ALAQ

STATISTICAL ANALYSIS PLAN

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
Trial Registration number: NCT04080895

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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.

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. 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.

2. STUDY OBJECTIVES AND ENDPOINTS

2.1 PRIMARY OBJECTIVE

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

2.2 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.

2.3 SECONDARY OBJECTIVES

a. To characterize the pharmacokinetic properties of artemether-lumefantrine and amodiaquine when given alone and in combination.
b. To evaluate the safety and tolerability of co-administered artemether-lumefantrine and amodiaquine. Protocol version 6.0 dated 22 June 2022 10 of 42.
c. To investigate pharmacogenetic polymorphisms affecting drug levels of artemetherlumefantrine and amodiaquine and their metabolites.

2.4 SECONDARY ENDPOINTS

a. 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.
b. Safety and tolerability parameters, including adverse events, electrocardiographic changes, vital signs and biochemical assessments. •
c. Pharmacogenetic polymorphisms in the case of unusual metabolizeStudy design

2.5 GENERAL

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 Artemether-lumefantrine followed by Amodiaquine or Amodiaquine followed Artemether-lumefantrine. There will be a washout period: > 6 weeks (after day 3) and then each of the participants will be given Artemether-lumefantrine + Amodiaquine. Thus, eEvery subject will have 1 screening and 3 admissions in the hospital.

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 day1 visit 2

3. ANALYSIS

3.1 GENERAL CONSIDERATIONS

The main strategy of analysis will be characterising the potential pharmacokinetic interactions of artemether - lumefantrine and amodiaquine . Safety analysis will also be done for potential signals and clinical importance. Means (SD) will be used to summarise normally distributed baseline variables. Non-normally distributed variables will be summarised using medians (IQR). Count data will be summarised using frequency counts and percentages. The percentages/proportions will be compared using Fisher's Exact test and a significance level of 5%. Detailed analysis approaches are provided in the relevant sections below.

3.1.1 Data integrity

This study will be conducted in compliance with the protocol, relevant Standard Operating Procedures (SOPs), Good Clinical Practice (GCP) and the applicable regulatory requirement(s). All the analyses will be performed on clean data only.

3.1.2 Data cleaning and verification

All data will be cleaned and verified prior to statistical analysis. The data manager will ensure that clean data is submitted to the statistician who will coordinate with the pharmacologist for analysis. The statistician and the pharmacologist will cross-check that the available data for analysis is clean. Any data cleaning queries will need to be resolved before statistical and pharmacological analyses.

3.1.3 Locking the dataset

After data cleaning and responding to all data queries, the clean data will be locked normally in the database that was used for data capturing. The data may also be locked and stored in other user-friendly formats such as MS Excel and Stata. The locked data will be stored at an identifiable secure place and should be available to the relevant researchers upon request following proper request procedures. The data will also be in other backup media such as CDs or tapes.

3.1.4 Data format and Analysis logs

Data will be given to the Trial Statistician in compatible excel format (e.g. csv) who will coordinate with the Pharmacologist. The statistical analyses will be done in STATA (version 18) or R software while the pharmacokinetic analyses will be performed using PK specialised software such as Phoenix 64 or NONMEM as appropriate. Do files and scripts will be developed by the statistician and pharmacologist for the respective analysis components to be used to run the analyses. This will ensure reproducibility of the analyses. Logs (output from the script/do-files) and do files/ scripts will be kept by the statistician backed up on the server in a study folder for all analyses and made available upon request.

3.2 NUMBER OF PARTICIPANTS

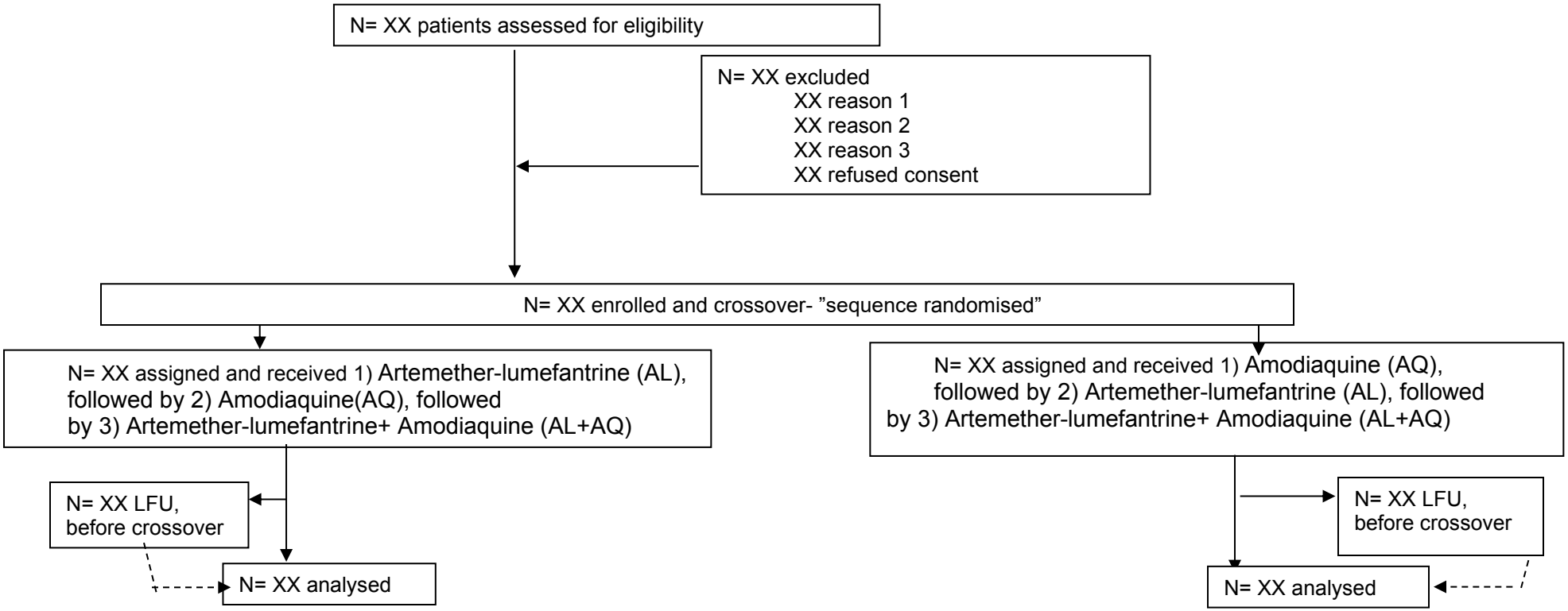
This study will enroll 16 healthy subjects both male and female, aged 18-60 years, at the pharmacokinetic unit Faculty of Tropical Medicine, Mahidol University. Subjects will be healthy HIV-1, hepatitis B and C uninfected individuals who comprehend the purpose of the study and have provided written consent.

3.3 STATISTICAL/ANALYTICAL ISSUES

3.3.1 Handling of dropouts or missing data

We expect most of the baseline variables to be collected as they form part of the inclusion/exclusion criteria. However, we expect to have some missing outcome data arising either from patients withdrawing from the study or being lost to follow-up. Available data analysis approach will be performed.

3.4 FIGURE 1 – TRIAL PROFILE



3.5 STUDY ASSESSMENTS

3.5.1 Demographics and other baseline characteristics

The baseline summaries will include the below variables (Table 1), stratified by group.

Table 1. Demographic and other baseline characteristics at the time of crossover

Characteristic	AL →AQ→AL+AQ	AQ →AL→AL+AQ
Age (years) Median (IQR)		
Sex: female n(%)		
Weight (kg) Median (IQR)		
Height (cm) Median (IQR)		
Body Mass Index (kg/m^2) Mean(sd)		
Haemoglobin (g/dL) Mean(sd)		
WBC (10^3/uL) Median (IQR)		
Platelet count (10^3/uL) Median (IQR)		
Neutrophil count (%) Median (IQR)		
ALT (U/L) Median (IQR)		
AST (U/L) Median (IQR)		
Creatinine (mg/dL) Median (IQR)		
XXXX		

3.5.2 Pharmacokinetic assessments

Individual pharmacokinetic parameters of artemether/dihydroartemisinin, lumefantrine/desbutyl-lumefantrine and amodiaquine/desethylamodiaquine will be calculated after each dose administration using a non-compartment approach.

Total exposure up to the last measured concentration (AUC_{LAST}) will be calculated using the linear trapezoidal method for ascending concentrations and the logarithmic trapezoidal method for descending concentrations. The terminal elimination half-life ($t_{1/2}$) will be estimated by the slope (λ_Z) of the best-fit log-linear regression of the observed concentrations in the terminal elimination phase. Drug exposure will be extrapolated from the last observed concentration to time infinity by C_{LAST}/λ_Z for each individual subject to compute total drug exposure (AUC_∞). Maximum concentration (C_{MAX}) and time to maximum concentration (T_{MAX}) will be taken directly from the observed data. Apparent oral elimination clearance (CL/F) and apparent volume of distribution (V/F) will be computed individually according to the equations below.

$$CL/F = \frac{Dose}{AUC_\infty} \qquad V/F = \frac{t_{1/2} \times CL/F}{ln2}$$

The pharmacokinetic parameters will be summarised for each drug stratified by arm (i.e., when administered alone or in combination). Similarly, descriptive statistics will be summarised by arm. Ln-transformed pharmacokinetic exposure parameters (C_{MAX}, AUC_{LAST} and $AUC_{0\infty}$) will be evaluated to assess potential drug-drug interactions. No drug-drug interaction is assumed if the 90% confidence intervals of the ratio (combination/alone) of C_{MAX}, AUC_{LAST} and AUC_∞ fall within 80% to 125% [10]. The 90% confidence intervals of the ratio (combination (COMB)/alone (ALONE)) will be calculated according to equation below.

$$Mean\ ratio\ (\%) = 100 \cdot e^{Parameter_{combination} - Parameter_{alone}}$$

where $Parameter_{combination}$ and $Parameter_{alone}$ are the least square mean of the natural logarithm of parameters derived for the combination and alone administration, respectively. The upper and lower boundary of the 90% confidence interval are calculated as follow:

$$90\%\ Confidence\ Interval = 100 \cdot e^{(Parameter_{combination} - Parameter_{alone}) \pm t_{0.95} \cdot SE_D}$$

where $t_{0.95}$ is taken from the student-t distribution, SE_D is the standard error of the difference of the test and reference least squares means. The pharmacokinetic parameters of combination to alone administration will be summarised in Table 2 below.

Table 2. Pharmacokinetic parameters of {Drug name} when administered alone and in combination.

Parameter	{Drug name} alone	{Drug name} combination	{Metabolite name} alone	{Metabolite name} combination
	Median (range)	Median (range)	Median (range)	Median (range)
C_{MAX} (ng/mL)				
T_{MAX} (h)				
CL (L/h)				
V (L)				
$t_{1/2}$ (h)				
AUC_{LAST} (ng×h/mL)				
AUC_{∞} (ng×h/mL)				

Abbreviations: C_{MAX}, maximum (peak) plasma concentration; T_{MAX}, time to maximum concentration; CL, elimination clearance; V, volume of distribution; AUC_{LAST}, area under the plasma concentration-time curve from time zero to last measurable drug concentration; AUC_{∞}, AUC from time zero extrapolated to infinity.

Drug-drug interaction results will be summarised in Table 3 below.

Table 3 Drug-drug interactions of artemether-lumefantrine and amodiaquine when administered in combination.

Parameter	ARM comb./alone administration	DHA comb./alone administration	LF comb./alone administration	DLF comb./alone administration	AQ comb./alone administration	DEAQ comb./alone administration
	Mean (90% CI)	Mean (90% CI)	Mean (90% CI)	Mean (90% CI)	Mean (90% CI)	Mean (90% CI)
C_{MAX} (ng/mL)						
AUC_{LAST}						

(ng×h/mL)						
AUC_{∞} (ng×h/mL)						

Abbreviations: C_{MAX}, maximum (peak) plasma concentration; AUC_{LAST}, area under the plasma concentration-time curve from time zero to last measurable drug concentration; AUC_{∞}, AUC from time zero extrapolated to infinity; ARM, Artemether; DHA, Dihydroartemisinin; LF, Lumefantrine; DLF, Desbutyl-lumefantrine; AQ, Amodiaquine; DEAQ, desethylamodiaquine.

3.5.3 Safety assessments

The incidence of AEs will be tabulated and reviewed for potential signals and clinical importance. The most prevalent AEs will be reported by group: (AL →AQ→AL+AQ) vs (AQ →AL→AL+AQ). Any Serious Adverse Events (SAEs) including any deaths will also be reported by group. Standard haematological parameters, serum chemistries, liver and renal function test results at screening and post-treatment will be summarised across the treatment groups. For the safety data such as adverse events, proportions will be compared using Fisher's exact test and a significance level of 5% where appropriate.