STUDY PROTOCOL

# Risk of Malaria in Children Who Have Reached the Age of Five Years and Are No Longer Eligible for Seasonal Malaria Chemoprevention (SMC) and or Seasonal Malaria Vaccination in Burkina Faso and Mali

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# 1. LIST OF ABBREVIATIONS AND ACRONYMS

| ABBREVIATION/ ACRONYM | DEFINITION |
|---|---|
| AZ | Azithromycin |
| CI | Confidence Interval |
| CVMNK | *Pfcrt* sensible haplotype |
| CVIET | *Pfcrt* resistant haplotype |
| CHUSS | Centre Hospitalier Universitaire Souro Sanou |
| DHFR | Dihydrofolate Reductase |
| DHPS | Dihydropteroate Synthase |
| GCP | Good Clinical Practice |
| GLP | Good Laboratory Practice |
| GPS | Global positioning system |
| HRP | Histidine-rich Protein |
| ICH GCP | International Conference of Harmonization Good Clinical Practice |
| ID | Identification |
| IRR | Incidence Rate Ratio |
| IRSS | Institut de Recherche en Science de la Santé |
| ITN | Insecticide Treated Bed Net |
| LSHTM | London School of Hygiene & Tropical Medicine |
| MRTC | Malaria Research and Training Center |
| ODK | Open Data Kit |
| pfCRT | *Plasmodium falciparum* chloroquine resistance transporter |
| pfDHFR | *Plasmodium falciparum* dihydrofolate reductase |
| pfDHPS | *Plasmodium falciparum* dihydropteroate synthase |
| pfMDR1 | *Plasmodium falciparum* multidrug resistance |
| RDT | Rapid Diagnosis Test |
| RTS,S | RTS,S/AS01$_E$ malaria vaccine |
| SAP | Statistical Analysis Plan |
| SMC | Seasonal Malaria Chemoprevention |
| SOP | Standard Operating Procedures |
| SP + AQ | Sulfadoxine-pyrimethamine plus Amodiaquine |
| WHO | World Health Organization |

# 2. STATEMENT OF COMPLIANCE

The study will be carried out in accordance with Good Clinical Practice (GCP) as required by the following:

- International Conference on Harmonisation (ICH) Guidance for GCP (E6)
- World Medical Association Declaration of Helsinki – Ethical Principles for Research Involving Human Subjects (Oct 2013 or subsequent amendments)

## 3. PROTOCOL SIGNATURE PAGE

The signatures below constitute the approval of this protocol and the attachments, and provide the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and ICH-GCP guidelines as outlined in the 'Statement of Compliance.

| | |
|---|---|
| Signature of Principal Investigator | Date |

Name:

Title:

# 4. PROTOCOL SUMMARY

Current, effective malaria control tools include seasonal malaria chemoprevention (SMC), which is tailored for the countries of the Sahel and sub-Sahel. This strategy involves the administration of full therapeutic doses of sulfadoxine-pyrimethamine plus amodiaquine once monthly over the highest malaria transmission period. SMC has the ability to clear a current infection and to prevent the acquisition of a new one. The RTS,S/AS01$_E$ malaria vaccine (hereafter referred to as RTS,S), recently approved for implementation by the WHO has recently been shown to provide synergistic protection against uncomplicated and severe malaria when combined with SMC in young children over a three-year period in Burkina Faso and Mali. This suppression of the parasite's presence in the body resulting from vaccine-induced immune protection against sporozoites as well as chemoprevention afforded by SMC may result in slowing down of the development of naturally-acquired immunity to repeated malaria infections. In prior research studies in which SMC was given over only a short period of time (no more than one transmission season), there was no consistent evidence of this impairment. However, for implementation programs in which children receive SMC from 3 months age through 5 years during the transmission season such crucial information is lacking, and it is important to evaluate whether an excess of clinical and severe malaria occurs when such interventions are stopped as children age beyond five years of age. In this protocol, we refer to this phenomenon as a "relative rebound effect" as we are only assessing changes in incident uncomplicated and severe malaria in children who received the combination of SMC plus RTS,S, SMC alone, or RTS,S alone. To investigate the issue of rebound we will conduct both case control studies (one for uncomplicated malaria and one for severe malaria) and passive case detection in children who have previously received either SMC, RTS,S, or the combination of both interventions and who have reached the age of 5 years when such interventions have been stopped. Two community controls will be recruited for each case in the uncomplicated malaria case control study while two community and two hospital controls will be recruited for each case in the severe malaria case control study. The participants will all be interviewed with regard to their use of Long-Lasting Insecticide Treated Nets (LLINs), their socio-economic status, SMC history, and housing. This information will be used to determine whether the risk of malaria is higher in children who have had a high level of coverage with SMC plus RTS,S compared to those who have been given either SMC or RTS,S, taking into account any potential confounding factors. There will also be passive follow-up of cases at health facilities. The project has the potential to accelerate progression toward malaria control and elimination in Burkina Faso and Mali and to indicate whether there is a need to provide extra protection to children in the period immediately after they are no longer eligible to receive chemoprevention and/or seasonal vaccination.

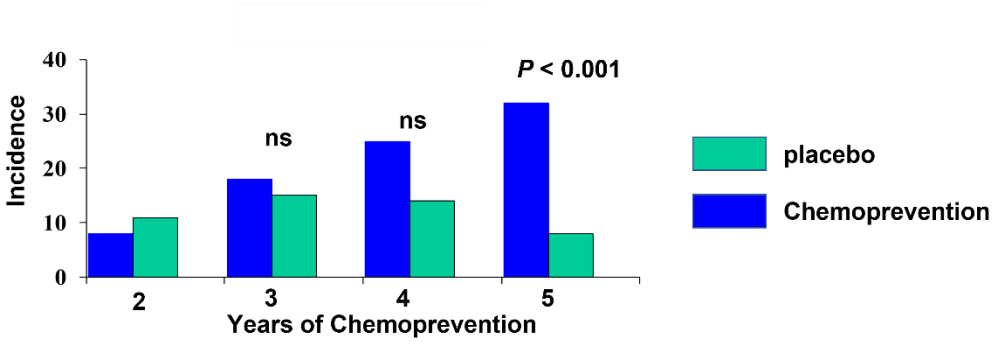
# 5. BACKGROUND

Seasonal malaria chemoprevention (SMC) has been shown to be a highly effective malaria control intervention in counties of the Sahel and sub-Sahel [1-2], and it is now being implemented widely in all malaria endemic countries of the Sahel with support from international donors and local governments following WHO's endorsement of SMC in March 2012 [3]. In Burkina Faso and Mali, implementation of SMC has achieved high coverage [4]. In each country, the delivery strategy is mainly through door-to-door campaigns. Community health workers visit the house and deliver the first dose of SMC and tick the SMC administration card. The remaining two doses of amodiaquine are given by mothers at home. SMC is tailored for children under five years old. Currently, all children in the areas where SMC is being delivered receive SMC from the age of 3 to 59 months, except in Senegal where administration continues until the age of 10 years. A landmark trial (NCT03143218) in Burkina Faso and Mali demonstrated for the first time that combining the RTS,S vaccine with the seasonal malaria chemoprevention (SMC) drugs, sulfadoxine-pyrimethamine plus amodiaquine, provided synergistic protection over three years against clinical malaria, severe malaria, and malaria deaths compared to either intervention when used alone [5]. Children, 5 to 17 months of age at the time of first RTS,S vaccination, were randomized into three groups, RTS,S alone, SMC alone, or RTS,S vaccination followed by SMC (RTS,S + SMC). Children randomized to the two RTS,S groups were vaccinated three times at monthly intervals immediately before the seasonal malaria transmission. SMC was provided at four monthly intervals beginning in July and ending in October of each year for the three years of the study. Since SMC is implemented in both countries through policy endorsed by the WHO, the study was designed to detect non-inferiority in terms of clinical malaria episodes in children randomized to the RTS,S alone group compared to those children randomized to the SMC alone group. The protective efficacy of RTS,S against clinical malaria was non-inferior to SMC (hazard ratio, 0.92 (95% confidence interval, 0.84 to 1.01)). The protective efficacy of the combination of RTS,S + SMC compared with SMC alone was 62.8% (95% CI, 58.4 to 66.8) against clinical malaria, 70.5% (95% CI, 41.9 to 85.0) against hospital admission with severe malaria, and 72.9% (95% CI, 2.9 to 92.4) against death from malaria [5].

An effective regimen of SMC and RTS,S vaccination is expected to clear existing parasites and to prevent new infections. It is a reasonable premise that the continuous suppression of *P. falciparum* infection impairs the development of naturally acquired immunity to malaria. Thus, children in the recently completed study in Burkina Faso and Mali who have stopped receiving SMC and/or RTS,S when they have reached the age of five years may be more susceptible to clinical malaria, including severe malaria. While we think it is more likely that there would be an increase in morbidity in the combined intervention group as a result of better protection over the intervention years of the trial, we cannot exclude the possibility that the combined group that had received both SMC and RTS,S may do better when the interventions stop, for example if prevention of multiple malaria episodes until the immune system has matured allows individuals to develop better protection against malaria. The inclusion of a control group who received neither SMC or RTS,S in order to measure true rebound would have been desirable but this was not possible on ethical grounds because SMC is standard of care in Mali and Burkina Faso.

Limited data are available on the possible rebound effects following discontinuation of chemoprevention. In Mali and Burkina Faso, there was no overall increase in risk in the period following the discontinuation of SMC which had been given for one year [6, 7], incidence rate ratios (IRR) of 0.98 (95%CI: 0.82–1.17) and 1.07 (95%CI: 0.90–1.27) were observed respectively. A meta-analysis of available data on IPT in children found only weak evidence of an increase in the incidence of malaria in the year post intervention (IRR=1.11; 95%CI 0.99–1.24; P=0.07). These inconclusive data describe the consequences of a short-term period of SMC administration. The risk of rebound malaria in children who have received SMC for five years is still unclear. However, in an early study of seasonal chemoprevention there was a substantial increase in the incidence of cases of clinical malaria in children who had received chemoprevention from the ages of 3 months to five years, but not in deaths, in the year after the intervention was stopped [8].

**Figure 1**. Incidence of uncomplicated malaria in children who had received Maloprim (pyrimethamine + dapsone) for a variable number of years in the year after they stopped receiving the intervention at the age of five years. The blue bars children indicate children who received chemoprevention and the green bars the children who received placebo [8].




The likelihood of malaria rebound in children who had received the RTS,S malaria vaccine during the Phase 3 trial of RTS,S in sub-Saharan Africa was assessed in a three-year extension study at three of the eleven original trial sites in Burkina Faso, Kenya, and Tanzania [9]. The incidence of severe malaria, clinical uncomplicated malaria, malaria hospitalizations, anemia, cerebral malaria, fatal malaria, and malaria parasite prevalence were assessed. The results of the extension trial showed no increased incidence of severe malaria overall or at each of the three trial sites. There was no evidence of rebound in clinical malaria in RTS,S vaccinated children when all three sites' data were combined. However, there was an increased incidence of clinical malaria (evidence of rebound) in the older children who received RTS,S in Nanoro, Burkina Faso, the one seasonal malaria transmission site included in the trial [9].

## 6. HYPOTHESIS

We hypothesize that children who had received both SMC and RTS,S may have a difference in the number of episodes of uncomplicated malaria compared to children who had received either SMC alone or RTS,S alone when they become too old (> 5 years of age) to receive the interventions that were previously randomly assigned to them during the RTS,S + SMC

clinical trial.

# 7. OBJECTIVES AND ENDPOINTS

## 7.1. Study Objectives

### 7.1.1. Primary objectives

A. Determination of whether the future risk of uncomplicated malaria in children who have previously received the combination of SMC plus RTS,S is different compared to children who have previously received SMC alone.
B. Determination of whether the future risk of uncomplicated malaria in children who have previously received the combination of SMC plus RTS,S is different compared to children who have previously received RTS,S alone.

### 7.1.2. Secondary objectives

1. Determination of whether children who have received the combination of SMC plus RTS,S for five years have a different risk of severe malaria up to three years after the interventions are stopped compared to children who have received either a) SMC alone or b) RTS,S alone as assessed using a passive case detection approach.

2. Determination of whether children who have received the combination of SMC plus RTS,S until five years of age have a different risk of severe malaria up to three years after the interventions are stopped compared to children who have received either SMC alone or RTS,S alone as assessed using a case-control approach.

3. Determination of whether children who have received the combination of SMC plus RTS,S until five years of age have a different risk of mortality at the end of the malaria transmission season up to three years after the interventions are stopped compared to children who have received either SMC alone or RTS,S alone.

4. Determination of whether children who have received the combination of SMC plus RTS,S until five years of age have a different risk of uncomplicated malaria up to three years after the interventions are stopped compared to children who have received either SMC alone or RTS,S alone according to:

a) adherence to the assigned intervention(s)
b) prior malaria history (number of clinical malaria episodes between 2017 and June 2021).

5. Determination of whether children who have received the combination of SMC plus RTS,S until five years of age have a different risk of asymptomatic malaria parasitemia at the end of the malaria transmission season up to three years after the interventions are stopped compared to children who have received either SMC alone or RTS,S alone.

6. Determination of whether children who have received the combination of SMC plus RTS,S until five years of age have a different risk of mild or moderate anemia at the end of the

malaria transmission season up to three years after the interventions are stopped compared to children who have received either SMC alone or RTS,S alone.

### 7.1.3. Exploratory objectives

1. Determination of the prevalence of molecular markers associated with resistance to sulfadoxine-pyrimethamine and amodiaquine after receiving SMC until five years of age.

2. Determination of whether receiving SMC plus RTS,S until five years of age results in a reduction in antibody titers to blood stage antigens of *P. falciparum* (see Appendix A for details) compared to children who had previously received either SMC or RTS,S.

3. Determination of whether it is possible to identify a pattern of antibodies to malaria pre-erythrocytic and blood stage antigens that can predict protection against malaria infection, uncomplicated clinical episodes of malaria or severe malaria which is valid in children who have previously been protected against malaria by an ITN, SMC or RTS,S or a combination of these interventions.

4. Determination of whether children who have received the combination of SMC plus RTS,S until five years of age have a different risk of malnutrition at the end of the malaria transmission season up to three years after the interventions are stopped compared to children who have received either SMC alone or RTS,S alone.

5. Determination of risk of uncomplicated malaria in children who have previously received the combination of SMC plus RTS,S differs compared to children who have previously received either SMC alone or RTS,S alone at different parasite thresholds for clinical malaria.

## 7.2. Study Endpoints

The period of follow-up that contributes to the primary and secondary end-points will vary depending upon whether children are in cohort 1 or cohort 2 (see below) but will be the same for each of the groups of children among whom comparisons are made.

### 7.2.1. Primary endpoints

A. Incidence of uncomplicated malaria (including multiple episodes within study children) in the combination group (RTS,S + SMC) compared to the incidence of uncomplicated malaria in the SMC group.
B. Incidence of uncomplicated malaria (including multiple episodes within study children) in the combination group (RTS,S + SMC) compared to the incidence of uncomplicated malaria in the RTS,S group.

### 7.2.2. Secondary Endpoints

1. Comparison of the incidence of severe malaria (multiple episodes within study children) in the RTS,S + SMC group compared to the SMC group.

2. Comparison of the incidence of severe malaria (multiple episodes within study children) in the RTS,S + SMC group compared to the RTS,S group.

3. The incidence rate of mortality for children in the combination group (SMC + RTS,S) compared to children in either the SMC group or RTS,S group as determined by passive surveillance.

4. Odds ratio as defined by the incidence of uncomplicated malaria cases and controls (see study design for details) for children in the combination group (SMC + RTS,S) compared to children in either the SMC group or RTS,S group according to confounding factors:

a) adherence to the assigned intervention(s)

b) prior malaria history (number of clinical malaria episodes between 2017 and June 2021).

5. Odds ratio as defined by asymptomatic malaria cases at the end of the transmission season (see study design for details) for children in the combination group (SMC + RTS,S) compared to children in either the SMC group or RTS,S group.

6. Odds ratio as defined by mild or moderate anemia at end of the transmission season (see study design for details) for children in combination group (SMC + RTS,S) compared to children in either the SMC group or RTS,S group.

7. Odds ratio as defined by malnutrition at the end of the transmission season (see study design for details) for children in the combination group (SMC + RTS,S) compared to children in either the SMC group or RTS,S group.

### 7.2.3. Exploratory endpoints

1. Prevalence of molecular markers associated with resistance to sulfadoxine-pyrimethamine and amodiaquine stratified by intervention group.

2. Antibody titer to selected *P. falciparum* blood stage malaria antigens measured by the Luminex assay (Appendix A).

3. Composite antibody response that predicts protection against malaria parasitemia, an uncomplicated episode of clinical malaria or severe malaria (Appendix A).

4. Odds ratio as defined by malnutrition at the end of the transmission season (see study design for details) for children in the combination group (SMC + RTS,S) compared to children in either the SMC group or RTS,S group.

5. Comparison of the hazard of malaria (including multiple episodes within study children) in the RTS,S + SMC group, RTS,S alone group; SMC alone group at different parasitemia thresholds.

# 8. STUDY DESIGN

The objectives of this study will be accomplished through a series of different approaches undertaken in the area where the previous intervention study was conducted including:

a. prospective matched cases-control study in years 1 (2021), 2 (2022) and – 3 (2023) for a) uncomplicated malaria and b) severe malaria

b. cohort surveillance years 1, 2 and- 3 (a) Passive case detection to capture children presenting to a health center with fever and b) Identification of those admitted to hospital with a possible diagnosis of severe malaria).

c. cross-sectional annual surveys in the area where the previous study was held at two time points (before the transmission season and at the end of the transmission season) in years 1, 2 and – 3.

## 8.1. Study site

This study will be supported by existing field centers set up in 2014 to conduct a multicenter clinical trial to investigate whether adding azithromycin to SMC is more effective than SMC alone in reducing the incidence of deaths and hospital admissions in African children [10]. This successful trial, which showed that the addition of AZ did not reduce deaths, was followed by the double blind, randomized clinical trial which investigated whether seasonal vaccination with the malaria vaccine RTS,S is non inferior to SMC with SP+AQ and whether the combination of the two interventions is superior to either intervention given alone (NCT03143218). This platform (site and study) offers an excellent setting in which to investigate the potential occurrence of relative rebound malaria, as an accurate record of the frequency with which children in this trial have received SMC drugs and/or RTS,S since enrollment in the trial is available.

The study site in Burkina Faso is the health district of Houndé, located 100 km from Bobo-Dioulasso, the second city in Burkina Faso where the CHUSS, the 2nd National Reference Hospital (University Hospital), is located and which is the also the base of the Institut de Recherche en Sciences de la Santé, Direction Régionale (IRSS). The study site in Mali is the district of Bougouni in the region of Sikasso, Mali, 150 km south of Bamako where the Malaria Research and Training Centre (MRTC) is based. Malaria, due mainly to *P. falciparum*, is highly seasonal in both districts with over 80% of cases occurring during the rainy season (July – October) and during the following month. The prevalence of *P. falciparum* malaria in school age children in December 2019 was 61% in Houndé and 22.5% in Bougouni. The main malaria vector in each study area is *Anopheles gambiae* ss. A high proportion of children sleep under a Long-Lasting Insecticide Treated Net (LLIN). The first line treatment for malaria in the public health system is artemether /lumefantrine in each district. Cases of uncomplicated malaria are treated at one of the health centers in the district and, in Bougouni, some cases are treated in the community by trained community health workers. Cases of severe malaria are managed in the district hospital.

Figure 2: Map of Mali and Burkina Faso showing the two sites.

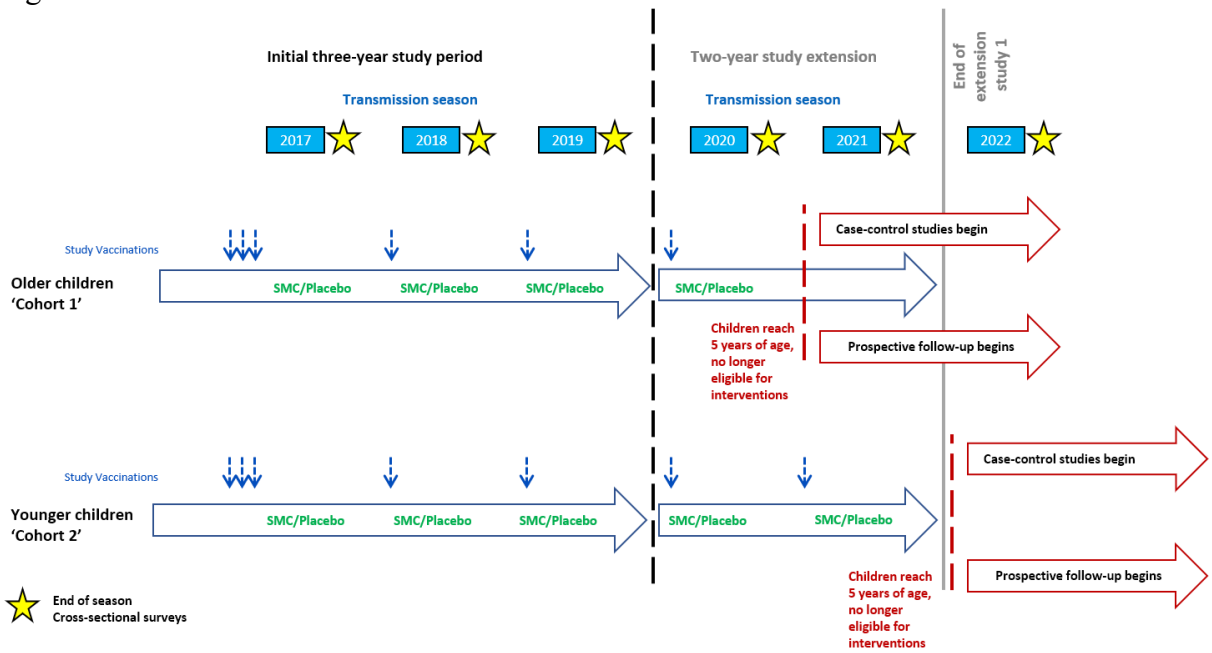

### 8.2. Study population

The study population comprises children recruited into the RTS,S + SMC trial in April 2017 in Houndé or Bougouni districts who have either received trial interventions up to 2020 (Cohort 1) or 2021 (Cohort 2) and who have reached the age of 59 months in July 2021 or 2022 respectively and are thus no longer eligible to receive SMC or seasonal vaccination with RTS,S. Approximately 60% of children (approximately 2,800 children) were eligible for enrollment in the follow-up and case control studies in 2021 (Cohort 1) and the remaining children, approximately 2,000, were eligible for recruitment to the study in 2022 (Cohort 2).

### 8.3. Extended follow-up

After the initial informed consent to enrol in the study has been obtained, a baseline finger prick blood sample will be collected onto filter paper, dried, and stored for serological analysis at a later time. Uncomplicated and severe malaria cases will be captured through a passive surveillance system set up at the participating health facilities to track and record any malaria episodes of children in cohort 1 and in cohort 2 who have previously completed the RTS,S + SMC trial interventions (because they are over 59 months of age and are no longer eligible for SMC). See Figure 3 for schematic.

Figure 3. Trial flow chart.




Project staff will identify study children through their ID card when they attend a health center or hospital and a new ID card will be given in addition to their old card but retaining the same ID. Children in cohort 1 will be followed through four consecutive transmission seasons, 2021 - 2024, and children in cohort 2 will be followed for three consecutive transmission seasons in 2022 - 2024, . If a child in the study presents to a health center with fever a RDT will be done for the purpose of deciding on treatment, a blood film will be obtained for subsequent microscopy and a blood spot obtained for potential molecular characterization of parasites if these are found and for serology. Any study child admitted to hospital with a possible diagnosis of severe malaria will be identified by a member of the study team and investigated appropriately. The study team will be blind to the prior treatment allocation.

## 8.4. Case control studies

The advantage of the case-control studies is that both cases and controls will be interviewed individually at the time an episode occurs. It will then be possible to ask more detailed questions about a list of risk factors at that time which are not available for analysis of the whole cohort. Some of these rapidly changing risk factors may be important and relevant to rebound, particularly for severe malaria.

In the first year of this follow-up study (2021 transmission season), case control studies were initiated in children who had received SMC alone, RTS,S alone, or the combination of RTS,S followed by SMC for four years and a second series of studies was done in the following year (2022) for those children who had received four or five years of SMC and/or RTS,S at that time point. In the third year (2023), case control studies will continue for the children who had received four or five years of SMC and/or RTS,S. In each case control study, cases and

controls are recruited in such a way as to cover the whole of the malaria transmission season.

### 8.4.1. Prospective matched case - control study for uncomplicated malaria

#### A. Cases

Cases for inclusion in this study will be children of either gender who are at least 5 years old, who have stopped receiving SMC and/or RTS,S and who present at a health facility with a febrile illness, with no other obvious cause of the fever, and who are positive for *P. falciparum* by microscopy with a parasitemia ≥ 5,000 parasites and < 250,000 per microliter.

A Rapid Diagnostic test (RDT) will be used for immediate care of the child. If the RDT is positive, informed consent will be obtained from the parent or the legally acceptable representative. If the blood film results obtained subsequently are negative, < 5,000 or > 250,000 parasites per microliter, the child will be excluded from the uncomplicated case control study.

The case's ID as reported on the ID card will be carefully recorded or scanned and this will allow access to the full history of that child's past experience of SMC or RTS,S vaccination and will also allow tracking of all morbidity records of that child during the previous four or five years. A dried blood spot will be collected and stored at the time of passive case detection.

Inclusion criteria for selection as a case include:

- A child of either sex enrolled in any of the three intervention groups of the SMC + RTS,S trial in April 2017 who has remained within the trial until the starting date of the extended follow-up study (April 2021 for cohort 1 and April 2022 for cohort 2).
- Age over 59 months.
- Diagnosis of uncomplicated falciparum malaria, defined as a temperature ≥ 37.5°C or a history of fever in the past 48 hours plus a positive blood film for *P. falciparum* malaria parasitemia with a parasite density ≥ 5,000 and < 250,000 parasites per microliter.
- Provision of signed informed consent for inclusion in the case control study from the parents or legally acceptable representative.

Exclusion criteria will be a failure to meet any of the above criteria.

#### B. Controls

Controls will be recruited from within the same community as the case with two controls per case. To achieve the required number of controls, a list of potential controls from the study cohort living in the same village as the case will be drawn from the study database and sorted in a random order. Children residing at least 2 compounds away from the case will be visited in the order of the list, in order to recruit two controls. If the designated control is not available, the next child on the list will be considered.

Inclusion criteria for selection as a control include:

- A child of either sex enrolled in any of the three intervention groups in the SMC +

RTS,S trial in April 2017 who has remained within the trial until the starting date of the extended follow-up study (April 2021 for cohort 1 and April 2022 for cohort 2).
- Residence in the same community as the case.
- Provision of signed informed consent for inclusion in the case control study from the parents or legally accepted representative.

The design of the case-control study is to recruit as controls either healthy children, or children whose symptoms were not sufficiently severe that the caregiver decided not to seek care. For this reason, exclusion criteria will be eligible children who have been taken to the clinic for treatment of fever within 24 hours of the time that the case was taken to the clinic. These children will not be included as controls.

### 8.4.2. Recruitment for the case-control study

Recruitment to the uncomplicated case-control study is undertaken as follows. Potential participants in the study attending a clinic who meet the inclusion criteria will have a finger prick blood sample collected for a malaria RDT at the clinic and for preparation of a blood smear. Preliminary enrolment, and treatment, is based on a positive RDT. A short questionnaire is completed at this time. Upon microscopic confirmation of the case, the child and the two controls, selected randomly from within the trial database, are visited at home to complete the questionnaires and to provide an opportunity to inspect bed-nets, home characteristics and any other environment features relevant to the risk of malaria such as residence near to a potential mosquito breeding site.

### 8.4.3. Measurement of exposures

The primary exposure of interest is the effect of the intervention group assignment. For instance, children previously enrolled in the RTS,S plus SMC group will be followed for the potential for clinical malaria rebound morbidity compared to children who had previously been assigned to either the RTS,S group or the SMC group. Additional data will be collected on other potentially important risk factors which might act as confounders or effect modifiers for the effect of SMC or RTS,S on rebound. These will include: adherence to the scheduled number of RTS,S vaccinations received and adherence to the scheduled number of SMC administrations received (both have been documented throughout the original trial and the extension study, with all doses of study vaccines and each daily dose of SMC medication administered as directly observed therapy and recorded using a tablet PC), the number of prior malaria episodes (derived from the study database for all children dating back to April 2017), parent/caregiver's socioeconomic status, the education level of the parent or legally accepted representative, the use of a bednet by the study child, the type of bednet, its quality and use the night before the interview, household size (number of children under 5 years old in the household), treatment prior to the current episode, the proximity to the nearest health facility (estimated using GPS), time taken to seek care, the availability of a community health worker in the case community. Home visits will be made for cases and controls to assess factors such as the condition of the house, use and condition of a bednet etc. unless this is not possible because of COVID-19 restrictions, in which case a telephone interview will be undertaken.

## 8.5. Case control study for severe malaria

The national malaria control program in both Burkina Faso and Mali requires that cases of severe malaria should be treated in hospital. Therefore, members of the study team are based at the district hospitals of Houndé and Bougouni for recruitment of cases of severe malaria for this case control study.

**A. Cases severe malaria**

Children admitted to a study hospital with a primary diagnosis of severe malaria based on symptoms and signs of severe malaria and a positive RDT or blood film are eligible for inclusion in this case control study. Children who are admitted to hospital with features of severe malaria, such as severe anemia, who have a negative RDT or blood film at the time of admission to hospital but who have been treated for malaria with confirmed records of a positive RDT or blood film within the previous two weeks are also eligible for inclusion.

Inclusion criteria for enrollment as a case include:

- Enrollment in any of the three intervention groups in the SMC + RTS,S trial in April 2017 and retention in the trial until the starting date of the extended follow-up study (April 2021 for cohort 1 and April 2022 for cohort 2).
- Intention to stay within the study area for the following year.
- Age over 59 months.
- Admission to hospital with a primary diagnosis of severe malaria confirmed by RDT or positive malaria blood film during the hospitalization, or admission to hospital with a confirmed record of a positive RDT or blood film prior to referral (see above).
- Provision of signed informed consent for inclusion in the case control study from the parents or legally accepted representative.

Exclusion criteria include children who do not meet these criteria.

**B. Controls severe malaria**

Two community and two hospital controls are recruited for each case in the severe malaria case control study. Two community controls are recruited from the community, selected from the database containing records of all children in the SMC+RTS,S trial as described above for the case control study of uncomplicated malaria. Inclusion and exclusion criteria for the community controls are not having a diagnosis of severe malaria with a positive RDT or blood film in the previous two weeks. Hospital controls are recruited to reduce the potential for health seeking bias. These controls are children admitted to hospital for a reason other than severe malaria. If more than two suitable cases are identified who meet these criteria, the first two families to be approached are selected at random.

Inclusion criteria for a child to be a hospital control include:

- Enrollment in the SMC + RTS,S in April 2017 and retention within the trial until the starting date of extended follow-up study (April 2021 for cohort 1 and April 2022 for cohort 2).
- Admission to hospital within one month of the case.
- Primary diagnosis is not severe malaria and no positive slide in the previous two weeks.
- Provision of signed informed consent for inclusion in the case control study from the

parents or legally accepted representative.

Children who do not meet the above criteria will not be considered to be eligible as a control. Residence in the same community as the case will not be required because of a potential restriction on the numbers of suitable hospital control cases available.

### 8.5.1. Measurement of exposures:

All risk factors collected in cases of uncomplicated *P. falciparum* malaria are also collected. In addition, information is collected on whether the mother travelled, died, or had experienced a severe illness, the nature of the first detectable sign of the illness, the time between the disease worsening and the first detectable signs of illness, whether the child had had any previous episode of convulsions, time taken to seek care, availability of transport to take the child to the clinic, the average time between arrival at the clinic and start of care, the immediate availability of health staff and antimalarial drugs at the district hospital, the identity of the person paying for child's care (father, mother, relative), the availability of free care (national policy) and nutritional status. A visit is made to the home of the case and all four controls to obtain additional information on the local surroundings unless this not possible because of COVID-19 restrictions, in which a telephone interview is undertaken.

## 8.6. Cross sectional survey

A cross-sectional survey was undertaken at the end of the transmission season in 2021 for children in Cohort 1 and will be undertaken in 2022 and 2023 for children in Cohorts 1 and 2. At these surveys, children have a clinical examination undertaken by a member of the study medical staff, temperature and anthropometric parameters measured and a finger prick blood sample collected for detection of malaria parasitemia and measurement of hemoglobin concentration using a hemocue machine. These studies will provide an opportunity to investigate the relationship between asymptomatic parasitemia or anemia with exposure to the intervention groups, and adherence to SMC, over the past four or five years.

## 8.7. Mortality surveillance

Any deaths in study children occurring during the study period are tracked and recorded. Field workers in close contact with the communities ensure that all deaths are identified and recorded correctly along with the subject ID. A verbal autopsy is undertaken to collect information on possible cause of death.

## 8.8. Management of the cohorts during national SMC campaigns

All of the children belonging to the trial will no longer be eligible to receive SMC or seasonal vaccination at the time when the national SMC campaign is ongoing and may be administering SMC to other members of their family. At the time of recruitment, the study team will explain to mothers that children over 59 months of age are no longer entitled to receive SMC but that the project will continue to provide care to children in the follow-up study. If a child does receive SMC incorrectly through the national program, this will be recorded and considered during the analysis.

### 8.9. Update on study activities completed, on-going, and planned

Activities under the protocol began in 2021 with study approval by ethical committees in Burkina Faso, Mali, and at the LSHTM. The study has been posted on the ISRCTN clinical trials registry https://doi.org/10.1186/ISRCTN12207852. This section of the protocol summarizes those activities for each of the two cohorts. Table 1 provides additional details.

A. Cohort 1.

- Informed consent for children participation in cohort 1 was obtained in 2021.
- Re-consenting for cohort 1 took place in May to June 2022 for these children to continue in the study until March 2023.
- Re-consenting for cohort 1 took place in March 2023 for these children to remain in the study until December 2023. Another reconsenting for continuing passive surveillance will be done in December 2023 for these children to remain in the study until December 2024.
- Children were administered a questionnaire in 2021, 2022 and 2023, to ask about variables that might influence susceptibility to malaria during the coming malaria transmission season such as house design, use of an ITN, access to a health center. Same questionnaire will be administered in quarters 2 and 4 in 2024.
- Passive surveillance for clinical and severe malaria was initiated in 2021 and is ongoing until December 2023. This will be continued until December 2024.
- Case-control for clinical and severe malaria was initiated in 2021 and finished in March 2023.
- Case-control for severe malaria was re-initiated in Q2 2023, and will continue until December 2024.
- Cross-sectional surveys were completed in quarter 4 of 2021 and 2022, and planned in 2023 and 2024.
- Blood filter samples for serology were collected in 2021 and 2022 (quarters 2 and 4), 2023 (quarter 2) and will be collected in quarter4 in 2023 and in quarters 2 and 4 in 2024
- Measurement of hemoglobin concentration was collected at the cross-sectional surveys in 2021 and 2022, and will be collected again in quarter 4, 2023 and in quarter 2024.
- Data entry was initiated in 2021 and is ongoing until quarter 1 in 2025.

B. Cohort 2.

- Informed consent for children participation in cohort 2 was obtained in 2022. Reconsenting will be done in December 2023 for these children to remain in the study until December 2024.
- Children were administered a questionnaire in 2022, and 2023 to ask about variables that might influence susceptibility to malaria during the coming malaria transmission season such as house design, use of an ITN, access to a health center. Same questionnaire will be administered in quarters 2 and 4 in 2024.
- Passive surveillance for clinical and severe malaria was initiated in 2022 and will continue until December 2024..

- Case-control for clinical malaria was initiated in 2022 and will continue until March 2023.
- Case-control for severe malaria was initiated in 2022 and will continue until December 2024.
- Cross-sectional surveys were completed in 2022 (quarters 2 and 4), and quarter 22023 and planned for quarter 4 in 2023 and quarters 2 and 4 in 2024
- Blood filter samples for serology were collected in quarters 2 and 4 of 2022, and quarter 2 of 2023 and will be collected in quarter 4 of 2023 and in quarters 2 and 4 in 2024.
- Measurement of hemoglobin concentration occured in quarter 4 of 2022 and will be measured in quarter 4 of 2023 and quarter 4 of 2024 at the cross-sectional surveys.
- Data entry will be initiated in 2022 and is ongoing until 2025.

# 9. LABORATORY PROCEDURES

## 9.1. Detection of *P. falciparum* parasites

A Histidine Rich Protein 2 (HRP2) based rapid diagnostic test is used for immediate care purposes and the cassettes are kept for further analysis. Slides dried and stained with Giemsa are read independently by two microscopists. If there is a discrepancy, a third reading is done, and the result reconciled following the standardized algorithm used in the main trial and described previously [12].

## 9.2. Measurement of hemoglobin concentration

Hemoglobin concentration is measured colorimetrically using a Hemocue colorimeter (Hemocue AB, Angelholm, Sweden).

## 9.3. Detection of molecular markers

Blood spots are taken onto filter paper (Whatman No3), dried and stored with desiccant, for later analysis of molecular markers of resistance. Used RDTs are also stored so that they can be used to assess the utility of used RDTs for monitoring molecular markers. Parasite DNA will be extracted using already published methods [13].

*Pfcrt genotyping*: a qPCR assay using three dual-labelled probes will be used to detect the three commonly known pfcrt haplotypes namely, CVMNK, CVIET and SVMNT [14], allowing detection of any return of sensitivity to chloroquine.

*PCR and sequencing of pfmdr1, pfdhfr and pfdhps*. All positive samples from qPCR will be used for the genotyping of Pfmdr-1 (86, 184, 1034, 1042, and 1246), pfDHFR (51/59/108/164), and pfDHPS (437/540/581/613). These three genes will be amplified by nested PCR using previously published primers [13, 14]. Genotyping of these genes will show the trends in the prevalence of these markers over several years of SMC implementation. Polymorphisms of the pfmdr1, dhfr and dhps genes will be determined by direct sequencing of amplified products (BigDye Terminator v3.1 cycle sequencing kits; ABI 3730 sequencer (Applied Biosystems)). The sequence chromatograms will be analyzed using Geneious

v10.1.3 (Biomatters, US) by alignment with the corresponding gene sequences of laboratory parasite line 3D7.

### 9.4. Malaria serology

In June 2021, children in cohort 1 were administered a questionnaire to ask about variables that might influence susceptibility to malaria during the coming malaria transmission season such as house design, use of an ITN, access to a health center etc. At the same time, a finger prick blood spot was obtained for measurement of antibodies to a wide range of malaria antigens and to determine the pattern of the immune response in relation to the level of exposure to SMC or RTS,S during the previous four or five years. The relationship between this pattern and the incidence of clinical episodes of malaria in the subsequent malaria transmission will be evaluated. The same procedure was be followed in June 2022 and June 2023 for children in cohorts 1 and 2 and will be followed in cohorts 1 and 2 children in June 2024At the cross-sectional survey at the conclusion of the transmission season described above, a finger prick blood spot is obtained for preparation of a blood film and determination of hemoglobin concentration. At the same time blood is obtained for measurement of antibodies to determine how the pattern has changed following one year of exposure to malaria (Appendix A).

Antibodies to a wide range of malaria pre-erythrocytic and blood stage antigens will be measured using the Luminex bead platform, following a protocol developed by Prof. Drakeley at the London School of Hygiene and Tropical Medicine [16]. Blood samples may also be sent to Prof Philip Rosenthal at the University of California, San Francisco. See Appendix A for details.

## 10.STATISTICAL CONSIDERATIONS

### 10.1. Statistical Analysis Plan

The statistical analysis will be conducted according to a Statistical Analysis Plan (SAP) which will be prepared and finalized before database lock. All data will be collected and verified prior to analysis. The following key statistical components and a detailed description will be documented in the SAP.

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

The number of subjects enrolled, completed, or withdrawn will be summarized. Reasons for

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

### 10.2. Statistical approach for analyzing endpoints

The co-primary and secondary endpoints will be assessed first in cohort 1 and then in cohort 2 after children have reached the age of five years using passive case detection and case-control methods. A consort diagram including the participation rate for each of the analyses will be provided.

#### 10.2.1. Passive case detection method

For the passive case-detection, the hazard (including multiple episodes within the same children) in groups will be defined according to:
i) prior intervention group (SMC group; RTS,S group; SMC + RTS,S group)
ii) adherence to the assigned intervention(s)
iii) prior malaria history (number of clinical malaria episodes between 2017 and June 2021 or June 2022, as applicable).

These analyses will use all the events recorded. It is known that children in the combination group were better protected than children in the single intervention groups during the first three years of the trial. If this situation persists over the remaining part of the intervention period, then children in the combined group might be less immune than children in the single intervention groups, due to a delay in acquisition of naturally-acquired immunity induced by repeated malaria episodes.

Taking the first comparison (intervention group) as an example, the hazard of malaria among 5-year-old children who previously received both SMC + RTS,S (2021) will be compared with the hazard of malaria among 5-year-old children who previously received RTS,S alone in order to indicate whether having also had SMC over the past 4 years increases the risk of rebound morbidity.

Likewise, comparing the combination group (SMC + RTS,S) to the SMC alone group will indicate whether having received RTS,S (in addition to SMC) increases the risk of rebound morbidity. Similar comparisons based on the extent of adherence (fully adherent compared to less adherent) and on the number of clinical malaria episodes captured during the intervention trial will indicate whether better adherence increases the risk of subsequent rebound, and whether more episodes of malaria decrease the risk of subsequent rebound due to enhanced acquisition of naturally-acquired immunity.

For each comparison, the hazard ratio will be estimated using Cox regression models, with a robust standard error (i.e., the Andersen-Gill extension of the Cox model) to account for potential clustering of multiple episodes within study children. The Efron method will be used for tied event times. Nelson-Aalen Cumulative hazards will be plotted for each group to show the mean number of events per child during the study and the timing of events, and Kaplan Meier failure estimates will be plotted to show the risks during different study periods.

Further details of the methods will be included in the analytical plan.

### 10.2.2. Case-control method

There are two prospective matched case-control studies; one analyzing episodes of uncomplicated malaria and a second analyzing episodes of severe malaria.

Both studies will use conditional logistic regression to estimate crude and covariate-adjusted odds ratios for each exposure of interest, comparing cases and controls. Because controls are recruited concurrently, cases and controls are matched on person time, and the odds ratio estimates the incidence rate ratio (and is interpretable as an IRR, even though uncomplicated malaria is not a rare outcome).

Taking prior intervention group as an example, the percentage of cases of uncomplicated malaria who had previously received the combination of RTS,S + SMC will be compared to the percentage of controls that had previously received the combination of RTS,S + SMC. If having received the combination of both RTS,S and SMC during the intervention period of the trial predisposes a child to acquiring more clinical malaria episodes when the intervention stops (i.e., better protection results in more rebound morbidity), then we would expect the prevalence of the exposure (being in the combined group) to be higher among cases than among the controls. This can be interpreted as exposure namely being in the combined group having increased the risk of malaria. It is possible that this association could work in the opposite direction, namely that having had better protection reduces later risk.

For the severe malaria case-control, all the logic and procedures will be the same as for the uncomplicated cases with the added aspect that two hospital-based controls will also be selected (children admitted for a reason other than severe malaria).

As an example, the percentage of cases of malaria who had previously received the combination of RTS,S + SMC would be compared to the percentage of controls who had previously received the combination. If having received both RTS,S and SMC during the intervention trial predisposes a child to acquiring more clinical malaria episodes when the intervention stops, then we would expect more of the cases to have been in the Combined RTS,S + SMC group than among the controls.

Likewise for adherence (for example a child having received all 4 SMC administrations per year), we might expect an increased incidence of clinical malaria in the year after SMC has been stopped in children compared to controls because of a delay in acquisition of naturally-acquired immunity. For the malaria history, we might expect that more clinical malaria cases in the year after interventions were stopped will have had relatively few episodes of malaria during the intervention trial (so have not developed immunity) compared to the controls. In addition, other exposures that are to be measured (data collected from questionnaires) include current bed net use, care-seeking behavior or barriers to care-seeking, knowledge of malaria, and household wealth.

For the severe malaria case-control analysis, all the logic and procedures will be the same as for the uncomplicated cases with the added aspect that two hospital-based controls will also

be selected (children admitted for a reason other than severe malaria).

### 10.2.3. Baseline Descriptive Statistics

Baseline demographics and characteristics, including age will be summarized by previous intervention group using descriptive statistics. Summaries of subject disposition will be prepared for all subjects, including the number and percent enrolled. A summary and listing of adherence to receipt of SMC administrations and RTS,S vaccinations will be prepared.

### 10.2.4. Planned Interim Analyses

There are no planned interim analyses.

### 10.2.5. Multiple Comparison/Multiplicity

Any adjustments to the significance level or the overall p value will be indicated in the SAP to account for any planned or unplanned subgroup analyses or multiple testing.

### 10.2.6. Exploratory Analyses

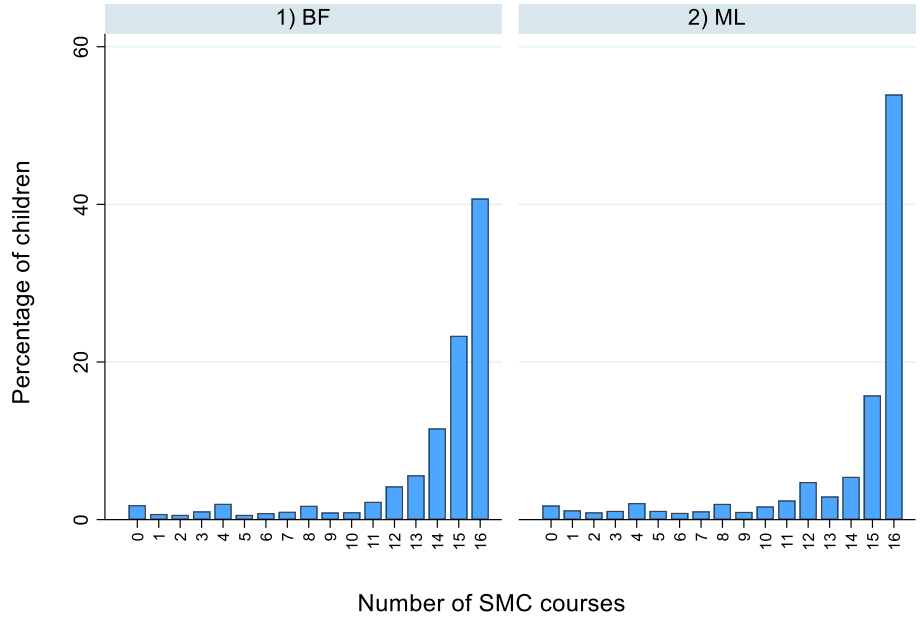
Exploratory analyses will be described in the Statistical and Analysis Plan when the exploratory assays have been finalized. The serological analysis procedure is described in Appendix A.

## 10.3. Sample Size

The target sample size for the uncomplicated malaria case control study is 450 cases of clinical malaria in each country for each year of follow-up with two controls per case. With the intention to assess both the prior randomization group (RTS,S alone, SMC alone, and combination RTS,S + SMC groups) and adherence to prior SMC as exposures, the sample size needs to consider both comparisons, and consider the fact that the study team will be blind to the prior treatment allocation. If 450 uncomplicated malaria cases are recruited per year in each country, then there will be approximately 150 cases per intervention group (SMC alone, RTS,S/AS01E alone and the combined group) in each year in each country and 300 per group over the two years in each country, a total of 1,800 cases Preliminary analysis, shown in the figure below, suggests that there will be a wide range of exposure to SMC in individual children allowing useful comparisons to be made.

In Mali, 706 cases from cohort 1 and 709 cases from cohort, and in Burkina Faso 1042 cases from cohort 1 and 917 from cohort 2 have been enrolled until the first week in October 2023. . This sample size will allow the analyses of the case-control study data in each country by each cohort separately. However, the number of severe malaria cases recruited until now is only 97, with 21 hospital controls. Thus the data from the two cohorts and two countries will be combined for the analysis of severe malaria case-control study.

Figure 4: Number of previous SMC courses in Burkina Faso and Mali up to the end of 2020.




### 10.3.1. Adherence to SMC as a risk factor for rebound

The calculations below are based on the prevalence of the exposure (full adherence to prior SMC) being 40%. This was 40.7% in Burkina Faso and 53.9% in Mali during the previous four years.

Assuming an odds ratio of 1.75, which would reflect a 35% relative increase in the exposure among the cases (to 54%), allowing for some correlation of the exposure between cases and controls due to the potential for adherence to be correlated within villages (up to 0.4), then with 5% significance, 300 cases within the SMC alone group over two years in each country will provide 90% power to detect a difference in SMC adherence between cases and controls. Although it is possible that the relative difference in exposure will be larger than 35% (i.e. a larger OR than 1.75), or that the correlation will be lower than 0.4, this sample size will ensure there is adequate precision around the effect estimate. Within each year, there will be 150 cases in the SMC alone group in each country in each year, giving 80% power to detect an OR of 2 at the 5% significance level, with other assumptions as above. As for the comparison over two years, the primary interest is in the precision of the effect size rather than detection of a difference; the sample size of 300 cases should ensure reasonable precision in each year.

### 10.3.2. Comparison of rebound according to prior intervention group

Considering prior receipt of a particular intervention (SMC alone, RTS,S/AS01E alone or the combination of the two) as the exposure, the prevalence of the exposure among the controls will be 33% for each randomization group. Prior intervention is not likely to be correlated between cases and controls within the villages used to define the matched sets, since this was randomized. A sample size of 300 cases in each prior intervention group in each country, will provide 90% power to detect an odds ratio of 1.6 between the prior intervention groups. A sample size of 300 cases within each year in each country will have 80% power to detect an OR of 1.77 (and thus reasonable precision around a larger treatment effect).

### 10.3.3. Severe malaria

Previous experience in the study area suggests that there will be there will be approximately 10-15 severe malaria episodes per 1000 child-years at risk among children who are no longer eligible for SMC, i.e., a total of between 30-50 severe cases in the first year after the intervention is stopped in both countries combined and 20-30 among children who are no longer eligible for SMC in the following year. After two years of follow-up in cohort 1 and one year of follow-up in cohort 2 a total of 78 severe cases have been recruited (22 in Mali and 56 in Burkina Faso). For this analysis, data from the two or three years of follow-up (cohorts 1 and 2 respectively) will be combined and considered separately for comparison with the community controls and the hospital controls. With 42 cases and two controls, the study will have 80% power to detect a risk factor found among 50% of controls, but in 75% of cases (i.e., a risk ratio of 1.5, or an odds ratio of 3.0). With 55 cases, the study would have 90% power to detect this difference. Analyses of the case-control study will be conducted in the two countries separately and within each country of the older and younger groups separately (4 groups).

Based on previous experience in the study areas, it is anticipated that the prevalence of malaria parasitemia in children aged five or six years old who have not been receiving SMC will be about 20% in Mali and 50% in Burkina Faso resulting in approximately 1,000 parasitemic children at the 2021 cross-sectional survey and a further 700 in 2022. The prevalence of moderate anemia defined as a hemoglobin 7.0 g/dl will be much less and likely to be in the range of 0.5% to 1.0% with about 30 cases detected in the first year of follow up and 15 in the following years so analysis for this end-point will utilize data from all years.

## 11.DATA MANAGEMENT

Passive surveillance data obtained in clinics or in the hospital is collected firstly onto registers, then immediately captured with electronic devices using the ODK (Open Data Kit) system in each site and for each activity. Laboratory technicians have laboratory books to record readings which are subsequently captured onto their electronic devices (Android). Database for the case control studies is kept and managed separately. Back up hard copies are made in case a device breaks down for any reason.

## 12.QUALITY ASSURANCE/QUALITY CONTROL

An independent monitor will be appointed to ensure that studies are being conducted according to the study protocol, and that appropriate ethical procedures are in place. The monitor will examine a random selection of clinical and laboratory records during each visit. Quality control procedures will be developed in each site, including supervisory checks on data recording by field staff and manual checks on accuracy of data entry.

## 13.ETHICS

Individual, written, informed consent for all children included in the rebound study was obtained for children in cohort 1 and cohort 2 from the family or legally recognized legally accepted representative of each child during surveys in March 2021 and May to June 2022, respectively, with clear mention that participants are free to leave the study at any time without the obligation to justify the reason why. Consent for children in cohort 1 to continue surveillance for a further year (until the end of December 2023) will be obtained in March 2023.The consent process includes provision of details on the reasons why the study is being undertaken and what agreeing to join the project might involve if their child is selected for enrollment in the case control study, including passive follow up, completing a questionnaire and collection of finger-prick blood samples. Once a child has been identified as case or control a further consent form is administered. In the case of a case this is usually done at the clinic or in hospital whilst consent from controls is obtained at the home visit. Ethical approval has been obtained from the Ethics Committee of Burkina Faso, Mali and LSHTM, United Kingdom.

Finger prick samples will be obtained to perform a rapid diagnostic test for malaria, blood smear and to obtain a few drops for preparation of filter paper blood spots prior to the malaria transmission season and at the cross-sectional survey. The project team (physicians, nurses and lab technicians) are well experienced and trained to carry out finger pricks with caution. The finger prick will cause minor pain. The materials to be used for finger prick will be sterile, for single use and disposable kits will be utilized.

All local guidelines on COVID-19 safety procedures are followed.

## 14. STUDY MANAGEMENT

INSTech will act as the study sponsor; an independent steering committee has been established to oversee the trial. and approval of the protocol will be sought before the start of the study. The trial will adhere to the principles outlined in the International Conference on Harmonization Good Clinical Practice (ICH GCP)

## 15. GOVERNANCE, RESPONSABILITY AND COLLABORATION

The overall co-ordination of the project will be undertaken by LSHTM. Dr Issaka Zongo, the study PI in Burkina Faso will be the main respondent for the day-to-day management of the study. He will contribute to all the scientific and technical aspects of the study and will be the main channel for any related communication. Data management and analysis will be done by Issaka Zongo in collaboration with MRTC scientists with support from a statistician and the data manager of the project. Advice will be sought from senior statisticians at the London School of Hygiene and Tropical Medicine if needed. Molecular markers of resistance assays will be done at a central laboratory in Mali and in Burkina Faso with support from Prof Philip Rosenthal from the University of California, San Francisco who has agreed to support this work.

## 16. CAPACITY DEVELOPMENT

The project will be an opportunity for capacity building. The project will give field study

experience to young scientists and contains provision for the support of one MSc and one PhD student linked to the project from the EDCTP. The MSc student will be registered for two full academic years from Oct 2021 to September 2023; the PhD student will register from January 2022 for graduation expected by the end of the fellowship. Finally, the principal investigator in this project will gain in-field study mentorship.

# 17. STUDY TIMELINES

Study timelines are indicated in the table below.

## 17.1. Budget

The study will be funded by grant from the EDCTP to Issaka Zongo (number TMA2019SFP-2834) and by PATH.

Table 1. Gantt Chart. Risk of Malaria in Children Who are no Longer Eligible for Seasonal Malaria Chemoprevention (SMC) or seasonal vaccination (SmV) in Burkina Faso and Mali

| | **2021** | | | | 2022 | | | | 2023 | | | | 2024 | | | | 2025 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **T1** | **T2** | **T3** | **T4** | T1 | T2 | T3 | T4 | T1 | T2 | T3 | T4 | T 1 | T2 | T3 | T4 | T1 | T2 |
| Ethics submission | | | | | | | | | | | | | | | | | | |
| Ethics approval | | | | | | | | | | | | | | | | | | |
| Ethics approval amendment | | | | | | | | | | | | x | | | | | | |
| Study registration | | | | | | | | | | | | | | | | | | |
| Passive surveillance- cohort 1 | | | | | | | | | | | | | x | x | x | x | | |
| Passive surveillance- cohort 2 | | | | | | | | | | | | | x | x | x | x | | |
| Reconsent cohort 1- passive surveillance | | | | | | | | | | | | x | | | | | | |
| Reconsent cohort 2- passive surveillance | | | | | | | | | | | | x | | | | | | |
| Case control cohort 1 recruitment- uncomplicated and severe cases | | | | | | | | | | | | | | | | | | |
| Case control cohort 1 recruitment- severe cases | | | | | | | | | | | | | x | x | x | x | | |
| Case control cohorts 2 recruitment- uncomplicated and severe cases | | | | | | | | | | | | | x | | | | | |
| Case control cohort 2 recruitment- severe cases | | | | | | | | | | | | | x | x | x | x | | |
| Cross sectional surveys | | | | | | | | | | | | | | x | | x | | |
| Collection of serology samples | | | | | | | | | | | | | | x | | x | | |
| Trial monitors visits | | | | | | | | | | | | | | | | x | | |
| Serology assays | | | | | | | | | | | | | | | x | x | | |
| Molecular marker assays | | | | | | | | | | | | | | | x | x | | |
| Data entry and cleaning | | | | | | | | | | | | | | | x | x | x | |
| Data analysis | | | | | | | | | | | | | | | | x | x | x |
| Manuscript writing | | | | | | | | | | | | | | | | | | |

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# 19. APPENDIX A. IMMUNE RESPONSES IN CHILDREN THAT HAD PREVIOUSLY RECEIVED SMC, RTS,S, OR THE COMBINATION OF SMC PLUS RTS,S.

## 19.1. Exploratory Serology Study 1:

Objective: The objective of this study is to evaluate the hypothesis that a potential change in antibody titers to blood stage antigens of *P. falciparum* among children who have received the combined intervention compared to titers among children who received RTS,S alone or SMC alone will have been resolved (i.e., the differences will no longer be observed) following exposure to malaria for one or two malaria transmission seasons after administration of the study interventions has ceased.

Endpoint: The study end-point will be the antibody titer to selected *P. falciparum* blood stage malaria antigens measured by the Luminex assay. The antibody panel will include antibodies associated with long term exposure to malaria (MSP2 CH150, MSP2 Dd2, PfAMA1, PfMSP119, GLURP R20), those associated with recent exposure (Etramp4 Ag2, Etramp5 Ag1, Gexp18, HSP40 Ag1, Hyp2, PfSEA, SBP1) and those with a putative protective function (EBA140 RIII-V, EBA175 RIII-V, EBA181 RIII-V,Rh2 2030, Rh4.2, Rh5.1).

Samples: Approximately 2,000 blood spot samples (1,000 from each country) were obtained from study children in cohort 1 at a cross-sectional survey conducted in November 2021.These children will have been exposed to malaria during one malaria transmission season since they ceased to receive RTS,S, SMC or the combination. Approximately 600 samples, 200 from children in each of the three study groups will be randomly selected for antibody measurement in both Burkina Faso and Mali (total 1,200). A similar number of samples were obtained from the same children during a cross sectional survey undertaken in November 2022, when the children from Cohort 1 had been exposed to two malaria transmission seasons since they ceased to receive RTS,S, SMC or both interventions and from a similar number of children in cohort 2. A similar number of samples from children in cohorts 1 and 2 will be obtained from the same children during a cross sectional survey undertaken in November 2023.

Sample size calculation: A study with 200 – 300 samples obtained from children in each intervention group obtained at each November survey one, two or three years after ceasing to receive the interventions will have sufficient power to demonstrate that the anticipated reduction in antibody titers to blood stage antigen in children who received RTS,S and SMC compared to titers in with children who received RTS,S or SMC at the time when they had first completed receipt of the interventions will no longer be present after one, two or three subsequent years of exposure to malaria.

Statistical analysis. Mean titers for each test antibody will be compared between the three study

groups in each country before and after one (Cohort 2) or two years (Cohort 1) after ceasing to receive RTS,S and SMC or RTS,S or SMC alone. In addition, antibodies will be ranked into terciles and the distribution by tercile compared between the study groups for each year.

### 19.2. Exploratory Serology study 2

Objective: This will an exploratory study with the objective of determining whether it is possible to identify a pattern of antibodies to malaria pre-erythrocytic and blood stage antigens that can predict protection against malaria infection, uncomplicated clinical episodes of malaria or severe malaria which is valid in children who have previously been protected against malaria by an ITN, SMC or RTS,S or a combination of these interventions.

Endpoint: The trial endpoint will be a composite antibody response that predicts protection against malaria parasitemia, an uncomplicated episode of clinical malaria or severe malaria. The antibody panel will include blood stage antibodies associated with long term exposure to malaria (MSP2 CH150, MSP2 Dd2, PfAMA1, PfMSP119, GLURP R20), those associated with recent exposure (Etramp4 Ag2, Etramp5 Ag1, Gexp18, HSP40 Ag1, Hyp2, PfSEA, SBP1) and those with a putative protective function (EBA140 RIII-V, EBA175 RIII-V, EBA181 RIII-V,Rh2 2030, Rh4.2, Rh5.1). In addition, antibodies to the circumsporozoite protein (CSP) will be measured. In addition to measurement of total antibody titer, additional analyses will be undertaken on antibody sub-class and avidity on selected samples using the Luminex platform.

Samples: Blood spot samples for this study were obtained from approximately 4,000 children who have previously received RTS,S, SMC or a combination of the two interventions for four years at a census undertaken in June 2021, prior to the malaria transmission season. Approximately a half of these children continued with the trial interventions (a booster of RTS,S in June, SMC throughout the 2021 malaria transmission season dose or a combination of both interventions) and a half received only standard care as they had reached the age of five years, the age after which SMC administration is no longer recommended. Approximately 8,000 further samples will be obtained during two additional surveys of study children undertaken in June 2022 and June 2023, prior to the malaria transmission seasons.

Sample size calculation. Formal sample size calculations are not indicated for this study which is primarily an exploratory one. However, the power of the study to identify patterns of antibody response that are associated with protection against different categories of malaria infection will be influenced by the number of cases detected during the two malaria transmission follow-up periods. It is estimated that in Mali there will be approximately 200 clinical cases of uncomplicated malaria and 10 cases of severe malaria in each of the two or three years of follow-up.. At the first cross-sectional survey undertaken in Mali in November 2021, it is estimated that about 20% of children (400) will be parasitemic with about 100 parasitemic children having received one or both of the interventions and 300 children having received only standard care. In November 2022 and 2023, when all children have received only standard of care during the preceding malaria transmission the percentage parasite prevalence is likely to be at least 30%

(approximately 600 children). In Burkina Faso, where malaria transmission is much higher, numbers are likely to be around double of those seen in Mali. Thus, the study will have a high degree of power to detect associations between antibody responses and the incidence of uncomplicated clinical cases of clinical malaria and with the prevalence of asymptomatic malaria parasitemia but much less power to detecting associations with severe malaria.

Analysis: The geometric mean titer and the range in antibody titer for each of the antibodies will be compared between children who experienced an uncomplicated clinical malaria episode or an episode of severe malaria during the malaria transmission season after the blood samples had been collected were collected with the geometric mean antibody titer and the antibody range found in children who did not experience an episode of uncomplicated clinical malaria or severe malaria. Similarly, antibody titers and antibody range in titer will be compared between children who were parasitemic at the time of the cross-sectional survey and those who were not parasitemic at this time. No adjustment for multiplicity will be performed.

Based on the initial findings, a model will be developed which incorporates the overall response to each antibody in order to define an antibody pattern that predicts protection against parasitemia, uncomplicated or severe malaria. In addition to evaluation of the IgG antibody response, additional analyses will be undertaken which investigate the potential protective role of antibody subclass and antibody avidity. The model will be applied initially to study all children and then for children in each of the three intervention groups to see whether prior preventive measures influence the accuracy of the model. The model will be developed first for findings in Mali and then applied to the samples in Burkina Faso, where transmission is higher, to see if the model is generalizable.