)**	**-**	**-**	**-**	

Numbers are included only to give an idea of layout / spacing.

Similar tables will be used to report the incidence of passively detected secondary outcomes.

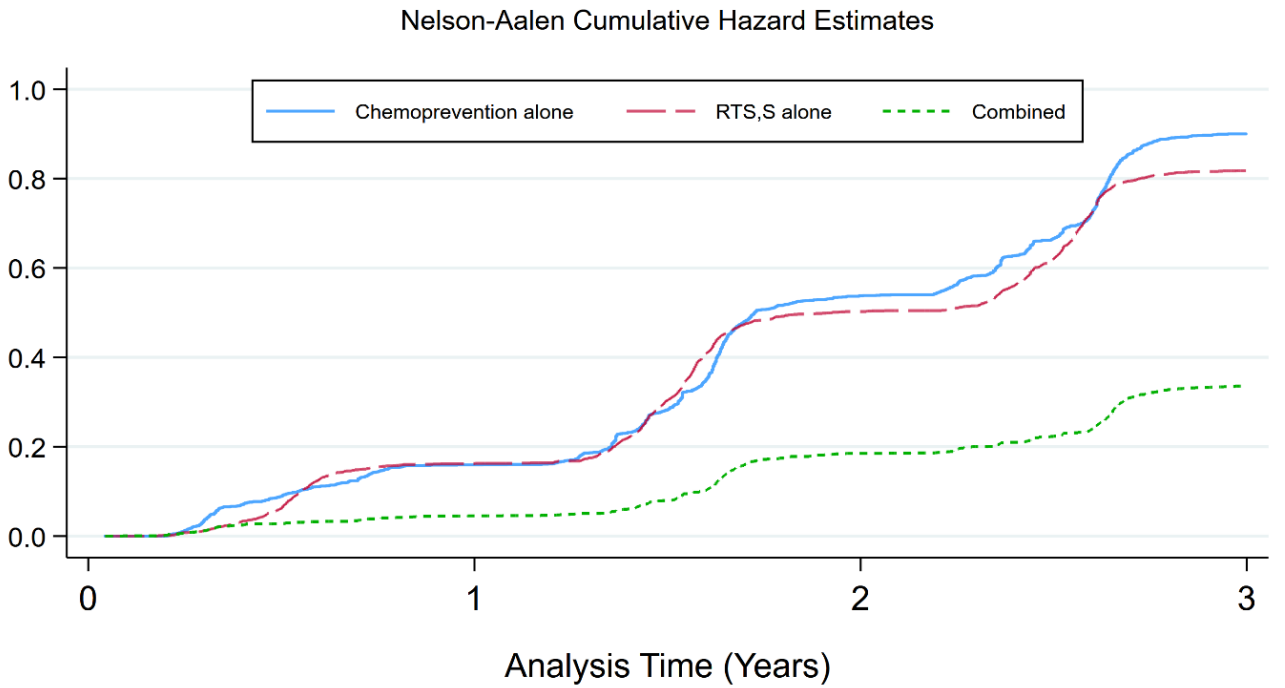
Dummy Table 3: Prevalence of *P. falciparum* infection at the end of malaria transmission season surveys: number of children tested; number with the outcome of interest; prevalence (95% CI); prevalence ratio (95% CI) and P-values from tests of homogeneity will be shown.

	No. children with result	No. with outcome	Prevalence (95% CI)	Prevalence Ratios (95% CI)		Test of homogeneity[1]
***P. falciparum* infection**						
All study children – 2020						
SMC	2000	200	10.0 (5.00, 15.0)	(Reference)		P=0.0
RTS,S	2000	200	10.0 (5.00, 15.0)	1.00 (0.90, 1.10)	Reference	
Combined	2000	200	10.0 (5.00, 15.0)	1.00 (0.90, 1.10)	1.00 (0.90, 1.10)	
Total	**6000**	**600**	**10.0 (5.00, 15.0)**	**-**	**-**	**-**
All study children – 2021						
SMC	2000	200	10.0 (5.00, 15.0)	(Reference)		P=0.0
RTS,S	2000	200	10.0 (5.00, 15.0)	1.00 (0.90, 1.10)	Reference	
Combined	2000	200	10.0 (5.00, 15.0)	1.00 (0.90, 1.10)	1.00 (0.90, 1.10)	
Total	**6000**	**600**	**10.0 (5.00, 15.0)**	**-**	**-**	**-**

Numbers are included only to give an idea of layout / spacing.

Similar tables will be used to report the prevalence of other secondary outcomes measured at the end of transmission season surveys, and weekly surveys.

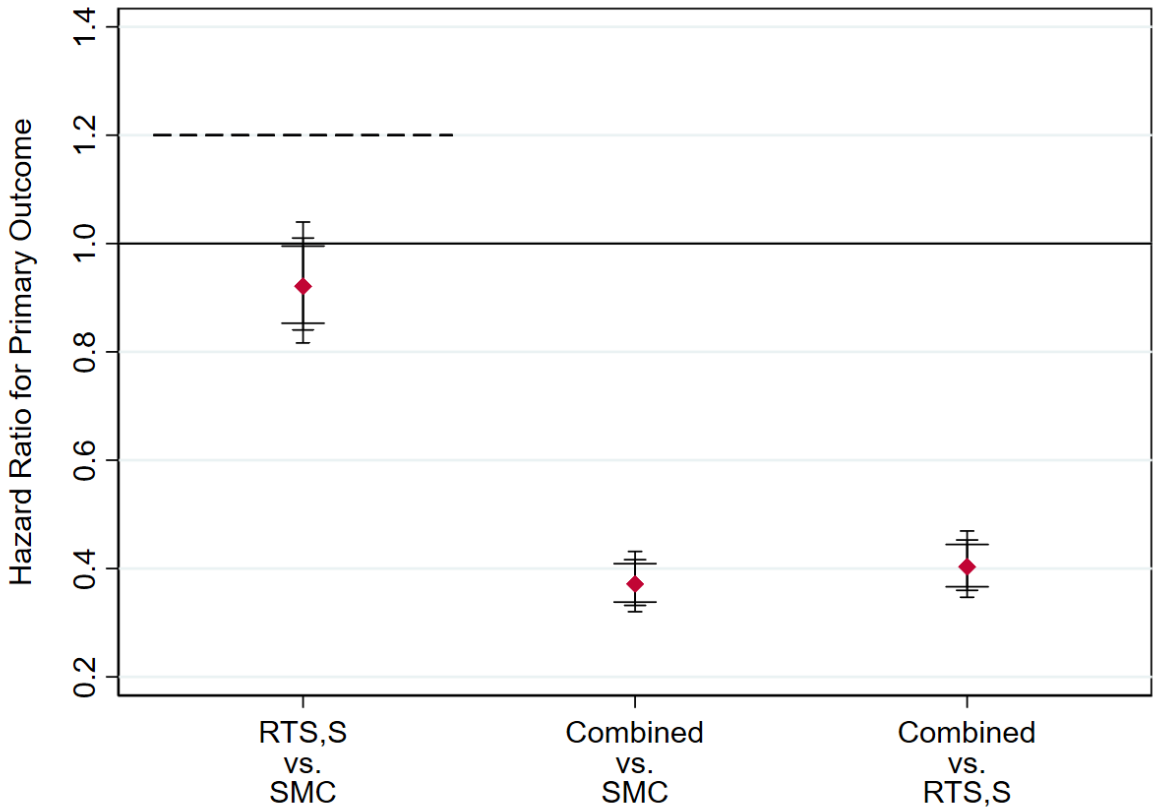
Dummy Figure 1. This will show i) cumulative hazards of malaria, by treatment group and ii) the risk of malaria, by treatment group. As an example, the plot from the first 3 years of the trial is shown.



Number at Risk

Chemoprevention	1904	1847	1716
RTS,S	1927	1882	1734
Combined	1919	1873	1740

Dummy Figure 2. This will show the hazard ratios for the primary outcome, including 90, 95 and 99% confidence intervals, and the non-inferiority margin for the comparison of RTS,S alone and SMC alone. As an example, the plot from the first 3 years of the trial is shown.



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