This supplement contains the following items.

1. Original protocol, final protocol, summary of changes.
2. Original statistical analysis plan, final statistical analysis plan, summary of changes.

Clinical trials registration no. NCT03143218

Original protocol approved by LSHTM Ethics Committee 5.1.2017

STUDY PROTOCOL

A PHASE IIIB COMPARATIVE TRIAL OF SEASONAL VACCINATION WITH THE MALARIA VACCINE RTS,S/AS01, SEASONAL MALARIA CHEMOPREVENTION AND OF THE TWO INTERVENTIONS COMBINED.

Study Identification number	207284 (MALARIA-099)
Brief Title:	RTS,S/AS01 and SMC Trial
Protocol Version & date:	Original protocol approved by LSHTM Ethics Committee 5.1.2017 (December 2016)
Sponsor:	London School of Hygiene & Tropical Medicine, Keppel Street, WC1E 7HT, London
Lead Principal Investigators:	Daniel Chandramohan & Brian Greenwood
Site Study Directors:	Jean Bosco Ouedraogo & Ogobara Doumbo

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The signatures below confirm agreement by the individuals authorised by the sponsor and principal participating institution at the clinical site that the study will be conducted in compliance with protocol version 2 dated September 2016.

Study Director in Mali	NAME (PRINTED)	DATE
……	……	……
Study Director in Burkina Faso	NAME (PRINTED)	DATE
……	……	……
Study Director in the UK	NAME (PRINTED)	DATE

LIST OF ABBREVIATIONS

ACT	Artemisinin Combination Therapy
AQ	Amodiaquine
CSP	Circumsporozoite protein.
DSMB	Data Safety and Monitoring Board
EMA	European Medicines Agency
GCP	Good Clinical Practice
IRSS	Institut de Recherche en Sciences de la Santé
ITN	Insecticide-treated bednet
LDH	Lactic dehydrogenase
LSHTM	London School of Hygiene & Tropical Medicine
MMV	Medicines for Malaria Venture
MPAC	Malaria Policy Advisory Committee
MRTC	Malaria Research and Training Centre
NMCP	National Malaria Control Programme
SMC	Seasonal Malaria Chemoprevention
RDT	Rapid Diagnostic Test
SP	Sulphadoxine/pyrimethamine
WHO	World Health Organization

PROTOCOL SUMMARY

Title	A comparative trial of seasonal vaccination with the malaria vaccine RTS,S/AS01, seasonal malaria chemoprevention and of the two interventions combined.
Study objective	This trial seeks to determine whether – 1. Seasonal vaccination following priming with the RTS,S/AS01 malaria vaccine would be non-inferior to Seasonal Malaria Chemoprevention (SMC) with sulphadoxine/pyrimethamine (SP) + amodiaquine (AQ) in preventing malaria in children in the areas of the Sahel and sub-Sahel of Africa where malaria is still a major public health challenge and whether RTS,S/AS01 would be easier to deliver than SMC. 2. RTS,S/AS01 would provide additional, useful and cost effective protection against malaria if given together with SMC in areas with highly seasonal malaria transmission and reduce the risk of the emergence of resistance to the antimalarials used for SMC.
Study design	This will be a double-blind, individually randomised trial with three study arms. The study groups are as follow: **Group1 (SMC)** **Group2 (RTSS)** **Group3 (RTSS+SMC)** **Year1**: Feb-April Rabies vaccine x 3 RTSS/AS01 x 3 RTSS/AS01 x 3 **Year1**: Aug-Nov SMC x 4 SMC placebo x 4 SMC x 4 **Year2**: Aug-Nov HepA vaccine x 1 RTSS/AS01 x 1 RTSS/AS01 x 1 SMC x 4 SMC placebo x 4 SMC x 4 **Year3**: Aug-Nov HepA vaccine x 1 RTSS/AS01 x 1 RTSS/AS01 x 1 SMC x 4 SMC placebo x 4 SMC x 4 A decision will be made by the steering committee in year 2 on whether all children in Groups 2 and 3 will receive a fractional dose of RTS,S/AS01 for their booster doses in years 2 and 3 or a full dose or whether children will be randomised to receive either a full or a fractional dose. This decision will be guided by the results of ongoing RTS,S/AS01 studies. Seasonal vaccination with either RTS,S/AS01 or control vaccine will be undertaken approximately one month before the start of the malaria transmission season and the first administration of SMC.
Study site	The trial will be conducted in Hounde district, Burkina Faso and in Bougouni district, Mali, sites of an on-going trial of the impact on

	mortality and hospital admissions of adding azithromycin to the SP + AQ used for SMC.
Study population	Children of either sex, 5-17 months of age on the scheduled date of administration of the first dose of RTS,S/AS01 vaccine (February 2017) who are living permanently in the study area will be eligible for inclusion in the trial provided the consent of a parent or legally acceptable representative is 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 received a malaria vaccine will be excluded from the trial. Children known to have received SMC during the year prior to enrolment will not be excluded from the trial but will be distributed equally between study groups at the time of randomisation.
Group1 - SMC arm	Children in the control group will be given three doses of rabies vaccine (February-April) and four rounds of SMC (SP+AQ) (August to November) at monthly intervals in year 1. In years 2 and 3 they will receive one dose of a control vaccine (Hepatitis A) in June and four rounds of SMC (August-November) at monthly intervals.
Group 2 – RTSS/ AS01 arm	Children in this intervention group one will be given three doses of RTS,S/AS01 vaccine (February-April) and four rounds of SMC placebo (August to November) at monthly intervals in year 1. In years 2 and 3 they will be given one dose of RTS,S/AS01 vaccine (June) and four rounds of SMC placebo (August-November) at monthly intervals.
Group 3 – SMC + RTS,S/AS01 arm	Children in the intervention group two will be given three doses of RTS,S/AS01 vaccine (February-April) and four rounds of SMC in year 1 (August to November) at monthly intervals. In years 2 and 3 they will be given one dose of RTS/AS01 vaccine (June) and four rounds of SMC (August-November) at monthly intervals.
Primary endpoint	The primary end-point for the trial will be 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 and which is accompanied by a positive blood film with a parasite density of 5,000 per µl or more.
Secondary endpoints	Secondary end-points for the trial will 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 which 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. Serious adverse events (SAEs), including any deaths, occurring at any time during the study with special reference to any cases of meningitis and cerebral malaria (WHO case definition). f. Anti-CSP antibody concentrations obtained after priming and after each booster dose, determined in a sub-sample of children. g. The presence of molecular markers of resistance to SP and AQ in parasite positive samples collected at each annual cross-sectional survey. h. The presence of polymorphisms in the *csp* gene of *Plasmodium falciparum* isolates from children who have received RTS,S/AS01 that differ from those of the isolate used in the preparation of the vaccine. Secondary end-point (b) will be important for the economic evaluation of the interventions. Particular attention will be paid to the occurrence of any cases of meningitis and cerebral malaria [end-point (e)] as an increase in the incidence of these conditions was identified as a potential safety signal in the phase 3 RTS,S/AS01 trial. The trial will not be large enough to measure an impact on mortality but all deaths will be recorded and investigated. Evaluation of the clinical and immunological response to a fifth dose of RTS,S/AS01 is highlighted as an important research objective by the WHO.
Sample size	Three thousand children will be recruited in Burkina Faso and in Mali (total 6,000) and these children will be followed for three years.
Study duration	October 2016 – June 2020

TABLE OF CONTENTS

1 BACKGROUND AND RATIONALE

The RTS,S/AS01 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 induces a strong antibody response to the *P. falciparum* CSP and high titres of anti-CSP antibody are associated with protection [2]. Following a long process of development, a phase 3 study of RTS,S/AS01 conducted in 15,439 children in 7 countries in Africa showed that three doses of RTS,S/AS01 given with a one month interval between doses, followed by a fourth dose 18 months post 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 given to infants at the age of 6-12 weeks [3]. RTS,S/AS01 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 [4]. 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, 4]. In July 2015, the European Medicines Agency reviewed efficacy and safety data on RTS.S/AS01 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 [5]. These included the pilot implementation of RTS,S/AS01 in children aged 5-17 months in 3-5 settings with moderate-to-high malaria transmission intensity, with a preference for areas where SMC is not being delivered, and evaluation of alternative approaches to deployment of the vaccine. Recent evidence [6] from challenge studies conducted in American adult volunteers suggests that a higher level of protection can be obtained when the third dose of the priming schedule is reduced to one fifth of the usual amount and delayed until approximately 6 months post dose 2, and when a reduced dose is used for boosting. In these studies, a vaccine efficacy of 86% was achieved three weeks following priming and 90% efficacy following boosting with a fractional dose. This encouraging result is now being followed in further studies.

SMC involves monthly administration of an antimalarial drug or drug combination in a full therapeutic course to children on three of four occasions during the period of highest risk of malaria infection. Studies undertaken in several countries in West Africa, including Burkina Faso and Mali, have shown 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% [7-9]. 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 [10]. Recent studies have defined the areas where SMC would be an appropriate intervention based on the seasonality and incidence of malaria [11]. These include most of the Sahel and sub-Sahel, population approximately 200 million, and possibly other areas in southern and eastern Africa. 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. 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 through the UNITAID supported SMC ACCESS programme and the support of other major donor organisations. 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).

SMC is effective but its delivery is demanding on the recipient and provider, requiring four contacts each malaria transmission season if anti-malarials are given to mothers to administer at home and 12 contacts if directly observed treatment is employed. In addition, SMC is threatened by the emergence of resistance to SP and AQ and there are currently no other combinations of licensed antimalarials that could be used to replace them. It is likely to be 5-10 years before novel antimalarials under development could be deployed for SMC. In contrast to SMC, seasonal vaccination with RTS,S/AS01 would require only one visit each transmission season after priming. RTS,S/AS01 may be a little less effective than SMC during the malaria transmission season but this may be balanced by provision of protection during the dry season, when some malaria transmission still occurs and when SMC would provide no benefit. There is, therefore, a need for a comparative

study of these two interventions. In some areas where SMC is currently being deployed, and other malaria control interventions such as long-lasting insecticide treated nets used widely, the incidence of malaria in young children remains high (0.4 episodes per year in children under the age of five years in SMC recipients in Burkina Faso). Thus, determining whether RTS,S/AS01 would provide added, useful protection to SMC in such situations is also important. It might also be able to protect some children who, because of side effects, are unable or unwilling to take SMC.

Although the EMA has given a positive opinion on RTS,S/AS01, it is not yet certain how this partially effective malaria vaccine can be used most effectively [12]. Three, large-scale pilot implementation studies are being planned by WHO but it is unlikely that, following WHO recommendations, any of these will be conducted in a country where SMC is being delivered. The WHO recommendations on RTS,S/AS01 indicate the need for research on alternative approaches to the delivery of this vaccine [13]. Exploration of the potential of the vaccine to prevent seasonal malaria, taking advantage of its high but rapidly waning efficacy, meets this recommendation and is, therefore, timely.

2 STUDY OBJECTIVES

The trial seeks to determine whether –

a. Seasonal vaccination following priming with the RTS,S/AS01 malaria vaccine would be non-inferior to SMC SP + AQ in preventing malaria in children in the areas of the Sahel and sub-Sahel of Africa where malaria is still a major public health challenge and whether it would be easier to deliver.
b. RTS,S/AS01 would provide additional, useful and cost effective protection against malaria if given together with SMC in areas with highly seasonal malaria transmission and reduce the risk of the emergence of resistance to the antimalarials used for SMC.

3 STUDY AREA

The trial will be conducted in Hounde health district, Burkina Faso and in Bougouni Koulikoro district, Mali. The Hounde 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 IRSS. The study site in Mali is the district of Bougouni in the region of Sikasso, Mali, 150 km south of Bamako where the MRTC is based.

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

The population of Hounde 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 *Plasmodium 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 2015 was 53% in Bugouni and 62% in Hounde. The main malaria vector in each study area is *Anopheles gambiae* ss. A high proportion of children sleep under an ITN in Bougouni (96%) but the percentage is less in Hounde. 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. During the two years of the SMC + AZ the incidence of clinically suspected meningitis in children aged 3 - 59

months in Bougouni was approximately 0.11 per 1,000 per year and that of cerebral malaria 0.88 per 1,000 per year.

During the past three years, Hounde and Bougouni districts have been the site of a trial, involving approximately 20,000 children aged 3-59 months, which has been evaluating the impact of adding azithromycin to the SP + AQ used for SMC in preventing overall mortality and hospital admissions with non-traumatic illnesses. This trial finishes at the end of 2016 which would allow a smooth transition to the new trial, incorporating many of the well trained staff who have conducted the previous study. Field laboratories, which are equipped to undertake parasitological and haematological investigations, have been established at each district hospital and efficient data management systems set up.

In Mali vaccines will be stored at MRTC, Bamako, Mali. The MRTC has a long history (> 12 years) of testing vaccines including in collaboration with GSK, the WRAIR, the NIAID/NIH and Sanaria. Vaccines will be stored in a cold room with continuous monitoring of the temperature devices with alarm and telephone SMS and 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. In Burkina Faso, vaccines will be stored at IRSS, in Bobo-Dioulasso where there is a dedicated storage room for drugs that 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 average with temperature and humidity controllers.

4 COMMUNITY SENSITISATION

The objectives of the study and the way in which it will be conducted have already been discussed with staff of the national malaria control progamme (NMCP) and expanded infant immunisation programme (EPI) in both Burkina Faso and Mali and their support for the trial obtained in principle. These discussions will be continued in the coming months. Community approval will be sought through meetings with leaders of the study communities and through open meetings held in the

study communities. Community leaders will be consulted prior to the start of the intervention on the best ways of achieving high compliance with vaccination, drug delivery and follow-up.

5 TRIAL POPULATION

After obtaining permission from the community leaders for the trial, a household census will be conducted in January 2017 and all households within the study areas with children 5-17 months of age on February 1st 2017 will be enumerated. At the census, a preliminary screening of potentially eligible children will be undertaken. Potentially eligible children and their caretakers will be visited again and written informed consent will be obtained from their caretakers for their inclusion in the trial before the administration of the first dose of study vaccines. Children entered into the trial will be assigned a unique ID number and their demographic data (date of birth and/or age, and gender), use of insecticide treated nets (ITN) and history of receiving SMC during the last transmission season will be collected. The census data will be updated in April/May 2018 and 2019 prior to the administration of the booster doses of vaccine. Eligible children will be allocated randomly to one of the three study arms in permuted blocks of 12 using standard randomisation procedures (table). Children who have a history of receiving SMC in the previous years will be distributed equally between the three study groups

A child will be eligible for inclusion in the trial if -

a. The child is a permanent resident of the study area and likely to remain a resident for the duration of the trial.
b. The child is 5 - 17 months of age at the time of first vaccination.
c. A parent or legally recognised guardian provides informed consent for the child to join the trial.

A child will be ineligible for inclusion in the trial if -

a. The child is a transient resident in the study area.
b. The child is in care.
c. The age of the child is outside the stipulated range.
d. The child has a history of an adverse reaction to SP or AQ.

e. The child has a serious underlying illness, including known HIV infection, unless this is well controlled by treatment, or severe malnutrition (weight for age or mid arm circumference Z scores < 3 SD).

f. The child is known to have an immune deficiency disease or is receiving an immunosuppressive drug.

g. The child has previously received a malaria vaccine.

h. The child is enrolled in another malaria intervention trial.

i. The parents or guardians do not provide informed consent.

Table. Study groups

	Group1 (SMC alone)	**Group2 (RTS,S alone)**	**Group3 (RTS,S+SMC)**
Year1: Feb-April	Rabies vaccine x 3	RTSS/AS01 x 3	RTSS/AS01 x 3
Aug-Nov	SMC x 4	SMC placebo x 4	SMC x 4
Year2: Aug-Nov	HepA vaccine x 1	RTSS/AS01 x 1	RTSS/AS01 x 1
	SMC x 4	SMC placebo x 4	SMC x 4
Year3: Aug-Nov	HepA vaccine x 1	RTSS/AS01 x 1	RTSS/AS01 x 1
	SMC x 4	SMC placebo x 4	SMC x 4

SMC or placebo will be given at monthly intervals on four occasions during the malaria transmission season.

6 SAMPLE SIZE

Three thousand children will be recruited in Burkina Faso and a similar number in Mali (total 6,000) and these children will be followed for three years. A low dropout rate of around 5 % per year (15% overall) is anticipated based on findings from the current SMC+AZ study, although it is possible that the dropout rate may be higher during a vaccine trial as a vaccine trial has not been conducted previously in the study districts. Since the relative efficacy of SMC and RTS,S/AS01 is uncertain, the conservative approach of recruiting equal numbers of children into each arm of the study (2,000 per arm) will be followed. Results obtained over a period of three years of observation in the two study sites will be combined for the primary analysis of the incidence of clinical malaria. An analysis

will also be undertaken of efficacy in years 2 and 3 combined ie. to the efficacy of administration of a booster dose. It is not anticipated that an unblinded interim analysis will be undertaken.

During the current SMC study in the proposed trial sites, the incidence of clinical episodes of malaria during the first year of the study (2014) was 442 and 382 per 1000 children in Mali and in Burkina Faso respectively. Complete morbidity data are not yet available for the second year of the study (2016) but malaria incidence was higher than in 2015. For the sample size calculations below, we have, therefore, assumed a conservative incidence rate of 300 cases per 1000 children over a calendar year. Using a two sided 95% confidence interval, a three-arm study which enrolled 2000 children in each arm (1000 per arm per centre) would have, for the non-inferiority comparisons, 90% power to exclude a relative difference in incidence between RTS,S/AS01 and SMC given alone of 16.1% over the three-year study period and of 28.6% or more for each individual year. The study would also have 80% power to exclude a relative difference in incidence of 13.9% over the whole study period and of 24.7% in each year. The differences that could be detected will be smaller if, as suspected, the incidence of clinical malaria is greater than 300 cases per 1,000 children per year. For the superiority comparisons of the combined interventions with SMC alone, using a two-sided 95% confidence interval, the study would have 90% power to detect a difference greater than 11.1% over the three years of the study and of 19.2% in each year.

For the analysis of the serological response to RTS,S/AS01, comparisons will be made between mean anti-CSP antibody titres pre and post the primary series of vaccination and before and after the two subsequent booster doses. Based on the standard deviations in antibody titres observed in children enrolled in the RTS,S/AS01 phase 2 and phase 3 trials, inclusion of around 160 individuals in each group (pre and post vaccination at each of the three time points) will give a study with approximately 80% power to detect a difference of 25% - 30% in mean titre between children who receive RTS,S/AS01 with or without co-administration of SMC.

7 THE INTERVENTIONS

RTS,S/AS01 vaccine: Three doses of RTS,S/AS01 will be given to children allocated to the RTSS or RTSS+SMC groups at monthly intervals during the 2017 dry season (February to April) followed by a fourth and fifth dose at the beginning of the 2018 and 2019 malaria transmission seasons. A

decision will be made by the steering committee in the first quarter of 2018, based on the information available from on-going studies at that time, whether or not to use a fractional dose for the booster immunisation.

The efficacy of RTS,S/AS01 against both severe and uncomplicated malaria in children vaccinated at the ages of 5 – 17 months and subsequently given a booster dose has been assessed by both the EMA and WHO to outweigh the vaccines side effects. The vaccine causes 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 common 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 issue related to administration of RTS,S/AS01 is the unexplained, statistically significant increase in meningitis observed in children given the vaccine at the age of 5-17 months during the phase 3 trial (about 4 per 1,000 during a four-year period of follow up); this was not observed when the vaccine was given at the age of 6-12 weeks.

Meningitis was caused by a variety of organisms and did not show any temporal relationship to vaccination and more than 40% of cases occurred at one centre in Malawi. The EMA concluded that the increase in cases of meningitis was probably a chance finding and that the benefits of the vaccine exceeded any safety issues. However, the agency recommended further evaluation of the incidence of meningitis in vaccine recipients when RTS,S/AS01 was deployed and this will be done in this study. Any study children admitted to hospital with suspected meningitis will be investigated as fully as possible and CSF samples obtained for microbiological diagnosis by PCR. In addition, there was a suggestion that the proportion of cases of severe malaria which were classified as cerebral malaria was increased in the RTS,S/AS01 recipients, although the incidence of severe malaria overall was reduced. Therefore, the incidence of cases of severe malaria meeting the WHO definition [ref] will be monitored carefully.

RTS,S/AS01 malaria vaccine should not be administered to subjects with known hypersensitivity to any component of the vaccine, to a previous dose of RTS,S/AS01 $_{E}$ malaria vaccine or who is known to be hypersensitive to hepatitis B vaccine.

Control vaccines: Rabies vaccine will be used as the control vaccine for the primary series of vaccinations during the dry season in year 1 (2017) for those allocated to the SMC group. The rabies vaccine used will be a licensed, WHO approved vaccine purchased through UNICEF. This is likely to be *Rabipur*^{R}*,* previously produced by Novartis but now by GSK. This vaccine is produced in chick embryo cells and contains polygeline and residues of chicken proteins, and it may contain traces of neomycin, chlortetracycline and amphotericin and it is, therefore, contraindicated in subjects with an history of a severe hypersensitivity to any of the ingredients in the vaccine. Minor local and systemic reactions are common (>1:100, <1.10) after vaccination with rabies vaccine and neurological complications including Guillain Barré syndrome have been described but are very rare (<1:10.000).

Hepatitis A vaccine (HAVRIX^{R}), a licensed inactivated hepatitis A vaccine produced by GSK, will be used for the booster dose in years 2 and 3. HAVRIX^{R} is contraindicated in subjects who have had an allergic reaction to prior administration of the vaccine or who are sensitive to neomycin. This vaccine may also cause minor local and systemic reactions ((>1:100, <1.10). Severe reactions, including anaphylaxis have been described but are very rare (<1.10,000).

Seasonal malaria chemoprevention: Four courses of SMC with SP+AQ will be given at monthly intervals during the malaria transmission season in line with WHO's recommendation and national policy [8]. A course of SMC for children aged over the age of one year will comprise a single treatment of SP (500mg/25 mg) and AQ 150mg on day 1 and AQ 150mg on days 2 and 3. Infants will receive half of these doses. SP and AQ and matching placebo will be obtained from a GMP certified supplier, if possible in a dispersible form. All treatments will be given under observation. Children who do not receive SMC will receive a matching placebo. Discussions are taking place with the manufacturer of the SP+AQ combination used for SMC (Guilin Pharamceuticals, Shanghai, Co) and the Medicines for Malaria Venture as to whether it will be possible for the company to prepare a dispersible, matching placebo. If this is not possible, tablets of SP and AQ and of matching placebo tablets produced by the same company will be used.

Approximately 5 million children have received SMC with SP + AQ during the 2015 rainy season across the Sahel and sub-Sahel (http://www.malariaconsortium.org/pages/access-smc.htm) and this drug combination has been shown to be remarkably safe [10]. 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. The possibility that SMC with SP+AQ might induce resistance to these drugs in *P. falciparum* has been investigated in a number of trials of SMC. Selection of parasites carrying mutations which confer resistance to pyrimethamine or sulphadoxine has been demonstrated in some but not all studies [7]. However, because the prevalence of parasitaemia in children who received SMC was substantially less than in the control group, the total number of parasites carrying resistance markers was less in children who had received SMC than in control children. Extensive use of SP for intermittent preventive treatment in pregnant women has not accelerated resistance to SP in West Africa. Preliminary analysis of samples obtained from children in the ongoing SMC trial in the study sites at the end of 2014 did not show levels of mutations in *dhfr* and *dhp*s genes likely to be associated with *in vivo* resistance to SP. The potential risk of inducing resistance to SP or AQ by SMC was reviewed carefully by a WHO Technical Expert Group and WHO's Malaria Policy Advisory Group and considered to be an acceptable risk in light of the major benefits conveyed by the intervention. Malaria parasites isolated at the end of the transmissions season will be tested for resistance markers to SP.

8 IMPLEMENTATION OF THE INTERVENTIONS

Vaccination. RTS,S/AS01 will be provided in a two-doses glass vial of lyophilized RTS,S antigen to be reconstituted with a two-dose glass vial of AS01 Adjuvant System. The final product for administration will be prepared by reconstitution of the lyophilized antigen with the liquid adjuvant to deliver two doses (1.0 ml). A single dose consists of 0.5 ml of RTS,S/AS01 final preparation. All vials of vaccine provided in this study are intended for single use only. After reconstitution the vaccine will be administered by slow IM injection, using a fresh 25G needle with length of one inch (25 mm), in the left deltoid. Vaccine will be injected within four hours of reconstitution (storage at +2°C to +8°C). Syringes containing RTS,S/AS01 or the control vaccine will be prepared by a pharmacist who takes no other part in the trial. Loading of syringes with vaccines and masking with

tape to blind the person administering the vaccine as to its nature 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.

Rabies vaccine, 1.0 ml in volume is given by intramuscular injection. The paediatric dose of HAVRIX ^{R} is 0.5 ml and it is also administered by intramuscular injection.

SMC. SMC drugs will be 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 four cycles of treatment required for one full malaria transmission season appropriate for the child's age. Treatment with each dose of SMC will be given by trained, paid volunteers at a central point in each study community under observation. Study children will be given an 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 will be scanned to ensure that the child is given the allocated intervention. Home visits will be made to children who miss treatment on the designated day and their parents/guardians will be asked if they would still like their child to receive SMC. If they agree, treatment will be given at home.

All children will be given an ITN at the commencement of the 2017 rainy season.

9 CONTRAINDICATIONS TO SUBSEQUENT VACCINATION

Contraindications to administration of a further dose of vaccine in any child include –

a. Anaphylaxis following administration of the first dose of the vaccine.

b. Any condition that in the judgment of the investigator would make intramuscular injection unsafe.

c. The occurrence of a new adverse event (AE) or the exacerbation of an existing AE that, in the opinion of the investigator, exposes the subject to unacceptable risk from subsequent vaccination.

d. An acute disease and/or fever at the time of vaccination. Fever is defined as temperature $\geq$ 37.5°C/99.5°F for oral, axillary or tympanic route, or $\geq$ 38.0°C/100.4°F for rectal route.

Subjects whose fever resolves with treatment may be vaccinated provided that revaccination falls within the stipulated time period.

Subjects with a minor illness (such as mild diarrhea, mild upper respiratory infection) without fever can be administered all vaccines.

10 FOLLOW-UP AND MEASUREMENT OF OUTCOMES

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

a. ***Passive surveillance for cases of uncomplicated and severe malaria***. In each country, project staff will be 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 health facilities. In Mali, cases of uncomplicated malaria may also be treated by community health workers who have been taught to diagnose malaria with a RDT and to treat RDT positive cases and these cases will also be recorded if they meet the inclusion criteria. Cases of suspected malaria (fever, history of fever within 48 hours or any other symptom/sign suggestive of malaria) will be tested with a RDT and managed on the basis of their RDT result and blood films will be obtained from all these cases for subsequent confirmation of the diagnosis.

b. ***Active surveillance for malaria.*** Each month during the malaria transmission season, 90 randomly selected children (30 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 parasitaemias. Any child who is febrile or who has other features suggestive of a diagnosis of malaria will have an RDT done and those who are positive will be treated with a full course of an ACT.

c. ***Prevalence of malaria parasitaemia and anaemia***. A survey of all study children will be done one month after the last round of SMC administration at the end 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 and those who are positive will be treated with a full course of an ACT. Finger prick blood samples will be collected for preparing blood slides and blood spots on filter paper from all

children. The prevalence of parasitaemia, including the presence of gametocytes, will be detected by microscopy.

d. ***Serious Adverse Events***. Project staff based at the district hospitals will be responsible for the identification of any child in the trial admitted to hospital and ensuring their referral to a study physician. Hospital staff will be provided with additional training on the recognition of cases of meningitis, cerebral malaria or immune deficiency diseases and standard operating procedures will be developed for management of children suspected of having one of these conditions. Definitions for meningitis and cerebral malaria, currently being developed by WHO for use in the pilot RTS,S/AS01 implementation trials will be used. The aetiology of cases of meningitis will be 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 will be investigated and, if this occurred in the community, a verbal autopsy will be done.

Surveillance of all children will be maintained throughout the study period for any Serious Adverse Events (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 a time decided by the DSMB, perhaps three monthly. Details of definitions of adverse events and SAEs and of reporting procedures are described in appendix 1.

e. ***Immune response to the vaccine***. Blood samples (2ml) will be collected from 160 children in each of the groups who receive RTS,S/AS01 prior to administering the first dose of vaccine and one month after third dose of the primary series of vaccination, and then in year 2 and 3 before giving the booster dose and one month after administration of the fourth and fifth doses of vaccine for measurement of anti-CSP antibodies.

f. ***Drug resistance****:* Dried blood spots from children who have malaria parasitaemia detected by microscopy at each annual cross-sectional survey will be used for analysis of molecular markers of resistance to SP and AQ at MRTC, Bamako and at IRSS, Bobo-Dioulasso. A

subset of samples will also be analysed at LSHTM as a test of quality control. A sub-set of positive samples will be tested for the presence of HRP2 deletions which may give rise to a false negative RDT.

***g. Polymorphisms in the P. falciparum CSP*.** DNA will be obtained from a randomly selected group of children with clinical episodes of malaria to measure polymorphisms in the *P. falciparum csp* gene to determine how closely these match the genetic structure of the isolate used in the production of the CSP vaccine as analysis of parasites collected during the phase 3 RTS,S trial showed that vaccine efficacy was higher against parasites with a CSP protein that was homologous with the strain used to produce the RTS,S/AS01 vaccine than against less well matched parasite strains [14].

11 LABORATORY PROCEDURES

a. ***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. Blood films collected at the same time will be read subsequently by two microscopists. All slides will be read twice by two separate readers following the guidelines developed for the phase 3 RTS.S/AS01 trial [15]. 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/µl) readings will be 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 will be 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 will be adopted. If the majority decision is positive, the final result will be the geometrical mean of the two positive readings. In the case of discrepancies in parasite density, the final result will be the geometric mean of the two geometrically closest readings.

b. ***Detection of markers of resistance to SP.*** Parasite DNA will be extracted from dried blood spots and nested PCR reactions will be 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 [16-18]. 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.

c. ***Measurement of haemoglobin concentration.*** Haemoglobin concentration will be measured colorimetrically using a Hemocue colorimeter (Hemocue AB, Angelholm, Sweden).

d. ***Measurement of anti-CSP concentration.*** Antibodies to CSP will be measured by a standardised ELISA at the University of Ghent in the laboratory of Professor Leroux-Roels as used in many previous trials of RTS,S/AS01.

e. ***Detection of polymorphisms in the csp gene.*** Sequencing of the C terminal region of the CSP protein will be undertaken using methods described previously for detecting polymorphisms in this region of the *csp* gene [14].

12 SOCIO-ECONOMIC STUDIES

Objectives. The objectives of the socio-economic component of the trial are to determine:

a. The cost-effectiveness of a) RTS, S vaccine versus SMC and b) the combination of both versus SMC alone.
b. The acceptability of the two interventions (separately and combined) to the health care deliverers and to the study communities.
c. The feasibility of introducing two malaria control strategies simultaneously from the health system perspective.

Economic Evaluation. Data on the costs of a clinical case of malaria and of a hospital admission with malaria to both the health care deliverers and recipients have already been collected in the two study areas during the course of the SMC+AZ trial and this information will be updated. The costs of adverse events, in particular of meningitis, will be estimated using published literature. Information on the costs of delivery of SMC outside an intervention study has also been gathered by the SMC ACCESS team and this will facilitate determination of the costs of this intervention. The costs of adding RTS,S/AS01 to the routine vaccination programme will require specific study through observations, key stakeholder interviews and review of relevant documents and files. The delivery costs will be estimated through a combination of a step-down and ingredients-approach costing methodology. A cost effectiveness analysis of the two interventions and their use in

combination will incorporate the results from the trial, the costs collected during this study as well as during previous studies and then model the cost effectiveness of the interventions from a societal perspective in a decision tree model. The final outcomes used in the model will be costs/DALYs averted. The costs of scaling up the optimum intervention at regional/ national level will be investigated

Acceptability. The acceptability of the two interventions to the health care deliverers and the preference of study communities for each of the interventions will be investigated through socioeconomic surveys and focus group discussions involving both the families of trial participants and those involved in administration of the trial interventions.

Feasibility. Investigating the feasibility of delivery of RTS,S/AS01 outside the routine EPI programme will be a major objective of the large pilot studies being planned by WHO. Experience gained on the logistic challenges posed to the national immunisation programme of delivering RTS,S/AS01 as a seasonal vaccine will be shared with both the national immunisation programmes in Burkina Faso and Mali and also with WHO.

13 DATA MANAGEMENT

To ensure data is fit for purpose, questionnaires will be tested prior to use and staff will receive training prior to and at regular intervals during data collection. Data will be managed using the DataFax system which has been set up for the SMC + AZ trial and which is working well. This system is based on electronic transfer of the CRFs from the research sites where the data are automatically captured and validated. The MRTC data management team are responsible for the training and support of the IRSS data management team, with overall support from the NIAID/NIH central data management team. Data will be uploaded to the DataFax system at the earliest opportunity to enable queries to be validated and issues resolved as soon as possible after data collection. Automatic checks will be performed on clinical and laboratory forms to ensure they are complete and contain valid responses prior to uploading by the local data managers at both sites.

An experienced independent GCP monitor (Raouf Osseni) currently monitoring the SMC + AZ trial will be contracted to ensure the quality of the data collected and that GCP standards are met. The monitor will conduct a trial initiation visit, a close out visit and at least one additional visit each year. The monitor will ensure that the trial is conducted according to the study protocol, that appropriate

ethical procedures are in place and s/he will examine a random selection of clinical and laboratory records during each visit to confirm their validity.

14 STUDY OUTCOMES

***Primary outcome*:** The primary outcome measure of the trial is the incidence of clinical 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 outcomes: Secondary outcomes include: -

a. Blood slide or rapid diagnostic test (RDT) positive malaria defined as a clinical episode 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 parasitemia) or a positive RDT.
b. Hospital admissions with malaria, including cases of severe malaria which meet WHO criteria for a diagnosis of severe malaria.
c. The prevalence of malaria infection (symptomatic or asymptomatic parasitaemia) 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. Serious adverse events (SAEs), including any deaths, occurring at any time during the study with special reference to any cases of meningitis, cerebral malaria or immune deficiency illness.
f. Anti-CSP concentrations obtained after priming and after each booster dose, determined in a sub-sample of children.
g. Comparison of the anti-CSP concentrations among children who received co-administration of SMC and RTS,S/AS01 versus those who received RTS,S/AS01 alone.
h. The presence of molecular markers of resistance to SP and AQ in parasite positive samples collected at each annual cross-sectional survey.

i. The match of polymorphisms in the *P. falciparu*m *csp* gene of parasite isolates obtained from children with clinical episodes of malaria to the genetic structure of the strain of parasite sued to develop the RTS.S/AS01 vaccine.

Particular attention will be paid to the occurrence of any cases of meningitis as this was identified as a potential safety signal in the phase 3 RTS,S/AS01 trial. The trial will not be large enough to measure an impact on mortality but all deaths will be recorded and investigated including by verbal autopsy if these occur at home. Safety data will be provided to GSK in the format requested by the company. Evaluation of the clinical and immunological response to a fifth dose of RTS,S/AS01 is highlighted as an important research objective by the WHO.

15 ANALYSIS

The primary endpoint of incidence of all episodes of clinical malaria over the study period will be analysed using Cox regression models with a robust standard error to account for clustering of episodes within individuals (i.e. the Andersen-Gill extension of the Cox model). This will estimate the total effect of the interventions. The proportion of children in each group who experience one episode of malaria will be compared as a secondary outcome using Kaplan-Meier estimates: evidence for provision of complete protection through the combination of SMC and vaccination each year will be explored using recently published methods [19, 20]. For the comparison of RTS,S/AS01 plus SMC to the other interventions, a standard superiority comparison will be performed, calculating two-sided 95% confidence intervals for the hazard ratio. For the non-inferiority comparison of RTS,S/AS01 to SMC, the two-sided 95% confidence interval for the hazard ratio will be compared to a pre-specified non-inferiority margin. The trial is powered to have more than 80% power to exclude a difference of 15% in the incidence of clinical malaria over the study period, a difference considered to be clinically important. Demonstration of a smaller difference would require a substantially larger trial and is considered to be not clinically relevant. If the lower limit of the 95% does not overlap zero (i.e. it is clear that RTS,S is superior to SMC) then a superiority comparison will be performed as for the combined intervention.

A formal analysis plan will be prepared and approved by the Data Safety Monitoring Board (DSMB) appointed for the trial before the study code is broken. 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 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 but the study is only powered to meet its primary and major secondary end-points if results from both countries are combined. Additional sub-analyses will include analysis by age, gender, bed net use during the transmission season, as determined by the history obtained at the cross-sectional surveys, and socio-economic status as determined by the educational level and occupation of the child's family.

16 ETHICS

Individual, written, informed consent will be obtained from the family or legally recognised guardian of each child entered into the trial. Ethical approval 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. 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 the district hospitals in the study areas.

17 TRIAL MANAGEMENT

The London School of Hygiene & Tropical Medicine will act as the main sponsor for the trial. Delegated responsibilities may 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, which will provide scientific oversight, has been established and their approval of the protocol will be obtained. The steering committee will hold teleconferencing or face-face meeting annually to monitor progress and advise on the scientific content of the study. In addition, a DSMB has been established to oversee the safety of the trial and

a clinical trial monitor will be appointed to ensure that the trial is conducted to GCP standards. Membership of the trial steering committee and the DSMB is shown in appendix 2.
The trial management committee will include the LSHTM PIs, site PIs and the trial administrator. The trial management committee is responsible for overseeing the trial and its members will communicate regularly by teleconferences.

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

18 ROLES OF THE INVESTIGATORS AND COLLABORATORS

The team from the IRSS, Burkina Faso which includes Jean Bosco Ouédraogo, Halidou Tinto and Issaka Zongo will be responsible for conducting the part of the trial undertaken in Burkina Faso and will participate in the analysis of the trial results. The team from MRTC which includes Alassane Dicko, Ogobara Doumbo, and Issaka Sagara will be 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, Irene Kuepfer, Matthew Cairns, Paul Milligan, Layla Yiannikaris, Karen Slater and Amit Bhasin) will provide epidemiological, statistical, administrative and financial management support. Silke Fernandes and Kara Hanson from the LSHTM will be responsible for the economic aspects of the study. A consultant will be recruited to assist the design of the acceptability and feasibility studies.

19 DISSEMINATION PLANS

Results from the trial will be presented at national and international conferences and in peer reviewed journals 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 in connection with the implementation of SMC and these

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

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21 ACTIVITIES SCHEDULE

Activities	2016				2017												2018												2019												2020	
	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	J	F	M	A	M	J	J	A	S	O	N	D	J-M	A-J
Protocol submission for ethics approval	x																																									
Ethics and regulatory approval				x																																					
Staff recruitment				x																																					
Trial registration				x																																					
Order study drugs		x																																							
Vaccine shipment				x																																					
Steering committee meeting	x					x												X												x											x	
IDMC meeting						x												X												x											x	
Census and consenting of children				x	x																																					
Randomisation					x																																					
Printing IDcards, labels (vaccine & drugs)					x																																					
Vaccination						x	x	x													x											x									
Administration of SMC												x	x	x	x									x	x	x	x									x	x	x	x			
Health facility based surveillance of malaria morbidity and mortality					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	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	x	x	x	x	x	x		
End of transmission surveys																x												x												x		
Repeat household census																		X												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	x	x	x	x	x	x	
PCR assays																		X	x										x	x											x	x	

Serology																																										
GCP monitoring					x							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	x	x	x	x	
Blinded analysis & report for committees																	x												x													
Locking of data																																									x	
Data analysis																																									xx	xx
Dissemination																																										x

22 APPENDICES

Appendix 1: Definitions of adverse and serious adverse events and reporting schedule

a. Definition of an adverse event and serious adverse event

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

a. results in death,
b. results in admission to hospital,
c. is life-threatening (the child was at risk of death at the time of the adverse event),
d. 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: Patients 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:

- Completely recovered : The child has fully recovered with no observable residual effects
- Not yet completely recovered : The child’s condition has improved, but still has some residual effects
- Deterioration : The child’s overall condition has worsened
- Permanent damage : The AE has resulted in a permanent impairment
- Death : The child died due to the AE
- Ongoing : The AE remains the same as at onset
- 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 and provided to the DSMB and GSK as requested. Any SAE potentially related to the vaccine or drug administration will be reported to the DSMB and institutional Ethics Committees within 72 hours and GSK within 96 hours.

Appendix 2. 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)

Morven Roberts MRC Head Office, London, (donor representative)

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

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

Data, Safety and Monitoring Board

Members of the Data Safety and Monitoring Board are -

Sheick Oumar Coulibaly, University de Ougadougou, Burkina Faso and WHO, Brazzaville.

Umberto D'Alessandro, MRC Unit, The Gambia.

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

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

Malcolm Molyneux, MLW Research Programme, College of Medicine, Blantyre, Malawi.

FINAL

April 2020

STUDY PROTOCOL

A PHASE IIIB COMPARATIVE TRIAL OF SEASONAL VACCINATION WITH THE MALARIA VACCINE RTS,S/AS01, SEASONAL MALARIA CHEMOPREVENTION AND OF THE TWO INTERVENTIONS COMBINED.

Study Identification number	207284 (MALARIA-099)
Brief Title:	RTS,S/AS01 and SMC Trial
Protocol Version & date:	**Final April 2020**
Sponsor:	London School of Hygiene & Tropical Medicine, Keppel Street, WC1E 7HT, London
Lead Principal Investigators:	Daniel Chandramohan & Brian Greenwood
Site Study Directors:	Jean Bosco Ouedraogo & Alassane Dicko

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The signatures below confirm agreement by the individuals authorised by the sponsor and principal participating institution at the clinical site that the study will be conducted in compliance with protocol version 4 dated 7 December 2017.

Study Director in Mali	NAME (PRINTED)	DATE
……	……	……
Study Director in Burkina Faso	NAME (PRINTED)	DATE
……	……	……
Study Director in the UK	NAME (PRINTED)	DATE

LIST OF ABBREVIATIONS

ACT	Artemisinin Combination Therapy
AQ	Amodiaquine
CSP	Circumsporozoite protein.
DSMB	Data Safety and Monitoring Board
EMA	European Medicines Agency
GCP	Good Clinical Practice
IRSS	Institut de Recherche en Sciences de la Santé
ITN	Insecticide-treated bednet
LDH	Lactic dehydrogenase
LSHTM	London School of Hygiene & Tropical Medicine
MMV	Medicines for Malaria Venture
MPAC	Malaria Policy Advisory Committee
MRTC	Malaria Research and Training Centre
NMCP	National Malaria Control Programme
SMC	Seasonal Malaria Chemoprevention
RDT	Rapid Diagnostic Test
SP	Sulphadoxine/pyrimethamine
WHO	World Health Organization

PROTOCOL SUMMARY

Title	A comparative trial of seasonal vaccination with the malaria vaccine RTS,S/AS01, seasonal malaria chemoprevention and of the two interventions combined.
Study objective	This trial seeks to determine whether – 1. Seasonal vaccination following priming with the RTS,S/AS01 malaria vaccine would be non-inferior to Seasonal Malaria Chemoprevention (SMC) with sulphadoxine/pyrimethamine (SP) + amodiaquine (AQ) in preventing malaria in children in the areas of the Sahel and sub-Sahel of Africa where malaria is still a major public health challenge and whether RTS,S/AS01 would be easier to deliver than SMC. 2. RTS,S/AS01 would provide additional, useful and cost effective protection against malaria if given together with SMC in areas with highly seasonal malaria transmission and reduce the risk of the emergence of resistance to the antimalarials used for SMC.
Study design	This is a double-blind, individually randomised trial with three study arms. The study groups are as follow: **Group1 (SMC)** **Group2 (RTSS)** **Group3 (RTSS+SMC)** **Year1**: April-June Rabies vaccine x 3 RTSS/AS01 x 3 RTSS/AS01 x 3 **Year1**: Aug-Nov SMC x 4 SMC placebo x 4 SMC x 4 **Year2**: Aug-Nov HepA vaccine x 1 RTSS/AS01 x 1 RTSS/AS01 x 1 SMC x 4 SMC placebo x 4 SMC x 4 **Year3**: Aug-Nov HepA vaccine x 1 RTSS/AS01 x 1 RTSS/AS01 x 1 SMC x 4 SMC placebo x 4 SMC x 4 Seasonal vaccination with either RTS,S/AS01 or control vaccine was undertaken approximately one month before the start of the malaria transmission season and the first administration of SMC.
Study site	The trial is being conducted in Houndé district, Burkina Faso and in Bougouni district, Mali, sites of a previous trial of the impact on mortality and hospital admissions of adding azithromycin to the SP + AQ used for SMC.
Study population	Children of either sex, 5-17 months of age on the scheduled date of administration of the first dose of RTS,S/AS01 vaccine (April 2017) who are living permanently in the study area were eligible for inclusion in the trial provided 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 received a malaria vaccine were excluded from the trial. Children known to have received SMC during the year prior to enrolment were not be excluded from the trial but distributed equally between study groups at the time of randomisation.
Group1 - SMC arm	Children in the control group were given three doses of rabies vaccine (April-June) and four rounds of SMC (SP+AQ) (August to November) at monthly intervals in year 1. In years 2 and 3 they received one dose of a control vaccine (Hepatitis A) in June and four rounds of SMC (August-November) at monthly intervals.
Group 2 – RTSS/ AS01 arm	Children in this group were given three doses of RTSS/AS01 vaccine (April-June) and four rounds of placebo-SMC (August to November) at monthly intervals in year 1. In years 2 and 3 they received one dose of a RTSS/AS)1 vaccine in June and four rounds of placebo-SMC (August-November) at monthly intervals.
Group 3 – SMC + RTS,S/AS01 arm	Children in the intervention group two were given three doses of RTS,S/AS01 vaccine (April-June) and four rounds of SMC in year 1 (August to November) at monthly intervals. In years 2 and 3 they were given one dose of RTS/AS01 vaccine (June) and four rounds of SMC (August-November) at monthly intervals.
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 per µl or more.
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. This activity to cease for the final 3 months of the study, April – June 2020 due to the COVID-19 outbreak.

	d. The prevalence of malaria parasitaemia, including gametocytaemia, moderate and severe anaemia and malnutrition at the end of the malaria transmission season. e. Serious adverse events (SAEs), including any deaths, occurring at any time during the study with special reference to any cases of meningitis and cerebral malaria (WHO case definition). f. Anti-CSP antibody concentrations obtained before and after priming and before and after each booster dose, determined in a sub-sample of children. g. The presence of molecular markers of resistance to SP and AQ in parasite positive samples collected at the final l cross-sectional survey h. The 28-day treatment outcome in children with asymptomatic malaria parasitaemia treated with SP+AQ. Secondary end-point (b) will be important for the economic evaluation of the interventions. Particular attention will be paid to the occurrence of any cases of meningitis and cerebral malaria [end-point (e)] as an increase in the incidence of these conditions was identified as a potential safety signal in the phase 3 RTS,S/AS01 trial. The trial will not be large enough to measure an impact on mortality but all deaths will be recorded and investigated. Evaluation of the clinical and immunological response to a fifth dose of RTS,S/AS01 is highlighted as an important research objective by the WHO.
Sample size	Approximately 3000 children have been recruited in Burkina Faso and in Mali (total 6,000) and these children will be followed for three years.
Study duration	October 2016 – June 2020

TABLE OF CONTENTS

1 BACKGROUND AND RATIONALE

The RTS,S/AS01 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 induces a strong antibody response to the *P. falciparum* CSP and high titres of anti-CSP antibody are associated with protection [2]. Following a long process of development, a phase 3 study of RTS,S/AS01 conducted in 15,439 children in 7 countries in Africa showed that three doses of RTS,S/AS01 given with a one month interval between doses, followed by a fourth dose 18 months post 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 given to infants at the age of 6-12 weeks [3]. RTS,S/AS01 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 [4]. 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, 4]. In July 2015, the European Medicines Agency reviewed efficacy and safety data on RTS.S/AS01 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 [5]. These included the pilot implementation of RTS,S/AS01 in children aged 5-17 months in 3-5 settings with moderate-to-high malaria transmission intensity, with a preference for areas where SMC is not being delivered, and evaluation of alternative approaches to deployment of the vaccine. Recent evidence [6] from challenge studies conducted in American adult volunteers suggests that a higher level of protection can be obtained when the third dose of the priming schedule is reduced to one fifth of the usual amount and delayed until approximately 6 months post dose 2, and when a reduced dose is used for boosting. In these studies, a vaccine efficacy of 86% was achieved three weeks following priming and 90% efficacy following boosting with a fractional dose. This encouraging result is now being followed in further studies.

SMC involves monthly administration of an antimalarial drug or drug combination in a full therapeutic course to children on three of four occasions during the period of highest risk of malaria infection. Studies undertaken in several countries in West Africa, including Burkina Faso and Mali, have shown 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% [7-9]. 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 [10]. Recent studies have defined the areas where SMC would be an appropriate intervention based on the seasonality and incidence of malaria [11]. These include most of the Sahel and sub-Sahel, population approximately 200 million, and possibly other areas in southern and eastern Africa. 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. 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 through the UNITAID supported SMC ACCESS programme and the support of other major donor organisations. 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).

SMC is effective but its delivery is demanding on the recipient and provider, requiring four contacts each malaria transmission season if anti-malarials are given to mothers to administer at home and 12 contacts if directly observed treatment is employed. In addition, SMC is threatened by the emergence of resistance to SP and AQ and there are currently no other combinations of licensed antimalarials that could be used to replace them. It is likely to be 5-10 years before novel antimalarials under development could be deployed for SMC. In contrast to SMC, seasonal vaccination with RTS,S/AS01 would require only one visit each transmission season after priming. RTS,S/AS01 may be a little less effective than SMC during the malaria transmission season but this may be balanced by provision of protection during the dry season, when some malaria transmission still occurs and when SMC would provide no benefit. There is, therefore, a need for a comparative study of these two interventions. In some areas where SMC is currently being deployed, and other

malaria control interventions such as long-lasting insecticide treated nets are used widely, the incidence of malaria in young children remains high (0.4 episodes per year in children under the age of five years in SMC recipients in Burkina Faso). Thus, determining whether RTS,S/AS01 would provide added, useful protection to SMC in such situations is also important. It might also be able to protect some children who, because of side effects, are unable or unwilling to take SMC.

Although the EMA has given a positive opinion on RTS,S/AS01, it is not yet certain how this partially effective malaria vaccine can be used most effectively [12]. Three, large-scale pilot implementation studies are now underway in Ghana, Kenya and Malawi. The WHO recommendations on RTS,S/AS01 indicate the need for research on alternative approaches to the delivery of this vaccine [13]. Exploration of the potential of the vaccine to prevent seasonal malaria, taking advantage of its high but rapidly waning efficacy, meets this recommendation and is, therefore, timely.

2 STUDY OBJECTIVES

The trial seeks to determine whether –

a. Seasonal vaccination following priming with the RTS,S/AS01 malaria vaccine would be non-inferior to SMC SP + AQ in preventing malaria in children in the areas of the Sahel and sub-Sahel of Africa where malaria is still a major public health challenge and whether it would be easier to deliver.
b. RTS,S/AS01 would provide additional, useful and cost effective protection against malaria if given together with SMC in areas with highly seasonal malaria transmission and reduce the risk of the emergence of resistance to the antimalarials used for SMC.

3 STUDY AREA

The trial will be conducted in Houndé health district, Burkina Faso and in Bougouni Koulikoro 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 IRSS. The study site in Mali is the district of Bougouni in the region of Sikasso, Mali, 150 km south of Bamako where the MRTC is based.

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

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 *Plasmodium 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 2015 was 53% in Bugouni and 62% in Houndé. The main malaria vector in each study area is *Anopheles gambiae* ss. A high proportion of children sleep under an ITN in Bougouni (96%) but the percentage is less in Houndé. 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. During the SMC + AZ trial the incidence of clinically suspected meningitis in children aged 3 - 59 months in Bougouni was approximately 0.11 per 1,000 per year and that of cerebral malaria 0.88 per 1,000 per year.

During 2014-2017, Houndé and Bougouni districts were the sites of a trial, involving approximately 20,000 children aged 3-59 months, which evaluated the impact of adding azithromycin to the SP +

AQ used for SMC in preventing overall mortality and hospital admissions with non-traumatic illnesses. The trial found that addition of azithromycin to the antimalarials used for SMC did not reduce the incidence of death or admission to hospital with a condition not due to trauma, its primary end-point, although it led to a modest reduction in the incidence of clinical attendances with respiratory, gastrointestinal or skin infections [14]. This trial finished at the end of 2016 allowing a smooth transition to the new trial, incorporating many of the well trained staff who had conducted the previous study. Field laboratories, which are equipped to undertake parasitological and haematological investigations, have been established at each district hospital and efficient data management systems set up.

In Mali, vaccines are stored at MRTC, Bamako, Mali. The MRTC has a long history (> 12 years) of testing vaccines including collaboration with GSK, the WRAIR, the NIAID/NIH and Sanaria. 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. In Burkina Faso, vaccines are stored at IRSS, in Bobo-Dioulasso where there is a dedicated storage room for drugs that 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 average with temperature and humidity controllers.

4 COMMUNITY SENSITISATION

The objectives of the study and the way in which it would be conducted were discussed with staff of the national malaria control progammes (NMCPs) and expanded infant immunisation programmes (EPIs) in both Burkina Faso and Mali and their support for the trial obtained in principle. These discussions have been continued. Community approval was sought through meetings with leaders of the study communities and through open meetings held in the study communities. Community leaders were consulted prior to the start of the intervention on the best ways of achieving high compliance with vaccination, drug delivery and follow-up.

5 TRIAL POPULATION

After obtaining permission from the community leaders for the trial, a household census was conducted in February – March 2017 and all households within the study areas with children 5-17 months of age on April 1st 2017 were enumerated. At the census, a preliminary screening of potentially eligible children was undertaken. Potentially eligible children and their caretakers were visited again and written informed consent was obtained from their caretakers for their inclusion in the trial before the administration of the first dose of study vaccines. Children entered into the trial were assigned a unique ID number and their demographic data (date of birth and/or age, and gender), use of insecticide treated nets (ITN) and history of receiving SMC during the last transmission season were collected. The census data was updated in April/May 2018 and 2019 prior to the administration of the booster doses of vaccine. Eligible children were allocated randomly to one of the three study arms in permuted blocks of 12 using standard randomisation procedures (table). Children who had a history of receiving SMC in the previous years were distributed equally between the three study groups

A child was eligible for inclusion in the trial if -

a. The child was a permanent resident of the study area and likely to remain a resident for the duration of the trial.
b. The child was 5 - 17 months of age at the time of first vaccination.
c. A parent or legally recognised guardian provided informed consent for the child to join the trial.

A child was ineligible for inclusion in the trial if -

a. The child was a transient resident in the study area.
b. The child was in care.
c. The age of the child was outside the stipulated range.
d. The child had a history of an adverse reaction to SP or AQ.
e. The child had a serious underlying illness, including known HIV infection, unless this was well controlled by treatment, or severe malnutrition (weight for age or mid arm circumference Z scores < 3 SD).
f. The child was known to have an immune deficiency disease or was receiving an immunosuppressive drug.

g. The child had previously received a malaria vaccine.

h. The child was enrolled in another malaria intervention trial.

i. The parents or guardians did not provide informed consent.

Table. Study groups

	Group1 (SMC alone)	**Group2 (RTS,S alone)**	**Group3 (RTS,S+SMC)**
Year1: April-June	Rabies vaccine x 3	RTSS/AS01 x 3	RTSS/AS01 x 3
Aug-Nov	SMC x 4	SMC placebo x 4	SMC x 4
Year2: Aug-Nov	HepA vaccine x 1	RTSS/AS01 x 1	RTSS/AS01 x 1
	SMC x 4	SMC placebo x 4	SMC x 4
Year3: Aug-Nov	HepA vaccine x 1	RTSS/AS01 x 1	RTSS/AS01 x 1
	SMC x 4	SMC placebo x 4	SMC x 4

SMC or placebo is given at monthly intervals on four occasions during the malaria transmission season.

6 SAMPLE SIZE

Approximately 3,000 children have been recruited in Burkina Faso and a similar number in Mali (total 6,000) and these children will be followed for three years. A low dropout rate of around 5 % per year (15% overall) is anticipated based on findings from the previous SMC+AZ study, although it is possible that the dropout rate may be higher during a vaccine trial as a vaccine trial has not been conducted previously in the study districts. Since the relative efficacy of SMC and RTS,S/AS01 is uncertain, the conservative approach of recruiting equal numbers of children into each arm of the study (2,000 per arm) has been followed. Results obtained over a period of three years of observation in the two study sites will be combined for the primary analysis of the incidence of clinical malaria. An analysis will also be undertaken of efficacy in each year of the trial to estimate the efficacy of administration of a booster dose. It is not anticipated that a formal unblinded interim analysis will be undertaken although a preliminary analysis by an independent statistician will be undertaken early in 2020 to provide guidance as to whether the trial should be extended.

During the SMC study in the trial sites, the incidence of clinical episodes of malaria during the first year of the study (2014) was 442 and 382 per 1000 children in Mali and in Burkina Faso respectively. Complete morbidity data was not available for the second year of the study (2016). For the sample size calculations, a conservative incidence rate of 300 cases per 1000 children over a calendar year was assumed. Using a two sided 95% confidence interval, a three-arm study which enrolled 2000 children in each arm (1000 per arm per centre) would have, for the non-inferiority comparisons, 90% power to exclude a relative difference in incidence between RTS,S/AS01 and SMC given alone of 16.1% over the three-year study period and of 28.6% or more for each individual year. The study would also have 80% power to exclude a relative difference in incidence of 13.9% over the whole study period and of 24.7% in each year. The differences that could be detected will be smaller if, as suspected, the incidence of clinical malaria is greater than 300 cases per 1,000 children per year. For the superiority comparisons of the combined interventions with SMC alone, using a two-sided 95% confidence interval, the study would have 90% power to detect a difference greater than 11.1% over the three years of the study and of 19.2% in each year.

For the analysis of the serological response to RTS,S/AS01, comparisons will be made between mean anti-CSP antibody titres pre and post the primary series of vaccination and before and after the two subsequent booster doses. Based on the standard deviations in antibody titres observed in children enrolled in the RTS,S/AS01 phase 2 and phase 3 trials, inclusion of around 160 individuals in each group (pre and post vaccination at each of the three time points) will give a study with approximately 80% power to detect a difference of 25% - 30% in mean titre between children who receive RTS,S/AS01 with or without co-administration of SMC.

7 THE INTERVENTIONS

RTS,S/AS01 vaccine: Three doses of RTS,S/AS01 were given to children allocated to the RTSS or RTSS+SMC groups at approximately monthly intervals (window 3-8 weeks) in April – June 2017 before the SMC administration period followed by a fourth and fifth dose at the beginning of the 2018 and 2019 malaria transmission seasons. A decision was made by the trial's steering committee not to use a fractional dose for the booster immunisation.

The efficacy of RTS,S/AS01 against both severe and uncomplicated malaria in children vaccinated at the ages of 5 - 17 months and subsequently given a booster dose has been assessed by both the

EMA and WHO to outweigh the vaccines side effects. The vaccine causes 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 common 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 issue related to administration of RTS,S/AS01 is the unexplained, statistically significant increase in meningitis observed in children given the vaccine at the age of 5-17 months during the phase 3 trial (about 4 per 1,000 during a four-year period of follow up); this was not observed when the vaccine was given at the age of 6-12 weeks.

Meningitis was caused by a variety of organisms and did not show any temporal relationship to vaccination and more than 40% of cases occurred at one centre in Malawi. The EMA concluded that the increase in cases of meningitis was probably a chance finding and that the benefits of the vaccine exceeded any safety issues. However, the agency recommended further evaluation of the incidence of meningitis in vaccine recipients when RTS,S/AS01 was deployed and this will be done in this study. Any study children admitted to hospital with suspected meningitis are being investigated as fully as possible and CSF samples obtained for microbiological diagnosis by PCR. In addition, there was a suggestion that the proportion of cases of severe malaria which were classified as cerebral malaria was increased in the RTS,S/AS01 recipients, although the incidence of severe malaria overall was reduced. Therefore, the incidence of cases of severe malaria meeting the WHO definition is monitored carefully.

RTS,S/AS01 malaria vaccine should not be administered to subjects with known hypersensitivity to any component of the vaccine, to a previous dose of RTS,S/AS01 $_{E}$ malaria vaccine or who is known to be hypersensitive to hepatitis B vaccine.

Control vaccines: Rabies vaccine has been used as the control vaccine for the primary series of vaccinations during the dry season in year 1 (2017) for those allocated to the SMC group. The rabies vaccine used was a licensed, WHO approved vaccine *Rabipur*^{R}*,* previously produced by Novartis but now by GSK. This vaccine is produced in chick embryo cells and contains polygeline and residues of chicken proteins, and it may contain traces of neomycin, chlortetracycline and amphotericin and it is, therefore, contraindicated in subjects with a history of a severe hypersensitivity to any of the

ingredients in the vaccine. Minor local and systemic reactions are common (>1:100, <1.10) after vaccination with rabies vaccine and neurological complications including Guillain Barré syndrome have been described but are very rare (<1:10.000).

Hepatitis A vaccine (HAVRIX^{R}), a licensed inactivated hepatitis A vaccine produced by GSK, is being used for the control booster dose in years 2 and 3. HAVRIX^{R} is contraindicated in subjects who have had an allergic reaction to prior administration of the vaccine or who are sensitive to neomycin. This vaccine may also cause minor local and systemic reactions ((>1:100, <1.10). Severe reactions, including anaphylaxis have been described but are very rare (<1.10,000).

Seasonal malaria chemoprevention: Four courses of SMC with SP+AQ will be given at monthly intervals during the malaria transmission season in line with WHO's recommendation and national policy [8]. 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. Infants receive half of these doses. SP and AQ and matching placebo have been obtained from Guilin Pharamceuticals, Shanghai, Co), a GMP certified supplier. All treatments are given under observation.

Approximately 5 million children have received SMC with SP + AQ during the 2015 rainy season across the Sahel and sub-Sahel (http://www.malariaconsortium.org/pages/access-smc.htm) and this drug combination has been shown to be remarkably safe [10]. 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. The possibility that SMC with SP+AQ might induce resistance to these drugs in *P. falciparum* has been investigated in a number of trials of SMC. Selection of parasites carrying mutations which confer resistance to pyrimethamine or sulphadoxine has been demonstrated in some but not all studies [7]. However, because the prevalence of parasitaemia in children who received SMC was substantially less than in the control group, the total number of parasites carrying resistance markers was less in children who had received SMC than in control children. Extensive use of SP for intermittent preventive treatment in pregnant women has not accelerated resistance to SP in West Africa. Preliminary analysis of samples obtained from children in an SMC trial in the study sites at the end of 2014 did not show levels of mutations in *dhfr* and *dhp*s genes

likely to be associated with *in vivo* resistance to SP. The potential risk of inducing resistance to SP or AQ by SMC was reviewed carefully by a WHO Technical Expert Group and WHO's Malaria Policy Advisory Group and considered to be an acceptable risk in light of the major benefits conveyed by the intervention. Malaria parasites isolated at the end of the transmissions season will be tested for resistance markers to SP.

8 IMPLEMENTATION OF THE INTERVENTIONS

Vaccination. RTS,S/AS01 is provided in a two-doses glass vial of lyophilized RTS,S antigen to be reconstituted with a two-dose glass vial of AS01 Adjuvant System. The final product for administration is prepared by reconstitution of the lyophilized antigen with the liquid adjuvant to deliver two doses (1.0 ml). A single dose consists of 0.5 ml of RTS,S/AS01 final preparation. All vials of vaccine provided in this study are intended for single use only. After reconstitution the vaccine was administered by slow IM injection, using a fresh 25G needle with length of one inch (25 mm), in the left deltoid. Vaccine was injected within four hours of reconstitution (storage at +2°C to +8°C). Syringes containing RTS,S/AS01 or the control vaccine were prepared by a pharmacist who took no other part in the trial. Loading of syringes with vaccines and masking with tape to blind the person administering the vaccine as to its nature was done by a person who took no further part in the trial. Vaccines were administered by a nurse or other category of health worker trained to give vaccines.

Rabies vaccine, 1.0 ml in volume was given by intramuscular injection. The paediatric dose of HAVRIX ^{R} is 0.5 ml and it was also administered by intramuscular injection.

SMC. SMC drugs are 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 four cycles of treatment required for one full malaria transmission season appropriate for the child's age. Treatment with each dose of SMC is given by trained, paid volunteers at a central point in each study community under observation. Study children have been given an 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.

All children were given an ITN at the commencement of the 2017 rainy season.

9 CONTRAINDICATIONS TO SUBSEQUENT VACCINATION

Contraindications to administration of a further dose of vaccine in any child included –

a. Anaphylaxis following administration of the first dose of the vaccine.

b. Any condition that in the judgment of the investigator would make intramuscular injection unsafe.

c. The occurrence of a new adverse event (AE) or the exacerbation of an existing AE that, in the opinion of the investigator, exposed the subject to an unacceptable risk from subsequent vaccination.

d. An acute disease and/or fever at the time of vaccination. Fever is defined as temperature ≥ 37.5°C/99.5°F for oral, axillary or tympanic measurements, or ≥ 38.0°C/100.4°F for rectal temperature. Subjects whose fever resolved with treatment were vaccinated provided that revaccination fell within the stipulated time period.

Subjects with a minor illness (such as mild diarrhea, mild upper respiratory infection) without fever were vaccinated.

10 FOLLOW-UP AND MEASUREMENT OF OUTCOMES

The following follow-up procedures required to measure the study outcomes are being undertaken

a. ***Passive surveillance for cases of 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 are responsible for identifying and documenting all cases of malaria who present to these health facilities. In Mali, cases of uncomplicated malaria may also be treated by community health workers who have been taught to diagnose malaria with a RDT and to treat RDT positive cases, and these cases are also being recorded if they meet the inclusion criteria. Cases of suspected malaria (fever, history of fever within 48 hours or any other symptom/sign suggestive of malaria) are tested with a RDT and managed on the basis of their RDT result and blood films

and filter paper strips are obtained from all these cases for subsequent confirmation of the diagnosis.

b. ***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 has an RDT done and those who are positive are treated with a full course of an ACT. This activity to cease for the final 3 months of the study, April – June 2020 due to the COVID-19 outbreak.

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

d. ***Serious Adverse Events***. Project staff based at the district hospitals are responsible for the identification of any child in the trial admitted to hospital and 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. Definitions for meningitis and cerebral malaria, currently being developed by WHO for use in the pilot RTS,S/AS01 implementation trials are being used. 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 is done.

   Surveillance of all children will be maintained throughout the study period for any Serious Adverse Events (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 are 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 are tabulated in a blinded fashion and provided to the DSMB and to GSK at the times requested by the DSMB. Details of definitions of adverse events and SAEs and of reporting procedures are described in appendix 1.

e. ***Immune response to the vaccine***. Blood samples (2ml) have been collected from approximately 160 children in each of the groups (80 per country) who received RTS,S/AS01 prior to administration of the first dose of vaccine and one month after third dose of the primary series of vaccination, and then in year 2 and 3 before and one month after administration of the fourth and fifth doses of vaccine for measurement of anti-CSP antibodies.

f. ***Drug resistance****:* Dried blood spots from children who have malaria parasitaemia detected by microscopy at each annual cross-sectional survey will be used for analysis of molecular markers of resistance to SP and AQ at MRTC, Bamako.

g. ***In vivo measurement of drug sensitivity to SP+AQ***: At the end of the 2019 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, an important factor in evaluation of the outcome of the trial. 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, from children sequentially until the required number of RDT positive children to meet the sample size at each site has been recruited. Blood smears will be examined the same day, or as soon as possible afterwards, for any child who has positive RDT test. Children who are parasitaemic but 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 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 (AL), the national first line treatment for uncomplicated malaria. Infections after day 7 (late parasitological failures) will be investigated to determine if the recurrent infection was due to a reinfection or a recrudescence. DNA from dried blood spots will be used for molecular characterisation of the parasite using polymorphisms in the *msp1, msp2* and CA1 polymerase genes.

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 155.. We anticipate that about 7.5% of 3000 children will carry malaria parasite at end transmission season in 2019, leading to 225 potentially eligible children per site.

h. ***Malaria endemicity in the study area and the trend in the distribution of molecular SP resistance markers*:** A survey of schoolchildren was conducted in Year 2 and will be repeated in Year 3 of the trial to assess the malaria parasite prevalence at the end of the transmission and to monitor the trend in the distribution of molecular SP resistance markers. 200 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 parasitemia at the end of each malaria transmissions season and to determine the overall level of malaria transmission and (ii) by PCR to assess the trend in the distribution of molecular markers of resistance to SP in the study area during the period of the trial. The sample size for this study is based on the following assumptions: (1) the prevalence of malaria parasite will be 50% and intra-cluster correlation coefficient (ICC) will be 0.1 (this is based on the observations from the surveys done in 2014, 2015 and 2016 as part of the recently completed seasonal malaria chemoprevention plus azithromycin trial); (2) the design effect will be 1.9 if the cluster size 10 children; (3) refusal to take part in the survey will be <5%; (3) the acceptable 95% precision of the prevalence will be +/-10%.

11 LABORATORY PROCEDURES

a. ***Detection of malaria.*** A histidine rich protein (HRP2) based Rapid Diagnostic Test (RDT) is be used for the initial diagnosis of malaria and to guide treatment. Blood films collected at the same time are read subsequently by two microscopists. All slides are read twice by two separate readers following the guidelines developed for the phase 3 RTS.S/AS01 trial [15]. Slides which are judged to be discordant for either positivity or parasite density are read by a third reader. For slides with high or medium density parasitaemia (> 400/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 applies. 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.

b. ***Detection of markers of resistance to SP.*** Parasite DNA is extracted from dried blood spots and nested PCR reactions are 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 [16-18]. 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.

c. ***Measurement of haemoglobin concentration.*** Haemoglobin concentration is measured colorimetrically using a Hemocue colorimeter (Hemocue AB, Angelholm, Sweden).

d. ***Measurement of anti-CSP concentration.*** Antibodies to CSP will be measured by a standardised ELISA at the University of Ghent in the laboratory of Professor Leroux-Roels as used in many previous trials of RTS,S/AS01.

12 SOCIO-ECONOMIC STUDIES

Objectives. The objectives of the socio-economic component of the trial are to determine:

a. The cost-effectiveness of a) RTS, S vaccine versus SMC and b) the combination of both versus SMC alone.
b. The acceptability of the two interventions (separately and combined) to the health care deliverers and to the study communities.
c. The feasibility of introducing two malaria control strategies simultaneously from the health system perspective.

Economic Evaluation. Data on the costs of a clinical case of malaria and of a hospital admission with malaria to both the health care deliverers and recipients have already been collected in the two study areas during the course of the SMC+AZ trial and this information will be updated. The costs of adverse events, in particular of meningitis, will be estimated using published literature. Information on the costs of delivery of SMC outside an intervention study has also been gathered by the SMC

ACCESS team and this will facilitate determination of the costs of this intervention. The costs of adding RTS,S/AS01 to the routine vaccination programme will require specific study through observations, key stakeholder interviews and review of relevant documents and files. The delivery costs will be estimated through a combination of a step-down and ingredients-approach costing methodology. A cost effectiveness analysis of the two interventions and their use in combination will incorporate the results from the trial, the costs collected during this study as well as during previous studies and then model the cost effectiveness of the interventions from a societal perspective in a decision tree model. The final outcomes used in the model will be costs/DALYs averted. The costs of scaling up the optimum intervention at regional/ national level will be investigated.

Acceptability. The acceptability of the two interventions to the health care deliverers and the preference of study communities for each of the interventions will be investigated through focus group discussions involving both the families of trial participants and those involved in administration of the trial interventions.

Feasibility. Investigating the feasibility of delivery of RTS,S/AS01 outside the routine EPI programme will be a major objective of the large pilot studies being planned by WHO. Experience gained on the logistic challenges posed to the national immunisation programme of delivering RTS,S/AS01 as a seasonal vaccine will be shared with both the national immunisation programmes in Burkina Faso and Mali and also with WHO.

13 DATA MANAGEMENT

To ensure data is fit for purpose, questionnaires were tested prior to use and staff received training prior to and at regular intervals during data collection. Data are managed using the DataFax system which was set up for the SMC + AZ trial and which is working well. This system is based on electronic transfer of the CRFs from the research sites where the data are automatically captured and validated. The MRTC data management team are responsible for the training and support of the IRSS data management team, with overall support from the NIAID/NIH central data management team. Data is uploaded to the DataFax system at the earliest opportunity to enable queries to be validated and issues resolved as soon as possible after data collection. Automatic checks are performed on clinical and laboratory forms to ensure they are complete and contain valid responses prior to uploading by the local data managers at both sites.

An experienced independent GCP monitor (Raouf Osseni) monitors the trial to ensure the quality of the data collected and that GCP standards are met. The monitor conducted a trial initiation visit, and at least one additional visit each year. He will also conduct a 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 STUDY OUTCOMES

***Primary outcome*:** The primary outcome measure of the trial is the incidence of clinical 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 outcomes: Secondary outcomes include: -

a. Blood slide or rapid diagnostic test (RDT) positive malaria defined as a clinical episode 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 parasitemia) or a positive RDT.
b. Hospital admissions with malaria, including cases of severe malaria which meet WHO criteria for a diagnosis of severe malaria.
c. The prevalence of malaria infection (symptomatic or asymptomatic parasitaemia) 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. Serious adverse events (SAEs), including any deaths, occurring at any time during the study with special reference to any cases of meningitis, cerebral malaria or immune deficiency illness.
f. Anti-CSP concentrations obtained after priming and after each booster dose, determined in a sub-sample of children.
g. Comparison of the anti-CSP concentrations among children who received co-administration of SMC and RTS,S/AS01 versus those who received RTS,S/AS01 alone.

h. The presence of molecular markers of resistance to SP and AQ in parasite positive samples collected at the final cross-sectional survey.
i. The 28-day treatment response of subjects with asymptomatic *P. falciparum* malaria to treatment with SP+AQ.

Particular attention will be paid to the occurrence of any cases of meningitis as this was identified as a potential safety signal in the phase 3 RTS,S/AS01 trial. The trial will not be large enough to measure an impact on mortality but all deaths will be recorded and investigated, including by verbal autopsy if these occur at home. Safety data are provided to GSK in the format requested by the company. Evaluation of the clinical and immunological response to a fifth dose of RTS,S/AS01 is highlighted as an important research objective by the WHO.

15 ANALYSIS

The primary endpoint of incidence of all episodes of clinical malaria over the study period will be analysed using Cox regression models with a robust standard error to account for clustering of episodes within individuals (i.e. the Andersen-Gill extension of the Cox model). This will estimate the total effect of the interventions. The proportion of children in each group who experience one episode of malaria will be compared as a secondary outcome using Kaplan-Meier estimates: evidence for provision of complete protection through the combination of SMC and vaccination each year will be explored using recently published methods [19, 20]. For the comparison of RTS,S/AS01 plus SMC to the other interventions, a standard superiority comparison will be performed, calculating two-sided 95% confidence intervals for the hazard ratio. For the non-inferiority comparison of RTS,S/AS01 to SMC, the two-sided 95% confidence interval for the hazard ratio will be compared to a pre-specified non-inferiority margin. The trial is powered to have more than 80% power to exclude a difference of 15% in the incidence of clinical malaria over the study period, a difference considered to be clinically important. Demonstration of a smaller difference would require a substantially larger trial and is considered to be not clinically relevant. If the lower limit of the 95% does not overlap zero (i.e. it is clear that RTS,S is superior to SMC) then a superiority comparison will be performed as for the combined intervention.

A formal analysis plan will be prepared and approved by the Data Safety Monitoring Board (DSMB) appointed for the trial before the study code is broken. 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 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 but the study is only powered to meet its primary and major secondary end-points if results from both countries are combined. Additional sub-analyses will include analysis by age, gender, bed net use during the transmission season, as determined by the history obtained at the cross-sectional surveys, and socio-economic status as determined by the educational level and occupation of the child's family.

A formal interim analysis will not be undertaken. However, a preliminary analysis of the key trial clinical trial end-points will be undertaken by an independent statistician early in 2020 in order to decide whether the trial should be continued until children reach the age of five years, the age at which SMC is no longer given.

16 ETHICS

Inclusion in the trial of an RTS,S/AS01 alone group even though SMC is recommended policy is justified on the grounds that RTS,S/AS01 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 has been obtained from the family or legally recognised guardian of each child entered into the trial. Ethical approval has been 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. 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 the district hospitals in the study areas.

17 TRIAL MANAGEMENT

The London School of Hygiene & Tropical Medicine acts as the main sponsor for the trial. Delegated responsibilities may 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 provides scientific oversight and holds regular meetings either through teleconferencing or face-face meetings .

A DSMB oversees the safety of subjects in the trial and a clinical trial monitor ensures that the trial is conducted to GCP standards. In order to ensure that children in the RTS,S/AS01 group are not put at any increased risk compared to children who receive SMC, the DSMB will monitor regularly the incidence of clinical cases of malaria in the three study groups. The DSMB has met on several occasions during the trial and at its meeting held in February2019 members of the board reviewed unblinded data on the occurrence of deaths, deaths due to malaria, hospital admissions overall and hospital admissions due to malaria in the three study groups and gave their permission for the trial to continue in 2019 retaining all three study groups. Membership of the trial steering committee and the DSMB is shown in appendix 2.
The trial management committee includes the LSHTM PIs, trial site PIs and the trial administrator. The trial management committee is responsible for overseeing the trial and its members communicate regularly by teleconferences.

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 trial has been registered on clinicaltrials.gov (NCT03143218).

18 ROLES OF THE INVESTIGATORS AND COLLABORATORS

The team from the IRSS, Burkina Faso which includes Jean Bosco Ouédraogo, Halidou Tinto and Issaka Zongo are 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 will be 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, Irene Kuepfer, Matthew Cairns, Paul Milligan, Karen Slater) provides epidemiological, statistical, administrative and financial management support. Silke Fernandes and

Kara Hanson from the LSHTM are responsible for the economic aspects of the study. A consultant may be recruited to assist the design of the acceptability and feasibility studies.

19 DISSEMINATION PLANS

Results from the trial will be presented at national and international conferences and in peer reviewed journals 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 in connection with the implementation of SMC and these links will facilitate the incorporation of RTS,S/AS01 vaccine into SMC/EPI programme if this is found to be a useful intervention. The study team has established good links with many other organisations involved in the delivery of SMC trials, including the SMC ACCESS programme coordinated by the Malaria Consortium and with the WHO staff responsible for conducting the RTS,S/AS01 implementation studies Thus, if it is found that RTS,S/AS01 vaccine is a useful replacement or addition to SMC regimens, routes have already been established through which this knowledge could be disseminated rapidly.

20 REFERENCES

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21 ACTIVITIES SCHEDULE

Activities	2016				2017												2018												2019												2020	
	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	J	F	M	A	M	J	J	A	S	O	N	D	J-M	A-J
Protocol submission for ethics approval	x																																									
Ethics and regulatory approval				x																																					
Staff recruitment				x																																					
Trial registration				x																																					
Order study drugs		x																																							
Vaccine shipment							x	x	x													x												x								
Steering committee meeting	x					x							x												x												x				x	
DSMB meeting						x					x		x						x									x							x				x	x	
Census and consenting of children							x	x																																		
Randomisation							x																																		
Printing IDcards, labels (vaccine & drugs)									x												x									x											
Vaccination								x	x	x												x												x							
Administration of SMC												x	x	x	x									x	x	x	x								x	x	x	x			
Health facility based surveillance of malaria morbidity and mortality								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	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		
End of transmission surveys																x												x												x		
Repeat household census																		x												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	x	x	
PCR assays																																								x	x	
Serology								x			x											x	x											x	x							
GCP monitoring					x							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	x	x	x	x	x	x	x	
Blinded analysis & report for committees																	x													x																
Locking of data																																												x		
Data analysis																																												xx	xx	
Dissemination																																													x	

22 APPENDICES

Appendix 1: Definitions of adverse and serious adverse events and reporting schedule

a. Definition of an adverse event and serious adverse event

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

a. results in death,
b. results in admission to hospital,
c. is life-threatening (the child was at risk of death at the time of the adverse event),
d. 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: Patients 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:

- Completely recovered : The child has fully recovered with no observable residual effects
- Not yet completely recovered : The child's condition has improved, but still has some residual effects
- Deterioration : The child's overall condition has worsened
- Permanent damage : The AE has resulted in a permanent impairment
- Death : The child died due to the AE
- Ongoing : The AE remains the same as at onset
- 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 and provided to the DSMB and GSK as requested. Any SAE potentially related to the vaccine or drug administration will be reported to the DSMB and institutional Ethics Committees within 72 hours and GSK within 96 hours.

Appendix 2. 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)

Morven Roberts MRC Head Office, London, (donor representative)

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

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

Data, Safety and Monitoring Board

Members of the Data Safety and Monitoring Board are -

Sheick Oumar Coulibaly, University de Ouagadougou, Burkina Faso and WHO, Brazzaville.

Umberto D'Alessandro, MRC Unit, The Gambia.

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

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

Malcolm Molyneux, MLW Research Programme, College of Medicine, Blantyre, Malawi.

RTS,S + SMC – Protocol amendments

Original Protocol dated December 2016 was approved by the LSHTM Ethics Committee on 5 January 2017.

Protocol Amendment 1 – May 2017

The following amendments to the protocol were approved by the LSHTM Ethics committee on 21 July 2017:

1. to include an interim, blinded analysis of the incidence of malaria in the three trial groups to be undertaken at the middle of the 2017 malaria transmission season as well as at the end to be reviewed by the DSMB;

2. to change the starting date for primary vaccination from February to April

3. to allow a window of 3-8 weeks between doses as opposed to the previous 'approximately one month' between doses

4. clarification that episodes of malaria that occur at least two weeks after the third dose of vaccine will contribute to the trial end-point.

Protocol Amendment 2 - December 2017

The following amendment to the protocol was approved by the LSTHM Ethics committee on 1 February 2018:

To continue the active surveillance during the dry season throughout the study period.

Protocol Amendment 3 – September 2018

The following amendment to the protocol was approved by the LSTHM Ethics committee on 26 October 2018:

To include a survey of schoolchildren aged 6-12 years to assess the malaria parasite prevalence at the end of the transmission season in Year 2 and Year 3 of the trial.

Protocol Amendment 4 – August 2019

The following amendments to the protocol was approved by the LSTHM Ethics committee on 18 October 2019

1. to include of a sub-study to measure the in vivo efficacy of the SMC drugs used for SMC

2. to include a preliminary analysis in Jan Feb 2020 at the request of the donors.

3. the decision to consider a fractionated dose was removed from the protocol

4. sub study to detect vaccine escape mutants dropped due to lack of funding.

Protocol amendment 5 - April 2020 (final protocol)

The following amendment to the protocol approved by the LSHTM Ethics Committee 7 April 2020

a) cessation of the home visits required for active detection of malaria infection during the remaining three months of the first phase of the study (April – June 2020) due to the COVID-19 outbreak.

RTS,S-SMC:

A comparative trial of seasonal vaccination with the RTS,S/AS01 malaria vaccine, seasonal malaria chemoprevention, and of the two interventions combined

Revisions to the Statistical Analysis Plan:

Revision Number	Date of Revision	Page Number(s)	Details

Study title
A comparative trial of seasonal vaccination with the malaria vaccine RTS,S/AS01, seasonal malaria chemoprevention, and of the two interventions combined.

Clinical trial registration
Clinicaltrials.gov Identifier: NCT03143218

Study duration
April 2017 – March 2020

Study site
The trial is being conducted in Houndé district, Burkina Faso and in Bougouni district, Mali, sites of a previous trial (NCT02211729), which evaluated the effectiveness of adding azithromycin to the drug combination used for seasonal malaria chemoprevention.

Study objectives
This trial seeks to determine

1. Whether seasonal vaccination with RTS,S/AS01 is 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 and SMC with SP+AQ) is superior to RTS,S/AS01 alone or SMC alone in preventing clinical malaria and other adverse outcomes.

These aspects are of equal priority.

Study groups
This is a double-blind, individually-randomised trial with three study arms.

The study groups, and abbreviated names used for ease of reference, are shown in the table below.

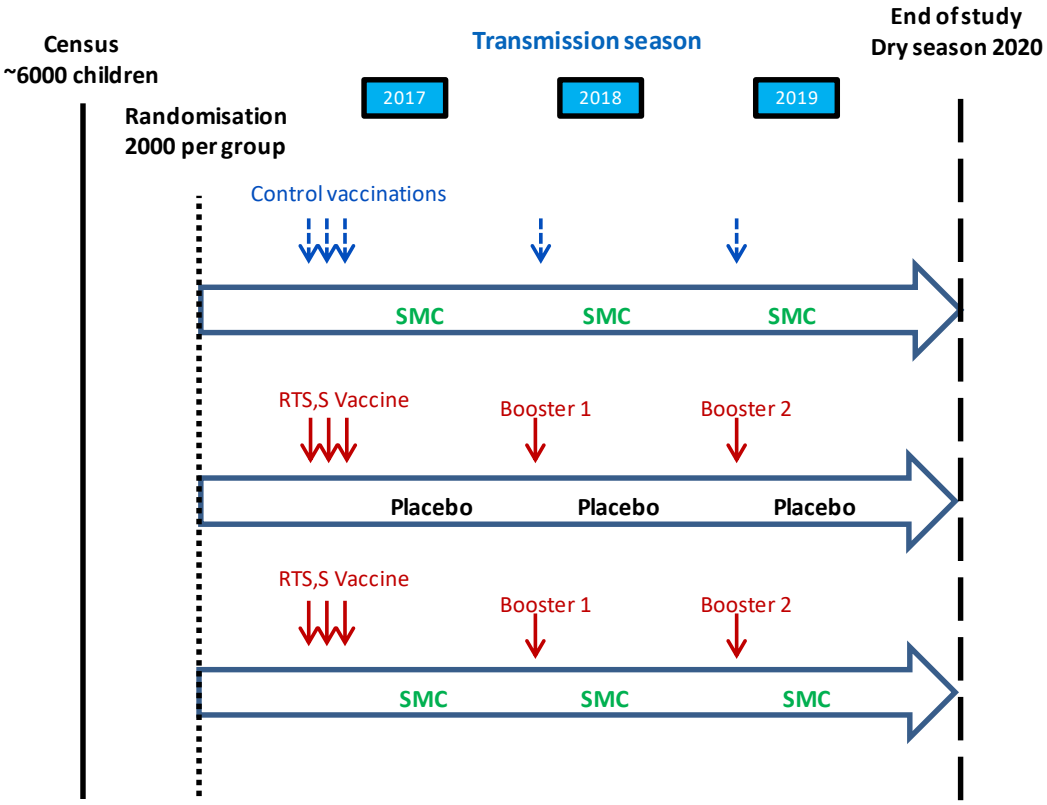
	Group		
	1) SMC 'SMC alone'	**2) RTS,S/AS01 'RTS,S alone'**	**3) RTS,S/AS01+SMC 'Combined group'**
April/May – June/July 2017	Rabies vaccine x 3	RTS,S/AS01 x 3	RTS,S/AS01 x 3
July/Aug – Oct/Nov 2017	SMC x 4	SMC placebo x 4	SMC x 4
June 2018	Hep A vaccine x 1	RTS,S/AS01 x 1	RTS,S/AS01 x 1
July-Oct 2018	SMC x 4	SMC placebo x 4	SMC x 4
June 2019	Hep A vaccine x 1	RTS,S/AS01 x 1	RTS,S/AS01 x 1
July-Oct 2019	SMC x 4	SMC placebo x 4	SMC x 4

Group 1 – SMC arm - 'SMC alone'
In 2017, children in the SMC group were given three doses of rabies vaccine 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.

Group 2 – RTSS/ AS01 arm – 'RTS,S alone'
In 2017, children in the RTS,S/AS01 group were given three doses of RTSS/AS01 vaccine and four rounds of placebo-SMC at monthly intervals. In 2018 and 2019, children received one dose of RTS,S/AS01 vaccine and four rounds of placebo-SMC at monthly intervals.

Group 3 – RTS,S/AS01+SMC arm – 'Combined arm'
In 2017, children in the combined group were given three doses of RTS,S/AS01 vaccine and four rounds of SMC with SP+AQ at monthly intervals. In 2018 and 2019, children received one dose of RTS,S/AS01 vaccine and four rounds of SMC with SP+AQ at monthly intervals.

Schematic of Key Study Activities



Precise timing of interventions
Administration of RTS,S/AS01 or control vaccine: 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 or control vaccine were given in the first two weeks of June.

Administration of SMC or placebo SMC: 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.

Study population

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.

Sample size and Study Design

Based on the calculations described below, the trial aimed to recruit approximately 3,000 children in Burkina Faso and 3,000 in Mali (total 6,000) who would be followed for three years. A low dropout rate of around 5% per year (15% overall) was anticipated based on findings from the previous trial conducted in the same study areas.

In Burkina Faso and Mali, 2777 and 3143 children, respectively, received the first dose of study vaccine.

Superiority comparison: The trial was designed to compare the two interventions combined with either used alone. The study is powered to i) assess the statistical evidence against the null hypothesis of no difference between the combined group and either the SMC alone or RTS,S alone group, and ii) estimate the efficacy of the combined group relative to the single intervention groups with a relatively high degree of precision. This latter aspect is important because if the combined intervention is to be used in practice, it is necessary to show that adding RTS,S/AS01 to SMC has a clinically significant benefit.

After two years of follow-up, incidence of clinical malaria, as defined below, was approximately 200 cases per 1000 child-years at risk. With approximately 2000 individuals in each arm, if the

incidence is at least 30% lower in the combined group, there is very high power over the three years of the study (close to 100%) to reject the null hypothesis of no difference between the combined group and the single treatment groups. If efficacy of the combined group relative to either of the other groups is at least 30%, there will be 90% power for the lower limit of the 95% confidence interval to exclude 15%, i.e. to address point ii) above, establishing that the protection from the combined group is at least 15% better than SMC or vaccination alone.

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 potential advantages of RTS,S over SMC in terms of ease of delivery and the potential to sustain high levels of coverage. A reduction in annual efficacy from 50% to 40% was considered the largest difference that would be likely to be acceptable. This translates to a 20% greater incidence in the RTS,S/AS01-alone arm compared to the SMC-alone arm. With the anticipated incidence rates, there is adequate power to reject a smaller margin, as detailed in the protocol. However, 20% was considered the largest difference that would be considered unimportant.

The study has 80% power to exclude, at the 2.5% significance level, a relative difference in the incidence of clinical malaria of 20% over the three year study period between the RTS,S/AS01 and SMC alone groups, if these two interventions were equally effective. Evidence of the effectiveness of the reference group – SMC alone - in these areas come from clinical trials in Mali and Burkina Faso in 2009 (1, 2), and more recently in case-control studies conducted in both countries in 2016. In the previous AZ-SMC trial in these two districts (3), the prevalence of molecular markers of resistance to SP and AQ were very low, and the curative efficacy of SP+AQ over 28 days was above 95% in both areas (Cairns et al., submitted).

Hypothesis testing 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.

For the non-inferiority comparison 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.

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 weekly and 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 burned to CD and a copy sent to the chair of the DSMB for archiving.

The randomisation codes will only be released 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.

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 temperature ≥ 37.5°C, or a history of fever within the past 48 hours), and a positive blood film, with a *P. falciparum* parasite density of 5,000 per µl or more. This cut-off has been used in previous studies of SMC in Burkina Faso and Mali, as well as 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. However, 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 the 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 surgery**

2.2.3 **Hospital admissions due to malaria.** Defined as hospital admissions where malaria was the primary diagnosis, supported by a positive blood smear, or positive RDT if no blood smear result was 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**

2.2.5 **Deaths due to any cause**

2.2.6 **Deaths due to any cause excluding external causes and surgery**

2.2.7 **Deaths due to malaria**. Defined as hospital admissions resulting in death, where malaria was recorded as the primary cause of death, and where parasitology results did not exclude malaria (i.e. a positive blood smear, if a slide result was available, or a positive RDT if no slide was done). Deaths in the community will also be included when malaria is assigned as the primary cause of death by verbal autopsy.

2.3 Outcomes measured at weekly surveys

A subset of children were selected to be sampled for parasitaemia during weekly surveys. Systematic random sampling, from lists sorted on age, was used to allocate children to be sampled in not more than one week per year, such that the sample of 24 children in each week,

in each country was balanced with respect to age and treatment group. Results from the weekly surveys will be analysed and presented separately for each malaria transmission season (2017, 2018 and 2019).

2.3.1 The prevalence of asexual stage *P. falciparum* infection of any density detected during the weekly home visits.
2.3.2 The prevalence of asexual stage *P. falciparum* infection with a density ≥ 5000 per ul detected during the weekly home visits.
2.3.3 The arithmetic mean *P. falciparum* parasite density, including samples which are parasite negatives as having a density of zero.

2.4 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 each malaria transmission season (2017, 2018 and 2019).

2.4.1 The prevalence of asexual stage *P. falciparum* infection of any density
2.4.2 The prevalence of asexual stage *P. falciparum* infection with density ≥ 5000 per ul
2.4.3 The prevalence of sexual stage *P. falciparum* infection (i.e. gametocytes)
2.4.4 The arithmetic mean *P. falciparum* parasite density, including samples which are parasite negatives as having a density of zero.
2.4.5 The prevalence of asexual stage infection of non-falciparum *Plasmodium* species.
2.4.6 The prevalence of sexual stage infection (i.e. gametocytes) of non-falciparum *Plasmodium* species.
2.4.7 The mean haemoglobin concentration in g/dL.
2.4.8 The prevalence of anaemia, defined as measured Hb < 10 g/dL.
2.4.9 The prevalence of moderate anaemia, defined as measured Hb < 7 g/dL.
2.4.10 The prevalence of severe anaemia, defined as measured Hb < 5 g/dL.

2.5 Other secondary outcomes

2.5.1 The prevalence of molecular markers of resistance to SP and AQ in children with *P. falciparum* infection, among samples collected at the final cross-sectional survey in December 2019. These 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.
2.5.2 The percentage of children with asymptomatic *P. falciparum* parasitaemia at the cross-sectional survey in December 2019, 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 (section 2.2), 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.

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 up to the 30th November 2019. A 95% confidence interval for the incidence rate of meningitis among children vaccinated with RTS,S/AS01 will be calculated.

The incidence of cerebral malaria among children vaccinated with RTS,S/AS01 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.

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. The subset of febrile convulsions that occurred within 7 days of vaccination will also be analysed.

An exploratory analysis will investigate if there is any evidence that RTS,S/AS01 increases mortality in girls. This will compare the incidence of deaths using Cox regression, with an interaction between a dummy variable indicating receipt of RTS,S/AS01 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 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.

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. 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 populations and person-years at risk

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 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 1) the date observation formally ended (31st March, 2020), 2) the date last seen if lost-to-follow-up (LTFU), 3) the date of permanent exit from the study area, or 4) 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).

As a secondary analysis, the primary outcome will also be analysed per protocol (PP). The PP population will be defined separately for each year of the study. Children who were seen at all scheduled vaccination contacts in a particular year (3 in 2017, 1 in 2018, 1 in 2019) 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 study drug administration but who did not receive SMC because they had malaria and were referred for treatment will be included in the per protocol analysis.

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

Trial profile

The trial profile will show 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. Reasons that children seen at the census were not eligible to join the study will be tabulated.

The profile will also show, 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. Reasons that children did not attend the first study contact for vaccination will be tabulated.

The number of children who exited the study population by the end of the first, second and third year of the study will be 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 will be produced for each of the two trial centres.

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 alone, and the combined group. Comparisons will also be made between RTS,S/AS01 alone and the combined group.

Primary endpoint

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, starting 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.

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.

As recommended in the updated CONSORT guidelines (8), the incidence rate differences (IRD) will also be calculated, as this gives an indication of the reduction in incidence attributable to the interventions, i.e. the absolute public health impact in similar contexts. The IRD will be calculated using ordinary least squares regression of transformed variables, 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.

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 adjust for 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

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 successive years of the study is also pre-specified, because it is possible that the efficacy of RTS,S/AS01 booster doses is different to the primary series. This will be assessed by exploring evidence for an interaction between intervention group and study year (2017-18, 2018-19, 2019-20).

Finally, 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. Of particular interest are participants who were young infants at the time of the first vaccination in 2017, as they may have had no exposure to malaria prior to enrolment in the trial.

Pre-specified Secondary analyses

As this is the first trial of seasonal vaccination, a number of secondary analyses are planned to investigate the effect of malaria event history, completeness of protection and protection over time. Lexis expansion will be used to stratify person-time since vaccination. This will enable regression splines to be fitted to obtain smooth estimates of protection over time from RTS,S/AS01.

Further analyses will explore the changing relative benefits of SMC and RTS,S/AS01 with age and transmission intensity (by comparing efficacy profiles with age between Burkina Faso, which has higher incidence rates, with Mali).

Ancillary studies to be reported separately

1. Evaluation of anti-CSP antibody concentrations obtained before and after priming and after each booster dose, determined in a sub-sample of children, and the relation of antibody concentration following vaccination to the subsequent risk of malaria.

2. The effect of the intervention on nutritional status at the end of season cross-sectional surveys.

Analysis of the preference of participants for an injection of vaccine or for multiple rounds of SMC was scheduled to take place during the last few months of this phase of the study. However, this has had to be postponed because of travel restrictions imposed by the COVID-19 crisis.

On the advice of the steering committee and one of the trial funders (PATH) the economic analysis of the two approaches to malaria control will be undertaken in the second year of an extension study. The extension study will observe children up to the age of five years when they will no longer be eligible to receive SMC, rather than stopping follow-up after three years, as had been proposed initially.

Planned main tables for published report

Table 1: Incidence of the primary outcome: 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.

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 1								
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)**	-	-	-	
Study Year 2								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)**	-	-	-	
Study Year 3								
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.

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

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 - 2017						
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 - 2018						
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 - 2019						
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.

Figure 1. This will show i) cumulative hazards of malaria, by treatment group and ii) the risk of malaria, by treatment group.

References

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RTS,S-SMC:

A comparative trial of seasonal vaccination with the RTS,S/AS01 malaria vaccine, seasonal malaria chemoprevention, and of the two interventions combined

Revisions to the Statistical Analysis Plan:

Revision Number	Date of Revision	Page Number(s)	Details
1	02-Jun-2020	6 10	Clarification of passive case detection of the primary outcome. Clarification of the rationale for the definition of the 'per protocol' population

Study title
A comparative trial of seasonal vaccination with the malaria vaccine RTS,S/AS01, seasonal malaria chemoprevention, and of the two interventions combined.

Clinical trial registration
Clinicaltrials.gov Identifier: NCT03143218

Study duration
April 2017 – March 2020

Study site
The trial is being conducted in Houndé district, Burkina Faso and in Bougouni district, Mali, sites of a previous trial (NCT02211729), which evaluated the effectiveness of adding azithromycin to the drug combination used for seasonal malaria chemoprevention.

Study objectives
This trial seeks to determine

1. Whether seasonal vaccination with RTS,S/AS01 is 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 and SMC with SP+AQ) is superior to RTS,S/AS01 alone or SMC alone in preventing clinical malaria and other adverse outcomes.

These aspects are of equal priority.

Study groups
This is a double-blind, individually-randomised trial with three study arms.

The study groups, and abbreviated names used for ease of reference, are shown in the table below.

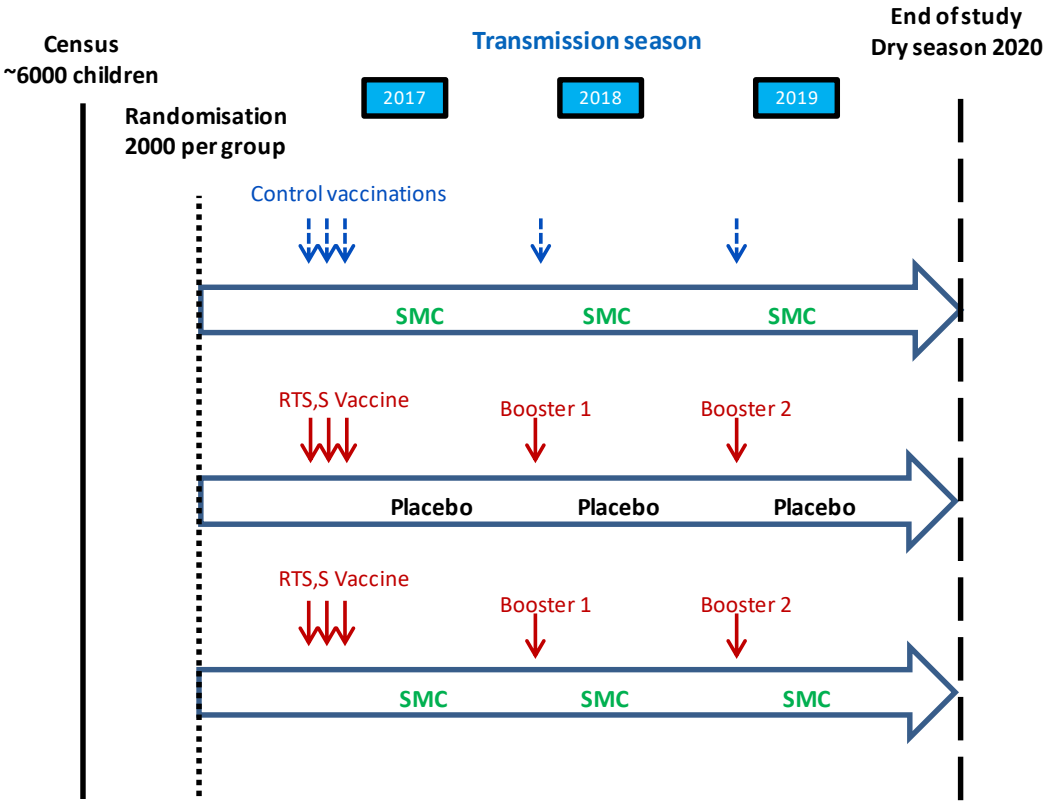
	Group		
	1) SMC 'SMC alone'	**2) RTS,S/AS01 'RTS,S alone'**	**3) RTS,S/AS01+SMC 'Combined group'**
April/May – June/July 2017	Rabies vaccine x 3	RTS,S/AS01 x 3	RTS,S/AS01 x 3
July/Aug – Oct/Nov 2017	SMC x 4	SMC placebo x 4	SMC x 4
June 2018	Hep A vaccine x 1	RTS,S/AS01 x 1	RTS,S/AS01 x 1
July-Oct 2018	SMC x 4	SMC placebo x 4	SMC x 4
June 2019	Hep A vaccine x 1	RTS,S/AS01 x 1	RTS,S/AS01 x 1
July-Oct 2019	SMC x 4	SMC placebo x 4	SMC x 4

Group 1 – SMC arm - 'SMC alone'
In 2017, children in the SMC group were given three doses of rabies vaccine 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.

Group 2 – RTSS/ AS01 arm – 'RTS,S alone'
In 2017, children in the RTS,S/AS01 group were given three doses of RTSS/AS01 vaccine and four rounds of placebo-SMC at monthly intervals. In 2018 and 2019, children received one dose of RTS,S/AS01 vaccine and four rounds of placebo-SMC at monthly intervals.

Group 3 – RTS,S/AS01+SMC arm – 'Combined arm'
In 2017, children in the combined group were given three doses of RTS,S/AS01 vaccine and four rounds of SMC with SP+AQ at monthly intervals. In 2018 and 2019, children received one dose of RTS,S/AS01 vaccine and four rounds of SMC with SP+AQ at monthly intervals.

Schematic of Key Study Activities



Precise timing of interventions
Administration of RTS,S/AS01 or control vaccine: 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 or control vaccine were given in the first two weeks of June.

Administration of SMC or placebo SMC: 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.

Study population

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.

Sample size and Study Design

Based on the calculations described below, the trial aimed to recruit approximately 3,000 children in Burkina Faso and 3,000 in Mali (total 6,000) who would be followed for three years. A low dropout rate of around 5% per year (15% overall) was anticipated based on findings from the previous trial conducted in the same study areas.

In Burkina Faso and Mali, 2777 and 3143 children, respectively, received the first dose of study vaccine.

Superiority comparison: The trial was designed to compare the two interventions combined with either used alone. The study is powered to i) assess the statistical evidence against the null hypothesis of no difference between the combined group and either the SMC alone or RTS,S alone group, and ii) estimate the efficacy of the combined group relative to the single intervention groups with a relatively high degree of precision. This latter aspect is important because if the combined intervention is to be used in practice, it is necessary to show that adding RTS,S/AS01 to SMC has a clinically significant benefit.

After two years of follow-up, incidence of clinical malaria, as defined below, was approximately 200 cases per 1000 child-years at risk. With approximately 2000 individuals in each arm, if the

incidence is at least 30% lower in the combined group, there is very high power over the three years of the study (close to 100%) to reject the null hypothesis of no difference between the combined group and the single treatment groups. If efficacy of the combined group relative to either of the other groups is at least 30%, there will be 90% power for the lower limit of the 95% confidence interval to exclude 15%, i.e. to address point ii) above, establishing that the protection from the combined group is at least 15% better than SMC or vaccination alone.

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 potential advantages of RTS,S over SMC in terms of ease of delivery and the potential to sustain high levels of coverage. A reduction in annual efficacy from 50% to 40% was considered the largest difference that would be likely to be acceptable. This translates to a 20% greater incidence in the RTS,S/AS01-alone arm compared to the SMC-alone arm. With the anticipated incidence rates, there is adequate power to reject a smaller margin, as detailed in the protocol. However, 20% was considered the largest difference that would be considered unimportant.

The study has 80% power to exclude, at the 2.5% significance level, a relative difference in the incidence of clinical malaria of 20% over the three year study period between the RTS,S/AS01 and SMC alone groups, if these two interventions were equally effective. Evidence of the effectiveness of the reference group – SMC alone - in these areas comes from clinical trials in Mali and Burkina Faso in 2009 (1, 2), and more recently in case-control studies conducted in both countries in 2016. In the previous AZ-SMC trial in these two districts (3), the prevalence of molecular markers of resistance to SP and AQ were very low, and the curative efficacy of SP+AQ over 28 days was above 95% in both areas (Cairns et al., submitted).

Hypothesis testing 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.

For the non-inferiority comparison 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.

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 weekly and 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 burned to CD and a copy sent to the chair of the DSMB for archiving.

The randomisation codes will only be released 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.

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 temperature ≥ 37.5°C, or a history of fever within the past 48 hours), and a positive blood film, with a *P. falciparum* parasite density of 5,000 per µl or more. This cut-off has been used in previous studies of SMC in Burkina Faso and Mali, as well as 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). Morbidity detected at contacts for which study children were visited at home (for serological sampling, and for the weekly parasitaemia survey) are excluded, as is vaccination, because some children were visited at home and brought to the clinic to be vaccinated.

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 the 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 surgery**

2.2.3 **Hospital admissions due to malaria.** Defined as hospital admissions where malaria was the primary diagnosis, supported by a positive blood smear, or positive RDT if no blood smear result was 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**

2.2.5 **Deaths due to any cause**

2.2.6 **Deaths due to any cause excluding external causes and surgery**

2.2.7 **Deaths due to malaria**. Defined as hospital admissions resulting in death, where malaria was recorded as the primary cause of death, and where parasitology results did not

exclude malaria (i.e. a positive blood smear, if a slide result was available, or a positive RDT if no slide was done). Deaths in the community will also be included when malaria is assigned as the primary cause of death by verbal autopsy.

2.3 Outcomes measured at weekly surveys

A subset of children were selected to be sampled for parasitaemia during weekly surveys. Systematic random sampling, from lists sorted on age, was used to allocate children to be sampled in not more than one week per year, such that the sample of 24 children in each week, in each country was balanced with respect to age and treatment group. Results from the weekly surveys will be analysed and presented separately for each malaria transmission season (2017, 2018 and 2019).

2.3.1 The prevalence of asexual stage *P. falciparum* infection of any density detected during the weekly home visits.
2.3.2 The prevalence of asexual stage *P. falciparum* infection with a density ≥ 5000 per ul detected during the weekly home visits.
2.3.3 The arithmetic mean *P. falciparum* parasite density, including samples which are parasite negatives as having a density of zero.

2.4 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 each malaria transmission season (2017, 2018 and 2019).

2.4.1 The prevalence of asexual stage *P. falciparum* infection of any density
2.4.2 The prevalence of asexual stage *P. falciparum* infection with density ≥ 5000 per ul
2.4.3 The prevalence of sexual stage *P. falciparum* infection (i.e. gametocytes)
2.4.4 The arithmetic mean *P. falciparum* parasite density, including samples which are parasite negatives as having a density of zero.
2.4.5 The prevalence of asexual stage infection of non-falciparum *Plasmodium* species.
2.4.6 The prevalence of sexual stage infection (i.e. gametocytes) of non-falciparum *Plasmodium* species.
2.4.7 The mean haemoglobin concentration in g/dL.
2.4.8 The prevalence of anaemia, defined as measured Hb < 10 g/dL.
2.4.9 The prevalence of moderate anaemia, defined as measured Hb < 7 g/dL.
2.4.10 The prevalence of severe anaemia, defined as measured Hb < 5 g/dL.

2.5 Other secondary outcomes

2.5.1 The prevalence of molecular markers of resistance to SP and AQ in children with *P. falciparum* infection, among samples collected at the final cross-sectional survey in December 2019. These 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.
2.5.2 The percentage of children with asymptomatic *P. falciparum* parasitaemia at the cross-sectional survey in December 2019, 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 (section 2.2), 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.

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 up to the 30th November 2019. A 95% confidence interval for the incidence rate of meningitis among children vaccinated with RTS,S/AS01 will be calculated.

The incidence of cerebral malaria among children vaccinated with RTS,S/AS01 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.

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. The subset of febrile convulsions that occurred within 7 days of vaccination will also be analysed.

An exploratory analysis will investigate if there is any evidence that RTS,S/AS01 increases mortality in girls. This will compare the incidence of deaths using Cox regression, with an interaction between a dummy variable indicating receipt of RTS,S/AS01 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 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.

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. 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 populations and person-years at risk

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 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 1) the date observation formally ended (31st March, 2020), 2) the date last seen if lost-to-follow-up (LTFU), 3) the date of permanent exit from the study area, or 4) 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).

As a secondary analysis, the primary outcome 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 2018, 1 in 2019) 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.

Trial profile

The trial profile will show 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. Reasons that children seen at the census were not eligible to join the study will be tabulated.

The profile will also show, 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. Reasons that children did not attend the first study contact for vaccination will be tabulated.

The number of children who exited the study population by the end of the first, second and third year of the study will be 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 will be produced for each of the two trial centres.

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 alone, and the combined group. Comparisons will also be made between RTS,S/AS01 alone and the combined group.

Primary endpoint

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, starting 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.

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.

As recommended in the updated CONSORT guidelines (8), the incidence rate differences (IRD) will also be calculated, as this gives an indication of the reduction in incidence attributable to the interventions, i.e. the absolute public health impact in similar contexts. The IRD will be calculated using ordinary least squares regression of transformed variables, 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.

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 adjust for 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

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 successive years of the study is also pre-specified, because it is possible that the efficacy of RTS,S/AS01 booster doses is different to the primary series. This will be assessed by exploring evidence for an interaction between intervention group and study year (2017-18, 2018-19, 2019-20).

Finally, 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. Of particular interest are participants who were young infants at the time of the first vaccination in 2017, as they may have had no exposure to malaria prior to enrolment in the trial.

Pre-specified Secondary analyses

As this is the first trial of seasonal vaccination, a number of secondary analyses are planned to investigate the effect of malaria event history, completeness of protection and protection over time. Lexis expansion will be used to stratify person-time since vaccination. This will enable regression splines to be fitted to obtain smooth estimates of protection over time from RTS,S/AS01.

Further analyses will explore the changing relative benefits of SMC and RTS,S/AS01 with age and transmission intensity (by comparing efficacy profiles with age between Burkina Faso, which has higher incidence rates, with Mali).

Ancillary studies to be reported separately

1. Evaluation of anti-CSP antibody concentrations obtained before and after priming and after each booster dose, determined in a sub-sample of children, and the relation of antibody concentration following vaccination to the subsequent risk of malaria.

2. The effect of the intervention on nutritional status at the end of season cross-sectional surveys.

Analysis of the preference of participants for an injection of vaccine or for multiple rounds of SMC was scheduled to take place during the last few months of this phase of the study. However, this has had to be postponed because of travel restrictions imposed by the COVID-19 crisis.

On the advice of the steering committee and one of the trial funders (PATH) the economic analysis of the two approaches to malaria control will be undertaken in the second year of an extension study. The extension study will observe children up to the age of five years when they will no longer be eligible to receive SMC, rather than stopping follow-up after three years, as had been proposed initially.

Planned main tables for published report

Table 1: Incidence of the primary outcome: 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.

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 1								
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)**	-	-	-	
Study Year 2								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)**	-	-	-	
Study Year 3								
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.

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

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 - 2017						
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 - 2018						
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 - 2019						
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.

Figure 1. This will show i) cumulative hazards of malaria, by treatment group and ii) the risk of malaria, by treatment group.

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Details of Amendments to the Statistical Analysis Plan

The Original SAP (dated 09-Apr-2020) was amended on one occasion (02-Jun-2020) and approved by the DSMB prior to locking and archiving of the trial database. In the amendment, two edits for clarification were made as detailed below.

Edit 1 - **Clarification of passive case detection of the primary outcome (Page 6):**

The text in bold italics was inserted as below.

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 temperature ≥ 37.5°C, or a history of fever within the past 48 hours), and a positive blood film, with a *P. falciparum* parasite density of 5,000 per µl or more. This cut-off has been used in previous studies of SMC in Burkina Faso and Mali, as well as 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). Morbidity detected at contacts for which study children were visited at home (for serological sampling, and for the weekly parasitaemia survey) are excluded, as is vaccination, because some children were visited at home and brought to the clinic to be vaccinated.***

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).

Edit 2 – Clarification of the rationale for the definition of the 'per protocol' population

The text in italics was inserted as below:

Page 10:

As a secondary analysis, the primary outcome 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 2018, 1 in 2019) 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.