A multi-centre, open-label randomised trial to assess the efficacy, safety and tolerability of the Triple ACT artemether-lumefantrine+amodiaquine (AL+AQ) compared to the ACT artemether-lumefantrine (AL) in uncomplicated falciparum malaria in Cambodia and Vietnam

A study by the Triple Artemisinin Combination Treatments (TACTs) Collaboration

Short title: Study to compare the Triple ACT AL+AQ with the ACT AL in Cambodia and Vietnam

ACRONYM: TACT-Cambodia-Vietnam

Protocol no.: MAL 17008

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Investigator Agreement and Conflict of Interest

"I have read this protocol and:

- agree to abide by all provisions set forth therein.
- agree to comply with the principles of the International Conference on Harmonisation Tripartite Guideline on Good Clinical Practice.
- and declare no conflict of interest, according to the current version of the Declaration of Helsinki"

Prof. Arjen M. Dondorp

Principal Investigator Principal Investigator's Signature Date :

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1. LIST OF ABBREVIATIONS

ACPR	Adequate clinical and parasitological response
ACT	Artemisinin-based combination therapy
AE	Adverse event
A/L	Artemether-lumefantrine
AQ	Amodiaquine
AUC	Area under the (plasma concentration-time) curve
CRF	Case record form
CTSG	Clinical Trials Support Group (MORU)
CYP3A4	Cytochrome P450 3A4
DHA	Dihydroartemisinin
DNA	Deoxyribonucleic Acid
DSMB	Data and Safety Monitoring Board
EDC	Electronic data capture
EDTA	Ethylene-diamine-tetra-acetic acid
G6PD	Glucose-6-phosphate dehydrogenase
GCP	Good Clinical Practice
Hb	Haemoglobin
Hct	Haematocrit
IM	Intramuscular
IV	Intravenous
MDR1	Multi-Drug Resistance Gene 1
MQ	Mefloquine
MORU	Mahidol-Oxford Research Unit
NMCP	National Malaria Control Programme
PCR	Polymerase Chain Reaction
PCT	Parasite Clearance Time
PD	Pharmacodynamic
PK	Pharmacokinetic
QA	Quality Assurance
QC	Quality Control
RNA	Ribonucleic acid
SAE	Serious Adverse Event
SNP	Single-nucleotide polymorphism
SOP	Standard Operating Procedure
TACT	Triple Artemisinin-based Combination Therapy
TRAC	Tracking Resistance to Artemisinin Collaboration
WHO	World Health Organisation
WWARN	Worldwide Antimalarial Resistance Network

2. SYNOPSIS

Study Title	**A multi-centre, open-label randomised trial to assess the efficacy, safety and tolerability of the Triple ACT artemether-lumefantrine+amodiaquine (AL+AQ) compared to the ACT artemether-lumefantrine (AL) in uncomplicated falciparum malaria in Cambodia and Vietnam**
ACRONYM	TACT-Cambodia-Vietnam
Trial Design	An open-label randomised trial comparing the Triple ACT (TACT) artemether-lumefantrine+amodiaquine with artemether-lumefantrine ACT), evaluating efficacy, safety, tolerability and artemisinin and partner drug resistance in four sites.
Trial Participants	Patients with acute uncomplicated *P. falciparum* malaria
Sample size	2 sites in Cambodia each recruiting 200 subjects 2 sites in Vietnam that will recruit a cumulative 200 subjects through competitive-enrollment Estimated total sample size 600 patients.
Inclusion Criteria	• Male or female, aged from 2 years to 65 years old • Acute uncomplicated *P. falciparum* malaria, confirmed by positive blood smear with asexual forms of *P. falciparum* (or mixed with non-falciparum species) • Asexual *P. falciparum* parasitaemia: 16 to 200,000/uL, determined on a thin or thick blood film • Fever defined as $\geq$ 37.5°C tympanic temperature or a history of fever within the last 24 hours • Written informed consent (by parent/guardian in case of children) • Willingness and ability of the patients or parents/guardians to comply with the study protocol for the duration of the study
Exclusion Criteria	• Signs of severe/complicated malaria (see chapter 5) • Haematocrit < 25% or Hb < 8 g/dL at screening • Acute illness other than malaria requiring treatment • For females: pregnancy, breast feeding • Patients who have received artemisinin or a derivative or an artemisinin-containing combination therapy (ACT) within the previous 7 days • History of allergy or known contraindication to artemisinins, lumefantrine or amodiaquine • Previous splenectomy • QTc-interval > 450 milliseconds at moment of presentation • Documented or claimed history of cardiac conduction problems • Previous participation in the current study or another study in the previous 3 months

Planned Trial Period	30 months (October 2017 – March 2020)
Primary Objective	To compare the efficacy of the TACT artemether-lumefantrine+amodiaquine versus the ACT artemether-lumefantrine as defined by the 42-day PCR corrected adequate clinical and parasitological response (ACPR).
Secondary Objectives	• To compare the efficacy of the TACT artemether-lumefantrine+amodiaquine versus ACT artemether-lumefantrine as defined by the 42-day PCR ACPR according to site/geographical region. • To assess and compare *P. falciparum* parasite clearance rates of the standard ACT and study TACT • To assess and compare fever clearance rates of the standard ACT and study TACT • To compare the safety and tolerability of TACT versus standard ACT • To compare changes in the electrocardiogram (such as prolongation of the QTc-interval) in patients treated with TACT versus ACT • To assess the spread of genetic markers of artemisinin (such as *Kelch13* mutations) and partner drug resistance and identify additional genetic determinants of drug resistance. • To assess and increase the representability/accuracy of parasite genome sequencing from dry blood spots for the genome sequencing results from leukocyte depleted blood samples • To identify differences at the transcriptome level in artemisinin and partner drug sensitive and resistant *P. falciparum* in order to increase the understanding of mechanisms of resistance • To compare clearance dynamics estimated with quantitative PCR measurements of parasite loads versus microscopy • To measure and compare the incidence and duration of gametocyte carriage in patients with antimalarial sensitive and resistant malaria before and after treatment with TACT and ACT • To develop DNA and RNA measurement methods for quantification of male and female gametocytes • To compare *ex vivo* susceptibility profiles of *P. falciparum* isolates across geographic regions • To assess pharmacokinetic and pharmacodynamic interactions between AL and AQ • To obtain additional safety data (in particular incidence and rate of haemolysis) on the deployment of single low dose primaquine, stratified according to G6PD status • To obtain additional data on the effect of the host genotype on the pharmacokinetics and pharmacodynamics of antimalarials. • To compare clearance dynamics estimated with plasma HRP2 levels of parasite loads versus microscopy

	• To obtain data on the place of residence, work, recent travel history and mobile phone usage in order to improve the understanding of locations of malaria transmission and possible routes spread of malaria and artemisinin resistance. • To obtain data and GPS mapping on a select group of participants and their peers relating to their understanding of the behaviours and risk factors associated with malaria infection in order to improve understanding of local malaria transmission
Primary endpoint	42-day PCR corrected efficacy defined as adequate clinical and parasitological response (ACPR). WHO definition: absence of parasitaemia at day 42 irrespective of axillary temperature and without previously meeting any of the WHO criteria for early or late treatment failure, or late parasitological failure.
Secondary endpoints	• 42-day PCR corrected efficacy defined as adequate clinical and parasitological response (ACPR) according to site/geographic region. • Parasite clearance half-life assessed by microscopy as primary parameter to determine parasite clearance • Additional parameters of parasite clearance dynamics • Fever clearance time (i.e. the time taken for the tympanic temperature to fall below 37.5˚C and remain there for at least 24 hours) • Incidence of adverse events and serious adverse events by study arms within the first 42 days. • Incidence of adverse events concerning markers of hepatic or renal toxicity such as bilirubin, ALT, AST, Alkaline Phosphatase and creatinine • Proportion of patients that reports completing a full course of observed TACT or ACT without withdrawal of consent or exclusion from study because of drug related serious adverse event • Incidence of prolongation of the QTc-interval above 500 ms or > 60 ms above baseline values. • Prolongation of QTc-interval compared to baseline at timepoint H4, H24, H28, H48, H52, H60 and H64 and between these timepoints • Change in haematocrit on day 1 to 7, 14, 21, 28, 35 and 42 according to geographical location and study arm, stratified for G6PD status • Correlation between the host genotype and the pharmacokinetics and pharmacodynamics of antimalarials • Prevalence of *Kelch13* mutations of known functional significance • Prevalence/incidence of other genetic markers of antimalarial drug resistance such as MDR1 copy number and MDR1 mutations • Genome wide association with in vivo/in vitro sensitivity parasite phenotype • Correlation between SNPs measured in dry blood spots and whole genome sequencing in leukocyte depleted blood samples • Transcriptomic patterns at t=0 and t=6h after start of treatment comparing sensitive and resistant parasites

	• Correlation between qPCR based versus microscopy based assessments of parasite clearance dynamics up to day 14 • Proportion of patients with gametocytemia before, during and after treatment with TACT or ACT, assessed at admission, up to day 14, stratified by presence of gametocytes at enrolment • Levels of RNA transcription coding for male or female specific gametocytes at admission up to day 14, stratified by the presence of gametocytes at enrolment • In vitro sensitivity of *P. falciparum* to artemisinins and partner drugs according to study sites and genotype • Pharmacokinetic profiles and interactions (Cmax and AUC) of artemisinin-derivatives and partner drugs in 20 ACT treated and 20 TACT treated patients of both study arms in Vietnam • Day 7 drug levels of partner drugs in association with treatment efficacy and treatment arm • Correlation between HRP2 based versus microscopy based assessments of parasite clearance dynamics • Data on the place of residence, work, recent travel history and mobile phone use. • Data and GPS mapping for a select group of participants and their peers in relation to behaviours and risk factors associated with malaria infection.
Drugs See Appendix 2 for dosing regimens	Artemether-lumefantrine for 3 days. VERSUS Artemether-lumefantrine for 3 days. plus: Amodiaquine for 3 days. **Primaquine** According to the WHO guideline, all patients except children under 10 kilograms will also be treated with a single dose of primaquine according to the local requirement and WHO treatments schedule as a gametocytocidal treatment.

3. BACKGROUND AND RATIONALE

Artemisinin combination therapies (ACTs) have been a major driving force behind substantial reductions in global malaria morbidity and mortality over recent years. However, further gains are threatened by the recent emergence of artemisinin resistance in Southeast Asia, a region which has been the epicentre for the evolution and spread of resistance to every important class of antimalarials.

3.1 Background

The Tracking Resistance to Artemisinin Collaboration (TRAC) study was focused on mapping the spread of artemisinin resistance by conducting a multi-centre clinical trial, coordinated by the Mahidol Oxford Research Unit (MORU) at sites in Asia (N=13) and Africa (N=3). This study measured individual parasite clearance rates, identifying locations where artemisinin resistance was present, as evidenced by slow parasite clearance (clearance half-life >5 hours) in a high proportion of cases[1]. This reduction in artemisinin sensitivity has left partner drugs within ACTs exposed to a much larger biomass of parasites and unsurprisingly partner drug resistance has followed [2].

Consequently, treatment failures after artemisinin combination therapies are becoming more widespread in Southeast Asia. Failures rates of up to >60% for dihydroartemisinin-piperaquine have been documented in Cambodia, Thailand and Vietnam[3-5](TRACII unpublished data).

Failure of first line ACTs will damage current control and elimination efforts and accelerate the emergence and spread of resistance. A major concern is that artemisinin and partner drug resistance may spread across a wider geographic area, as chloroquine resistance did in the 1960s and 1970s, moving from Southeast Asia to the Indian subcontinent and Africa. Furthermore, the resistance itself could ‘deepen’ by extending beyond the ring stage of the asexual cycle (to which the resistance phenotype is currently limited).

There is an urgent need to evaluate alternative treatments where standard courses of ACTs are failing, and to develop combinations of existing drugs which will not fall rapidly to resistance and can be deployed immediately. New drugs are at least five years away. The TRACII study has examined the safety, tolerability and efficacy of different TACT combinations such as DHA-piperaquine+mefloquine and artemether-lumefantrine+amodiaquine. The preliminary results of this study show full efficacy of the TACTs DHA-piperaquine+mefloquine in Cambodia, Thailand and Vietnam, even though the efficacy of the ACT DHA-piperaquine is poor. In other sites, the TACT artemether-lumefantrine+amodiaquine is as effective as the standard ACT artemether-lumefantrine. Furthermore, the preliminary results indicate a good safety and tolerability profile of both the TACTs.

Artemisinin and piperaquine resistant parasites are now well established in Cambodia, Vietnam and Thailand and most likely all originate from the same region on the Thai-Cambodian border [6]. The first line treatment for the uncomplicated *P. falciparum* malaria has recently been changed to artesunate-mefloquine. Hopefully this change of partner drug will contribute to a diminishing of the resistance to piperaquine. However, it is possible that mefloquine resistance will develop on top of the existing artemisinin and piperaquine resistance, which would lead to the development and most likely spread of truly multi-resistant *P. falciparum* in Southeast Asia.

Therefore, although the efficacy of the TACT DHA-piperaquine+mefloquine in Vietnam and Cambodia so far has been excellent, there is the potential of the occurrence of parasites that are resistant to artemisinin, mefloquine and piperaquine, which could lead to treatment failures after treatment with the TACT DHA-piperaquine+mefloquine.

We propose to trial the efficacy of artemether-lumefantrine, an ACT that has not been used in Vietnam and Cambodia yet. Furthermore, we propose to assess the efficacy, safety and tolerability of the TACT artemether-lumefantrine+amodiaquine. Both this ACT and the TACT could potentially play a role in the treatment of multidrug resistant malaria in this region if found to be effective, safe and tolerable.

3.2 Study Rationale

The principle that multiple drugs with independent mechanisms of action prevent the emergence of drug resistance is proven in a range of human diseases. In HIV and tuberculosis for example, the occurrence and spread of drug resistance can be prevented by use of a combination of three or more antiretroviral or antimycobacterial therapies respectively, but until now this was not thought necessary in malaria. In malaria, there is a fortuitous inverse correlation between susceptibility to amodiaquine and lumefantrine which will be exploited in the TACTs.

The global spread of chloroquine (CQ) resistance was also associated with cross-resistance to amodiaquine. However, amodiaquine continued to be used in some areas of Southeast Asia, such as Myanmar, owing to its low cost. Relatively few trials of the ACT artesunate-amodiaquine have been conducted. The largest showed borderline efficacy in Myanmar with recrudescence rates of about 10%. Studies in Vietnam, as well as India and Africa, have reported cure rates above 95%[7].

Transfection data provide the most reliable means of understanding the molecular basis of amodiaquine resistance. Fidock and colleagues introduced two major patterns of mutations (Dd2 and 7G8) into wild-type Pfcrt-encoding parasites and were able to confirm that these mutations were causative factors in CQ resistance.[8]. Notably there was a clear inverse correlation between the IC50 values for CQ and mefloquine (MQ) as well as for amodiaquine and MQ. In other words, resistance to chloroquine/amodiaquine caused by specific Pfcrt-mutations was associated with mefloquine hypersensitivity.

A second locus, Pfmdr1, also appears to be involved in amodiaquine resistance, with the N86Y mutation associated with resistance in vitro and increased clinical failures with amodiaquine monotherapy[9]. A paper by Venkatesan et al. suggests that recurrent infection after artemether-lumefantrine and artesunate-amodiaquine is associated with specific mutations in the Pfmdr1 gene, but that the two partner drugs select alternative and drug resistance opposing alleles[10].

In theory, the concurrent use of lumefantrine combined with amodiaquine exploits these opposing selection pressure effects. Potentially, this will prevent or delay emergence of high-level resistance to both drugs at the same time. This would be expected to prolong or maintain the overall efficacy of both partner drug components [11].

3.3 Proposed activities

Artemether-lumefantrine+amodiaquine TACT study

The study of artemether-lumefantrine or artemether-lumefantrine combined with amodiaquine will be a two-arm randomised open label comparative study. Based on the relatively safe and limited side effect profiles of both drugs, no life-threatening interactions between lumefantrine and amodiaquine are expected. In fact, the preliminary data of the TRACII trial indicates that the TACT artemether-lumefantrine+amodiaquine has a comparable safety profile to the use of artemether-lumefantrine alone. Currently, recrudescence rates after treatment with artemether-lumefantrine are low in Laos, India, Myanmar and Bangladesh.

If it transpires that the efficacy of artemether-lumefantrine in Cambodia and Vietnam is high, it may be too early to see improvement in efficacy after the addition of amodiaquine. However, it is important to rule out a negative effect of amodiaquine on the overall efficacy, as well as obtaining further knowledge of the safety and tolerability of the artemether-lumefantrine+amodiaquine.

Once safety and tolerability are verified, studies can be conducted to assess the long-term efficacy of this novel TACT.

Activities/outcomes

The main activity proposed is a series of detailed *in vivo* clinical, parasitological and pharmacological assessments in 600 subjects across 2 sites in Cambodian and 2 sites in Vietnam. In Cambodia 400 subjects will be recruited and in Vietnam, the 2 sites will recruit a cumulative 200 subjects (the sample size for this country) through competitive-recruitment due to the expected low recruitment

rates at each site. The subjects will be randomized between the ACT artemether-lumefantrine and the TACT artemether-lumefantrine+amodiaquine.

The sites have been chosen based on current information on resistance patterns and incidence of malaria, presence of established clinical research programme and feasibility to perform the proposed research activities.

Parasite clearance rates will be assessed by repeated assessments of the parasite counts after the start of the antimalarial treatments. Efficacy, safety and tolerability of ACTs and TACTs will be assessed through weekly follow up visits where vital signs, symptom questionnaires, physical examinations, blood smears, biochemistry assays and full blood counts will be performed.

E*x vivo* assessments of parasite susceptibility to artemisinins and partner drugs will be measured and compared to historical data, clinical phenotype and other sites in an effort to identify artemisinin and partner drug resistance.

The in vivo and ex vivo data on artemisinin and partner drug sensitivity will be used to identify new genetic or transcriptomic markers/patterns of artemisinin or partner drug resistance. Also efforts will be made to identify a 'genetic backbone', which is thought to be a specific characteristic of South East Asian malaria parasites leading to an increased tendency to develop artemisinin or partner drug resistance[12].

This study will obtain data on the effect of antimalarials on the QTc-intervals. In addition, we will assess the effects of antimalarials on factors such as post-treatment haematocrit and haemoglobin levels. Extensive pharmacokinetic analysis will allow for an assessment of drug-drug interactions.

The pharmacogenetics of antimalarial agents are poorly known despite the fact that application of pharmacogenetics might be critical in optimizing treatment of malaria in individuals but also populations at large. Blood samples (dried blood blots) for human genotyping will be obtained and stored from all subjects recruited with subject's consent. Data that could be indicative of (abnormal) pharmacokinetics (i.e. low/high drug blood levels) resulting in altered pharmacodynamics such as haemolysis and/or prolongation of the QTc-interval will be obtained. For instance, abnormal absorption, distribution and/or clearance of the study drug (hence altered pharmacokinetics) might lead to increased drug blood levels and a measured prolongation of the QTc-interval. Genotyping will be performed on the samples of subjects with suspected abnormal pharmacokinetics or pharmacodynamics (for instance in case of unexpected adverse events such as substantial prolongation of the QTc-interval). Targeted genotyping could provide insight in the genetic origins of abnormal pharmacokinetics and/or pharmacodynamics in patients participating in the study. In addition, samples from all individuals will be used for whole genome association studies of the clinical phenotype and the subjects' genotype. For example, changes in the QTc-interval of the individual subjects could be correlated with the genotype of all subjects in the form of a whole genome association study in order to identify genes that are potentially involved in an abnormal prolongation of the QTc-interval after administration of the antimalarials. Alternative examples of whole genome association studies are the correlation of blood drug levels with the host genotype.

Plasma HRP2 levels (a marker of parasite biomass) could potentially serve for the estimation of parasitaemia dynamics before and after treatment. Plasma that is left over after antimalarial drug level measurements will be used for HRP2 measurements and subsequent modelling of parasite dynamics.

All the organisations in this collaboration will work closely with local counterparts including the National Malaria Control Programmes (NMCPs), non-governmental and other relevant organisations. Training is an integral part of this collaborative working relationship, and the building of local research capacity is an essential component of all research plans.

All research-related activities, from study design, planning, implementation through to analysis and writing of reports will be performed jointly with local counterparts. Both on-the-job training and formal training will be provided when needed, in particular for Good Clinical Practice (GCP) skills.

The close interaction between WHO and its regional offices will ensure that new knowledge is disseminated efficiently and effectively throughout the region. The Worldwide Antimalarial Resistance Network website (www.wwarn.org) will be also used as a medium to disseminate information.

The trial will be overseen by a Data and Safety Monitoring Board (DSMB) and will be monitored regularly. (See section 11.2)

4. OBJECTIVES

4.1. Primary Objective

To compare the efficacy of the TACT artemether-lumefantrine+amodiaquine versus the ACT artemether-lumefantrine as defined by the 42-day PCR corrected adequate clinical and parasitological response (ACPR).

4.2. Secondary Objectives

- To compare the efficacy of the TACT artemether-lumefantrine+amodiaquine versus ACT artemether-lumefantrine as defined by the 42-day PCR ACPR according to site/geographical region.
- To assess and compare *P. falciparum* parasite clearance rates of the standard ACT and study TACT
- To assess and compare fever clearance rates of the standard ACT and study TACT
- To compare the safety and tolerability of TACT versus standard ACT
- To compare changes in the electrocardiogram (such as prolongation of the QTc-interval) in patients treated with TACT versus ACT
- To assess the spread of genetic markers of artemisinin (such as *Kelch13* mutations) and partner drug resistance and identify additional genetic determinants of drug resistance.
- To assess and increase the representability/accuracy of parasite genome sequencing from dry blood spots for the genome sequencing results from leukocyte depleted blood samples
- To identify differences at the transcriptome level in artemisinin and partner drug sensitive and resistant *P. falciparum* in order to increase the understanding of mechanisms of resistance
- To compare clearance dynamics estimated with quantitative PCR measurements of parasite loads versus microscopy
- To measure and compare the incidence and duration of gametocyte carriage in patients with antimalarial sensitive and resistant malaria before and after treatment with TACT and ACT
- To develop DNA and RNA measurement methods for quantification of male and female gametocytes
- To compare *ex vivo* susceptibility profiles of *P. falciparum* isolates across geographic regions
- To assess pharmacokinetic and pharmacodynamic interactions between AL and AQ
- To obtain additional safety data (in particular incidence and rate of haemolysis) on the deployment of single low dose primaquine, stratified according to G6PD status
- To obtain additional data on the effect of the host genotype on the pharmacokinetics and pharmacodynamics of antimalarials.

- To compare clearance dynamics estimated with plasma HRP2 levels of parasite loads versus microscopy
- To obtain data on the place of residence, work, recent travel history and mobile phone usage in order to improve the understanding of locations of malaria transmission and possible routes spread of malaria and artemisinin resistance.
- To obtain data and GPS mapping on a select group of participants and their peers relating to their understanding of the behaviours and risk factors associated with malaria infection in order to improve understanding of local malaria transmission.

5. TRIAL DESIGN

5.1 Study sites

The study will take place at 2 sites in Cambodian and 2 sites in Vietnam (Appendix 3).

5.2 Summary of trial design

An open-label randomised trial comparing the Triple ACT (TACT) artemether-lumefantrine+amodiaquine with artemether-lumefantrine ACT treatment, evaluating efficacy, safety, tolerability and artemisinin and partner drug resistance in four sites.

5.3 Study duration

The recruitment phase of the study is expected to last 24 months once a site starts to recruit. The first sites intend to start recruiting patients in October 2017. Training will precede study execution by up to 1 month. Data management and analysis, sample analysis (PK, *in vitro*, molecular markers) and report writing are expected to take about 6 months per site. Therefore, the total time to complete the study will be about 30 months.

5.4 Primary and secondary endpoints

5.4.1. Primary Endpoint

42-day PCR corrected efficacy defined as adequate clinical and parasitological response (ACPR). WHO definition: absence of parasitaemia at day 42 irrespective of axillary temperature and without previously meeting any of the WHO criteria for early or late treatment failure, or late parasitological failure.

5.4.2. Secondary Endpoints

- 42-day PCR corrected efficacy defined as adequate clinical and parasitological response (ACPR) according to site/geographic region.
- Parasite clearance half-life assessed by microscopy as primary parameter to determine parasite clearance
- Additional parameters of parasite clearance dynamics
- Fever clearance time (i.e. the time taken for the tympanic temperature to fall below 37.5˚C and remain there for at least 24 hours)
- Incidence of adverse events and serious adverse events by study arms within the first 42 days.
- Incidence of adverse events concerning markers of hepatic or renal toxicity such as bilirubin, ALT, AST, Alkaline Phosphatase and creatinine
- Proportion of patients that reports completing a full course of observed TACT or ACT without withdrawal of consent or exclusion from study because of drug related serious adverse event

- Incidence of prolongation of the QTc-interval above 500 ms or > 60 ms above baseline values.
- Prolongation of QTc-interval compared to baseline at timepoint H4, H24, H28, H48, H52, H60 and H64 and between these timepoints
- Change in haematocrit on day 1 to 7, 14, 21, 28, 35 and 42 according to geographical location and study arm, stratified for G6PD status
- Correlation between the host genotype and the pharmacokinetics and pharmacodynamics of antimalarials
- Prevalence of *Kelch13* mutations of known functional significance
- Prevalence/incidence of other genetic markers of antimalarial drug resistance such as MDR1 copy number and MDR1 mutations
- Genome wide association with in vivo/in vitro sensitivity parasite phenotype
- Correlation between SNPs measured in dry blood spots and whole genome sequencing in leukocyte depleted blood samples
- Transcriptomic patterns at t=0 and t=6h after start of treatment comparing sensitive and resistant parasites
- Correlation between qPCR based versus microscopy based assessments of parasite clearance dynamics up to day 14
- Proportion of patients with gametocytemia before, during and after treatment with TACT or ACT, assessed at admission, up to day 14, stratified by presence of gametocytes at enrolment
- Levels of RNA transcription coding for male or female specific gametocytes at admission up to day 14, stratified by the presence of gametocytes at enrolment
- In vitro sensitivity of *P. falciparum* to artemisinins and partner drugs according to study sites and genotype
- Pharmacokinetic profiles and interactions (Cmax and AUC) of artemisinin-derivatives and partner drugs in 20 ACT treated and 20 TACT treated patients of both study arms in Vietnam
- Day 7 drug levels of partner drugs in association with treatment efficacy and treatment arm
- Correlation between HRP2 based versus microscopy based assessments of parasite clearance dynamics
- Data on the place of residence, work, recent travel history and mobile phone use.
- Data and GPS mapping for a select group of participants and their peers in relation to behaviours and risk factors associated with malaria infection.

5.5 Trial Participants

5.5.1 Overall Description of Trial Participants

Male and non-pregnant female patients aged between 2 years and 65 years with acute uncomplicated falciparum malaria are the target study population. All study patients must meet the applicable inclusion and exclusion criteria.

5.5.2 Inclusion criteria

- Male or female, aged from 2 years to 65 years old.

- Acute uncomplicated *P. falciparum* malaria, confirmed by positive blood smear with asexual forms of *P. falciparum* (or mixed with non-falciparum species)
- Asexual *P. falciparum* parasitaemia: 16 to 200,000/uL, determined on a thin or thick blood film
- Fever defined as ≥ 37.5°C tympanic temperature or a history of fever within the last 24 hours
- Written informed consent (by parents/guardian in case of children)
- Willingness and ability of the patients or parents/guardians to comply with the study protocol for the duration of the study

Note on parasitaemia calculation:

Parasitaemia = N parasites per 2000 red blood cells on the thin smear x Hct x 62.8

Or

Parasitaemia = N parasites per 500 White blood cells on the thick smear by WBC count x16.

5.5.3 Exclusion criteria

- Signs of severe/complicated malaria
- Haematocrit < 25% or Hb < 8 g/dL at screening
- Acute illness other than malaria requiring treatment
- For females: pregnancy, breast feeding
- Patients who have received artemisinin or a derivative or an artemisinin-containing combination therapy (ACT) within the previous 7 days
- History of allergy or known contraindication to artemisinins, or to the ACT or TACT to be used at the site
- Previous splenectomy
- QTc-interval > 450 milliseconds at moment of presentation
- Documented or claimed history of cardiac conduction problems
- Previous participation in the current study or another study in the previous 3 months

Criteria for severe malaria (Adjusted from criteria used in SEAQUAMAT trial)

- Glasgow coma scale <11/15 in adults, or Blantyre coma scale ≤3/5 in children.
- Severe prostration
- Shock, assessed by admitting physician (low blood pressure and cool peripheries)
- Lactate >4.0 mmol/L (if measured through point of care test)
- Blood bicarbonate <15 mmol/L (if measured)
- Haematocrit <20% and *P. falciparum* parasitaemia >100000/µL
- Visible jaundice and P. *falciparum* parasitaemia >100000/µL
- Blood urea nitrogen >17 mmol/L (if measured)
- Asexual *P. falciparum* parasitaemia >10%
- Plasma glucose <2.2 mmol/L (if measured)
- Respiratory distress (>32 breaths per min)

6. PROCEDURES

Study procedures will be performed according to the schedule of assessments (Appendix 1). This will require that participants are admitted to the hospital for at least 72 hours and continue to be followed up on a weekly basis for 6 weeks. This differs from what would be standard management of uncomplicated malaria in Cambodia and Vietnam.

6.1. Informed Consent

The patient (or witness if illiterate) or the parent/guardian of a minor must personally sign and date the latest approved version of the informed consent form before any study specific procedures are performed. Written and verbal versions of the participant information and informed consent in the local language will be presented to the participants detailing no less than: the exact nature of the study; the implications and constraints of the protocol; the known side effects and any risks involved in taking part. It will be clearly stated that participation is voluntary and that the participant or guardian is free to withdraw from the study at any time for any reason without prejudice to future care, and with no obligation to give the reason for withdrawal.

The patient or parent/guardian will be allowed as much time as possible to consider the information and take the opportunity to question the Investigator, or other independent parties to decide whether they will (or allow his/her charge to) participate in the study. However, no more than two hours should elapse between presentation and treatment either on or off the study. Written informed consent will then be obtained by means of participant or guardian dated signature or thumb print (if unable to write) and dated signature of the person who presented and obtained the informed consent.

If required by local ethics committees, children will be asked to sign an assent form. A copy of the signed informed consent/assent document(s) will be given to the patients/parents.

6.2. Screening, Eligibility and Baseline Assessments

Patients who present at the participating sites will be screened to assess eligibility. Full consent (and assent if required) will be obtained before any enrolment procedures are conducted. It will be made clear from the outset that refusal to participate will not jeopardise subsequent antimalarial treatment. A screening log will be kept.

6.2.1. Demographics and Medical History

Basic demographic and epidemiological data (e.g. sex, age, address, bed net use, malaria risk factors, prior treatment and previous participation in this or previous studies), and a full medical history will be recorded by the study staff.

6.2.2. Physical Examination and Vital Signs

Physical examination will be conducted by a qualified study team member. Weight, height, pulse, blood pressure, respiratory rate, temperature, spleen and liver size will be recorded if palpable.

6.2.3. Drug history

All prescribed or over-the-counter and traditional medications used within the last 7 days will be recorded. Any drug allergies will be recorded.

6.2.4. Screening tests

These will be EDTA-anticoagulated blood for:

- A parasite count from Giemsa or Field stained thick and thin blood films
- Haematocrit

Urine pregnancy test for females of child bearing potential

Electrocardiograph to assess the QTc-interval

6.3. Randomisation and blinding

Patients who fulfil all the inclusion criteria and have none of the exclusion criteria will be randomised 1:1 to one of the two treatment arms according to a randomisation schedule. Randomisation will be in blocks of 8-12. Allocation will be done by drawing the next sequential numbered opaque envelope, which contains the study number and treatment allocation.

The patients will be assigned a study arm through a computer-generated randomisation schedule. Individual, sealed and sequentially numbered envelopes will be provided for each trial site with one envelope per patient, indicating the treatment allocation.

This is an open-label study so the blinding of investigators and patients is not applicable. However, the randomisation procedure allows for adequate drug allocation concealment before envelopes are opened. All laboratory investigations will be performed without knowledge of the treatment allocation.

6.4. Blood sampling

6.4.1 On admission

Patients will have an intravenous catheter inserted for the first 24 hours. An SOP will be provided instructing how this is to be kept patent and how to take blood for protocol tests.

On study admission, immediately before drug administration, blood will be collected for the following:

- Repeat parasite count (thick and thin films). If it is found subsequently that this parasitaemia no longer meets the inclusion criterion for parasitaemia the patient will be kept in the study.
- Dry blood blots (400 microlitres, 4 spots collected on filter papers for:
    - Parasite DNA genotyping for development of genetic surveillance.
    - PCR parasite barcoding (using 24-200 SNPs) to compare genotypes in case of recurrence of infection.
    - host genetics (G6PD genotyping) and haemoglobin electrophoresis
    - host genetics (genotyping of host factors affecting pharmacokinetics and pharmacodynamics of antimalarial drugs)
- Full blood count or WBC differential and/ Hct and Hb if possible. (EDTA-anticoagulated)
- Parasite DNA and RNA measurements (Up to 10 ml for adult, up to 5 ml for child) EDTA-anticoagulated blood.
- Validation of qPCR measurements by microscopy results. (2 ml of EDTA-anti-coagulated blood for adult and child).
- DNA/RNA for gametocyte quantification and characterisation (3 ml of heparinised blood for adult, 2.0 ml for child, some sites).
- *Ex vivo* parasite cultivation for drug sensitivity and transcriptomic assays (3 ml adult, 2 ml child, heparinised blood, some sites).
- Parasite cryopreservation (3 ml adult, 2 ml child, heparinised blood, all sites).
- Baseline biochemistry assessments including bilirubin, ALT, AST, Alkaline Phosphatase and creatinine levels (heparinised blood)

6.4.2. During hospitalisation

During hospitalisation, patients will have blood taken for malaria films at 4h, 6h, 8h and 12h and thereafter every 6 hours until parasite clearance (when two consecutive malaria slides are negative). In every case, malaria films will be performed at H4, H6, H8, H12, H24, H48 and H72 (even if two consecutive negative blood smears have been seen before these timepoints) Patients may be discharged if the final ECG has been performed and parasite clearance has been achieved, but not earlier than HR72. Haematocrits will be done whenever a malaria blood film is made.

During hospitalisation, blood will also be taken for:

- Day 0 H12, day 1 H24 and day 2 H48: Dry blood blots (100 microlitres, 1 spots collected on filter papers)
    - Parasite DNA genotyping for development of genetic surveillance.
- D0 H6: Parasite cryopreservation for transcriptomic analysis: EDTA-anticoagulated blood, (1 ml for adult, 1 ml for child, all sites).
- Day 1 H24 and Day 2 H48: Parasite DNA/RNA measurements: EDTA-anticoagulated blood (1 ml for adult, 1 ml for child).
- Day 3 H72: Validate qPCR measurements versus microscopy results: EDTA-anticoagulated blood (2 ml for adult and child).
- Day 3 H72: DNA/RNA for gametocyte quantification and characterization: heparinized blood (3 ml of for adult, 2.0 ml for child).
- Day 3 H72: biochemistry assessments including bilirubin, ALT, AST, Alkaline Phosphatase and creatinine levels: heparinised blood.
- Day 3 H72: Full blood count or WBC differential and Hct and Hb if possible. (EDTA-anticoagulated)

6.4.3. Pharmacokinetic Study Sampling

PK sampling using EDTA-anticoagulated blood (1 ml per sampling occasion). Venous plasma will be used to evaluate the drug exposure to artemether, lumefantrine, and amodiaquine (and metabolites) using a conventional non-compartment analysis (first dose pharmacokinetics). All collected samples will also be analysed using nonlinear mixed-effects modelling to assess the possible drug-drug interactions and the relationship between drug concentrations and treatment outcome. Drug levels collected at 4 hours after the first and last dose will also be used to evaluate a direct relationship between possible QTc-prolongation and drug exposures. A SOP will be written to detail the technique for specimen collection, handling and storage. Samples will be centrifuged immediately and the plasma stored at minus 80°C in properly labelled cryotubes.

Pharmacology analysis can be divided into two different approaches:

1. Pharmacokinetic profiles and interactions of artemisinin-derivatives and partner drugs in 20 ACT treated and 20 TACT treated patients of both study arms in Vietnam

Up to 15 samples taken from both adults and children giving a total blood volume of up to 15 ml. The PK schedule is presented below:

- Dense samples will be collected through an indwelling venous cannula during the first 24 hours: at 0 hr (pre-dose), 1 hr, 2 hr, 4 hr, 6 hr, 8 hr, 12 hr, 24 hr. Additional samples will be collected by venipuncture at 4 hours after last dose (52 hours) and at day 4, 7, 14, and 28.

2. A venous day 0 (pre-dose) and day 7 sample will be collected from all patients. EDTA blood (1 ml in adults and children).

Additional PK blood samples (1 ml EDTA) will be taken in the following situations:

- In case of an abnormal QTc-interval. In that case a blood sample will be obtained at the moment of the first identification of the abnormal QTc-interval and 6 and 12 hours after the first abnormal QTc-interval in order to assess the correlation between the prolonged QTc-interval and the drug levels that could be leading to the prolonged QTc-interval. (Maximum 3 ml EDTA blood).

- In case of a recurrent infection at the day of recurrence.

6.4.4. Plasma HRP2 measurements

Plasma HRP2-levels will be measured on enrollment and subsequent timepoints and used for modelling of parasite dynamics on the residues of samples obtained for PK analysis (some sites). Therefore, no additional blood sampling will be needed for these measurements and analysis.

6.4.5. Host genotyping

Blood samples (dried blood blots) for human genotyping will be obtained and stored from all subjects recruited with subject's consent. Genotyping will be performed on the samples of subjects with suspected abnormal pharmacokinetics or pharmacodynamics (for instance in case of unexpected adverse events such as severe prolongation of the QTc-interval). In addition, all samples will be used for whole genome association studies of the clinical phenotype (QTc-interval prolongation, drug levels, haemolysis (among others) and the subjects' genotype.
Potential targets for targeted genotyping are polymorphisms of Cytochrome 450 (e.g. CYP2D6 and CYP3A4) and other enzymes related to drug metabolism and genes and mutations predisposing to long QT-syndrome such as but not limited to the genes KCNQ1 (LQT1), KCNH2 (LQT2), and SCN5A (LQT3).
Genetic samples (in the form of dried blood blots or extracted DNA) will be stored (for a maximum of 10 years) and genotyped at the Molecular Tropical Medicine Laboratory, Bangkok, Thailand. In case a particular genetic test cannot be performed at the Molecular Tropical Medicine Laboratory, Bangkok, Thailand, the genetic samples may be transferred to another institute (which might be a foreign country) for genotyping. The subject will be asked for consent for this transfer during the initial informed consent process. A material transfer agreement will be in place if required before any samples are shipped.
The results of the genotyping will not be reported back to the subjects unless the abnormality is found through a method with a clear diagnostic certificate and the finding is judged to be of clear clinical importance to the subject.

6.4.6. Blood volumes

The blood volumes for the protocol mandated tests are detailed in the study schedule (Appendix 1) and will vary slightly between sites depending on whether sites can perform all of the tests of this protocol e.g. not all sites will be able to do the full blood count, store the plasma PK samples or do the *ex vivo* parasite culture. Also, it is likely that only a minority of patients will develop a recurrent parasitaemia and be requested to give further blood samples.

Concerning blood volumes, an age of above 12 years will be referred to as ′adult′, an age of 12 years and younger will be referred to as ′child′.

Maximum blood volumes are presented below for adults and children for 42 days of follow up. The maximum blood volume is the total amount taken if the patients remained in hospital for 3 days, had all blood samples taken and where part of the pharmacokinetic as well as other subsets (like qPCR validation and sampling for gametocyte genetics) and had one recurrent parasitaemia during follow up. The maximum blood volume will be approximately **97.9 ml for adults** and **75.9 ml for children with a weight above 20 kg** (less than 10% of total blood volume taken over 8 weeks as recommended by WHO- *Bulletin of the World Health Organization 2011:89:46-53)*.

We will exclude children **below 20 kilograms** from dense PK, DNA and RNA measurements on day 3, day 5, day 7, day 14, day 21, day 28, day 35 and day 42 thereby decreasing their maximum drawn blood volume approximately to **33.9 ml** over 42 days.

The maximum blood volume that will be drawn in children below the age of 12 (above a weight of 20 kilograms) is 25.3 ml in the first 24 hours of the study. This is below the 3 ml/kg in the first 24 hours as recommended by the WHO Bulletin 2011.

Allowing for the possibility that we may need to repeat blood tests, we may add 2 mL to these estimated maximum blood volumes.

These volumes become less if:

- There is no recurrent parasitaemia (minus 20 mL adults, minus 12 mL children)

6.5 Study drug regimens

Overview TRACII drug regimens	
TACT-arm	**ACT-arm**
Artemether-lumefantrine x 3 days PLUS Amodiaquine x 3 days	**Artemether-lumefantrine x 3 days**
Patients will receive Primaquine on day 1 according to the WHO guidelines and local requirement	

Patients will be treated with weight-based doses according to the schedule in appendix 2.

The study drugs will be administered by study medical or nursing staff while the patients are hospitalised. The study drug will be taken with at least 1.2 grams of fat to optimize absorption (comparable to 80-100 ml of a MILO milk carton or an equivalent fat containing beverage).

If the patient vomits within half an hour after intake of the antimalarial drugs, the dose will be repeated. If vomiting occurs between half and one hour, half of the dose will be repeated. If vomiting occurs more than one hour after drug administration, no repeat dosing will be done. Repeat doses will be recorded on the CRF. If vomiting within 1 hour occurs more than one time, no repeat dosing is allowed. The patient will be treated at the discretion of the investigator.

6.5.1 Artemether-lumefantrine

Currently available as dispersible or standard tablets containing 20 mg of artemether and 120 mg of lumefantrine, in a fixed-dose combination formulation. The flavoured dispersible tablet paediatric formulation facilitates use in young children.

Target dose/range:
The dose of artemether-lumefantrine is administered according to the treatment schedule in appendix 2, thereby approaching the WHO-recommended target ranges of artemether 5-24 mg/kg and lumefantrine 29-144 mg/kg over 3 days.

6.5.2. Amodiaquine

Amodiaquine is available in tablets of 150, 300 and 600 mg. The weight-based treatment schedule in appendix 2 aims for a dosage of approximately 10mg (4.5-15mg)/kg/day amodiaquine for three days, thereby approaching the WHO target dose and range of 10 mg (7.5-15mg)/kg/day.

6.5.3. Primaquine

Primaquine is available in tablets of 7.5 and 15 base mg. The weight-based treatment schedule in appendix 2 aims for a dosage of approximately 0.15-0.375 mg/kg on day one thereby approaching the 0.25 mg base/kg single dose recommended by the WHO.

6.6. Procedures during hospitalisation

A physical examination, measurement of vital signs will be performed daily and every six hours on indication. A symptom questionnaire will be taken daily to help identify adverse events. Follow up procedures

After patients are discharged, they will be followed up at Day 5 and 7 for:

- Day 5 and 7: Malaria blood slide
- Day 5 and 7: Haematocrit/Hb (EDTA-anticoagulated blood).
- Day 5 and 7: Validation of qPCR measurements versus microscopy results: EDTA-anticoagulated (2 ml for adults, 2 ml for child).

- Day 5 and 7: DNA/RNA for gametocyte quantification and characterization: Heparinised blood (3 ml for adults, 2 ml for child).
- Day 7: Basic biochemistry assessments including bilirubin, ALT, AST, Alkaline Phosphatase and creatinine levels: heparinised blood
- Day 7: Full blood count or WBC differential and Hct and Hb if possible (EDTA-anticoagulated)
- Day 7: ECG
- Physical examination and recording of symptoms and adverse events.

After day 7, they will be next seen weekly until Day 42 for:

- Malaria blood slide
- Haematocrit/Hb (EDTA-anticoagulated blood).
- Day 14, 28 and 42: Validation of qPCR measurements versus microscopy results: EDTA-anticoagulated (2 ml for adults, 2 ml for child).
- Day 14, 21, 28, 35 and 42: DNA/RNA for gametocyte quantification and characterization (3 ml for adults, 2 ml for child, heparinised blood).
- Day 28: Basic biochemistry assessments including bilirubin, ALT, AST, Alkaline Phosphatase and creatinine levels. (Heparinised blood)
- Day 28: Full blood count or WBC differential and Hct and Hb if possible (EDTA-anticoagulated)
- Day 28: ECG
- Physical examination and recording of symptoms and adverse events.

6.6.1 Time windows

The time-window for the visit on Day 7 is + 1 day and for the visits on Days 14 - 42 is –1 to +2 days. If a patient does not attend, a home-visitor from the study team will try to locate the patient and bring them to the clinic.

6.6.2 Additional visits

Patients presenting to the clinic with a fever or other symptoms on unscheduled days will be assessed by the study physician. Their temperature will be recorded and blood smear will be made for any patient with a documented fever (tympanic temperature ≥ 37.5˚C) or a history of fever. Patients will be treated as clinically indicated.

In the event that a patient becomes pregnant, on study, additional visits will be added at 3 months, 6 months and 9 months (after birth), to allow documentation of the outcome of the pregnancy.

6.6.3 Patients with recurrent parasitaemia

Patients with a recurrent falciparum parasitaemia (including mixed with another malaria species) during follow up will have blood taken for the following:

- Repeat parasite count (thick and thin films).
- Dry blood blots (400 microlitres, 4 spots collected on Whatman FTA cards) for:
    - Parasite DNA genotyping for development of genetic surveillance.
    - PCR parasite barcoding (using 24-200 SNPs) to compare genotypes in case of recurrence of infection.
- Full blood count or WBC differential and Hct and Hb if possible (EDTA-anticoagulated)
- Parasite DNA and RNA measurements (Up to 10 ml for adult, up to 5 ml for child) EDTA-anticoagulated blood.

- Validation of qPCR measurements by microscopy results. (2 ml of EDTA-anti-coagulated blood for adult and child,).
- DNA/RNA for gametocyte quantification and characterisation (3 ml of Heparinised blood for adult, 2.0 ml for child).
- *Ex vivo* parasite cultivation for drug sensitivity and transcriptomic assays (3 ml adult, 2ml child, heparinised blood)
- Parasite cryopreservation (3 ml adult, 2 ml child, heparinised blood)

In case of a recurrent infection, subjects should still be followed up according to the study protocol until day 42. An additional questionnaire on the place of living, work and travel and mobile phone use in the period between the initial infection and the recurrent infection will be completed.

Patients who develop a non-falciparum parasitaemia during follow up will be treated according to local guidelines.

6.7. Epidemiological data on place of residence, work, travel history and mobile phone use

In order to have a greater understanding of the possible sites of malaria transmission, and to relate genetic diversity to geographical location, patients or their guardians will be asked a short set of questions on their place of residence, place of work and their history of travel in the last 2 months. In addition, basic questions on use of mobile phones will be asked. These questions will help in understanding the use of mobile phones in each country in subjects prone to malaria infections. In separate studies, we plan to use anonymised, aggregated data on mobile phone use to mode population movement and predict potential routes of spread of malaria and antimalarial drug resistance. We will use the mobile phone usage survey to understand how relevant these movement patterns are to patients with malaria.

We will review these travel histories obtained in the hospital and then select a representative sample of patients for additional in-depth interviews with them and their peers to be conducted in their villages. This is to obtain a detailed understanding of the behaviours and risk factors for malaria infection. We will GPS the households of all patients and their places of work and places where their infection may have occurred, such as forests, farms or plantations. We will collect all available local malaria treatment records to describe how the study population compares to the overall population who receive treatment for malaria and this will allow us to better understand local malaria epidemiology and transmission patterns. All personal information will be anonymised so that no individual can be identified from their treatment records, through interviews, or from mapping data.

6.8. Rescue treatment

The indication for rescue treatment is:

- the development of any danger signs or signs of severe malaria at any point.

Rescue treatment will consist of parenteral artesunate, 2.4 mg/kg IV/IM STAT, followed by 2.4 mg/kg IV/IM at 12 hours and 24 hours and then daily until able to take oral medication.

Parenteral quinine will be added given in standard doses: 20 mg/kg (in 5% dextrose) IV over 4 hours, followed by 10 mg/kg every 8 hours until the patient is able to take oral medication.

Patients with persistent asexual parasitaemia on day 7 or who develop a recurrent parasitaemia after day 7 with no signs of severity will be treated with either another ACT (Artesunate-mefloquine in Cambodia according to National treatment guideline which is now current first line treatment for uncomplicated falciparum malaria) or a different combination e.g. quinine and doxycycline or clindamycin, in accordance with local recommendations.

Patients who develop a non-falciparum parasitaemia during follow up will be treated according to local guidelines.

6.9. Analysis

6.9.1 Drug assays

Partner drug concentrations will be measured in plasma samples. These assays will be performed at the Department of Clinical Pharmacology, MORU, Bangkok, Thailand.

6.9.2 Analysis of PK data

Pharmacokinetic analyses will be performed by the Department of Clinical Pharmacology, MORU, Bangkok, Thailand at the end of the study period or earlier when requested, for instance by the DSMB.

6.9.3 Ex vivo drug sensitivity assay

An *ex vivo* assay will be performed to measure parasite responses to artemisinin derivates and partner drugs according to the latest standards at the time of assessment. Parasites will be also be cryopreserved for future studies.

6.9.4 Molecular studies

Parasite DNA will be used for genomic studies including but not limited to microsatellite typing to identify parasite clones and single nucleotide polymorphisms (SNP) typing/whole genome sequencing to generate data for genome-wide association studies of *in vivo*, *ex vivo*, and *in vitro* responses of parasites to artemisinin and controls. Parasite RNA will be used for transcriptome analyses.

6.9.5 PCR for quantitative parasitaemia

One of the objectives of this study is to measure assess the parasite clearance dynamics by PCR and compare these with clearance rates estimated using this method to microscopy.

6.10. Discontinuation/ Withdrawal of Participants from the Study

Each participant has the right to discontinue the study drug or the study at any time. Data accrued up until the time of discontinuation will be used in the analysis.

In general, the investigator must make every effort to perform the study procedure until day 42, including in the following situations:

- Significant non-compliance with treatment regimen or study requirements
- An adverse event which requires discontinuation of the study medication or results in inability to continue to comply with study procedures
- Disease progression which requires discontinuation of the study medication or results in inability to continue to comply with study procedures
- Development of severe malaria
- Recurrent parasitaemia
- Loss to follow up (every attempt should be made to re-contact the participant)
- Discontinuation of the study drug

However, the investigator may discontinue participation in the study of a participant if he or she considers it necessary.

In addition, the participants always have the right to withdraw consent in writing or verbally.

The reason for withdrawal or discontinuation, if available, will be recorded in the CRF. If the study drug or participation in the study is discontinued due to an adverse event, the investigator will arrange for follow-up visits at least until the adverse event has resolved or stabilised.

Any pregnancy must be reported to the Principal Investigator within one working day of awareness. The PI must take all reasonable efforts to discover the outcome of the pregnancy and fill out the pregnancy form. If there is a congenital abnormality or a still born baby, this needs to be reported as a serious adverse event.

6.11. Source Data

Source documents are original documents, data, and records from which participants' CRF data are obtained. These include, but are not limited to, hospital records (from which medical history and previous and concurrent medication may be summarised into the CRF), clinical and office charts, laboratory and pharmacy records, diaries, microfiches, radiographs, and CRFs.

CRF entries will be considered source data if the CRF is the site of the original recording (e.g., there is no other written or electronic record of data). In this study, the CRF will be used as the source document for most of the data points.

All documents will be stored safely in confidential conditions. On all study-specific documents, other than the signed consent form, the participant will be referred to by the patient number and initials, not by name.

7. STUDY DRUGS

7.1 Storage of Study Drugs

All efforts will be made to store the study drugs in accordance with the manufacturers' recommendations in a secure area. This may be difficult at some sites where air-conditioned storage rooms are not available. The ACTs and TACTs should be stored between 15°C to 30°C (59°F to 86°F).

Where this is not possible and monitored storage, conditions do not meet the recommendations the artemisinin-derivatives and partner drug content of batches of ACTs and TACTs will be retested at the end of the study.

7.2 Compliance with Study Drugs

Study drugs will be administered as Directly-Observed-Therapy. If the patient vomits, and is re-dosed; this will be recorded in the CRF. If vomiting within 1 hour occurs again after retreatment, no repeat dosing is allowed. In this case the patient will be treated at the discretion of the Investigator. Each patient should be followed up until day 42.

Once the course of the study drugs is completed, patients will be discharged. All drug doses will be recorded in the CRF.

7.3 Accountability of the Study Treatment

All movements of study medication will be recorded. Both study medication of individual patient and overall drug accountability records will be kept up to date by the study staff.

7.4 Concomitant Medication

Throughout the study, investigators may prescribe concomitant medications or treatments deemed necessary (e.g. antipyretics or anti-emetics) to provide adequate supportive care except for antibiotics with antimalarial activity unless unavoidable (e.g. doxycycline, azithromycin). If these are required the patients will be kept in the study and this will be noted as a protocol deviation. Anti-emetics should not be prescribed as a prophylaxis if no nausea or vomiting is present. If anti-emetics are indicated metoclopramide is the preferred anti-emetic as this drug has the least QTc-interval prolonging effect of the anti-emetics that are commonly prescribed.

Antimalarials for recurrent infections (see Rescue treatment) and non-falciparum malaria (if applicable) will be prescribed as described above. Any medication, other than the study medication taken during the study will be recorded in the CRF.

8. SAFETY REPORTING

This trial will use drugs that have either been registered or evaluated extensively.

To allow for comparison of safety and tolerability of both new TACTs compared to the ACTs we will record and review all Adverse Events (AEs) and Serious Adverse Events, (SAEs), that occur in the study.

A symptom questionnaire will be performed daily during hospitalisation and at each subsequent visit to the health care center, to aid in the identification of adverse events. An additional questionnaire will be administered to patients who report symptoms such as suicide ideation and symptoms that might be attributable to a psychosis, to collect further data.

The investigator is responsible for the detection and documentation of events meeting the criteria and definition of an adverse event (AE) or serious adverse event (SAE), as provided in this protocol.

All SAEs and AEs will be promptly documented from the moment of inclusion in the study to discontinuation of the patient from study participation. Any events occurring between screening and randomization will be considered as baseline, preexisting conditions.

All adverse events must be recorded in the AE/SAE CRF. To avoid colloquial expressions, the adverse event should be reported in standard medical terminology. Whenever possible, the adverse event should be evaluated and reported as a diagnosis rather than as individual signs or symptoms. If a definitive diagnosis is not possible, the individual symptoms and signs should be recorded. Whenever possible, the aetiology of the abnormal findings will be documented on the CRF. Any additional relevant laboratory results obtained by the Investigator during the course of this study will be recorded on the CRF.

If the event meets the criteria for “serious”, the SAE must be reported to the TACT-Cambodia-Vietnam safety team within 24 hours from the time that the event was identified. If further data is required, additional documentation can be submitted. All SAEs must be followed until resolution, or until the SAE is deemed permanent or leads to death.

8.1 Definitions

8.1.1. Adverse Event (AE)

An AE is any undesirable event or clinical deterioration that occurs to a study participant during the course of the study; that is, from the time of administration of study drugs until study ends (i.e., until the follow up visit) whether or not that event is considered related to the study drugs, or to a concomitant drug or procedure: e.g.

- any unfavourable and unintended symptom
- physical sign
- abnormal laboratory result
- an illness

Any new clinical sign or clinical deterioration that occurs between signing the consent form and the administration of study drugs is not an AE. This information will be recorded in the medical records, as a pre-existing condition.

8.1.2 Serious Adverse Event

A serious adverse event is an AE that:

- results in death
- is life-threatening i.e. the patient was at risk of death at the time of the AE
- requires inpatient hospitalisation or prolongation of existing hospitalisation
- results in persistent or significant disability/incapacity
- is a congenital anomaly/birth defect
- QTc interval longer than 500 milliseconds

- QTc interval > 60 milliseconds from baseline
- Any other significant medical condition

All of the above criteria apply to the case as a whole and should not be confused with the outcomes of individual reactions/events. More than one of the above criteria can be applicable to the one event. Important medical events that may not be immediately life-threatening or result in death or hospitalisation may be considered an SAE when, based upon appropriate medical judgment, they may jeopardize the patient or require medical or surgical intervention to prevent one of the outcomes listed in the definition above. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in hospitalisation, or development of drug dependency or drug abuse.

8.1.3 QTc Interval

Electrocardiograms (ECGs) will be recorded at screening, D0H0, D0H4, D1H24, D1H28, D2H48, D2H52, D2H60 and D2H64, D7 and D28 in order to measure the QTc-interval.

The screening ECG can be used as the baseline (D0H0) ECG, if the time for administration of the D0H0 study drug is within 30 minutes of the time of the screening ECG. If the time between the screening ECG and the time for administration of the D0H0 study drug is longer than 30 minutes a separate baseline (D0H0) ECG must be performed.

A QTcBazett interval longer than 450 milliseconds at the screening or D0H0 will lead to exclusion or discontinuation from the study. The automated electronic readings at screening or baseline will not be confirmed by manual reading.

At the timepoints D0H4, D1H24, D1H28, D2H48 and D2H52, D2H60 and D2H64 an abnormal automated electronic reading (QTc-interval > 500 milliseconds or QTc > 60 milliseconds from baseline) will be confirmed by manual readings. At these timepoints, a QTc-interval > 500 milliseconds or a prolongation compared to baseline > 60 milliseconds will be considered a Serious Adverse Event and will lead to discontinuation of the study drug but not discontinuation of the patient from the trial. A specific SOP will describe procedures such as study drug replacement and follow up of the QTc-interval until normalization. QTc intervals will be measured every six hours after the first abnormal QTc-interval is recorded until the QTc-interval has normalized in two consecutive ECGs. Additional blood samples will be taken for pharmacological measurements at the moment of the identification of the abnormal QTc-interval and 6 and 12 hours after the first identification of the abnormal QTc-interval in order to assess the correlation between the prolonged QTc-interval and the drug levels that could be leading to the prolonged QTc-interval.

8.1.4 Biochemical assessments

In order to assess the safety of the novel TACTs basic biochemical assessments of markers related to hepatic and renal toxicity will be performed on day 0 (baseline) day 3, day 7 and 28. If found to be abnormal the values will be graded according to the Division of AIDS table mentioned in section 8.3.1 and followed up according to the local investigators preference and in accordance with section 8.3.4. In any case, increased ALTs and bilirubin levels found on day 7 and/or day 28 will be followed up on day 42.

Screening is performed without assessing biochemistry values. At baseline blood will be drawn for biochemistry assessments just minutes before baseline drug administration. Initial treatment at baseline will therefore be administered without knowledge of the baseline biochemistry values. If baseline biochemistry values turn out to be abnormal the study drugs must be discontinued and subjects will be treated according to the standard treatment or artesunate intravenously (if complicated or severe malaria is suspected based on the obtained laboratory values). An abnormal baseline biochemistry value is defined as falling within grade 3 or 4 of the DAIDS grading table. In any case the subject will still be followed up according to the study protocol until day 42).

8.2 Reporting Procedures for Serious Adverse Events

All SAEs must be reported by the site investigator to the Sponsor, within one day of his or her awareness of the SAE. The CRF documenting the SAE, should be faxed or emailed to the CTSG, (Fax No +66 (0) 2 354 9169; email TACTCV@tropmedres.ac).

Further reports should be submitted, if required, until the SAE is resolved.

The site investigator must also report the SAEs to the local ethics committee in accordance with local requirements.

8.3 Evaluating Adverse Events and Serious Adverse Events

8.3.1 Assessment of Intensity

Each adverse event will be graded according to the Division of AIDS table for grading the severity of ADULT AND PEDIATRIC adverse Events Version 2.0, November 2014 which will be included in the safety monitoring SOP.

If an adverse event is not listed in the Division of AIDS table, the Investigator will assess the severity using the following guidelines:

1 = Mild: awareness of sign or symptom, but easily tolerated

2 = Moderate: enough discomfort to cause interference with usual activity

3 = Severe: incapacitating with inability to work or do usual activity

4 = Life-Threatening

8.3.2 Clarification of the difference in meaning between 'severe' and 'serious'

The term "severe" is often used to describe the intensity (severity) of a specific event (as in mild, moderate, or severe myocardial infarction); the event itself, however, may be of relatively minor medical significance (such as severe headache). This is not the same as "serious", which is based on the outcome or criteria defined under the serious adverse event definition. An event can be considered serious without being severe if it conforms to the seriousness criteria, similarly severe events that do not conform to the criteria are not necessarily serious. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations.

8.3.3 Assessment of relatedness

The investigator is obligated to assess the relationship between study drug and the occurrence of each AE/SAE using the following categories of relatedness:

- Definite: clear-cut temporal association
- Probable: clear-cut temporal association, with improvement upon drug withdrawal, and not reasonably explained by the patient's known clinical state or other aetiology.
- Possible: less clear temporal association; other aetiologies are possible. (Other possible aetiologies should be recorded on the CRF).
- Not related: no temporal association with the study drug; assessed as related to other aetiologies such as concomitant medications or conditions, or patient's known clinical state.

The investigator will provide the assessment of causality as per the AE/SAE data collection tool.

8.3.4 Outcome

The investigator will follow-up the AE and SAE until resolution or until no further medically relevant information can be expected. AE and SAE outcome will be classified as follows:

- Continuing/ongoing
- Resolved
- Resolved with sequelae
- Permanent
- Fatal

9 STATISTICAL CONSIDERATIONS

9.1 Sample size justification

The sample size calculation is based on the primary endpoint of the 42-day PCR corrected ACPR. Our hypothesis is that artemether-lumefantrine+amodiaquine is superior to artemether-lumefantrine. Earlier efficacy studies of artemether-lumefantrine found an efficacy of 82.4 and 86.5% [13, 14]. Artesunate-mefloquine has not been used for almost a decade in Cambodia (up to 2016, when it became the first line treatment again). This has decreased the pfMDR1 copy number counts in the Cambodian population [15]. Given the association of pfMDR1 copy number and lumefantrine sensitivity this has potentially increased the efficacy of artemether-lumefantrine, despite increasing levels of artemisinin resistance [16]. Therefore, we assume the efficacy of artemether-lumefantrine to be 90%. Because of the additive therapeutic effect of amodiaquine we assume superiority of artemether-lumefantrine+amodiaquine with an efficacy of 99%.

A sample size of 100 patients per arm (power 0.80 and alpha=0.05) would allow us to detect this superiority. This leads to a total of 200 subjects needed per study site. In Vietnam, the 2 sites will recruit a cumulative 200 subjects (the sample size for this country) through competitive-recruitment due to the expected low recruitment rates at each site. A total of 600 patient will be recruited for the study.

The following Stata command*: "sampsi 0.99 0.90, alpha (0.05) power (0.8) nocontinuity"* was used (Stata 14.0).

9.2 Statistical Analyses

Analysis of other endpoints will be described in a Statistical Analysis Plan. A brief overview is given below.

9.2.1 Proportions

These will be compared using chi squared or Fisher's exact test, as appropriate. Crude proportions will be calculated with the exact 95% confidence intervals (CI), where relevant.

9.2.2 Continuous data

These will be summarised by medians (IQR, ranges) and means (standard deviations, 95% CIs), as appropriate, and will include the parasite counts and laboratory parameters. Comparisons of continuous data will be assessed using the paired/unpaired t tests or the sign rank/Mann Whitney U tests, as appropriate.

Analyses of the parasite clearance data will be conducted to look for geographical and temporal differences.

9.2.3 Pharmacokinetic data

The pharmacokinetic parameters of lumefantrine and amodiaquine during the first 24 hours will be estimated with a non-compartment analysis and will include standard PK parameters such as Cmax, Tmax and AUC. Nonlinear mixed-effects modelling will be employed to integrate all available data for an in-depth analysis of the pharmacokinetic properties of the above drugs as well as their relationship to treatment outcome.

9.2.4 Safety analysis

Safety analyses will be based on the whole population that get administered the study drug. Safety and tolerability of TACTs versus ACTs will be assessed by comparing the frequency (%) of adverse events and serious adverse events, with particular attention to abdominal pain, appetite perturbation, biochemical markers of hepatic and renal toxicity and QT interval prolongation, using the

Fisher's exact test. Safety data will be presented in tabular and/or graphical format and summarized descriptively. Any clinically relevant abnormalities or values of potential clinically concern will be described. Patients will be analysed according to an intention to treat and a per protocol method where appropriate.

9.2.5 Adverse events

Adverse events will be graded according to Division of AIDS table for grading the severity of ADULT AND PEDIATRIC adverse Events Version 2.0, November 2014 which will be included in the safety monitoring SOP.

All adverse event summaries will refer to treatment emergent adverse events, i.e. adverse events that newly started or increased in intensity after the study drug administration. AE summaries will be generated for all AEs that occurred after study drug administration, until the end of the study.

9.2.6 Assessment of relationship between PK parameters and ECG findings.

In addition to the results of the safety analysis an exploratory analysis of the ECG data will be conducted. The ECG parameters will be compared with the PK properties of the drugs using appropriate statistical methods.

9.2.7 Interim analyses

Interim analysis reports on the safety of TACTs will be provided by the Project Coordinator in collaboration with the Trial Statistician (Dr Mavuto Mukaka, e-mail: Mavuto@tropmedres.ac) after the first 60 and 180 patients. To assess the safety of the novel TACTs the DSMB will review the interim analysis reports.

10 DIRECT ACCESS TO SOURCE DATA/DOCUMENTS

Direct access will be granted to authorised representatives from the sponsor and host institution and the regulatory authorities, if applicable, to permit trial-related monitoring and inspections.

11 QUALITY CONTROL AND QUALITY ASSURANCE PROCEDURES

The study will be conducted in accordance with the current approved protocol, ICH GCP, any national regulations that may apply to this study and standard operating procedures. The WWARN will be engaged in assuring QA/QC of study execution in collaboration with the MORU Clinical Trials Support Group (CTSG). Their role will include but not be limited to monitoring adherence to SOPs for collection of clinical data and laboratory specimens and quality checks (curation) of clinical and laboratory data according to standard methodologies. Malaria slide QC will be performed by WWARN.

11.1 Monitoring

Study sites may have in place a system for internal monitoring. In addition, regular external monitoring of all sites will be performed by the MORU CTSG according to ICH GCP and a Monitoring Plan. Data will be evaluated for compliance with the protocol and accuracy in relation to source documents. The monitors will check whether the clinical trial is conducted and data are generated, documented and reported in compliance with the protocol, GCP and the applicable regulatory requirements. Evaluation of on-site monitoring schemes, such as a reciprocal monitoring scheme, may be undertaken at selected sites by CTSG and WWARN.

11.2 DSMB

An independent Data Safety and Monitoring Board (DSMB) will be set up consisting of qualified volunteers with the necessary knowledge of clinical trials. The DSMB will receive summary reports, prior to each meeting. All data reviewed by the DSMB will be in the strictest confidence. A DSMB charter will outline its responsibilities and how it will operate.

The DSMB will meet formally at the following timepoints:

- before the study starts
- after the first 60 patients have been accrued into the study
- after the first 180 patients have been accrued into the study
- At additional time-points before the planned interim analyses, as indicated by the DSMB after their review, if deemed necessary
- At the end of the study (i.e. after the last patient has finished follow up)

Unscheduled meetings can be held on the initiative of the Medical Monitor (Dr Lorenz Von Seidlein, e-mail: Lorenz@tropmedrs.ac), Principal Investigator or Executive committee to consider e.g. serious adverse events as they are reported OR if an earlier safety review is thought to be indicated.

12 ETHICS

12.1 Declaration of Helsinki

The Investigator will ensure that this study is conducted in compliance with the current revision of the Declaration of Helsinki (Fortaleza 2013).

12.2 ICH Guidelines for Good Clinical Practice

The Investigator will ensure that this study is conducted according to any National Regulations and that it will follow the principles of the ICH Guidelines for Good Clinical Practice 1996.

12.3 Approvals

The study protocol and its associated documents will be submitted to the Oxford Tropical Research Ethics Committee (OxTREC) and the appropriate local ethics committees for written approval.

The Investigator will submit and, where necessary, obtain approval from the above parties for all substantial amendments to the original approved documents.

12.4 Risks

This study will use drugs that have been studied thoroughly and their toxicities are well described. In general, they are all well tolerated.

12.4.1 Risks of artemether-lumefantrine

Reported A/L side effects have generally been mild. The main side effects are GI upset: anorexia (~18%), nausea (~5%), vomiting (~18%), abdominal pain (~5%), and diarrhoea (~10%), headache (~10%), dizziness (~4%), fatigue (~1%) and sleep disturbance (~2%). Other symptoms reported infrequently include palpitations, myalgia, arthralgia (all of which could be disease related), and rash. A/L does not cause prolongation of the QTc interval.

12.4.2 Risks of amodiaquine

The main side-effects of amodiaquine are nausea, vomiting and fatigue which are mild to moderate in nature. When the drug was used for prophylaxis, rare adverse reactions of agranulocytosis and hepatoxicity were observed.

12.4.3 Risks of new partner drug combinations

No interactions between lumefantrine and amodiaquine are expected. Based on the relatively safe and limited side effect profiles of both drugs, no life-threatening interactions between lumefantrine and amodiaquine are expected. In addition, the preliminary results of the TRACII trial indicate that the TACT artemether-lumefantrine+amodiaquine has a good safety and tolerability profile.

12.4.4 Risk of phlebotomy & finger stick

The primary risks of phlebotomy include local discomfort, occasional bleeding or bruising of the skin at the site of needle puncture, and rarely haematoma or infection.

12.5 Benefits

Malaria is a disease that needs to be treated promptly. All patients will benefit from receiving efficacious treatment at no cost. They will be followed up closely and will be given rescue treatment if clinically indicated.

12.6 Alternatives to Study Participation

Patients are able to decline freely participation in this study. If so, they will receive standard care for their malaria.

12.7 Incentives & Compensation

Study patients or their guardian in the case of children will be compensated for time lost from work. Patients will be reimbursed for the time lost from work as a result of hospitalisation, the cost of local

transport to attend for the follow up visits and will receive a per diem to cover the costs of meals on those days. The amounts in monetary terms will be determined by each site. The study will pay for treatment for drug-related SAEs or other research-related injuries. The study cannot pay for long term care for disability after hospital discharge resulting from complications of the illness.

12.8 Confidentiality

The trial staff will ensure that the participants' anonymity is maintained. The participants will be identified only by initials and a study number on the CRF and the MACRO EDC database. All documents will be stored securely and be accessible to trial staff and authorised personnel only.

13 SAMPLE SHARING AND STORAGE

Samples collected will be used for the purpose of this study as stated in the protocol and stored for future use. Consent will be obtained from patients for sample storage and/or shipment of specific samples to collaborating institutions for investigations that cannot be performed locally. Any proposed plans to use samples other than for those investigations detailed in this protocol will be submitted to the relevant ethics committees prior to any testing. Material transfer agreements will be arranged and signed where appropriate/needed.

14 DATA HANDLING AND RECORD KEEPING

All study data will be recorded on standard Case Report Forms (CRF), at the study sites. The CRFs will be sent to the Clinical Trial Support Group, Data Management team and will be entered on the MACRO EDC database in accordance with standard operating procedures. All data management activities will be carried out to ensure data quality, in accordance with the data management plan.

The participants will be identified by a study specific participant number and/or code in any database. The name and any other identifying detail will NOT be included in any study data electronic file. Data may be used alone or in combination with data from related studies in secondary analyses.

The data from this study will be shared with WWARN with the consent of the study sites (The WWARN terms of submission are detailed in Appendix 4). The Principal Investigators from each site will have the opportunity to review and sign these terms of submission document to indicate their agreement.

15 SPONSORSHIP AND INSURANCE

The University of Oxford is the study sponsor and will obtain the necessary insurance.

16 PUBLICATION POLICY

Any data published in the peer-reviewed medical literature will protect the identity of the patients. This trial will be registered in a web based protocol registration scheme. All those who have made a substantial contribution will be co-authors on publications. The sites have the right to publish their data individually and to include members of the sponsor's team who have made a significant contribution. There will also publications of pooled data which will be coordinated by the MORU group. All sites will have the opportunity to contribute to these publications.

All the research findings from the programme and from relevant research outside the Programme will be analysed and integrated, and through the WWARN site and the WHO Global Malaria Programme will be disseminated to policy makers, National Malaria Control Programmes (NMCPs) and other researchers.

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18. APPENDIX 1. STUDY SCHEDULES D42 FOLLOW UP

TEST/APPLICATION	SAMPLE	SCR	D0								D1					D2						D3				D4				D5				D6				D7	D14	D21	D28	D35	D42	DREC
			H0	H1	H2	H4	H6	H8	H12	H18	H24	H28	H30	H36	H42	H48	H52	H54	H60	H64	H66	H72	H78	H84	H90	H96	H102	H108	H114	H120	H126	H132	H138	H144	H150	H156	H162	H168						
Blood Smear [1]	EDTA, WB	X	X			X	X	X	X	X	X		X	X	X	X		X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
HCT/Hb/FBC	EDTA	X	X			X	X	X	X	X	X		X	X	X	X		X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Packed cells (qPCR)	EDTA, Buffy-coat dep.		X																			X								X								X	X		X		X	X
Dried Blood Spot	EDTA, WB		X						X		X					X																												X
RNA	EDTA, WBC-dep.		X								X					X																												X
DNA	EDTA, WBC-dep.		X								X					X																												X
PK	EDTA		X	X	X	X	X	X	X		X						X									X												X	X		X		X	X
Packed cells (gamet)	Heparin		X																			X								X								X	X		X		X	X
Biochemistry	Heparin		X																			X																X			X			
Cryopreservation	Heparin		X				X																																					X
Ex vivo test	Heparin		X																																									X
Pregnancy (Urine)	Urine	X																																										
						Maximum volume drawn for child <20 kilograms: 33.9 mL (approx.)																																						
						Maximum volume drawn for child ≤ 12 years old: 75.9 mL (approx.)																																						
						Maximum volume drawn for adult >12 years old, no PK: 85.9 mL (approx.)																																						
						Maximum volume drawn for adult >12 years old: 97.9 mL (approx.)																																						
Informed consent		X																																										
Demographic data/Medical history		X																																										
Travel survey/mobile phone use survey			X																																									X
Drug history		X																																										
Temperature (Untill two cons. meas.<37.5 degrees C		X					X		X	X	X		X	X	X	X		X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X						X
Vital signs		X					X*		X*	X*	X		X*	X*	X*	X		X*	X*		X*	X	X*	X*	X*	X	X*	X*	X*	X	X*	X*	X*	X	X*	X*	X*	X	X	X	X	X	X	X*
Height		X																																										
Weight		X																																										X
Physical examination		X									X		X**	X**	X**	X		X**	X**		X**	X	X**	X**	X**	X	X**	X**	X**	X	X**	X**	X**	X	X**	X**	X**	X	X	X	X	X	X	X**
Randomisation & assign study ID			X																																									
ACT/TACT (ACT/TACT doses to be given supervised according to dosage schedule)			X					X			X			X		X			X																									
Symptom questionnaire			X								X					X						X				X				X				X				X	X	X	X	X	X	X
Electrocardiographic recording		X	X			X					X	X				X	X		X	X																		X			X			
1- Patients to be sampled per schedule unti parasites have cleared (2 consecutive slides are negative). Sampling is done once daily as outpatients.																																												
*Vital signs if clinically indicated																																												
Physical examinations if clinically indicated																																												

ADULT (MAX:) PK and genetics — 97.9 mL

TEST/APPLICATION	SAMPLE (VOL, mL)	SCR	D0								D1					D2						D3				D4				D5				D6				D7	D14	D21	D28	D35	D42	DREC
			H0	H1	H2	H4	H6	H8	H12	H18	H24		H30	H36	H42	H48	H52	H54	H60		H66	H72	H78	H84	H90	H96	H102	H108	H114	H120	H126	H132	H138	H144	H150	H156	H162	H168						
Blood Smear [1]	EDTA, WB (0.05)	0.1mL				0.1mL	0.1mL	0.1mL	0.1mL	0.1mL	0.1mL		0.1mL	0.1mL	0.1mL	0.1mL		0.1mL	0.1mL		0.1mL		0.1mL	0.1mL	0.1mL	0.1mL	0.1mL	0.1mL	0.1mL		0.1mL	0.1mL	0.1mL	0.1mL	0.1mL	0.1mL	0.1mL			0.1mL		0.1mL		
HCT/Hb/FBC	EDTA (0.1)																																											
Packed cells (qPCR/ZB)	EDTA, Buffy-coat dep. (3)		12.5 mL																			2.1mL								2.1mL								2.1mL	2.1mL		2.1mL		2.1mL	11.5 mL
Dried Blood Spot	EDTA, WB (0.4)								0.1mL		0.1mL					0.1mL																												
RNA	EDTA, WBC-dep. (1)										1.0 mL					1.0 mL																												
DNA	EDTA, WBC-dep. (5)																																											
PK	EDTA (1)/Dry blood			1.0 mL	1.0 mL	1.0 mL	1.0 mL	1.0 mL	1.0 mL		1.0 mL						1.0 mL									1.0 mL												1.0 mL	1.0 mL		1.0 mL		1.0 mL	
Packed cells (gams)	Heparin, (3)																					3.0 mL								3.0 mL								3.0 mL	3.0 mL		3.0 mL		3.0 mL	
Biochemistry	Heparin (1ml)		11.0 mL																			1.0 mL																1.0 mL			1.0 mL			10.0 mL
Cryopreservation	Heparin, (3)						1.0 mL																																					
Ex vivo test (TMI, ZB)	Heparin, (3)																																											
Pregnancy (Urine)	Plain (10)	10.0 ml																																										0.0 mL
			23.5 mL	1.0 mL	1.0 mL	1.1mL	2.1mL	1.1mL	1.2 mL	0.1mL	2.2 mL	0.0 mL	0.1mL	0.1mL	0.1mL	1.2 mL	1.0 mL	0.1mL	0.1mL	0.0 mL	0.1mL	6.1mL	0.1mL	0.1mL	0.1mL	1.1mL	0.1mL	0.1mL	0.1mL	5.1mL	0.1mL	0.1mL	0.1mL	0.1mL	0.1mL	0.1mL	0.1mL	7.1mL	6.1mL	0.1mL	7.1mL	0.1mL	6.1mL	21.5 mL
						Total	31.1mL					Total	2.5 mL				Total	2.5 mL					Total	6.4 mL			Total	1.4 mL			Total	5.4 mL			Total	0.4 mL		7.1mL	6.1mL	0.1mL	7.1mL	0.1mL	6.1mL	

ADULT (MAX:) NO PK — 85.9 mL

TEST/APPLICATION	SAMPLE (VOL, mL)	SCR	D0								D1					D2						D3				D4				D5				D6				D7	D14	D21	D28	D35	D42	DREC
			H0	H1	H2	H4	H6	H8	H12	H18	H24		H30	H36	H42	H48	H52	H54	H60		H66	H72	H78	H84	H90	H96	H102	H108	H114	H120	H126	H132	H138	H144	H150	H156	H162	H168						
Blood Smear [1]	EDTA, WB (0.05)	0.1mL				0.1mL	0.1mL	0.1mL	0.1mL	0.1mL	0.1mL		0.1mL	0.1mL	0.1mL	0.1mL		0.1mL	0.1mL		0.1mL		0.1mL	0.1mL	0.1mL	0.1mL	0.1mL	0.1mL	0.1mL		0.1mL	0.1mL	0.1mL	0.1mL	0.1mL	0.1mL	0.1mL			0.1mL		0.1mL		
HCT/Hb/FBC	EDTA (0.1)																																											
Packed cells (qPCR/ZB)	EDTA, Buffy-coat dep. (3)		12.5 mL																			2.1mL								2.1mL								2.1mL	2.1mL		2.1mL		2.1mL	11.5 mL
Dried Blood Spot	EDTA, WB (0.4)								0.1mL		0.1mL					0.1mL																												
RNA	EDTA, WBC-dep. (1)										1.0 mL					1.0 mL																												
DNA	EDTA, WBC-dep. (5)																																											
PK	EDTA (1)/Dry blood																																					1.0 mL						
Packed cells (gams)	Heparin, (3)																					3.0 mL								3.0 mL								3.0 mL	3.0 mL		3.0 mL		3.0 mL	
Biochemistry	Heparin (1ml)		11.0 mL																			1.0 mL																1.0 mL			1.0 mL			10.0 mL
Cryopreservation	Heparin, (3)						1.0 mL																																					
Ex vivo test (TMI, ZB)	Heparin, (3)																																											
Pregnancy (Urine)	Plain (10)	10.0 ml																																										0.0 mL
			23.5 mL	0.0 mL	0.0 mL	0.1mL	1.1mL	0.1mL	0.2 mL	0.1mL	1.2 mL	0.0 mL	0.1mL	0.1mL	0.1mL	1.2 mL	0.0 mL	0.1mL	0.1mL	0.0 mL	0.1mL	6.1mL	0.1mL	0.1mL	0.1mL	0.1mL	0.1mL	0.1mL	0.1mL	5.1mL	0.1mL	0.1mL	0.1mL	0.1mL	0.1mL	0.1mL	0.1mL	7.1mL	5.1mL	0.1mL	6.1mL	0.1mL	5.1mL	21.5 mL
						Total	25.1mL					Total	1.5 mL				Total	1.5 mL					Total	6.4 mL			Total	0.4 mL			Total	5.4 mL			Total	0.4 mL		7.1mL	5.1mL	0.1mL	6.1mL	0.1mL	5.1mL	

CHILD (<12 YO) >20KG	75.9 mL																																											
TEST/APPLICATION	SAMPLE (VOL, mL)	SCR	D0								D1					D2						D3				D4				D5				D6				D7	D14	D21	D28	D35	D42	DREC
			H0	H1	H2	H4	H6	H8	H12	H18	H24		H30	H36	H42	H48	H52	H54	H60		H66	H72	H78	H84	H90	H96	H102	H108	H114	H120	H126	H132	H138	H144	H150	H156	H162	H168						
Blood Smear [1]	EDTA, WB (0.05)	0.1mL				0.1mL	0.1mL	0.1mL	0.1mL	0.1mL	0.1mL		0.1mL	0.1mL	0.1mL	0.1mL		0.1mL	0.1mL		0.1mL		0.1mL	0.1mL	0.1mL	0.1mL	0.1mL	0.1mL	0.1mL		0.1mL	0.1mL	0.1mL	0.1mL	0.1mL	0.1mL	0.1mL			0.1mL		0.1mL		
HCT/Hb/FBC	EDTA (0.1)																					2.1mL								2.1mL								2.1mL	2.1mL		2.1mL		2.1mL	
Packed cells (qPCR/ZB)	EDTA, Buffy-coat dep. (3)		7.5 mL																																									6.5 mL
Dried Blood Spot	EDTA, WB (0.4)								0.1mL		0.1mL					0.1mL																												
RNA	EDTA, WBC-dep.										1.0 mL					1.0 mL																												
DNA	EDTA, WBC-dep. (3)																																											
PK	EDTA (1)/Dry blood			1.0 mL	1.0 mL	1.0 mL	1.0 mL	1.0 mL	1.0 mL		1.0 mL						1.0 mL									1.0 mL												1.0 mL	1.0 mL		1.0 mL		1.0 mL	
Packed cells (gams)	Heparin, (0)																					2.0 mL								2.0 mL								2.0 mL	2.0 mL		2.0 mL		2.0 mL	
Biochemistry	Heparin (1ml)		8.0 mL																			1.0 mL																1.0 mL			1.0 mL			7.0 mL
Cryopreservation	Heparin, (2.5)						1.0 mL																																					
Ex vivo test (TMI, ZB)	Heparin, (2.5)																																											
Pregnancy (Urine)	Plain (10)	0 ml	0.0 mL																																									0.0 mL
			15.5 mL	1.0 mL	1.0 mL	1.1mL	2.1mL	1.1mL	1.2 mL	0.1mL	2.2 mL	0.0 mL	0.1mL	0.1mL	0.1mL	1.2 mL	1.0 mL	0.1mL	0.1mL	0.0 mL	0.1mL	5.1mL	0.1mL	0.1mL	0.1mL	1.1mL	0.1mL	0.1mL	0.1mL	4.1mL	0.1mL	0.1mL	0.1mL	0.1mL	0.1mL	0.1mL	0.1mL	6.1mL	5.1mL	0.1mL	6.1mL	0.1mL	5.1mL	13.5 mL
						Total	23.1mL					Total	2.5 mL				Total	2.5 mL					Total	5.4 mL			Total	1.4 mL			Total	4.4 mL			Total	0.4 mL		6.1mL	5.1mL	0.1mL	6.1mL	0.1mL	5.1mL	

CHILD (<12 YO, <20 KG)	33.9 mL																																											
TEST/APPLICATION	SAMPLE (VOL, mL)	SCR	D0								D1					D2						D3				D4				D5				D6				D7	D14	D21	D28	D35	D42	DREC
			H0	H1	H2	H4	H6	H8	H12	H18	H24	H28	H30	H36	H42	H48	H52	H54	H60	H64	H66	H72	H78	H84	H90	H96	H102	H108	H114	H120	H126	H132	H138	H144	H150	H156	H162	H168						
Blood Smear [1]	EDTA, WB (0.05)	0.1mL				0.1mL	0.1mL	0.1mL	0.1mL	0.1mL	0.1mL		0.1mL	0.1mL	0.1mL	0.1mL		0.1mL	0.1mL		0.1mL		0.1mL	0.1mL	0.1mL	0.1mL	0.1mL	0.1mL	0.1mL		0.1mL	0.1mL	0.1mL	0.1mL	0.1mL	0.1mL	0.1mL			0.1mL		0.1mL		
HCT/Hb/FBC	EDTA (0.1)																					0.1mL								0.1mL								0.1mL	0.1mL		0.1mL		0.1mL	
Packed cells (qPCR/ZB)	EDTA, Buffy-coat dep. (2)		5.0 mL																																									5.0 mL
Dried Blood Spot	EDTA, WB (0.4)								0.1mL		0.1mL					0.1mL																												
RNA	EDTA, WBC-dep. (5)										0.0 mL					0.0 mL																												
DNA	EDTA, WBC-dep. (3)																																											
PK	EDTA (1)/Dry blood																																					1.0 mL						
Packed cells (gams)	Heparin, (2)																													0.0 mL								0.0 mL	0.0 mL		0.0 mL		0.0 mL	
Biochemistry	Heparin (1ml)		8.0 mL																			1.0 mL																1.0 mL			1.0 mL			7.0 mL
Cryopreservation	Heparin, (2)						1.0 mL																																					
Ex vivo test (TMI, ZB)	Heparin, (2)																																											
Pregnancy (Urine)	Plain (10)	0 ml	0.0 mL																																									0.0 mL
			13.0 mL	0.0 mL	0.0 mL	0.1mL	1.1mL	0.1mL	0.2 mL	0.1mL	0.2 mL	0.0 mL	0.1mL	0.1mL	0.1mL	0.2 mL	0.0 mL	0.1mL	0.1mL	0.0 mL	0.1mL	1.1mL	0.1mL	0.1mL	0.1mL	0.1mL	0.1mL	0.1mL	0.1mL	0.1mL	0.1mL	0.1mL	0.1mL	0.1mL	0.1mL	0.1mL	0.1mL	2.1mL	0.1mL	0.1mL	1.1mL	0.1mL	0.1mL	12.0 mL
						Total	14.6 mL					Total	0.5 mL				Total	0.5 mL					Total	1.4 mL			Total	0.4 mL			Total	0.4 mL			Total	0.4 mL		2.1mL	0.1mL	0.1mL	1.1mL	0.1mL	0.1mL	

19 APPENDIX 2. DOSING SCHEDULES

19.1 Artemether-lumefantrine

Artemether-lumefantrine dosing schedule						
One tablet A/L contains 20mg artemether and 120mg lumefantrine						
	No. of tablets recommended at approximate timing of dosing					
Weight: Kilo-gram	0 h	8 h	24 h	36 h	48 h	60 h
5-14.9	1	1	1	1	1	1
15-24.9	2	2	2	2	2	2
25-34.9	3	3	3	3	3	3
≥35	4	4	4	4	4	4

19.2 Amodiaquine

Amodiaquine dosing schedule						
One tablet contains 150mg Amodiaquine						
	No. of tablets recommended at approximate timing of dosing					
Weight: Kilo-gram	0 h	8 h	24 h	36 h	48 h	60 h
5-14.9	0.5	0	0.5	0	0.5	0
15-24.9	0.5	0.5	0.5	0.5	0.5	0.5
25-34.9	1	1	1	1	1	1
≥35	1.5	1.5	1.5	1.5	1.5	1.5

19.3 Primaquine

Primaquine dosing schedule	
Weight: Kilogram	Tab/day (Tab=7.5mg)
<25	0.5 tablet on day 1
25-50	1 tablet on day 1
>50	2 tablets on day 1

20 APPENDIX 3. LIST OF STUDY SITES & PRINCIPAL INVESTIGATORS

CAMBODIA

CNM: Dr Huy Rekol (Principal investigator) Dr Chea Nguon (Co-Principal Investigator) Dr Lek Dysoley (Co-Principal Investigator)

MORU : Dr. Rupam Tripura (Local investigator), Dr. James Callery (Local investigator), Dr. Tom Peto (Local investigator), Professor AM Dondorp (Principal Investigator), MORU, Thailand.

Site 1: **Pailin Referral Hospital**, Pailin Province,

Site 2: **Stung Treng Referral Hospital/ Siem Pang Health Center**, Stung Treng Province:

VIETNAM

Country PI:

Hien Tran Tinh MD, PhD Oxford University Clinical Research Unit (OUCRU), Vietnam,

Ho Dang Trung Nghia MD, PhD Oxford University Clinical Research Unit (OUCRU), Vietnam.

Site 1: **Phuoc Long Hospital,** Phuoc Province: Co-PI: Le Thanh Long, MD, Director

Site 2: **Hospital for Tropical Diseases of Khanh Hoa**, Khanh Hoa Province: Co-PI: Nguyen Dong MD, Director,

21 APPENDIX 4: WWARN TERMS OF SUBMISSION

TERMS

The WWARN project (http://www.wwarn.org) is dependent on the submission of data about individual patients' responses to anti-malarial drug treatment. WWARN respects the rights of researchers and institutions who may wish to share their data with WWARN.

These terms of submission (which form part of the WWARN website's terms of use) provide a framework for sharing data with WWARN, explaining the terms on which you may use the site to submit data to WWARN and how WWARN will use that data. Please read these terms carefully before submitting data to WWARN: by submitting data to WWARN, you indicate that you accept these terms and you agree to abide by them.

WWARN

WWARN is a project developed by an international collaboration of research institutions and which is currently hosted by the University of Oxford. The collaborative group consists of five WWARN project teams, each of which will have responsibility for the analysis of submitted data in relation to a defined topic (each a WWARN module).

By submitting data to WWARN you agree that the WWARN project teams will have access to your data on substantially the same terms as granted in these terms of submission.

DATA

In order to ensure compliance with its legal and ethical obligations, WWARN requires that all submitted data:

has been obtained in accordance with any laws and ethical approvals applicable in its country of origin;

has been obtained with the knowledge and consent of the individual to which it relates; and

has been anonymised, so that any individual to which it relates cannot be identified from it.

You must have the authority to submit the data to be used in accordance with these terms of submission.

WWARN reserves the discretionary right to delete any submitted data, in whole or in part, if it contains any information that would identify an individual.

In order to protect your data and safeguard the rights of the individuals to which it relates, WWARN will take appropriate technical and organisational measures against unauthorised or unlawful processing of any submitted data and against the accidental loss or destruction of, or damage to, submitted data.

RIGHTS

You will continue to own your data. WWARN will not claim any rights of ownership in any data you may submit but, in order to allow WWARN to operate, it requires your permission to use the data for the purposes set out in these terms of submission.

By submitting data, you grant to WWARN a right to use, copy, reformat and to make further analyses of your data in accordance with these terms of submission.

You may, at any time and for any reason, withdraw WWARN's right to use your data, but you accept that your data may have been used to prepare and/or create reports and analyses and that these reports and analyses cannot be withdrawn once they have been made available.

In the event that WWARN transfers from the University of Oxford to another entity, the rights you grant to WWARN will be transferred to that entity.

USE OF DATA

Your data will be stored on a secure server and will be accessible only to you, anyone you may nominate and WWARN.

Your data may be further processed by WWARN and used in combination with other data to create aggregated data.

WWARN may use aggregated data to create reports and analyses, including:

summary reports to define geospatial and temporal trends in anti-malarial drug resistance and drug quality; and

specialised analyses of pooled studies (for example, analyses in relation to a particular drug, patient group or time span).

In the event that WWARN receives a request to contribute data to an external project or collaborative analysis, it will not contribute or grant access to your data without your express permission.

PUBLICATION OF DATA

Your submission of data to WWARN does not affect your right to publish those data, which you may do entirely at your own discretion and without consent from or reference to WWARN.

Data relating to anti-malarial drug resistance is only useful to those involved in the treatment of malaria if it is up-to-date and, for that reason, WWARN encourages you to submit your data before publication. WWARN will only use your data in an aggregated form, except that where you provide your express permission WWARN may include a high-level summary of your research on the WWARN Explorer.

Reports and analyses created by WWARN may be published or otherwise made publicly accessible, including through the WWARN website.

Your contribution to any WWARN publication will be acknowledged in accordance with the guidelines of the International Committee of Medical Journal Editors (www.icmje.org/ethical_1author.html).

YOUR CONCERNS

If you have any concerns about these terms of submission, please contact info@wwarn.org.

I accept and agree to abide by these Terms of Submission

Signature…….

Name……

Date……..