DeTACT-ASIA

A multi-centre, randomised, controlled, non-inferiority trial to compare the efficacy, safety and tolerability of Triple Artemisinin-based Combination Therapies versus first-line ACTs+placebo for the treatment of uncomplicated *Plasmodium falciparum* malaria in Asia

Short title: A study by the Development of Triple Artemisinin-based Combination Therapies (DeTACT) Project

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A study by the Development of Triple Artemisinin Combination Therapies (DeTACT) Collaboration

ACRONYM: DeTACT-ASIA study

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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:

Site Principal investigator	Site 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
AL	Artemether-lumefantrine
AQ	Amodiaquine
AS	Artesunate
ASMQ	Artesunate-mefloquine
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
ERB	Ethics Review Board
G6PD	Glucose-6-phosphate dehydrogenase
GCP	Good Clinical Practice
Hb	Haemoglobin
Hct	Haematocrit
IM	Intramuscular
IV	Intravenous
MORU	Mahidol-Oxford Research Unit
MQ	Mefloquine
NMCP	National Malaria Control Programme
OxTREC	Oxford Tropical Research Ethics Committee
PBO	Placebo
PCR	Polymerase Chain Reaction
PCT	Parasite Clearance Time
PD	Pharmacodynamic
Pfmdr1	*P. falciparum* Multi-Drug Resistance Gene 1
PK	Pharmacokinetic
PPQ	Piperaquine
QA	Quality Assurance
QC	Quality Control
RNA	Ribonucleic acid
SAE	Serious Adverse Event
SNP	Single-nucleotide polymorphism
TACT	Triple Artemisinin-based Combination Therapy
TRAC	Tracking Resistance to Artemisinin Collaboration
WBC	White Blood Cells
WHO	World Health Organisation
WI	Work Instruction
WWARN	Worldwide Antimalarial Resistance Network

2. SYNOPSIS

Study Title	A multi-centre, randomised, controlled non-inferiority trial to compare the efficacy, safety and tolerability of Triple Artemisinin-based Combination Therapies versus first-line ACTs + placebo for the treatment of uncomplicated *Plasmodium falciparum* malaria in Asia
ACRONYM	DeTACT-Asia
Trial Design	A partially blinded randomised controlled non-inferiority trial comparing the efficacy, tolerability and safety of Triple ACTs artemether-lumefantrine+amodiaquine (AL+AQ) and artesunate- mefloquine+piperaquine (ASMQ+PPQ) and the ACTs artemether-lumefantrine+placebo (AL+PBO), artesunate-mefloquine+placebo (ASMQ+PBO) (with single-low dose primaquine in some sites) for the treatment of uncomplicated *Plasmodium falciparum* malaria to assess and compare their efficacy, safety, tolerability.
Trial Participants	Subjects with acute uncomplicated *P. falciparum* malaria
Sample size	6 sites (5 countries) in Asia each site recruiting 228 subjects Currently identified sites are 1 each in Cambodia, Myanmar, Bangladesh, Indonesia and 2 sites in India. Note: In India, 2 locations in Rourkela and Midnapore will function as one site. In Cambodia, three locations Kravanh,Siem Pang and Chambak will function as one site. (detail in Appendix 3) Estimated sample size up to 1,368 subjects.
Inclusion Criteria	• Male or female, ≥ 6 months • Ability to take oral medication • Acute uncomplicated *P. falciparum* monoinfection • Asexual *P. falciparum* parasitaemia: 96 to 200,000/µL, determined on a peripheral blood film • Fever defined as ≥ 37.5°C tympanic temperature or a history of fever within the last 24 hours • Written informed consent by the subject or by the parent/guardian in case of children lower than the age of consent and assent if required (per local regulations) • Willingness and ability of the subjects or parents/guardians to comply with the study protocol for the duration of the study

Exclusion Criteria	• Signs of severe malaria (adapted from WHO criteria see section 8.4) • Patients not fulfilling criteria for severe malaria but with another indication for parenteral antimalarial treatment at the discretion of the treating physician • Hematocrit < 20% at screening • Subjects who have received artemisinin or a derivative within the previous 7 days OR lumefantrine or amodiaquine within the previous 14 days OR mefloquine or piperaquine within the previous 30 days • Acute illness other than malaria requiring systemic treatment • Severe acute malnutrition • Known HIV infection • Known tuberculosis infection • For females: pregnant, trying to get pregnant or are lactating • History of allergy or known contraindication to any of the study drugs, including neuropsychiatric disorders and epilepsy • Previous splenectomy • Enrolment in DeTACT in the previous 3 months • Participation in another interventional study in the previous 3 months
Planned Trial Period	Projected recruitment of 20 months March 2021 – October 2022; study projected to finish in March 2023.
Primary Objective	To compare the efficacy of the ACTs and TACTs as defined by the 42-day PCR corrected adequate clinical and parasitological response (ACPR) within each individual site
Secondary Objectives	• To compare the safety and tolerability of ACTs and TACTs within and across sites and regions, including comparisons of 'non-matching' ACTs vs TACTs (AL+PBO vs ASMQ+PPQ, ASMQ+PBO vs AL+AQ) • To compare additional measures of treatment efficacy between treatment arms, including the 63-day ACPR, the post-treatment prophylactic effect of ACTs and TACTs defined as the 42-day and 63-day PCR uncorrected ACPR, gametocyte carriage, parasite clearance rates and fever clearance. • To assess pharmacokinetic and pharmacodynamic interactions between antimalarials in TACTs

Exploratory Objectives	• To assess molecular genetic and transcriptomic correlates for artemisinin and partner drug resistance of the infecting *P. falciparum* strains and to assess their *ex vivo* and *in vitro* drug susceptibility. • To compare the selective effect of ACTs versus TACTs on parasites carrying mutations associated with resistance to antimalarial drugs • To obtain additional safety data (in particular incidence, rate and magnitude of haemolysis) on the deployment of single low dose primaquine, stratified according to G6PD status/genotype • To obtain additional data on the effect of the host genotypes known to affect pharmacokinetics and pharmacodynamics of antimalarials • To obtain data on the place of residence, work, recent travel history and mobile phone usage in order to improve the understanding of local malaria transmission by identifying behaviours and risk factors associated with malaria infection and possible routes of spread of malaria and artemisinin resistance • To assess new methods for determination of gametocytaemia, parasite phenotypes and genotypes • To assess the correlation between anti-*Plasmodium falciparum* antibodies and drug efficacy measures • To assess the prevalence of parasites carrying complete or partial deletions in *pfhrp2* gene
Primary endpoint	42-day efficacy defined as PCR-corrected adequate clinical and parasitological response (ACPR).
Secondary endpoints	• <u>For safety and tolerability</u>: Incidence of adverse events and serious adverse events within the first 42 days including markers of hepatic, renal or bone marrow toxicity; cardiotoxicity, in particular QT or QTc-interval above 500 ms at timepoint H4 and H52/H64 and between these time points; change in haemoglobin concentration at day 3, 7, 28, stratified for G6PD status/genotype; proportion of subjects requiring retreatment due to vomiting within 1 hour after administration of the study drugs; proportion of subjects 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

	• For efficacy: 63-day PCR corrected and uncorrected efficacy; 42-day PCR uncorrected efficacy; parasite clearance half-life assessed by microscopy as primary parameter to determine parasite clearance; proportion of subjects with microscopically detectable *P. falciparum* parasitaemia at Day 3; fever clearance time (i.e. the time taken for the tympanic temperature to fall below 37.5 ºC in patients who were febrile at inclusion); proportion of subjects with gametocytaemia during and after treatment stratified by presence of gametocytes at enrolment. • For PK/PD interactions: Pharmacokinetic profiles and interactions (including C_{max} and AUC) of artemisinin-derivatives and partner drugs in ACT and TACT treated subjects in correlation with pharmacodynamics measures of drug efficacy; day 7 plasma levels of partner drugs in correlation with treatment efficacy and treatment arm
Exploratory endpoints	• Comparison of 63-day vs 42-day PCR corrected and uncorrected efficacy of ACTs vs TACTs • Proportions of recurrent infections with parasites carrying mutations of known functional significance • Proportions of specimens collected at baseline with parasites carrying mutations of known functional or operational significance (*pfkelch13*, *pfcrt*, *pfmdr1*, *pfdhfr*, *pfdhps*, *pfplasmepsin2 ,* partial or complete deletions of *pfhrp2* and other current parasite genetic markers associated with resistance or identified over the course of the study) • Candidate markers of resistance identified through genome wide association studies with *in vivo* or *in vitro* parasite drug sensitivity phenotypes • *In vitro* sensitivity of *P. falciparum* to artemisinins and partner drugs according to study sites and genotype • Accuracy of SNPs assessment from dry blood spots versus from whole genome sequencing in leukocyte depleted blood samples • Correlation between qPCR based versus microscopy based assessments of parasite clearance dynamics • Correlation of parasite clearance metrics as assessed by microscopy versus digital microscopy • Comparison of transcriptomic patterns of drug sensitive and resistant parasites before treatment and 6, 12 and 24 hours after start of treatment • 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

	• Correlation between the host genotype (e.g., CYP2D6, CYP3A4, KCNQ1/LQT1, KCNH2/LQT2, SCN5A/LQT3) and the pharmacokinetics and pharmacodynamics of antimalarials. • Correlations between the place of residence, work, recent travel history assessed by interview and mobile phone records to identify behaviours and risk factors associated with malaria infection. • Correlation between specific antibody titres and measures of drug efficacy
Drugs See Appendix 2 for dosing regimens	Artemether-lumefantrine+placebo for 3 days. VERSUS Artemether-lumefantrine+amodiaquine for 3 days. VERSUS Artesunate-mefloquine+placebo for 3 days VERSUS Artesunate-mefloquine+piperaquine for 3 days Primaquine According to the WHO guideline, all subjects except children under 10 kilograms will also be treated with a single dose of primaquine according to the local requirements or guidelines and WHO treatments schedule as a gametocytocidal treatment. Primaquine will not be deployed in study sites with median to high levels of malaria transmission (estimated Annual Parasite Incidence ≥ 50 per 1000 population per year according to the WHO World Malaria Report 2017)

3. BACKGROUND, RATIONALE AND PROJECT STRUCTURE

3.1. Background

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

The Tracking Resistance to Artemisinin Collaboration (TRAC), coordinated by the Mahidol Oxford Research Unit (MORU) at sites in Asia and Africa, mapped areas of artemisinin resistance, defined as slow parasite clearance (clearance half-life >5 hours) [1]. In many areas with artemisinin resistance, this has now been compounded by ACT partner drug resistance [2]. Consequently, treatment failures after artemisinin combination therapies are becoming more widespread in Southeast Asia. Failures rates of >60% for dihydroartemisinin-piperaquine have been documented in Cambodia, Thailand and Vietnam [3-5], (TRACII project unpublished data).

A lineage of artemisinin and piperaquine resistant parasites originating in Western Cambodia is now well established in Cambodia, Vietnam and Thailand [6]. In Cambodia, the first line treatment for uncomplicated *P. falciparum* malaria has recently been changed to artesunate-mefloquine, but it is feared that the current high efficacy of this combination can be lost rapidly.

Loss of efficacy of first line ACTs jeopardizes current malaria control and elimination efforts and will accelerate the spread of drug 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 subsequently to Africa, which bears the vast majority of the global malaria burden. Furthermore, artemisinin resistance could worsen by extending beyond the current ring stage parasite resistance, although this has until now not been observed.

New antimalarial drugs are not expected to come to the market within the next 5 years. There is an urgent need to evaluate alternative treatments using combinations of existing drugs which will not fall rapidly to resistance and can be deployed immediately. These treatments are needed now for areas in the Greater Mekong Subregion where ACTs are increasingly failing. In addition, regions where ACTs are currently still effective, including Myanmar, Bangladesh, India and Sub-Saharan Africa, will need to be prepared with alternative treatments in case multidrug resistant falciparum malaria is imported or emerges *de novo*. For these regions it is important to develop strategies to prevent the spread or delay the emergence of artemisinin and ACT partner drug resistance and deployment of Triple Artemisinin Combination Treatments (TACT) could be part of such a strategy.

The underlying scientific rationale for TACTs is similar to that underlying current ACTs and the treatment of tuberculosis, Human Immunodeficiency Virus, *Helicobacter pylori*, and several other infections. If drugs with different targets or resistance mechanisms are combined, then the probability that resistance will emerge to the combination will usually equate to the product of the

individual components' resistance probabilities. As these individual probabilities are very small, combining drugs is a powerful method of reducing the emergence of resistance [7]. The emergence and survival of a mutant organism which is spontaneously (i.e. *de novo*) resistant to two or more drugs is rare.

There appears to be a fortuitous inverse relationship between the resistance mechanisms to lumefantrine and amodiaquine. A similar inverse relationship might exist for piperaquine and mefloquine. Furthermore both drug pairs have reasonable well matched elimination kinetics providing mutual protection against resistance throughout the elimination phase of the remaining parasitaemia. Evidence supporting the counteracting resistance mechanisms of amodiaquine versus lumefantrine and piperaquine versus mefloquine comes from molecular genetic and genetic epidemiological studies. Transfection experiments in *Plasmodium falciparum* showed that introduction of N86Y into the parasite gene *pfmdr1* confers amodiaquine resistance *in vitro* and this mutation is associated with increased rates of clinical failure after amodiaquine monotherapy [8, 9]. Yet this mutation increases *in vitro* drug sensitivity to lumefantrine. Whereas the mutant allele is more commonly seen in recurrent infections after artesunate-amodiaquine, the wild-type allele is selected after artemether-lumefantrine [10].

Amplification of the *pfmdr1* (wild type) gene is associated with mefloquine resistance [11]. In Cambodia, where mefloquine resistance was widespread, it was observed that as piperaquine resistance emerged, sensitivity to mefloquine was restored and prevalent *P. falciparum* strains reversed to a single copy *pfmdr1* genotype [12, 13]. Although this coincided with the decrease in mefloquine drug pressure, the observation that the marker for piperaquine resistance (amplification of the *pfplasmepsin2* gene) is rarely detected in conjunction with the marker of mefloquine resistance (amplification of the *pfmdr1* gene) suggests opposing resistance mechanisms [14, 15].

Whether this 'mutual exclusiveness' represents a role of *pfmdr1* in piperaquine resistance (for instance by restricting the amount of piperaquine entering the food vacuole) or is simply the effect of a relative low mefloquine pressure in the last decade in Cambodia and Vietnam needs further investigation. Importantly, although in low frequency, parasites bearing both markers of mefloquine resistance and piperaquine resistance (*pfmdr1* and *pfpfplasmepsin2-3* amplification, respectively) have been found in field isolates [16], and its prevalence was recently reported to have increased in Preah Vihear province in Cambodia, after the reintroduction of artesunate-mefloquine as first line treatment [17]. The consequence of the double amplification of *pfmdr1* and *pfplasmepsin2-3* for efficacy of the TACT DHA-piperaquine-mefloquine has not been established.

An increase in the QTc-interval following intake of the antimalarials piperaquine and amodiaquine has been described [18-22]. In the recent TRACII study conducted primarily in adults, we also observed that the QTc interval increased after administration of DHA-piperaquine by a mean of around 20 milliseconds. Reassuringly, the addition of mefloquine to DHA-piperaquine did not lead to a further increase of the QTc-interval. Further, the incidence of a QTc prolongation following treatment of more than 60 milliseconds was comparable between subjects treated with DHA-piperaquine and DHA-piperaquine + mefloquine. Data from the TRACII study also confirmed that a treatment with artemether-lumefantrine does not prolong the QTc-interval whereas the addition of amodiaquine to artemether-lumefantrine prolonged the QTc-interval by about 10 milliseconds

over the first 64 hours, which conforms to what has been previously described for amodiaquine. Amodiaquine did not increase the incidence of a QTc-prolongation >60 milliseconds above baseline, which is rare in the treatment with artemether-lumefantrine alone (about 1 in 300 treatments). Any antimalarial treatment is associated with a drop in the heart rate in the first day of treatment. As the fever and/or clinical illness subsides the heart rate generally decreases. The TRACII study confirmed previous findings that amodiaquine use is associated with a bigger decrease in heart rate compared to other antimalarials and a higher incidence of bradycardia (<54 beats/minute), both in adults and children.

3.2. Study rationale

The TRACII study has examined the safety, tolerability and efficacy of different Triple Artemisinin based Combination Therapies (TACTs) such as DHA-piperaquine+mefloquine (DHA-PPQ+MQ) and artemether-lumefantrine+amodiaquine (AL+AQ). The preliminary results of this study show that the TACT artemether-lumefantrine+amodiaquine is as effective and safe as the standard ACT artemether-lumefantrine, even in children recruited in the Democratic Republic of Congo. Further, the TACT DHA-PPQ+MQ was found to be highly efficacious at study sites in Cambodia, Thailand and Vietnam, even though the efficacy of the ACT DHA-PPQ was poor at these sites. However, in a study assessing the pharmacokinetics of the TACT DHA-PPQ+MQ in healthy volunteers, a significant drug-drug interaction was observed wherein the addition of MQ to the ACT DHA-PPQ significantly reduced the absorption of DHA (van der Pluijm et al.; manuscript in preparation). Similarly, a substantial reduction in the absorption of DHA was also observed in malaria patients included in the TRACII study though this did not affect parasite clearance half-lives. Taken together, these data indicate that there is an increased risk of sub-therapeutic exposure to DHA at its current dosage when co-administered with piperaquine and mefloquine as a TACT. Therefore, drug combinations wherein DHA (target dose of 2.4 mg/ kg in the current fixed dose combination) is replaced with artesunate (target dose of 4 mg/kg), which is completely metabolised to DHA very rapidly after absorption [23], will be tested in this study as artesunate-mefloquine (ASMQ) with or without PPQ (ASMQ+PPQ) along with AL with or without AQ.

Co-blistered or co-formulated TACTs could be rapidly deployed in areas with multidrug resistant malaria. For other regions, the safety and efficacy of TACTs should be assessed in the local setting in order to guarantee rapid deployment in case resistance starts affecting treatment efficacy. Furthermore, deployment of TACTs on a wide scale could prevent or delay the emergence and spread of antimalarial resistance in areas where this has not yet happened. The efficacy, safety and tolerability of TACTs need extensive evaluation in adults and children before policy recommendations can change and widespread deployment can be considered.

Therefore, we aim to conduct a large scale randomized clinical trial in 5 Asian countries to compare the efficacy, safety and tolerability of the TACTs artemether-lumefantrine+amodiaquine and artesunate-mefloquine+piperaquine and the standard ACTs artemether-lumefantrine+placebo, and artesunate-mefloquine+placebo. Given that the observations of low incidence of QTc prolongation are from a relatively small population, they need further monitoring and confirmation. Accordingly, in this study electrocardiograph measurements (ECGs) will also be performed at baseline and several time points thereafter to assess the effect of antimalarials on the QT or QTc interval.

In addition to the main clinical outcomes of the study, we aim to assess the extent of the spread or the *de novo* emergence of resistance to the antimalarials in the combinations through detailed *in vivo* and *in vitro* assessments and to study the pharmacokinetics and inter-drug interactions of the drugs, the parasite- and host-related factors affecting treatment outcomes. Finally, we aim to gain insights into the spread of resistance mediated by population movements and parasite gene flows in general through travel surveys and geographic localization of parasite genetic data.

4. OBJECTIVES

4.1. Primary objectives

To compare the efficacy of the matching ACTs and TACTs as defined by the 42-day PCR corrected adequate clinical and parasitological response (ACPR) within each individual site

Matching comparisons of ACT and TACT are as follows:

- AL+PBO versus AL+AQ
- ASMQ+PBO versus ASMQ+PPQ

4.2. Secondary objectives

- To compare the safety and tolerability of ACTs and TACTs within and across sites and regions, including comparisons of 'non-matching' ACTs vs TACTs (AL+PBO versus ASMQ+PPQ, ASMQ+PBO versus AL+AQ).
- To compare additional measures of treatment efficacy between treatment arms, including the 63-day ACPR, the post-treatment prophylactic effect of ACTs and TACTs defined as the 42-day and 63-day PCR uncorrected ACPR, gametocyte carriage, parasite clearance rates and fever clearance.
- To assess pharmacokinetic and pharmacodynamic interactions between antimalarials in TACTs

4.3. Exploratory objectives

- To assess molecular genetic and transcriptomic correlates for artemisinin and partner drug resistance of the infecting *P. falciparum* strains and to assess their *ex vivo* and *in vitro* drug susceptibility.
- To compare the selective effect of ACTs versus TACTs on parasites carrying mutations associated with resistance to antimalarial drugs
- To obtain additional safety data (in particular incidence, rate and magnitude of haemolysis) on the deployment of single low dose primaquine, stratified according to G6PD status/genotype
- To obtain additional data on the effect of the host genotypes known to affect pharmacokinetics and pharmacodynamics of antimalarials

- To obtain data on the place of residence, work, recent travel history and mobile phone usage in order to improve the understanding of local malaria transmission by identifying behaviours and risk factors associated with malaria infection and possible routes of spread of malaria and artemisinin resistance

- To assess new methods for determination of gametocytaemia, parasite phenotypes and genotypes

- To assess the correlation between anti-*Plasmodium falciparum* antibodies and drug efficacy measures

- To assess the prevalence of parasites carrying complete or partial deletions in *pfhrp2* gene

5. PRIMARY, SECONDARY AND EXPLORATORY ENDPOINTS

5.1. Primary endpoint

42-day efficacy defined as PCR corrected adequate clinical and parasitological response (ACPR).

5.2. Secondary endpoints

- For safety and tolerability: Incidence of adverse events and serious adverse events within the first 42 days including markers of hepatic, renal or bone marrow toxicity; cardiotoxicity, in particular QT or QTc-interval above 500 ms at timepoint H4 and H52/H64 and between these time points; change in hemoglobin concentration at D3, 7, 28 stratified for G6PD status/genotype; proportion of subjects requiring retreatment due to vomiting within 1 hour after administration of the study drugs; proportion of subjects 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

- For efficacy: 63-day PCR corrected and uncorrected efficacy; 42-day PCR uncorrected efficacy; parasite clearance half-life assessed by microscopy as primary parameter to determine parasite clearance; proportion of subjects with microscopically detectable *P. falciparum* parasitaemia at Day 3; fever clearance time (i.e. the time taken for the tympanic temperature to fall below 37.5 ºC in patients who were febrile at inclusion); proportion of subjects with gametocytaemia during and after treatment stratified by presence of gametocytes at enrolment.

- For PK/PD interactions: Pharmacokinetic profiles and interactions (including C_{max} and AUC) of artemisinin-derivatives and partner drugs in ACT and TACT treated subjects in correlation with pharmacodynamics measures of drug efficacy; day 7 plasma levels of partner drugs in correlation with treatment efficacy and treatment arm

5.3. Exploratory endpoints

- Comparison of 63-day vs 42-day PCR corrected and uncorrected efficacy of ACTs vs TACTs
- Proportions of recurrent infections with parasites carrying mutations of known functional significance
- Proportions of specimens collected at baseline with parasites carrying mutations of known functional or operational significance (*pfkelch13*, *pfcrt*, *pfmdr1*, *pfdhfr*, *pfdhps*, *pfplasmepsin2 ,* partial or complete deletions of *pfhrp2* and other current parasite genetic markers associated with resistance or identified over the course of the study)
- Candidate markers of resistance identified through genome wide association studies with *in vivo* or *in vitro* parasite drug sensitivity phenotypes
- *In vitro* sensitivity of *P. falciparum* to artemisinins and partner drugs according to study sites and genotype
- Accuracy of SNPs assessment from dry blood spots versus from whole genome sequencing in leukocyte depleted blood samples
- Correlation between qPCR based versus microscopy based assessments of parasite clearance dynamics
- Correlation of parasite clearance metrics as assessed by microscopy versus digital microscopy
- Comparison of transcriptomic patterns of drug sensitive and resistant parasites before treatment and 6, 12 and 24 hours after start of treatment
- 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
- Correlation between the host genotype (e.g., CYP2D6, CYP3A4, KCNQ1/LQT1, KCNH2/LQT2, SCN5A/LQT3) and the pharmacokinetics and pharmacodynamics of antimalarials.
- Correlations between the place of residence, work, recent travel history assessed by interview and mobile phone records to identify behaviours and risk factors associated with malaria infection.
- Correlation between specific antibody titres and measures of drug efficacy

6. TRIAL DESIGN

6.1. Study sites

The study will take place in Cambodia, Myanmar, Bangladesh, Indonesia and 2 sites in India. Two sites in India, one will be in Agartala and the second will be constituted of two institutions in India, located in Rourkela and Midnapur, which will function as a single site and will recruit a total of 228 subjects through competitive enrollment. Similarly three locations in Cambodia located in Kravanh,Siem Pang and Chambak, will function as a single site and will recruit a total 228 subjects through competitive enrollment.

Estimated sample size is 1368 subjects.

6.2. Summary of trial design

A partially blinded randomised controlled non-inferiority trial of the Triple ACTs artemether-lumefantrine + amodiaquine (AL+AQ) and artesunate- mefloquine+piperaquine (ASMQ+PPQ) with the ACTs artemether-lumefantrine + placebo (AL+PBO) and artesunate- mefloquine + placebo (ASMQ+PBO) for the treatment of uncomplicated *Plasmodium falciparum* malaria to assess and compare their efficacy, safety, tolerability. (See sub-section 9.4 for further details regarding partial blinding).

6.3. Study duration

Projected recruitment duration of 20 months March 2021 – October 2022; study projected finish in March 2023.

7. PROPOSED ACTIVITIES

Subjects will be randomized to up to four arms: artemether-lumefantrine + amodiaquine, artemether-lumefantrine + placebo, artesunate-mefloquine + piperaquine and artesunate-mefloquine + placebo. As a contingency measure in case of significant differences in the efficacy or safety of one of the combinations being tested and/or study drug expiry or unavailability, subjects may be randomised to 2 arms with a matching ACT-TACT pair, i.e., with artemether-lumefantrine + placebo or artemether-lumefantrine + amodiaquine OR artesunate-mefloquine + placebo or artesunate-mefloquine + piperaquine.

Some sites may randomize between 2 arms only with matching ACT-TACT pairs, i.e., artemether-lumefantrine + placebo or artemether-lumefantrine + amodiaquine OR artesunate-mefloquine + placebo or artesunate-mefloquine + piperaquine.

In the control arms, the ACT will be co-packed with a matched (appearance) placebo.

In lower transmission settings (Annual Parasite Incidence <50 per 1000 population per year) the treatment will include a single 0.25 mg/kg gametocytocidal dose of primaquine as recommended by the WHO for children ≥10 kg. All drug administrations will be observed.

Subjects will be treated in an in-patient unit for 3 days and followed up weekly up to D63. Microscopy to detect and quantify malaria parasitaemia will be performed daily (more frequently in patients with parasite density of >5000/µL at inclusion) during hospitalization, at all weekly and unscheduled visits. A physical examination and measurements of vital signs along with a symptom questionnaire for tolerability will be performed and recorded through a standardized

method at baseline, daily during admission and weekly during follow up through D42 and at all unscheduled visits. Physical exam, vital sign measurements and assessments of symptoms will be performed on D49, D56, and D63 only for patients who are parasitaemic or those who report fever or other symptoms. Electrocardiographs will be performed during admission (H0, H4, H52, or H64) and day 42 of follow up to assess and compare the effect of ACTs and TACTs antimalarials on QT or QTc-intervals.

Safety assessments will be performed by measuring markers of renal and hepatic toxicity, haemoglobin, platelet counts, absolute and differential white blood cell counts and ECGs. Pharmacokinetic profiles in a subset of subjects will be linked to measures of efficacy and toxicity. To avoid extensive blood sampling, especially in children, a population pharmacokinetic approach will be followed, requiring sparse blood sampling reducing the burden of sampling for the patient [21].

Blood samples for parasite genotyping as well as genomic, transcriptomic and immunology studies will be collected at baseline and at selected time-points on follow-up and also on the day of a recurrent infection.

In patients with parasite densities >5000 per µL at screening, parasite clearance rates will be assessed by repeated assessments of the parasite counts after the start of the antimalarial treatments. At each visit a capillary blood sample will be taken for parasite count.

If a patient presents with a new uncomplicated falciparum malaria infection after 3 months or longer after the initial enrolment, the patient or relative (in case of a child) will be asked to re-enter the patient in the trial.

In selected study sites, *P. falciparum* parasites will be cryopreserved for *in vitro* antimalarial drug sensitivity testing. Where possible, e*x vivo* or *in vitro* assessments of parasite susceptibility to artemisinins and partner drugs will be assessed.

The *in vivo* and *ex vivo* data on artemisinin and partner drug sensitivity will potentially be used to identify new genetic or transcriptomic markers/patterns of artemisinin or partner drug resistance.

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.

Subjects 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 which can inform future malaria studies or interventions.

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.

8. TRIAL PARTICIPANTS

8.1. Overall description of trial participants

Asian sites: Male and non-pregnant female subjects aged 6 months and above with acute uncomplicated falciparum malaria are the target study population. All study subjects must meet the applicable inclusion and exclusion criteria.

8.2. Inclusion criteria

- Male or female, ≥ 6 months
- Ability to take oral medication
- Acute uncomplicated *P. falciparum* monoinfection
- Asexual *P. falciparum* parasitaemia: 96 to 200,000/µL, determined on a peripheral blood film
- Fever defined as ≥ 37.5°C tympanic temperature or a history of fever within the last 24 hours
- Written informed consent by the subject or parent/guardian in case of children lower than the age of consent and assent if required (per local regulations)
- Willingness and ability of the subjects or parents/guardians to comply with the study protocol for the duration of the study

8.3. Exclusion criteria

- Signs of severe malaria (adapted from WHO criteria see section 8.4)
- Patients not fulfilling criteria for severe malaria but with another indication for parenteral antimalarial treatment at the discretion of the treating physician
- Haematocrit < 20% at screening
- Subjects who have received artemisinin or a derivative within the previous 7 days OR lumefantrine or amodiaquine within the previous 14 days OR mefloquine or piperaquine within the previous 30 days
- Acute illness other than malaria requiring systemic treatment
- Severe acute malnutrition
- Known HIV infection

- Known tuberculosis infection
- For females: pregnant, trying to get pregnant or are lactating
- History of allergy or known contraindication to any of the study drugs, including neuropsychiatric disorders and epilepsy
- Previous splenectomy
- Enrolment in DeTACT in the previous 3 months
- Participation in another interventional study in the previous 3 months

8.4. Criteria for severe malaria

- Impaired consciousness (Glasgow Coma Scale, Blantyre Coma Scale)
- Prostration
- Respiratory distress (defined as maximal respiratory rate, by age)
- ≥2 convulsions in the past 24 hours
- Circulatory collapse
- Pulmonary oedema
- Abnormal bleeding
- Visible jaundice
- Haemoglobinuria (blackwater)
- Hyperparasitaemia (>10%)

9. PROCEDURES

Study procedures will be performed according to the schedule of assessments (Appendix 1). Potential patients will be screened. If eligible and they have agreed to enter the trial, they will be admitted to the hospital for at least 72 hours and continue to be followed up as outpatients on a weekly basis until Day 63 (9 weeks).

9.1. Informed consent

Full consent (and assent if required) will be obtained before any study specific enrolment procedures are conducted. It will be made clear from the outset that refusal to participate will not jeopardise subsequent antimalarial treatment.

The patient (or witness if illiterate) and/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 and/or parent/guardian will be allowed as much time as possible to consider the information and to 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. As far as possible, no more than two hours should elapse between presentation and treatment either within or outside of the study. For sites where this time window is not sufficient to complete all study-related procedures required before treatment, local approval will be sought to increase the time allowed for pre-treatment procedures.

Written informed consent will be obtained by means of the participant's or the participant's guardian's dated signature or thumb print (if unable to write), and the consent form must be signed and dated by a literate witness 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 or affix a thumbprint on an assent form. A copy of the signed informed consent/assent document(s) will be given to the subjects/parents.

9.2. Screening procedures

Subjects who present at the participating sites will be screened to assess eligibility using the inclusion and exclusion criteria summarized in section 8.2 and 8.3.

A screening form will be completed for all subjects. A screening log will be kept.

EDTA-anticoagulated blood (0.5 ml) will be collected for:

- Parasite count from Giemsa stained thick and thin blood films with or without malaria rapid diagnostic test
- Haematocrit

After obtaining consent a urine pregnancy test for females of child bearing potential will be performed.

9.3. Baseline assessments

9.3.1. Demographic 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.

9.3.2. Drug history

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

9.4. Randomization and blinding

In Asia, subjects who fulfil all the inclusion criteria and have none of the exclusion criteria will be randomised in an ACT:TACT:TACT:ACT ratio of 1:1:1:1, with the ACT arms consisting out of an ACT and placebo.

The study drug will be provided by the pharmaceutical company in co-blistered or co-packed form in boxes.

The boxes containing artemether-lumefantrine+amodiaquine and artemether-lumefantrine +placebo will be identical, and a combination of both study drugs boxes will be sequentially numbered.

Also, the boxes containing artesunate- mefloquine+piperaquine and artesunate-mefloquine +placebo will be identical, and a combination of both study drugs boxes will be sequentially numbered.

The absorption of lumefantrine is increased if the artemether-lumefantrine is taken with fat, which increases the efficacy. Therefore artemether-lumefantrine containing arms should be taken with fat.

The absorption of piperaquine is also increased if it is taken with fat and can hence lead to increased prolongation of the QT or QTc interval. Therefore artesunate-mefloquine containing arms (with PPQ or PBO) should not be taken with fat.

For this reason it is important that the investigator knows whether the allocated treatment should be taken with a fatty snack or drink or not. Therefore the randomization envelope will state that the allocated treatment potentially contains artemether-lumefantrine or artesunate-mefloquine. However it will not state whether a second partner drug (amodiaquine or piperaquine is included in the treatment). Furthermore the boxes containing either artemether-lumefantrine(+/-amodiaquine) or artesunate-mefloquine(+/-piperaquine) will be clearly distinguishable.

The subjects 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 whether the treatment for the subject should be taken from the set of artemether-lumefantrine+/-amodiaquine or the artesunate-mefloquine+/-piperaquine containing set. Block randomisation will be used.

This study will be partially blinded since it will not be known to the investigators nor the participants at the time of randomisation or drug administration whether they have been assigned to an ACT or a TACT arm. However, they will know whether they have been assigned to the AL or ASMQ based arms for the reasons outlined above and also given the differences in dosing, i.e., 6 doses for AL based arms vs 3 doses for ASMQ based arms.

9.5. Clinical procedures

9.5.1. Clinical procedures at baseline

- Standardized symptom questionnaire
- Physical examination
- Weight, height, pulse, blood pressure, respiratory rate, temperature.

- Assessment of malnutrition status: Mid-Upper Arm Circumference (MUAC), presence and grading of (nutritional) oedema (if present)
- Spleen and liver size will be recorded if palpable.

9.5.2. Clinical procedures during hospitalization

Daily until day of discharge:

- Standardized symptom questionnaire
- Physical examination
- Pulse, blood pressure, respiratory rate
- Tympanic temperature (every 6 hours until two times <37.5 degrees Celsius)
- Recording of vomiting and retreatment
- Recording of adverse events

9.5.3. 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, subjects or their guardians will be asked a short set of questions on their place of residence, place of work/location of school if applicable 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 will use anonymised, aggregated data on mobile phone use to model 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 subjects with malaria.

We will review these travel histories obtained in the hospital and then select a representative sample of subjects 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.

In some sites, we will GPS the households of all subjects 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 de-identified so that no individual can be identified from their treatment records, through interviews, or from mapping data.

9.5.4. Clinical procedures during follow up

Day 7, 14, 21, 28, 35 and 42

- Standardized symptom questionnaire
- Physical examination

- Pulse, blood pressure, respiratory rate
- Tympanic temperature
- Recording of adverse events

Day 49, 56 and 63

In principle follow up at day 49, 56 and 63 will consist only of performing a tympanic temperature measurement, blood smear, and dried blood spot. If the subject indicates new symptoms and/or a positive blood smear is found the subject will be seen by the study clinician and the following will be performed. A urine pregnancy test will be performed for women of child-bearing age at D63 only.

- Standardized symptom questionnaire
- Physical examination
- Pulse, blood pressure, respiratory rate
- Recording of adverse events

9.5.5. Clinical procedures in case of a recurrent infection

- Standardized symptom questionnaire
- Physical examination
- Weight, height, pulse, blood pressure, respiratory rate
- Tympanic temperature
- Completion of recurrent infection form
- Treatment of recurrent infection.
- Recording of treatment for recurrent infection in the concomitant medication form

Subjects 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 the same or another ACT or a different combination e.g. quinine and doxycycline or clindamycin, in accordance with local recommendations.

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

In case of a recurrent infection, subjects should still be followed up according to the study protocol until day 63. 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.

9.5.6. Clinical procedures in case of unscheduled visits

Subjects presenting to the clinic with a fever or other clinical symptoms on unscheduled days will be assessed by the study physician. In any case the following will be recorded:

- Standardized symptom questionnaire
- Physical examination
- Pulse, blood pressure, respiratory rate
- Tympanic temperature
- Recording of adverse events

Subjects will be treated and further investigations conducted 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.

9.6. Electrocardiographic recording

Electrocardiograms (ECGs) will be recorded at D0H0, D0H4, D2H52 (for treatments containing ASMQ) or D2H64 (for treatments containing AL) and D42 in order to assess changes in the QT or QTc-interval.

At the timepoint D0H4 and D2H52/D2H64 an abnormal automated electronic reading (QT or QTc-interval > 500 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 work instruction will describe procedures such as identifying other causes of QT/QTc-prolongation, study drug replacement and follow up of the QT/QTc-interval until normalization. QT/QTc intervals will be measured every six hours after the first abnormal QT/QTc-interval is recorded until the QT/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 QT/QTc-interval and 6 and 12 hours after the first identification of the abnormal QT/QTc-interval in order to assess the correlation between the prolonged QT/QTc-interval and the drug levels that could be leading to the prolonged QT/QTc-interval.

9.7. Blood sampling

9.7.1. On admission/baseline

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 (200 microlitres, 4 spots collected on chromatography papers) for parasite and host genotyping (see section 14.3 for details on parasite genotyping and section 9.7.6 for details on host genotyping).
- Full blood count OR WBC differential, HCT, Hb (0.5 mL EDTA-anticoagulated)

- Parasite DNA and RNA measurements (packed cells from up to 10 ml for adult, up to 5 ml for child) EDTA-anticoagulated blood; an aliquot of plasma will be stored for immunological assessments.
- *Ex vivo/in vitro* parasite cultivation for drug sensitivity and transcriptomic assays (3 ml adult, 2 ml child, heparinised blood, some sites).
- Baseline biochemistry assessments including bilirubin, ALT, AST, Alkaline Phosphatase and creatinine levels (1 mL heparin)
- Baseline pharmacological measurement (1 mL heparin)

9.7.2. During hospitalization

Blood smears and hematocrit

Subjects with a parasite density of >5000 per µL will have blood taken for malaria films at, 6h, 12h, 18h, 24h and thereafter every 12 hours until parasite clearance (when two consecutive malaria slides are negative).

In all cases, malaria films and hematocrit measurements will be performed at H24, H48 and H72 on all subjects (even if two consecutive negative blood smears have been seen before these timepoints)

Other sampling during hospitalization

- D0 H6, Day 0 H12: Parasite DNA/RNA measurements: EDTA-anticoagulated blood (1 mL). (some sites)
- Day 1 H24: Hct measurement (0.5 mL EDTA-anticoagulated)
- Day 1 H24: Parasite DNA/RNA measurements & qPCR: EDTA-anticoagulated blood (1 mL). (some sites)
- Day 2 H48: Hct measurement (0.5 mL EDTA-anticoagulated)
- D2 H48: For qPCR: EDTA-anticoagulated blood (1 mL). (some sites)
- D2H52 (for artesunate-mefloquine based arms) OR D2H64 (for artemether-lumefantrine based arms) – Pharmacological measurement sample (1 mL Heparin)
- Day 3 H72: biochemistry assessments including bilirubin, ALT, AST, Alkaline Phosphatase and creatinine levels: 1 mL heparinised blood
- Day 3 H72: For qPCR: EDTA-anticoagulated blood (1 mL). (some sites)
- Day 3 H72: Full blood count OR WBC differential, Hct and Hb. (0.5 mL EDTA-anticoagulated)

9.7.3. Blood sampling during follow up

Day 7

After subjects are discharged, they will be followed up at Day 7 for:

- Day 7: Malaria blood slide
- Day 7: Full blood count OR WBC differential, Hct and Hb (0.5 mL, EDTA-anticoagulated blood)
- Day 7: DNA/RNA for qPCR gametocyte quantification and characterization (0.5 mL Heparin & 0.5 mL EDTA blood in children and 1 mL each in adults),) (some sites)
- Day 7: Basic biochemistry assessments including bilirubin, ALT, AST, Alkaline Phosphatase and creatinine levels: 1 mL heparin blood
- Day 7: Pharmacological measurement (1 ml, heparin)

Day 14, 21, 28, 35 and 42

After day 7, subjects will be followed up weekly until Day 42 for:

- Malaria blood slide
- Day 14, 21, 28, 35 and 42: DNA/RNA for qPCR gametocyte quantification and characterization: (0.5 mL Heparin & 0.5 mL EDTA blood in children and 1 mL each in adults)) (some sites)
- Day 28: Basic biochemistry assessments including bilirubin, ALT, AST, Alkaline Phosphatase and creatinine levels. (1 mL heparin blood)
- Day 28: Full blood count OR WBC differential, Hct and Hb (0.5 mL EDTA-anticoagulated)

Day 49, 56 and 63; Unscheduled visits

After day 42, subjects will be followed up weekly until Day 63 for:

- Malaria blood slide
- Dried blood spot

9.7.4. Blood sampling in case of a recurrent infection

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

- Thick and thin films: parasite count.
- Dried blood spots (200 microlitres, 4 spots collected on chromatography paper) for parasite DNA genotyping
- Parasite DNA and RNA measurements (packed cells from up to 10 ml for adult, up to 5 ml for child EDTA-anticoagulated blood); an aliquot of plasma will be stored for immunological assessments.

- *Ex vivo/in vitro* parasite cultivation for drug sensitivity and transcriptomic assays (3 ml adult, 2 ml child, heparinised blood, some sites).
- Pharmacological measurement (1 ml, heparin)

9.7.5. Blood sampling for pharmacokinetics

PK sampling (1 ml whole blood in heparin per sampling occasion). Venous plasma will be used to evaluate the drug exposures to antimalarial drugs used in this study (E.g. artesunate, artemether, lumefantrine, amodiaquine, piperaquine, mefloquine, and their metabolites). A work instruction will be written to detail the technique for specimen collection, handling and storage. Samples will be centrifuged immediately and the plasma stored frozen at minus 80°C or lower in properly labelled cryotubes.

A maximum of seven samples for PK/PD analyses will be collected in each patient over a total sampling period of 42 days:

- One fixed day 0 sample will be collected for lumefantrine, amodiaquine, piperaquine, and mefloquine (and their metabolites) concentration measurements in all subjects.
- One sample will be collected systematically at the same time as the ECG at H52 (for ASMQ containing arms) and H64 (for AL containing arms)
- One fixed day 7 sample will be collected for lumefantrine, amodiaquine, piperaquine, and mefloquine (and their metabolites) concentration measurements in all subjects
- Two samples at random time-points during the first 3 days of dosing will be collected for artemether, artesunate, lumefantrine, amodiaquine, piperaquine, and mefloquine (and their metabolites) concentration measurements in 300 patients
- Two samples within random time windows during the 42 days of follow up will be collected for lumefantrine, amodiaquine, piperaquine, and mefloquine (and their metabolites) concentration measurements in 300 patients
- In addition to the sampling mentioned above, up to 3 additional samples will be collected during the 42 days of follow up from adults who weigh >70 kg (~5% of the patient population, based on the TRACII study) and receiving AL+amodiaquine or AL+placebo

Additional PK blood samples will be collected in the following situations:

- In case of an abnormal QT/QTc-interval at H4, D42. A blood sample will be obtained at the moment of the first identification of the abnormal QT/QTc-interval.
- At 6 and 12 hours after the first abnormal QT/QTc-interval at any time-point (H4, H52, H64, D42) in order to assess the correlation between the prolonged QT-interval and the drug levels that could result in a prolonged QT/QTc-interval (maximum 3 ml heparin blood).

- In case of a recurrent infection at the day of recurrence (1 ml heparin blood).

9.7.6. Host genotyping

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. Potential targets for targeted genotyping are polymorphisms in the G6PD genes coding for enzymes related to drug metabolism (e.g. cytochrome P450 enzymes) and genes and mutations predisposing to long QT-syndrome such as but not limited to the genes KCNQ1 (LQT1), KCNH2 (LQT2), and SCN5A (LQT3).

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 when 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 or therapeutic importance to the subject.

9.8. Special procedures related to baseline biochemistry measurements

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 (grade 3 or 4 of the CTCAE grading table) the study physician in consultation with the medical monitor will decide how to continue treatment and further management of the patient. In any case the subject will still be followed up according to the study protocol until day 63).

9.9. Time window for follow up visits

The time-window for the visit on Day 7 is + 1 day and for the visits on Days 14 - 63 is –1 to +2 days.

9.10. 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 subjects will develop a recurrent parasitaemia and be requested to give further blood samples.

Concerning blood volumes, an age of 12 years and older will be referred to as ‘adult’, an age of less than 12 years will be referred to as ‘child’.

Maximum blood volumes are presented below for adults and children for 63 days of follow up. The maximum blood volume is the total amount taken if the subjects 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 and/or unscheduled visit during follow up. The maximum blood volume will be approximately **69.5 ml for adults** and **48.5 ml for children**, which is less than 10% of total blood volume generally recommended as a maximum volume to be taken over 8 weeks.

The maximum blood volume that will be drawn in children below the age of 12 is **17.5 ml** in the first 24 hours of the study. This is below the 3 ml/kg in the first 24 hours as recommended only if subjects are ≥6 kg [24]. Accordingly, samples collected from children of <6 kg will only be those which are specifically required for the efficacy, safety and tolerability assessments, viz., blood smears, dried blood spots, venous samples for clinical biochemistry, haematology and PK samples.

10. STUDY DRUG

10.1. Study drug regimens

Overview DeTACT drug regimens	
TACT-arm	**ACT-arm**
In all sites	
Artemether-lumefantrine x 3 days **PLUS** **Amodiaquine x 3 days**	**Artemether-lumefantrine x 3 days** **PLUS** **Placebo x 3 days**
In all sites	
Artesunate-mefloquine x 3 days **PLUS** **Piperaquine x 3 days**	**Artesunate-mefloquine x 3 days** **PLUS** **Placebo x 3 days**
In low transmission settings subjects will receive a single low dose primaquine on day 1 according to the WHO guidelines and local regulations	

Subjects 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 subjects are hospitalised.

The study drugs containing artemether-lumefantrine+/-placebo 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 or snack).

No meals or snacks must be consumed for 1 hour before and after intake of study drugs containing artesunate-mefloquine+/-piperaquine. In case any food is taken, it must be non-fatty snacks (e.g., small quantity of rice, bread, crackers etc)

10.2. Drug administration procedures in case of vomiting

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. In this case the patient will be treated at the discretion of the investigator, for instance with intravenous artesunate.

10.3. Treatment in case of a recurrent infection

Subjects 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 an ACT or a different combination e.g. quinine and doxycycline or clindamycin, in accordance with local recommendations.

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

10.4. 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 (per WHO Treatment Guidelines, 3rd edition, 2015) as below:

- For subjects <20 kg bodyweight, 3 mg/kg IV/IM STAT, followed by 3 mg/kg IV/IM at 12 hours and 24 hours and then daily until able to take oral medication
- For subjects ≥20 kg bodyweight, 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.

If needed, the rescue treatment will be adjusted to local guidelines.

We will encourage the addition of parenteral quinine to the treatment of severe malaria in areas where artemisinin resistance is clearly established and will be 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.

10.5. 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 subjects 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 and non-falciparum malaria will be prescribed as described above. Any medication, other than the study medication taken during the study will be recorded in the CRF.

10.6. Study drug details

In the first year the Triple ACTs will consist out of co-blistered or co-packaged combinations of an ACT and a matching partner drug. If healthy volunteer studies comparing co-blistered/co-packaged and co-formulated formulations of Triple ACTs prove bioequivalence of all drugs the Triple ACT arms will be changed if possible to co-formulated formulations in the second year.

10.6.1. Artemether-lumefantrine

Currently available as dispersible 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.

10.6.2. Amodiaquine

Amodiaquine is available as dispersible tablets of 40 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.

10.6.3. Artesunate

Artesunate will be administered according to an optimised dosing schedule using tablets of 32 or 100 mg artesunate with a dosing target of 4 mg/kg/day (see dosing schedule in Appendix 2)

10.6.4. Mefloquine

Mefloquine will be administered according to an optimised dosing schedule using tablets of 70 or 220 mg mefloquine hydrochloride with a dosing target of 8.3 mg/kg/day (see dosing schedule in Appendix 2).

10.6.5. Piperaquine

Piperaquine will be administered according to an optimised dosing schedule using tablets of 160 or 500 mg of piperaquine tetraphosphate. t. The weight-based treatment schedule in appendix 2 aims for a dosage of approximately

- 24 mg/kg/day in patients <25 kg (range16.0 – 32.0 mg/kg) piperaquine for three days, thereby approaching the WHO-recommended target range of 20 – 32 mg/kg per day.
- 18 mg/kg/day in patients ≥25 kg (range 15.0 – 29.4 mg/kg) piperaquine for three days, thereby approaching the WHO-recommended target range of 16 – 27 mg/kg per day.

10.6.6. 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.

10.6.7. Placebo for piperaquine

Placebo tablets for piperaquine are currently under development. They will be identical in size, shape and colour. We aim for a similar taste of piperaquine and the placebo.

10.6.8. Placebo for amodiaquine

Placebo tablets for amodiaquine are currently under development. They will be identical in size, shape and colour. We aim for a similar taste of amodiaquine and the placebo.

10.7. 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 study drugs should be stored between 15°C to 30°C (59°F to 86°F).

In case of doubts on storage conditions meeting the recommendations the artemisinin-derivatives and partner drug content of batches of ACTs and TACTs will be retested at the end of the study.

10.8. Accountability of the study drugs

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.

11. DISCONTINUATION/ WITHDRAWAL OF PARTICIPANTS FROM THE STUDY

Each participant has the right to discontinue the study drug or withdraw from the study at any time. Data accrued up until the time of discontinuation/withdrawal will be used in the analysis, unless the subject wishes to withdraw even the data which has already been collected in which case those data will be excluded as well.

In general, the investigator must make every effort to perform the study procedure until day 63.

In principle the following situations are NOT reasons to discontinue the patient from the study:

- 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.

12. 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 facility, to aid in the identification of adverse events. An additional questionnaire will be administered to subjects who report psychological symptoms.

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 randomization in the study to discontinuation of the patient from study participation. Any events occurring between screening and randomization will be considered as baseline, pre-existing conditions. Any conditions that were present at baseline will not be considered adverse events unless the grade of the condition deteriorates.

Each adverse event will be graded according Common Terminology Criteria for Adverse Events

(CTCAE) version 5.0, adapted for paediatric populations where needed

https://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_50

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. If a definitive diagnosis is possible, then it 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 safety team within 24 hours from the time that the event was identified. The safety team will consist out of the Principal investigator, the study coordinator, a representative of the CTSG and the Medical Monitor. If further data is required, additional documentation can be submitted, but an initial report should be submitted within the timeframe. All SAEs must be followed until resolution, or until the SAE is deemed permanent or leads to death. SAEs must also be reported to the ECs and the Regulatory Authorities as per required by local guidelines.

12.1. Definitions

12.1.1. Adverse events (AEs)

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 pre-existing condition that has worsened in grade

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.

12.1.2. Grading of laboratory abnormalities

Basic biochemical assessments of markers related to hepatic and renal toxicity will be performed on day 0 (baseline) day 3, day 7 and 28.
In addition haemoglobin, platelet counts, white blood cell counts and white blood cell differentiation will be obtained at day 0, day 3, day 7 and day 28.
If found to be abnormal the values will be graded according to the CTCAE table and followed up until resolved or deemed to be permanent.

12.1.3. Serious adverse events (SAEs)

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
- Any other significant medical condition

More than one of the above criteria can be applicable to the one event. Other significant medical conditions 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.

12.1.4. Study defined SAEs

For the purpose of this study, the following are considered SAEs

- Prolongation of the QT or QTc interval >500 milliseconds
- AST/ALT >10 times ULN
- ALT or AST >3 times ULN with Total Bilirubin >2 times ULN (Hy's Law)
- Creatinine >3.5 times ULN

12.2. Reporting procedures for Serious Adverse events

All SAEs must be reported by the site investigator within one day of his or her awareness of the SAE to the Study PI, Study Coordinators, Medical Monitor and the CTSG by faxing or emailing the CRF documenting the SAE (Fax No.: +66 (0) 2 354 9169; email: DETACTsafetyteam@tropmedres.ac).

Further reports should be submitted, if required, until the SAE is resolved, is deemed permanent or results in death.

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

12.3. Evaluating adverse events and Serious adverse events

12.3.1. Assessment of intensity of AEor SAE

Each adverse event will be graded according Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 (adapted for paediatric populations as needed). This will be included in the safety monitoring plan.

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

1 = Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.

2 = Moderate: minimal, local or noninvasive intervention indicated; limiting age appropriate instrumental ADL*

3 = Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self care ADL**

4 = Life-Threatening consequences; urgent intervention indicated

5 = Death related to AE

Activities of Daily Living (ADL)

*Age-appropriate instrumental ADL which will be defined in study SOPs.

**Age-appropriate self-care ADL which will be defined in study SOPs.

12.3.2. Assessment of relatedness of AE or SAE to study drugs

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 case record form.

12.3.3. Outcome of AE and SAEs

The investigator will follow-up the AE and SAE until resolution, death, deemed permanent and 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

12.4. Clarification of the difference in the meaning of '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 and the defined study specific SAEs. 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.

13. 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.

14. LABORATORY STUDIES

14.1. Drugs concentration assays

Antimalarial drug concentrations will be measured in plasma samples at the end of the study period or earlier if requested, for instance by the DSMB. All assays will be performed using validated LC-MS/MS methodologies at the Department of Clinical Pharmacology, MORU, Bangkok, Thailand. Observed antimalarial drug measurements will be used for initial statistical analyses and group comparisons to assess potential drug-drug interactions between drugs, relationship between drug concentrations and safety parameters, and relationship between drug concentrations and efficacy parameters.

All collected PK 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 outcomes (i.e. safety and efficacy outcomes). Individually predicted drug levels at the time of QT-measurements will also be used to evaluate a direct relationship between possible QTc-prolongation and drug exposures.

14.2. Ex vivo and in vitro sensitivity assays

Ex vivo and in *vitro* assays will be performed to measure parasite responses to artemisinin derivatives and partner drugs according to the latest standards at the time of assessment. Parasites will be also be cryopreserved for future studies.

14.3. Molecular studies

Parasite DNA will be used for genetic studies including but not limited to microsatellite typing and single nucleotide polymorphisms (SNP) typing whole genome sequencing to identify parasite clones, to measure proportions of parasites carrying mutations of functional and/or operational relevance (genetic markers associated with drug resistance, complete or partial deletions in *pfhrp2* gene), to generate data for genome-wide association studies with *in vivo*, *ex vivo*, and *in vitro* responses of parasites to artemisinin and controls. Further, the genotype data from different sample types will be compared to develop methods for sample collection methods adapted to resource-poor settings.

Parasite RNA will be used for transcriptome analyses and for RNA-based detection, characterization and/or quantitation of gametocytes.

Blood samples (dried blood blots) for host genotyping will be obtained and stored from all subjects recruited with their 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). Potential targets for targeted genotyping are polymorphisms in genes coding for enzymes related to drug metabolism (e.g. cytochrome P450 enzymes) and genes and mutations predisposing to long QT-syndrome such as but not limited to the genes KCNQ1 (LQT1), KCNH2 (LQT2), and SCN5A (LQT3).

14.4. 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.

14.5. Immunity

On enrolment and in case of recurrent parasitaemia, quantitative assessments for a panel of antibodies against *Plasmodium falciparum* will be performed. Antibody levels will be correlated with markers of disease outcome such as parasite clearance rates, 42 day efficacy and day of recrudescence or reinfection.

15. STATISTICAL CONSIDERATIONS

15.1. Sample size justification

These studies will have a non-inferiority trial design. Each site will be powered for the efficacy endpoint. For the safety and tolerability endpoint, the sample size will be pooled across the DeTACT-Africa and DeTACT-Asia studies to achieve the desired power of at least 80% to detect rare events.

For sample size calculations, the following assumptions have been used: The expected efficacy of ACTs (AL or ASMQ) in these countries is expected to be approximately 96%. The matching TACT efficacy is assumed to be at least 90% under the null hypothesis of inferiority based on the WHO 90% efficacy threshold. However preliminary TRACII study data suggest that TACTs potentially may have a better efficacy by about 1% to 3% higher in favour of TACT (i.e. 97%-99% efficacy) in the Asian countries.

A one-sided alpha of 0.025 has been used in sample size calculations as non-inferiority is in one direction. A sample size of 47 subjects in the ACT plus placebo arm and 47 subjects in the matching TACT arm would be needed to achieve approximately 80% power to detect a non-inferiority margin difference between the group efficacy of -6%. With an additional 20% to cover for loss to follow up, we would therefore need 57 subjects in the ACT plus placebo arm and 57 subjects in the matching TACT arm in each of the sites. Since there will be 4 arms per site, it means that we will need 228 subjects per site with 114 subjects in the two ACT arms combined and 114 subjects in the two TACT arms combined.

For the safety and tolerability endpoints, we will perform a pooled analysis of the data obtained in the DeTACT-Africa and DeTACT-Asia. A total of 1707 subjects in ACT arms (918 and 789 subjects in the AL and ASMQ arms respectively) and 2787 subjects in h TACT arms (1494 and 1293 subjects in the AL+AQ and ASMQ+PPQ arms respectively) recruited in both studies combined, will allow us to detect increases of rare adverse events from incidences of 1 in 100 subjects to 3 in 100 subjects, also with more than 80% power

The sample size calculations were performed using a specialized sample size calculation software, PASS 15.

15.2. Statistical analysis

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

15.2.1. 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.

15.2.2. 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.

15.2.3. Pharmacokinetic data

Pharmacokinetic parameters such as C_{max}, T_{max} and AUC of artemether, lumefantrine, amodiaquine, artesunate, piperaquine, and mefloquine (and their metabolites) will be estimated using nonlinear mixed-effects modelling. All available study data will be pooled and integrated for a population modeling approach for an in-depth analysis of the pharmacokinetic properties of the above drugs as well as their relationship to treatment outcomes (i.e. safety and efficacy outcomes).

15.2.4. 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. Subjects will be analysed according to an intention to treat and a per protocol method where appropriate.

16. DIRECT ACCESS TO SOURCE DATA/DOCUMENTS

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

17. 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 Clinical Trials Support Group (CTSG) or their designees will be engaged in assuring QA/QC of study execution in collaboration with the WWARN Asia Regional Centre. Their role will include but not be limited to monitoring adherence to WIs 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. A project steering committee will oversee the project.

17.1. Monitoring

Study sites may have in place a system for internal monitoring. In addition, regular external monitoring of all sites will be coordinated 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.

18. DSMB AND PROJECT STEERING COMMITTEE

18.1. 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 subjects have been accrued into the study

- after the first 500 subjects 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 (Contact details will be summarized in DSMB Charter and investigators site file), Principal Investigator or DSMB to consider e.g. serious adverse events as they are reported OR if an earlier safety review is thought to be indicated.

18.2. Project steering committee

Project Steering Committee Composition

1. Ric Price (Academia representative) Chair
2. Sarthak Das (APLMA)
3. Louis Da Gama (CSO representative)
4. Melanie Renshaw (ALMA)
5. Nick White
6. Arjen Dondorp
7. Peter Olumese (WHO Representative, Observer)

Tasks of the Project Steering Committee

The overall task of the committee is to provide high-level guidance to the Project Management Group for the proper execution of the project.

This includes:

1. Review the progress of the DeTACT project towards its interim and overall objectives (see attached table)

2. To review progress of the trial in the context of relevant information from other sources, e.g. on the development of antimalarial drug resistance in the regions of the project

3. Consider recommendations of the Data Safety Monitoring Board (DSMB), and when applicable the Ethics Committees. This includes recommendations of the DSMB regarding stopping or continuation of the clinical trial.

4. Provide independent guidance for problem resolution when they arise during the project.

5. Assist in communication of project findings to the respective constituencies of the committee members.

Meeting Frequency

The Project Steering Committee will meet at an annual basis, coinciding with the annual DeTACT investigators' meeting. In addition the committee will meet at regular intervals 6 monthly either virtual or in person, or at an ad hoc basis whenever needed.

Reporting
The Project Steering Committee will provide a written report with recommendations to the Project Management Group on a yearly basis for the duration of the project. Interim recommendations will be provided if necessary.

Conflicts of Interests
Project Steering Committee members will have to declare potential or perceived conflicts of interests. For this purpose, a Conflict of Interests form will need to be completed.

19. ETHICS

19.1. 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).

19.2. 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.

19.3. 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. If required by participating countries Regulatory Authority, the protocol will also be submitted for approval The Investigator will submit and, where necessary, obtain approval from the above parties for all substantial amendments to the original approved documents.

19.4. Risks

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

19.4.1. Risks of artemether-lumefantrine

Reported AL 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. AL does not cause prolongation of the QTc interval.

19.4.2. 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 hepatotoxicity were observed.

19.4.3. Risks of artesunate-mefloquine

Side effects such as nausea and vomiting, dizziness and headache have shown to be consistently associated with ASMQ. Other adverse effects following treatments with ASMQ are fatigue,

nightmares and anxiety. Palpitations are also relatively more common with AS+MQ. Mild prolongations of QTc intervals have been reported following ASMQ treatments but these are less frequent as compared to DHA-PPQ. From an individual patient meta-analysis of 5,487 subjects treated for *P. falciparum* with ASMQ, 49% of patients had at least one adverse event, 3.4% of subjects had early vomiting and the incidence of serious neurological reaction was 2 per 1,000.

19.4.4. Risks of piperaquine

Piperaquine is generally very well tolerated. Occasional abdominal discomfort and diarrhoea have been reported in clinical trials of DHA-PPQ which may have resulted from piperaquine. Although piperaquine slightly prolongs the electrocardiogram QT interval, there is no evidence for clinically significant cardiovascular toxicity at the doses which will be administered in this study.

19.4.5. Risks of new partner drug combinations

No interactions between lumefantrine and amodiaquine or piperaquine and mefloquine 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. Also no life-threatening interactions between piperaquine and mefloquine are expected.

In addition, the preliminary results of the TRACII trial indicate that the TACTs artemether-lumefantrine+amodiaquine and DHA-piperaquine+mefloquine have a good safety and tolerability profile, although both TACTs were found to induce slightly higher vomiting rates within the first hour after administration of the study drugs, compared to their matching ACTs. The safety profile of ASMQ +/- PPQ is expected to be similar to that of DHA-PPQ +/- MQ.

19.4.6. 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.

19.5. Benefits

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

19.6. Alternatives to study participation

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

19.7. Incentives & Compensation

Study subjects or their guardian in the case of children will be compensated for time lost from work. Subjects and/or their guardians 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 meals or 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.

19.8. Confidentiality

The trial staff will ensure that the participants' anonymity is maintained. All documents will be stored securely and be accessible to trial staff and authorised personnel only. In the future, with the consent of the participants, or parents/guardians in the case of a child, anonymized specimens and data may be shared with other research groups.

20. 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 subjects 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.

21. DATA HANDLING AND RECORD KEEPING

All study data will be recorded on standard Case Report Forms (CRF) and entered to MACRO EDC©, a GCP-compliant data management system. The database is password-protected and includes internal quality checks to identify data that appear inconsistent, incomplete, or inaccurate.

Study participants will be identified by a unique participant number in the database. The study data management plan outlines all activities that will be carried out to ensure security and quality of the data.

Subject records at site will, taking into account the ability of the sites, be stored in binders or scanned and stored electronically. Adult's records will be retained for five years following completion of the study while for children, the records will be retained until the youngest child participating in the trial reaches 21 or five years following completion of the study, whichever is longer. The study database will be retained indefinitely.

With participant's consent, participant's data and results from blood analyses stored in the database may be shared according to the terms defined in the MORU data sharing policy with data repositories such as the WorldWide Antimalarial Resistance Network (WWARN, terms of submission here: http://www.wwarn.org/tools-resources/terms-submission) or other researchers to use in the future. All personal information will be anonymised so that no individual can be identified from their treatment records, through interviews, or from mapping data.

22. SPONSORSHIP AND INSURANCE

The University of Oxford is the study sponsor and has a specialist insurance policy in place, which would operate in the event of any participant suffering harm as a result of their involvement in the research (Newline Underwriting Management Ltd, at Lloyd's of London). For the sites in India local insurance will be obtained.

23. PUBLICATION POLICY

Any data published in the peer-reviewed medical literature will protect the identity of the subjects. 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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APPENDIX 1.
STUDY SCHEDULE

DeTACT-Asia Project Clinical Activity Schedule

Test/application	SCR	D0						D1				D2						D3 (*see footnote for D4-D6)				D7	D14	D21	D28	D35	D42	D49	D56	D63	DREC	Unsch.
		H0	H4	H6	H8	H12	H18	H24	H30	H36	H42	H48	H52	H54	H60	H64	H66	H72	H78	H84	H90	H168										
Verbal consent (if applicable)	X																															
Informed consent	X																															
Demographic data/Medical history	X	X																														
Drug history	X	X																														
Temperature (until 2 measurements<37.5)	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
Pregnancy test for females of child bearing potential	X																													X		
Height/weight	X	X																													X	
MUAC and other assesments of nutritional status	X	X																														
Physical examination	X	X						X				X						X				X	X	X	X	X	X	[X]	[X]	[X]	X	X
Symptom questionaire		X						X				X						X				X	X	X	X	X	X	[X]	[X]	[X]	X	X
Place of residence, work, travel history and data on mobile phone use		X																													X	
Adverse/serious adverse events recording		X						X				X						X				X	X	X	X	X	X	[X]	[X]	[X]	X	X
Randomisation to study drug		X																														
Study drug administration (ASMQ+/-PPQ) (no fat)		X						X				X																				
Study drug administration (AL+/-AQ) (with fat)		X			X			X		X		X			X																
Study drug administration (Primaquine)								X																								
Electrocardiographic recording		X	X										{X}			{X}											X					

[X] On day 49, 56 and 63 follow up will consist of temperature mesaurement, blood smear and dried blood spot. However, if new symptoms and/or positive malaria smears then do all activities indicated as [X]. Additional examinations may be performed at the discretion of the clinician.
{X} ECG will be performed at H52 for patients in the ASMQ+/-PPQ arms and at H64 for the AL +/- AQ arms.
If a recurrent infection is found or the subject reports symptoms spontaneously additional procedures will be performed:
Temperature, Vital signs, Symptom questionaire and Physical examination
*** All patients should remain in hospital a minimum of 72 hours; If patient still in hospital after Day 3: continue doing daily symptom questionaire, physical examination, pulse, heart rate, blood pressure, temperature, and adverse/serious events recording; if still febrile continue temperature every 6 hours until two consecutive measurements of <37.5 degrees Celsius**
DREC= Day of recurrent P. falciparum infection (including mixed P. falciparum with another species); if patient blood smear is positive for P. falciparum during follow up then perform all indicated study activities and blood collection.
Unsch= Unscheduled visit; Time window for scheduled visits: at Day 7, +1 day; at Days 14-63 is -1 to +2 days.

BLOOD SAMPLING SCHEDULE

DeTACT-Asia Project Blood Sampling Schedule (Children <12 years)

Sampling type and volume (ml)	SCR	D0						D1				D2						D3 (*see footnote for D4-D6)				D7	D14	D21	D28	D35	D42	D49	D56	D63	DREC	Unsch.	Total (ml)
		H0	H4	H6	H8	H12	H18	H24	H30	H36	H42	H48	H52	H54	H60	H64	H66	H72	H78	H84	H90	H168											
Blood smear (*)	0.5	0.5 (Hb & HCT if FBC not done)		0.5		0.5	0.5	0.5		0.5		0.5			(*)			0.5 (Hb & HCT if FBC not done)		(*)		0.5 (Hb & HCT if FBC not done)	0.5	0.5	0.5 (Hb & HCT if FBC not done)	0.5	0.5	0.5	0.5	0.5	0.5	(*)	9.5
HCT (EDTA)																																	
Hb (EDTA)																																	
Dried Blood Spot (EDTA) (aliquot of blood collected for microscopy)		(0.5)																									0.5	0.5	0.5	0.5			0
In vitro/ex vivo sensitivity assays (Heparinized)		2																												2			4
DNA/RNA/Immunological assesments (EDTA/Heparin)		5		1		1		1				1						1				1	1	1	1	1	1				5		21
PK sampling (**)		1		1				1					0.5			0.5						1		1		1					1		8
Biochemistry (heparinised blood)		1																1				1			1								4
Full blood count (EDTA)		0.5																0.5				0.5			0.5								2
Total volumes per time point (ml)	0.5	10	0	2.5	0	1.5	0.5	2.5	0	0.5	0	1.5	0.5	0	0	0.5	0	3	0	0	0	4	1.5	2.5	3	2.5	1.5	0.5	0.5	0.5	8.5	0	**48.5**

*** Patients with >5000 parasites/µL at inclusion will have blood smears at 6h, 12h, 18h, 24h and thereafter every 12 hours until parasite clearance (when two consecutive blood smears are negative) even if blood smears stay positive longer than 72h. In all cases, blood smears must be collected at 24h, 48h, 72h and then at all visits, including unscheduled visits. If parasitemia <=5000 parasites/ul at inclusion, blood smears must still be perfomed at 24h, 48, and 72h.**
**** Day 0, day 7 and day of recurrence samples will be obtained in all subjects. In 300 subjects, two samples will be taken at a random timepoint in the first 72 hours and two samples will be taken at a random timepoint in the first 42 days.**
PK samples have been included in this table to calculate the total volume of blood to be collected but may not be collected at the time-points indicated on this table (e.g., H6, H24, D21, D35 PK samples). An additional PK sample of 1 mL will be collected at H52 or at H64 but has been inlcuded in this table as 0.5 mL at both time-points, again to calculate the total volume.

DeTACT-Asia Project Blood Sampling Schedule (Adults ≥ 12 years)

Sampling type and volume (ml)	SCR	D0						D1				D2						D3 (*see footnote for D4-D6)				D7	D14	D21	D28	D35	D42	D49	D56	D63	DREC	Unsch.	Total (ml)
		H0	H4	H6	H8	H12	H18	H24	H30	H36	H42	H48	H52	H54	H60	H64	H66	H72	H78	H84	H90	H168											
Blood smear (*)	0.5	0.5 (Hb & HCT if FBC not done)		0.5		0.5	0.5	0.5		0.5		0.5			(*)			0.5 (Hb & HCT if FBC not done)		(*)		0.5 (Hb & HCT if FBC not done)	0.5	0.5	0.5 (Hb & HCT if FBC not done)	0.5	0.5	0.5	0.5	0.5	0.5	(*)	9.5
HCT (EDTA)																																	
Hb (EDTA)																																	
Dried Blood Spot (EDTA) (aliquot of blood collected for microscopy)		(0.5)																									(0.5)	(0.5)	(0.5)	(0.5)			0
In vitro/ex vivo sensitivity assays (Heparin)		3																												3			6
DNA/RNA/Immunological assesments (EDTA/Heparin)		10		1		1		1				1						1				2	2	2	2	2	2				10		37
PK sampling (**)		1		1				1					0.5			0.5						1		1		1					1		8
Biochemistry (heparinised blood)		1																1				1			1								4
Full blood count (EDTA)		0.5																0.5				0.5			0.5								2
Total volumes per time point (ml)	0.5	16	0	2.5	0	1.5	0.5	2.5	0	0.5	0	1.5	0.5	0	0	0.5	0	3	0	0	0	5	2.5	3.5	4	3.5	2.5	0.5	0.5	0.5	14.5	0	**66.5**

*** Patients with >5000 parasites/µL at inclusion will have blood smears at 6h, 12h, 18h, 24h and thereafter every 12 hours until parasite clearance (when two consecutive blood smears are negative) even if blood smears stay positive longer than 72h. In all cases, blood smears must be collected at 24h, 48h, 72h and then at all visits, including unscheduled visits. If parasitemia <=5000 parasites/ul at inclusion, blood smears must still be perfomed at 24h, 48, and 72h.**
**** Day 0, day 7 and day of recurrence samples will be obtained in all subjects. In 300 subjects, two samples will be taken at a random timepoint in the first 72 hours and two samples will be taken at a random timepoint in the first 42 days.**
PK samples have been included in this table to calculate the total volume of blood to be collected but may not be collected at the time-points indicated on this table (e.g., H6, H24, D21, D35 PK samples). An additional PK sample of 1 mL will be collected at H52 or at H64 but has been inlcuded in this table as 0.5 mL at both time-points, again to calculate the total volume. In patients >70 kg, three additional samples will be taken at random timepoints in the first 42 days. For these patients the maximum total volume of blood will be 69.5 mL.

APPENDIX 2. DOSING SCHEDULES

Artemether-lumefantrine + amodiaquine or placebo

Artemether-lumefantrine +/- Amodiaquine (or placebo) dosing schedule		
One tablet of AL contains 20 mg artemether and 120 mg lumefantrine; One tablet of AQ contains 40 mg amodiaquine; Placebo contains no active pharmaceutical ingredient.		
Body weight (kg)	**Number of tablets per timepoint (0h, 8h, 24h, 36h, 48h, 60h)**	
	AL	**Amodiaquine / PBO**
	Tablets	**Tablets**
5 to 9.9	1	1
10 to 15.9	2	2
16 to 29.9	3	3
30 to 54.9	5	5
≥ 55	6	6

Artemether-lumefantrine + Amodiaquine or placebo

Artemether-lumefantrine + Amodiaquine (or placebo) dosing schedule			
Body weight (kg)	**Dose administered at 0h, 8h, 24h, 36h, 48h, 60h**		
	Artemether (mg)	**Lumefantrine (mg)**	**Amodiaquine (mg) / PBO**
5 to 9.9	20	120	40 / 0
10 to 15.9	40	240	80 / 0
16 to 29.9	60	360	120 / 0
30 to 54.9	100	600	200 / 0
≥ 55	120	720	240 / 0

Artesunate-mefloquine + piperaquine or placebo

Artesunate-mefloquine + piperaquine (or placebo) dosing schedule			
Body weight (kg)	**Dose administered at 0h, 24h, 48h**		
	Artesunate (mg)	**Mefloquine HCl (mg)**	**Piperaquine TP (mg) / PBO**
5 to 10.9	32	70	160/ 0
11 to 17.9	64	140	320 / 0
18 to 33.9	100	220	500 / 0
34 to 64.9	200	440	1000 / 0
≥ 65	300	660	1500/ 0

Artesunate-mefloquine + piperaquine or placebo

Artesunate-mefloquine + piperaquine (or placebo) dosing schedule One Paediatric tablet of AS contains 32 mg artesunate, one Adult tablet contains 100 mg; One Paediatric tablet of MQ contains 70 mg mefloquine, one Adult tablet contains 220 mg; One Paediatric tablet of PPQ contains 160 mg piperaquine, one Adult tablet contains 500 mg; Paediatric and Adult placebos contain no active pharmaceutical ingredient			
Body weight (kg)	**Number of tablets per timepoint (0h, 24h, 48h)**		
	Artesunate	**Mefloquine HCl**	**Piperaquine TP/ PBO**
	Tablets	**Tablets**	**Tablets**
5 to 10.9	1 (Paed)	1 (Paed)	1 (Paed)
11 to 17.9	2 (Paed)	2 (Paed)	2 (Paed)
18 to 33.9	1 (Adult)	1 (Adult)	1 (Adult)
34 to 64.9	2 (Adult)	2 (Adult)	2 (Adult)
≥ 65	3 (Adult)	3 (Adult)	3 (Adult)

Primaquine

Primaquine dosing schedule			
One tablet of Primaquine contains either 7.5 or 15 mg of Primaquine			
Body weight (kg)	**Dose administered at 24 h**		
	Primaquine (mg)	**7.5 mg tablet**	**15 mg tablet**
<25	3.75	0.5	0.25
25 – 50	7.5	1	0.5
>50	15	2	1

APPENDIX 3. LIST OF POTENTIAL STUDY SITES

Potential Sites' Locations
Kravanh,Siem Pang and Chambak, Cambodia (counted as one site)
Ramu, Bangladesh
Agartala, India
Midnapore and Rourkela, India (counted as one site)
Myanmar (site to be determined)
Sumba, Indonesia