# A Randomized, Open-Label Study to Evaluate Potential Pharmacokinetic Interactions of Orally Administered Artemether-lumefantrine and Amodiaquine in Healthy Adult Subjects

**Short Title:** Pharmacokinetic study of artemether-lumefantrine and amodiaquine in Healthy Subjects

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## Table of Contents

ABBREVIATIONS

| | |
|---|---|
| AE | Adverse event |
| AL or A/L | Artemether-lumefantrine |
| ALP | Alkaline phosphatase |
| ALT | Alanine aminotransferase |
| AQ | Amodiaquine |
| AST | Aspartate aminotransferase |
| AUC (0-Th) | Area under the drug concentration curve from time 0 to time T hours |
| AUC (0-last) | Area under the drug concentration curve from time 0 to last time-point |
| AUC (0-∞) | Area under the drug concentration curve from time 0 to infinity |
| BUN | Blood urea nitrogen |
| BW | Body weight |
| BP | Blood pressure |
| Clcr | Creatinine clearance |
| Cmax | Maximum observed drug concentration |
| CK | Creatine kinase |
| CQ | Chloroquine |
| CRF | Case report form |
| ECG | Electrocardiogram |
| EDTA | Ethylenediaminetetraacetic acid |
| FBS | Fasting blood sugar |
| GCP | Good clinical practice |
| HBsAg | Hepatitis B surface antigen |
| HIV | Human immunodeficiency virus |
| HR | Heart rate |
| IC50 | Half maximal inhibitory concentration |
| EC | Ethics committee |
| IRB | Institutional review board |
| kg | Kilograms |
| MetHb | Methemoglobin |
| mg | Milligram |
| mL | Milliliter |
| mmHg | Millimeters of mercury |
| MORU | Mahidol-Oxford Tropical Medicine Research Unit |

| | |
|---|---|
| MQ | Mefloquine |
| PGx | Pharmacogenetic |
| PK | Pharmacokinetics |
| Pfcrt | *Plasmodium falciparum* chloroquine resistance transporter |
| Pfmdr1 | *Plasmodium falciparum* multidrug resistance protein-1 |
| PO | Per os (by mouth or orally) |
| RBC | Red blood cell |
| SAE | Serious adverse event |
| Scr | Serum creatinine |
| TG | Triglyceride |
| Tmax | Time to maximum observed drug concentration |
| t½ | Terminal elimination half-life |
| ULN | Upper limit of normal |
| WBC | White blood cell |
| Wt | Weight |
| Vd | Volume of distribution based on the terminal phase |
| λz | Terminal elimination rate constant |

## 1. INTRODUCTION

Artemisinin combination therapies (ACTs) have been a major driving force behind substantial reductions in global malaria morbidity and mortality over recent years. However, further gains are threatened by the recent emergence of artemisinin resistance in Southeast Asia (1), a region that has been the epicentre for the evolution and spread of resistance to every important class of antimalarials.

ACT is the simultaneous use of two or more blood schizontocidal drugs with independent modes of action. The rationale behind ACT is that the chance of parasites simultaneously developing resistance to two drugs with different modes of action is much lower than the chance of parasites developing resistance to single drugs. Rapid clearance of parasitemia and resolution of symptoms is dependent on artemisinin while the complete clearance of the remaining parasites is dependent on the partner medicine (2). With the emergence of artemisinin resistance, partner drugs have been left exposed to a much larger biomass of parasites and unsurprisingly partner drug resistance has followed (3).

Treatment failures after artemisinin combination therapies are becoming more widespread in Southeast Asia. Failures rates of around 30% for dihydroartemisinin-piperaquine and mefloquine-artesunate have been documented in western Cambodia (4) and western Thailand border areas (5) respectively. Failure of first line ACTs will damage current control and elimination efforts and accelerate the emergence and spread of resistance. A major concern is that artemisinin and partner drug resistance may spread across a wider geographic area, as chloroquine resistance did in the 1960s and 1970s, moving from Southeast Asia to the Indian subcontinent and Africa. Furthermore, while the resistance phenotype is currently limited to the ring stage of the asexual cycle, there is a chance that the resistance itself could ‘deepen’ by extending beyond this stage, causing more problems in the management of malaria .

There is an urgent need to evaluate alternative treatments where standard courses of ACTs are failing, and to develop combinations of drugs which will not fall rapidly to resistance and can be deployed immediately. As new drugs are at least five years away, we propose to combine artemisinin derivatives with two existing slowly-eliminated partner drugs; triple artemisinin combination therapy (TACT).

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

### Choosing partner drug combinations

In general the ‘malaria world’ can be divided into different areas, based on current resistance patterns and current antimalarial drug use.

The decision on which two partner drugs to combine in any new regimen must take into account pharmacokinetic and pharmacodynamic interactions, locally existing resistance profiles to artemisinin derivatives and the individual potential partner drugs, and the current antimalarial treatment policy.

The global spread of chloroquine (CQ) resistance was also associated with cross-resistance to amodiaquine. However amodiaquine continued to be used in some areas of Southeast Asia, such as Myanmar, owing to its low cost. Relatively few trials of the ACT artesunate-

amodiaquine have been conducted. The largest showed borderline efficacy in Myanmar with recrudescence rates of about 10%. Studies in Vietnam, as well as India and Africa, have reported cure rates above 95% (6-10).

Transfection data provide the most reliable means of understanding the molecular basis of amodiaquine resistance. Fidock and colleagues introduced two major patterns of mutations (Dd2 and 7G8) into wild-type *pfcrt*-encoding parasites and were able to confirm that these mutations were causative factors in CQ resistance (11). Notably there was a clear inverse correlation between the IC50 values for CQ and mefloquine (MQ) as well as for amodiaquine and MQ. In other words, resistance to chloroquine/amodiaquine caused by specific *pfcrt*-mutations was associated with mefloquine hypersensitivity.

A second locus, *pfmdr1*, also appears to be involved in amodiaquine resistance, with the N86Y mutation associated with resistance in vitro and increased clinical failures with amodiaquine monotherapy (12-14). A recent paper by Venkatesan et al. suggests that recurrent infection after artemether-lumefantrine and artesunate-amodiaquine is associated with specific mutations in the *pfmdr1* gene, but that the two partner drugs select alternative and drug resistance opposing alleles (15).

In theory, the concurrent use of lumefantrine combined with amodiaquine exploits these opposing selection pressure effects. Potentially, this will prevent or delay emergence of high-level resistance to both drugs at the same time. This would be expected to prolong or maintain the overall efficacy of both partner drug components (16).

To assess the safety and tolerability and pharmacological interactions of the combination of artemether-lumefantrine and amodiaquine we propose an open label sequential trial of artemether-lumefantrine combined with amodiaquine.

As we propose to combine artemether-lumefantrine, and amodiaquine, it is necessary that the potential interactions of these drugs be characterized. These drugs are metabolized by cytochrome P450 (abbreviated CYP) enzymes which potentially causes pharmacokinetic alteration, resulting in clinically significant drug-drug interactions that can cause unanticipated adverse reactions or therapeutic failures because of the suboptimal exposure of the parasite.

**Artemether-lumefantrine data**

Following administration of artemether-lumefantrine to both healthy volunteers and patients with malaria, artemether is absorbed with peak plasma concentrations reached about 2 hours after dosing. Absorption of lumefantrine, a highly lipophilic compound, starts after a lag-time of up to 2 hours, with peak plasma concentrations about 6 to 8 hours after administration. Food enhances the absorption of both artemether and lumefantrine. In healthy volunteers, the relative bioavailability of artemether was increased between 2- to 3-fold, and that of lumefantrine 16-fold when artemether-lumefantrine tablets were taken after a high-fat meal compared to under fasted conditions. Artemether and lumefantrine are both highly bound to human serum proteins in vitro (95.4% and 99.7%, respectively). In human liver microsomes and recombinant CYP450 enzymes, the metabolism of artemether was catalyzed predominantly by CYP3A4/5. Artemether is cleared from plasma with an elimination half-life of about 2 hours. Lumefantrine is eliminated more slowly, with an elimination half-life of 3 to 6 days in healthy volunteers and in patients with falciparum malaria. Demographic characteristics such as sex and weight appear to have no clinically relevant effects on the pharmacokinetics of artemether and lumefantrine (17).

**Amodiaquine data**

Amodiaquine by oral administration is absorbed rapidly, and then extensively metabolized to N-desethylamodiaquine (active metabolite) and 2-hydroxy-desethyl-amodiaquine by hepatic first-pass effect. $C_{max}$ of amodiaquine in plasma, whole blood and packed cells is 32±3ng/ml, 60±10ng/ml and 42±6ng/ml respectively; Tmax of amodiaquine is 0.5±0.03h, 0.5±0.1h and 0.5±0.1h respectively; $AUC_{0-8}$ of amodiaquine in plasma, whole blood and packed cells is 99±19ng·ml$^{-1}$h, 148±25ng·ml$^{-1}$h and 167±72ng·ml$^{-1}$h respectively. Cmax of desethylamodiaquine in plasma, whole blood and packed cells is 181±26ng/ml (19 fold higher than those of the parent drug), 561±70ng/ml and 561±143ng/ml respectively; Tmax for desethylamodiaquine is 3.4±0.8h, 2.2±0.5h and 3.6±1.1h respectively; and AUC0-24 for desethylamodiaquine in plasma, whole blood and packed cells is 2304±37ng·ml$^{-1}$h, 6811±752ng·ml$^{-1}$h and 5713±1269ng·ml$^{-1}$h respectively (whole blood AUC was higher than plasma AUC, P<0.001). The trial of $^{14}$C-labelled amodiaquine administered orally to Wistar rats showed that amodiaquine can be distributed widely in body with the main organs of accumulation being kidney, liver, bone marrow and spleen. Amodiaquine is not observed in plasma 8 hours after administration, whereas the concentration of desethylamodiaquine in plasma at 96h is 29±8ml$^{-1}$. The half-life of amodiaquine in plasma is 5.2±1.7h, and elimination half-life ($\beta_{t1/2}$) of desethylamodiaquine in plasma is 9-31 days. The bioavailability of amodiaquine is decreased due to first-pass hepatic metabolism. The plasma protein binding rate of amodiaquine and desethylamodiaquine is greater than 90%. The $T_{max}$ of amodiaquine taken orally by patients with falciparum malaria is 1.75h, distinctly longer than the $T_{max}$ of healthy volunteers (18).

**Potential interactions through hepatic metabolism**

All study drugs are metabolized in the liver. Artemether and lumefantrine are primarily metabolized via CYP3A4 (19). Artemether metabolism through CYP3A4 produces an active metabolite, dihydroartemisinin (DHA) that contributes substantially to its antimalarial activity. Artemether is also a CYP3A4 inducer, while lumefantrine inhibits CYP2D6 (20). The clearance of amodiaquine and metabolism to its main metabolite, N-desethylamodiaquine, is catalyzed primarily by CYP2C8 (21). As some of these drugs share the same enzymes or have effects on the enzymes for other drug metabolism, the co-administration may result in clinically significant drug-drug interactions that can cause unanticipated adverse reactions or therapeutic failures. To evaluate whether such effects take place, we propose to co-administer these drugs in this healthy volunteer study.

## 2. OBJECTIVE(S)

### 2.1. Primary Objective

- To characterize the potential pharmacokinetic interactions of artemether - lumefantrine and amodiaquine in healthy adult subjects.

### 2.2. Secondary Objectives

- To characterize the pharmacokinetic properties of artemether-lumefantrine and amodiaquine when given alone and in combination.
- To evaluate the safety and tolerability of co-administered artemether-lumefantrine and amodiaquine.

- To investigate pharmacogenetic polymorphisms affecting drug levels of artemether-lumefantrine and amodiaquine and their metabolites.

### 2.3. Exploratory Objective

- To evaluate independent renal markers against standard blood urea nitrogen/ creatinine levels as markers of renal toxicity.

## 3. ENDPOINT(S)

### 3.1. Primary Endpoint

- Area under the concentration-time curve (AUC0-∞ and AUC0-last) and maximum concentration (Cmax) of artemether, lumefantrine and amodiaquine and their metabolites when given alone and in combination.

### 3.2. Secondary Endpoints

- Elimination clearance (CL/F), terminal elimination half-life (t1/2) and apparent volume of distribution (Vd) of artemether, lumefantrine and amodiaquine and their metabolites when given alone and in combination.

- Safety and tolerability parameters, including adverse events, electrocardiographic changes, vital signs and biochemical assessments.
- Pharmacogenetic polymorphisms in the case of unusual metabolizer.

### 3.3. Exploratory Endpoint

- Independent renal marker blood levels and standard blood urea nitrogen/creatinine levels.

## 4. STUDY DESIGN

This is an open-label pharmacokinetic study in 16 healthy Thai subjects. Subjects will be admitted in the inpatient ward and will be randomized to **group A** or **group B** to receive 3 drug regimens described in the following table 1. Every subject will have 1 screening and 3 admissions in the hospital.

**Table 1**: Study Schedule

| **Visit 1** | **Screening** | |
|---|---|---|
| Within 14 days | | |
| | **Group A (n = 8)** | **Group B ( n = 8)** |
| **Visit 2** | **Regimen 1**<br>Artemether-lumefantrine<br>on Day 0, 1, and 2 | **Regimen 2**<br>Amodiaquine<br>on Day 0, 1 and 2 |
| Washout period: > 6 weeks (after day 3) | | |
| **Visit 3** | **Regimen 2**<br>Amodiaquine | **Regimen 1**<br>Artemether-lumefantrine |

| | | |
|---|---|---|
| | on Day 0, 1 and 2 | on Day 0, 1 and 2 |
| Washout period: > 6 weeks (after day 3) | | |
| **Visit 4** | **Regimen 3**<br>Artemether-lumefantrine + Amodiaquine on Day 0, 1 and 2 | |

The total duration for each subject's participation in the study is approximately 6 - 8 months depend on washout period. Study duration is approximately 12 months.

## 5. STUDY POPULATION

### 5.1. Number of Subjects

This study will enroll 16 healthy subjects both male and female, aged 18-60 years, at the pharmacokinetic unit Faculty of Tropical Medicine, Mahidol University. Subjects will be healthy HIV-1, hepatitis B and C uninfected individuals who comprehend the purpose of the study and have provided written consent. All subjects will undergo screening assessments (visit 1). Screening assessments (visit 1) may be carried out over more than one day, provided that all required assessments are completed within the 14 days prior to visit 2. If the interval between screening (visit 1) and day -1 visit 2 is three days or less, the clinical laboratory screening test result and serum pregnancy test result can be used for enrolment evaluation on day -1 visit 2. In such cases, these tests would not need to be repeated at day-1 visit 2.

### 5.2. Inclusion Criteria

In order to be eligible to enter the protocol, subjects must meet the following criteria:

1. Healthy as judged by a responsible physician with no abnormality identified on a medical evaluation including medical history and physical examination.
2. Male or female non-smoker aged between 18 years to 60 years.
3. A female is eligible to enter and participate in this study if she is:
    - of non-childbearing potential including pre-menopausal females with documented (medical report verification) hysterectomy or double oophorectomy
    - or postmenopausal defined as 12 months of spontaneous amenorrhea or 6 months of spontaneous amenorrhea with serum follicle stimulating hormone levels >40 mIU/mL or 6 weeks postsurgical bilateral oophorectomy with or without hysterectomy
    - or of childbearing potential, has a negative serum pregnancy test at screening and prior to start the study drug in each period, and agrees to abstain from sexual intercourse or use effective contraceptive methods (e.g., intrauterine device, hormonal contraceptive drug, tubal ligation or female barrier method with spermicide) during the study until completion of the follow-up procedures
4. A male is eligible to enter and participate in this study if he: agrees to abstain from sexual intercourse with females of childbearing potential or lactating females; or is willing to use a condom/spermicide, during the study until completion of the follow-up procedures.

5. Normal electrocardiogram (ECG) with QTc <450 msec.
6. Willingness and ability to comply with the study protocol for the duration of the trial.

### 5.3. Exclusion Criteria

Subjects will not be enrolled into the study if they meet any of the following exclusion criteria:

1. Females who are pregnant, trying to get pregnant, or are lactating.
2. The subject has evidence of active substance abuse that may compromise safety, pharmacokinetics, or ability to adhere with protocol instructions.
3. A positive pre-study hepatitis B surface antigen, positive hepatitis C antibody, or positive human immunodeficiency virus-1 (HIV-1) antibody result at screening.
4. Subjects with a personal history of cardiac disease, symptomatic or asymptomatic arrhythmias, syncopal episodes, or additional risk factors for torsades de pointes (heart failure, hypokalemia) or with a family history of long QT syndrome, Brugada syndrome, or sudden cardiac death.
5. Abnormal serum creatinine (Scr) and estimated glomerular filtration rate (eGFR) <70 mL/min as determined by CKD-EPI equation
6. History of alcohol or substance abuse or dependence within 6 months of the study.
7. Use of prescription or non-prescription drugs except paracetamol at doses of up to 2 grams/day, including vitamins, herbal and dietary supplements (including St. John's Wort) within 7 days (or 14 days if the drug is a potential enzyme inducer) or 5 times the drug half-life (whichever is longer) prior to the first dose of study medication until the completion of the follow-up procedure, unless in the opinion of investigator, the medication will not interfere with the study procedures or compromise subject safety; the investigator will take advice from the manufacturer representative as necessary.
8. The subject has participated in a clinical trial and has received a drug or a new chemical entity within 30 days or 5 x half-life, or twice the duration of the biological effect of any drug (whichever is longer) prior to the first dose of study medication.
9. The subject is unwilling to abstain from ingesting alcohol within 48 hours prior to the first dose of study medication until collection of the final pharmacokinetic sample during each regimen.
10. Subjects who have donated blood to the extent that participation in the study would result in more than 300 mL blood donated within a 30-day period. Note: This does not include plasma donation.
11. Subjects who have a history of allergy to the study drug or drugs of this class, or a history of drug or other allergy that, in the opinion of the investigator, contraindicates participation in the trial. In addition, if heparin is used during pharmacokinetic sampling, subjects with a history of sensitivity to heparin or heparin-induced thrombocytopenia should not be enrolled.
12. Lack of suitability for participation in this study, including but not limited to, unstable medical conditions, systemic disease manifested by tendency to granulocytopenia e.g. rheumatoid arthritis and lupus erythematosus that in the opinion of the investigator would compromise their participation in the trial.

13. AST or ALT >1.5 times the upper limit of normal (ULN)
14. Subjects with history of renal disease, hepatic disease, and/or cholecystectomy
15. Abnormal methemoglobin level (more than 3 mg/dL).
16. History of antimalarial drugs use including but not limited to mefloquine, chloroquine, primaquine, artesunate, piperaquine and pyronaridine treatment within 6 months.
17. Subject who have received quinacrine in last 30 days.
18. Subject has been included in other clinical trials in last 6 months.

## 6. STUDY ASSESSMENTS AND PROCEDURES

Each subject will have adequate time to provide informed consent of his or her own free will and prior to conducting any study specific procedures. Each subject will be provided a copy of the signed informed consent prior to the initiation of any study procedures.

After a subject has provided written informed consent, the investigator or other study personnel will determine if the subject is eligible for enrolment in the study. This will be done by reviewing the inclusion and exclusion criteria and completing all of the screening assessments.

The pharmacokinetic blood samples for a subject should be obtained at the scheduled times relative to when the subject was dosed.

### 6.1 Study Procedures (Appendix 2)

#### 6.1.1 **Visit 1 (Screening visit)**

Subjects will be screened to assess eligibility. Full consent will be obtained before any screening procedures are conducted.

- Basic demographics including age, date of birth, sex, height, and body weight
- Physical Examination and symptoms questionnaire assessment
- Vital signs (blood pressure (BP), respiratory rate (RR), body temperature (BT) and heart rate (HR)) and medical history
- ECG
- Clinical laboratory assessment:
    - chemistry: Albumin, ALP, ALT, AST, total and direct bilirubin, BUN, creatinine, creatine kinase, potassium, sodium, magnesium and calcium
    - hematology: hemoglobin, hematocrit, white blood cell count with differential and platelet count
    - fasting blood sugar (FBS) (at screening and during admission)
    - urinalysis: white blood cell, red blood cell and protein
    - *TSH (at screening visit only)
    - HIV antibody, hepatitis B surface antigens, and hepatitis C antibody tests. (Pre and Post-test counseling will be performed for HIV test) (These tests

will be done at screening visit only)

Note: The remaining serum will be frozen and stored for use in an exploratory analysis evaluating independent renal markers against standard blood urea nitrogen/ creatinine levels as markers of renal toxicity.

All laboratory assessments (chemistry, hematology, FBS and urinalysis) must be drawn in the fasting state (8 hours fast). Blood collection for clinical laboratory assessment is approximately 10 mL.

- Serum pregnancy testing (if appropriate)

6.1.2 **Visit 2-4**

16 healthy subjects who fulfill the eligibility criteria will be recruited and randomized to the study.

Procedures on each admission (Appendix 2):

**Visit 2**

Day -1 (admission)

- Physical Examination and symptoms questionnaire assessment
- Chemistry, Hematology, FBS and urinalysis assessment
- 24-hour urine for creatinine clearance test
- Genetic sample collection (Genetic sample can be collected at any of the subsequent days or visits, if it is missed today)
- Vital signs will be checked every 4 hours.
- ECG monitoring according to table 2
- Holter monitoring (~30 mins baseline recording)
- Urine pregnancy test (if applicable)

Day 0-3 (admission)

- Daily physical examination and symptoms questionnaire assessment
- Vital signs will be checked before and every 4 hours after first dose.
- ECG monitoring according to table 2
- Continuous Holter monitoring during admission.
- Pharmacokinetic samples will be collected according to table 2
- Study drug administration (Artemether-lumefantrine OR Amodiaquine) will be given according to table 1.
- 24-hour urine for creatinine clearance test (Day 2)
- Chemistry, Hematology, FBS and urinalysis assessment before discharge

Day 4 (+1) (follow up)

- Physical Examination, vital signs and symptoms questionnaire assessment

- Pharmacokinetic sample collection
- ECG

Day 7 (+/-2d) (follow up)

- Physical Examination, vital signs and symptoms questionnaire assessment
- Chemistry, Hematology and urinalysis assessment
- Pharmacokinetic sample collection
- ECG
- Holter monitoring (~ 30 mins)

Day 14 (+/-2d) (follow up)

- Physical Examination, vital signs and symptoms questionnaire assessment
- Chemistry, Hematology and urinalysis assessment
- Urine pregnancy test (if applicable)
- Pharmacokinetic sample collection
- ECG

Day 21 (+/-2d) (follow up)

- Physical Examination, vital signs and symptoms questionnaire assessment
- Chemistry, Hematology and urinalysis assessment
- ECG

Day 28 (+/-2d) (follow up)

- Physical Examination, vital signs and symptoms questionnaire assessment
- Chemistry, Hematology and urinalysis assessment
- Urine pregnancy test (if applicable)
- Pharmacokinetic sample collection
- ECG
- Holter monitoring (~ 30 mins)

Day 35 (+/-2d) (follow up) (for Amodiaquine only)

- Physical Examination, vital signs and symptoms questionnaire assessment
- Chemistry, Hematology and urinalysis assessment
- ECG

Day 42 (+/-2d) (follow up) (for Amodiaquine only)

- Physical Examination, vital signs and symptoms questionnaire assessment
- Chemistry, Hematology and urinalysis assessment
- Urine pregnancy test (if applicable)
- Pharmacokinetic sample collection

- ECG

**Visit 3**

Day -1 (admission)

- Physical Examination and symptoms questionnaire assessment
- Chemistry, Hematology, FBS and urinalysis assessment
- 24-hour urine for creatinine clearance test
- Vital signs will be checked every 4 hours.
- ECG monitoring according to table 2
- Holter monitoring (~30 mins baseline recording)
- Urine pregnancy test (if applicable)

Day 0-3 (admission)

- Daily physical examination and symptoms questionnaire assessment
- Vital signs will be checked before and every 4 hours after first dose.
- ECG monitoring according to table 2
- Continuous Holter monitoring during admission.
- Pharmacokinetic samples will be collected according to table 2
- Study drug administration (Amodiaquine OR Artemether-lumefantrine) will be given according to table 1.
- 24-hour urine for creatinine clearance test (Day 2)
- Chemistry, Hematology, FBS and urinalysis assessment before discharge

Day 4 (+1) (follow up)

- Physical Examination, vital sign and symptoms questionnaire assessment
- Pharmacokinetic sample collection
- ECG

Day 7 (+/-2d) (follow up)

- Physical Examination, vital signs and symptoms questionnaire assessment
- Chemistry, Hematology and urinalysis assessment
- Pharmacokinetic sample collection
- Holter monitoring (~ 30 mins)
- ECG

Day 14 (+/-2d) (follow up)

- Physical Examination, vital signs and symptoms questionnaire assessment
- Chemistry, Hematology and urinalysis assessment
- Urine pregnancy test (if applicable)

- Pharmacokinetic sample collection
- ECG

Day 21 (+/-2d) (follow up)

- Physical Examination, vital signs and symptoms questionnaire assessment
- Chemistry, Hematology and urinalysis assessment
- ECG

Day 28 (+/-2d) (follow up)

- Physical Examination, vital signs and symptoms questionnaire assessment
- Chemistry, Hematology and urinalysis assessment
- Urine pregnancy test (if applicable)
- Pharmacokinetic sample collection
- Holter monitoring (~ 30 mins)
- ECG

Day 35 (+/-2d) (follow up) (for Amodiaquine only)

- Physical Examination, vital signs and symptoms questionnaire assessment
- Chemistry, Hematology and urinalysis assessment
- ECG

Day 42 (+/-2d) (follow up) (for Amodiaquine only)

- Physical Examination, vital signs and symptoms questionnaire assessment
- Chemistry, Hematology and urinalysis assessment
- Urine pregnancy test (if applicable)
- Pharmacokinetic sample collection
- ECG

**Visit 4**

Day -1 (admission)

- Physical Examination and symptoms questionnaire assessment
- Chemistry, Hematology, FBS and urinalysis assessment
- 24-hour urine for creatinine clearance test
- Vital signs will be checked every 4 hours.
- ECG monitoring according to table 2
- Holter monitoring (~30 mins baseline recording)
- Urine pregnancy test (if applicable)

Day 0-3 (admission)

- Daily physical examination and symptoms questionnaire assessment
- Vital signs will be checked before and every 4 hours after first dose.
- ECG monitoring according to table 2
- Continuous Holter monitoring during admission.
- Pharmacokinetic samples will be collected according to table 2
- Study drug administration (Artemether-lumefantrine AND Amodiaquine) will be given according to table 1.
- 24-hour urine for creatinine clearance test (Day 2)
- Chemistry, Hematology, FBS and urinalysis assessment before discharge

Day 4 (+1) (follow up)

- Physical Examination, vital signs and symptoms questionnaire assessment
- Pharmacokinetic sample collection
- ECG

Day 7 (+/-2d) (follow up)

- Physical Examination, vital sign and symptoms questionnaire assessment
- Chemistry, Hematology and urinalysis assessment
- Pharmacokinetic sample collection
- ECG
- Holter monitoring (~ 30 mins)

Day 14 (+/-2d) (follow up)

- Physical Examination, vital signs and symptoms questionnaire assessment
- Chemistry, Hematology and urinalysis assessment
- Urine pregnancy test (if applicable)
- Pharmacokinetic sample collection
- ECG

Day 21 (+/-2d) (follow up)

- Physical Examination, vital signs and symptoms questionnaire assessment
- Chemistry, Hematology and urinalysis assessment
- ECG

Day 28 (+/-2d) (follow up)

- Physical Examination, vital signs and symptoms questionnaire assessment
- Chemistry, Hematology and urinalysis assessment
- Urine pregnancy test (if applicable)
- Pharmacokinetic sample collection

- ECG
- Holter monitoring (~ 30 mins)

Day 35 (+/-2d) (follow up)

- Physical Examination, vital signs and symptoms questionnaire assessment
- Chemistry, Hematology and urinalysis assessment
- ECG

Day 42 (+/-2d) (follow up)

- Physical Examination, vital signs and symptoms questionnaire assessment
- Chemistry, Hematology and urinalysis assessment
- Urine pregnancy test (if applicable)
- Pharmacokinetic sample collection
- ECG

All laboratory assessments (chemistry, hematology, FBS and urinalysis) must be drawn in the fasting state (8 hours fast). Results of these tests are to be available and reviewed prior to each subject receiving the study drug on day 0.

Note: At all visits the remaining blood serum will be frozen and stored for use in an exploratory analysis evaluating independent renal markers against standard blood urea nitrogen/ creatinine levels as markers of renal toxicity.

**6.3 Safety Assessments**

The physician or qualified clinical study staff will review the data at intervals throughout each study period.

6.3.1. Adverse Events

Adverse events (AEs) and serious adverse events (SAEs) will be monitored. Refer to Section on AEs and SAEs.

6.3.2. Vital Signs (BP, RR, BT and HR)

Subjects must have been resting quietly for at least 10 minutes prior to taking measurements.

Blood pressure (BP) and heart rate (HR) for visit 2-4 will be measured in both supine standing position in each time-points.

6.3.3. ECGs

Subjects must have been resting quietly for at least 20 minutes prior to taking measurements. Each ECG measurement will be done in triplicate at each time point to avoid outlier values due to random variation.

6.3.4 Holter monitor

During each admission, subjects will undergo continuous Holter monitoring to record changes in heart rhythm and cardiac conduction times. Simple non-invasive autonomic function tests i.e. heart-rate response to postural change (further detailed in an SOP) will be undertaken during the time that the Holter monitor is fitted (including during follow-up if a Holter monitor is available).

6.3.5. Clinical Laboratory Assessments

The following hematology, clinical chemistry, FBS, and urinalysis tests will be performed at each admission.

- Hematology: hemoglobin, hematocrit, white blood cell (WBC) count with differential, and platelet count
- Chemistry: Albumin, alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), total and direct bilirubin, blood urea nitrogen (BUN), creatinine, creatine kinase (CK), potassium, sodium, magnesium and calcium
- FBS: Fasting blood sugar (at screening and during admission)
- Urinalysis: white blood cell (WBC), red blood cell count (RBC), and protein.
- *TSH (for screening visit only)

  Note: The remaining blood serum will be frozen and stored for use in an exploratory analysis evaluating independent renal markers against standard blood urea nitrogen/ creatinine levels as markers of renal toxicity.

Any results falling outside of the reference range will be repeated at the discretion of the investigator. If there is more than one laboratory result for the same analyte, the last value will be recorded in the CRF.

6.3.6. Pregnancy

For female subjects, serum pregnancy test will be performed at visit 1, and urine pregnancy test will be performed before the first dose of each regimen and biweekly during follow up visits.

Action to Be Taken if Pregnancy Occurs in a Female

If any female subject or the female partner of a male subject who becomes pregnant while participating in this study that is after enrolment until the last follow up visit, the following procedures will be followed:

- The subject must be immediately discontinued from study drug.
- The subject must be referred to antenatal clinic for follow up.
- The pregnancy will be monitored to determine the outcome of the pregnancy. Any premature termination of the pregnancy will be reported.

While pregnancy itself is not considered to be an AE or SAE, any pregnancy complication or elective termination of a pregnancy for medical reasons will be recorded as an AE or SAE (see AE/SAE section of the protocol for definitions and a description of monitoring).

A spontaneous abortion is always considered to be an SAE and will be reported as such. Furthermore, any SAE occurring as a result of a post-study pregnancy and is considered reasonably related to the investigational product by the investigator, will be reported. All pregnancies occurring during the study period and including follow up period should be reported to PI as soon as possible.

## 6.4. Pharmacokinetics

Blood for pharmacokinetic assessments may be obtained by individual needle stick or IV

cannula. If a cannula is used for collection of the whole blood samples, the cannula will be inserted into an arm vein within sufficient time before dosing and may be kept patent using a dilute heparin solution as a flush. An initial volume of approximately 1.0 mL of blood will be collected and discarded prior to collection of each whole blood sample to ensure that the saline or heparin solution does not artificially dilute the samples. If PK samples are collected via individual needle sticks rather than through a cannula, then no extra blood needs to be collected and discarded prior to the collection of the sample.

Each blood sample will be labeled with subject's number, schedule date and time, collection date and time. The actual time of each blood sample will be recorded on the case report form (CRF).

**Table 2** Pharmacokinetic collection schedule for each study regimens

| **Regimen** | **Blood sampling** | **ECG measurements** |
|---|---|---|
| **Regimen 1**: Artemether-lumefantrine (AL)<br><br>Dosing: 0h, 8h, 24 h, 36 h, 48 h, 60 h | **Day 0**: Hr. 0 (pre-dose), 0.25, 0.5, 1, 2, 3, 4, 6, 8 (pre-dose), 10, 12, 14<br>**Day 1**: Hr. 24 (pre-dose), 26, 28, 30, 36 (pre-dose), 38, 40, 42<br>**Day 2**: Hr. 48 (pre-dose), 48.25, 48.5, 49, 50, 51, 52, 54, 56, 60 (pre-dose), 61, 62, 64, 66<br>**Day 3**: Hr. 72<br>**Day 4**, **Day 7, Day 14, Day 28** | **Day -1**: Hr. 0, 1, 2, 4, 6, 8, 10, 12, 14<br>**Day 0**: Hr. 0 (pre-dose), 1, 2, 4, 6, 8 (pre-dose), 10, 12, 14<br>**Day 1**: Hr. 24 (pre-dose), 26, 28, 30, 36 (pre-dose), 38, 40, 42<br>**Day 2**: Hr. 48 (pre-dose), 49, 50, 52, 54, 60 (pre-dose), 61, 62, 64, 66<br>**Day 3**: Hr. 72<br>**Day 4, Day 7, Day 14, Day 21, Day 28** |
| **Regimen 2:** Amodiaquine (AQ)<br><br>Dosing: 0h, 8h, 24 h, 36 h, 48 h, 60 h | **Day 0**: Hr. 0 (pre-dose), 0.25, 0.5, 1, 2, 3, 4, 6, 8 (pre-dose), 10, 12, 14<br>**Day 1**: Hr. 24 (pre-dose), 26, 28, 30, 36 (pre-dose), 38, 40, 42<br>**Day 2**: Hr. 48 (pre-dose), 48.25, 48.5, 49, 50, 51, 52, 54, 56, 60 (pre-dose), 61, 62, 64, 66<br>**Day 3**: Hr. 72<br>**Day 4**, **Day 7, Day 14, Day 28, Day 42** | **Day -1**: Hr. 0, 1, 2, 4, 6, 8, 10, 12, 14<br>**Day 0**: Hr. 0 (pre-dose), 1, 2, 4, 6, 8 (pre-dose), 10, 12, 14<br>**Day 1**: Hr. 24 (pre-dose), 26, 28, 30, 36 (pre-dose), 38, 40, 42<br>**Day 2**: Hr. 48 (pre-dose), 49, 50, 52, 54, 60 (pre-dose), 61, 62, 64, 66<br>**Day 3**: Hr. 72<br>**Day 4, Day 7, Day 14, Day 21, Day 28, Day 35, Day 42** |
| **Regimen 3:** Artemether-lumefantrine + amodiaquine (AL+AQ) | **Day 0**: Hr. 0 (pre-dose), 0.25, 0.5, 1, 2, 3, 4, 6, 8 (pre-dose), 10, 12, 14<br>**Day 1**: Hr. 24 (pre-dose), 26, 28, 30, 36 (pre-dose), 38, 40, 42<br>**Day 2**: Hr. 48 (pre-dose), 48.25, 48.5, | **Day -1**: Hr. 0, 1, 2, 4, 6, 8, 10, 12, 14<br>**Day 0**: Hr. 0 (pre-dose), 1, 2, 4, 6, 8 (pre-dose), 10, 12, 14<br>**Day 1**: Hr. 24 (pre-dose), 26, 28, 30, 36 |

| | | |
|---|---|---|
| Dosing: 0h, 8h, 24 h, 36 h, 48 h, 60 h | 49, 50, 51, 52, 54, 56, 60 (pre-dose), 61, 62, 64, 66<br>**Day 3**: Hr. 72<br>**Day 4**, **Day 7, Day 14, Day 28, Day 42** | (pre-dose), 38, 40, 42<br>**Day 2**: Hr. 48 (pre-dose), 49, 50, 52, 54, 60 (pre-dose), 61, 62, 64, 66<br>**Day 3**: Hr. 72<br>**Day 4, Day 7, Day 14, Day 21, Day 28, Day 35, Day 42** |

The time-window for the visit on Day 4 is + 1 day and for the visits on Days 7 - 42 is + 2 or - 2 days

Total blood collection for pharmacokinetic analysis will be around 165 mL.

The total blood collections for both laboratory assessments and pharmacokinetic sampling on each visit was summarized in Appendix 4.

6.4.1. Pharmacokinetic Samples (Plasma Matrix)

Blood samples (1 mL for regimen 1, 2 and 2 mL for regimen 3) will be collected into properly labeled 2 mL sodium heparin blood collection tubes at the study times shown in Table 2.

Sample Handling

Upon collection, blood samples will be centrifuged for 7 minutes at 2000g at 4°C. After centrifugation, the plasma of each sample will be transferred via pipette to a pre-labeled sample storage tube and stored upright until analyzed.

Sample Storage

Samples will be immediately frozen in the storage tubes in the upright position in a non-self-defrosting freezer at -70°C or lower.

**6.5. Storage of Genetic Samples**

If the subject consents for genetic sample collection and testing, the genetic samples (10 mL) will be collected.

In case of any abnormal metabolisms in individuals, genotyping for the identification of polymorphisms of Cytochrome 450 (e.g. CYP2D6, CYP3A4) and other enzymes related to drug metabolism will be performed. DNA will be extracted from blood and PCR will be performed. SNP typing will be used to determine the gene mutation by comparing subject with normal metabolism activities to subject with abnormal metabolism activities. These tests will determine if there is any genetic abnormality. Such data may be useful in predicting those who may not obtain the full therapeutic or prophylactic benefit of study drugs.

Genetic samples will be stored (approximately 10 years) and tested at the Molecular Tropical Medicine Laboratory, Faculty of Tropical Medicine, Mahidol University. In case that particular genetic test is unable to be performed at Molecular Tropical Medicine Laboratory, Faculty of Tropical Medicine, Mahidol University, genetic sample may be transferred to another institute with subject consent.

A MTA (Material Transfer Agreement) will also be in place before any samples are sent to another institution for testing.

Any proposed plans to use this sample other than for the purposes of this study will be submitted to the IRB/EC prior to any genetic testing being performed.

## 7. LIFESTYLE AND/OR DIETARY RESTRICTIONS

- Subjects should refrain from all illicit drugs throughout the study.
- Subjects should refrain from the ingestion of grapefruit containing products throughout the study.
- Subjects must fast from all food and drink for at least 8 hours prior to clinical laboratory assessment.
- Subject should not consume alcohol within 48 hours prior to study drug administration and throughout the study.
- Subjects will be allowed to eat normally during out-patient/washout time and will receive standard meal during inpatient stay. All drugs will be taken with light meal. They will be given a similar light standard meal within 30 minutes before each drug dosing (similar calories, carbohydrate, protein and fat ratio). Subjects will be allowed to eat 4 hours after study drug administration. Water or soft drinks without caffeine may be consumed 2 hours post-dose (after autonomic function testing). All subjects will receive the same meal.
- Total fluid intake may not exceed 3L per day during the dosing period.

## 8. PRODUCTS

- **Artemether-lumefantrine**

  Artemether-lumefantrine is in the form of dispersible or standard tablets of 20 mg of artemether and 120 mg of lumefantrine in a fixed-dose combination formulation. The weight-based dosing schedule in appendix 3.

- **Amodiaquine**

  Amodiaquine is in the form of dispersible tablets containing 76.5 mg amodiaquine co-blistered with sulfadoxine/pyrimethamine 250 mg/12.5 mg dispersible tablet (SPAQ-CO® Disp).

  Amodiaquine dispersible tablets are tablets with yellow core debossed with “AM” on one side and a score line on the other side.

  Sulfadoxine/pyrimethamine dispersible tablets are white round tablets, debossed with “SP” on one side and a score line on the other side.

  This study will only use the amodiaquine. The weight-based dosing schedule in appendix 3.

  All sulfadoxine/pyrimethamine tablets, unused Amodiaquine and unused Artemether-lumefantrine will be disposed following hospital standard procedures after study completion.

## 9. CONCOMITANT MEDICATIONS

The Principal Investigator must be informed as soon as possible about any medications taken by the subject from the time of screening until the last follow up visit or withdrawal visit. If a subject takes any concomitant medications taken during the study, it will be recorded in the CRF. Drug name, dose, frequency, route of administration and the start and end dates of administration are to be recorded

### 9.1. Prohibited Concomitant Medications

Use of prescription drugs including but not limited to drugs with antimalarial activities and any drug contraindicated with the investigational drugs e.g. quinacrine, mefloquine or non-prescription drug, including, vitamins, herbal and dietary supplements (including St. John's Wort) within 7 days (or 14 days if the drug is a potential enzyme inducer) or 5 half-lives (whichever is longer) prior to the first dose of study medication and for the duration of the trial including follow-up will be prohibited, unless in the opinion of the investigator, the medication will not interfere with the study procedures or compromise subject safety; the investigator will take advice from the manufacturer representative as necessary,

Subject should discuss with study team member before using over-the-counter medications during the study period except as needed to treat an adverse event.

If a subject takes a prohibited medication during the course of the study, the investigator and the statistician will determine if the subject should be withdrawn from the study or from the final pharmacokinetic analysis.

## 10. SUBJECT COMPLETION AND WITHDRAWAL

### 10.1. Subject Completion

Subjects who complete all the study visits and follow up per protocol, will be considered as having completed the study.

### 10.2. Subject withdrawn from the study

This includes the following:

- Subject voluntarily withdrew consent
- The Principal Investigator, sponsor withdrawing the study subject at his/her discretion for the benefit of the subject.

The reasons for withdrawal, or failure to provide a reason, will be documented by the investigator in the Completion/Withdrawal section of the CRF.

If a subject is prematurely discontinued from participation in the study for any reason, the investigator must make every effort to perform the follow up visit assessments, unless they have withdrawn consent.

### 10.3 Subject discontinues the study drugs

Such subjects will be followed up for safety data collection. Reasons for this scenario may include:

- Pregnancy
- Subject requests to discontinue study drugs
- Abnormal renal and hepatic laboratory result including;
    - o Any creatinine above 1.5 x ULN OR increase 0.3 mg/dL above baseline
    - o Any ALT or AST above 3 x ULN
    - o Any elevation in total and direct bilirubin 2x ULN
    - o Any AST or ALT above 2xULN and TBL >1.5xULN
    - o Any AST or ALT above 2xULN with the appearance of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash and/or eosinophilia (eosinophil percent or count above the ULN)

If the abnormalities are potentially caused by one of the study drug the research physician will decide whether the other regimen of the study drugs should be continued. All laboratory abnormalities will be assessed by a trained physician. In case of abnormalities, a medical history to assess exposure to substances (medication, alcohol and others) with known toxicity for instance liver or kidney will be performed. In addition, the physician can perform additional investigations that are deemed necessary to explain the laboratory abnormalities (such as hepatitis serology or urine analysis). The liver function and creatinine abnormalities will be monitored until resolution or the stabilization of the condition. In any case all laboratory values should have returned to normal (within the normal range in the case of full blood count values and at least <1.5 times the ULN in case of the biochemistry values) before the administration of the next treatment round.

- QTc interval abnormalities;
- QTc or QT interval longer than 500 milliseconds

A case by case judgement will be made on whether the subjects should be discontinued from the study completely. All other drugs known to prolong the QTc interval will be stopped. QTc intervals will be monitored continuously until normalisation of the QTc interval to below 450 milliseconds. Magnesium and potassium levels will be measured and deficiencies will be supplemented through intravenous or oral medication.

Unevaluable subjects may be replaced, according to the discretion of the investigators with another subject of the same population, if either sample size or completeness of dataset is compromised. All data will be listed and will be valid for clinical safety and tolerability evaluation.

### 10.4 Study Halting Rules

When three subjects experience adverse events of grade 3 or above that are "probably related or related" to the study drug, administration of study drug(s) will be halted and the investigator team will stop recruiting new study subjects promptly. Enrolled subjects who do not experience adverse event of grade 3 or above will receive study drug(s) until the study ends.

## 11. ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

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 reported from first dose of study drug administration to discontinuation of the subject from study participation. Any events occurring between screening and first dose of study drug administration will be considered as baseline, preexisting conditions.

### 11.1. Definition of an Adverse Event

An AE is any undesirable event that occurs to a study subject 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. Therefore, an AE can be absent at baseline but newly develops, or was present at baseline and worsens and includes:

- any unfavorable and unintended symptom
- physical sign
- abnormal laboratory result

- an illness

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

### 11.2. Definition of a Serious Adverse Event

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

a. Results in death

b. Is life-threatening

NOTE: The term 'life-threatening' in the definition of 'serious' refers to an event in which the subject was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, if it were more severe.

c. Requires hospitalization or prolongation of existing hospitalization

NOTE: In general, hospitalization signifies that the subject has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or out-patient setting. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfills any other serious criteria, the event is serious. When in doubt as to whether "hospitalization" occurred or was necessary, the AE should be considered serious.

Hospitalization for elective treatment of a pre-existing condition that did not worsen from baseline is not considered an AE.

d. Results in disability/incapacity

NOTE: The term disability means a substantial disruption of a person's ability to conduct normal life functions. This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, and accidental trauma (e.g., sprained ankle) which may interfere or prevent everyday life functions but do not constitute a substantial disruption.

e. Results in a congenital anomaly/birth defect

Medical or scientific judgment should be exercised in deciding whether reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These should also be considered serious.

Examples of such events are invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias, or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

### Laboratory Abnormalities and Other Abnormal Assessments as Adverse Events or Serious Adverse Events

Abnormal laboratory findings (e.g., biochemistry, hematology, urinalysis) or other abnormal assessments (e.g., BP, RR, BT, HR and ECGs) detected during the study or are present at baseline and worsen following the start of the study will be reported as AEs or SAEs.

Subjects with an adverse event will be managed clinically. This may include stopping study

drug, further investigations, treatment, referral to a specialist, etc.

### 11.3 Prompt Reporting of Serious Adverse Events

All SAEs should be reported promptly to medical monitor for the study within 24 hours for all SAEs to e-mail address: ALAQsafety@tropmedres.ac.

If all information is not yet available then an initial report must be made within the timeframe. Further interim reports may be made if necessary or a final report when all relevant information is available.

Faculty of Tropical Medicine Ethic committee (FTM EC) will be notified within 5 official days for all SAEs.

### 11.4 Evaluating Adverse Events and Serious Adverse Events

11.4.1. Assessment of Intensity

All adverse events will be evaluated by the investigators and graded according to Common Terminology Criteria for Adverse Events (CTCAE) version 5.0. For lab results that are not available in Common Terminology Criteria for Adverse Events (CTCAE) version 5.0, the investigator should make a determination as to whether or not the laboratory abnormality is clinically significant. If PI believes lab abnormality is clinically significant, it should be reported as an adverse event and a grade should be identified to the best of their ability.

If an adverse event is not listed in the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0, 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 non-invasive 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.

Clarification of the difference in meaning between “severe” and “serious”

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

11.4.2. Assessment of Relatedness

The investigator is obligated to assess the relationship between study drug and the occurrence

of each AE/SAE using the following categories of relatedness:

- Definite: clear-cut temporal association, with a positive re-challenge test or laboratory confirmation.
- Probable: clear-cut temporal association, with improvement upon drug withdrawal, and not reasonably explained by the subject's known clinical state.
- Possible: less clear temporal association; other etiologies are possible. Other possible etiologies should be recorded on the CRF.
- None: no temporal association with the study drug; related to other aetiologies such as concomitant medications or conditions, or subject's known clinical state.

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

11.4.3. Action Taken

- No action taken
- Study drug is changed
- Study drug is discontinued
- No concomitant medication required
- Concomitant medication discontinued
- Concomitant medication is required
- Hospitalization required or prolonged
- Other

11.4.4. Outcome

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

- Resolved
- Resolved with sequelae
- Continuing
- Death

## 12. DATA ANALYSIS AND STATISTICAL CONSIDERATIONS

The primary focus of statistical analysis is to verify the pharmacokinetic interactions of artemether, lumefantrine and amodiaquine in terms of $AUC_{0-\infty}$, $AUC_{0-LAST}$ and $C_{MAX}$. Comparisons across groups will be made using an analysis of variance (ANOVA) of logarithmically transformed pharmacokinetic parameters (i.e. $AUC_{0-LAST}$, $AUC_{0-\infty}$, $C_{MAX}$). The criteria for assuming no interaction or equivalence when co-administered is met if confidence interval (90% CI) values for geometric mean ratios of $AUC_{0-\infty}$, $AUC_{0-LAST}$ and $C_{MAX}$ fall within 80% to 125% for the two groups. The point estimate of the geometric mean ratio and the residual variability from the ANOVA will be used to calculate the 90% confidence intervals around the mean.

### 12.1 General Considerations

All data will be summarized descriptively for the appropriate analysis sets defined below.

### 12.2 Analysis Sets

Safety Set

All subjects who receive study drug(s) will be included in the safety population.

Full Analysis Set

Subjects who receive the full dose of study drug without vomiting with enough samples for characterization of the concentration-time profile will be included in the pharmacokinetic analysis.

Subjects who vomit study drug or who do not have enough time points for characterization of their PK profile or with protocol violations will be replaced at the discretion of the study investigator so that the study sample size is retained for analysis.

## 12.3 Subject Disposition

The number of subjects recruited and receiving study drug(s) will be presented by study drug groups, as will be the number of subjects included in the various analysis populations.

Protocol violations will be listed per subject, describing the nature of the violation and specifying whether it is a major or a minor violation.

## 12.4 Determination of sample size

A sample size of at least 12 is recommended sample size for determining bioequivalence test (22).Therefore, we will recruit 16 healthy volunteers to ensure that we have higher power of the bioequivalence test.

## 12.5 Methods of statistical analysis

12.5.1 Pharmacokinetic assessments

Individual concentration-time data will be evaluated using a non-compartmental analysis approach as implemented in WinNonlin (Pharsight Corporation, California, USA). Total exposure up to the last measured concentration ($AUC_{0\text{-}LAST}$) will be calculated using the linear trapezoidal method for ascending concentrations and the logarithmic trapezoidal method for descending concentrations. Exposure will be extrapolated from the last observed concentration to time infinity by $C_{LAST}/\lambda_Z$ for each individual subject to compute total drug exposure ($AUC_{0\text{-}\infty}$). The terminal elimination half-life ($t_{1/2}$) will be estimated by log-linear regression of the terminal phase. Maximum plasma concentration ($C_{MAX}$) and time to maximum concentration ($T_{MAX}$) will be taken directly from the observed data. Apparent volume of distribution ($V_D/F$) and oral clearance (CL/F) will be computed individually according to standard procedures and summarized for each treatment arm.

Pharmacokinetic parameters (i.e. $AUC_{0\text{-}LAST}$, $AUC_{0\text{-}\infty}$, $C_{MAX}$, $T_{MAX}$, CL/F, $V_D/F$, and $t_{1/2}$) will be described using means (SD or 95% CI) and medians (range) as appropriate.

12.5.2 Safety Analysis

Safety analyses will be based on the safety set. Safety and tolerability of artemether, lumefantrine and amodiaquine will be assessed by comparing the frequency (%) of adverse events and serious adverse events, with particular attention to abdominal pain, appetite perturbation 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 as treated.

An additional exploratory analysis evaluating independent renal markers against standard blood urea nitrogen/ creatinine levels will be conducted using appropriate statistical methods.

Adverse Events

All adverse event summaries will refer to treatment emergent adverse events, i.e. adverse

events that newly started or increased in intensity after the study drug administration. AE summaries will be generated for all AEs that occurred after study drug administration, until the end of the study.

### 12.6 Assessment of the relationship between pharmacokinetic parameters and ECG findings.

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

### 12.7. Interim analyses

No formal interim analysis is planned.

## 13. STUDY CONDUCT CONSIDERATIONS

### 13.1. Regulatory and Ethical Considerations, Including the Informed Consent Process

The study will be conducted in accordance with all applicable regulatory requirements. The study will also be conducted in accordance with good clinical practice (GCP), all applicable subject privacy requirements, and, the guiding principles of the Declaration of Helsinki.

### 13.2. Risks

#### Artemether-lumefantrine

Reported Artemether-lumefantrine side effects have generally been mild. The common side effects are headache (56%), anorexia (40%), dizziness (39%), asthenia (38%), GI disorders (loss of appetite 40%, nausea 26%, vomiting 17%, abdominal pain 17%), arthralgia (34%), myalgia (32%), sleep disorder (22%), palpitations (18%), fatigue (17%). Rare but could be serious side effects are hepatomegaly (9%) and anemia (4%).

#### Amodiaquine

The main side effects of amodiaquine are nausea, vomiting and fatigue which are mild to moderate in nature. Transient sinus bradycardia and QTc prolongation were reported in malaria patients treated with amodiaquine (16 and 7 of 20 patients, respectively), but did not result in any symptoms (Ngouesse et al., 2001). Transient neurological problems were reported in drug overdose. When the drug was used for a long period such as in malaria prophylaxis (now not recommended), rare adverse reactions of agranulocytosis (1:2000-2200), hepatoxicity (1:15600), yellow pigmentation, and ocular changes including irreversible retinal abnormalities were observed.

#### Risk of Blood Collection

Sample blood taking may cause some pain, bleeding and bruising. Rarely, a local skin infection may develop. Occasionally, a subject may feel lightheaded or faint when blood is drawn. Blood for the pharmacokinetic study will be drawn via an indwelling catheter whenever possible to minimize the number of venipunctures.

### 13.3. Benefit

There are no anticipated direct benefits to the subjects in this study. Knowledge gained from this study is expected to help find the drug dosage for optimal treatment regimen.

### 13.4. Confidentiality

The information obtained during the conduct of this clinical study is confidential. The results of the research study may be published, but subject names or identities will not be revealed. Records will remain confidential. To maintain confidentiality, the principal investigators at the study site will keep records in locked cabinets and subjects will be only identified by subject number and initial to prevent association with the subject's name.

The monitor(s), the auditor(s), members of the Ethics Committee, and regulatory authorities may be able to access your medical and study records. However, any information is still confidential and your name will not be identified in the publications of this research outcome.

With subject's consent, subject's clinical data and results from blood analyses stored in our database may be shared with other researchers to use in the future. However, the other researchers will not be given any information that could identify the subject.

### 13.5. Compensation

Subjects will receive compensation for traveling costs associated with traveling to the study site and loss of work time during their stays in the hospital. The amount of compensation will be 500 baht/day for screening visit (visit 1) and each follow up visit after admission visit 2-4 and 6,000 baht for 4 nights admission (visit 2-4). Total compensation will be around 28,000 baht for screening, all admission visits and follow up visits.

If the subject is withdrawn from the study before the study ends, the subject will be compensated for the time that the subject is actually enrolled in the study.

### 13.6. Data Management

The data collection tool for this study will be approved case report forms (CRFs). Subject data necessary for analysis and reporting will be entered/transmitted into a validated database or data system. Clinical data management will be performed in accordance with applicable standards and data cleaning procedures.

Study records will be stored for a period of 10 years after completion of the study, while electronic data will be retained indefinitely.

### 13.7. Study Monitoring

The trial will be conducted in compliance with this protocol, International Conference on Harmonization (ICH) Guidelines for Good Clinical Practice (GCP) and any applicable regulatory requirement(s). Monitors will visit the clinical research sites to monitor all aspects of the study in accordance with the appropriate regulations. The objectives of a monitoring visit will be:

1) to verify the prompt reporting of all data points, including reporting SAEs, checking availability of signed informed consent

2) to compare individual subject records and the source documents (supporting data, laboratory specimen records and medical records to include physician progress notes, nurses' notes, subjects' hospital charts)

3) to ensure protection of study subjects, compliance with the protocol, and accuracy and completeness of records

The monitors will be involved with the site initiation visit, study close out visit and interim monitoring visits the number of which will be determined by the quality of the site. In addition, the monitor will inspect the clinical sites regulatory files to ensure that regulatory requirements are being followed. During the monitoring visits, the investigator (and/or designee) and other

study personnel will be available to discuss the study progress and monitoring visit.

### 13.8. Protocol Deviation and Violation

A protocol deviation is a variance in a research activity between that described in the protocol that has been reviewed and approved by the IRB/EC and the actual activities or procedures that were performed. A protocol deviation may be classified as minor or significant. A "significant" protocol deviation is also referred to as a protocol violation.

Only the site Investigator may institute emergency departures from the protocol that eliminate an apparent immediate hazard to a subject and that is deemed crucial for the safety and wellbeing of that subject. The site IRB/EC must be made aware of these departures from the protocol in accordance with local requirements.

**Protocol Deviation**

A protocol deviation is an activity performed that deviates from the approved protocol but does not significantly affect the subject's safety and/or efficacy assessment of the trials (no immediate hazard or data falsification). These deviations are to be noted on the "Protocol Deviation /Violation Form" which will be reviewed by the monitor during monitoring visits.

The following are examples of protocol deviations:

- Missed tests or study procedures
- Late or missed visit by a subject.

**Protocol Violation**

A protocol violation is an activity that has the potential to or is affecting the safety, rights and wellbeing of subjects and/or efficacy assessment of the trial. If a protocol violation is identified by either site staff or the monitor, the site Investigator needs to complete the Protocol Deviation/Violation Form.

The following are some examples of activities that deviate from the protocol that will be considered protocol violations that should be reported:

- Omission or inadequate administration of informed consent form
- Error of study drug administration
- Enrolling a subject who does not meet the study criteria for enrollment
- Subject who developed withdrawal criteria during the study but was not withdrawn
- Any activity that has the potential to or is affecting the subject's rights, safety or welfare

### 13.9 Insurance Policy

The University of Oxford has a specialist insurance policy in place: - Newline Underwriting Management Ltd, at Lloyd's of London – which would operate in the event of any Subject suffering harm as a result of their involvement in the research."

### 13.10. 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.

## 14. RESEARCH USE OF STORED HUMAN SAMPLES/SPECIMENS

### 14.1. Intended Use of the Samples/Specimens

Samples and data collected under this protocol will be used to study the pharmacokinetic parameters of artemether-lumefantrine and amodiaquine.

### 14.2. Storage of Samples/Specimens

Samples will be stored no longer than 10 years using codes assigned by the investigators or their designee(s). Access to research samples will be limited using either a locked room or a locked freezer.

In the future, investigators from this protocol and other investigators besides those listed in this protocol may wish to study these samples. A proposal of the planned research will be submitted to the IRB for their consideration.

### 14.3. Storage of Genetic Sample

Any proposed plans to use this sample other than for the purposes of this study will be submitted to the IRB/EC prior to any genetic testing being performed. Genetic samples will remain in the country of collection until (if applicable) the IRB/EC approves shipment of genetic material for specific testing. A MTA (Material Transfer Agreement) will also be in place before any samples are sent to another institution for testing.

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**Appendix 1**
Protocol Approval and Signature Page

I have read and approve protocol titled A Randomized, **Open-Label Study to Evaluate Potential Pharmacokinetic Interaction of Orally Administered Artemether-lumefantrine and Amodiaquine in Healthy Adult Subjects**. I confirm that this study will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality.

I will conduct this study in accordance with all applicable regulations and Good Clinical Practice (GCP).

_____ _____

(Assistant Professor Borimas Hanboonkunupakarn) Date

Principal Investigator

## Appendix 2

Time and Events Table

| Procedure | Visit 1 | Within 14 days[11] | Visits 2-4 | | | | | Follow up visit[2,4,5,6,9,10,12] |
|---|---|---|---|---|---|---|---|---|
| | Screening | | Day -1 | Day 0 | Day 1 | Day 2 | Day 3 | |
| Informed Consent | × | | | | | | | |
| Demographics | × | | | | | | | |
| Medical Hx/ Medication Hx | × | | × | | | | | |
| Check in to unit | | | × | | | | | |
| Physical Exam and symptoms questionnaire | × | | × | × | × | × | × | |
| Vital signs | × | | ×[1] | ×[1] | ×[1] | ×[1] | ×[1] | |
| Pregnancy test | ×[2] | | ×[3] | | | | | |
| ECG | × | | ×[4] | ×[4] | ×[4] | ×[4] | ×[4] | |
| Holter monitoring | | | × | × | × | × | × | |
| Clinical Lab[5]<br>- Hematology<br>- Chemistry<br>- FBS<br>- Urinalysis | × | | × | | | | × | |
| 24-hour urine for creatinine clearance test | | | × | | | × | | |
| PK blood collection[6] | | | | × | × | × | × | |
| Study Drug Administration[7] | | | | × | (×) | (×) | | |
| PGx sample[8] | | | (×) | (×) | (×) | (×) | (×) | |
| Adverse event[9] | | | | × | × | × | × | |
| Concomitant medication[10] | | | | × | × | × | × | |
| Discharge from unit | | | | | | | × | |

1. Vital signs will be measured before dosing and every 4 hours after first dose. Blood pressure (BP) and heart rate (HR) for visit 2-4 will be measured in both the supine and standing positions in each time-points
2. Serum pregnancy test will be done at screening visit
3. Urine pregnancy test will be done at day -1 for each admission visit and biweekly during follow up visit
4. ECG (x3) will be performed according to table 2
5. Clinical lab assessment will be performed at screening visit (visit 1), day -1 and day 3 of each admission visit (visit 2-4) and weekly during follow up visit (see appendix 4 for the detail)
6. PK sample will be collected according to table 2 and appendix 4
7. Study drug administration will be administered according to table 1 and 2 and appendix 3
8. Genetic sample can be collected at any of the subsequent visit if the blood draw at the visit 2 is missed
9. Adverse events will be monitored until the last follow up visit.
10. Concomitant medications will be monitored until last follow up visit.
11. If interval between screening (visit 1) and on day -1 visit 2 is three days or less, the clinical laboratory screening test result and serum pregnancy test result can be used for enrolment evaluation on day -1 visit 2. In such cases, these tests would not need to be repeated at day-1 visit 2.
12. Holter monitoring will be performed on day -1, admission visit, D7 and D28 of visit 2, visit 3 , and visit 4

## Appendix 3

Drug dosing schedule

| Artemether-lumefantrine dosing schedule | | | | | | |
|---|---|---|---|---|---|---|
| **One tablet A/L contains 20 mg artemether and 120 mg lumefantrine.** | | | | | | |
| | No. of tablets recommended at approximate timing of dosing | | | | | |
| Weight: | 0 h | 8 h | 24 h | 36 h | 48 h | 60 h |
| ≥ 35 Kg | 4 | 4 | 4 | 4 | 4 | 4 |

| Amodiaquine dispersible tablets dosing schedule | | | | | | |
|---|---|---|---|---|---|---|
| **One tablet contains 76.5 mg Amodiaquine.** | | | | | | |
| | No. of tablets recommended at approximate timing of dosing | | | | | |
| Weight: | 0h | 8h | 24h | 36h | 48h | 60h |
| 35-41 Kg | 2 | 2 | 2 | 2 | 2 | 2 |
| 42-55 Kg | 3 | 3 | 3 | 3 | 3 | 3 |
| ≥ 56 Kg | 4 | 4 | 4 | 4 | 4 | 4 |

## Appendix 4

Total Blood Collection

| visit | | laboratory assessments | PK Sampling | Blood sampling |
|---|---|---|---|---|
| Visit 1 | Screening visit<br>Clinical lab (Chemistry and Serum pregnancy test, HIV, Hep B, Hep C and TSH 6 mL, Hematology 2 mL, FBS 2 mL) (Total 10 mL) | 10 mL | 0 | 10 mL |
| Visit 2 | Day-1 (admission)<br>Clinical lab (Chemistry 5 mL, Hematology 2 mL, and FBS 2 mL) (Total 9 mL) | 9 mL | 0 | 85 mL<br>(Artemether - lumefantrine only)<br>**Or**<br>100 mL (for amodiaquine only) |
| | Day 0-3 (admission)<br>Clinical lab (Chemistry 5 mL, Hematology 2 mL, and FBS 2 mL) (Total 9 mL)<br>Pharmacokinetic samples at<br>**Day 0**: Hr. 0 (pre-dose), 0.25, 0.5, 1, 2, 3, 4, 6, 8 (pre-dose), 10, 12, 14<br>**Day 1**: Hr. 24 (pre-dose), 26, 28, 30, 36 (pre-dose), 38, 40, 42<br>**Day 2**: Hr. 48 (pre-dose), 48.25, 48.5, 49, 50, 51, 52, 54, 56, 60 (pre-dose), 61, 62, 64, 66<br>**Day** 3: Hr. 72<br>total 35 time-points (1 mL each time- points) | 9 mL | 35 mL | |
| | Day 4 (+1) (follow up)<br>Pharmacokinetic samples (1mL) | 0 | 1 mL | |
| | Day 7 (+/-2d) (follow up)<br>Clinical lab (Chemistry 5 mL and Hematology 2 mL) (Total 7 mL)<br>Pharmacokinetic sample collection (1mL) | 7 mL | 1 mL | |
| | Day 14 (+/-2d) (follow up)<br>Clinical lab (Chemistry 5 mL and Hematology 2 mL) (Total 7 mL)<br>Pharmacokinetic sample collection | 7 mL | 1 mL | |

| visit | | laboratory assessments | PK Sampling | Blood sampling |
|---|---|---|---|---|
| | (1mL) | | | |
| | Day 21 (+/-2d) (follow up)<br>Clinical lab (Chemistry 5 mL and Hematology 2 mL) (Total 7 mL) | 7 mL | 0 | |
| | Day 28 (+/-2d) (follow up)<br>Clinical lab (Chemistry 5 mL and Hematology 2 mL) (Total 7 mL)<br>Pharmacokinetic sample collection (1 mL) | 7 mL | 1 mL | |
| | Day 35 (+/-2d) (follow up) (for Amodiaquine only)<br>Clinical lab (Chemistry 5 mL and Hematology 2 mL) (Total 7 mL) | 7 mL | 0 | |
| | Day 42 (+/-2d) (follow up) (for Amodiaquine only)<br>Clinical lab (Chemistry 5 mL and Hematology 2 mL) (Total 7 mL)<br>Pharmacokinetic sample collection (1 mL) | 7 mL | 1 mL | |
| Visit 3 | Day-1 (admission)<br>Clinical lab (Chemistry 5 mL, Hematology 2 mL, and FBS 2 mL) (Total 9 mL) | 9 mL | 0 | 85 mL<br>(Artemether - lumefantrine only)<br>**Or**<br>100 mL (for amodiaquine only) |
| | Day 0-3 (admission)<br>Clinical lab (Chemistry 5 mL, Hematology 2 mL, and FBS 2mL) ( Total 9 mL)<br>Pharmacokinetic sample collection at<br>**Day 0**: Hr. 0 (pre-dose), 0.25, 0.5, 1, 2, 3, 4, 6, 8 (pre-dose), 10, 12, 14<br>**Day 1**: Hr. 24 (pre-dose), 26, 28, 30, 36 (pre-dose), 38, 40, 42<br>**Day 2**: Hr. 48 (pre-dose), 48.25, 48.5, 49, 50, 51, 52, 54, 56, 60 (pre-dose), 61, 62, 64, 66<br>**Day 3**: Hr. 72<br>Total 35 time-points (1 mL each time- points) | 9 mL | 35 mL | |

| visit | | laboratory assessments | PK Sampling | Blood sampling |
|---|---|---|---|---|
| | Day 4 (+1) (follow up)<br>Pharmacokinetic sample collection (1 mL) | 0 | 1 mL | |
| | Day 7 (+/-2d) (follow up)<br>Clinical lab (Chemistry 5 mL and Hematology 2 mL) (Total 7 mL)<br>Pharmacokinetic sample collection (1 mL) | 7 mL | 1 mL | |
| | Day 14 (+/-2d) (follow up)<br>Clinical lab (Chemistry 5 mL and Hematology 2 mL) (Total 7 mL)<br>Pharmacokinetic sample collection (1 mL) | 7 mL | 1 mL | |
| | Day 21 (+/-2d) (follow up)<br>Clinical lab (Chemistry 5 mL and Hematology 2 mL) (Total 7 mL) | 7 mL | 0 | |
| | Day 28 (+/-2d) (follow up)<br>Clinical lab (Chemistry 5 mL and Hematology 2 mL) (Total 7 mL)<br>Pharmacokinetic sample collection (1mL) | 7 mL | 1 mL | |
| | Day 35 (+/-2d) (follow up) (for Amodiaquine only)<br>Clinical lab (Chemistry 5 mL and Hematology 2 mL) (Total 7 mL) | 7 mL | 0 | |
| | Day 42 (+/-2d) (follow up) (for Amodiaquine only)<br>Clinical lab (Chemistry 5 mL and Hematology 2 mL) (Total 7 mL)<br>Pharmacokinetic sample collection (1 mL) | 7 mL | 1 mL | |
| Visit 4 | Day-1 (admission)<br>Clinical lab (Chemistry 5 mL, Hematology 2 mL, and FBS 2 mL) (Total 9 mL) | 9 mL | 0 mL | 140 mL |
| | Day 0-3 (admission)<br>Clinical lab (Chemistry 5 mL, | 9 mL | 70 mL | |

| visit | | laboratory assessments | PK Sampling | Blood sampling |
|---|---|---|---|---|
| | Hematology 2mL ,and FBS 2mL) (Total 9 mL)<br>Pharmacokinetic sample collection at<br>**Day 0**: Hr. 0 (pre-dose), 0.25, 0.5, 1, 2, 3, 4, 6, 8 (pre-dose), 10, 12, 14<br>**Day 1**: Hr. 24 (pre-dose), 26, 28, 30, 36 (pre-dose), 38, 40, 42<br>**Day 2**: Hr. 48 (pre-dose), 48.25, 48.5, 49, 50, 51, 52, 54, 56, 60 (pre-dose), 61, 62, 64, 66<br>**Day 3**: Hr. 72<br>Total 35 time-points (2 mL each time- points) (1 mL for Artemether -lumefantrine and 1 mL for Amodiaquine | | | |
| | Day 4 (+1) (follow up)<br>Pharmacokinetic sample collection (2 mL) | 0 | 2 mL | |
| | Day 7 (+/-2d) (follow up)<br>Clinical lab (Chemistry 5 mL and Hematology 2mL) (Total 7 mL)<br>Pharmacokinetic sample collection (2 mL) | 7 mL | 2 mL | |
| | Day 14 (+/-2d) (follow up)<br>Clinical lab (Chemistry 5 mL and Hematology 2 mL) (Total 7 mL)<br>Pharmacokinetic sample collection (2 mL) | 7 mL | 2 mL | |
| | Day 21 (+/-2d) (follow up)<br>Clinical lab (Chemistry 5 mL and Hematology 2mL) (Total 7 mL) | 7 mL | 0 | |
| | Day 28 (+/-2d) (follow up)<br>Clinical lab (Chemistry 5 mL and Hematology 2mL) (Total 7 mL)<br>Pharmacokinetic sample collection (2 ml) | 7 mL | 2 mL | |
| | Day 35 (+/-2d) (follow up)<br>Clinical lab (Chemistry 5 mL and | 7 mL | 0 | |

| visit | | laboratory assessments | PK Sampling | Blood sampling |
|---|---|---|---|---|
| | Hematology 2mL) (Total 7 mL) | | | |
| | Day 42 (+/-2d) (follow up)<br>Clinical lab (Chemistry 5 mL and Hematology 2 mL) (Total 7 mL)<br>Pharmacokinetic sample collection (2 mL) | 7 mL | 2 mL | |
| Genetic sampling (10 mL)<br>Genetic sample can be collected at any schedule visits. | | 10 mL | | 10 mL |
| **Total** | | | | **345 mL** |