Malaria chemoprevention efficacy study protocol

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ABBREVIATIONS

ACT	artemisinin-based combination therapy
ANC	antenatal care
CI	confidence interval
CPES	chemoprevention efficacy studies
DBS	dried blood spot
IPTi	intermittent preventive treatment of malaria in infants
IPTp	intermittent preventive treatment of malaria in pregnancy
IPTsc	intermittent preventive treatment of malaria in school-aged children
MDA	mass drug administration
PMC	perennial malaria chemoprevention
RDT	rapid diagnostic test
SMC	seasonal malaria chemoprevention
SP	sulfadoxine-pyrimethamine
TES	therapeutic efficacy studies
WHO	World Health Organization

1. INTRODUCTION

Preventive chemotherapy is the use of medicines, either alone or in combination, to prevent malaria infection and its consequences. It requires giving a full treatment course of an antimalarial medicine, often at predefined intervals to individuals who have not been diagnosed with malaria.[1] By providing antimalarial medicine to vulnerable populations, existing undiagnosed malaria infections are treated, and the medicine provides a period of protection against new infections.

Chemoprevention[2] strategies currently recommended by the World Health Organization (WHO) and covered by this guidance are described in Annex 1. These include intermittent preventive treatment of malaria in pregnancy (IPTp), perennial malaria chemoprevention (PMC), seasonal malaria chemoprevention (SMC), and intermittent preventive treatment of malaria in school-aged children (IPTsc) *(1)*.[3] Information on the efficacy of the medicines as used in these malaria chemoprevention strategies is critical for ensuring that the strategies remain effective in different settings with different levels of drug resistance.

WHO has prepared a standard protocol for therapeutic efficacy studies (TES), and tools for data analysis and monitoring the parasitological and therapeutic responses to treatment *(2)*. TES are prospective evaluations of patients' clinical and parasitological responses to treatment for uncomplicated malaria. TES are considered the gold standard for assessing antimalarial drug treatment efficacy, and the resulting data are used to inform national malaria treatment policy in malaria-endemic countries. Unfortunately, systematic reviews have demonstrated that even when TES are done at the same time and place, they do not accurately predict the efficacy of chemoprevention strategies *(3)*. In TES, the therapeutic efficacy in clearing asexual blood-stage parasites is assessed in subjects with uncomplicated malaria. In chemoprevention, the drug regimen given can work by clearing both asexual blood-stage parasites and pre-erythrocytic stage parasites in asymptomatic subjects. In both treatment and chemoprevention, many factors other than drug resistance impact the efficacy, including immunity, drug absorption and metabolism. A mutation that can significantly impact the efficacy of a drug in clearing asexual blood-stage parasites may have a different impact on the drug's efficacy in clearing pre-erythrocytic stage parasites. Additionally, a lower number of asexual blood-stage parasites in an asymptomatic recipient of chemoprevention may be easier for a drug to clear compared to a high number of asexual blood-stage parasites in a symptomatic patient enrolled in TES. Specific protocols are therefore needed for monitoring chemoprevention efficacy.

This document is intended as a guide for studies of chemoprevention efficacy and was developed based on reviews of protocols of ongoing studies. The document adapts some of the principles and practices underlying treatment efficacy monitoring to provide standardized approaches for monitoring and evaluating the efficacy of medicines used for malaria chemoprevention. This guide will be updated once additional experience is gained from studies of chemoprevention efficacy.

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1 Those identified with malaria at the time of the intervention are treated as per the national treatment guidelines. Only individuals with symptoms are diagnosed by rapid diagnostic test or microscopy.

2 The term chemoprevention is used as equivalent to and interchangeable with the term preventive chemotherapy.

3 Mass drug administration (MDA) and post-discharge malaria chemoprevention (PDMC) are not addressed in this document.

2. STUDIES OF CHEMOPREVENTION EFFICACY

2.1 Definition

Drug efficacy is defined as the capacity of an antimalarial medicine to achieve the therapeutic objective when administered at the recommended dose *(4)*. Chemoprevention efficacy and impact, as understood in this document, are defined in Box 1.

Box 1: Definition of chemoprevention efficacy and impact

Chemoprevention efficacy: ability of a course of an antimalarial medicine to clear existing parasitaemia and prevent any new infections for a short period (e.g. 28 days).

Chemoprevention impact: impact of chemoprevention on uncomplicated malaria, anaemia, low birthweight, severe malaria and death.

The WHO-recommended chemoprevention strategies discussed here (IPTp, PMC, SMC and IPTsc) are all intended to clear infections that are present at the time of treatment and to provide a period of post-treatment prophylaxis after the medicine is given. Potential recipients of chemoprevention with symptoms of malaria would be referred for parasitological diagnosis by rapid diagnostic test (RDT) or microscopy and treated as per the national treatment guidelines if malaria diagnosis is confirmed. All malaria chemoprevention strategies are for areas with moderate to high transmission (seasonal or perennial) where a significant proportion of the population may present with asymptomatic parasitaemia. Chemoprevention efficacy studies (CPES) aim to evaluate the ability of antimalarial medicines to clear parasitaemia among asymptomatic individuals and prevent parasitaemia for a predefined period of follow-up. Parasitaemia detected during the follow-up period can be caused either by the failure to clear an infection present at the time of enrolment or by the failure to prevent a new infection. The study methodology can be modified to focus on the efficacy of the medicine given to prevent infection (described in section 4.1).

CPES are meant to be routine studies of the efficacy of medicines used for malaria chemoprevention; the purpose is not to evaluate long-term outcomes for the treated individuals. However, studies that link efficacy and longer term outcomes could help to inform policy-makers about the likely public health impact of the chemoprevention strategies and establish relationships between chemoprevention efficacy and specific outcomes.

Drug resistance is only one of several factors that may reduce chemoprevention efficacy. Other factors include incorrect dosage, poor patient adherence to treatment, poor drug quality, drug interactions, poor absorption, rapid elimination (e.g. diarrhoea or vomiting) or poor metabolism of prodrugs. Some of these factors can be ruled out in an efficacy study when drug administration is supervised, and the origin and quality of the drugs are verified. The outcome of the study is influenced by a combination of factors, including those related to the human (e.g. immunity) and parasite (e.g. drug resistance); individual variation leading to differences in the availability of the drug (e.g. pharmacokinetics) *(5)*; and the intensity of malaria transmission.

Although detection of declining chemoprevention efficacy may signal the presence of resistance to an antimalarial drug, confirmation and characterization of parasite resistance require additional tools in addition to TES (e.g. in vitro or ex vivo tests, measurement of drug concentrations in the blood, and analysis of molecular markers). For further information, see the TES protocol and related documents *(6–10)*.

2.2 Molecular markers of resistance to antimalarial drugs

Once genetic changes in the parasite are identified and validated as markers of clinically relevant forms of resistance, drug resistance can be monitored using molecular techniques. These analyses can be performed reliably and inexpensively.

While the markers have been found to be imperfectly associated with treatment outcomes, their value in predicting the efficacy and usefulness of a medicine for chemoprevention is still unclear. Therefore, it is currently not possible to rely on surveillance of these markers alone to make decisions on chemoprevention efficacy.

Molecular markers of *Plasmodium falciparum* resistance to antimalarial drugs that have been found to be associated with reduced treatment response and are useful in surveillance for resistance include:

- *Pfcrt (P. falciparum chloroquine resistance transporter)* point mutations conferring resistance to chloroquine and piperaquine;
- *Pfdhfr (P. falciparum dihydrofolate reductase)* point mutations conferring resistance to pyrimethamine;
- *Pfdhps (P. falciparum dihydropteroate synthase)* point mutations conferring resistance to sulfadoxine and other sulfa drugs;
- Increased copy numbers of *Pfmdr1 (P. falciparum multidrug resistance 1 protein*) associated with *P. falciparum* resistance to mefloquine;
- Increased copy numbers of *Pfpm2–3 (P. falciparum plasmepsin 2–3)* and/or point mutations in *Pfcrt* associated with *P. falciparum* resistance to piperaquine;
- *PfKelch13* gene point mutations associated with *P. falciparum* partial resistance to artemisinins.

A more comprehensive summary of molecular markers for drugs currently or potentially being used for chemoprevention is provided in Annex 2.

3. CPES STUDY DESIGN

CPES are single-arm studies[4] that aim to evaluate the ability of one or more rounds of chemoprevention to clear any pre-existing parasitaemia and prevent infection for a predefined period of follow-up.

3.1 Study population

The population groups targeted for enrolment consist of asymptomatic individuals eligible for the chemoprevention intervention. Those with malaria symptoms must be tested for malaria (by RDT or microscopy). If malaria is confirmed, they should be treated with the recommended treatment as per national treatment guidelines and excluded from the study. The prophylactic drug is expected to be able to clear low parasitaemia at inclusion (< 2000 asexual parasites/µL in moderate to high transmission area), but easy access to treatment must be ensured.

Inclusion and exclusion criteria are listed in Table 1.

3.2 Study site

CPES should be done in areas where chemoprevention interventions are conducted or are being considered for introduction/implementation. The specific sites selected must be based on:

- a sufficient number of individuals targeted for the chemoprevention intervention in the site to make it feasible to reach the target sample size;
- up-to-date information on the local epidemiology of malaria;
- trained, motivated personnel capable of recruiting and following up study participants, collecting samples and providing malaria treatment as needed;
- the availability of facilities to store samples and supplies securely, and stain slides; and
- access to a laboratory equipped for blood film examination.

The site can consist of one or more facilities or villages, including schools for IPTsc where the intervention is being provided. If supervision of treatment and follow-up is only feasible in a few of the villages covered by a certain health facility, the site can be defined as these villages.

The number of sites needed in a country depends on both the size of the area targeted for chemoprevention interventions and the distribution of drug resistance. If data are available showing markedly different levels of drug resistance in different areas, studies should be planned to cover these different settings.

4 The option of having the study as a two-arm study is covered in section 4.2.

3.3 Sample size

Not having parasitaemia during the follow-up period may be the result of the chemoprevention working as intended or it may be due to the study participant not being exposed to infective bites. This poses a challenge in terms of both the conclusions that can be drawn and the sample size needed.

The hypothesis tested is that the proportion of individuals infected at the end of the study will be significantly lower than it would have been if no chemoprevention had been given. In many settings, it will not be possible to have a control group. It would not be ethical to withhold chemoprevention from individuals who are eligible for a recommended intervention with the aim of having a control group. Therefore, having a control group may be possible only in settings where, for instance, the aim of the study is to assess the efficacy of an intervention before it is generally recommended in an area. Some general guidance on sample size calculations is given in Annex 3.

Table 1. Inclusion and exclusion criteria

Study population	Individuals eligible for chemoprevention as per the recommendations with no malaria symptoms
Inclusion criteria	
All CPES	▪ Eligible for chemoprevention for SMC, PMC, IPTp and IPTsc as per the current recommendations ▪ Able and willing to comply with the study protocol and follow-up schedule ▪ Provides informed consent/parent or guardian provides informed consent on behalf of child
Additional intervention-specific inclusion criteria	
IPTp	▪ For women ≥ 18 years[a] after the first trimester of pregnancy
Exclusion criteria	
All CPES	▪ Symptoms of malaria (axillary fever ≥ 37.5 °C and/or history of fever in the past 48 hours) ▪ Known allergy to the medicine provided ▪ Sulfadoxine-pyrimethamine (SP) should not be given to individuals receiving a sulfa-based medication for treatment or prophylaxis, including co-trimoxazole (trimethoprim–sulfamethoxazole). This medicine is widely used in HIV-positive individuals (infants and pregnant women) as prophylaxis against opportunistic infections. ▪ Individuals receiving azithromycin should be excluded from the study due to the antimalarial activity of azithromycin.
Additional intervention-specific exclusion criteria	
PMC, SMC, IPTsc	▪ Presence of severe malnutrition according to WHO's child growth standards
IPTsc[b]	▪ Females of menstruation age (> 12 years) unable or unwilling to take pregnancy test due to socio-cultural constrains

[a] Could include adolescents between 12 and 17 years of age but may need specific ethical approval and informed consent from parents/guardian.

[b] Could include adolescents between 12 and 15 years of age but may need specific ethical approval and informed consent from parents/guardian for pregnancy testing.

3.4 Treatment

Those enrolled in the study will receive the recommended treatment course for chemoprevention under direct supervision. How this is organized can vary among study sites, but supervised treatment is needed to ensure that failure is not caused by lack of adherence. If the individual vomits within one hour of receiving the treatment, the treatment should be repeated and vomiting recorded.

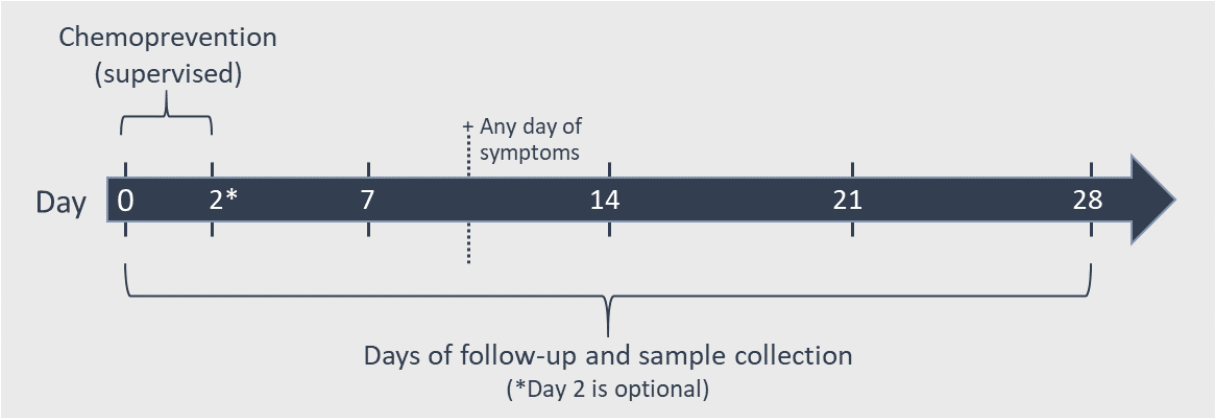
Study participants identified with asexual parasitaemia, as measured by microscopy between day 4 (in the case of development of clinical symptoms) and day 28 of the follow-up period, will be reported as chemoprevention failure and must be treated with an alternative regimen known to be efficacious – typically the recommended first-line treatment.

3.5 Follow-up

Study participants must be followed up with malaria blood films and dried blood spots (DBS) collected weekly for at least four weeks (days 0, 7, 14, 21 and 28), as well as on any day with symptoms. Additionally, day 2 can be added as a day of follow-up and sample collection; this is optional, but as this is the last day of administration for several of the currently recommended chemoprevention medicines, collecting samples on this day may be relatively easy (see Fig. 1).

With the currently recommended chemoprevention medicines, a significant level of protection is expected to be maintained for three to four weeks after administration of each course, but will likely decay rapidly thereafter. Where chemoprevention is administered monthly, follow-up for one course of chemoprevention for longer periods (e.g. 42 or 63 days) would require the next course of chemoprevention to be postponed, potentially increasing the risk to study participants. Therefore, this should only be considered when any risk to the study participants can be minimized through follow-up and easy access to care, and when data from a longer follow-up period are considered essential.

Fig. 1. Days of follow-up



3.6 Information and samples collected

On day 0 and all days of follow-up, a thick blood smear should be collected. In addition, in order to minimize discomfort to the patient due to repeated finger pricks, two or three drops of blood should be collected on filter paper each time a blood smear is required. Additionally, the temperature should be recorded (see Annexes 4 and 5 for details).

Patients with symptoms should always be tested for malaria; where this cannot be done with microscopy, an RDT can be used. If neither is available, the patient should be referred to a health facility.

For slides read retrospectively, asymptomatic study participants found to have parasitaemia from day 7 should be traced and treated as per the national treatment guidelines.

3.7 Diagnosis and molecular analysis

The presence of parasitaemia on day 0 and all days of follow-up should be determined primarily by microscopy. Nucleic acid amplification tests for the detection of low-density parasitaemia are considered experimental at this time and are not recommended for classifying study participants as parasitaemic. If parasites are detected by microscopy, the parasitaemia count should be recorded. If parasitaemia is detected on day 0 and any day of follow-up, PCR genotyping is needed to distinguish between reinfection and recrudescence *(11)*. DBS collected on days with parasitaemia (day 0 or during follow-up) should be analysed for relevant molecular markers of antimalarial drug resistance *(12)*. Recruitment will occur in asymptomatic study participants who are either uninfected or have parasitaemia. For study participants with low parasitaemia, genotyping at day 0 might be difficult.

3.8 Reporting and analysing study results

CPES aim to evaluate the ability of the provided medicine to clear any existing parasitaemia and/or subsequently prevent parasitaemia for a predefined period of follow-up. Chemoprevention efficacy will be a combination of curative and preventive efficacy. The main study outcome to be reported across interventions is chemoprevention failures based on study participants identified with asexual parasitaemia, as measured by microscopy from day 4 to the end of follow-up (see Table 2). Chemoprevention failure rates can be reported as the cumulative failure rate using Kaplan-Meier analysis or as the proportion using a per protocol analysis. A time-to-event (parasitaemia) analysis can be a helpful additional analysis for interpreting results.

Failure can be classified as clinical failure or parasitological failure, as in TES (see Annex 6), based on the presence of symptoms. Early treatment failures are likely to be rare, as the treatment is given to study participants with no parasitaemia, or with low parasitaemia but no symptoms; however, these should be reported separately. For the same reason, failures from days 4 to 7 will be rare, and they will be recorded if study participants seek care outside of scheduled days of follow-up.

Failure rates where chemoprevention efficacy is less than 100% will be a function of the prevalence of asymptomatic parasitaemia at the time of treatment and the curative efficacy, plus the risk of reinfection and preventive efficacy. Therefore, care needs to be taken in extrapolating conclusions across sites and seasons. Conclusions may be informed by knowledge of the local epidemiology and molecular findings.

Table 2. Main study outcomes to be reported

Chemoprevention failures
▪ Study participants identified with asexual parasitaemia by microscopy during the follow-up period from day 4 to day 28
Additional details when reporting chemoprevention failures
▪ Clinical failures: study participants identified with parasitaemia during the follow-up period from day 4 to day 28 with axillary temperature ≥ 37.5°C ▪ Parasitological failures: study participants identified with parasitaemia during the follow-up period from day 7 to day 28 with axillary temperature < 37.5°C ▪ Study participants identified with a new infection during the follow-up period ▪ Study participants identified with recrudescence during the follow-up period ▪ Geometric mean and range of asexual parasitaemia in study participants with chemoprevention failures ▪ Days to reported failure (median and range)
Prevalence of molecular marker(s)
▪ Prevalence of molecular marker(s) of resistance to chemoprevention drug(s) at day 0 and in any parasites detected after day 4 (# of infections carrying markers of interest / # of successfully genotyped infections)
Note: In the retrospective analysis, children found to have a parasitemia > 2000 asexual parasites/µL blood can be excluded and reported on separately.

4. ADDITIONS AND MODIFICATIONS TO THE STANDARD METHODOLOGY

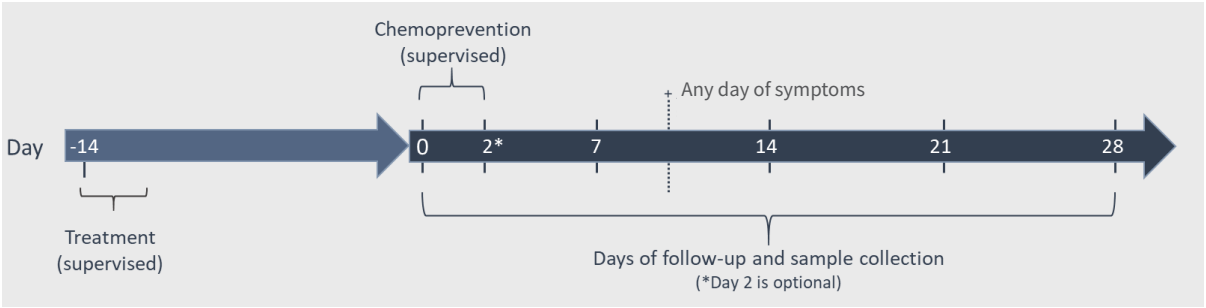
4.1 Modification to study new infection only

In some circumstances, there could be an interest in only studying the efficacy of chemoprevention to prevent infection. This could, for instance, be as a follow-up study in areas where a high number of chemoprevention failures have been found and further analysis as to the mechanism of failure is warranted.

If the objective of the study is to focus on the efficacy of chemoprevention to prevent infection, it is recommended to treat study participants with a highly efficacious antimalarial with a relatively short half-life 14 days prior to enrolment (see Fig. 2). Currently, the most suitable candidate for the pre-study treatment is artemether-lumefantrine, as lumefantrine has a shorter half-life than the other available partner drugs in artemisinin-based combination therapies (ACTs).

Studies with pre-study treatment enable more precise estimates of the efficacy of chemoprevention to prevent new infections. Studies without pre-study treatment report the proportion of participants with new infections during the follow-up period based on a PCR classification of parasitaemia as recrudescence or a new infection. This estimate is likely to be confounded by intra-host competition affecting the risk of new infection when parasites are already present, incorrect PCR classification, and failure of the PCR to pick up more than one infection.

Fig. 2. Study modified to focus on new infection



4.2 Conducting CPES as a two-arm study

In some settings, conducting CPES as a two-arm study can be done to compare groups receiving different drugs or, in an area where the chemoprevention strategy may be introduced, to compare a group receiving chemoprevention with a group not receiving chemoprevention. Steps need to be taken to ensure comparability between groups and to address any ethical concerns.

4.3 Monitoring of antimalarial drug resistance

Preferably, chemoprevention efficacy is monitored at sentinel sites where treatment efficacy and the prevalence of key molecular markers are also monitored. In-depth knowledge of the changing epidemiology at these sites will help inform the design of studies and interpretation of the results.

WHO recommends that first- and second-line treatments be monitored through TES at sentinel sites at least every two years. In general, chemoprevention interventions should not employ the same medicines used for treatment. However, if this is done, treatment efficacy needs to be monitored more closely.

Changes in the prevalence of relevant molecular markers should continuously be monitored alongside the use of chemoprevention. In areas where chemoprevention has not yet been introduced and in areas where a change of the drugs used for chemoprevention is planned, a molecular marker survey should be done prior to introducing or changing drugs.

The purpose of tracking molecular markers is to track changes potentially caused by the use of the chemoprevention, particularly if pre-intervention data are available. This tracking enables analysis of the association between the efficacy of chemoprevention and the general prevalence of relevant molecular markers. It can provide early evidence and serve as an early warning sign of resistance. Unfortunately, molecular markers of drug resistance are available for only some antimalarial medicines (Annex 2). The correlation between molecular markers and the therapeutic efficacy of many antimalarials is imperfect and should be interpreted with caution.

The survey of molecular markers can be done by collecting filter paper with DBS from patients seeking treatment in health facilities after confirmed malaria diagnosis and after the patient has consented to be part of the study.

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ANNEX 1. MALARIA CHEMOPREVENTION STRATEGIES COVERED BY CPES

	Current WHO recommendation
Intermittent preventive treatment of malaria in pregnancy (IPTp)	In malaria-endemic areas, pregnant women of all gravidities should be given antimalarial medicine at predetermined intervals to reduce disease burden in pregnancy and adverse pregnancy and birth outcomes. SP has been widely used for malaria chemoprevention during pregnancy and remains effective in improving key pregnancy outcomes. IPTp-SP should start as early as possible in the second trimester and not before week 13 of pregnancy. Doses should be given at least one month apart, with the objective of ensuring that at least three doses are received. Antenatal care (ANC) contacts remain an important platform for delivering IPTp. Where inequities in ANC service and reach exist, other delivery methods (such as the use of community health workers) may be explored, ensuring that ANC attendance is maintained and underlying inequities in ANC delivery are addressed.
Perennial malaria chemoprevention (PMC) – formerly intermittent preventive treatment of malaria in infants (IPTi)	In areas of moderate to high perennial malaria transmission, children belonging to age groups at high risk of severe malaria can be given antimalarial medicines at predefined intervals to reduce disease burden. PMC schedules should be informed by the age pattern of severe malaria admissions, the duration of protection of the selected drug, and the feasibility and affordability of delivering each additional PMC course. SP has been widely used for chemoprevention in Africa, including for PMC. ACTs have been effective when used for PMC, but evidence is limited on their safety, efficacy, adherence to multi-day regimens, and cost-effectiveness in the context of PMC.
Seasonal malaria chemoprevention (SMC)	In areas of seasonal malaria transmission, children belonging to age groups at high risk of severe malaria should be given antimalarial medicines during peak malaria transmission seasons to reduce disease burden. Eligibility for SMC is defined by the seasonality of malaria transmission and age groups at risk of severe malaria. The added value of a seasonally targeted intervention is likely to be greatest where transmission is intensely seasonal. Monthly cycles of SP plus amodiaquine (SP+AQ) have been widely used for SMC in African children under 5 years old and have been shown to be efficacious, safe, well tolerated, available and inexpensive.
Intermittent preventive treatment of malaria in school-aged children (IPTsc)	School-aged children living in malaria-endemic settings with moderate to high perennial or seasonal transmission can be given a full therapeutic course of antimalarial medicine at predetermined times as chemoprevention to reduce disease burden. IPTsc has been evaluated in children aged 5–15 years. The burden of malaria and benefits of IPTsc may vary across this age range, but evidence is limited. National malaria programmes can consider IPTsc if resources allow for its introduction among school-aged children without compromising chemoprevention interventions for those carrying the highest burden of severe disease, such as children < 5 years old. Schools may provide a low-cost means to deliver chemoprevention to school-aged children. However, seasonal variation in malaria transmission and the timing of school terms, as well as equity concerns, may mean alternative delivery channels are needed to maximize impact.

For more information see:

WHO recommendations for malaria chemoprevention and elimination. In: Global Malaria Programme [website]. Geneva: World Health Organization (https://www.who.int/news/item/03-06-2022-updated-who-recommendations-for-malaria-chemoprevention-and-elimination, accessed 3 June 2022).

ANNEX 2. MOLECULAR MARKERS OF RESISTANCE

Drug	Molecular markers	
	Gene	Mutation
4-aminoquinolines		
Chloroquine	*Pfcrt*	*K76T + different sets of mutations at other codons*
	Pfmdr1 (in combination with *Pfcrt* mutations only)	*N86Y, Y184F and D1246Y*
Amodiaquine	Yet to be validated	Studies show that amodiaquine selects for *Pfmdr1* mutations
Piperaquine	*Pfpm2–3*	*Pfpm2–3 increased copy number*
	Pfcrt	*Detected in vivo: T93S, H97Y, F145I, I218F and C350R*
Antifolates		
Pyrimethamine	*Pfdhfr*	*N51I, C59R, S108N and I164L*
Sulfadoxine	*Pfdhps*	*S436A/F, A437G, K540E, A581G and A613T/S*
Proguanil	*Pfdhfr*	*A16V, N51I, C59R, S108N and I164L*
Amino-alcohols		
Lumefantrine	Yet to be validated	Studies show that lumefantrine selects for *Pfmdr1 mutations (N86)*
Mefloquine	*Pfmdr1*	*Pfmdr1 increased copy number*
Quinine	Yet to be validated	
Mannich base		
Pyronaridine	Yet to be validated	
Naphthoquinone		
Atovaquone	*Pfcytochrome b*	*Y268N/S/C*
Sesquiterpene lactones		
Artemisinin and its derivatives	*PfKelch13*	List of candidate and validated markers (WHO, 2020)
Antibiotics		
Doxycycline	Resistance not documented	
Clindamycin	Resistance not documented	
8-aminoquinolines		
Primaquine	Resistance not documented	
Tafenoquine	Resistance not documented	

For more information see:

Report on antimalarial drug efficacy, resistance and response: 10 years of surveillance (2010–2019). Geneva: World Health Organization; 2016 (https://apps.who.int/iris/handle/10665/336692, accessed 3 June 2022).

ANNEX 3. SAMPLE SIZE CALCULATIONS

The hypothesis tested is that the proportion of individuals infected at the end of the study (i.e. 28 days post-intervention) will be significantly lower than it would have been if no chemoprevention had been given. Two different study designs have been considered to test this hypothesis: a single-arm study where all participants receive chemoprevention, and a two-arm study with a chemoprevention group and a control group (no chemoprevention).

Single-arm study design

In this study design, the focus is on the proportion of participants infected at the end of the follow-up period compared to a theoretical value. This theoretical value can be calculated from a variety of data sources ranging from data generated from previous infection studies in the area of interest to more generalized region-specific data sources.

Estimating incidence rate and prevalence in individuals not receiving chemoprevention

The theoretical proportion infected at the end of the follow-up period will be a composite of the expected prevalence at the start of the study and the expected number of new infections. Therefore, it is essential to have an estimate of the proportion of individuals who are likely to become infected within 28 days with no chemoprevention or antimalarial treatment (that is, the cumulative incidence), or the incidence rate. There are several approaches to obtain the required baseline data, each with its own strengths and limitations. The incidence can be based on information from a modest study where a small cohort is followed up over a predefined period (e.g. 28 days). Alternatively, the incidence rate can be estimated indirectly from prevalence data using micro-simulation models *(1)*.[1]

Once the incidence rate has been estimated, the corresponding 95% confidence interval (CI) should be determined. It is suggested that the lower CI be adopted as the indicator of likely new infections.

Where the incidence rate is calculated from a baseline study, the lower 95% CI can be calculated as (log incidence rate, or the Rothman/Greenland method):

$$\text{Lower 95\% CI} = \frac{\text{Incidence rate}}{EF}$$

$$\text{where } EF = e^{1.96/\sqrt{(\text{number of new infections in baseline study})}}$$

1 Cameron et al. *(1)* published modelled prevalence (PfPR2-10) vs. incidence curves for infants and young children (0–5 yrs), older children (5–15 yrs) and adults (> 15 yrs) in areas with low and high seasonal malaria transmission. The source data were derived from 13 countries in Africa. Thus, data on site-specific prevalence could be converted to estimated incidence rates. However, it should be noted that estimated incidence rates are given per person-year of observation. This needs to be converted to "per 28-person-days" to predict the number of individuals expected to become infected during the study follow-up period. In areas with little seasonality, the incidence rate can be converted from person-years to 28-person-days by linear scaling. However, in areas with high seasonality, this scaling should take into account the length of the transmission season and the timing of the study relative to the transmission season. To illustrate this point in a highly simplified example, if transmission only occurred in four months of the year with a relatively constant incidence across those four months, and the proposed study had a follow-up period within those four months, then the estimated value of 1 infection/person-year could be scaled to 0.23 infections/28-person-days (calculated as (1 infection/4-person-months)/ 121 days * 28 days) *(1)*.

Where the incidence rate is estimated based on prevalence, the published 95% lower CI can be used for the corresponding prevalence rate *(1)*. Since these values are a compilation of results from various studies, rather than an estimate from the specific site of interest, there is greater potential for this theoretical value to deviate from reality.

If the proportion of individuals infected at the start of the study is relatively high, it is likely that many of the new infections will be additional infections within already infected individuals. When additional infection occurs, the proportion of individuals infected will not increase. The theoretical proportion of individuals infected at the end of the study should be adjusted for this.

Sample size calculation

Various online calculators are available to calculate required sample sizes. For a single-arm study, the online calculator should test a one-sample proportion; a one-sided test is appropriate, since the hypothesis is that the proportion of individuals infected at the end of the CPES will be significantly lower than it would have been if no chemoprevention had been given. One suitable online calculator can be found at http://powerandsamplesize.com/Calculators/Test-1-Proportion/1-Sample-1-Sided. Sample size calculations are normally performed using 80% power and 5% significance (or Type 1 error). Table A3.1 contains sample calculations of required sample sizes.

Table A3.1. Required sample sizes for a single-arm study – calculations performed with 80% power and 5% statistical significance and no loss to follow-up

Required sample size		Theoretical proportion parasitaemic at end of follow-up period with no chemoprevention					
		0.01	0.05	0.1	0.15	0.2	0.25
Expected proportion parasitaemic at end of follow-up period after chemoprevention	0.001	447	62	28	17	12	9
	0.005	1987	87	34	20	14	10
	0.01		123	42	23	16	11
	0.02		252	59	30	19	14
	0.03		630	83	38	23	16
	0.04		2,737	121	47	27	18
	0.05			184	60	32	21
	0.06			301	77	38	24
	0.07			557	101	46	27
	0.08			1301	136	55	31
	0.09			5,386	191	67	36
	0.1				282	83	42
	0.12				823	136	58
	0.14				7,724	251	84
	0.16					584	129
	0.18					2,405	219
	0.2						440

Two-arm study design

In this study design, the proportions of participants found to be parasite-positive during the follow-up period (from day 4) are compared between the chemoprevention group and the control group (not receiving chemoprevention). This is a more robust study design, as it directly compares the proportions of infected individuals between the two groups, removing the uncertainty around what the anticipated incidence rate would be in the absence of chemoprevention.

The sample size calculation can be based on the overall proportion found to be infected at the end of the study.

To calculate the required sample size, initial estimates of the prevalence and incidence rate (without chemoprevention) during the follow-up period are required, as is an estimate of the effect size for the chemoprevention. However, these initial estimates are to guide the sample size calculations only and do not feature in the analysis of the data. In contrast to the single-arm study, the estimate of the incidence rate can be used in the calculations, instead of the lower 95% CI.

A sample size calculator comparing two proportions using a one-sided test is appropriate to test the hypothesis that the proportion of individuals with new infections in the follow-up period will be significantly lower in the chemoprevention group than in the control group. The lowest overall sample sizes will be obtained when there are equal numbers in the chemoprevention and control groups, but individual study sites may need to move away from this balanced design for a variety of ethical and logistical reasons.

Table A3.2 provides estimated sample sizes for a balanced sample design for a range of different anticipated incidence rates. Alternate values for unbalanced designs can be calculated using http://powerandsamplesize.com/Calculators/Compare-2-Proportions/2-Sample-1-Sided, or another similar online calculator.

Table A3.2. Minimum required sample sizes (in each group) to be able to detect a statistically significant reduction in the proportion of individuals infected following chemoprevention compared to a control group, with 80% power, 5% significance, a balanced design and no loss to follow-up

Required sample size in each group		Expected proportion parasitaemic at end of follow-up period with no chemoprevention					
		0.01	0.05	0.1	0.15	0.2	0.25
Expected proportion parasitaemic at end of follow-up period after chemoprevention	0.001	831	125	58	36	26	19
	0.005	3,675	161	65	39	27	20
	0.01		222	77	44	30	22
	0.02		461	106	54	35	25
	0.03		1,183	151	68	41	28
	0.04		5,305	221	85	48	32
	0.05			340	109	57	37
	0.06			566	141	69	42
	0.07			1,065	186	83	49
	0.08			2,526	254	101	56
	0.09			10,615	360	124	65
	0.1				538	155	77
	0.12				1,600	257	108
	0.14				15,309	481	158
	0.16					1,137	246
	0.18					4,749	423
	0.2						859

Reference

1. Cameron E, Battle KE, Bhatt S, Weiss DJ, Bisanzio D, Mappin B, et al. Defining the relationship between infection prevalence and clinical incidence of *Plasmodium falciparum* malaria. Nat Commun. 2015;6:8170. doi:10.1038/ncomms9170.

ANNEX 4. OVERVIEW OF ACTIVITIES BY DAY

Day 0

Screening

- Clinical assessment, including measurement of temperature; for those with symptoms of malaria conduct diagnosis by RDT or microscopy
- Informed consent and assent

Enrolment

- Supervised treatment, first dose
- Collect thick blood smear
- Collect DBS on filter paper
- Collect general information (age, weight, height, gender), information on vector control use, and intervention-specific information:
    - IPTp: gravidity, last menstrual period (to exclude first trimester) previous use of antimalarial medicine in current pregnancy
    - PMC: previous use of antimalarial medicine in past six weeks, if child is being breastfed
    - SMC: previous use of antimalarial medicine in current transmission season, if child is being breastfed
    - IPTsc: previous use of antimalarial medicine in past six weeks

Day 1

- Supervised treatment (as needed, depending on medicine given)

Day 2

- Supervised treatment (as needed, depending on medicine given)

Optional

- Record temperature
- Collect thick blood smear
- Collect DBS on filter paper

Day 7

- Record temperature
- Collect thick blood smear
- Collect DBS on filter paper

Optional

- Collect blood to test drug blood level

Days 14, 21 and 28, and any other day

- Record temperature
- Collect thick blood smear
- Collect DBS on filter paper

ANNEX 5. MANDATORY AND OPTIONAL ACTIVITIES

Activity	Mandatory	Optional
Treatment		
Pre-study curative treatment		Administer pre-study curative treatment, two weeks prior to the chemoprevention regimen
Chemoprevention	Provide preventive chemotherapy as per recommendations	
Supervision of treatment	All medicine must be given under direct supervision	
Clinical malaria	Study participants identified as having clinical malaria at enrolment must receive first-line treatment as per the national treatment guidelines	
Patient follow-up		
Follow-up period	28 days after start of chemoprevention administration with a drug with a short half-life 42 days after start of treatment with a drug with a long half-life	42 days after start of treatment with a drug with a short half-life. Requires any additional chemoprevention doses to be delayed 56 days after start of treatment with a drug with a long half-life
Days of patient follow-up	Weekly follow-up (days 7, 14, 21, and 28) + any day that study participants have symptoms	Additional follow-up on day 2 and days 35, 42, 49 and 56
Malaria parasitaemia	Study participant with chemoprevention failure (early failures, malaria parasitaemia with symptoms after day 4 or malaria parasitaemia without symptoms after day 7 – see Annex 6) must receive first-line treatment as per the national treatment guidelines	
Information and samples collected		
Information collected at enrolment	• Temperature, symptoms of malaria, antimalarial treatment in past, use of vector control + intervention-specific information: • IPTp: last menstrual period, gravidity, previous use of antimalarial medicine in current pregnancy • PMC: previous use of antimalarial medicine in past six weeks and if child is being breastfed • SMC: previous use of antimalarial medicine in current transmission season, and if child is being breastfed • IPTsc: previous use of antimalarial medicine in past six weeks. For females of >12 years ability and willingness to take pregnancy test.	
Information collected on days of follow-up	Temperature, symptoms of malaria	

Samples collected	DBS and thick blood smear on day of enrolment and all days of follow-up	Venous blood sample on day 7 to measure drug blood level
Molecular analysis		
Reinfection/ recrudescence markers	Blood collected in DBS on day 0 and day of failure for analysis of markers of new infection/recrudescence	
Drug resistance markers	Analysis of markers of drug resistance in DBS collected on day 0 for study participants with parasitaemia Analysis of markers of drug resistance in DBS collected for study participants with parasitaemia during follow-up	

ANNEX 6. TES DEFINITIONS OF FAILURE

Early treatment failure (ETF)

- danger signs or severe malaria on day 1, 2 or 3, in the presence of parasitaemia;
- parasitaemia on day 2 higher than on day 0, irrespective of axillary temperature;
- parasitaemia on day 3 with axillary temperature ≥37.5 °C; and
- parasitaemia on day 3 ≥25% of count on day 0.

Late clinical failure (LCF)

- danger signs or severe malaria in the presence of parasitaemia on any day between day 4 and day 28 (day 42) in patients who did not previously meet any of the criteria of early treatment failure; and
- presence of parasitaemia on any day between day 4 and day 28 (day 42) with axillary temperature ≥37.5 °C in patients who did not previously meet any of the criteria of early treatment failure.

Late parasitological failure (LPF)

- presence of parasitaemia on any day between day 7 and day 28 (day 42) with axillary temperature <37.5 °C in patients who did not previously meet any of the criteria of early treatment failure or late clinical failure.

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