BMJ Open

Seasonal Malaria Vaccination: protocol of a Phase III trial of seasonal vaccination with the RTS,S/AS01 vaccine, seasonal malaria chemoprevention and the combination of vaccination and chemoprevention

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2019-035433
Article Type:	Protocol
Date Submitted by the Author:	19-Nov-2019
Complete List of Authors:	Chandramohan, Daniel; London School of Hygiene and Tropical Medicine, Disease Control Dicko, Alassane; MRTC Zongo, Issaka; Institut de Recherche en Sciences de la Santé (IRSS) Sagara, Issaka; MRTC Cairns, Matthew; London School of Hygiene and Tropical Medicine, Disease Control Kuepfer, Irene; London School of Hygiene and Tropical Medicine, Disease Control Diarra, Modibo; MRTC Tapily, Amadou; MRTC Issiaka, Djibrilla; MRTC Sanogo, Koualy; MRTC Mahamar, Almahamoudou; MRTC Sompougdou , Frederic; Institut de Recherche en Sciences de la Santé (IRSS) Yerbanga, Serge; Institut de Recherche en Sciences de la Santé (IRSS) Thera, Ismaila; MRTC Milligan, Paul; London School of Hygiene and Tropical Medicine Tinto, Halidou; Institut de Recherche en Sciences de la Santé (IRSS) Ouedraogo, Jean-Bosco; Institut de Recherche en Sciences de la Santé (IRSS) Greenwood, B.; London School of Hygiene and Tropical Medicine, Disease Control
Keywords:	Immunology < TROPICAL MEDICINE, Tropical medicine < INFECTIOUS DISEASES, Epidemiology < TROPICAL MEDICINE

I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our licence.

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which Creative Commons licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

SEASONAL MALARIA VACCINATION: PROTOCOL OF A PHASE III TRIAL OF SEASONAL VACCINATION WITH THE RTS,S/AS01 VACCINE, SEASONAL MALARIA CHEMOPREVENTION AND THE COMBINATION OF VACCINATION AND CHEMOPREVENTION

Daniel Chandramohan PhD[1], Alassane Dicko MD[2], Issaka Zongo PhD[3], Issaka Sagara MD[2], Matthew Cairns PhD[1], Irene Kuepfer PhD[1], Modibo Diarra MD[2], Amadou Tapily MD[2], Djibrilla Issiaka MD, Koualy Sanogo MD, Almahamoudou Mahamar PharmD, Frederic Sompougdou MD[3], Serge Yerbanga PhD[3], Ismaila Thera MPH[2], Paul Milligan PhD[1], Halidou Tinto PhD[3], Jean-Bosco Ouedraogo PhD[3], Brian Greenwood MD[1]

[1]London School of Hygiene & Tropical Medicine, London, United Kingdom; [2]Malaria Research and Training Centre (MRTC), University of Science, Techniques and Technologies of Bamako, Bamako, Mali; [3] Institut de Recherche en Sciences de la Santé (IRSS), Bobo-Dioulasso, Burkina Faso.

Corresponding Author: Prof Daniel Chandramohan, Faculty of Infectious and Tropical Diseases, London School of Hygiene& Tropical Medicine, Keppel St., London WC1E 7HT, UK. Email: Daniel.chandramohan@lshtm.ac.uk

ABSTRACT

Introduction: Seasonal malaria chemoprevention (SMC), with sulphadoxine-pyrimethamine plus amodiaquine (SP+AQ) is effective but does not provide complete protection. The RTS,S/AS01 malaria vaccine provides high level of protection against malaria shortly after vaccination but this wanes rapidly. Such a vaccine could be an alternative or additive to SMC. This trial aims to determine whether seasonal vaccination with RTS,S/AS01 vaccine could an alternative to SMC and whether a combination of the two interventions would provide added benefits.

Methods and Analysis: This is an individually randomised, double-blind, placebo-controlled trial. 5920 children aged 5-17 months were enrolled in April 2017 in Mali and Burkina Faso. Children in group 1 received three priming doses of RTS,S/AS01 vaccine before the start of the 2017 malaria transmission season and a booster dose at the beginning of two subsequent transmission seasons. In addition, they received SMC SPAQ placebo on four occasions each year. Children in group 2 received three doses of rabies vaccine in year one and Hepatitis A vaccine in years 2 and 3 together with four cycles of SMC SPAQ each year. Children in group 3 received RTS,S/AS01 vaccine and four courses of SMC SPAQ. Incidence of clinical malaria is determined by case detection at health facilities. Weekly active surveillance for malaria is undertaken in a sub-set of children. The prevalence of malaria is measured in surveys at the end of each transmission season. The primary endpoint is the incidence of clinical malaria confirmed by a positive blood film with a minimum parasite density of 5000 per µl. Primary analysis will be by modified intention to treat defined as children who have received the first dose of the vaccine.

Ethics and Dissemination: Approved by the national ethics committees and the London School. The results will be presented to all stakeholders and published in open access journals.

Registration

The trial is registered with clinicaltrials.gov. (https://www.clinicaltrials.gov/ct2/show/NCT03143218?term=NCT03143218&rank=1).

Strengths and limitations of this study

- One major strength of this trial is that it is exploring a novel approach of seasonal vaccination to malaria control in an area where the burden of malaria remains very high despite a high coverage of currently recommended interventions.
- Another strength is its large size which makes the study adequately powered to test the study hypotheses.
- A third strength is that the clinical impact of the two interventions is supported by laboratory studies which will measure the impact of SMC on drug resistance and of RTS,S/AS01 on potential vaccine escape parasites.
- Despite establishment of an intensive surveillance system some episodes of malaria and some serious adverse events may be missed but because the trial is individually randomised these should be distributed equally among study groups.
- To apply the trial results to areas where the seasonal malaria transmission duration and pattern is different, modelling may be needed.

INTRODUCTION

The RTS,S/AS01 malaria vaccine is a recombinant protein vaccine that contains a component of the circumsporozoite protein of *Plasmodium falciparum* fused to hepatitis B surface antigen (HBsAg), 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]. A phase 3 trial of RTS,S/AS01 conducted in 15,439 children in 7 countries in Africa showed that three doses of RTS,S/AS01 given at monthly intervals, followed by a fourth dose 18 months later, gave 36.5 % [95% CI 31%,41%] protection against clinical attacks of malaria in children aged 5-17 months who were followed for 48 months [2]. RTS,S/AS01 provides a high level of protection during the first three months after vaccination [3], estimated to be 68% in the phase 3 trial [4]. The main safety issues related to administration of RTS,S/AS01 detected in the phase 3 trial were 1) an unexplained, statistically significant, increase in cases of meningitis observed in children given the vaccine at the age of 5-17 months, 2) a possible increase in the proportion of severe cases of malaria that were classified as cerebral malaria and 3) a gender imbalance in the small number of deaths recorded during the trial [5].

Seasonal Malaria Chemoprevention (SMC) involves monthly administration of a full therapeutic dose of an antimalarial drug or drug combination to children on three of four occasions during the period of highest risk of malaria infection. Studies undertaken in several countries in West Africa have shown that SMC with a combination of sulphadoxine/pyrimethamine (SP) and amodiaquine (AQ) (SP+AQ) is highly effective in reducing the incidence of severe and uncomplicated malaria in areas where the transmission of malaria is markedly seasonal [6-8]. SMC with SP+AQ is very safe [9]. Many countries in the Sahel and sub-Sahel region of West Africa have now incorporated SMC into their national malaria control programme achieving high levels of coverage [10] but malaria continues to be a major cause of mortality and morbidity in these countries and additional control tools are needed. Adding azithromycin to the anti-malarial drugs used for SMC did not reduce hospital admissions or deaths from non-traumatic causes [11]. Thus, determining whether adding RTS,S/AS01 would provide valuable additional protection is important. The primary objectives of the trial are to determine - (1) whether seasonal vaccination following priming with the RTS,S/AS01 malaria vaccine is non-inferior in preventing malaria to SMC with SP + AQ in children living in the areas of the Sahel and sub-Sahel of Africa where

malaria transmission is highly seasonal (2) Whether RTS,S/AS01 can provide additional, useful and cost effective protection against malaria if given together with SMC in areas with highly seasonal malaria transmission.

METHODS

This is an individually randomised, double-blind, placebo controlled trial with three groups. The trial will be conducted and reported according to CONSORT guidelines [12].

Study Setting

The trial is being conducted in Houndé district, Burkina Faso and in Bougouni district, Mali (Figure 1). Malaria, due predominantly to *P. falciparum,* is highly seasonal in both districts. The prevalence of *P. falciparum* malaria in school age children in December 2016 was 53% in Bougouni and 50% in Houndé [11]). The main malaria vector in both study areas is *Anopheles gambiae* ss. In 2016, a high proportion of children slept under an ITN in Bougouni (95%) but the percentage was slightly lower in Houndé (79.9%). The first line treatment for malaria in the public health system in both districts is artemether -lumefantrine. The incidence of parasite confirmed malaria was 1068 per 1000 during the transmission season among 3-59 month old children who were allocated to the SMC group in a trial conducted in Bougouni and Houndé districts in 2014-2016 [11]. Malaria is seasonal, with 79.6% of annual cases occurring over 5 months of the year from July to November in Bougouni and 81.0% in between July to November in Hounde.

Study population

All households within the study areas with children 5-17 months of age on April 1st 2017 were enumerated in February-March 2017 and children were screened for their eligibility to enter the trial. A child was considered eligible 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 on April 1st 2017. A child was deemed to be ineligible if (a) the child was a transient resident; (b) the age of the child was outside the stipulated range; (c) the child had a history of an adverse reaction to SP or AQ; (d) 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); (e) the child was known to have an immune deficiency disease or was receiving an immunosuppressive drug;

(f) the child had previously received a malaria vaccine; (g) the child was enrolled in another malaria intervention trial. Written, informed consent was obtained from their caretakers of eligible children prior to their inclusion in the trial.

Each eligible child was assigned a unique census identification number (ID) 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 were updated in April/May 2018 and 2019 prior to the administration of the booster doses of vaccine.

Randomisation

Eligible children were allocated randomly to one of the three study groups (Table 1). The randomization list was prepared using permuted blocks after sorting the list of eligible children by age, gender, area of residence, and prior receipt of SMC, to ensure these characteristics would be balanced between the three groups.

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 followed by a fourth and fifth dose given in June 2018 and June 2019 prior to the malaria transmission seasons.

Control vaccines: Rabies vaccine *Rabipur*^{R}, produced by GSK was used as the control vaccine for the primary series of vaccinations during the dry season of 2017 for those allocated to the SMC alone group. Hepatitis A vaccine (HAVRIX^{R}), a licensed inactivated hepatitis A vaccine produced by GSK, was used for the booster dose in years 2 and 3.

Seasonal malaria chemoprevention: Four courses of SMC with SP+AQ were given at monthly intervals during the malaria transmission season in line with WHO's recommendation and national policy. A course of SMC for a child over the age of one year comprises a single treatment of SP (500mg sulphadoxine/25 mg pyrimethamine) and AQ 150mg on day 1 and AQ 150mg on days 2 and 3. Infants receive half of these doses. Children in the RTS,S alone group received a matching SP+AQ placebo. Dispersible form of SP and AQ and matching placebo were obtained from Guilin Pharamceuticals, Shanghai, a GMP certified supplier. All treatments were given under observation.

Implementation of the interventions

Vaccines: 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 was done by a person who also took no further part in the trial. Vaccines were administered by study health staff trained to give vaccines.

Vaccination was undertaken in fixed or temporary centres established near study health centres or hospitals. The families of children scheduled for vaccination on a particular day were notified the day before this was due to take place and asked to bring their child to the vaccination centre on that day. In the case of families who lived a long distance from a vaccination centre, project transport was provided to bring the children and caretakers to the vaccination centre and to take them home. Children were retained at the vaccination centre for at least 30 minutes after vaccination to ensure that there were no immediate adverse events. Home visits were made to the families of children who missed vaccination on the designated day and their caretakers were asked if they would still like their child to receive vaccination (or SMC). If they agreed, they were asked to bring their children to the vaccination/drug administration centre. Some children were unable to attend for vaccination at the time that this took place in their community because their family had traveled or due to an acute illness. A protocol amendment, approved by the data and safety monitoring board, allowed these children to receive the booster vaccination in 2018 and in 2019 if they presented for SMC administration later in the malaria transmission season.

SMC: SMC drugs were pre-packed by a pharmacist who took no further part in the trial, in re-sealable envelopes bearing the child's unique number and containing tablets for the four cycles of treatment appropriate for the child's age. Each dose of SMC or placebo was administered by trained project staff at a central point in the study village. Study children were given an identity card containing their photograph, 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.

Insecticide treated bednets (ITNs): All study children were given an ITN at enrolment in 2017 before the malaria transmission season.

Study outcomes

The primary trial outcome is clinical malaria, defined as an episode of illness characterised by fever (temperature >= 37.5° C), or a history of fever within the previous 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 include the following:

(1) Blood slide or rapid diagnostic test (RDT) positive malaria defined as a clinical episode of an uncomplicated febrile illness (temperature >= 37.5° C), or a history of fever within the previous 48 hours, with a positive blood film (any level of asexual parasitemia) or a positive Rapid Diagnostic Test (RDT).

(2) Hospital admissions with malaria, including cases of severe malaria which meet WHO criteria for a diagnosis of severe malaria.

(3) Malaria infection (symptomatic or asymptomatic parasitaemia) in a subset of randomly selected children seen during home visits.

(4) Malaria parasitaemia (including gametocytaemia), moderate and severe anaemia, and malnutrition at the end of the malaria transmission season.

(5) Serious adverse events (SAEs), including any deaths, with special reference to meningitis, cerebral malaria or immune deficiency illnesses.

(6) Anti-CSP concentrations obtained after priming and after each booster dose, determined in a sub-sample of children.

(7) The presence of molecular markers of resistance to SP and AQ in parasite positive samples collected at the last cross-sectional survey.

(8) 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 used to develop the RTS.S/AS01 vaccine.

Sample Size

Based on the sample size 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 a previous trial conducted in the same study areas [12]. Based on the results of a blinded interim analysis of an SMC+ azithromycin study [12], it was assumed that the incidence of clinical malaria confirmed by blood slide would be 300 cases per 1000 children over a calendar year in the SMC alone group. Results obtained over a period of three years of observation in the two study sites will be combined for the comparison of the primary endpoint but secondary analyses will evaluate impact at each study site separately.

Superiority: The trial is designed to be able to estimate the efficacy of the two interventions combined compared to either used alone, with a relatively high degree of precision. Based on the incidence of events observed in the trial after two years of follow-up, with approximately 2000 individuals in each arm, there is 90% power for the lower limit of confidence to be above 15% if the efficacy is 30% or more, over the three years of the study, and 80% power to detect an efficacy of 25% in each individual year, in pooled analysis of both sites.

Non-inferiority: SMC for 4 months of the year has an efficacy, assuming receipt of all 4 monthly cycles, of about 85%; if, without intervention, that 4 months would account for 60% of 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 we would be willing to accept, or would consider unimportant, if RTSS were to replace SMC. A reduction in efficacy from 50% to 40% translates to a 20% greater incidence in the RTSS-alone arm compared to the SMC-alone arm. The analysis plan will specify a margin of 20%, this takes into account the potential advantages of RTS,S over SMC in terms of ease of delivery and hence the likelihood of being able to sustain high levels of coverage. The trial has 80% power to exclude, at the 2.5% significance level, a relative difference in the incidence of clinical episodes of malaria between the RTS,S/AS01 and SMC alone groups of 20% over the three-year study period, if the two interventions were equally effective.

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 each subsequent booster dose. 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) will give 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 at each time point.

Follow-up and measurement of outcomes

Passive surveillance for cases of uncomplicated and severe malaria: Project staff based in the study hospitals and health centres that serve the study communities identify and document all cases of malaria who present to these health facilities. In Mali, some cases of uncomplicated malaria are treated by community health workers who have been taught to diagnose malaria with a RDT and to treat positive cases. Blood films and filter paper strips are obtained from all these cases for subsequent confirmation of the diagnosis by microscopy.

Active surveillance for malaria: Each week, 24 randomly selected children (8 from each arm of the study) in each country are visited at home, their temperature measured and a blood film collected. Any child who is febrile or who has other features suggestive of malaria is tested with an RDT and treated with a full course of an artemisinin combination therapy (ACT) if positive.

Prevalence of malaria parasitaemia and anaemia: A survey of all study children is undertaken at least one month after the last round of SMC administration at the end each malaria transmission season. During these surveys anthropometric measurements are taken and a finger prick blood samples is collected for preparing blood slides and blood spots. Children who are febrile are tested with an RDT and treated with an ACT if positive.

Malaria endemicity in the study area: A survey of schoolchildren aged 6-12 years resident in the same areas as the study children is conducted at the end of each malaria transmission season. Two hundred randomly selected school children per country (total 400) aged 6-12 years who are well and have not received SMC are tested for malaria by microscopy.

Measurement of the immune response to vaccination. Blood samples (2ml) have been collected from approximately 160 children in each of the groups (80 per country) who receive RTS,S/AS01 prior to administering the first dose of vaccine and one month after the third dose of the primary series of vaccination had been given In years 2 and 3 samples have been collected before and one month after administration of the fourth and fifth doses of vaccine for measurement of anti-CSP antibodies.

Measurement of resistance to the antimalarial drugs used for SMC. Dried blood spots from a randomly group of children who have malaria parasitaemia detected by microscopy at the last cross-sectional survey will be used for analysis of molecular markers of resistance to SP and AQ.

Serious Adverse Events (SAEs): Project staff based at the study hospitals have been provided with additional training on the recognition of meningitis, cerebral malaria and 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 developed by WHO for use in the pilot RTS,S/AS01 implementation trials are being applied. All deaths occurring outside the health facilities are assessed by verbal autopsy using the WHO 2016 verbal autopsy questionnaire [13]. Any SAEs that are (a) considered by the investigators 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 quarterly.

Laboratory methods

Detection of malaria: A histidine rich protein (HRP2) based RDT is used for the initial diagnosis of malaria and to guide treatment. Blood films collected at the same time are read subsequently by two readers following the guidelines developed for the phase 3 RTS.S/AS01 trial [14]. Slides which are judged to be discordant for either positivity or parasite density are read by a third reader, and the discrepancy resolved following the algorithm in the above study [14].

Measurement of haemoglobin concentration: Haemoglobin concentration is measured colourimetrically using a Hemocue colorimeter (Hemocue AB, Angelholm, Sweden).

Detection of markers of resistance to SP and amodiaquine: 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.

Measurement of anti-CSP concentration: Antibodies to CSP will be measured by a standardised ELISA at the University of Ghent laboratory using a method applied in previous trials of RTS,S/AS01.

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 [15].

Investigation of suspected cases of meningitis. The aetiology of cases of meningitis is determined by microscopical examination of cerebrospinal fluid samples for bacteria and white blood cells at the district hospital and then by subsequent PCR testing at a reference laboratory.

Data management

Data are collected using electronic case record forms (CRF) developed using Open Data Kit (ODK) software. This system is based on electronic transfer of the CRFs from the research sites. Automatic checks are performed on clinical and laboratory forms to ensure that they are complete and contain valid responses prior to transferring data.

Analysis plan

The primary endpoint of the trial is the incidence of all episodes of blood slide confirmed clinical malaria over the study period and this will be evaluated 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).

The primary analysis will be based on data for both sites combined, with site as a stratification factor, but site-specific analyses will also be undertaken. A test of interaction will be used to determine if there is any evidence that efficacy of RTSS varies between the two trial sites. An analysis will also be undertaken to assess efficacy of the booster dose of the vaccine each year.

The proportion of children in each group who experience at least one episode of malaria will be compared as a secondary outcome using Kaplan-Meier estimates of the risk. Evidence for provision of complete protection through the combination of SMC and vaccination each year will be explored using published methods [16, 17]. For the comparison of RTS,S/AS01 plus

SMC versus the other two study groups, two-sided 95% confidence intervals for the hazard ratio will be calculated. For the non-inferiority comparison of RTS,S/AS01 to SMC, two-sided 90%, 95% and 99% confidence intervals for the rate ratio will be calculated, to indicate the degree of confidence with which the margin of 1.20 (a relative increase of 20%) can be excluded. Primary analysis will be by modified ITT. According to protocol (ATP) analyses will also be performed.

An analysis plan will be prepared, for approval by the Data Safety Monitoring Board (DSMB) before the study code is broken. Primary analyses will be by modified ITT. ATP analyses will also be undertaken. Efforts would be made during the trial to ensure high levels of adherence to minimise differences between ITT and ATP populations. All children who received the first dose of vaccine will be included in the intention to treat analysis. (Children who did not attend for the first vaccine dose were withdrawn from the study). 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, and all scheduled doses of vaccine, will be included in the per protocol analysis for each year of the study. Additional sub-analyses will include analysis by age, gender, bed net use during the transmission season and socio-economic status.

Trial management

The London School of Hygiene & Tropical Medicine (LSHTM) is the main sponsor for the trial. Delegated responsibilities are assigned locally. The LSHTM holds Public Liability (‘’negligent harm’’) and Clinical Trial (“non-negligent harm’’) insurance policies which apply to this trial. An independent trial steering committee provides scientific oversight and has approved the protocol. The steering committee holds teleconferencing or face-face meeting annually to monitor progress and advise on the scientific content of the study. The quality of data and the GCP standards of the trial are monitored by an independent, experienced GCP monitor.

ETHICS AND DISSEMINATION

Ethics

Inclusion in the trial of an RTS,S/AS01 alone group is justified, even though SMC is recommended policy, on the grounds that RTS,S/AS01 could provide some added protection outside the main transmission season when SMC is not being given and that it may be easier

to administer than SMC. Individual, written, informed consent has been obtained from the family or legally recognised guardian of each child entered into the trial. Conduct of the trial does not impose any additional costs on the local health services. The project contributes to the costs of routine clinical care of study subjects during the trial and to strengthening of clinical care at the district hospitals in the study areas.

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. The trial has also been approved by the regulatory authorities in Burkina Faso and Mali. An independent Data Safety Monitoring Board (DSMB) monitors regularly the safety of children in the trial, especially those in the RTS,S/AS01 alone group who are not receiving SMC which is standard of care. In February 2018, the board reviewed unblinded data on the incidence of serious adverse events (SAEs) and SAEs due to malaria in each study group and gave permission for the trial to proceed for a further year retaining all three study arms.

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 is registered on clinicaltrials.gov (https://www.clinicaltrials.gov/ct2/show/NCT03143218?term=NCT03143218&rank=1).

Patient and Public Involvement

No patients are involved in this trial. Healthy children were enrolled with the consent of their care takers. They were not involved in the development of the research question and study design.

Dissemination plans

Results from the trial will be discussed with the study communities at the end of the study, presented at national and international conferences and in peer reviewed, open access journals. Trial results will be shared with the WHO's technical expert groups and Malaria Policy Advisory Group. Strong links have been established already with the Ministries of Health, NMCPs and EPI programmes in Burkina Faso and Mali. The trial team has also

established good links with many other organisations involved in the delivery of SMC trials including the SMC ACCESS programme 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.

AUTHORS CONTRIBUTION

DC and BG wrote the first draft of the manuscript, AD, IZ, IS, HT, and JB reviewed the manuscript and made significant contributions, MC, IK, IS and PM wrote sections on sample size, data management and analysis plan, MD, AT, DI, KS, AM, FS, and SY reviewed the paper and are involved in the field operations of the study.

FUNDING

The trial is being funded by the UK Joint Global Health Trials The trial is being funded by the UK Joint Global Health Trials (The Department of Health and Social Care, the Department for International Development, the Global Challenges Research Fund, the Medical Research Council and the Wellcome Trust) grant no. MR/P006876/1, with supplementary funding from PATH MVI, grant no. 18269.

REFERENCES

1. Cohen J, Nussenzweig V, Nussenzweig R et al. From the circumsporozoite protein to the RTS,S/AS candidate vaccine. *Human Vaccin* 2010; 6:90-6.
2. RTS,S Clinical Trials Partnership. Efficacy and safety of the RTS,S/AS01 malaria vaccine with or without a booster dose in infants and children in Africa: final results of a phase 3, individually randomised, controlled trial. *Lancet* 2015; 386: 31-45.
3. Bojang K, Milligan PJ, Pinder M et al. Efficacy of RTS,S/AS02 malaria vaccine against *Plasmodium falciparum* infection in semi-immune adult men in The Gambia: a randomised trial. *Lancet.* 2001;358:1927-34.
4. https://www.who.int/immunization/sage/meetings/2015/october/1_Final_malaria_vaccine_background_paper_v2015_09_30.pdf
5. Klein S, Shann F, Moss W, Benn C, Aaby P. RTS,S Malaria Vaccine and Increased Mortality in Girls. mBio. 2016; 7(2):e00514-16.
6. Wilson AL. A systematic review and meta-analysis of the efficacy and safety of intermittent preventive treatment in children (IPTc). Plos One 2011; 6: e16976.
7. Dicko A, Diallo AI, Tembine I et al. Intermittent preventive treatment of malaria provides substantial protection against malaria in children already protected by an insecticide-treated bednet in Mali: a randomised, double-blind, placebo-controlled trial. *PLoS Med* 2011; 8: 1000407.
8. Konate AT, Yaro JB, Ouedraogo AZ et al. Intermittent preventive treatment of malaria provides substantial protection against malaria in children already protected by an insecticide-treated bednet in Burkina Faso: a randomised, double-blind, placebo-controlled trial. *PLoS Med* 2011; 8: e1000408.
9. Ndiaye JL, Cissé B, Ba EH et al. Safety of seasonal intermittent preventive treatment against malaria with sulfadoxine pyrimethamine + amodiaquine when delivered to children under 10 years of age by district health staff in Senegal. *PLoS One*, in press.
10. (http://www.malariaconsortium.org/pages/access-smc.htm).
11. Chandramohan D, Dicko A, Zongo I et al. Effect of Adding Azithromycin to Seasonal MalariaChemoprevention. N Engl J Med 2019;380:2197-206.
12. Piaggio G, Elbourne DR, Altman DG, Pocock SJ, Evans SJ. Reporting of noninferiority and equivalence randomized trials: an extension of the CONSORT statement. Jama. 2006 Mar 8;295(10):1152-60. PubMed PMID: 16522836.

13. Leitao J, Chandramohan D, Byass P, et al. Revising the WHO verbal autopsy instrument to facilitate routine cause-of-death monitoring. Glob Health Action. 2013;6:21518

14. Swysen C, Vekemans J, Bruls M et al. Development of standardized laboratory methods and quality processes for a phase iii study of the RTS,S/AS01 candidate malaria vaccine. *Mal J 2011;* 10: e223.

15. 15. Neafsey DE, Juraska M, Bedford T,et al Genetic diversity and protective efficacy of the RTS,S/AS01 malaria vaccine. N Engl J Med 2015; 373: 2025-2037.

16. Xu Y, Lam KF, Cheung YB. Estimation of intervention effects using recurrent event time data in the presence of event dependence and a cured fraction. Stat Med. 2014 Jan 22. PubMed PMID: 24449504. Epub 2014/01/23. Eng.

17. Cairns M, Cheung YB, Xu Y, Asante KP, Owusu-Agyei S, Diallo D, et al. Analysis of Preventive Interventions for Malaria: Exploring Partial and Complete Protection and Total and Primary Intervention Effects. Am J Epidemiol. 2015 Jun 15;181(12):1008-17. PubMed PMID: 26022663. Pubmed Central PMCID: PMC4462336. Epub 2015/05/30.eng.

TABLES AND FIGURES

Table 1. Study groups and the interventions that they received.

	Group1	**Group2**	**Group3**
	RTS,S/AS01 alone	**SMC alone**	**SMC + RTS,S/AS01**
Year 1 April-June July-Oct	 RTSS/AS01 x 3 SMC placebo* x 4	 Rabies vaccine x 3 SMC* x 4	 RTSS/AS01 x 3 SMC* x 4
Year 2 June July-Oct	 RTSS/AS01 x 1 SMC placebo* x 4	 HepA vaccine x 1 SMC* x 4	 RTSS/AS01 x 1 SMC* x 4
Year 3 June July-Oct	 RTSS/AS01 x 1 SMC placebo* x 4	 HepA vaccine x 1 SMC* x 4	 RTSS/AS01 x 1 SMC* x 4

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

Figure 1. Study districts in Mali and Burkina Faso

BMJ Open

Seasonal Malaria Vaccination: protocol of a Phase III trial of seasonal vaccination with the RTS,S/AS01 vaccine, seasonal malaria chemoprevention and the combination of vaccination and chemoprevention

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2019-035433.R1
Article Type:	Protocol
Date Submitted by the Author:	18-Feb-2020
Complete List of Authors:	Chandramohan, Daniel; London School of Hygiene and Tropical Medicine, Disease Control Dicko, Alassane; MRTC Zongo, Issaka; Institut de Recherche en Sciences de la Santé (IRSS) Sagara, Issaka; MRTC Cairns, Matthew; London School of Hygiene and Tropical Medicine, Disease Control Kuepfer, Irene; London School of Hygiene and Tropical Medicine, Disease Control Diarra, Modibo; MRTC Tapily, Amadou; MRTC Issiaka, Djibrilla; MRTC Sanogo, Koualy; MRTC Mahamar, Almahamoudou; MRTC Sompougdou , Frederic; Institut de Recherche en Sciences de la Santé (IRSS) Yerbanga, Serge; Institut de Recherche en Sciences de la Santé (IRSS) Thera, Ismaila; MRTC Milligan, Paul; London School of Hygiene and Tropical Medicine Tinto, Halidou; Institut de Recherche en Sciences de la Santé (IRSS) Ofori-Anyinam, Opokua; GlaxoSmithKline Biologicals SA Ouedraogo, Jean-Bosco; Institut de Recherche en Sciences de la Santé (IRSS) Greenwood, B.; London School of Hygiene and Tropical Medicine, Disease Control
Primary Subject Heading:	Epidemiology
Secondary Subject Heading:	Epidemiology, Global health
Keywords:	Immunology < TROPICAL MEDICINE, Tropical medicine < INFECTIOUS DISEASES, Epidemiology < TROPICAL MEDICINE

I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our licence.

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which Creative Commons licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

SEASONAL MALARIA VACCINATION: PROTOCOL OF A PHASE III TRIAL OF SEASONAL VACCINATION WITH THE RTS,S/AS01 VACCINE, SEASONAL MALARIA CHEMOPREVENTION AND THE COMBINATION OF VACCINATION AND CHEMOPREVENTION

Daniel Chandramohan PhD[1], Alassane Dicko MD[2], Issaka Zongo PhD[3], Issaka Sagara MD[2], Matthew Cairns PhD[1], Irene Kuepfer PhD[1], Modibo Diarra MD[2], Amadou Tapily MD[2], Djibrilla Issiaka MD, Koualy Sanogo MD, Almahamoudou Mahamar PharmD, Frederic Sompougdou MD[3], Serge Yerbanga PhD[3], Ismaila Thera MPH[2], Paul Milligan PhD[1], Halidou Tinto PhD[3], Opokua Ofori-Anyinam[4], Jean-Bosco Ouedraogo PhD[3], Brian Greenwood MD[1]

[1]London School of Hygiene & Tropical Medicine, London, United Kingdom; [2]Malaria Research and Training Centre (MRTC), University of Science, Techniques and Technologies of Bamako, Bamako, Mali; [3] Institut de Recherche en Sciences de la Santé (IRSS), Bobo-Dioulasso, Burkina Faso. [4] GlaxoSmithKline Biologicals SA (Vaccines)

Corresponding Author: Prof Daniel Chandramohan, Faculty of Infectious and Tropical Diseases, London School of Hygiene& Tropical Medicine, Keppel St., London WC1E 7HT, UK. Email: Daniel.chandramohan@lshtm.ac.uk

ABSTRACT

Introduction: Seasonal malaria chemoprevention (SMC), with sulphadoxine-pyrimethamine plus amodiaquine (SP+AQ) is effective but does not provide complete protection against clinical malaria. The RTS,S/AS01 malaria vaccine provides high level of protection shortly after vaccination but this wanes rapidly. Such a vaccine could be an alternative or additive to SMC. This trial aims to determine whether seasonal vaccination with RTS,S/AS01 vaccine could be an alternative to SMC and whether a combination of the two interventions would provide added benefits.

Methods and Analysis: This is an individually randomised, double-blind, placebo-controlled trial. 5920 children aged 5-17 months were enrolled in April 2017 in Mali and Burkina Faso. Children in group 1 received three priming doses of RTS,S/AS01 vaccine before the start of the 2017 malaria transmission season and a booster dose at the beginning of two subsequent transmission seasons. In addition, they received SMC SP+AQ placebo on four occasions each year. Children in group 2 received three doses of rabies vaccine in year one and Hepatitis A vaccine in years 2 and 3 together with four cycles of SMC SP+AQ each year. Children in group 3 received RTS,S/AS01 vaccine and four courses of SMC SP+AQ. Incidence of clinical malaria is determined by case detection at health facilities. Weekly active surveillance for malaria is undertaken in a randomly selected sub-set of children. The prevalence of malaria is measured in surveys at the end of each transmission season. The primary endpoint is the incidence of clinical malaria confirmed by a positive blood film with a minimum parasite density of 5000 per µl. Primary analysis will be by modified intention to treat defined as children who have received the first dose of the malaria or control vaccine.

Ethics and Dissemination: Approved by the national ethics committees of Mali and Burkina Faso and the London School of Hygiene & Tropical Medicine. The results will be presented to all stakeholders and published in open access journals.

Registration

The trial is registered with clinicaltrials.gov. (https://www.clinicaltrials.gov/ct2/show/NCT03143218?term=NCT03143218&rank=1).

Strengths and limitations of this study

- One major strength of this trial is that it is exploring a novel approach of seasonal vaccination to malaria control in an area where the burden of malaria remains very high despite a high coverage of currently recommended interventions.
- The study is adequately powered to test the non-inferiority hypotheses which requires a large sample size.
- A third strength is that the clinical impact of the two interventions is supported by laboratory studies which will measure the impact of SMC on drug resistance and of RTS,S/AS01 on potential vaccine escape parasites.
- Despite establishment of an intensive surveillance system some episodes of malaria and some serious adverse events may be missed but because the trial is individually randomised these should be distributed equally among study groups.
- To apply the trial results to areas where the seasonal malaria transmission duration and pattern is different, modelling may be needed.

INTRODUCTION

The RTS,S/AS01 malaria vaccine is a recombinant protein vaccine that contains a component of the circumsporozoite protein of *Plasmodium falciparum* fused to hepatitis B surface antigen (HBsAg), 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]. A phase 3 trial of RTS,S/AS01 conducted in 15,439 children in 7 countries in Africa showed that three doses of RTS,S/AS01 given at monthly intervals, followed by a fourth dose 18 months later, gave 36.5 % [95% CI 31%,41%] protection against clinical attacks of malaria in children aged 5-17 months who were followed for 48 months [2]. RTS,S/AS01 provides a high level of protection during the first three months after vaccination [3], estimated to be 68% in the phase 3 trial [4]. The main safety issues related to administration of RTS,S/AS01 detected in the phase 3 trial were 1) an unexplained, statistically significant, increase in cases of meningitis observed in children given the vaccine at the age of 5-17 months, 2) a possible increase in the proportion of severe cases of malaria that were classified as cerebral malaria and 3) a gender imbalance in the small number of deaths recorded during the trial [5].

Seasonal Malaria Chemoprevention (SMC) involves monthly administration of a full therapeutic dose of an antimalarial drug or drug combination to children on three or four occasions during the period of highest risk of malaria infection. Studies undertaken in several countries in West Africa have shown that SMC with a combination of sulphadoxine/pyrimethamine (SP) and amodiaquine (AQ) (SP+AQ) is highly effective in reducing the incidence of severe and uncomplicated malaria in areas where the transmission of malaria is markedly seasonal [6-8]. SMC with SP+AQ is very safe [9]. Many countries in the Sahel and sub-Sahel region of West Africa have now incorporated SMC into their national malaria control programme achieving high levels of coverage [10] but malaria continues to be a major cause of mortality and morbidity in these countries and additional control tools are needed. Adding azithromycin to the anti-malarial drugs used for SMC did not reduce hospital admissions or deaths from non-traumatic causes [11]. Thus, determining whether adding RTS,S/AS01 would provide valuable additional protection is important. The primary objectives of the trial are to determine - (1) whether seasonal vaccination following priming with the RTS,S/AS01 malaria vaccine is non-inferior in preventing malaria to SMC with SP + AQ in children living in the areas of the Sahel and sub-Sahel of Africa where

malaria transmission is highly seasonal (2) Whether RTS,S/AS01 can provide additional, useful and cost effective protection against malaria if given together with SMC in areas with highly seasonal malaria transmission.

METHODS

This is an individually randomised, double-blind, placebo controlled trial with three groups. The trial will be conducted and reported according to CONSORT guidelines [12].

Study Setting

The trial is being conducted in Houndé district, Burkina Faso and in Bougouni district, Mali (Figure 1). Malaria, due predominantly to *P. falciparum,* is highly seasonal in both districts. The prevalence of *P. falciparum* malaria in school age children in December 2016 was 53% in Bougouni and 50% in Houndé [11]. The main malaria vector in both study areas is *Anopheles gambiae* ss. In 2016, a high proportion of children slept under an insecticide treated bednet (ITN) in Bougouni (95%) but the percentage was slightly lower in Houndé (79.9%). The first line treatment for malaria in the public health system in both districts is artemether-lumefantrine. The incidence of parasite confirmed malaria was 1068 per 1000 child years at risk during the transmission season among 3-59 month old children who were allocated to the SMC group in a trial conducted in Bougouni and Houndé districts in 2014-2016 [11]. Malaria is seasonal, with 79.6% of annual cases occurring over 5 months of the year from July to November in Bougouni and 81.0% in between July to November in Hounde.

Study population

All households within the study areas with children 5-17 months of age on April 1st 2017 were enumerated in February-March 2017 and children were screened for their eligibility to enter the trial. A child was considered eligible 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 on April 1st 2017. A child was deemed to be ineligible if (a) the child was a transient resident; (b) the age of the child was outside the stipulated range; (c) the child had a history of an adverse reaction to SP or AQ; (d) the child had a serious underlying illness (self-reported or obtained from health records), 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); (e) the child was known to have an immune deficiency disease or was receiving an immunosuppressive drug; (f) the child had previously received a malaria vaccine; (g) the child was enrolled in another malaria intervention trial. Written, informed consent was obtained from their caretakers of eligible children prior to their inclusion in the trial.

Each eligible child was assigned a unique census identification number (ID) 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 were updated in April/May 2018 and 2019 prior to the administration of the booster doses of vaccine.

The study started on February 1st 2017 and is expected to be competed on June 30th 2020. (Figure2)

Patient and Public Involvement

No patients are involved in this trial. Healthy children were enrolled with the consent of their care takers. They were not involved in the development of the research question and study design.

Randomisation

Eligible children who were consented soon after the census were allocated randomly to one of the three study groups (Table 1). The randomization list was prepared using permuted blocks after sorting the list of eligible children by age, gender, area of residence, and prior receipt of SMC, to ensure these characteristics would be balanced between the three groups. An independent information technology consultant loaded the randomisation list on four Tablet PCs and handed over two PCs each to the chief vaccine administrators in Mali and Burkina. The chief vaccinators used these PCs to scan the QR code containing child's name and study ID printed on the ID cards of children to find out the vaccine allocated to each child. These PCs were locked in the vaccine room and were accessible to the chief vaccinators only.

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 followed by a fourth and fifth dose given in June 2018 and June 2019 prior to the malaria transmission seasons.

Control vaccines: Rabies vaccine *Rabipur*^{R}, produced by GSK was used as the control vaccine for the primary series of vaccinations during the dry season of 2017 for those allocated to the SMC alone group. Hepatitis A vaccine (HAVRIX^{R}), a licensed inactivated hepatitis A vaccine produced by GSK, was used for the booster dose in years 2 and 3.

Seasonal malaria chemoprevention: Four courses of SMC with SP+AQ were given at monthly intervals during the malaria transmission season in line with WHO's recommendation and national policy. A course of SMC for a child over the age of one year comprises a single treatment of SP (500mg sulphadoxine/25 mg pyrimethamine) and AQ 150mg on day 1 and AQ 150mg on days 2 and 3. Infants receive half of these doses. Children in the RTS,S alone group received a matching SP+AQ placebo. Dispersible form of SP and AQ and matching placebo were obtained from Guilin Pharmaceuticals, Shanghai, a GMP certified supplier. All doses of SMC were given under observation.

Implementation of the interventions

Vaccines: Syringes containing RTS,S/AS01 or the control vaccine were prepared by the chief vaccinator who took no other part in the trial. Loading of syringes with vaccines and masking with tape to blind the person administering the vaccine was done by a person who also took no further part in the trial. Vaccines were administered by study health staff trained to give vaccines.

Vaccination was undertaken in fixed or temporary centres established near study health centres or hospitals. The families of children scheduled for vaccination on a particular day were notified the day before this was due to take place and asked to bring their child to the vaccination centre on that day. In the case of families who lived a long distance from a vaccination centre, project transport was provided to bring the children and caretakers to the vaccination centre and to take them home. Children were retained at the vaccination centre for at least 30 minutes after vaccination to ensure that there were no immediate adverse events. Home visits were made to the families of children who missed vaccination on the designated day and their caretakers were asked if they would still like their child to receive vaccination (or SMC). If they agreed, they were asked to bring their children to the vaccination/drug administration centre. Some children were unable to attend for vaccination at the time that this took place in their community because their family had traveled or due to

an acute illness. A protocol amendment, approved by the data and safety monitoring board, allowed these children to receive the booster vaccination in 2018 and in 2019 if they presented for SMC administration later in the malaria transmission season.

SMC: SMC drugs were pre-packed by a pharmacist who took no further part in the trial, in re-sealable envelopes bearing the child's unique number and containing tablets for the four cycles of treatment appropriate for the child's age. Each dose of SMC or placebo was administered by trained project staff at a central point in the study village. Study children were given an identity card containing their photograph, 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. The Ministry of Health (MoH) introduced administration of SMC in the study areas in 2018. A memebr of the study team accompanied the MoH team to prevent the risk of study children receiving SMC from the routine system.

Insecticide treated bednets : All study children were given an ITN at enrolment in 2017 before the malaria transmission season.

Study outcomes

The primary trial outcome is clinical malaria, defined as an episode of illness characterised by fever (temperature >= 37.5^{o} C), or a history of fever within the previous 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 include the following:

(1) 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 previous 48 hours, with a positive blood film (any level of asexual parasitemia) or a positive Rapid Diagnostic Test (RDT).

(2) Hospital admissions with malaria, including cases of severe malaria which meet WHO criteria for a diagnosis of severe malaria.

(3) Malaria infection (symptomatic or asymptomatic parasitaemia) in a subset of randomly selected children seen during home visits.

(4) Malaria parasitaemia (including gametocytaemia), moderate and severe anaemia, and malnutrition at the end of the malaria transmission season.

(5) Serious adverse events (SAEs), including any deaths, with special reference to meningitis, cerebral malaria or immune deficiency illnesses.

(6) Anti-CSP concentrations obtained after priming and after each booster dose, determined in a sub-sample of children.

(7) The presence of molecular markers of resistance to SP and AQ in parasite positive samples collected at the last cross-sectional survey.

(8) The match of polymorphisms in the *P. falciparum circumsporozite* (*csp)* gene of parasite isolates obtained from children with clinical episodes of malaria to the genetic structure of the strain of parasite used to develop the RTS.S/AS01 vaccine.

Sample Size

Based on the sample size 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 a previous trial conducted in the same study areas [12]. Based on the results of a blinded interim analysis of an SMC+ azithromycin study [12], it was assumed that the incidence of clinical malaria confirmed by blood slide would be 300 cases per 1000 children over a calendar year in the SMC alone group. Results obtained over a period of three years of observation in the two study sites will be combined for the comparison of the primary endpoint but secondary analyses will evaluate impact at each study site separately.

Superiority: The trial is 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 used alone, and ii) estimate the efficacy of the combined group with a relatively high degree of precision. This latter aspect is important because it is necessary to show that adding RTS,S/AS01 to SMC has clinically significant benefit.

Based on the incidence of events observed in the trial after two years of follow-up, with approximately 2000 individuals in each arm, if the efficacy is 30% or more, 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 treatment groups. There will be 90% power for the lower limit of the confidence interval to exclude 15%, i.e. to establish that the protection from the combined group is at least 15% better than SMC or vaccination alone.

Non-inferiority: SMC for 4 months of the year has an efficacy, assuming receipt of all 4 monthly cycles, of about 85%. If, without intervention, the peak 4 months 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 we would be willing to accept, or would consider unimportant, if RTSS were to replace SMC. A reduction in efficacy from 50% to 40% translates to a 20% greater incidence in the RTSS-alone arm compared to the SMC-alone arm. The analysis plan will specify a margin of 20%, this takes into account the potential advantages of RTS,S over SMC in terms of ease of delivery and hence the likelihood of being able to sustain high levels of coverage. The trial has 80% power to exclude, at the 2.5% significance level, a relative difference in the incidence of clinical episodes of 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.

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 each subsequent booster dose. 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) will give 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 at each time point.

Follow-up and measurement of outcomes

Passive surveillance for cases of uncomplicated and severe malaria: Project staff based in the study hospitals and health centres that serve the study communities identify and document

all cases of malaria who present to these health facilities. Community health workers referred all suspected malaria cases to study health facilities. Blood films and filter paper strips are obtained from all these cases for subsequent confirmation of the diagnosis by microscopy.

Active surveillance for malaria: Each week, 24 randomly selected children (8 from each arm of the study) in each country are visited at home, their temperature measured and a blood film collected. Any child who is febrile or who has other features suggestive of malaria is tested with an RDT and treated with a full course of an artemisinin combination therapy (ACT) if positive.

Prevalence of malaria parasitaemia and anaemia: A survey of all study children is undertaken at least one month after the last round of SMC administration at the end of each malaria transmission season. During these surveys anthropometric measurements are taken and a finger prick blood samples is collected for preparing blood slides and blood spots. Children who are febrile are tested with an RDT and treated with an ACT if positive.

Malaria endemicity in the study area: A survey of schoolchildren aged 6-12 years resident in the same areas as the study children is conducted at the end of each malaria transmission season. Two hundred randomly selected school children per country (total 400) aged 6-12 years who are well and have not received SMC are tested for malaria by microscopy.

Measurement of the immune response to vaccination. Blood samples (2ml) have been collected from approximately 160 children in each of the groups (80 per country) who receive RTS,S/AS01 prior to administering the first dose of vaccine and one month after the third dose of the primary series of vaccination had been given In years 2 and 3 samples have been collected before and one month after administration of the fourth and fifth doses of vaccine for measurement of anti-CSP antibodies.

Measurement of resistance to the antimalarial drugs used for SMC. Dried blood spots from a randomly group of children who have malaria parasitaemia detected by microscopy at the last cross-sectional survey will be used for analysis of molecular markers of resistance to SP and AQ.

Serious Adverse Events (SAEs): Project staff based at the study hospitals have been provided with additional training on the recognition of meningitis, cerebral malaria and 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 developed by WHO for use in the pilot RTS,S/AS01 implementation trials are being applied. All deaths occurring outside the health facilities are assessed by verbal autopsy using the WHO 2016 verbal autopsy questionnaire [13]. Any SAEs that are (a) considered by the investigators 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 quarterly.

Laboratory methods

Detection of malaria: A histidine rich protein (HRP2) based RDT is used for the initial diagnosis of malaria and to guide treatment. Blood films collected at the same time are read subsequently by two readers following the guidelines developed for the phase 3 RTS.S/AS01 trial [14]. Slides which are judged to be discordant for either positivity or parasite density are read by a third reader, and the discrepancy resolved following the algorithm in the above study [14].

Measurement of haemoglobin concentration: Haemoglobin concentration is measured colourimetrically using a Hemocue colorimeter (Hemocue AB, Angelholm, Sweden).

Detection of markers of resistance to SP and amodiaquine: 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. 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.

Measurement of anti-CSP concentration: Antibodies to CSP will be measured by a standardised ELISA at the University of Ghent laboratory using a method applied in previous trials of RTS,S/AS01.

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 to look for the selection effect of RTS,S vaccine on malaria parasites [15].

Investigation of suspected cases of meningitis. The aetiology of cases of meningitis is determined by microscopical examination of cerebrospinal fluid samples for bacteria and white blood cells at the district hospital and then by subsequent PCR testing at a reference laboratory.

Data management

Data are collected using electronic case record forms (eCRF) developed using Open Data Kit (ODK) software. Tablet PCs loaded with eCRFs are available at all study health centres that provide treatment. This system is based on electronic transfer of the CRFs from the research sites. Automatic checks are performed on clinical and laboratory forms to ensure that they are complete and contain valid responses prior to transferring data.

Analysis plan

The primary endpoint of the trial is the incidence of all episodes of blood slide confirmed clinical malaria over the study period and this will be evaluated 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).

The primary analysis will be based on data for both sites combined, with site as a stratification factor, but site-specific analyses will also be undertaken. A test of interaction will be used to determine if there is any evidence that efficacy of RTSS varies between the two trial sites. An analysis will also be undertaken to assess efficacy of the booster dose of the vaccine each year.

The proportion of children in each group who experience at least one episode of malaria will be compared as a secondary outcome using Kaplan-Meier estimates of the risk. Evidence for provision of complete protection through the combination of SMC and vaccination each year will be explored using published methods [16, 17]. For the comparison of RTS,S/AS01 plus SMC versus the other two study groups, two-sided 95% confidence intervals for the hazard ratio will be calculated. For the non-inferiority comparison of RTS,S/AS01 to SMC, two-sided 90%, 95% and 99% confidence intervals for the rate ratio will be calculated, to indicate the degree of confidence with which the margin of 1.20 (a relative increase of 20%) can be

excluded. Primary analysis will be by modified ITT. According to protocol (ATP) analyses will also be performed.

An analysis plan will be prepared, for approval by the Data Safety Monitoring Board (DSMB) before the study code is broken. Primary analyses will be by modified ITT. ATP analyses will also be undertaken. Efforts would be made during the trial to ensure high levels of adherence to minimise differences between ITT and ATP populations. All children who received the first dose of vaccine will be included in the intention to treat analysis. (Children who did not attend for the first vaccine dose were withdrawn from the study). 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, and all scheduled doses of vaccine, will be included in the per protocol analysis for each year of the study. Additional sub-analyses will include analysis by age, gender, bed net use during the transmission season and socio-economic status.

Trial management

The London School of Hygiene & Tropical Medicine (LSHTM) is the main sponsor for the trial. Delegated responsibilities are assigned locally. The LSHTM holds Public Liability ("negligent harm'') and Clinical Trial ("non-negligent harm'') insurance policies which apply to this trial. An independent trial steering committee provides scientific oversight and has approved the protocol. The steering committee holds teleconferencing or face-face meeting annually to monitor progress and advise on the scientific content of the study. The quality of data and the GCP standards of the trial are monitored by an independent, experienced GCP monitor.

ETHICS AND DISSEMINATION

Ethics

Inclusion in the trial of an RTS,S/AS01 alone group is justified, even though SMC is recommended policy, on the grounds that RTS,S/AS01 could provide some added protection outside the main transmission season when SMC is not being given and that it may be easier to administer than SMC. Individual, written, informed consent has been obtained from the family or legally recognised guardian of each child entered into the trial. Conduct of the trial does not impose any additional costs on the local health services. The project contributes to

the costs of routine clinical care of study subjects during the trial and to strengthening of clinical care at the district hospitals in the study areas.

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. The trial has also been approved by the regulatory authorities in Burkina Faso and Mali. An independent Data Safety Monitoring Board (DSMB) monitors regularly the safety of children in the trial, especially those in the RTS,S/AS01 alone group who are not receiving SMC which is standard of care. In February 2018, the board reviewed unblinded data on the incidence of serious adverse events (SAEs) and SAEs due to malaria in each study group and gave permission for the trial to proceed for a further year retaining all three study arms.

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 is registered on clinicaltrials.gov (https://www.clinicaltrials.gov/ct2/show/NCT03143218?term=NCT03143218&rank=1).

Dissemination plans

Results from the trial will be discussed with the study communities at the end of the study, presented at national and international conferences and in peer reviewed, open access journals. Trial results will be shared with the WHO's technical expert groups and Malaria Policy Advisory Group. Strong links have been established already with the Ministries of Health, NMCPs and EPI programmes in Burkina Faso and Mali. The trial team has also established good links with many other organisations involved in the delivery of SMC trials including the SMC ACCESS programme 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.

AUTHORS CONTRIBUTION

DC and BG wrote the first draft of the manuscript, AD, IZ, IS, HT, OO and JB reviewed the manuscript and made significant contributions to the protocol, MC, IK, IS and PM wrote sections on sample size, data management and analysis plan, MD, IT, AT, DI, KS, AM, FS, and SY reviewed the paper and are involved in the field operations of the study.

FUNDING

The trial is being funded by the UK Joint Global Health Trials (The Department of Health and Social Care, the Department for International Development, the Global Challenges Research Fund, the Medical Research Council and the Wellcome Trust) grant no. MR/P006876/1, with supplementary funding from PATH MVI, grant no. 18269.

COMPETING INTERESTS

All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf and declare: no support from any organisation for the submitted work; PM is in receipt of a grant for Statistical support for the Malaria Vaccine Implementation Project; OO-A declares that she is an employee of the GlaxoSmithKline group of companies and is an owner of GSK stocks and stock options; no other relationships or activities that could appear to have influenced the submitted work.

REFERENCES

1. Cohen J, Nussenzweig V, Nussenzweig R et al. From the circumsporozoite protein to the RTS,S/AS candidate vaccine. *Human Vaccin* 2010; 6:90-6.
2. RTS,S Clinical Trials Partnership. Efficacy and safety of the RTS,S/AS01 malaria vaccine with or without a booster dose in infants and children in Africa: final results of a phase 3, individually randomised, controlled trial. *Lancet* 2015; 386: 31-45.
3. Bojang K, Milligan PJ, Pinder M et al. Efficacy of RTS,S/AS02 malaria vaccine against *Plasmodium falciparum* infection in semi-immune adult men in The Gambia: a randomised trial. *Lancet.* 2001;358:1927-34.
4. https://www.who.int/immunization/sage/meetings/2015/october/1_Final_malaria_vaccine_background_paper_v2015_09_30.pdf
5. Klein S, Shann F, Moss W, Benn C, Aaby P. RTS,S Malaria Vaccine and Increased Mortality in Girls. mBio. 2016; 7(2):e00514-16.
6. Wilson AL. A systematic review and meta-analysis of the efficacy and safety of intermittent preventive treatment in children (IPTc). Plos One 2011; 6: e16976.
7. Dicko A, Diallo AI, Tembine I et al. Intermittent preventive treatment of malaria provides substantial protection against malaria in children already protected by an insecticide-treated bednet in Mali: a randomised, double-blind, placebo-controlled trial. *PLoS Med* 2011; 8: 1000407.
8. Konate AT, Yaro JB, Ouedraogo AZ et al. Intermittent preventive treatment of malaria provides substantial protection against malaria in children already protected by an insecticide-treated bednet in Burkina Faso: a randomised, double-blind, placebo-controlled trial. *PLoS Med* 2011; 8: e1000408.
9. Ndiaye JL, Cissé B, Ba EH et al. Safety of seasonal intermittent preventive treatment against malaria with sulfadoxine pyrimethamine + amodiaquine when delivered to children under 10 years of age by district health staff in Senegal. *PLoS One*, in press.
10. (http://www.malariaconsortium.org/pages/access-smc.htm).
11. Chandramohan D, Dicko A, Zongo I et al. Effect of Adding Azithromycin to Seasonal MalariaChemoprevention. N Engl J Med 2019;380:2197-206.
12. Piaggio G, Elbourne DR, Altman DG, Pocock SJ, Evans SJ. Reporting of noninferiority and equivalence randomized trials: an extension of the CONSORT statement. Jama. 2006 Mar 8;295(10):1152-60. PubMed PMID: 16522836.

13. Leitao J, Chandramohan D, Byass P, et al. Revising the WHO verbal autopsy instrument to facilitate routine cause-of-death monitoring. Glob Health Action. 2013;6:21518

14. Swysen C, Vekemans J, Bruls M et al. Development of standardized laboratory methods and quality processes for a phase iii study of the RTS,S/AS01 candidate malaria vaccine. *Mal J 2011;* 10: e223.

15. Neafsey DE, Juraska M, Bedford T,et al Genetic diversity and protective efficacy of the RTS,S/AS01 malaria vaccine. N Engl J Med 2015; 373: 2025-2037.

16. Xu Y, Lam KF, Cheung YB. Estimation of intervention effects using recurrent event time data in the presence of event dependence and a cured fraction. Stat Med. 2014 Jan 22. PubMed PMID: 24449504. Epub 2014/01/23. Eng.

17. Cairns M, Cheung YB, Xu Y, Asante KP, Owusu-Agyei S, Diallo D, et al. Analysis of Preventive Interventions for Malaria: Exploring Partial and Complete Protection and Total and Primary Intervention Effects. Am J Epidemiol. 2015 Jun 15;181(12):1008-17. PubMed PMID: 26022663. Pubmed Central PMCID: PMC4462336. Epub 2015/05/30.eng.

TABLES AND FIGURES

Table 1. Study groups and the interventions that they received.

	Group1	**Group2**	**Group3**
	RTS,S/AS01 alone	**SMC alone**	**SMC + RTS,S/AS01**
Year 1 April-June July-Oct	 RTSS/AS01 x 3 SMC placebo* x 4	 Rabies vaccine x 3 SMC* x 4	 RTSS/AS01 x 3 SMC* x 4
Year 2 June July-Oct	 RTSS/AS01 x 1 SMC placebo* x 4	 HepA vaccine x 1 SMC* x 4	 RTSS/AS01 x 1 SMC* x 4
Year 3 June July-Oct	 RTSS/AS01 x 1 SMC placebo* x 4	 HepA vaccine x 1 SMC* x 4	 RTSS/AS01 x 1 SMC* x 4

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

Figure 1: Study districts in Mali and Burkina Faso

Figure 2: study design

Figure 1: Study districts in Mali and Burkina Faso

Figure 2
Study Design

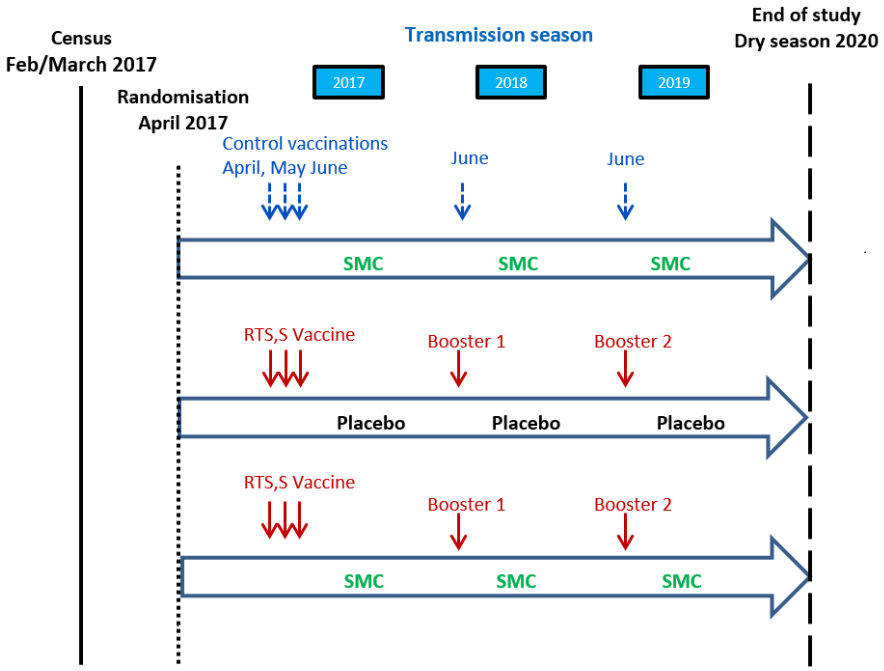

Figure 2: Study Design

BMJ Open

Seasonal Malaria Vaccination: protocol of a Phase III trial of seasonal vaccination with the RTS,S/AS01E vaccine, seasonal malaria chemoprevention and the combination of vaccination and chemoprevention

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2019-035433.R2
Article Type:	Protocol
Date Submitted by the Author:	27-May-2020
Complete List of Authors:	Chandramohan, Daniel; London School of Hygiene and Tropical Medicine, Disease Control Slater, Karen; London School of Hygiene and Tropical Medicine Faculty of Infectious and Tropical Diseases, Dicko, Alassane; Malaria Research and Training Center Zongo, Issaka; Institut de Recherche en Sciences de la Santé (IRSS) Sagara, Issaka; Malaria Research and Training Center Cairns, Matthew; London School of Hygiene and Tropical Medicine, Infectious Disease Epidemiology Kuepfer, Irene; London School of Hygiene and Tropical Medicine, Disease Control Diarra, Modibo; Malaria Research and Training Center Tapily, Amadou; Malaria Research and Training Center Issiaka, Djibrilla; Malaria Research and Training Center Sanogo, Koualy; Malaria Research and Training Center Mahamar, Almahamoudou; Malaria Research and Training Center Sompougdou , Frederic; Institut de Recherche en Sciences de la Santé (IRSS) Yerbanga, Serge; Institut de Recherche en Sciences de la Santé (IRSS) Thera, Ismaila; Malaria Research and Training Center Milligan, Paul; London School of Hygiene and Tropical Medicine, Infectious Disease Epidemiology Tinto, Halidou; Institut de Recherche en Sciences de la Santé (IRSS) Ofori-Anyinam, Opokua; GlaxoSmithKline Biologicals SA Ouedraogo, Jean-Bosco; Institut de Recherche en Sciences de la Santé (IRSS) Greenwood, B.; London School of Hygiene and Tropical Medicine, Disease Control
Primary Subject Heading:	Epidemiology
Secondary Subject Heading:	Epidemiology, Global health
Keywords:	Immunology < TROPICAL MEDICINE, Tropical medicine < INFECTIOUS DISEASES, Epidemiology < TROPICAL MEDICINE

I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our licence.

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which Creative Commons licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

SEASONAL MALARIA VACCINATION: PROTOCOL OF A PHASE III TRIAL OF SEASONAL VACCINATION WITH THE RTS,S/AS01$_E$ VACCINE, SEASONAL MALARIA CHEMOPREVENTION AND THE COMBINATION OF VACCINATION AND CHEMOPREVENTION

Daniel Chandramohan PhD[1], Alassane Dicko MD[2], Issaka Zongo PhD[3], Issaka Sagara MD[2], Matthew Cairns PhD[1], Irene Kuepfer PhD[1], Modibo Diarra MD[2], Amadou Tapily MD[2], Djibrilla Issiaka MD[2], Koualy Sanogo MD[2], Almahamoudou Mahamar PharmD[2], Frederic Sompougdou MD[3], Serge Yerbanga PhD[3], Ismaila Thera MPH[2], Paul Milligan PhD[1], Halidou Tinto PhD[3], Opokua Ofori-Anyinam[4], Jean-Bosco Ouedraogo PhD[3], Brian Greenwood MD[1]

[1]London School of Hygiene & Tropical Medicine, London, United Kingdom; [2]Malaria Research and Training Centre (MRTC), University of Science, Techniques and Technologies of Bamako, Bamako, Mali; [3] Institut de Recherche en Sciences de la Santé (IRSS), Bobo-Dioulasso, Burkina Faso. [4] GlaxoSmithKline Biologicals SA (Vaccines)

Corresponding Author: Prof Daniel Chandramohan, Faculty of Infectious and Tropical Diseases, London School of Hygiene& Tropical Medicine, Keppel St., London WC1E 7HT, UK. Email: Daniel.chandramohan@lshtm.ac.uk

ABSTRACT

Introduction: Seasonal malaria chemoprevention (SMC), with sulphadoxine-pyrimethamine plus amodiaquine (SP+AQ) is effective but does not provide complete protection against clinical malaria. The RTS,S/AS01 malaria vaccine provides a high level of protection shortly after vaccination but this wanes rapidly. Such a vaccine could be an alternative or additive to SMC. This trial aims to determine whether seasonal vaccination with RTS,S/AS01$_E$ vaccine could be an alternative to SMC and whether a combination of the two interventions would provide added benefits.

Methods and Analysis: This is an individually randomised, double-blind, placebo-controlled trial. 5920 children aged 5-17 months were enrolled in April 2017 in Mali and Burkina Faso. Children in group 1 received three priming doses of RTS,S/AS01$_E$ vaccine before the start of the 2017 malaria transmission season and a booster dose at the beginning of two subsequent transmission seasons. In addition, they received SMC SP+AQ placebo on four occasions each year. Children in group 2 received three doses of rabies vaccine in year one and Hepatitis A vaccine in years 2 and 3 together with four cycles of SMC SP+AQ each year. Children in group 3 received RTS,S/AS01$_E$ vaccine and four courses of SMC SP+AQ. Incidence of clinical malaria is determined by case detection at health facilities. Weekly active surveillance for malaria is undertaken in a randomly selected sub-set of children. The prevalence of malaria is measured in surveys at the end of each transmission season. The primary endpoint is the incidence of clinical malaria confirmed by a positive blood film with a minimum parasite density of 5000 per µl. Primary analysis will be by modified intention to treat defined as children who have received the first dose of the malaria or control vaccine.

Ethics and Dissemination: The protocol was approved by the national ethics committees of Mali and Burkina Faso and the London School of Hygiene & Tropical Medicine. The results will be presented to all stakeholders and published in open access journals.

Registration

The trial is registered with clinicaltrials.gov. (https://www.clinicaltrials.gov/ct2/show/NCT03143218?term=NCT03143218&rank=1).

Strengths and limitations of this study

- One major strength of this trial is that it is exploring a novel approach of seasonal vaccination to malaria control in an area where the burden of malaria remains very high despite a high coverage of currently recommended interventions.
- The study is adequately powered to test the non-inferiority hypotheses which requires a large sample size.
- A third strength of the trial is that the clinical impact of the two interventions is supported by laboratory studies which will measure the impact of SMC on drug resistance and of RTS,S/AS01$_E$ on potential vaccine escape parasites.
- Despite establishment of an intensive surveillance system, some episodes of malaria and some serious adverse events may be missed but because the trial is individually randomised these should be distributed equally among study groups.
- To apply the trial results to areas where the seasonal malaria transmission duration and pattern is different, modelling may be needed.

INTRODUCTION

The RTS,S/AS01$_E$ malaria vaccine is a recombinant protein vaccine that contains a component of the circumsporozoite protein of *Plasmodium falciparum* fused to hepatitis B surface antigen (HBsAg), 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$_E$ [1]. A phase 3 trial of RTS,S/AS01$_E$ conducted in 15,439 children in seven countries in Africa showed that three doses of RTS,S/AS01$_E$ given at monthly intervals, followed by a fourth dose 18 months later, gave 36.5 % [95% CI 31%,41%] protection against clinical attacks of malaria in children aged 5-17 months who were followed for 48 months [2]. RTS,S/AS01$_E$ provides a high level of protection during the first three months after vaccination [3], estimated to be 68% in the phase 3 trial [4]. The main safety issues related to administration of RTS,S/AS01$_E$ detected in the phase 3 trial were 1) an unexplained, statistically significant, increase in cases of meningitis observed in children given the vaccine at the age of 5-17 months, 2) a possible increase in the proportion of severe cases of malaria that were classified as cerebral malaria and 3) a gender imbalance in the small number of deaths recorded during the trial [5].

Seasonal Malaria Chemoprevention (SMC) involves monthly administration of a full therapeutic dose of an antimalarial drug or drug combination to children on three or four occasions during the period of highest risk of malaria infection. Studies undertaken in several countries in West Africa have shown that SMC with a combination of sulphadoxine/pyrimethamine (SP) and amodiaquine (AQ) (SP+AQ) is highly effective in reducing the incidence of severe and uncomplicated malaria in areas where the transmission of malaria is markedly seasonal [6-8]. SMC with SP+AQ is very safe [9]. Many countries in the Sahel and sub-Sahel region of West Africa have now incorporated SMC into their national malaria control programme achieving high levels of coverage [10] but malaria continues to be a major cause of mortality and morbidity in these countries and additional control tools are needed. Adding azithromycin to the anti-malarial drugs used for SMC did not reduce hospital admissions or deaths from non-traumatic causes [11]. Thus, determining whether adding RTS,S/AS01$_E$ would provide valuable additional protection is important. The primary objectives of the trial are to determine - (1) whether seasonal vaccination following priming with the RTS,S/AS01$_E$ malaria vaccine is non-inferior in preventing malaria to SMC with SP + AQ in children living in the areas of the Sahel and sub-Sahel of Africa where malaria transmission is highly seasonal (2) whether RTS,S/AS01$_E$ can provide additional,

useful and cost effective protection against malaria if given together with SMC in areas with highly seasonal malaria transmission.

METHODS

This is an individually randomised, double-blind, placebo controlled trial with three groups. The trial will be conducted and reported according to CONSORT guidelines [12].

Study Setting

The trial is being conducted in Houndé district, Burkina Faso and in Bougouni district, Mali (Figure 1). Malaria, due predominantly to *P. falciparum,* is highly seasonal in both districts. The prevalence of *P. falciparum* malaria in school age children in December 2016 was 53% in Bougouni and 50% in Houndé [11]. The main malaria vector in both study areas is *Anopheles gambiae* ss. In 2016, a high proportion of children slept under an insecticide treated bednet (ITN) in Bougouni (95%) but the percentage was slightly lower in Houndé (79.9%). The first line treatment for malaria in the public health system in both districts is artemether-lumefantrine. The incidence of parasite confirmed malaria was 1068 per 1000 child years at risk during the transmission season among 3-59 month old children who were allocated to the SMC group in a trial conducted in Bougouni and Houndé districts in 2014-2016 [11]. Malaria is seasonal, with 79.6% of annual cases occurring over 5 months of the year from July to November in Bougouni and 81.0% between July to November in Houndé.

Study population

All households within the study areas with children 5-17 months of age on April 1st 2017 were enumerated in February-March 2017 and children were screened for their eligibility to enter the trial. A child was considered eligible 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 on April 1st 2017. A child was deemed to be ineligible if (a) the child was a transient resident; (b) the age of the child was outside the stipulated range; (c) the child had a history of an adverse reaction to SP or AQ; (d) the child had a serious underlying illness (self-reported or obtained from health records), 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); (e) the child was known to have an immune deficiency disease or was receiving an immunosuppressive drug; (f) the child had previously received a

malaria vaccine; (g) the child was enrolled in another malaria intervention trial. Written, informed consent (supplement) was obtained by study staff from their caretakers of eligible children prior to their inclusion in the trial.

Each eligible child was assigned a unique census identification number (ID) 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 were updated in April/May 2018 and 2019 prior to the administration of the booster doses of vaccine.

The study started on February 1st 2017 and is expected to be completed on June 30th 2020. (Figure2)

Patient and Public Involvement

No patients are involved in this trial. Healthy children were enrolled with the consent of their care takers. Care takers were not involved in the development of the research question or study design.

Randomisation

Eligible children who were consented soon after the census were allocated randomly to one of the three study groups (Table 1). The randomization list was prepared using permuted blocks after sorting the list of eligible children by age, gender, area of residence, and prior receipt of SMC, to ensure these characteristics would be balanced between the three groups. An independent information technology consultant loaded the randomisation list on four Tablet PCs and handed over two PCs each to the chief pharmacist in Mali and Burkina. The chief pharmacists used these PCs to scan the QR code containing the child's name and study ID printed on the ID cards of children to find out the vaccine allocated to each child. These PCs were locked in the vaccine room and were accessible to the chief pharmacists only.

Interventions

RTS,S/AS01$_E$ *vaccine:* Three doses of RTS,S/AS01$_E$ were given to children allocated to the RTSS or RTSS+SMC groups at approximately monthly intervals (window 3-8 weeks) in April – June 2017 followed by a fourth and fifth dose given in June 2018 and June 2019 prior to the malaria transmission seasons.

Control vaccines: Rabies vaccine *Rabipur*^{R}, produced by GSK, was used as the control vaccine for the primary series of vaccinations during the dry season of 2017 for those allocated to the SMC alone group. Hepatitis A vaccine (HAVRIX^{R}), a licensed inactivated hepatitis A vaccine produced by GSK, was used for the booster dose in years 2 and 3.
Seasonal malaria chemoprevention: Four courses of SMC with SP+AQ were given at monthly intervals during the malaria transmission season in line with WHO's recommendation and national policy. A course of SMC for a child over the age of one year comprises a single treatment of SP (500mg sulphadoxine/25 mg pyrimethamine) and AQ 150mg on day 1 and AQ 150mg on days 2 and 3. Infants receive half of these doses. Children in the RTS,S alone group received a matching SP+AQ placebo. Dispersible forms of SP and AQ and matching placebo were obtained from Guilin Pharmaceuticals, Shanghai, a GMP certified supplier. All doses of SMC were given under observation.

Implementation of the interventions

Vaccines: Syringes containing RTS,S/AS01$_{E}$ or the control vaccine were prepared by the chief 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 was done by a member of the study staff who also took no further part in the trial. Vaccines were administered by study health staff trained to give vaccines.

Vaccination was undertaken in fixed or temporary centres established near study health centres or hospitals. The families of children scheduled for vaccination on a particular day were notified the day before this was due to take place and asked to bring their child to the vaccination centre on that day. In the case of families who lived a long distance from a vaccination centre, project transport was provided to bring the children and caretakers to the vaccination centre and to take them home. Children were retained at the vaccination centre for at least 30 minutes after vaccination to ensure that there were no immediate adverse events. Home visits were made to the families of children who missed vaccination on the designated day and their caretakers were asked if they would still like their child to receive vaccination (or SMC). If they agreed, they were asked to bring their children to the vaccination/drug administration centre. Some children were unable to attend for vaccination at the time that this took place in their community because their family had traveled or due to an acute illness. A protocol amendment, approved by the data and safety monitoring board,

allowed these children to receive the booster vaccination in 2018 and in 2019 if they presented for SMC administration later in the malaria transmission season.

SMC: SMC drugs were pre-packed by a pharmacist who took no further part in the trial, in re-sealable envelopes bearing the child's unique number and containing tablets for the four cycles of treatment appropriate for the child's age. Each dose of SMC or placebo was administered by trained project staff at a central point in the study village. Study children were given an identity card containing their photograph, study identity number and date of birth. At the time of vaccination and/or SMC administration, a child's photo ID card was scanned to ensure that the child was given the allocated intervention. The Ministry of Health (MoH) introduced administration of SMC in the study areas in Mali and Burkina Faso in 2018. A member of the study team accompanied the MoH team to prevent the risk of study children receiving SMC again from the routine system.

Insecticide treated bednets(TTN): All study children were given an ITN at enrolment in 2017 before the malaria transmission season.

Study outcomes

The primary trial outcome is clinical malaria, defined as an episode of illness characterised by fever (temperature >= 37.5^{o} C), or a history of fever within the previous 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 include the following:

(1) 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 previous 48 hours, with a positive blood film (any level of asexual parasitemia) or a positive Rapid Diagnostic Test (RDT).

(2) Hospital admissions with malaria, including cases of severe malaria which meet WHO criteria for a diagnosis of severe malaria.

(3) Malaria parasitaemia (symptomatic or asymptomatic) in a subset of randomly selected children seen during home visits.

(4) Malaria parasitaemia (asymptomatic or symptomatic), moderate or severe anaemia, and malnutrition at the end of the malaria transmission season.

(5) Serious adverse events (SAEs), including any deaths, with special reference to meningitis, cerebral malaria or immune deficiency illnesses.

(6) Anti-CSP concentrations obtained after priming and after each booster dose, determined in a sub-sample of children.

(7) The presence of molecular markers of resistance to SP and AQ in parasite positive samples collected at the last cross-sectional survey.

(8) The match of polymorphisms in the *P. falciparum circumsporozite* (*csp)* gene of parasite isolates obtained from children with clinical episodes of malaria to the genetic structure of the strain of parasite used to develop the RTS.S/AS01 vaccine.

Sample Size

Based on the sample size 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 a previous trial conducted in the same study areas [12]. Based on the results of a blinded interim analysis of an SMC+ azithromycin study [12], it was assumed that the incidence of clinical malaria confirmed by blood slide would be 300 cases per 1000 children over a calendar year in the SMC alone group. Results obtained over a period of three years of observation in the two study sites will be combined for the comparison of the primary endpoint but secondary analyses will evaluate impact at each study site separately. 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$_E$ to SMC has a clinically significant benefit.

Superiority: The trial is 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 used alone, and ii) estimate the efficacy of the combined group with a relatively high degree of precision. This

latter aspect is important because it is necessary to show that adding RTS,S/AS01$_E$ to SMC has clinically significant benefit.

Based on the incidence of events observed in the trial after two years of follow-up, with approximately 2000 individuals in each arm, if the efficacy is 30% or more, 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 treatment groups. There will be 90% power for the lower limit of the confidence interval to exclude 15%, i.e. to establish that the protection from the combined group is at least 15% better than SMC or vaccination alone.

Non-inferiority: SMC for 4 months of the year has an efficacy, assuming receipt of all 4 monthly cycles, of about 85%. If, without intervention, the peak 4 months 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 we would be willing to accept, or would consider unimportant, if RTSS were to replace SMC. A reduction in efficacy from 50% to 40% translates to a 20% greater incidence in the RTSS-alone arm compared to the SMC-alone arm. The analysis plan will specify a margin of 20%, this takes into account the potential advantages of RTS,S over SMC in terms of ease of delivery and hence the likelihood of being able to sustain high levels of coverage. The trial has 80% power to exclude, at the 2.5% significance level, a relative difference in the incidence of clinical episodes of malaria of 20% over the three year study period between the RTS,S/AS01$_E$ and SMC alone groups, if these two interventions were equally effective.

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

Follow-up and measurement of outcomes

Passive surveillance for cases of uncomplicated and severe malaria: Project staff based in the study hospitals and health centres that serve the study communities identify and document

all cases of malaria who present to these health facilities. Community health workers refer all suspected malaria cases to study health facilities. Blood films and filter paper strips are obtained from all these cases for subsequent confirmation of the diagnosis by microscopy.

Active surveillance for malaria: Each week, 24 randomly selected children (8 from each arm of the study) in each country are visited at home, their temperature measured and a blood film collected. Any child who is febrile or who has other features suggestive of malaria is tested with an RDT and treated with a full course of an artemisinin combination therapy (ACT) if positive.

Prevalence of malaria parasitaemia and anaemia: A survey of all study children is undertaken at least one month after the last round of SMC administration at the end of each malaria transmission season. During these surveys, anthropometric measurements are taken and a finger prick blood samples is collected for preparing blood slides and blood spots. Children who are febrile are tested with an RDT and treated with an ACT if positive.

Malaria endemicity in the study area: A survey of schoolchildren aged 6-12 years resident in the same areas as the study children is conducted at the end of each malaria transmission season. Two hundred randomly selected school children per country (total 400) aged 6-12 years who are well and have not received SMC are tested for malaria by microscopy.

Measurement of the immune response to vaccination. Blood samples (2ml) have been collected from approximately 160 children in each of the groups (80 per country) who received RTS,S/AS01$_E$ prior to administering the first dose of vaccine and one month after the third dose of the primary series of vaccination had been given. In years 2 and 3 samples have been collected before and one month after administration of the fourth and fifth doses of vaccine for measurement of anti-CSP antibodies.

Measurement of resistance to the antimalarial drugs used for SMC. Dried blood spots from a randomly group of children who have malaria parasitaemia detected by microscopy at the last cross-sectional survey will be used for analysis of molecular markers of resistance to SP and AQ.

Serious Adverse Events (SAEs): Project staff based at the study hospitals have been provided with additional training on the recognition of meningitis, cerebral malaria and 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 developed by WHO for use in the pilot RTS,S/AS01$_E$ implementation trials are being applied. All deaths occurring outside the health facilities are assessed by verbal autopsy using the WHO 2016 verbal autopsy questionnaire [13]. Any SAEs that are (a) considered by the investigators 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 quarterly.

Laboratory methods

Detection of malaria: A histidine rich protein (HRP2) based RDT is used for the initial diagnosis of malaria and to guide treatment. Blood films collected at the same time are read subsequently by two readers following the guidelines developed for the phase 3 RTS,S/AS01$_E$ trial [14]. Slides which are judged to be discordant for either positivity or parasite density are read by a third reader, and the discrepancy resolved following the algorithm used in the above study [14].

Measurement of haemoglobin concentration: Haemoglobin concentration is measured colourimetrically using a Hemocue colorimeter (Hemocue AB, Angelholm, Sweden).

Detection of markers of resistance to SP and amodiaquine: 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. 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.

Measurement of anti-CSP concentration: Antibodies to CSP will be measured by a standardised ELISA at the University of Ghent laboratory using a method applied in 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 to look for the selection effect of RTS,S/AS01$_E$ vaccine on malaria parasites [15].

Investigation of suspected cases of meningitis. The aetiology of cases of meningitis is determined by microscopical examination of cerebrospinal fluid samples for bacteria and white blood cells at the district hospital and then by subsequent PCR testing at a reference laboratory.

Data management

Data are collected using electronic case record forms (eCRF) developed using Open Data Kit (ODK) software. Tablet PCs loaded with eCRFs are available at all study health centres that provide treatment. This system is based on electronic transfer of the CRFs from the research sites. Automatic checks are performed on clinical and laboratory forms to ensure that they are complete and contain valid responses prior to transferring data. Electronic case record forms will be deposited with the study data on the LSHTM Data Compass System (http://datacompass.lshtm.ac.uk). All personal identifiers will be removed from the study database before archiving. The data will be assigned a Digital Object Identifier. 12 months after completing the analysis the data will be made available in the London School of Hygiene & Tropical Medicine data depository. A Data Access Group will review requests to share archived data.

Analysis plan

The primary analysis will be by modified intention to treat (mITT). The mITT population will include all children who were screened and who received the first dose of RTS,S/AS01$_E$ or control vaccine, irrespective of the number of doses of subsequent vaccines or SMC/SMC placebo received. The primary endpoint of the trial is the incidence of all episodes of blood slide confirmed clinical malaria over the study period and this will be evaluated 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). 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. This preserves the overall type I error rate at 5%. There were no formal interim analyses planned or conducted.

The primary analysis will be based on data for both sites combined, with site as a stratification factor, but site-specific analyses will also be undertaken. A test of interaction will be used to determine if there is any evidence that efficacy of RTS,S varies between the two trial sites. An analysis will also be undertaken to assess efficacy of the booster dose of the vaccine each year.

The proportion of children in each group who experience at least one episode of malaria will be compared as a secondary outcome using Kaplan-Meier estimates of the risk. Evidence for provision of complete protection through the combination of SMC and vaccination each year will be explored using published methods [16, 17]. For the comparison of RTS,S/AS01$_E$ plus SMC versus the other two study groups, two-sided 95% confidence intervals for the hazard ratio will be calculated. For the non-inferiority comparison of RTS,S/AS01$_E$ to SMC, two-sided 90%, 95% and 99% confidence intervals for the rate ratio will be calculated, to indicate the degree of confidence with which the margin of 1.20 (a relative increase of 20%) can be excluded.

An analysis plan will be prepared, for approval by the Data Safety Monitoring Board (DSMB) before the study code is broken. Primary analyses will be by modified ITT. ATP analyses will also be undertaken. Efforts would be made during the trial to ensure high levels of adherence to minimise differences between ITT and ATP populations. All children who received the first dose of vaccine will be included in the intention to treat analysis. (Children who did not attend for the first vaccine dose were withdrawn from the study). 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, and all scheduled doses of vaccine, will be included in the per protocol analysis for each year of the study. Additional sub-analyses will include analysis by age, gender, bed net use during the transmission season and socio-economic status.

Trial management

The London School of Hygiene & Tropical Medicine (LSHTM) is the main sponsor for the trial. Delegated responsibilities are assigned locally. The LSHTM holds Public Liability (“negligent harm’’) and Clinical Trial (“non-negligent harm’’) insurance policies which apply to this trial. An independent trial steering committee provides scientific oversight and has approved the protocol. The steering committee holds teleconferencing or face-face meeting annually to monitor progress and advise on the scientific content of the study. The quality of

data and the GCP standards of the trial are monitored by an independent, experienced GCP monitor.

ETHICS AND DISSEMINATION

Ethics

Inclusion in the trial of an RTS,S/AS01$_E$ alone group is justified, even though SMC is recommended policy, on the grounds that RTS,S/AS01$_E$ could provide some added protection outside the main transmission season when SMC is not being given and that it may be easier to administer than SMC. Individual, written, informed consent has been obtained from the family or legally recognised guardian of each child entered into the trial. Conduct of the trial does not impose any additional costs on the local health services. The project contributes to the costs of routine clinical care of study subjects during the trial and to strengthening of clinical care at the district hospitals in the study areas.

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 (protocol version 2; dated 09 December 2016). The trial has also been approved by the regulatory authorities in Burkina Faso and Mali. An independent Data Safety Monitoring Board (DSMB) monitors regularly the safety of children in the trial, especially those in the RTS,S/AS01$_E$ alone group who are not receiving SMC which is standard of care. In February 2018, the board reviewed unblinded data on the incidence of serious adverse events (SAEs) and SAEs due to malaria in each study group and gave permission for the trial to proceed for a further year retaining all three study arms.

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 is registered on clinicaltrials.gov (https://www.clinicaltrials.gov/ct2/show/NCT03143218?term=NCT03143218&rank=1).

Dissemination plans

Results from the trial will be discussed with the study communities at the end of the study, presented at national and international conferences and in peer reviewed, open access

journals. Trial results will be shared with the WHO's technical expert groups and Malaria Policy Advisory Group. Strong links have been established already with the Ministries of Health, NMCPs and EPI programmes in Burkina Faso and Mali. The trial team has also established good links with many other organisations involved in the delivery of SMC trials including the SMC ACCESS programme and with the WHO staff responsible for conducting the RTS,S/AS01$_E$ implementation studies Thus, if it is found that RTS,S/AS01$_E$ vaccine is a useful replacement or addition to SMC regimens, routes have already been established through which this knowledge could be disseminated rapidly.

AUTHORS CONTRIBUTION

DC and BG wrote the first draft of the manuscript, AD, IZ, IS, HT, OO-A and J-BO reviewed the manuscript and made significant contributions to the protocol, MC, IK, IS and PM wrote sections on sample size, data management and analysis plan, MD, IT, AT, DI, KS, AM, FS, and SY reviewed the paper and are involved in the field operations of the study.

FUNDING

The trial is being funded by the UK Joint Global Health Trials (The Department of Health and Social Care, the Department for International Development, the Global Challenges Research Fund, the Medical Research Council and the Wellcome Trust) grant no. MR/P006876/1, with supplementary funding from PATH MVI, grant no. 18269. The trial is sponsored by the London School of Hygiene and Tropical Medicine (LSHTM), Keppel Street, London WC1E 7HT. The investigators from the LSHTM are responsible for the design, data collection, analysis, interpretation and writing the report. The funding organisations have no role in these aspects.

COMPETING INTERESTS

All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf and declare: no support from any organisation for the submitted work; PM is in receipt of a grant for Statistical support for the Malaria Vaccine Implementation Project; OO-A declares that she is an employee of the GlaxoSmithKline group of companies and is an owner of GSK stocks and stock options; no other relationships or activities that could appear to have influenced the submitted work.

REFERENCES

1. Cohen J, Nussenzweig V, Nussenzweig R et al. From the circumsporozoite protein to the RTS,S/AS candidate vaccine. *Human Vaccin* 2010; 6:90-6.
2. RTS,S Clinical Trials Partnership. Efficacy and safety of the RTS,S/AS01 malaria vaccine with or without a booster dose in infants and children in Africa: final results of a phase 3, individually randomised, controlled trial. *Lancet* 2015; 386: 31-45.
3. Bojang K, Milligan PJ, Pinder M et al. Efficacy of RTS,S/AS02 malaria vaccine against *Plasmodium falciparum* infection in semi-immune adult men in The Gambia: a randomised trial. *Lancet.* 2001;358:1927-34.
4. https://www.who.int/immunization/sage/meetings/2015/october/1_Final_malaria_vaccine_background_paper_v2015_09_30.pdf
5. Klein S, Shann F, Moss W, Benn C, Aaby P. RTS,S Malaria Vaccine and Increased Mortality in Girls. mBio. 2016; 7(2):e00514-16.
6. Wilson AL. A systematic review and meta-analysis of the efficacy and safety of intermittent preventive treatment in children (IPTc). Plos One 2011; 6: e16976.
7. Dicko A, Diallo AI, Tembine I et al. Intermittent preventive treatment of malaria provides substantial protection against malaria in children already protected by an insecticide-treated bednet in Mali: a randomised, double-blind, placebo-controlled trial. *PLoS Med* 2011; 8: 1000407.
8. Konate AT, Yaro JB, Ouedraogo AZ et al. Intermittent preventive treatment of malaria provides substantial protection against malaria in children already protected by an insecticide-treated bednet in Burkina Faso: a randomised, double-blind, placebo-controlled trial. *PLoS Med* 2011; 8: e1000408.
9. Ndiaye JL, Cissé B, Ba EH et al. Safety of seasonal intermittent preventive treatment against malaria with sulfadoxine pyrimethamine + amodiaquine when delivered to children under 10 years of age by district health staff in Senegal. *PLoS One*, in press.
10. (http://www.malariaconsortium.org/pages/access-smc.htm).
11. Chandramohan D, Dicko A, Zongo I et al. Effect of Adding Azithromycin to Seasonal MalariaChemoprevention. N Engl J Med 2019;380:2197-206.
12. Piaggio G, Elbourne DR, Altman DG, Pocock SJ, Evans SJ. Reporting of noninferiority and equivalence randomized trials: an extension of the CONSORT statement. Jama. 2006 Mar 8;295(10):1152-60. PubMed PMID: 16522836.

13. Leitao J, Chandramohan D, Byass P, et al. Revising the WHO verbal autopsy instrument to facilitate routine cause-of-death monitoring. Glob Health Action. 2013;6:21518

14. Swysen C, Vekemans J, Bruls M et al. Development of standardized laboratory methods and quality processes for a phase iii study of the RTS,S/AS01 candidate malaria vaccine. *Mal J 2011;* 10: e223.

15. Neafsey DE, Juraska M, Bedford T,et al Genetic diversity and protective efficacy of the RTS,S/AS01 malaria vaccine. N Engl J Med 2015; 373: 2025-2037.

16. Xu Y, Lam KF, Cheung YB. Estimation of intervention effects using recurrent event time data in the presence of event dependence and a cured fraction. Stat Med. 2014 Jan 22. PubMed PMID: 24449504. Epub 2014/01/23. Eng.

17. Cairns M, Cheung YB, Xu Y, Asante KP, Owusu-Agyei S, Diallo D, et al. Analysis of Preventive Interventions for Malaria: Exploring Partial and Complete Protection and Total and Primary Intervention Effects. Am J Epidemiol. 2015 Jun 15;181(12):1008-17. PubMed PMID: 26022663. Pubmed Central PMCID: PMC4462336. Epub 2015/05/30.eng.

TABLES AND FIGURES

Table 1. Study groups and the interventions that they received.

	Group1	**Group2**	**Group3**
	RTS,S/AS01 alone	**SMC alone**	**SMC + RTS,S/AS01**
Year 1			
April-June	RTSS/AS01 x 3	Rabies vaccine x 3	RTSS/AS01 x 3
July-Oct	SMC placebo* x 4	SMC* x 4	SMC* x 4
Year 2			
June	RTSS/AS01 x 1	HepA vaccine x 1	RTSS/AS01 x 1
July-Oct	SMC placebo* x 4	SMC* x 4	SMC* x 4
Year 3			
June	RTSS/AS01 x 1	HepA vaccine x 1	RTSS/AS01 x 1
July-Oct	SMC placebo* x 4	SMC* x 4	SMC* x 4

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

Figure 1: Study districts in Mali and Burkina Faso

Figure 2: study design

Figure 1: Study districts in Mali and Burkina Faso

Figure 2
Study Design

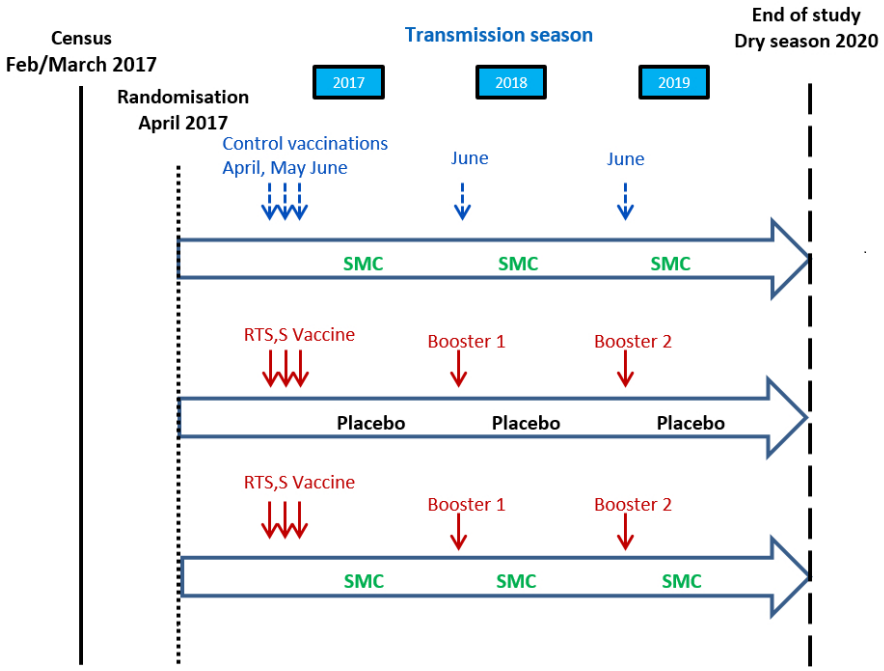

Figure 2: Study Design

Version 2

09.12.16

Draft information sheet (to be translated and distributed before screening)

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

Information Sheet

Introduction

Malaria is a serious problem in Burkina Faso/Mali and affects mainly children. Malaria is spread by mosquitoes and can be partially prevented by sleeping under a mosquito net that incorporates an insecticide that kills mosquitoes, an ITN. All children should be encouraged to sleep under an ITN. However, ITNs are not perfect and some children who sleep under an ITN still get malaria.

Malaria can also be prevented by taking drugs. Research conducted in several countries in Africa, including Burkina Faso and Mali, has shown that when children take antimalarial medicines three or four times during the rainy season, they gain at least 50% protection against malaria even if they are already sleeping under an ITN. Therefore, the World Health Organisation now recommends that all children under the age of five years living in countries like Burkina Faso and Mali, where malaria occurs mainly in the short rainy season, should be given a combination of two antimalarial medicines (SP and amodiaquine) three or four times during the rainy season to prevent malaria, even if they are well. This is called seasonal malaria chemoprevention (SMC) which you probably know about.

Recently, a study conducted in several countries in Africa showed that three doses of a malaria vaccine called RTS,S/AS01 given to 5-17 month old children, provided about 30% protection against malaria during the four years after vaccination. Protection was much higher (about 70%) in the first few months after vaccination but after that, the vaccine gradually lost its effect. Children who received a booster dose of the vaccine when they were 18 months old had more protection than those who did not. The malaria vaccine caused some side effects, so WHO has recommended further testing of this vaccine in children before it is introduced into the regular vaccination programmes. We propose to investigate whether the vaccine could be used to prevent seasonal malaria in countries, such as Burkina Faso and Mali, where malaria occurs during only a few months of the year.

Giving three doses of SP+AQ four times every year requires a lot of time and effort from the caretakers and health care providers. In addition, malaria parasite may become resistant to SP+AQ in few years time and then SMC with SP+AQ would become ineffective because these drugs no longer kill the malaria parasite. In contrast, seasonal vaccination with a malaria vaccine would require only one visit just before the rainy season and would be less demanding for children and their parents. Therefore, we are planning to do a study of these two possible ways of protecting children from malaria and to see which is best and whether

using both approaches together might provide some extra protection against malaria. We need your help in addressing these important questions.

The trial

If you agree that your child can join the trial, your child will be allocated to one of the three study groups by lottery.

Group 1. In year one of the study, children in group 1 will receive 3 doses of the malaria vaccine and 4 rounds of dummy tablets that look like SP+AQ. In years 2 and 3 of the study, children in this group will receive one dose of the malaria vaccine and 4 rounds of dummy tablets that look like SP+AQ.

Group 2. In year one of the study children in group 2 will receive 3 doses of a vaccine that protects against rabies and 4 rounds of SP+AQ. In years 2 and 3 of the study, children in this group will receive 1 dose of hepatitis A vaccine that can protect against jaundice and 4 rounds of SP+AQ

Group 3. In year one of the study children in group 3 will receive 3 doses of the malaria vaccine and 4 rounds of SP+AQ. In years 2 and 3 children in this group will receive one dose of the malaria vaccine and 4 rounds of SP+AQ.

Your child will be eligible to receive any other vaccines appropriate for their age recommended by the national EPI programme.

Druing year one of the study you will be asked to bring your child to a study health centre once a month for three months (from April to June in 2017) to receive the study vaccines. In addition, during the rainy season you will be asked to bring your child to a central place in the village on four occasions, at monthly intervals, where the children will be given either SP and AQ or matching dummy tablets depending on their study group.

In years 2 and 3 of the study you will be asked to bring your child once, in June, to receive either the malaria vaccine or a vaccine against hepatitis A depending on the group of your child. Then you will need to bring your child to a central place in the village four times during the rainy season each year to receive SP+AQ or dummy tablets.

To test whether the tablets and the vaccine are having a good effect against malaria and other severe illnesses and are safe, children in the trial will be investigated for malaria and other illnesses when they attend a clinic because they are sick. Some children will be visited at home to check if they have malaria and all children will be examined at the end of the rainy season each year to check for malaria.

The funds needed to conduct this trial are being provided by a grant from the UK's Medical Research Council, Department for International Development and the Wellcome Trust. The IRSS, Bobo, and Malaria Research and Training Centre, Bamako working with the London School of Hygiene & Tropical Medicine are responsible for the conduct of the trial.

What happens if your child becomes sick?

If your child becomes sick you will be asked to take him/her to a local clinic and show the nurse or doctor your vaccination card. He/she will examine your child to find out what is wrong with him/her and treat the child with the correct medicines. As part of the examination, few drops of blood sample will be collected by pricking the child's finger to see if the child has malaria parasites in the blood or is anaemic (thinning of blood). A sample of blood on paper will be stored to test whether the malaria parasites in your community are still sensitive to the drugs used for SMC. If the child has either malaria or anaemia s/he will be given effective treatment against these conditions. Any child with a severe illness will be referred to hospital and receive appropriate treatment, for example investigation and treatment of meningitis, according to national guidelines.

Will my child be followed up in other ways?

Malaria can be present without making a person feel sick. Therefore, 90 children will be visited at home each month to check if they have malaria by testing a drop of blood even if they are feeling well. In addition, all children will be asked to attend a survey at the end of the rainy season. During these surveys, all children will be examined by a doctor and a finger prick blood sample will be collected to see if malaria parasites are present or if the child is anaemic. If either is found, the child will be treated with effective antimalarial medicines and/or medicines that treat anaemia.

In addition, a sub-group of about 160 children will be selected through a lottery to help us in determining how well the vaccine is producing changes in the blood that provide protection against malaria. In these children, a teaspoonful (2 ml) of blood will be taken from a vein, using a small needle, twice each year. In year 1, a sample will be taken before the first dose of the vacccine is given and then one month after the third dose of vaccine is given. In years 2 and 3, a teaspoonful of blood will be taken from these children before and one month after a booster dose of vaccine is given.

Are the drugs and vaccines that will be given to my child safe?

Both of the antimalarial drugs that will be given to your child are very safe and are recommended by WHO for the prevention of malaria in children, although they may occasionally cause some minor side effects such as dizziness and vomiting and, very rarely, SP can cause a serious skin rash.

Like nearly all vaccines, the malaria, rabies and hepatitis vaccines that will be used in this study may sometimes cause redness, pain and swelling at the site of the injection and some fever. A few children, about 1 per 1,000, who received the malaria vaccine developed a convulsion associated with fever after their injection but all these children recovered rapidly and none had any permanent harm. In a large trial of the malaria vaccine a few more children than expected developed meningitis (brain fever) – it is not known whether this was a chance finding or related in some way to the vaccine. Therefore, we will follow carefully each child to detect any such cases and treat them promptly. The rabies and hepatitis A vaccines that will be given to children who do not receive the malaria vaccine are licensed vaccines which only very rarely cause serious side effects, such as an allergic reaction to the vaccine, (less than 1:1,000 children vaccinated).

What will happen to the information collected during the trial?

Personal information obtained about children in the trial will be available only to the team of scientists involved in conducting the research, including some who are based in Europe. Once the results of the trial have been obtained, a member of the research team will come to your community and explain what was found. Once the trial is finished, information about how the drugs and vaccines affected the health of children who have participated in the trial, for example how many got malaria, may be made available to other scientists interested in the results who have obtained approval to look at this information but these scientists will not be able to identify your child becaue all personal information that could be used to identify your child will be removed when sharing data with other scientists.

What will happen to the blood samples collected during the trial?

The blood will be used to measure how your child responds to the malaria vaccines and drugs. It will also be used in tests to learn more about the parasites that are causing malaria. Any remaining blood samples left after doing these tests will be stored in a repository and might be used to carry out tests against other infections as well as malaria in the future, if a good reason for doing this occurs.

What happens if I decide that my child should not join the trial?

You are completely free to decide if you would like your child to be join this trial or not. If you agree initially that your children can join the trial, you are free to withdraw him/her from the trial at any time if you wish to do so. If you decide not to let your child join the trial, s/he will continue to receive all the treatment and routine vaccinations that your child is eligible to receive, including SMC.

Will I receive payment for allowing my child to enter the trial?

You will not receive any direct payment for allowing your child to enter the trial. However, if your child becomes sick and needs treatment the costs of this, either in the health centre or hospital, will be met by the study and you will not have to pay for these expesnses.

What happens after the trial?

After the trial is over your child should continue to receive the antimalarial drugs SP and AQ during the rainy season from the National Malaria Control programme as this is now national policy. The Ministry of Health and WHO will be asked to review the results of the trial and on the basis of these results decide what is the best way of preventing malaria in children in your area and whether this should include the use of the malaria vaccine being tested in this study.

Further questions

Do you have any questions about the trial? If you want more information now or at any time during the trial please contact [To follow with contact telephone numbers]

CONSENT FORM
(to be adapted at each site)

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

I confirm that the nature of this study on the malaria vaccine RTSS and seasonal malaria prevention with SP and amodiaquine has been explained to me in a language that I understand, that I have had an opportunity to consider this information, to ask questions and to have these answered to my satisfaction.

I understand that I am under no obligation to enter my child into the trial and that if I do not do so, my child will not be penalised in any way. I understand that I can withdraw my child from the trial at any time if I wish to do so without a penalty of any kind.

I understand that my child will receive 3 doses of either the malaria vaccine or rabies vaccine in year one of the study and one dose of the malaria vaccine or hepatitis A vaccine in years 2 and 3. In addition my child will receive antimalarial treatment (SP+AQ) or similar looking dummy tablets that do not contain any medicines against malaria, on four occasions during the malaria season over the three year study period. I also understand that it will be necessary to take a small, finger-prick blood sample from my child once a year at the end of the rainy season and whenever the child is sick and brought to a clinic to see if your child has malaria or anaemia even though s/he seems to be well. In addition about 160 children selected by lottery will be asked to provide a small blood sample (a teaspoonful) before and after vaccination each year (a total of six samples) to see whether the vaccine has led to the production of substances in the blood that protect against malaria and to learn more about what causes malaria and I understand that my child might be one of those selected.

I understand that the information collected about my child will be available only to the medical staff who are undertaking the trial and to doctors in London who are co-ordinating this study. I understand that once the trial is finished, information about children who have participated in the trial may be made available to other scientists interested in the results who have obtained approval to do so, but these scientists will not be able to identify your child.

I agree that my child [NAME] can enter this study on new ways of preventing malaria.

Printed name of the parent or legal guardian giving consent:

Signature or thumbprint of the above:

Date:

I certify that the nature of this trial has been explained to the respondent in a language that s/he understands, that s/he has been given an opportunity to ask questions about the study and that s/he has given her/his consent for her/his child to enter the trial.

Printed name of witness:

Signature of witness:

Date:

SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description
Administrative information		
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym *Added the study sites Mali and Burkina Faso to the title (page 1)*
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry *Shown in the abstract (page 2) and main text (page 15)*
	2b	All items from the World Health Organization Trial Registration Data Set *Not applicable*
Protocol version	3	Date and version identifier *Version 2 date 09 December 2016 (page 15)*
Funding	4	Sources and types of financial, material, and other support *Shown in Funding section, paragraph 1; page 16*
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors *Shown in Authors contribution section, paragraph1; page 16*
	5b	Name and contact information for the trial sponsor *Shown in Funding section, paragraph 1;page 16*
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities *Shown in Funding section, paragraph 1;page 16*

	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee) *Shown in Trial management section, paragraph 1; page 14*
Introduction		
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention *Shown in Introduction section, paragraph 1 and 2; page 4*
	6b	Explanation for choice of comparators *Shown in Introduction section, paragraph2; page 4*
Objectives	7	Specific objectives or hypotheses *Shown in Introduction section, paragraph2; page 4*
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory) *Described in Methods section, paragraph 1; page 5*
Methods: Participants, interventions, and outcomes		
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained *Described in study setting section, paragraph 1; page 5 and Figure 1*
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists) *Described in study population section, paragraph 1; pages 5 and 6*
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered *Described in interventions section, paragraphs 1 and 2; pages 6 and 7*

	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)
		Not applicable
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)
		No specific procedures to improve adherence were done
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial
		Not applicable
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended
		Described in study outcomes section, paragraphs 1 and 2; pages 8 and 9
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)
		Shown in Figure 2
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations
		Described in sample size section, paragraphs 1 to 5; pages page 9 and 10
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size
		Described in section study populations paragraphs 1; page 5

Methods: Assignment of interventions (for controlled trials)

Allocation:

Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions *Described randomisation section, paragraph 1; in page 6*
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned *Describe in randomisation section, paragraph 1; page 6*
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions *Described in randomisation section, paragraph 1; page 6*
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how *Described in implementation of interventions section, paragraphs 1 to 3; pages 7 and 8*
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial *Need for unblinding was determined by the data safety monitoring board.*
Methods: Data collection, management, and analysis		
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol *Described in data management section, paragraph1; page 13. Data collection tools (case record forms) will be deposited with the study data on the LSHTM Data Compass system (http://datacompass.lshtm.ac.uk/).*

	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols *Not applicable*
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol *Described in analysis plan section, paragraph 1 to 4; page 13 and 14. These procedures are described in more detail in the Statistical Analysis Plan (supplement).*
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol *Described in analysis plan section, paragraph 1 to 3; page 13 and 14. More details are provided in the Statistical Analysis Plan (supplement).*
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses) *Described in analysis plan paragraph 4; page 14. More details are provided in the statistical analysis plan (supplement).*
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation) *Described in analysis plan section, paragraph 4; page 14. More details are provided in the statistical analysis plan (supplement)*
Methods: Monitoring		
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed *Has an independent DMC. DMC charter is available on request from the principal investigators (Daniel.chandramohan@lshtm.ac.uk)*

	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial *No formal interim analysis was planned.*
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct *Collecting, managing and reporting spontaneously reported adverse and serious adverse events are described in the section follow-up and measurement of outcomes; paragraph 7; pages 11 and 12.*
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor *Monitoring of good clinical practice was done by an independent monitor (section trial management, paragraph 1, page 14).*
Ethics and dissemination		
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval *Described in ethics section, paragraph 1 and 2; pages 14 and 15*
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators) *Described in ethics section, page 15*
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32) *Described in the study population section paragraph 1, page 6 and in the patient and public involvement section, page 6*
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable *An ancillary study to assess sensitivity of the SMC drugs (sulphadoxine-pyrimethamine and Amodiaquine) was conducted in November-December 2019. For this study additional consent was obtained.*

Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial *Described in the data management section, page 13*.
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site *Described in page the competing interest section; page16*
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators *Described in the Data management section, page 13.*
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation *Not applicable*
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions *Described in the dissemination plan section; page 15*
	31b	Authorship eligibility guidelines and any intended use of professional writers *No professional writers will be involved. Manuscript(s) will be written by the investigators. (page 16)*
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code *Full protocol, locked dataset and statistical codes will be granted public access through the Data Access group. (Data management section, page 13)*.
Appendices		
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates *Submitted as supplement*

Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable *Described in the Laboratory methods section; page 12*

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.