ZANZIBAR MALARIA ELIMINATION PROGRAMME

STUDY PROTOCOL:

EFFICACY AND SAFETY OF ARTESUNATE + AMODIAQUINE COMBINED WITH A SINGLE LOW DOSE OF PRIMAQUINE (0.25 MG/KG) FOR THE TREATMENT OF UNCOMPLICATED PLASMODIUM FALCIPARUM MALARIA IN ZANZIBAR

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COVER SHEET FOR THERAPEUTIC EFFICACY TEST PROTOCOL

Study Title/Acronym	Efficacy and safety of artesunate + amodiaquine combined with a single low dose of primaquine (0.25 mg/kg) for the treatment of uncomplicated plasmodium infection in Zanzibar (ACO V)
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Study dates	April to October 2017
Sponsors	Ministry of Health, Zanzibar, Tanzania P.O. Box 236, Zanzibar, Tanzania. Global Fund: QNB-M-MOH Karolinska Institutet and Uppsala University, Sweden
Clinical trial registration	The study will be registered at ClinicalTrials.gov prior to commencement of the trial

SUMMARY

Title: Efficacy and safety of artesunate + amodiaquine combined with a single low dose of primaquine (0.25 mg/kg) for the treatment of uncomplicated *Plasmodium falciparum* malaria in Zanzibar.

Background: Zanzibar introduced artemisinin combination therapy (ACT) for the treatment of uncomplicated *P. falciparum* malaria in 2003 with artesunate + amodiaquine as first-line treatment. Following wide scale deployment of ACT together with strengthened vector control with long lasting insecticidal nets (LLIN) and indoor residual spraying (IRS) the burden of malaria has declined markedly in Zanzibar to a level equivalent with malaria pre-elimination. Artesunate+amodiaquine has previously shown to be safe and highly efficacious with polymerase chain reaction (PCR) corrected cure rates >90% by day 42 in clinical trials conducted 2003/4 and 2005. Recently, and in line with WHO recommendations for low transmission settings, a single low dose of primaquine (0.25 mg/kg), a *P. falciparum* gametocytocidal drug, has been introduced as policy. However, no updated efficacy data of the firstline treatment for uncomplicated malaria are available from Zanzibar.

Objective: To assess the efficacy and safety of artesunate+amodiaquine combined with a single low dose of primaquine (0.25 mg/kg) for the treatment of uncomplicated *P. falciparum* malaria in Zanzibar.

Methods: A one-arm prospective antimalarial drug efficacy trial will be conducted.

Participants will be recruited from febrile patients, i.e. documented axillary temperature ≥37.5 °C or history of fever during the past 48 hours, of 3 months and above years old, presenting at primary health care facilities in Zanzibar, with microscopy confirmed uncomplicated *P. falciparum* infection. Enrolled patients will receive directly observed treatment with artersunate + amodiaquine once daily for 3 consecutive days according to the national malaria treatment guidelines. A single low dose of primaquine (0.25 mg/kg) will be administerad together with the first artesunate + amodiaquine dose. Clinical and parasitological as well as safety parameters will be monitored over a 28-day follow-up period. The study will be conducted between April and October 2017. The results will be used to assist the Zanzibar Ministry of Health in assessing the current national treatment guidelines for uncomplicated *P. falciparum* malaria.

BACKGROUND

Zanzibar was among the first regions in sub-Saharan Africa to introduce artemisinin combination therapy (ACT) for the treatment of uncomplicated *P. falciparum* malaria in 2003 as a response to reports of unacceptable high treatment failure with chloroquine. The wide scale deployment of ACT, free of charge to all malaria patients through public health care facilities, together with strengthened vector control with long lasting insecticidal nets (LLIN) and indoor residual spraying (IRS) have resulted in a marked decline in burden of malaria in Zanzibar (Bhattarai et al 2007).

Since September 2003 the first line-treatment of uncomplicated malaria in Zanzibar is artesunate + amodiaquine. Presently, a fixed dose combination of artesunate + amodiaquine (Artesunate Amodiaquine Winthrop®, Sanofi Aventis), prequalified by the WHO, is deployed for treatment of uncomplicated malaria infection once daily for 3 consecutive days in all primary health care facilities. The present alternative treatment in case of known intolerance to artesunate + amodiaquine is artemether-lumefantrine (ALU); however, in some cases other artemisinin based combination therapy are prescribed particularly in private facilities. ALU is rarely used. Artesunate injection is a drug of choice for severe malaria, if not available Artemether or quinine are acceptable alternatives, whereas Artesunate and amodiaquine is also used for intermittent screening and treatment in pregnancy (IST).

Artesunate + amodiaquine as well as artemether-lumefantrine have previously shown to be safe and highly efficacious, with polymerase chain reaction **(**PCR) corrected cure rates >90% by day 42 in clinical trials conducted 2003/4 (Mårtensson et al 2005) and 2005 (Holmgren et al 2007) for the treatment of children with uncomplicated *P. falciparum* malaria in Zanzibar. Zanzibar has recently, and in line with WHO recommendations for low transmission settings, introduced as policy for uncomplicated malaria a single low dose of primaquine (0.25 mg/kg), a *P. falciparum* gametocytocidal drug, in addition to standard ATC. However, no recent efficacy data of the presently recommended first-line treatment are available.

The aim of this study is therefore to provide policymakers with updated efficacy and safety data of artesunate + amodiaquine in combination with a single low dose of primaquine (0.25 mg/kg) and data on genetic markers of ACT tolerance/resistance, proposed as an early warning system for development and spread of antimalarial drug resistance, in Zanzibar. The study protocol is based on the new WHO guidelines for surveillance of antimalarial drug efficacy (WHO 2014).

OBJECTIVES

The general objective of this study is to assess the therapeutic efficacy and safety of artesunate + amodiaquine combined with a single low dose of primaquine (0.25 mg/kg) for the treatment of uncomplicated *P. falciparum* malaria patients in Zanzibar.

The specific objectives are:

- to determine the clinical and parasitological efficacy of artesunate + amodiaquine and primaquine in the treatment of uncomplicated *Plasmodium falciparum* infection;
- to differentiate recurrent infections during follow-up, i.e. recrudescence from new infections, by PCR analysis;
- to evaluate the incidence of adverse events, particularly with regards to potential

hematological adverse events of primaquine; and

- to determine the polymorphism of molecular markers associated with artesunate + amodiaquine tolerance/resistance.
- to formulate recommendations, which will enable the Zanzibar Ministry of Health to make informed decisions about whether the current national antimalarial treatment guidelines should be updated or not.
- To determine efficacy rate of the first line treatment compared to the first efficacy trial thirteen years ago.

3. METHODS

3.1 Study design and clinical trial registration

This surveillance study is designed as a one-arm prospective evaluation of the clinical and parasitological responses to directly observed treatment for uncomplicated malaria WHO, 2003 and 2009). People with uncomplicated malaria, who meet the study inclusion criteria will be enrolled, treated on site with artesunate + amodiaquine and a single low dose of primaquine (0.25 mg/kg) and monitored for 28 days. The follow-up will consist of a fixed schedule of check-up visits and corresponding clinical and laboratory examinations. On the basis of the results of these assessments, the patients will be classified as having therapeutic failure (early or late) or an adequate response. Blood samples from patients experiencing therapeutic failure during the follow-up period will be used to estimate the efficacy of the study drugs based on PCR analysis to distinguish between recrudescence (treatment failures) and reinfection (new infections).

This study will be registered at the ClinicalTrials.gov registry platform before commencement of the trial.

3.2 Study sites

The study will screen patients from 14 selected primary health care facilities (see below) in three different three districts of Zanzibar Islands. These facilities will classified as sattelite study sites and recruitment sites. The satellite facilities will be used for initially screening the eligible patients for the inclusion criterias; these includes signs of uncomplicated malaria, positive confimed using malaria Rapid Diagnostic Tests (mRDT), verbal consent for patients above 18 years and verbal asent from parent (s) for the children below 18 years. Any patient who will show eligibility to participate will be transferred to the recruitment sites.

In each district there will be one recruitment study site of which patients will enrolled in based on the set eligibility criteria. Recruitment sites will equiped with microscope with all consumables, haemaque machine for hemaglobin and well trained technicians that recently has undergone a malaria microscopy refresher course to ensure the highest possible quality of microscopy service throughout the study. Responsible Clinicians will be oriented to the study protocol and assigned to recruit, treat, fill the case record form and follow up details inclusion and exclusion criterias.

The 14 primary health care facilities included in the study are from 2 districts (West and Central and one district (Micheweni) of Unguja and Pemba Islands respectively. These facilities were chosen based on higher malaria cases detected on the previous three months

but also because they are located nearby to the one of the proposed recruitment sites. The proposed study sites are the following:

Micheweni, Tumbe, Shumba viamboni (Micheweni District); Chukwani, Kizimbani, Shakani, Kombeni, Bububu, Selem, Magogoni (West District); Mwera, Miwani, Machui and Uzini (Central District).

Proper communication between settalites and recruitment study sites will be established to make sure all screened patients with initial eligible criterias to be able transferred to the recruitment sites for detailed screening for study inclusion criterias. All eligible patients will be given written consent and asent forms to sign at the recruitment sites. Treatment, observation and follow up to the enrolled patients will be conducted in all three proposed recruitment study sites namely Micheweni (Pemba) and Bububu Jeshini and Uzini of Unguja Island.

3.3 Study population

The population will consist of patients of all ages presenting at the study sites with symptom and signs compatible with uncomplicated P. falciparum malaria. All adult patients will be informed about the study and asked to sign the consent form for participation prior to enrolment. Parents or guardians will give informed consent on behalf of their children. Children over 12 years of age will sign an informed assent form.

3.4 Timing and duration of study

The study is planned to be conducted for six months, from April to October 2017.

3.5 Inclusion criteria

- Age 3 months and above;
- *P. falciparum* infection detected by mRDT and confirmed by microscopy;
- presence of *P. falciparum* malaria asexual parasitaemia (any level);
- presence of axillary ≥37.5 °C or history of fever during the past 48 hours
- ability to swallow oral medication;
- ability and willingness to comply with the study protocol for the duration of the study and to comply with the study visit schedule; and
- informed consent from the patient or from a parent or guardian in the case of children.

3.6 Exclusion criteria

- Presence of general danger signs in children aged under 5 years or signs of severe falciparum malaria according to the definitions of WHO (Appendix 1);
- mono-infection with a Plasmodium species other than *P. falciparum* detected by microscopy;
- presence of febrile conditions other than malaria (e.g. measles, acute lower respiratory tract infection, severe diarrhoea with dehydration) or other known underlying chronic or severe diseases (e.g. severe malanutrition, cardiac, renal and hepatic diseases,

HIV/AIDS);

- regular medication, which may interfere with the study drugs;
- history of hypersensitivity reactions or contraindications to any of the study medicines; and
- preganacy

3.7 Loss to follow-up

Loss to follow-up occurs when, despite all reasonable efforts, an enrolled patient does not attend the scheduled visits and cannot be found. No treatment outcome will be assigned to these patients. Every effort must be made to schedule a follow-up visit for patients who fail to return to the study site, especially during but also after administration of the study drug. These patients will be classified as lost to follow-up and censored or excluded from the analysis. Patients who are lost to follow-up but who subsequently return to the study site before day 28 will not be turned away and will be encouraged to return for check-up visits. The principal investigator will decide whether the patient is to be definitely classified as lost to follow-up on the basis of his or her history or is to be maintained for the analysis.

3.8 Patient discontinuation or protocol violation

Study patients who meet any of the following criteria will be classified as withdrawn.

- Withdrawal of consent. A patient may withdraw consent at any time, without prejudice for further follow-up or treatment at the study site.
- Persistent vomiting of the treatment. A patient who vomits the study medication twice will be withdrawn from the study and given rescue treatment.
- Failure to attend the scheduled visits during the first 3 days.
- Serious adverse events necessitating termination of treatment before the full course is completed. A patient can be discontinued from the study if the principal investigator decides so due to an adverse event of adequate nature or intensity. In this case, information on the adverse event and symptomatic treatment given must be recorded on a case report form. If the adverse event is serious, the principal investigator must notify the sponsor or its designee immediately and follow the reporting procedures described in section 5.3.
- Enrolment violation:
- severe malaria on day 0; or
- erroneous inclusion of a patient who does not meet the inclusion criteria.
- voluntary protocol violation: self- or third-party administration of antimalarial drug (or antibiotics with antimalarial activity) (Appendix 2);
- involuntary protocol violation:
- occurrence during follow-up of concomitant disease that would interfere with a clear classification of the treatment outcome;
- detection of mono-infection with another malaria species during follow-up; or
- misclassification of a patient due to a laboratory error (parasitaemia), leading to administration of rescue treatment.

Patients who are withdrawn will be followed up until recovery or to the end of follow-up, no treatment outcome will be assigned to these patients, and they will be censored or excluded from the analysis. The reasons for discontinuation or protocol violation will be recorded on the case report form.

4. TREATMENT

4.1 Antimalarial treatment

Artesuate + amodiaquine will be administered orally as a fixed dose combination, i.e. Artesunate/Amodiaquine Winthrop®, prequalified by the WHO, at a dose of approximately artesunate 4 mg/kg + amodiaquine 10mg/kg once daily for 3 consecutive days.

Primaquine will be administered orally, i.e. ®, prequalified by the WHO, as a single dose (0.25 mg/kg) together with the first artesunate + amodiaquine dose of approximately artesunate 4 mg/kg + amodiaquine 10mg/kg once daily for 3 consecutive days.

The study drugs, represent the present drugs deployed to public health care facilities by the Zanzibar Ministry of Health.

The investigators will be instructed to record the batch number and expiry date of the test medicines. The correct drug dosages will be determined from the dosing charts (Appendix 3), which are in accordance with the national treatment guidelines in Zanzibar.

All doses of medicine will be administered under direct supervision of a qualified member of the staff designated by the principal investigator. The study patients will be observed for 30 minutes after medicine administration for adverse reactions or vomiting. Any patient who vomits during this observation period will be re-treated with the same dose of medicine and observed for an additional 30 minutes. If the patient vomits again after the second study drug administration, he/she will be withdrawn and offered rescue therapy (see below section 4.3 Rescue treatment).

4.2 Concomitant treatment and medication that should not be used

Fever ≥38.5°C will be treated with paracetamol according to national treatment guidelines. Prior treatment with antimalarial drugs will not be considered an exclusion criterion; however, during follow-up, if infections other than malaria require the administration of medicines with antimalarial activity, the patient will be withdrawn from the study. Patients given tetracycline as an eye ointment will not be excluded (Appendix 2). Patients will be withdrawn from the study in the case of self-medication or if an antimalarial drug or an antibiotic with antimalarial activity is administered by a third party.
Adverse events requiring treatment can be treated according to local practice in Zanzibar. If there is a clinical indication for any additional medication during the course of the study, including medication given to treat an adverse event related to the study medicine, the name of the medicine, the dosage and the date and time of administration must be recorded on the case report form.

The use of herbal remedies during the study should be avoided, and participants should be encouraged to return to the study site for treatment if they feel unwell. If any herbal remedies are taken during the study, this should be captured on the case report form, under ‘study medication administration’.

4.3 Rescue treatment

If a patient vomits a dose of the study medicine twice, he/she will be withdrawn from the study and receive Artesunate IV (preferred route) or IM p according to national treatment guidelines

Women who are found to be pregnant at enrolment will be treated with tablets quinine 10 mg/kg body weight 8 hourly for 7days if she is in the first trimester or AS 4mg/kg +AQ 10 mg/kg body weight once a day for 3 days if she is in the second and third trimester will be used according to national treatment guidelines.

Any patient with signs of severe or complicated malaria will be hospitalized and receive parenteral therapy with Artesunate according to national treatment guidelines. Initially, an intravenous loading dose of 2.4 mg/kg will be given at admission then at 12 hrs and 24 hrs, thereafter once a day. The total duration of treatment with parenteral Artesunate should be a minimum of 24hrs and a maximum of 7 days if the patient is still unable to tolerate oral medication for up to 7 days.Together with relevant supportive treatment, such as administration of glucose to avoid hypoglycaemia, antipyretic and other physical methods to control fever, blood transfusion if indicated and/or broad spectrum antibiotics if meningitis/septicaemia cannot be ruled out. .If the patient is re-infected with malaria species, he/she will receive artesunate 4 mg/kg + amodiaquine 10 mg/kg body weight once daily for 3 days according to the national treatment guidelines.

5. EVALUATION CRITERIA

The study end-point is the classification assigned to a patient. Valid study end-points include: treatment failure, completion of the follow-up period without treatment failure, loss to follow-up, withdrawal from study, and protocol violation. At all times, the well-being of the patient will take priority over his or her continuation in the study.

5.1 Efficacy and safety evaluation

5.1.1 Classification of treatment outcomes

Treatment outcomes will be classified on the basis of an assessment of the parasitological and clinical outcome of antimalarial treatment according to the latest WHO guidelines (WHO 2005). Thus, all patients will be classified as having early treatment failure, late clinical failure, late parasitological failure or an adequate clinical and parasitological response, as defined in Appendix 4.

As parasitological cure is the goal of antimalarial therapy, all study patients who show treatment failure will be given rescue treatment. Follow-up will continue until recovery. The results from these patients do not need to be recorded systematically for the purpose of the surveillance study.

5.1.2 Safety end-points

The incidence of any adverse event will be documented. All patients will be asked routinely at each follow-up visit about previous symptoms and about any symptom that have emerged since the previous visit. When clinically indicated, patients will be evaluated and treated

appropriately. All adverse events will be recorded on the case report form. Serious adverse events (see definitions below section 5.3 Safety assessment) must be reported to the sponsor.

5.2 Clinical evaluation

All patients will be evaluated clinically as described below.

5.2.1 Physical examination

A standard physical examination will be performed at baseline (day 0 before drug administration) and on days 1, 2, 3, 7, 14, 21 and 28. A complete medical history, demographic information and contact details will be taken at baseline.

5.2.2 Body weight

Body weight will be recorded on day 0 to the nearest kilogram using a Salter scale, a hanging or electronic weighing scale for young children. The scales will be properly calibrated. Patients should not wear excessive clothing while being weighed as this can overestimate their true weight. All young children should only wear undergarments while being weighed. The screening weight will be used to satisfy the inclusion or exclusion for nutrition status as well as to calculate the dose (number of tablets) to be administered. The reliability of the scales will be verified before the study begins and checked at regular intervals.

The circumference of the left mid-upper arm will be measured, at the mid-point between the elbow and the shoulder, and will be recorded to the nearest 0.2 cm.

Oedema will be assessed by thumb pressure for 3 s on the dorsal surface of both feet.

5.2.3 Body temperature

Axillary temperature will be measured at baseline (day 0 before drug administration) and on days 1, 2, 3, 7, 14, 21, 28. Temperature will be measured with a thermometer that has a precision of 0.1 °C. Temperature will also be measured as clinically indicated. If the result is <36.0 °C, the measurement will be repeated. The same route for assessing temperature should be used throughout the study.

The quality of the temperature-taking technique and the thermometers should be assessed regularly. Thermometers should be tested in a water-bath of known temperature before the study begins and at regular intervals thereafter.

5.2.4 Microscopic blood examination

A thick film for asexual and gametocyte counts and species identification should be obtained and examined at screening on day 0 to confirm adherence to the inclusion and exclusion criteria. Thick blood films, again assessed for asexual and gametocyte parasitemia, will be also examined on days 1, 2, 3, 7, 14, 21, 28 or on any other day if the patient returns spontaneously and parasitological reassessment is required. Specimens will be labelled anonymously (screening number or study number, day of follow-up, date).

A fresh Giemsa stain dilution will be prepared at least once a day and possibly more often, depending on the number of slides to be processed. Giemsa-stained thick films will be

examined at a magnification of 1000× (using x10 ocular lens and x100 objective) to identify the parasite species and to determine asexual and gametocyte parasite density.

The study number of the patient, the date and the day of follow-up will be recorded either on the frosted edge of the slide or on the glass with a permanent glass pen.

The thick blood smear will be used to calculate the parasite density, by counting the number of asexual parasites in a set number of white blood cells (typically 200) with a hand tally counter. Once a field has been started, it must be counted to completion; the final number of white blood cells will therefore rarely be exactly 200. If more than 500 parasites have been counted before 200 white blood cells have been reached, the count will be stopped after the reading of the last field has been completed. Parasite density, expressed as the number of asexual parasites per µl of blood, will be calculated by dividing the number of asexual parasites by the number of white blood cells counted and then multiplying by an assumed white blood cell density (typically 8000 per µl).

$$\text{Parasite density (per µl)} = \frac{\text{number of parasites counted} \times (8000)}{\text{Number of leukocytes counted}}$$

The same technique will be used to establish the parasite count on each subsequent blood film. When the number of asexual parasites is less than 10 per 200 white blood cells in follow-up smears, counting will be done against at least 500 white blood cells (i.e. to completion of the field in which the 500th white blood cell is counted). A blood slide will be considered negative when examination of 1000 white blood cells reveals no asexual parasites. The presence of gametocytes on an enrolment or follow-up slide will be recorded.

In addition, 100 fields of the second thick film will be examined to exclude mixed infections; in case of any doubt, the thin film will be examined for confirmation. If examination of the thin film is not conclusive, the patient will be excluded from the analysis after complete treatment and follow-up.

Two qualified microscopists will read all the slides independently, and parasite densities will be calculated by averaging the two counts. Blood smears with discordant results (differences between the two microscopists in species diagnosis, in parasite density of > 50% or in the presence of parasites) will be re-examined by a third, independent microscopist, and parasite density will be calculated by averaging the two closest counts.

5.2.5 Genotyping of malaria parasites

In order to differentiate a recrudescence (treatment failure/same parasite strain) from a newly acquired infection (reinfection/different parasite strain) among recurrent parasitemias found during follow-up, a genotype analysis will be conducted. This analysis is based on the extensive diversity in the following *P. falciparum* genes: *the merozoite surface protein 1* (*msp1*) and *2* (*msp2*), and the *glutamine-rich protein* (*glurp*) (WHO 2008). The genotypic profiles of pre- and post-parasite strains are compared in a stepwise manner to distinguish recrudescence from reinfection.

In order to minimize discomfort to the patient due to repeated finger pricks, two to three drops of blood will be collected on a 3MM (Whatman) filter paper during screening or enrolment and each time blood smears are required according to the protocol from day 7.

Specimens will be labelled anonymously (study number, day of follow-up, date), kept in individual plastic bags with desiccant pouches and protected from light, humidity and extreme temperature until analysed. Paired filter paper samples from day on enrolment (day 0) and initial day of recurrent parasitemia during the 28 day follow-up period will be analysed by stepwise genotyping using previously described nested-PCR protocols at the Malaria Research Unit, Karolinska Institutet, Stockholm, Sweden (Snounou et al 2002). Unused filter papers will be destroyed immediately after the study.

The identification of genetic alterations, i.e. single nucleotide polymorphisms (SNPs) and gene amplifications, associated with tolerance/resistance to ACTs (Sisowath et al 2005; Holmgren et al, 2006), has enabled a new potential surveillance tool, which may be of particular importance in areas with very low malaria transmission such as Zanzibar, as an early warnings system of development and spread of antimalarial drug resistance. Several markers of tolerance/resistance to antimalarial drugs, e.g. amodiaquine and lumefantrine, have been described, i.e. in the *P. falciparum* chloroquine resistance transporter gene (*pfcrt*) and the multidrug resistance gene 1 (*pfmdr1*). Furthermore, key markers of artemisinin tolerance/resistance in the K13 propeller region will also be analysed based on previously described protocols (Ariey et al, 2014).

Analysis of SNPs and gene amplifications associated with tolerance/resistance to ACT will be performed according to established protocols at Karolinska Institutet, Stockholm, Sweden, from the previously described blood spots collected on 3MM filter paper. Thus, these parasite genetic analyses will not require any additional blood sampling from enrolled patients. The results will be compared with historical data from Zanzibar from clinical trials of artesunate + amodiaquine conducted 2003/4 and 2005 using trend analysis.

5.2.6 Pregnancy test

Female patients aged 15-49 years will be asked about their menstrual history according to standard and context specific case management of women of child-bearing age in Zanzibar.

Women who are found to be pregnant at screening, and thus not enrolled, and estimated to be in the first trimester will be treated with oral quinine 10 mg/kg body weight 8 hourly for 7 days, whereas pregnant women in the second and third trimester will receive artesunate 4 mg/kg + amodiaquine 10 mg/kg treatment once daily for 3 days according to national treatment guidelines.

Female study participants of child-bearing age, defined as above (menstruating women above 49 years also will be considered), will be appropriately counselled about the risks of becoming pregnant and exposing the fetus to the study medicines and encouraged to use barrier methods for contraception during the entire study period.

5.2.7 Haematological assessment haemoglobin

Haemoglobin will be assessed systematically on all participants on days 0, 3, 7, 14 and 28 using Hemocue, and at any time in case of clinical suspicion of anaemia, i.e. pallor, according to standard case management of malaria in Zanzibar.

5.3 Safety assessment

Safety will be assessed by recording the nature and incidence of adverse events and serious adverse events. Adverse events will be assessed by direct questioning. An adverse event is defined as any unfavourable, unintended sign, symptom, syndrome or disease that develops or worsens with the use of a medicinal product, regardless of whether it is related to the medicinal product. All adverse events must be recorded on the case report form.

A serious adverse event is defined as any untoward medical occurrence that at any dose:

- results in death, is life threatening;
- requires hospitalization or prolongation of hospitalization;
- results in a persistent or significant disability or incapacity; or
- is a congenital anomaly or birth defect.

'Life-threatening' means that the person was at immediate risk of death; it does not refer to a adverse event that might have caused death if it were more severe. 'Persistent or significant disability or incapacity' means that a person's ability to carry out normal life functions is substantially disrupted.

All serious adverse events occurring during the study must be recorded and reported by the principal investigator to the sponsor, regardless of whether the principal investigator considers the events to be related to the investigated medicine.

The investigator will collect information on all people who become pregnant while participating in this study and will record the information on the appropriate form. The person will also be followed to determine the outcome of the pregnancy. Generally, follow-up will be no longer than 6–8 weeks after the estimated delivery date. Any premature termination of pregnancy will be reported. While pregnancy itself is not considered an adverse event or a serious adverse event, any complication of pregnancy or elective termination for medical reasons will be recorded as an adverse event or a serious adverse event. A spontaneous abortion is always considered a serious adverse event and will be reported as such.

6. STUDY ASSESSMENT

6.1 Screening and enrolment

All patients who meet the basic enrolment criteria (fever or history of fever within the last 48 hours, symptoms of malaria, absence of danger signs in children in relation to malaria—child unable to drink or breastfeed, vomiting everything, recent history of convulsions, lethargic or unconscious state, unable to sit or stand, difficulty in breathing—, absence of signs of severe malaria, absence of severe malnutrition, pregnancy) during screening will be assigned a consecutive number and evaluated in greater depth by the health care staff at the study sites. In children, care will be taken to detect early signs of febrile diseases other than malaria, as their presence will necessitate exclusion from the evaluation. The most frequent confounding condition is a lower respiratory tract infection: cough or difficult breathing, together with fast breathing, is an indicator for exclusion. Fast breathing is defined as a respiratory frequency > 50/min in infants under 12 months of age and > 40/min in children aged 12–59 months. Other relatively common febrile conditions are otitis media, tonsillitis, measles and abscesses.

Patients with these conditions will not be enrolled but should be treated for both malaria (if they have parasitaemia) and the other infection, as appropriate.

The screening record form (Appendix 5) will be used to record the general information and the clinical observations on each patient being screened. If the patient meets the clinical criteria, he/she will be examined for presence of parasitaemia, initially with a RDT for malaria. For all screening patients with a positive RDT test, a confirmatory blood slide will be conducted to ensure presence of *P. falciparum* mono-infection. Once the patient meets all the enrolment criteria, he/she or a parent or guardian in case of children will be asked for consent to participate in the study.

6.2 Follow-up

Patients who meet all the enrolment criteria will be given a personal identification number and will receive treatment only after the study has been fully explained to them and they have willingly provided informed consent. Any person who decides not to participate in the study will be examined, treated and followed-up by the health facility staff according to the standard of care established by the Zanzibar Ministry of Health.

The basic follow-up schedule is summarized in Appendix 6. A case report form (Appendix 7) and a serious adverse event report form (Appendix 8) will be used to record the general information and clinical observations on each patient enrolled into the study. The appointment schedule will be clearly explained, and a follow-up card with a personal identification number will be provided.

The day a patient is enrolled and receives the first dose of study medicine is designated ‘day 0’. All antimalarial treatment will be given by a study team member under supervision. Enrolled patients will be observed for at least 30 min after treatment to ensure that they do not vomit the medicine. If vomiting occurs within 30 min of treatment, the full treatment dose will be repeated. Ancillary treatment, such as antipyretics, will be provided if necessary to patients by the study team and documented on the case report form. Patients with persistent vomiting (i.e. necessitating more than a single repeat dose) will be excluded from the study and immediately referred to the health facility staff for appropriate management.

Thereafter, patients are required to undergo regular clinical reassessment. Blood films for asexual and gametocyte parasite counts will be made on days 2, 3 and 7 and then weekly for the remainder of the follow-up period, i.e. on days 14, 21 and 28. Patients will be advised to return on any day during the follow-up period if symptoms return and not to wait for the next scheduled visit day. In particular, parents or guardians should be instructed to bring children to the centre at any time if they show any sign of danger (unable to drink or breastfeed, vomiting everything, presenting with convulsions, lethargic or unconscious, unable to sit or stand, presenting with difficult breathing), if they are still sick or if there is any cause for worry. Clinical reassessment will be sufficiently thorough to ensure patient safety and will include assessment not only for potential treatment failure but also for potential adverse reactions to the medicine. Additionally, blood films will be obtained whenever parasitological reassessment is requested by the clinical staff.

Because many medicines have to be given over several days, the initial visits are critical not only for assessing efficacy but also for ensuring patient safety; defaulters at this stage will not have received a complete course of treatment and may be at risk for clinical deterioration. All reasonable efforts will be made to find defaulters to ensure complete treatment. Similarly, the ultimate success of the study rests on minimizing loss to follow-up. While patients are encouraged to return on their own for scheduled follow-up visits, it is essential that

provisions be made ahead of time for locating patients at home if they do not attend as requested. This requires obtaining detailed directions to the home during enrolment, and study team members familiar with the community will be responsible for home visits and means of transport for the patients. Health attendant (community mobiliser) of each facility will be involved in this study. His/her main responsibility will be to take detail record of each enrolled patient, if patient did not show up at health facility on his/her scheduled day then active follow up is done by community mobiliser. The schedule of treatment and follow-up examinations given in this protocol must be followed to ensure data integrity. Patients who fail to return on days 1 and 2 and miss one dose of the treatment will be withdrawn from the study definitively. After day 3, patients who fail to return on day 7 but are present on day 6 or 8 (likewise days 13/15, days 20/22, days 27/29 may still be included in the analysis. Deviation from the protocol of more than 1 day should, however, be avoided (see also section 3.7).

7. DATA MANAGEMENT

The principal investigator will ensure that the study protocol is strictly adhered to and that all data are collected and recorded correctly on the case report form. Laboratory and clinical data will be recorded on a daily basis on the case report form designed for the study. Data derived from source documents should be consistent with the source documents, or the discrepancies should be explained. Any change or correction to a case report form should be dated and explained and should not obscure the original entry. All case report forms will be checked for completeness.

After the study has been completed, data will be entered into a database by double independent data entry, according to WHO standard procedures (WHO/GMP). The trial data will be stored in a computer database, maintaining confidentiality.

The principal investigator is responsible for keeping all screening forms, the case report form and the completed subject identification code list in a secure location.

8. STATISTICAL METHODS

8.1 Sample size

Sample size calculations is based on an assumed PCR corrected treatment failure rate of artesunate and amodiaquine by day 28 of 5%, a confidence level of 95% and a precision of 5%. This corresponds to a sample of 73 + 20% to account for attrition, in total 90 patients.

8.2 Analysis of data

Microsoft Excel-2007 program World Health Organization 2008 will be used for data management and analysis. Data will be double entered, validated and analysed by two methods: the Kaplan-Meier method and per-protocol analysis in accordance with WHO recommendations. In addition to the reasons for withdrawal listed in section 3.8, patients will be considered withdrawn from the analysis if the PCR results are unclassifiable or if the results of PCR indicate that the failure is due to reinfection with *P. falciparum* or *P. vivax.*

The final analysis and report will include:

- a description of all patients screened and the distribution of reasons for non-inclusion in the study;
- a description of all the patients included in the study;
- the proportion of adverse events and serious adverse events in all the patients included in the study;
- the proportion of patients lost to follow-up or withdrawn, with 95% confidence intervals and a list of reasons for withdrawal;
- the cumulative incidence of success and failure rates at day 28, PCR-uncorrected and PCR-corrected; and
- the proportion of early treatment failure, late clinical failure, late parasitological failure and adequate clinical and parasitological response at day 28, with 95% confidence intervals, PCR-uncorrected and PCR-corrected, as well as gametocyte carriage at each sampling occasion.
- polymorphism of genetic markers related to artesunate + amodiaquine tolerance/resistance.

Guidelines on calculating the cumulative success or failure rate, the proportion of adequate clinical and parasitological response and treatment failure are given in Appendix 9.

8.3 Dissemination of results

At the end of the study, the principal investigator will submit a report on the study and its main outcome. This report will be shared with the Zanzibar Malaria Elimination Programme and the Zanzibar Ministry of Health.

The results of the study will be presented during scientific meetings and submitted for publication in scientific journals.

The patient data will be made available to WWARN, a global database on antimalarial drug efficacy/resistance. To ensure patient confidentially no patient names will be used; instead only the patient identification number, country of original (Zanzibar) and age will be used.

All health facilities and their respective health authorities will be informed about the outcome of the study.

8.4 Amendments to the protocol

After the protocol has been accepted, no change may be made without the agreement of the principal investigator, the sponsor(s) and the ethical review boards.

9. ETHICAL CONSIDERATIONS

9.1 Approval by the national ethical committee

Before the study, official ethical approval to conduct the study will be obtained from Zanzibar Medical Research Ethical Committee (ZAMREC).

9.2 Informed consent

Patients will be included in the study only if they, or a parent or guardian of children give informed consent. The consent request, available in English and translated into Swahili, will be read entirely to the patient, parent or guardian. Details about the trial and its benefits and potential risks will be explained. Once any questions have been answered, a signature will be requested on the document (Appendix 10). If the patient is illiterate, a literate witness must sign; if possible, the signatory will be selected by the participant and will have no connection to the research team. The principal investigator must also obtain the assent of children over the age of 12 years, but their assent should be accompanied by the consent of a parent or guardian. Consent statement for the pregnancy test is also required for female participants of child-bearing age who are sexually active, please see above section 5.2.6.

9.3 Confidentiality

All information on patients will remain confidential and be shared only by the study team. Unique identifiers will be used for computer-based data entry and blood samples. In all cases, the principal investigator will ensure that screening forms, the case report form and the completed identification code list are kept in locked files.

9.4 Health-care services

Free health care throughout follow-up for any illness related to malaria will be provided to the study patients regardless of treatment outcome; this includes any expenses related to hospital admission and to adverse medicine reactions, if required.

When prospective or actual participants are found to have diseases unrelated to malaria, the study team should advise them to obtain, or refer them for, medical care.

Any person who decides not to participate or who cannot be enrolled into the study because he/she does not meet the criteria will be referred to the health facility staff. Such people will be treated with the official first-line treatment, i.e. Artesunate Amodiaquine Winthrop®, once daily for 3 days combined with a single low dose of primaquine (0.25 mg/kg) and followed-up according to the standard of care established by the Zanzibar Ministry of Health and Social Welfare.

If a patient is withdrawn from the study before he/she has completed the full course of the treatment, the clinician must make all necessary arrangements to provide the patient with the full dose of the medicine being tested or in case of intolerance to artesunate + amodiaquine with a full treatment course of artemether 20 mg – lumefantrine 120 mg (Alu) according to body weight at 0, 8, 24, 36, 48, and 60 hours as recommended in the national treatment guidelines.

9.5 Inducement

Subjects will be reimbursed for their transport to attend all visits to the health centre.

9.6 Community

Prior to study commencement, the health facilities catchment areas (Shehia) will be gathered together through their local leaders (Sheha). In these meetings investigators will brief the community leaders about the study and stress the importance of completion of the entire follow-up schedule of 28 days. They will be invited to ask questions and full explanation will be provided to any raised issue. A copy of the study consent form will be provided to each Sheha to enable any community member to read more about the study.

10. BUDGET TEMPLATE

Human resources	**Tsh**	**USD**
· professional scientific staff 3pple*300,000/- per month*12 months	10 800 000,00	7 448,28
· technical staff 40pple*100,000/- per month*12 months	48 000 000,00	33 103,45
· local support 20pple*50,000/- per month*12 months	12 000 000,00	8 275,86
Sub-total	**70 800 000,00**	**48 827,59**
Transport for Local supervision		
24 visits*25lts*1,650/- per ltr 1st phase	1 980 000,00	1 365,52
144 visits*25lts*1,650/- per ltr 2nd phase	11 880 000,00	8 193,10
360 visits*25lts*1,650/- per ltr 3rd phase	29 700 000,00	20 482,76
Sub-total	**43 560 000,00**	**30 041,38**
Equipment and supplies		
· equipment (thermometers, lab equips ets)	8 900 000,00	6 137,93
· supplies (slides, cotton, spirit, lancets, giemsa etc)	11 200 000,00	7 724,14
· operational costs (space rental, communication)	8 500 000,00	5 862,07
Sub-total	**28 600 000,00**	**19 724,14**
Contingency fees for clinical trials		
· ethical review	450 000,00	310,34
Sub-total	**450 000,00**	**310,34**
Patient incentive costs (bus fares for follow up and free LLIN)		
Patients bus fares (100pts*1,000per trip*9trips)	2,250 000,00	1,551.52
Free LLINs (7,000/- pet net*100 nets)	700 000,00	482,76
Sub-total	**2 950 000,00**	**2,033.28**
Technical assistance		
Training for 20 Lab technicians	25 000 000,00	17 241,38
(training, support for research institutions, capacity building)	1 450 000,00	1 000,00
Sub-total	**26 450 000,00**	**18 241,38**
Supervision		
Local Supervisors	3 120 000,00	2 151,72
Sub-total	**3 120 000,00**	**2 151,72**
Quality assurance system		
(data validation, slides cross-check)		
Sub-total	**2 300 000,00**	**1 586,21**
Data management		
(data entry, data analysis, report writing)		
Sub-total	**7 270 500,00**	**5 014,14**
Laboratory support		
(genotyping)		

Sub-total	0	-
Miscellaneous		
Sub-total	**2 500 000,00**	**1 724,14**
GRAND TOTAL	186,200,500	128,414

11. CURRICULUM VITAE OF THE PRINCIPAL INVESTIGATOR

Mwinyi I. Msellem, CMLT, ADMLS, MSc

Date and place of birth	14 Dec 1966, Zanzibar
Nationality	Tanzanian
Civil status	Married with four children
Address	P.O.BOX 503 Zanzibar, Tanzania
Telephone	+255 77 743 29 11 and +255 71 543 29 11
E-mail	mmwinyi@hotmail.com

Employment

November 2016 to date: Training and Research Officer, Mnazi mmoja hospital, Zanzibar Ministry of Health

Responsible for coordinating researches and trainings to be conducted in Mnazi mmoja hospital. Also to make sure eligible staffs are participating in capacity building programmes inside and outside of Zanzibar. To work very close with other members of Training and Research unit of Mnazi mmoja hospital and of the Ministry of Health to develop training plans based on priorities and available resources. To advise the Executive Director and its Board on resource mobilization, collaborations and partnership with other organisations outside of the hospital for trainings and researches programmes which will improve and develop hospital management and its services.

February, 2011 to November 2016: Assistant Program Manager, Zanzibar Malaria Programme under the Zanzibar Ministry of Health,

I was supporting the Program Manager (PM) in coordinating and implementing program strategies to increase efficiency, maintain quality, and ensure continuous improvement in programme implementation. Assist the PM to review of all grant agreements with implementers to ensure that they are fully aligned with the grant agreements signed between the ZAMEP Program and the Donors; ensure the program units and implementers prepare the quarterly and annual programmatic and financial reports; manage staff and support functions to promote the effective functioning of the Program, build capacity of program staff through training, supervise line managers and undertake regular monitoring of program field activities. To support Zanzibar Malaria Control Program and later Elimination Programme in controlling and eliminate malaria as a public health problem and obstacle to socio-economic development, through providing technical guidance to local authorities, national and international partners.

August, 2001 to February 2011: Head of Diagnostic unit of Zanzibar Malaria Programme under the Zanzibar Ministry of Health,

Leader on technical coordination, convening and facilitating mechanism in the ZAMEP that aims to: advise, provide technical support, review, encourage harmonization and pooling of efforts for faster uptake and scale up of malaria recommended quality diagnostic techniques in Zanzibar.

October, 1992 to August 2001: Laboratory technician in Laboratories of Zanzibar Malaria Programme.
Execution of all laboratory procedures aimed to provide accurate finding of malaria parasites detection on patient samples. Participating in all laboratory based operational studies. To perform quality control and quality assurance of other laboratories with their respective technicians

January 1984 to October, 1992: Entomological aid in Entomology unit of Zanzibar Malaria Programme.
Responsible for collection of mosquitoes using different techniques (human bait, window traps, cattle traps, CDC light traps etc). Under guidance of the Programmes and partners Entomologists to assist on insecticide susceptibility tests, bioassay tests, dissection of mosquitoes for different studies.

Education

2005 -2007 Licentiate Degree in Karolinska Instituttet, Stockholm –Sweden: Trained in Research and training. Defended thesis "Efficacy of artemisinin based combination therapy and effectiveness of Rapid Diagnostic Test for management of patients with *Plasmodium falciparum* malaria in Zanzibar"

1998 –2000 Advanced diploma in Medical Laboratory Sciences (Muhimbili University, College of Health Sciences, Dar es Salaam – Tanzania). Trained in Parasitology and Medical Entomology. Learned and conducted research titled "Factors associated with *Plasmodium falciparum* gametocytomia to the young children aged 5-60 months in Zanzibar Island"

1989 – 1992 Certificate in Medical Laboratory Technology (College Of Health Sciences – Zanzibar). Trained in general Medical Laboratory Sciences.

1987 – National Ordinary School Certificate (Lumumba Secondary School).

1983 – School Leaving Certificate 'O' level (Haile Sellassie Secondary School)

1973 – 1982 Primary and Secondary School Certificate (Muyuni School)

Trainings and International workshops

2009- WHO Technical Consultation on Parasitological Confirmation Of Malaria Diagnosis – Geneva
2008, 2009 and 2010 Malaria Review and Planning Meetings for East and Southern Africa jointly organized by WHO IST, EARN, -Zambia, Namibia and Kenya
2004 –Participant in East Africa Regional Workshop on Protozoan Pathogens, Focused on Pregnant Host Morogoro –Tanzania
2003 Training of Trainers for Malaria Diagnosis procedures for anglophone countries Ndola - Zambia
2002 – Participant in MIM/TDR Workshop on Invitro Drug Susceptibility Testing of Plasmodium falciparum to antimalarial drugs, Cotonou –Benin
2001 Facilitator on Basic Malaria Microscopy to Zanzibar Laboratory Technicians

Other responsibilities

In 2013 –Member of Proposal Development Task Force: under the guidance of the ZGFCCM, main responsibilities was management and coordination of Global Fund proposal development and applications for funding in Zanzibar.

From February, 2011 to October 2016: Clinical researches coordinator in Zanzibar Malaria Programme: In collaboration with Programme partners to coordinate and conduct clinical based and community based operational researches in different study sites.

Since 2009 to date: Executive Secretary in Professional non-government Organisation: responsible for the management and administration of the Zanzibar Association for Medical Laboratory Scientific Officers (ZAMELSO)

Since 2006 to 2016: National IRS Supervisor in Zanzibar Malaria Programme: organize and coordinate day to day Indoor Residual house Spraying (IRS) activities, to conduct supervision, monitoring and evaluation of IRS activities, to work with community leadership, district IRS technical committee etc (ensure participation and ownership which will lead to the success of IRS activities). Participate in the recruitment of spray operators. Ensure proper record keeping and documentation of spraying activities. Ensure safe and quality spraying.

2005 to date: Clinical Trials Coordinator, in the collaborative Institution (Zanzibar Malaria Research Unit Karolinska Institute (ZAMRUKI)) between Karolinska University of Sweden and Zanzibar Ministry of Health through Malaria Programme. Among other duties I coordinate and act as local supervisor to the Medical, Undergraduate and postgraduate students from Karolinska Instituttet in all operational researches implemented in Zanzibar as per training plans and Malaria Programme Strategic plan.

Consultancies

In 2009: I contracted by WHO as local consultant for the development of Round 8 Global Fund malaria and health systems strengthening proposals for Zanzibar. To assist local consultant to identify key gaps and priorities; national level consultation and consensus building on key priorities; develop mechanisms for CSO inclusion; lead writer on the proposal and budget.

October 2013: Lead on ZAMELSO proposal write up and then project implementation on HIV&AIDS prevention, testing and behaviour change communication interventions among Intravenous drug users in Unguja Island

From 2008 and 2013: Member of writing team on the formulation of Zanzibar Malaria Strategic Plans II and lll (2008, 2013)

August 2014: Team leader on ZAMELSO proposal writing and project implementation titled Improve HIV&AIDS prevention, testing, adherence in Care & Treatment Centres and rehabilitation support among people with inject drugs in Unguja Island

Publications (*About 22 publications in international peer reviewed journals)*

1. *Using low-cost drones to map malaria vector habitats.* Andy Hardy, Makame Makame, Dónall Cross, Silas Majambere and **Mwinyi Msellem**. *Parasites & Vectors*, **DOI:** 10.1186/s13071-017-1973-3. 14 January 2017

2. *Influence of Rapid Malaria Diagnostic Tests on Treatment and Health Outcome in Fever Patients, Zanzibar—A Crossover Validation Study.* Mwinyi I. Msellem, Andreas Mårtensson, Guida Rotllant, Achuyt Bhattarai, Johan Strömberg, Elizeus Kahigwa, Montse Garcia, Max Petzold, Peter Olumese, Abdullah Ali, Anders Björkman Less. *PLoS medicine2009*. PLoS Medicine, vol 6(4), 27 April 2009.

3. *Loop-mediated isothermal amplification (LAMP) for point-of-care detection of asymptomatic low-density malaria parasite carriers in Zanzibar.* Jackie Cook, Berit Aydin-Schmidt, Iveth J González, David Bell, Elin Edlund, Majda H Nassor, Mwinyi Msellem, Abdullah Ali, Ali K Abass, Andreas Mårtensson, Anders Björkman. *Malaria Journal2015*

4. *The Usefulness of Rapid Diagnostic Tests in the New Context of Low Malaria Transmission in Zanzibar.* Delér Shakely, Kristina Elfving, Berit Aydin-Schmidt, Mwinyi I. Msellem, Ulrika Morris, Rahila Omar, Xu Weiping, Max Petzold, Bryan Greenhouse, Kimberly A. Baltzell, Abdullah S. Ali, Anders Björkman, Andreas Mårtensson. *PloS one2013*

5. *Field deployment of loop-mediated isothermal amplification for centralized mass-screening of asymptomatic malaria in Zanzibar: a pre-elimination setting.* Ulrika Morris, Mwinyi Khamis, Berit Aydin-Schmidt, Ali K Abass, Mwinyi I Msellem, Majda H Nassor, Iveth J González, Andreas Mårtensson, Abdullah S Ali, Anders Björkman, Jackie Cook. *Malaria Journal2015*

Languages

SWAHILI AND ENGLISH

Referees

PROF. ANDERS BJORKMAN
MALARIA RESEARCH LABORATORY
KAROLINSKA INSTITUTE
STOCKHOLM SWEDENDR.

DR. ALI SALIM ALI
EXECUTIVE DIRECTOR
MNAZI MMOJA HOSPITAL
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MR. ABDULLAH S. ALI
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ZANZIBAR MALARIA ELIMINATION PROGRAMME,
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ZANZIBAR TANZANIA

Appendix 1. DEFINITION OF SEVERE FALCIPARUM MALARIA[1]

Severe manifestation of *P. falciparum* malaria in adults and children

Clinical manifestations

- prostration,
- impaired consciousness,
- respiratory distress (metabolic acidosis),
- multiple convulsions,
- circulatory collapse,
- pulmonary oedema (radiological),
- abnormal bleeding,
- jaundice,
- haemoglobinurea.

Laboratory findings

- severe anaemia (haemoglobin < 5 g/dl, haematocrit < 15%),
- hypoglycaemia (blood glucose < 2.2 mmol/l or 40 mg/dl),
- acidosis (plasma bicarbonate < 15 mmol/l),
- hyperlactataemia (venous lactic acid > 5 mmol/l),
- hyperparasitaemia (> 4% in non-immune patients),
- renal impairment (serum creatinine above normal range for age).

Classification of severe malaria in children

Group 1: children at increased risk for death

- prostration
- respiratory distress

Group 2: children at risk for clinical deterioration

- haemoglobin < 5 g/dl, haematocrit < 15%
- two or more convulsions within 24 h

Group 3: children with persistent vomiting

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[1] World Health Organization. Severe falciparum malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 2000, 94(Suppl. 1):1–90.

Appendix 2. MEDICATIONS (WITH ANTIMALARIAL ACTIVITY) THAT SHOULD NOT BE USED DURING THE STUDY PERIOD

- chloroquine, amodiaquine;
- quinine, quinidine;
- mefloquine, halofantrine, lumefantrine;
- artemisinin and its derivatives (artemether, arteether, artesunate, dihydroartemisinin);
- proguanil, chlorproguanil, pyrimethamine;
- sulfadoxine, sulfalene, sulfamethoxazole, dapsone;
- primaquine;
- atovaquone;
- antibiotics: tetracycline*, doxycycline, erythromycin, azythromycin, clindamycin, rifampicin, trimethoprim;
- pentamidine.

* Tetracycline eye ointments can be used.

Appendix 3. DOSING CHART OF ARTESUNATE + AMODIAQUINE AND PRIMAQUINE

A fixed dose combination of artesunate + amodiaquine (Artesunate Amodiaquine Winthrop®, Sanofi Aventis), prequalified by the WHO, will be used as study drug. Tablets containing 25/67.5 mg, 50/135mg and 100/270 mg, base of artesunate/amodiaquine will be administered according to the dosing chart below:

Body weight	Number of tablets of Artesunate (AS) + Amodiaquine (AQ)		
	Day 0	Day 1	Day 2
<10 kg	**1 tab** (AS 25 mg + AQ 67.5 mg)	**1 tab** (AS 25 mg + AQ 67.5 mg)	**1 tab** (AS 25 mg + AQ 67.5 mg)
10-20 kg	**1 tab** (AS 50 mg + AQ 135 mg)	**1 tab** (AS 50 mg + AQ 135 mg)	**1 tab** (AS 50 mg + AQ 135 mg)
21-30 kg	**2 tab** (AS 50 mg + AQ 135 mg)	**2 tab** (AS 50 mg + AQ 135 mg)	**2 tab** (AS 50 mg + AQ 135 mg)
>30 kg	**2 tab** (AS 100 mg + AQ 270 mg)	**2 tab** (AS 100 mg + AQ 270 mg)	**2 tab** (AS 100 mg + AQ 270 mg)

DOSAGE AND ADMINISTRATION OF PRIMAQUINE			
	WEIGHT	**AGE**	**Single low dose [0 hrs]**
1	**5-9 kg**	1-4 yrs	2.5 mg
2	**10-20 kg**	5-8 yrs	5 mg
3	**20-40 kg**	9-14 yrs	10 mg
4	**>40 kg**	15+ yrs	15 mg

Pediatric dosing of SLD primaquine:
To dissolve tablets in Water:

a. Based on patients weight dissolve one tablet(s) (15mg) of Primaquine in 5 ml of water.
b. Ensure that the solution is thoroughly mixed.
c. Measure the volume (cc) of solution to give to the participant based on the participant's weight. The drug will be mixed with equal volume of juice to mask its bitter test.

Picture 1: Sanofi product, Primaquine phosphate USP Tablets 26.3 mg (15 mg equivalent base)

Picture 2: Example of mortar, pestle and graduated syringes

The extemporaneous preparation should be conducted as follows:

- Define number of 15mg (primaquine base) tablets required based on patient's age or weight.
- Crush tablets in mortar with pestle.
- Pour powder into the clean container to be used for dilution (preferably glass container as there is no data on interaction with plastic).
- Based on 15 mL of water for 1 tablet, measure in a graduated syringe the required volume of water needed to dissolve the tablets. Use a fraction of water from the syringe to rinse the mortar and pestle, and pour contents into the container.
- In the container, mix the powder and liquid thoroughly with a spoon.
- Leave for 1 minute to rest. Some residuals (excipients not dissolved such as talc) will appear on the bottom of the container and do not need to be withdrawn.
- Based on patient's weight, withdraw required volume with graduated syringe, avoiding the excipient at the bottom of the container. See table 1 below for examples.
- The prepared solution can be stored in the glass container for a maximum of 3 days at 40°C or 7 days at 30°C, protected from light.
- Do not store the prepared solution inside the syringe as there are no data available on potential interactions between syringe materials and primaquine.

Age (years)	**Weight (kg)**	**Dosage (mg)** (0.25 x weight)	**Volume to be withdrawn (ml)** (for a solution of 1mg/ ml)	**Syringes recommended for administration**
1	9	2.25	2.25	5 ml
6	18	4.5	4.5	10 ml
14	36	9.0	9.0	10 ml (or 20 ml)

Appendix 4. CLASSIFICATION OF TREATMENT OUTCOMES[2]

Early treatment failure

- 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 ºC;
- parasitaemia on day 3 ≥ 25% of count on day 0.

Late treatment failure

Late clinical failure

- danger signs or severe malaria in the presence of parasitaemia on any day between day 4 and day 28 in patients who did not previously meet any of the criteria of early treatment failure;
- presence of parasitaemia on any day between day 4 and day 28 with axillary temperature ≥ 37.5 ºC or history of fever in patients who did not previously meet any of the criteria of early treatment failure

Late parasitological failure

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

Adequate clinical and parasitological response

- absence of parasitaemia on day 28, irrespective of axillary temperature, in patients who did not previously meet any of the criteria of early treatment failure, late clinical failure or late parasitological failure

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[2] WHO. *Susceptibility of* Plasmodium falciparum *to antimalarial drugs. Report on global monitoring 1996–2004*. Geneva, World Health Organization, 2005 (WHO/HTM/MAL/2005.110) (http://www.who.int/malaria/resistance).

Appendix 5. CASE SCREENING FORM

Case screening form	
Health centre name: District: Sheiha	Study number: Patient screening number: Date of visit (dd-mmm-yyyy):
Demographic data	
Date of birth (dd-mmm-yyyy): or estimated age: in: ☐ months or ☐ years Height (cm): Weight (kg): Sex: ☐ Male ☐ Female If female, is the patient pregnant? ☐ Yes ☐ No ☐ Unknown If pregnant, provide the date of the last menstrual period (dd-mmm-yyyy):	
Pre-treatment temperature	
History of fever in previous 48 h? ☐ Yes ☐ No Axillary temperature: ºC	
Screening test for presence of *P. falciparum* with Rapid Diagnostic Test	
RDT result ☐ Positive ☐ Negative **(If negative, patient is not eligible)**	
Thick and thin blood smears for estimation of *P. falciparum* parasite counts	
Species: ☐ *P. falciparum* ☐ *P. vivax* ☐ *P. ovale* ☐ *P. malariae* Were species other than *P. falciparum* present? ☐ Yes ☐ No **(If yes, patient is not eligible).** Presence of *P. falciparum* asexual parasites: ☐ Yes ☐ No **(If no, patient is not eligible)** Presence of *P. falciparum* gametocytes? ☐ Yes ☐ No	
Has a blood sample for PCR been collected? ☐ Yes ☐ No	
Urinary analysis (pregnancy test for female patients)	
Result of pregnancy test: ☐ Positive ☐ Negative ☐ Not done **(If positive, patient is not eligible)**	
Inclusion criteria	
• age 3 months and above • mono-infection with *P. falciparum* detected by microscopy; • presence of *P. falciparum* malaria asexual parasitaemia; • presence of axillary ≥37.5 °C or history of fever during the past 24 hours • ability to swallow oral medication; • ability and willingness to comply with the study protocol for the duration of the study and to comply with the study visit schedule; and • informed consent from the patient or from a parent or guardian in the case of children.	
Does the patient meet all the inclusion criteria? ☐ Yes ☐ No **(If no, patient is not eligible)**	

Case screening form (page 2)	
Exclusion criteria	
• presence of general danger signs in children aged under 5 years or signs of severe falciparum malaria according to the definitions of WHO (Appendix 1); • mono-infection with a Plasmodium species other than *P. falciparum* detected by microscopy; • presence of severe malnutrition (defined as a child whose growth standard is below –3 z-score, has symmetrical oedema involving at least the feet or has a mid-upper arm circumference < 110 mm); • presence of febrile conditions due to diseases other than malaria (e.g. measles, acute lower respiratory tract infection, severe diarrhoea with dehydration) or other known underlying chronic or severe diseases (e.g. cardiac, renal and hepatic diseases, HIV/AIDS); • regular medication, which may interfere with antimalarial pharmacokinetics; • history of hypersensitivity reactions or contraindications to any of the study medicines; and • pregnancy	
Does the patient meet any of the exclusion criteria? ☐ Yes ☐ No **(If yes, the patient is not eligible)**	
If yes, please specify the reason for exclusion:	
Patient informed consent and assent	
Consent form signed: ☐ Yes ☐ No Assent form signed: ☐ Yes ☐ No	Patient identity number: Date (dd-mmm-yyyy):

Appendix 6. SCHEDULE OF FOLLOW-UP ACTIVITIES

	Day 0	1	2	3	7	14	21	28	Any other day
Procedure									
Clinical assessment	X	X	X	X	X	X	X	X	X
Temperature	X	X	X	X	X	X	X	X	X
Blood slide for parasite count	X	X	X	X	X	X	X	X	X
Urine sample	(X)							(X)	
Blood for:									
Genotyping/ molecular markers	X	X	X	X	X	X	X	X	X
Hb	X			X	X	X		X	
Treatment									
Artesunate + amodiaquine	X	X	X						
Primaquine	X								

Day 0

Screening

- clinical assessment, including measurement of weight and height; referral in cases of severe malaria or danger signs;
- measurement of temperature;
- parasitological assessment;
- pregnancy test (if necessary);
- informed consent.

Enrolment

- treatment, first dose;
- blood sampling for genotyping/molecular markers of drug resistance;
- haemoglobin

Day 1

- clinical assessment; referral in cases of severe malaria or danger signs;
- measurement of axillary temperature;
- parasitological assessment
- treatment, second dose or alternative treatment in case of early treatment failure.

Day 2

- clinical assessment; referral in cases of severe malaria or danger signs;
- measurement of axillary temperature;
- parasitological assessment;
- treatment, third dose or alternative treatment in case of early treatment failure.

Day 3, day 7, day 14, day 21, day 28, any other day

- clinical assessment; referral in cases of severe malaria or danger signs;
- measurement of axillary temperature;
- parasitological assessment;
- alternative treatment in cases of treatment failure;
- pregnancy test at the end of follow-up (if necessary);
- blood sampling for genotyping to distinguish between recrudescence and reinfection and molecular markers of drug resistance in cases of treatment failure after day 7,
- haemoglobin will be routinely assessed at enrolment, day 3, 7, 14 and 28.

Appendix 7. CASE REPORT FORMS

Case report form: Day of enrolment = day 0	
Health centre name: District: Sheiha: Household card identification number:	Study number: Patient identity number: Date of visit (dd-mmm-yyyy):
Demographic data	
Date of birth (dd-mmm-yyyy): or estimated age: in: ☐ months or ☐ years Height (cm): Weight (kg): Sex: ☐ Male ☐ Female If female, is the patient pregnant? ☐ Yes ☐ No ☐ Unknown **(If yes, patient is not eligible)** If pregnant, provide the date of the last menstrual period (dd-mmm-yyyy):	
Overnight travel away from home within past month? ☐ Yes ☐ No If yes, please list all places where at least on night was spend:	
Pre-treatment temperature	
History of fever in previous 48 h? ☐ Yes ☐ No Axillary temperature: ºC	
Thick blood smears for *P. falciparum*: asexual and gametocyte counts; haemoglobin assessment	
Average number of asexual *P. falciparum* parasites/µl: Presence of *P. falciparum* gametocytes? ☐ Yes ☐ No Were species other than *P. falciparum* present? ☐ Yes ☐ No **(If yes, patient is not eligible).** If yes, which species? ☐ *P. vivax* ☐ *P. ovale* ☐ *P. malariae* Hemoglobin	
Has blood sample for PCR been collected? ☐ Yes ☐ No	
Prior medication	
All prior medication, including natural remedies and homeopathic medicines, taken within the previous 14 days should be reported in this section. Has the patient taken any prior antimalarial medication? ☐ Yes ☐ No. If yes, please specify below. Either the date of stopping or the 'ongoing' box should be checked.	

Medicine name (generic name)	Dates	Ongoing (Yes = ☒)	Total daily dose and unit (e.g. 400 mg)	Route of administration	Indication for use
	Start: Stop:	☐			
	Start: Stop:	☐			
	Start: Stop:	☐			

Case report form: Day of enrolment = day 0 (page 2)				
Medication administration				
Name(s) of antimalarial drug(s)	Time of dose (hh:min)	Number of tablets	Did the patient vomit?	Time of vomiting (hh:min)
			☐ Yes ☐ No	
			☐ Yes ☐ No	
Name(s) of other medicine(s)				
			☐ Yes ☐ No	
			☐ Yes ☐ No	

Case report form: follow-up day 1

Study number:

Patient identity number:

Date of visit (dd-mmm-yyyy):

Clinical status

Presence of danger signs or signs of severe or complicated malaria? ☐ Yes ☐ No

Axillary temperature: ºC

Thick blood smears for estimation of *P. falciparum* parasitemia

Average number of asexual *P. falciparum* parasites/µl:

Presence of *P. falciparum* gametocytes? ☐ Yes ☐ No

Were species other than *P. falciparum* present? ☐ Yes ☐ No

If yes, which species? ☐ *P. vivax* ☐ *P. ovale* ☐ *P. malariae*

Adverse events

Presence of an adverse event? ☐ Yes ☐ No

If yes, name the adverse event:

Is it a serious adverse event? ☐ Yes ☐ No. If yes, inform the sponsor.

Medication administration

Name(s) of antimalarial drug(s)	Time of dose (hh:min)	Number of tablets	Did the patient vomit?	Time of vomiting (hh:min)
			☐ Yes ☐ No	
			☐ Yes ☐ No	
Name(s) of other medicine(s)				
			☐ Yes ☐ No	
			☐ Yes ☐ No	

Case report form: follow-up day 2

Study number:

Patient identity number:

Date of visit (dd-mmm-yyyy):

Clinical status

Presence of danger signs or signs of severe or complicated malaria? ☐ Yes ☐ No

Axillary temperature: ºC

Thick blood smears for estimation of *P. falciparum* parasitemia

Average number of asexual *P. falciparum* parasites/µl:

Presence of *P. falciparum* gametocytes? ☐ Yes ☐ No

Were species other than *P. falciparum* present? ☐ Yes ☐ No

If yes, which species? ☐ *P. vivax* ☐ *P. ovale* ☐ *P. malariae*

Adverse events

Presence of an adverse event? ☐ Yes ☐ No

If yes, name the adverse event:

Is it a serious adverse event? ☐ Yes ☐ No. If yes, inform the sponsor.

Medication administration

Name(s) of antimalarial drug(s)	Time of dose (hh:min)	Number of tablets	Did the patient vomit?	Time of vomiting (hh:min)
			☐ Yes ☐ No	
			☐ Yes ☐ No	
Name(s) of other medicine(s)				
			☐ Yes ☐ No	
			☐ Yes ☐ No	

Case report form: follow-up day 3

Study number:

Patient identity number:

Date of visit (dd-mmm-yyyy):

Clinical status

Presence of danger signs or signs of severe or complicated malaria? ☐ Yes ☐ No

Axillary temperature: ºC

Thick blood smears for estimation of *P. falciparum* parasitemia; Hemoglobin assessment

Average number of asexual *P. falciparum* parasites/µl:

Presence of *P. falciparum* gametocytes? ☐ Yes ☐ No

Were species other than *P. falciparum* present? ☐ Yes ☐ No

If yes, which species? ☐ *P. vivax* ☐ *P. ovale* ☐ *P. malariae*

Hemoglobin

Adverse events

Presence of an adverse event? ☐ Yes ☐ No

If yes, name the adverse event:

Is it a serious adverse event? ☐ Yes ☐ No. If yes, inform the sponsor.

Medication administration

Name(s) of antimalarial drug(s)	Time of dose (hh:min)	Number of tablets	Did the patient vomit?	Time of vomiting (hh:min)
			☐ Yes ☐ No	
			☐ Yes ☐ No	
Name(s) of other medicine(s)				
			☐ Yes ☐ No	
			☐ Yes ☐ No	

Case report form: follow-up day 7

Study number:

Patient identity number:

Date of visit (dd-mmm-yyyy):

Clinical status

Presence of danger signs or signs of severe or complicated malaria? ☐ Yes ☐ No

History of fever within previous 48 h? ☐ Yes ☐ No

Axillary temperature: ºC

Thick blood smears for estimation of *P. falciparum* parasitemia; Hemoglobin assessment

Average number of asexual *P. falciparum* parasites/µl:

Presence of *P. falciparum* gametocytes? ☐ Yes ☐ No

Were species other than *P. falciparum* present? ☐ Yes ☐ No

If yes, which species? ☐ *P. vivax* ☐ *P. ovale* ☐ *P. malariae*

Hemoglobin

Has a blood sample for PCR been collected? ☐ Yes ☐ No

Adverse events

Presence of an adverse event? ☐ Yes ☐ No

If yes, name the adverse event:

Is it a serious adverse event? ☐ Yes ☐ No. If yes, inform the sponsor.

Medication administration

Name(s) of antimalarial drug(s)	Time of dose (hh:min)	Number of tablets	Did the patient vomit?	Time of vomiting (hh:min)
			☐ Yes ☐ No	
			☐ Yes ☐ No	
Name(s) of other medicine(s)				
			☐ Yes ☐ No	
			☐ Yes ☐ No	

Case report form: follow-up day 14

Study number:

Patient identity number:

Date of visit (dd-mmm-yyyy):

Clinical status

Presence of danger signs or signs of severe or complicated malaria? ☐ Yes ☐ No

History of fever within previous 48 h? ☐ Yes ☐ No

Axillary temperature: ºC

Thick blood smears for estimation of *P. falciparum* parasitemia; Hemoglobin assessment

Average number of asexual *P. falciparum* parasites/µl:

Presence of *P. falciparum* gametocytes? ☐ Yes ☐ No

Were species other than *P. falciparum* present? ☐ Yes ☐ No

If yes, which species? ☐ *P. vivax* ☐ *P. ovale* ☐ *P. malariae*

Hemoglobin

Has a blood sample for PCR been collected? ☐ Yes ☐ No

Adverse events

Presence of an adverse event? ☐ Yes ☐ No

If yes, name the adverse event:

Is it a serious adverse event? ☐ Yes ☐ No. If yes, inform the sponsor.

Medication administration

Name(s) of antimalarial drug(s)	Time of dose (hh:min)	Number of tablets	Did the patient vomit?	Time of vomiting (hh:min)
			☐ Yes ☐ No	
			☐ Yes ☐ No	
Name(s) of other medicine(s)				
			☐ Yes ☐ No	
			☐ Yes ☐ No	

Case report form: follow-up day 21

Study number:

Patient identity number:

Date of visit (dd-mmm-yyyy):

Clinical status

Presence of danger signs or signs of severe or complicated malaria? ☐ Yes ☐ No

History of fever within previous 48 h? ☐ Yes ☐ No

Axillary temperature: ºC

Thick blood smears for estimation of *P. falciparum* parasitemia

Average number of asexual *P. falciparum* parasites/µl:

Presence of *P. falciparum* gametocytes? ☐ Yes ☐ No

Were species other than *P. falciparum* present? ☐ Yes ☐ No

If yes, which species? ☐ *P. vivax* ☐ *P. ovale* ☐ *P. malariae*

Has a blood sample for PCR been collected? ☐ Yes ☐ No

Adverse events

Presence of an adverse event? ☐ Yes ☐ No

If yes, name the adverse event:

Is it a serious adverse event? ☐ Yes ☐ No. If yes, inform the sponsor.

Medication administration

Name(s) of antimalarial drug(s)	Time of dose (hh:min)	Number of tablets	Did the patient vomit?	Time of vomiting (hh:min)
			☐ Yes ☐ No	
			☐ Yes ☐ No	
Name(s) of other medicine(s)				
			☐ Yes ☐ No	
			☐ Yes ☐ No	

Case report form: follow-up day 28

Study number:

Patient identity number:

Date of visit (dd-mmm-yyyy):

Clinical status

Presence of danger signs or signs of severe or complicated malaria? ☐ Yes ☐ No

History of fever within previous 48 h? ☐ Yes ☐ No

Axillary temperature: ºC

Thick blood smears for estimation of *P. falciparum* parasitemia; Hemoglobin assessment

Average number of asexual *P. falciparum* parasites/µl:

Presence of *P. falciparum* gametocytes? ☐ Yes ☐ No

Were species other than *P. falciparum* present? ☐ Yes ☐ No

If yes, which species? ☐ *P. vivax* ☐ *P. ovale* ☐ *P. malariae*

Hemoglobin

Has a blood sample for PCR been collected? ☐ Yes ☐ No

Adverse events

Presence of an adverse event? ☐ Yes ☐ No

If yes, name the adverse event:

Is it a serious adverse event? ☐ Yes ☐ No. If yes, inform the sponsor.

Medication administration

Name(s) of antimalarial drug(s)	Time of dose (hh:min)	Number of tablets	Did the patient vomit?	Time of vomiting (hh:min)
			☐ Yes ☐ No	
			☐ Yes ☐ No	
Name(s) of other medicine(s)				
			☐ Yes ☐ No	
			☐ Yes ☐ No	

Case report form: day ___(any other day that is not part of regular follow-up)				
Study number: Patient identity number: Date of visit (dd-mmm-yyyy):				
Clinical status				
Presence of danger signs or signs of severe or complicated malaria? ☐ Yes ☐ No History of fever within previous 48 h? ☐ Yes ☐ No Axillary temperature: ºC				
Thick blood smears for estimation of *P. falciparum* parasitemia; Hemoglobin assessment				
Average number of asexual *P. falciparum* parasites/µl: Presence of *P. falciparum* gametocytes? ☐ Yes ☐ No Were species other than *P. falciparum* present? ☐ Yes ☐ No If yes, which species? ☐ *P. vivax* ☐ *P. ovale* ☐ *P. malariae* Hemoglobin				
Has a blood sample for PCR been collected? ☐ Yes ☐ No				
Adverse events				
Presence of an adverse event? ☐ Yes ☐ No If yes, name the adverse event: Is it a serious adverse event? ☐ Yes ☐ No. If yes, inform the sponsor.				
Medication administration				
Name(s) of antimalarial drug(s)	Time of dose (hh:min)	Number of tablets	Did the patient vomit?	Time of vomiting (hh:min)
			☐ Yes ☐ No	
			☐ Yes ☐ No	
Name(s) of other medicine(s)				
			☐ Yes ☐ No	
			☐ Yes ☐ No	

Case report form: follow-up day 28 (=final day of study)
Overall assessment
Outcome: ☐ adequate clinical and parasitological response ☐ early treatment failure ☐ late clinical failure ☐ late parasitological failure ☐ lost to follow-up ☐ withdrawn
Outcome occurred on follow-up day: (e.g. 1, 2, 3, 7, 14, 21 and 28)
PCR: ☐ *P. falciparum* recrudescence ☐ *P. falciparum* reinfection ☐ other species ☐ mixed with *P. falciparum* recrudescence ☐ mixed with *P. falciparum* reinfection ☐ unknown
PCR corrected results: ☐ adequate clinical and parasitological response ☐ early treatment failure ☐ late clinical failure ☐ late parasitological failure ☐ lost to follow-up ☐ withdrawn
Reason for withdrawal:
Other comments:

Appendix 8. SERIOUS ADVERSE EVENT REPORT FORM

Serious adverse event report form	
Health centre name: District: Sheiha:	Study number: Patient identity number: Date of visit (dd-mmm-yyyy): Follow-up day:
Demographic data	
Date of birth (dd-mmm-yyyy): or estimated age: in: ☐ months or ☐ years Height (cm): Weight (kg): Sex: ☐ Male ☐ Female If female, is the patient pregnant? ☐ Yes ☐ No ☐ Unknown If pregnant, provide the date of the last menstrual period (dd-mmm-yyyy):	
Serious adverse event	
Type of event: ☐ Death ☐ Life-threatening ☐ Hospitalization or prolongation of hospitalization ☐ Permanent disability ☐ Congenital anomaly or birth defect Date of occurrence (dd-mmm-yyyy):	
Describe the serious adverse event (include all relevant laboratory results):	
Describe how the reaction was treated:	

Serious adverse event report form (page 2)

Comments (e.g. relevant medical history, drug allergies, previous exposure to similar drugs, other laboratory data, whether reaction abated after stopping the drug, whether reaction reappeared after reintroduction):

Outcome

☐ Recovered completely

☐ Not yet recovered

☐ Recovered with long-term consequences

If patient recovered, provide date of recovery (dd-mmm-yyyy):

Medicines (list the **medicine suspected of causing** the serious adverse event as well as all **concomitant medicines**)

Brand name, batch number, manufacturer name (list suspected medicine first)	Daily dose	Route	Start date	End date	Indications for use

Reporting officer

Name:

Qualification:

Address:

Phone:

Fax:

Email:

Signature: Date:

Appendix 9. GUIDELINES FOR ANALYSIS OF RESULTS

End-point for day X (X = 28 or 42)	PCR-uncorrected results	
	Cumulative success or failure rate (Kaplan-Meier analysis)	Proportion (per-protocol analysis)
Adequate clinical and parasitological response on day X	Success	Success
Early treatment failure	Failure	Failure
Late clinical failure before day 7	Failure	Failure
Late clinical failure or late parasitological failure on or after day 7	Failure	Failure
Other species infection	Censored day of infection	Excluded from analysis
Lost to follow-up	Censored last day of follow-up according to timetable	Excluded from analysis
Withdrawal and protocol violation	Censored last day of follow-up according to timetable before withdrawal or protocol violation	Excluded from analysis

End-point for day X (X = 28 or 42)	PCR-corrected results	
	Cumulative success or failure rate (Kaplan-Meier analysis)	Proportion (per-protocol analysis)
Adequate clinical and parasitological response at day X	Success	Success
Early treatment failure	Failure	Failure
Late clinical failure before day 7	Failure	Failure
Late clinical failure or late parasitological failure on or after day 7		
• falciparum recrudescence*	Failure	Failure
• falciparum reinfection*	Censored day of reinfection	Excluded from analysis
• other species mixed with falciparum recrudescence	Failure	Failure
• other species mixed with falciparum reinfection	Censored day of reinfection	Excluded from analysis
• other species infection	Censored day of infection	Excluded from analysis
• undetermined or missing PCR	Excluded from analysis	Excluded from analysis
Lost to follow-up	Censored last day of follow-up according to timetable	Excluded from analysis
Withdrawal and protocol violation	Censored last day of follow-up according to timetable before protocol violation or withdrawal	Excluded from analysis

* WHO. *Methods and techniques for clinical trials on antimalarial drug efficacy: genotyping to identify parasite populations*. Geneva, World Health Organization, 2008 (http://www.who.int/malaria/resistance).

Appendix 10. CONSENT AND ASSENT FORMS[3]

Efficacy and safety of artesunate + amodiaquine combined with a single low dose of primaquine (0.25 mg/kg) for the treatment of uncomplicated plasmodium infection in Zanzibar

Informed consent form for adults

This informed consent form is for adults 18 years of age and above who attend any of the 20 primary health care facilities in Zanzibar used as study sites in the above mentioned trial, and who have been invited to participate in the study to evaluate the efficacy of artesunate +amodiaquine for the treatment of uncomplicated falciparum malaria.

Name of principal investigator:	Mwinyi I. Msellem
Name of organization:	Zanzibar Malaria Elimination Programme
Name of sponsor:	The Global Funds against AIDS, Tuberculosis and Malaria
Name of proposal and version:	Efficacy and safety of artesunate + amodiaquine combined with a single low dose of primaquine (0.25 mg/kg) for the treatment of uncomplicated *Plasmodium falciparum* infection in Zanzibar

This informed consent form has two parts:

I. Information sheet (to share information about the study with you)

II. Certificate of consent (for signatures if you agree to take part)

You will be given a copy of the full informed consent form.

Part I. Information sheet

My name is..... I work for the Zanzibar Ministry of Health. We are presently doing a study in this primary health care facility on the treatment of malaria. Malaria is a dangerous disease; however, it can be treated with medicine. The purpose of this study is to confirm that the medicine presently recommended as first-line treatment for malaria in Zanzibar, is still effective for curing malaria infection on the Isles.

We have examined your blood and it was found that you have been infected with malaria parasites. Therefore, I am going to give you information about our ongoing study and invite you to participate in it. Before you decide whether to participate or not, you are free to talk to anyone you feel comfortable with about the study. There may be some words that you do not understand.

[3] http://www.who.int/rpc/research_ethics/en/

Please ask me to stop as we go through the information, and I will take time to explain. If you have questions later, you can ask me, the study doctor or the staff.

Your participation in this study is entirely voluntary. If you choose not to participate, all the service you may need from this clinic will continue as usual. Even if you agree to participate now but decide later to change your mind and withdraw your consent, the services you receive at the clinic will continue.

You will receive medicine once daily for 3 days to treat the malaria parasites you have in your blood. The medicine we are testing is a fixed dose combination of artesunate + amodiaquine combined with a single low doise of primaquine (0.25 mg/kg). This is the recommended first-line treatment for uncomplicated malaria infection by the Zanzibar Ministry of Health and Social Welfare. The Ministry through the Zanzibar Malaria Elimination Programme regularly conducts studies to make sure that the recommended malaria medicines are still working. The malaria medicines are known to be very effective, but you should know that some people experience minor side-effects from the medicine: weakness, headache, nausea, vomiting, abdominal pain, diarrhoea and itching may occur. Primaquine has in rare cases been associated with anemia. However, these symptoms are usually transient and of mild or moderate intensity.

If we find that the medicine is not able to cure the malaria infection that you are infected with, we will instead give you what is called 'rescue medicine'. The rescue medicine is called quinine and is given orally at a dose of 10 mg/kg body weight trice daily to complete a total of 21 doses in 7 days. You should also know that this medicine has some minor side-effects: such as tinnitus, muffled hearing, sometimes vertigo or dizziness. In case you develop signs of severe malaria infection we will provide in-patient care for you and treat you with quinine given directly into your blood using a drip.

In this study we will ask all women aged between 15-49 years about their menstrual history. If the last menstrual period is 4 weeks ago or more we will ask oral permission to conduct a urine test for pregnancy. Pregnant women will not be enrolled in this study since Artesunate + Amodiaquine Winthrop® is not recommended for treatment during the first trimester of the pregnancy.

All patients in this study will be asked to come back to the clinic for review on day 1, 2, 3, 7, 14, 21 and 28 after enrolment, but also any time if fever persists. At each visit in the study, a small amount of blood (equivalent to a few drops) will be taken from your finger. In total blood will be taken 8 times during the study, including today's visit. You may experience a bit of pain or fear when your finger is pricked. The pain normally disappears within 1 day. The blood will be dropped onto a glass slide and a small piece of paper. The blood sample collected on the glass slide will be examined with the use of a microscope to study if the malaria medicine is effective in curing the infection and in preventing malaria parasites from coming back into your blood. These examinations will be done directly at the clinic during each follow-up visit. The blood samples that will be collected on small pieces of paper will be used to study the malaria parasites in your blood. The examination of these blood samples will be done after the completion of the study in Sweden in collaboration with colleagues at the medical university in Stockholm namely Karolinska Institutet. Since these analyses will be done after the study is completed they will not be available to guide or evaluate the treatment. Except for the above described analyses no other tests will be done with your blood.

The study period for each patient in this study is 28 days. During that time, we would like you to come to the health facility, which in total will be 8 visits including today's visit. Each follow-up visit will last for about 1 hour. At each visit, you will be asked a few questions about your health condition, including if you have developed any new symptoms since last visit and if you have used any other medicines than the study medication. In addition you will also undergo a brief physical examination by study clinician at every visit.

As previously mentioned the medicine can have some unwanted or unexpected effects that we are not currently aware of; however, we will follow you closely and keep track of these effects, if they arise, and of any other problems. We will give you a telephone number to call if you notice anything out of the ordinary or if you have concerns or questions, please see below.. You can also come to this health facility at any time and ask to see (.....give name of nurse, doctor). If you experience side-effects, we may use some other medicine, free of charge, which will help to reduce the symptoms or reactions, or we may stop the medicine. If this is necessary we will discuss it together. You will always be consulted before we move to the next step.

Today, we took a few drops of blood for testing. The first dose of treatment will be given here at the facility once when you accept to participate.

On the

2nd visit: you will receive the 2nd dose of treatment plus a blood test.

3rd visit: you will receive the 3rd dose of treatment plus a blood test.

4th, 5th, 6th, 7th and 8th visit, you will have a blood test taken.

If you decide to participate in this study, any illnesses related to malaria or to the malaria treatment will be treated at no charge to you. Your participation will help us to understand if the medicine is still effective, and this may benefit the society and future generations. You will be reimbursed one thousand (1,000) Tanzania shillings for your travel expenses for every visit and at the end (day 28) of study.

We will not disclose your identity and your participation in the study with anyone. The information that we collect from this study will be kept confidential. Any information collected about you will have a number on it instead of your name. Only the study team members will know what your number is and we will lock that information up.

We will share the knowledge that we get from this study with you before it is made available to the public. Confidential information will not be shared. There will be small meetings in the community where members of the study team will inform about the results of the study, and these meetings will be announced in advance. Afterwards, we will publish the results and make them available so that other interested people may learn from our study.

This proposal has been reviewed and approved by Zanzibar Medical Research Ethical Committee (ZAMREC). The committee has carefully assessed the study in advance to make sure that study participants are protected from any harm. If you wish to find about more about the institutional review board, you may contact Dr. Jamala Taib, Chairperson of ZAMREC telephone No 077 743 24 65

***If you have any further questions or for any other reason would like to contact the responsible persons for the study you are most welcome to do so, please find our contact information below*:**

Part II. Certificate of consent

I _____have been invited to participate in a study of a medicine used to treat malaria.

I have read the above information, or it has been read to me. I have had the opportunity to ask questions, and any questions that I have asked have been answered to my satisfaction. I consent voluntarily to participate in this study.

Print name of participant: _____

Signature of participant: _____

Date: _____
(dd/mmm/yyyy)

Witness' signature: (A witness' signature and the patient's thumbprint are required only if the patient is illiterate. In this case, a literate witness must sign. If possible, this person should be selected by the participant and should have no connection with the study team.)

I have witnessed the accurate reading of the consent form to the potential participant, who has had the opportunity to ask questions. I confirm that the participant has given consent freely.

Print name of witness: _____ and thumbprint of participant:

Signature of witness: _____ ☐

Date: _____
(dd/mmm/yyyy)

Investigator's signature:

I have accurately read or witnessed the accurate reading of the consent form to the potential participant, who has had the opportunity to ask questions. I confirm that the participant has given consent freely.

Print name of investigator: _____

Signature of investigator: _____

Date: _____
(dd/mmm/yyyy)

A copy of this informed consent form has been provided to the participant. _____ (initials of the principal investigator or assistant).

Informed consent form for children or minors

This informed consent form is for parents or guardians of children aged up to 18 years who attend any of the 20 primary health care facilities in Zanzibar used as study sites in the above mentioned trial, and who have been invited to participate in the study to evaluate the efficacy of artesunate +amodiaquine for the treatment of uncomplicated falciparum malaria.

Name of principal investigator:	Mwinyi I. Msellem
Name of organization:	Zanzibar Malaria Elimination Programme
Name of sponsor:	GFATM: QNB-M-MOH, Karolinska Instituttet
Name of proposal and version:	Efficacy and safety of artesunate + amodiaquine combined with a single low dose of primaquine (0.25 mg/kg) for the treatment of uncomplicated *Plasmodium falciparum* infection in Zanzibar

This informed consent form has two parts:

I. Information sheet (to share information about the study with you)

II. Certificate of consent (for signatures if you agree to take part)

You will be given a copy of the full informed consent form.

Part I: Information sheet

My name ……., and I work for the Ministry of Health. We are doing a study on the treatment of malaria. Malaria is a dangerous disease; however, it can be treated with medicine. The purpose of this study is to confirm that the medicine presently recommended as first-line treatment for malaria in Zanzibar is still effective for curing malaria infection on the Isles.

We are inviting all adults and children 3 months and above living in this area to take part in this study.

I am going to give you information and invite you to consent to have your child participate in this study. Before you decide whether you want your child to participate, you can talk to anyone you feel comfortable with. There may be some words that you do not understand. Please ask me to stop as we go through the information, and I will take time to explain. If you have questions later, you can ask me, the study doctor or the staff.

Your decision to have your child participate in this study is entirely voluntary. If you choose not to consent, all the services your child receives at this clinic will continue as usual. Even if you agree now but decide to change your mind and withdraw later, the services your child receives at the clinic will continue.

Your child will receive medicine once a day over 3 days. The malaria medicines are the recommended first-line treatment for uncomplicated malaria infection by the Zanzibar Ministry of Health and Social Welfare. As the parasites that cause malaria can become resistant to the medicine, the Ministry regularly does studies to make sure the medicine is still working. The medicines are known to be very effective, but you should know that it has some minor side-

effects: weakness, headache, nausea, vomiting, abdominal pain, diarrhoea and itching may occur. Primaquine has in rare cases been associated with anemia. However, these symptoms are usually transient and of mild or moderate intensity.

If we find that the medicine is not able to cure the malaria infection that you are infected with, we will instead give you what is called 'rescue medicine'. The rescue medicine is called quinine and is given orally at a dose of 10mg/kg body weight trice daily to complete a total of 21 doses in 7 days. You should also know that this medicine has some minor side-effects: such as tinnitus, muffled hearing, sometimes vertigo or dizziness. In case you develop signs of severe malaria infection we will provide in-patient care for you and treat you with quinine given directly into your blood using a drip.

A small amount of urine will be taken once. It will be tested for the presence of other medicines used to treat malaria in your child's body. During the follow-up, a small amount of blood will be taken 7/9 times from your child's finger or heel. Your child may experience a bit of pain or fear when the finger is pricked. The pain should disappear within 1 day. The blood will be dropped onto a slide or a small piece of paper. The blood samples will be used to study the malaria in your child's blood. The examination of the blood samples will be done after the study and it will not affect the success of the treatment. Nothing else will be done with the blood.

The study period for each patient in this study is 28 days. During that time, your child will have to come to the health facility for 1 hour, which in total will be 8 visits including today's visit. At each visit, you will be asked a few questions about your child's health condition, including if he/she have developed any new symptoms since last visit and if he/she was any other medicines than the study medication. In addition your child will also undergo a brief physical examination by study clinician at every visit. You may stay with your child during each of the visits and during the procedures.

As previously mentioned the medicine can have some unwanted or unexpected effects that we are not currently aware of; however, we will follow your child closely and keep track of these effects, if they arise, and of any other problems. We will give you a telephone number to call if you notice anything out of the ordinary or if you have concerns or questions, please see below. You can also come to this health facility at any time and ask to see (give name of nurse, doctor). If your child experience side-effects, we may use some other medicine, free of charge, which will help to reduce the symptoms or reactions, or we may stop the medicine. If this is necessary we will discuss it together. You will always be consulted before we move to the next step.

Today, we will take few drops of blood for testing. Your child will receive the first dose of treatment.

On the

- 2nd visit, your child will receive the 2nd dose of treatment.
- 3rd visit, your child will receive the 3rd dose of treatment plus a blood test.
- 4th, 5th, 6th, 7th, 8th visits, your child will have a blood test.

If you decide that your child will participate in this study, any illnesses related to malaria or to the malaria treatment will be treated at no charge to you. Your child's participation will help us to make sure the medicine is still working, and this will benefit society and future generations. You will be reimbursed two thousand and five hundred (2,500) Tanzania shillings for your travel expenses for every visit and at the end (day 28) of the study.

We will not share the identity of participants in the study with anyone. The information that we collect from this study will be kept confidential. Any information collected about your child will have a number on it instead of your child's name. Only the study team members will know what the number is, and we will lock that information up.

We will share the knowledge that we get from this study with you before it is made available to the public. Confidential information will not be shared. There will be small meetings in the community where members of the study team will inform about the results of the study, and these meetings will be announced in advance afterwards, we will publish the results and make them available so that other interested people may learn from our study.

This proposal has been reviewed and approved by Zanzibar Medical Research Ethical Committee (ZAMREC). The committee has carefully assessed the study in advance to make sure that study participants are protected from any harm. If you wish to find about more about the institutional review board, you may contact Dr. Jamala Taib, Chairperson of ZAMREC telephone No 077 743 24 65

If you have any further questions or for any other reason would like to contact the responsible persons for the study you are most welcome to do so, please find our contact information below:

Part II: Certificate of consent

I have been invited to have my child participate in a study of a medicine used to treat malaria.

I have read the above information, or it has been read to me. I have had the opportunity to ask questions, and any questions that I have asked have been answered to my satisfaction. I consent voluntarily to my child's participation in this study.

Print name of participant: _____

Print name of parent or guardian: _____

Signature of parent or guardian: _____

Date: _____
(dd/mmm/yyyy)

Witness' signature: (A witness' signature and the thumbprint of the participant's parent or guardian are required only if the parent or guardian is illiterate. In this case, a literate witness must sign. If possible, this person should be selected by the participant's parent or guardian and should have no connection with the study team.)

I have witnessed the accurate reading of the consent form to the potential participant's parent or guardian, who has had the opportunity to ask questions. I confirm that the participant's parent or guardian has given consent freely.

Print name of witness: _____ and thumbprint of parent/guardian:

Signature of witness: _____

Date: _____
(dd/mmm/yyyy)

Investigator's signature:

I have accurately read or witnessed the accurate reading of the consent form to the potential participant's parent or guardian, who has had the opportunity to ask questions. I confirm that the participant's parent or guardian has given consent freely.

Print name of investigator: _____

Signature of investigator: _____

Date: _____
(dd/mmm/yyyy)

A copy of this informed consent form has been provided to participant's parent or guardian. _____ (initials of the principal investigator/assistant).

Informed assent forms will _____ or will not _____ be completed.

Example of an informed assent form

This informed assent form is for children aged 12–17 years who attend any of the 20 primary health care facilities in Zanzibar used as study sites in the above mentioned trial, and who have been invited to participate in the study to evaluate the efficacy of artesunate +amodiaquine for the treatment of uncomplicated falciparum malaria.

Name of principal investigator:	Mwinyi I. Msellem
Name of organization:	Zanzibar Malaria Elimination Programme
Name of sponsor:	GFATM: QNB-M-MOH, Karolinska Instituttet
Name of proposal and version:	Efficacy and safety of artesunate + amodiaquine combined with a single low dose of primaquine (0.25 mg/kg) for the treatment of uncomplicated *Plasmodium falciparum* infection in Zanzibar

This informed assent form has two parts:

I. Information sheet (to share information about the study with you)

II. Certificate of assent (for signatures if you agree to take part)

You will be given a copy of the full informed assent form.

Part I. Information sheet

My name is ....., and I work for the Zanzibar Malaria Elimination Programme. We are presently doing a study in this primary health care facility on the treatment of malaria. Malaria is a dangerous disease; however, it can be treated with medicine. The purpose of this study is to confirm that the medicine presently recommended as first-line treatment for malaria in Zanzibar is still effective for curing malaria infection on the Isles.

We are inviting all adults and children living in this area to take part in this study.

I am going to give you information and invite you to participate in this study. You can choose whether you want to participate. We have discussed this study with your parent(s) or guardian, and they know that we are also asking you for your agreement. If you decide to participate in the study, your parent(s) or guardian also has to agree. If you do not wish to take part in the study, you do not have to, even if your parents have agreed. It is your choice. If you decide not to participate, nothing will change; this is still your clinic. Even if you say 'Yes' now you can change your mind later and it will still be okay. You may discuss anything in this form with your parents or friends or anyone else you feel comfortable talking to. There may be some words you do not understand or things that you want me to explain more because you are interested or concerned. Please ask me to stop at any time, and I will take time to explain.

Interviewer: I have checked with the child, and he or she understands that participation is voluntary. _____ (initials)

You will receive medicine once a day over 3 days. The medicine is a fixed dose combination of artesunate + amodiaquine combined with a single low dose of primaquine (0.25 mg/kg). This is the recommended first-line treatment for uncomplicated malaria infection by the Zanzibar Ministry of Health and Social Welfare. The Ministry through the Zanzibar Malaria Elimination

Programme regularly conducts studies to make sure that the recommended malaria medicines are still working. The malaria medicines are known to be very effective, but you should know that some people experience minor side-effects from the medicine: weakness, headache, nausea, vomiting, abdominal pain, diarrhoea and itching may occur. Primaquine has in rare cases been associated with anemia. However, these symptoms are usually transient and of mild or moderate intensity.

All patients in this study will be asked to come back to the clinic for review on day 1, 2, 3, 7, 14, 21 and 28 after enrolment, but also any time if fever persists. At each visit in the study, a small amount of blood will be taken from your finger. You may experience a bit of pain or fear when your finger is pricked. The pain normally disappears within 1 day. The blood sample collected on the slide will be examined with the use of a microscope to study if the malaria medicine is effective in curing the infection and in preventing malaria parasites from coming back into your blood. The blood samples that will be collected on small pieces of paper will be used to study the malaria parasites in your blood. The examination of these blood samples will be done after the completion of the study in Sweden in collaboration with colleagues at the medical university in Stockholm namely Karolinska Institutet. Since these analyses will be done after the study is completed they will not be available to guide or evaluate the treatment. Except for the above described analyses no other tests will be done with your blood.

The study period for each patient in this study is 28 days. During that time, we would like you to come to the health facility, which in total will be 8 visits including today's visit. Each follow-up visit will last for about 1 hour. At each visit, you will be asked a few questions about your health condition, including if you have developed any new symptoms since last visit and if you have used any other medicines than the study medication. In addition you will also undergo a brief physical examination by study clinician at every visit.

Interviewer: I have checked with the child, and he or she understands the procedures. _____ (initials)

As previously mentioned the medicine can have some unwanted or unexpected effects that we are not currently aware of; however, we will follow you closely and keep track of these effects, if they arise, or of any other problems. If anything unusual happens to you, we need to know, and you should feel free to call us any time with your concerns or questions. If you get sick or have concerns or questions between scheduled visits to clinic, you should let me or the staff nurse know. You do not have to wait for a scheduled visit. We have also given your parents information about what to do if you are hurt or get sick during the study.

Interviewer: I have checked with the child, and he or she understands the risks and discomforts. _____ (initials)

If you decide to participate in this study, any illnesses related to malaria or to the malaria treatment will be treated at no charge to you. Your participation will help us to make sure the medicine is still working, and this will benefit society and future generations.

Interviewer: I have checked with the child, and he or she understands the benefits. _____ (initials)

Because you live quite far from the clinic, we will give your parents or guardian enough money to pay for the trip here and back.

We will not tell other people that you are participating in this study, and we will not share information about you with anyone who does not work in the study. Information about you that will be collected from the study will be put away, and no one but the study team will be able to see it. Any information about you will have a number on it instead of your name. Only the study team will know what your number is, and we will lock that information up.

When we have finished the research, I will sit down with you and your parent or guardian and tell you about what we learnt. Afterwards, we will be telling more people, scientists and others, about the study and what we found. We will do this by writing and sharing reports and data and by going to meetings with people who are interested in the work we do.

You can ask me questions now or later. You can ask the nurse questions. I have written a number and address where you can reach us or, if you are nearby; you can come and see us. If you want to talk to someone else whom you know, like your teacher, doctor or auntie, that is okay too.

Part II: Certificate of assent

I have been invited to participate in a study of the efficacy of an antimalarial medicine. I have read this information (or had the information read to me), and I understand it. I have had my questions answered and know that I can ask questions later if I have them. I agree to take part in the study. _____ (initials) or I do not wish to take part in the study and I have not signed the assent below. _____ (initials)

Child's signature (only if the child assents):

Print name of child: _____

Signature of child: _____

Date: _____
(dd/mmm/yyyy)

Witness' signature: (A witness' signature and the child's thumbprint are required only if the child is illiterate. In this case, a literate witness must sign. If possible, this person should be selected by the participant and should have no connection with the study team.)

I have witnessed the accurate reading of the assent form to the potential participant, who has had the opportunity to ask questions. I confirm that the participant has given consent freely.

Print name of witness: _____ and thumbprint of the child or minor:

Signature of witness: _____ ☐

Date: _____
(dd/mmm/yyyy)

Investigator's signature:

I have accurately read or witnessed the accurate reading of the assent form to the potential participant, who has had the opportunity to ask questions. I confirm that the participant has given consent freely.

Print name of investigator: _____

Signature of investigator: _____

Date: _____
(dd/mmm/yyyy)

A copy of this informed assent form has been provided to the participant. _____ (initials of the principal investigator or assistant).

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