DeTACT-ASIA STATISTICAL ANALYSIS PLAN

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

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

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

ACRONYM: DeTACT-ASIA study

Version 1
Date 11th March 2024

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PRINCIPAL INVESTIGATOR

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1. Analysis Considerations

1.1 General Analysis Approach

The main analysis strategy for the primary outcome will be the intention-to-treat (ITT) principle. In this analysis, patients will be analysed according to the arm of randomisation, irrespective of the treatment

that was actually given. Complete Case Analysis will be used in the ITT analysis population (Groenwold et al 2012; Mukaka et al 2016; Sullivan et al 2018). Furthermore, the estimates of efficacy using survival analysis will be done. This approach will help in handling missing data as all patients will be included in the analysis and censored at their last follow-up time. These ITT analyses will be followed by the per-protocol (PP) analysis. In this analysis, only those who adhered to the protocol with respect to the primary outcome will be included for analysis of the primary outcome.

Key secondary endpoints such as parasite clearance parameters (e.g. half-lives), safety and tolerability data will be analysed similar to the ITT approach for the main primary outcome. In this analysis, patients will be analysed according to the arm of randomisation irrespective of the treatment that was actually given, and all patients will be included in the analyses irrespective of their follow-up status as long as they have the data. Withdrawals and losses to follow-up will not affect the analyses of this data as long as the relevant data needed for these analyses is available prior to withdrawal or loss to follow-up.

Data analysis will mainly be performed using Stata 17 or higher, StataCorp, 4905 Lakeway Drive College Station, Texas 77845 USA or in R software.

1.2 Data cleaning and verification

All data will be cleaned and verified prior to statistical analysis. The study site will be visited by the Study Monitor periodically at times agreed on with the Investigator. At the time of each monitoring visit, the Study Monitor will review the completed CRFs to ascertain that all items have been completed and that the data provided are accurate and obtained in the manner specified in the protocol. The Study Monitor will also check that the data in the CRF are consistent with the clinical records (Source Data Verification [SDV]) and that study results are recorded completely and correctly. The Data Manager will ensure that clean data is submitted to the Trial Statistician for analysis. The Trial Statistician will cross-check that the available data for analysis is clean. Any data-cleaning queries will need to be resolved before statistical analyses.

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.

1.4 Data format and Analysis logs

Prior to dispensing data to the Trial Statistician, the head of Data Management will make sure that the data to be sent to the Trial Statistician is clean. This will help the statistician to provide the analysis results in a timely manner as there will be a reduced number of queries if clean data is provided. Data will be given to the Trial Statistician by the head of Data Management (or designated person) in a format that is compatible with statistical software reading. Statistical analyses will be performed in Stata, version 17 or higher or R software. Statistical programs and output logs will be kept for all analyses and made available upon request.

1.5 Interim analyses

We plan to have at least 2 interim analyses on clinical, laboratory and electrocardiographic data to assess the safety of the triple artemisinin-based combination therapies (TACTs). The interim reports will be reviewed by the DSMB. The interim analyses will be performed after the first 100 patients, the

first 500 patients and at additional time points before the planned interim analyses, as indicated by the DSMB after their review, if deemed necessary. After this, a safety analysis will be performed based on recommendations by the DSMB. No stopping rules, either statistical or clinical will be specified. The need to stop the trial will be based on the perception of the accumulating data by the DSMB. The main strategy of analysis will focus on safety data although efficacy data may also be presented.

The Trial Coordinator in collaboration with the Trial Statistician will produce the report for the DSMB. Only relevant data included in a specific interim report will be made available to the DSMB members at the time of sending the report. During a DSMB meeting, the report will be presented to the members by the Trial Coordinator in line with the meeting agenda. For a normally scheduled DSMB meeting, the report will be sent out to the members at least a week before the meeting.

2. Introduction

This is a multicentre study with 3 centres in 2 different countries. The study was designed in a way that each centre has 80% power to detect a difference in the efficacy outcomes if it exists.

Artemisinin-based combination therapies (ACTs) have been a major contributor to the substantial reductions in global malaria morbidity and mortality over the last decade. However, further gains are threatened by the recent emergence of artemisinin and partner drug resistance in Southeast Asia, a region which has been the epicentre for the evolution and spread of resistance to every important class of antimalarials. Loss of efficacy of first-line ACTs jeopardizes current malaria control and elimination efforts and will accelerate the spread of drug resistance. A major concern is that artemisinin and partner drug resistance may spread across a wider geographic area, as chloroquine resistance did in the 1960s and 1970s, moving from Southeast Asia to the Indian subcontinent and subsequently to Africa, which bears the vast majority of the global malaria burden. Furthermore, artemisinin resistance could worsen by extending beyond the current ring stage parasite resistance, although this has until now not been observed. We aim to assess the extent of the spread or the *de novo* emergence of resistance to the antimalarials in the combinations through detailed *in vivo* and *in vitro* assessments and to study the pharmacokinetics and inter-drug interactions of the drugs, the parasite- and host-related factors affecting treatment outcomes. We also aim to gain insights into the spread of resistance mediated by parasite gene flow in general through geographic localization of parasite genetic data. 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 the use of a combination of three or more antiretroviral or antimycobacterial therapies respectively, but until now this was not thought necessary in malaria. In malaria there is a fortuitous inverse correlation between susceptibility to lumefantrine and amodiaquine or mefloquine and piperaquine which will be exploited in the TACTs.

2.1. Study objectives and endpoints

2.1.1. Primary objective

To compare the efficacy of the ACTs and TACTs as defined by the 42-day PCR corrected adequate clinical and parasitological response (ACPR) within each individual country for artemether-lumefantrine+amodiaquine vs artemether-lumefantrine+placebo (AL+AQ vs AL+PBO).

2.1.2. Primary endpoint

The Primary endpoint is the 42-day efficacy defined as PCR-corrected adequate clinical and parasitological response (ACPR).

(NB, WHO definition of ACPR: absence of parasitaemia at day 42 irrespective of axillary temperature and without previously meeting any of the WHO criteria for early or late treatment failure, or late parasitological failure.)

2.1.3. Secondary objectives and endpoints

- To compare the safety and tolerability of ACTs and TACTs within and across countries and regions.
    - Incidence of adverse events and serious adverse events within the first 42 days including markers of hepatic, renal or bone marrow toxicity; cardiotoxicity, in particular QT or QTc-interval above 500 ms at timepoint H4 and H52/H64 and between these time points; change from baseline in haemoglobin at days 3, 7, 28, stratified for G6PD status/genotype; proportion of subjects requiring retreatment due to vomiting within 1 hour after administration of the study drugs; proportion of subjects that reports completing a full course of observed TACT or ACT without withdrawal of consent or exclusion from study because of drug-related serious adverse event
    - Changes in the electrocardiogram (such as prolongation of the QTc-interval) in patients treated with TACT versus ACT
- To compare additional measures of treatment efficacy between treatment arms, including the 63-day ACPR, the post-treatment prophylactic effect of ACTs and TACTs defined as the 42-day & 63-day PCR uncorrected ACPR, gametocyte carriage, parasite clearance rates and fever clearance.
    - Comparing 63-day PCR corrected and uncorrected efficacy; 42-day PCR uncorrected efficacy between ACT and TACT
    - Parasite clearance half-life assessed by microscopy as primary parameter to determine parasite clearance; proportion of subjects with microscopically detectable *P. falciparum* parasitaemia at Day 3; fever clearance time (i.e. the time taken for the tympanic temperature to fall below 37.5 ºC in patients who were febrile at inclusion); proportion of subjects with gametocytaemia during and after treatment stratified by presence of gametocytes at enrolment.
- To assess pharmacokinetic and pharmacodynamic interactions between antimalarials in ACT and TACTs
    - Pharmacokinetic profiles and interactions (including Cmax and AUC) of artemisinin-derivatives and partner drugs in ACT and TACT treated subjects in correlation with pharmacodynamics measures of drug efficacy; day 7 plasma levels of partner drugs in correlation with treatment efficacy and treatment arm

2.1.4. Exploratory objectives and endpoints

- To compare measures of treatment efficacy between treatment arms
    - Comparison of 63-day vs 42-day PCR corrected and uncorrected efficacy of ACTs vs TACTs
- To assess molecular genetic and transcriptomic correlates for artemisinin and partner drug resistance of the infecting *P. falciparum* strains
    - Whole genome sequence and transcriptome analysis of artemisinin and/or partner drug-resistant parasites compared with drug-sensitive parasites
- To compare *in vitro* susceptibility profiles of *P. falciparum* isolates across geographic regions
    - In vitro sensitivity of *P. falciparum* to artemisinins and partner drugs according to study sites and genotype
- To compare the selective effect of ACTs versus TACTs on parasites carrying mutations associated with resistance to antimalarial drugs

  - Proportions of recurrent infections with parasites carrying mutations of known functional significance
  - Proportions of specimens collected at baseline with parasites carrying mutations of known functional or operational significance (pfkelch13, pfcrt, pfmdr1, pfdhfr, pfdhps, pfplasmepsin2, partial or complete deletions of pfhrp2 and other current parasite genetic markers associated with resistance or identified over the course of the study)

- To obtain additional safety data (in particular incidence, rate and magnitude of haemolysis) on the deployment of single low dose primaquine, stratified according to G6PD status/genotype
  - Change in hematocrit on day 1 to 7, 14, 21, 28, 35 and 42 according to geographical location and study arm, stratified for G6PD status

- To obtain additional data on the effect of the host genotypes known to affect pharmacokinetics and pharmacodynamics of antimalarials
  - Correlation between the host genotype (e.g., CYP2D6, CYP3A4, KCNQ1/LQT1, KCNH2/LQT2, SCN5A/LQT3) and the pharmacokinetics and pharmacodynamics of antimalarials.
- To assess new methods for the determination of gametocytaemia, parasite phenotypes and genotypes
  - Novel assays to be tested
- To assess the correlation between anti-*Plasmodium falciparum* antibodies and drug efficacy measures
  - Correlation between specific antibody titres and measures of drug efficacy
- To assess and increase the representability/accuracy of parasite genome sequencing from dry blood spots for the genome sequencing results from leukocyte-depleted blood samples
  - Accuracy of SNPs assessment from dry blood spots versus from whole genome sequencing in leukocyte-depleted blood samples
  - Candidate markers of resistance identified through genome wide association studies with in vitro parasite drug sensitivity phenotypes
  - Correlation between qPCR-based versus microscopy-based assessments of parasite clearance dynamics
  - Correlation of parasite clearance metrics as assessed by microscopy versus digital microscopy
- To identify differences at the transcriptome level in artemisinin and partner drug sensitive and resistant *P. falciparum* in order to increase the understanding of mechanisms of resistance
  - Comparison of transcriptomic patterns of drug sensitive and resistant parasites before treatment and 6, 12 and 24 hours after the start of treatment
- To develop DNA and RNA measurement methods for quantification of male and female gametocytes
  - Levels of RNA transcription coding for male or female-specific gametocytes at admission up to day 14, stratified by the presence of gametocytes at enrolment

2.2. Study design

Brief Description

This is a multi-centre, partially blinded, randomised, controlled non-inferiority trial of the Triple ACT artemether-lumefantrine + amodiaquine (AL+AQ) with the standard ACT artemether-lumefantrine+placebo (AL+PBO) for the treatment of uncomplicated *Plasmodium falciparum* malaria to assess and compare their efficacy, safety and tolerability. The study sites are shown in Figure 1 below. Patients will be randomized at the ratio 1:1 to the following arms: AL+ PBO: AL+AQ.

Figure 1 Study sites

There are 3 centres of recruitment in 2 different countries (Cambodia and Bangladesh). 114 patients will be recruited from Bangladesh and two locations in Cambodia located in Kravanh, Siem Pang functioning as a single site and will recruit a total of 114 subjects through competitive enrolment.

2.3 Determination of sample size

These studies will have a non-inferiority trial design. Each site will be powered for the efficacy endpoint. For the safety and tolerability endpoint, the sample size will be pooled across the DeTACT-Africa and DeTACT-Asia studies to achieve the desired power of at least 80% to detect rare events. For sample size calculations, the following assumptions have been used: The expected efficacy of ACT in these countries is expected to be approximately 96%. The matching TACT efficacy is assumed to be at least 90% under the null hypothesis of inferiority based on the WHO 90% efficacy threshold. However preliminary TRACII study data suggest that TACTs potentially may have a better efficacy by about 1% to 3% higher in favour of TACT (i.e. 97%-99% efficacy) in the Asian countries.

A one-sided alpha of 0.025 has been used in sample size calculations as non-inferiority is in one direction. A sample size of 47 subjects in the ACT plus placebo arm and 47 subjects in the matching TACT arm would be needed to achieve approximately 80% power to detect a non-inferiority margin difference between the

group efficacy of -6%. With an additional 20% to cover for loss to follow-up, we would therefore need 57 subjects in the ACT plus placebo arm and 57 subjects in the matching TACT arm in each of the sites.

3. Data Analysis

3.1 Trial Profile

The number of patients who will be screened, reasons for non-enrolment, number of patients randomized, number of patients lost to follow up and the number of patients assessed for the 42-day endpoint will be summarised in a CONSORT flow diagram, figure 2, below.

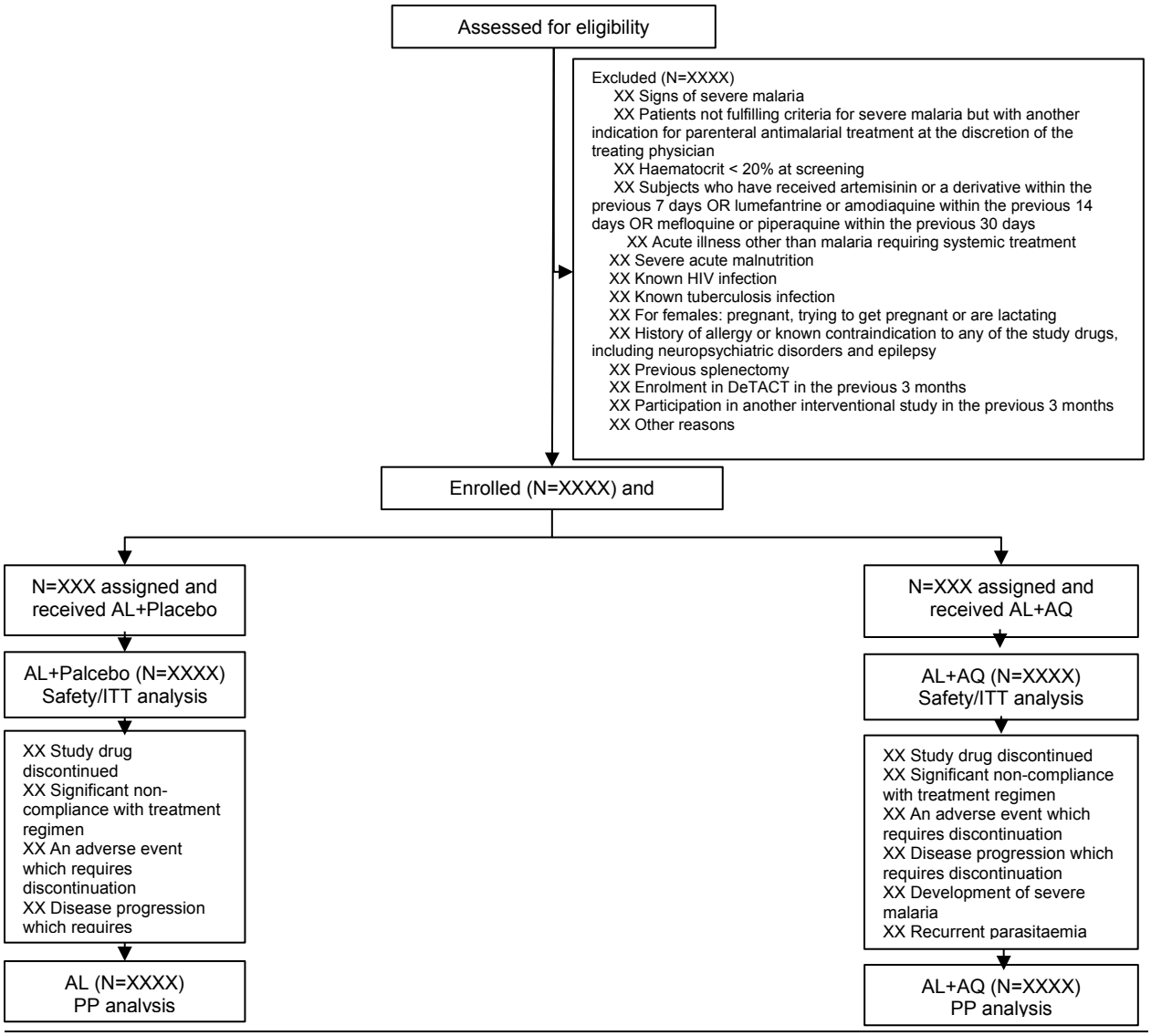

Figure 2 Consort Trial Profile by Arms

3.2 Demographics and other baseline characteristics

The following baseline characteristics will be described by study arm in Table 1 (below). Variables such as age, heart rate, and respiratory rate will be summarized using median and interquartile range (IQR). Continuous variables such as weight, height, systolic and diastolic blood pressure, QT-intervals, haematocrit and haemoglobin will be summarized using mean and standard deviation or median (IQR) as appropriate. Parasitaemia at baseline will be described as a geometric mean with range. Categorical variables such as sex, presence of fever, bed net use and gametocytaemia at baseline will be summarized using frequencies and percentages.

Table 1. Baseline characteristics for the participants per study intervention across the sites

Characteristics	AL+Placebo (N=XX)	AL+AQ (N=XX)	Total (N=XX)
Female: n (%)	XX(XX.X)	XX(XX.X)	XX(XX.X)
Age (years): median (IQR; range)	XX.X (XX.X-XX.X)	XX.X (XX.X-XX.X)	XX.X (XX.X-XX.X)
Age < 12 years: n (%)	XX(XX.X)	XX(XX.X)	XX(XX.X)
Pf asexual parasitaemia at hour 0 (/uL): Geometric mean (range)**	XX.X (XX.X-XX.X)	XX.X (XX.X-XX.X)	XX.X (XX.X-XX.X)
Pf asexual parasitaemia at screening (/uL): Geometric mean (range)	XX.X (XX.X-XX.X)	XX.X (XX.X-XX.X)	XX.X (XX.X-XX.X)
Gametocyte presence; n(%)	XX(XX.X)	XX(XX.X)	XX(XX.X)
Haematocrit (%): mean (SD)	XX.X (XX.X)	XX.X (XX.X)	XX.X (XX.X)
Weight (kg): median (IQR)	XX.X (XX.X-XX.X)	XX.X (XX.X-XX.X)	XX.X (XX.X-XX.X)
Temperature (ºC): median (IQR)	XX.X (XX.X-XX.X)	XX.X (XX.X-XX.X)	XX.X (XX.X-XX.X)
Fever (>=37.5 ºC) n (%)	XX(XX.X)	XX(XX.X)	XX(XX.X)
Respiratory rate (breaths/min): median (IQR)	XX.X (XX.X-XX.X)	XX.X (XX.X-XX.X)	XX.X (XX.X-XX.X)
Heart rate (beats/min): median (IQR)	XX.X (XX.X-XX.X)	XX.X (XX.X-XX.X)	XX.X (XX.X-XX.X)
Systolic blood pressure (mmHg): median (IQR)	XX.X (XX.X-XX.X)	XX.X (XX.X-XX.X)	XX.X (XX.X-XX.X)
Diastolic blood pressure (mmHg): median (IQR)	XX.X (XX.X-XX.X)	XX.X (XX.X-XX.X)	XX.X (XX.X-XX.X)
Haemoglobin (g/dL): mean (SD)	XX.X (XX.X)	XX.X (XX.X)	XX.X (XX.X)
Creatinine (µMol/L): median (IQR)	XX.X (XX.X-XX.X)	XX.X (XX.X-XX.X)	XX.X (XX.X-XX.X)
ALT/SGPT (IU/L): median (IQR)	XX.X (XX.X-XX.X)	XX.X (XX.X-XX.X)	XX.X (XX.X-XX.X)
AST/SGOT(IU/L): median (IQR)	XX.X (XX.X-XX.X)	XX.X (XX.X-XX.X)	XX.X (XX.X-XX.X)

Total Bilirubin (µMol/L): median (IQR)	XX.X (XX.X-XX.X)	XX.X (XX.X-XX.X)	XX.X (XX.X-XX.X)
Alkaline Phosphatase (IU/L): median (IQR)	XX.X (XX.X-XX.X)	XX.X (XX.X-XX.X)	XX.X (XX.X-XX.X)
QT interval on monitor (milliseconds): median (IQR)	XX.X (XX.X-XX.X)	XX.X (XX.X-XX.X)	XX.X (XX.X-XX.X)
Calculated QTc Bazett interval (milliseconds): median (IQR)	XX.X (XX.X-XX.X)	XX.X (XX.X-XX.X)	XX.X (XX.X-XX.X)

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

Efficacy will be reported as cumulative ACPR cure rates by day 42 estimated as day 42 PCR corrected efficacy and compared between ACT vs TACT. The Kaplan Meier/survival methods will be used to visually show the time to recurrence. Non-inferiority will be assessed using 95% one-sided confidence intervals.

Handling of missing data

In the ITT analysis, efficacy will be reported as proportions of outcomes using Complete case Analysis.

In the PP analysis in which efficacy will be reported as proportions of outcomes, patients in which study drugs are discontinued and/or endpoints are not available due to other reasons (such as withdrawal from the study, loss to follow up, *P falciparum* (Pf) or *P vivax* (Pv) reinfections and inconclusive PCR correction) will be excluded from the analysis.

In the Kaplan-Meier/survival analysis subjects in which study drugs are discontinued and/or endpoints are not available due to other reasons (such as withdrawal from the study, loss to follow-up, Pf or Pv reinfections and inconclusive PCR correction) will be censored or treated as competing risks, as appropriate, from the moment of occurrence of one of these events.

Similar analyses as secondary endpoints will be performed for day 63 PCR corrected efficacy and day 42 and day 63 for PCR uncorrected data as well. Where applicable, we will also report the cumulative reinfection rate. The treatment efficacy, 95% confidence intervals and p-values for the comparison of ACTs with matching TACTs will be presented as outlined in Tables 2, 3, 4 and 5 below.

Table 2a Comparison of Day 42 PCR corrected efficacy as proportions of ACT with TACT by site

Site	PCR corrected ACPR at day 42 n/N, (%)		Risk difference (95% CI, p-value)
	AL+Placebo (N=XXXX)	AL+AQ (N=XXXX)	AL+Placebo vs AL+AQ
Cambodia	XX/XXX (XX.X)	XX/XXX (XX.X)	XX.X (XX.X - XX.X, 0.XXX)
Cambodia (Kravanh)	XX/XXX (XX.X)	XX/XXX (XX.X)	XX.X (XX.X - XX.X, 0.XXX)
Cambodia (Siem Pang)	XX/XXX (XX.X)	XX/XXX (XX.X)	XX.X (XX.X - XX.X, 0.XXX)

Bangladesh	XX/XXX (XX.X)	XX/XXX (XX.X)	XX.X (XX.X - XX.X, 0.XXX)
All sites	XX/XXX (XX.X)	XX/XXX (XX.X)	XX.X (XX.X - XX.X, 0.XXX)

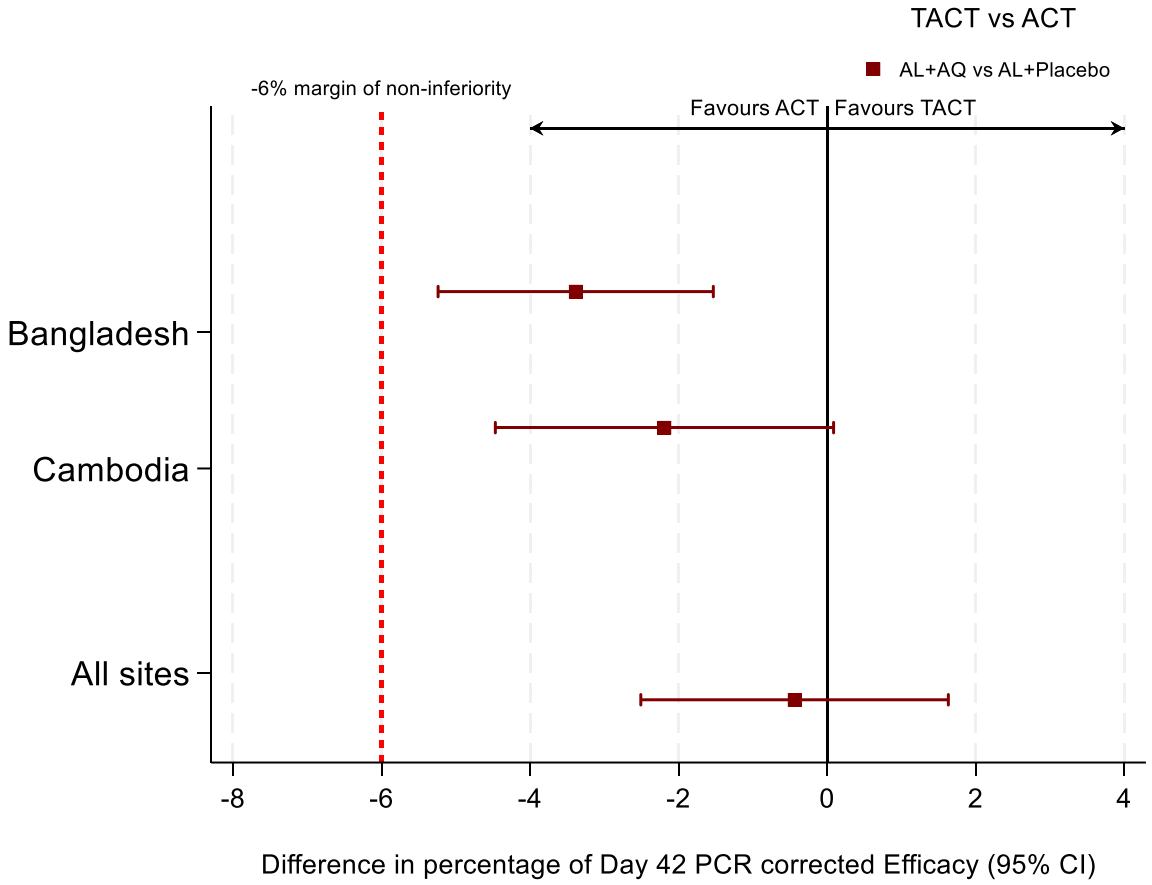

Figure 3 The difference between TACT vs ACT of Day 42 PCR corrected efficacy as proportions by site

Table 2b Comparison of Day 42 PCR corrected efficacy using survival methods of ACT with TACT by site

Site	PCR corrected ACPR at day 42 Cumulative recurrent infection free percentage (efficacy), % (95% CI)		Hazard ratio (95% CI, p-value)
	AL+Placebo (N=XXXX)	AL+AQ (N=XXXX)	AL+Placebo vs AL+AQ

Cambodia	XX.X (XX.X - XX.X)	XX.X (XX.X - XX.X)	XX.X (XX.X - XX.X, 0.XXX)
Cambodia (Kravanh)	XX/XXX (XX.X)	XX/XXX (XX.X)	XX.X (XX.X - XX.X, 0.XXX)
Cambodia (Siem Pang)	XX/XXX (XX.X)	XX/XXX (XX.X)	XX.X (XX.X - XX.X, 0.XXX)
Bangladesh	XX.X (XX.X - XX.X)	XX.X (XX.X - XX.X)	XX.X (XX.X - XX.X, 0.XXX)
All sites	XX.X (XX.X - XX.X)	XX.X (XX.X - XX.X)	XX.X (XX.X - XX.X, 0.XXX)

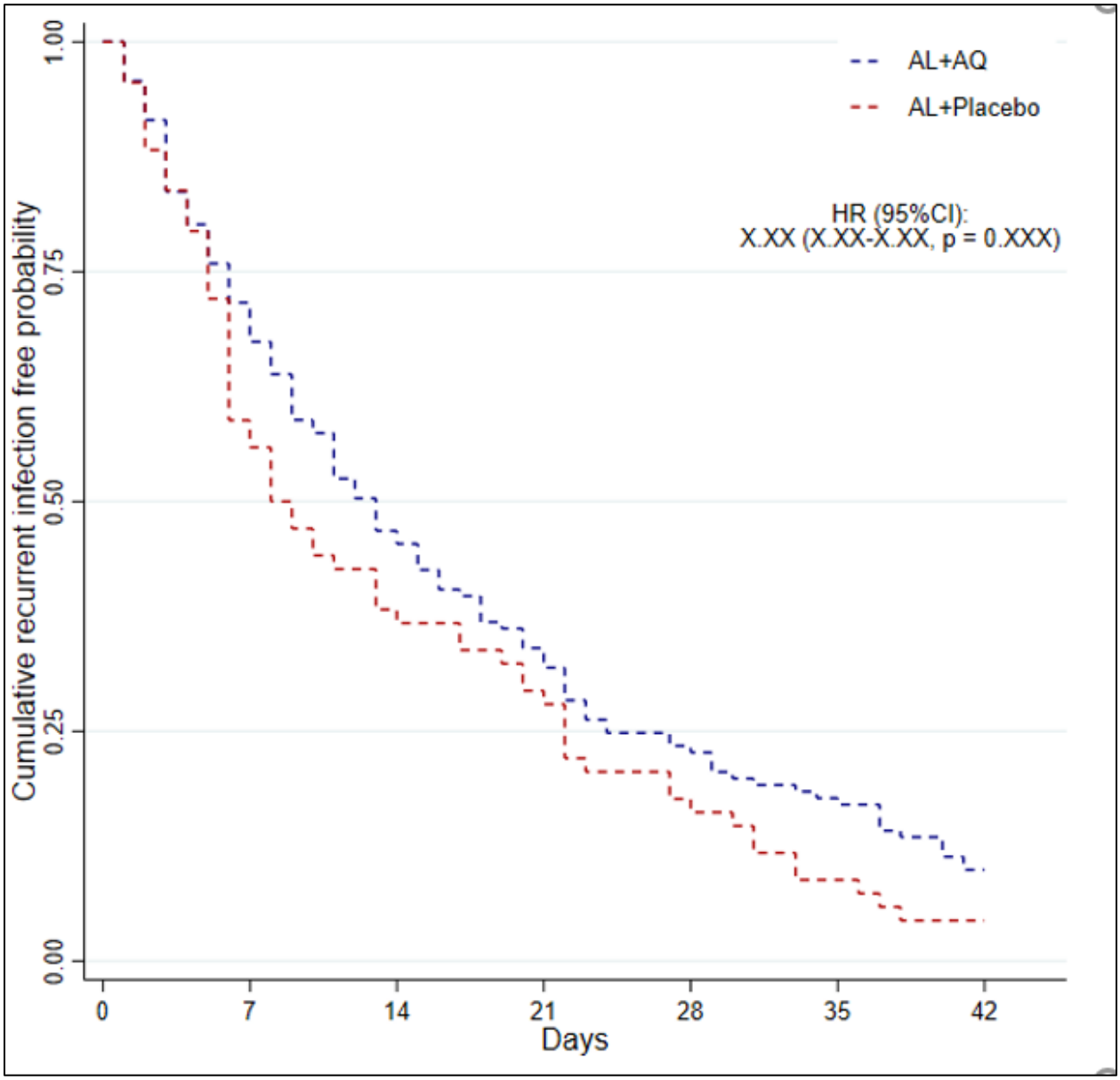

Figure 4.1 Cumulative recurrent infection-free probability until day 42 from PCR corrected efficacy by treatment in Cambodia

Figure 4.2 Cumulative recurrent infection-free probability until day 42 from PCR corrected efficacy by treatment in Cambodia (Kravanh)

Figure 4.3 Cumulative recurrent infection-free probability until day 42 from PCR corrected efficacy by treatment in Cambodia (Siem Pang)

Figure 4.4 Cumulative recurrent infection-free probability until day 42 from PCR corrected efficacy by treatment in Bangladesh

Table 3a Comparison of Day 63 PCR corrected efficacy as proportions of ACT with TACT by sites

Site	AL+Placebo (N=XXXX)	AL+AQ (N=XXXX)	AL+Placebo vs AL+AQ
Cambodia	XX/XXX (XX.X)	XX/XXX (XX.X)	XX.X (XX.X - XX.X, 0.XXX)
Cambodia (Kravanh)	XX/XXX (XX.X)	XX/XXX (XX.X)	XX.X (XX.X - XX.X, 0.XXX)
Cambodia (Siem Pang)	XX/XXX (XX.X)	XX/XXX (XX.X)	XX.X (XX.X - XX.X, 0.XXX)
Bangladesh	XX/XXX (XX.X)	XX/XXX (XX.X)	XX.X (XX.X - XX.X, 0.XXX)
All sites	XX/XXX (XX.X)	XX/XXX (XX.X)	XX.X (XX.X - XX.X, 0.XXX)

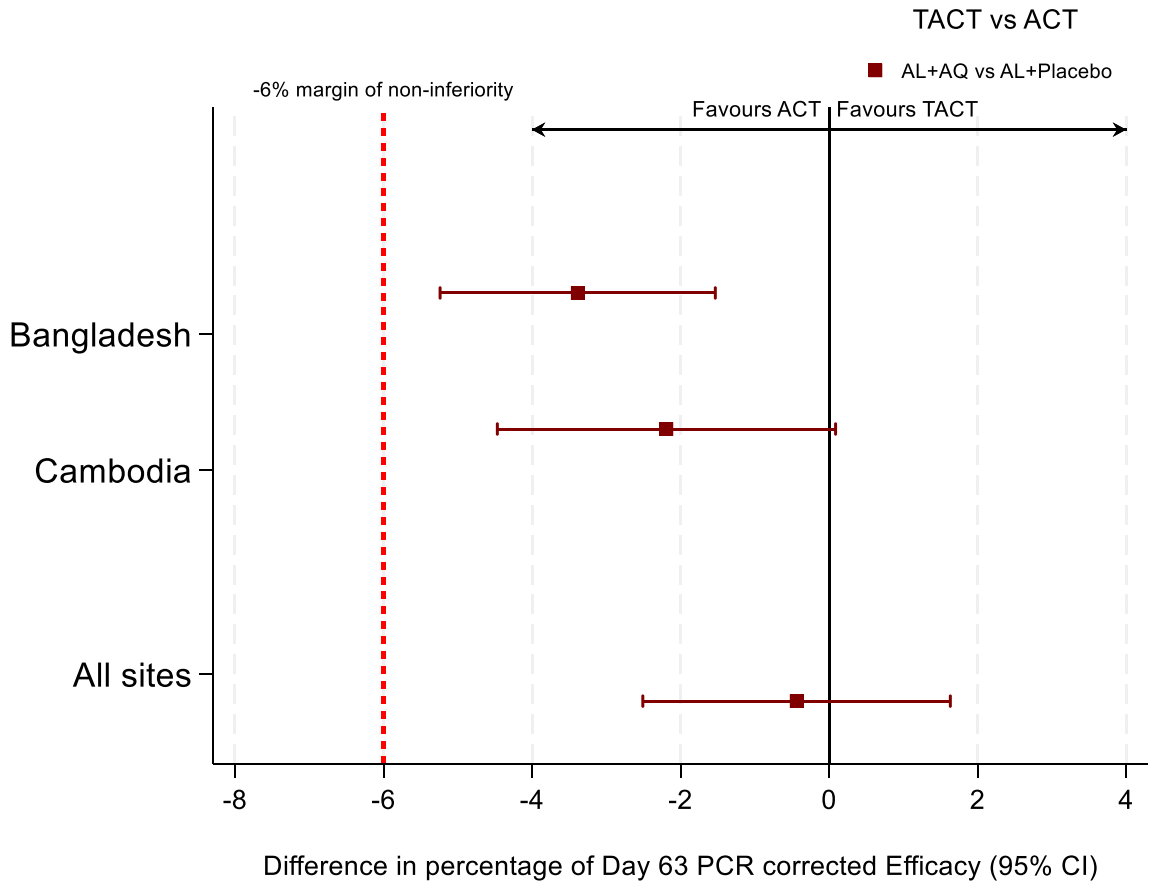

Figure 5 The difference between TACT vs ACT of Day 63 PCR corrected efficacy as proportions by site

Table 3b Comparison of Day 63 PCR corrected efficacy using survival methods of ACT with TACT by site

Site	PCR corrected ACPR at day 63 Cumulative recurrent infection free percentage (efficacy), % (95% CI)		Hazard ratio (95% CI, p-value)
	AL+Placebo (N=XXXX)	AL+AQ (N=XXXX)	AL+Placebo vs AL+AQ
Cambodia	XX.X (XX.X - XX.X)	XX.X (XX.X - XX.X)	XX.X (XX.X - XX.X, 0.XXX)

Cambodia (Kravanh)	XX/XXX (XX.X)	XX/XXX (XX.X)	XX.X (XX.X - XX.X, 0.XXX)
Cambodia (Siem Pang)	XX/XXX (XX.X)	XX/XXX (XX.X)	XX.X (XX.X - XX.X, 0.XXX)
Bangladesh	XX.X (XX.X - XX.X)	XX.X (XX.X - XX.X)	XX.X (XX.X - XX.X, 0.XXX)
All sites	XX.X (XX.X - XX.X)	XX.X (XX.X - XX.X)	XX.X (XX.X - XX.X, 0.XXX)

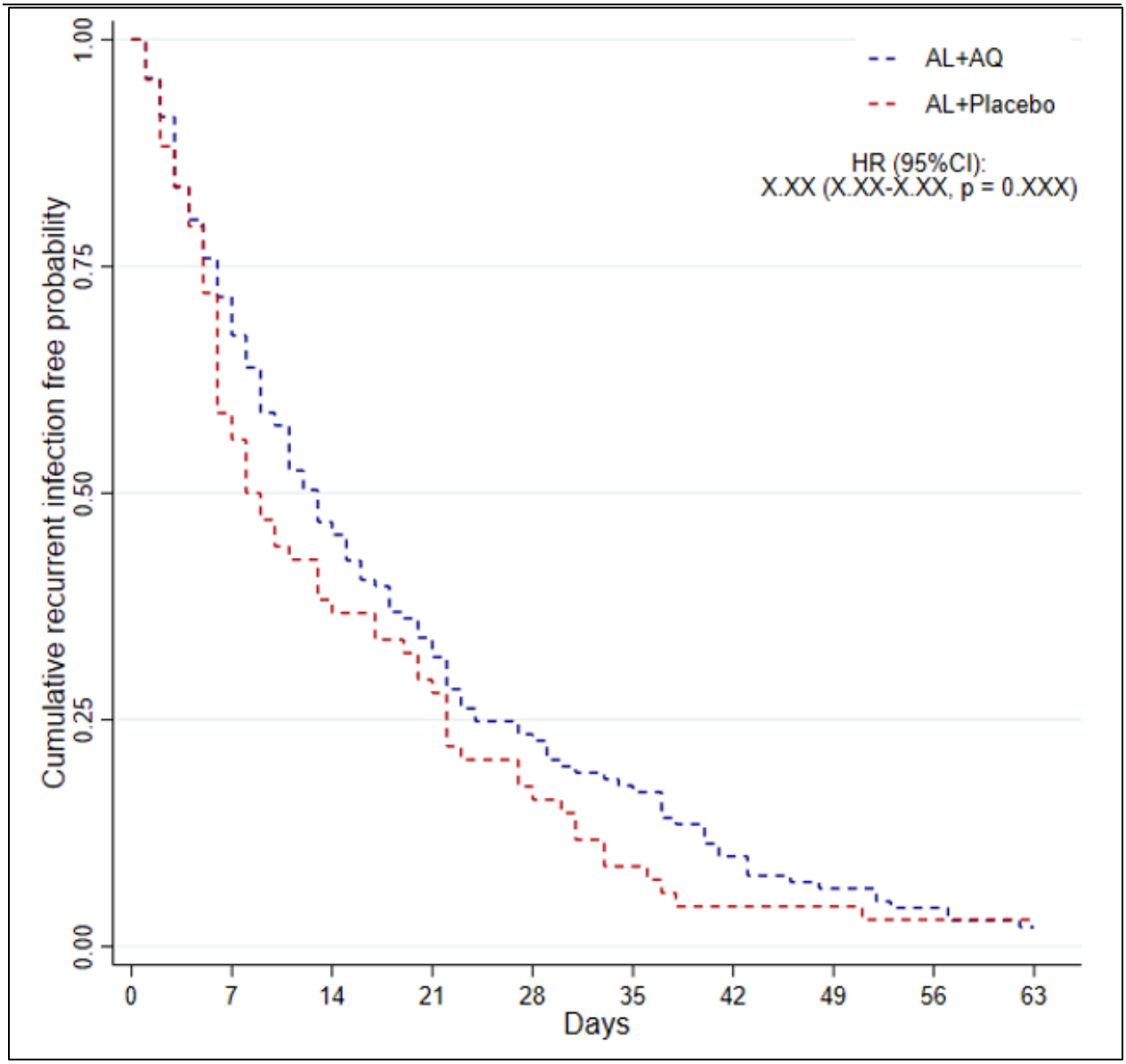

Figure 6.1 Cumulative recurrent infection-free probability until day 63 from PCR corrected efficacy and days by treatment in Cambodia

Figure 6.2 Cumulative recurrent infection-free probability until day 63 from PCR corrected efficacy and days by treatment in Cambodia (Kravanh)

Figure 6.3 Cumulative recurrent infection-free probability until day 63 from PCR corrected efficacy and days by treatment in Cambodia (Siem Pang)

Figure 6.4 Cumulative recurrent infection-free probability until day 63 from PCR corrected efficacy and days by treatment in Bangladesh

Table 4a Comparison of Day 42 PCR uncorrected efficacy as proportions of ACT with TACT by sites

Site	PCR uncorrected ACPR at day 42 n/N, (%)		Risk difference (95% CI, p-value)
	AL+Placebo (N=XXXX)	AL+AQ (N=XXXX)	AL+Placebo vs AL+AQ
Cambodia	XX/XXX (XX.X)	XX/XXX (XX.X)	XX.X (XX.X - XX.X, 0.XXX)
Cambodia (Kravanh)	XX/XXX (XX.X)	XX/XXX (XX.X)	XX.X (XX.X - XX.X, 0.XXX)
Cambodia (Siem Pang)	XX/XXX (XX.X)	XX/XXX (XX.X)	XX.X (XX.X - XX.X, 0.XXX)
Bangladesh	XX/XXX (XX.X)	XX/XXX (XX.X)	XX.X (XX.X - XX.X, 0.XXX)
All sites	XX/XXX (XX.X)	XX/XXX (XX.X)	XX.X (XX.X - XX.X, 0.XXX)

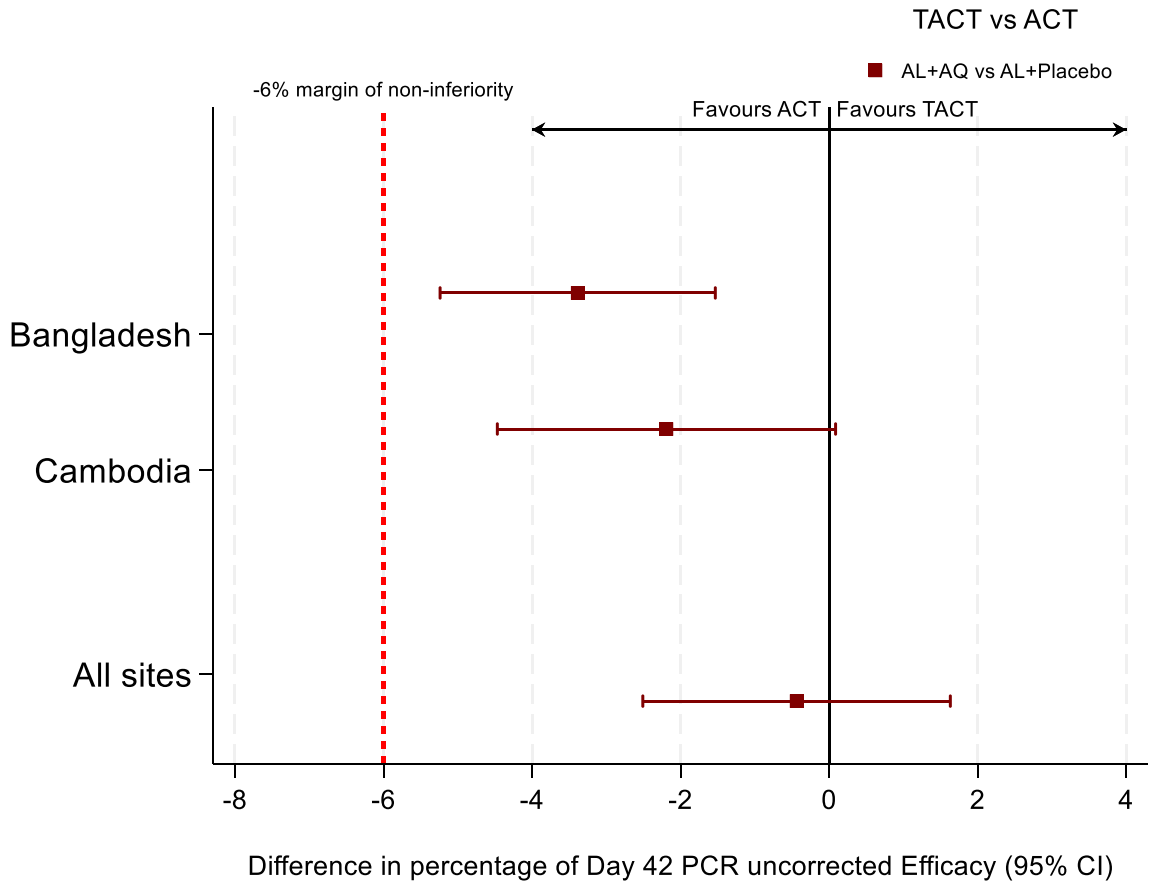

Figure 7 The difference between TACT vs ACT of Day 42 PCR uncorrected efficacy as proportions by site

Table 4b Comparison of Day 42 PCR uncorrected efficacy using survival methods of ACT with TACT by site

Site	PCR uncorrected ACPR at day 42 Cumulative recurrent infection free percentage (efficacy), % (95% CI)		Hazard ratio (95% CI, p-value)
	AL+Placebo (N=XXXX)	AL+AQ (N=XXXX)	AL+Placebo vs AL+AQ
Cambodia	XX.X (XX.X - XX.X)	XX.X (XX.X - XX.X)	XX.X (XX.X - XX.X, 0.XXX)
Cambodia (Kravanh)	XX/XXX (XX.X)	XX/XXX (XX.X)	XX.X (XX.X - XX.X, 0.XXX)
Cambodia (Siem Pang)	XX/XXX (XX.X)	XX/XXX (XX.X)	XX.X (XX.X - XX.X, 0.XXX)

Bangladesh	XX.X (XX.X - XX.X)	XX.X (XX.X - XX.X)	XX.X (XX.X - XX.X, 0.XXX)
All sites	XX.X (XX.X - XX.X)	XX.X (XX.X - XX.X)	XX.X (XX.X - XX.X, 0.XXX)

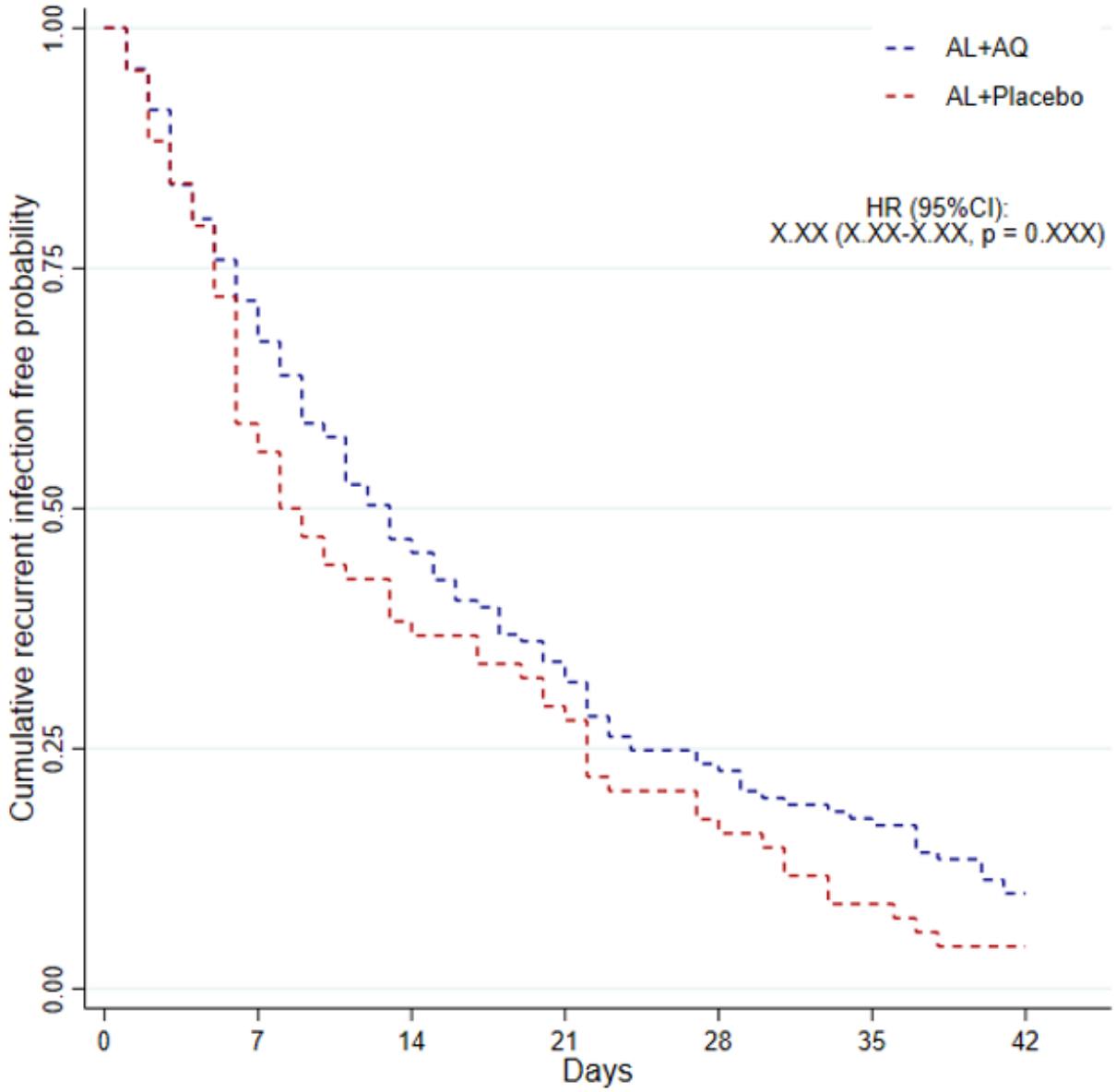

Figure 8.1 Cumulative recurrent infection-free probability until day 42 from PCR uncorrected efficacy and days by treatment in Cambodia

Figure 8.2 Cumulative recurrent infection-free probability until day 42 from PCR uncorrected efficacy and days by treatment in Cambodia

Figure 8.3 Cumulative recurrent infection-free probability until day 42 from PCR uncorrected efficacy and days by treatment in Cambodia

Figure 8.4 Cumulative recurrent infection-free probability until day 42 from PCR uncorrected efficacy and days by treatment in Bangladesh

Table 5a Comparison of Day 63 PCR uncorrected efficacy as proportions of ACT with TACT by site

	PCR corrected ACPR at day 42 n/N, (%)		Risk difference (95% CI, p-value)
	AL+Placebo (N=XXXX)	AL+AQ (N=XXXX)	AL+Placebo vs AL+AQ
Cambodia	XX/XXX (XX.X)	XX/XXX (XX.X)	XX.X (XX.X - XX.X, 0.XXX)
Cambodia (Kravanh)	XX/XXX (XX.X)	XX/XXX (XX.X)	XX.X (XX.X - XX.X, 0.XXX)
Cambodia (Siem Pang)	XX/XXX (XX.X)	XX/XXX (XX.X)	XX.X (XX.X - XX.X, 0.XXX)
Bangladesh	XX/XXX (XX.X)	XX/XXX (XX.X)	XX.X (XX.X - XX.X, 0.XXX)
All sites	XX/XXX (XX.X)	XX/XXX (XX.X)	XX.X (XX.X - XX.X, 0.XXX)

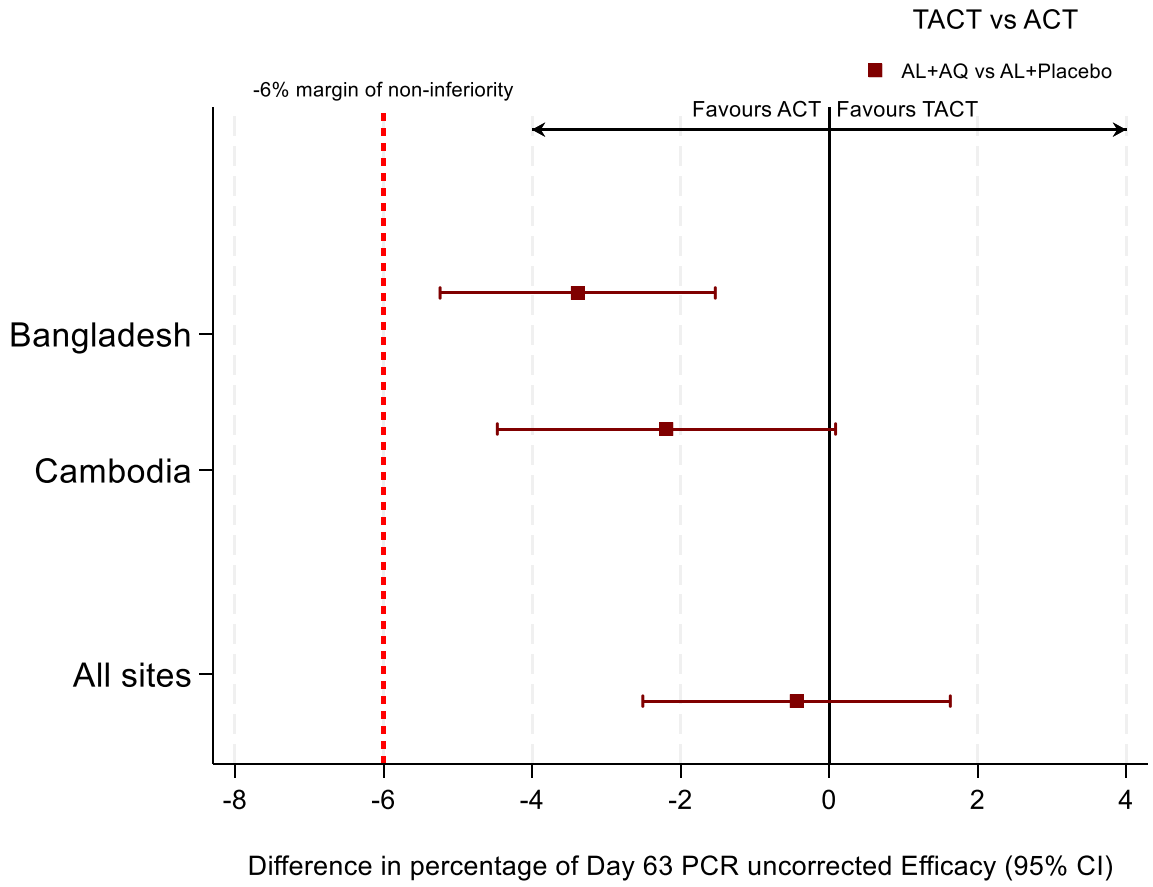

Figure 9 The difference between TACT vs ACT of Day 63 PCR uncorrected efficacy as proportions by site

Table 5b Comparison of Day 63 PCR uncorrected efficacy using survival methods of ACT with TACT by site

Site	PCR uncorrected ACPR at day 63 Cumulative recurrent infection free percentage (efficacy), % (95% CI)		Hazard ratio (95% CI, p-value)
	AL+Placebo (N=XXXX)	AL+AQ (N=XXXX)	AL+Placebo vs AL+AQ
Cambodia	XX.X (XX.X - XX.X)	XX.X (XX.X - XX.X)	XX.X (XX.X - XX.X, 0.XXX)
Cambodia (Kravanh)	XX/XXX (XX.X)	XX/XXX (XX.X)	XX.X (XX.X - XX.X, 0.XXX)
Cambodia (Siem Pang)	XX/XXX (XX.X)	XX/XXX (XX.X)	XX.X (XX.X - XX.X, 0.XXX)

Bangladesh	XX.X (XX.X - XX.X)	XX.X (XX.X - XX.X)	XX.X (XX.X - XX.X, 0.XXX)

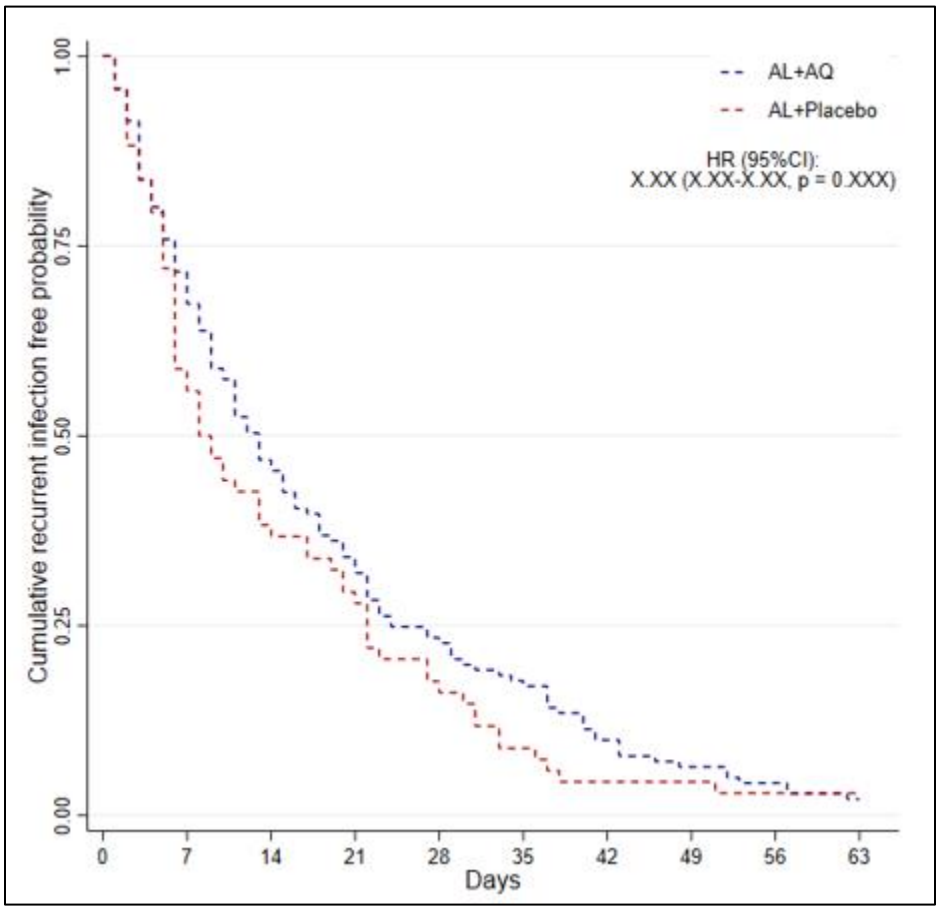

Figure 10.1 Cumulative recurrent infection-free probability until day 63 from PCR uncorrected efficacy and days by treatment in Cambodia

Figure 10.2 Cumulative recurrent infection-free probability until day 63 from PCR uncorrected efficacy and days by treatment in Cambodia (Kravanh)

Figure 10.1 Cumulative recurrent infection-free probability until day 63 from PCR uncorrected efficacy and days by treatment in Cambodia (Siem Pang)

Figure 10.2 Cumulative recurrent infection-free probability until day 63 from PCR uncorrected efficacy and days by treatment in Bangladesh

3.4 Safety and tolerability of ACTs and TACTs within and across sites and regions.

Safety analyses will be based on the whole population that gets administered the study drug. That is, the safety and tolerability data will be pooled from all the sites that received the same antimalarial treatment. Safety and tolerability of TACTs versus ACTs will be assessed by comparing the frequency (%) of adverse events and serious adverse events, with particular attention to abdominal pain, appetite perturbation, biochemical markers of hepatic and renal toxicity and QT interval prolongation, using the Fisher's exact test. Safety data will be presented in tabular and/or graphical format and summarized descriptively. Any

clinically relevant abnormalities or values of potential clinical concern will be described. Patients will be analysed following the intention to treat analysis. 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. Adverse events will be graded according to Common Terminology Criteria for Adverse Events (CTCAE) version 5.0, adapted for paediatric populations where needed. The relevant CTCAE table is appended to this document. The safety and tolerability summaries will be presented in Table 6.

The frequency and proportion of subjects that report completing a full course of observed TACT or ACT without withdrawal of consent or exclusion from study because of drug-related serious adverse events will be summarized within and across sites and regions. The 95% confidence intervals will be reported for the proportions. The proportions will be compared between matching drugs. The analyses will be summarised in Table 7.

3.5 Changes in the electrocardiogram (such as prolongation of the QTc-interval) in patients treated with TACT versus standard ACT

Incidence of prolongations of the QTc-interval (Bazett) >500 milliseconds at least once (at H4, H48, H52) will be summarized in Table 5. Absolute changes in the QTc-interval will be summarized as in Table 8 and will be graphically displayed by arm as in Figure 5. Comparisons on the changes in the QTc-interval will be made using the unpaired t-test for each specific time point.

Table 6 Adverse events within the first 42 days

Adverse events	AL+Placebo		AL+AQ	
Number of participants	XXXX		XXXX	
Vomiting/number of treatments, n/N, (%)	XXX/XXXX (XX.X)		XXX/XXXX (XX.X)	
Serious adverse events (SAEs), n/N, (%)	XX/XXXX (XX.X)		XX/XXXX (XX.X)	
Possible, probable or definite drug related SAEs, n/N, (%)	XX/XXXX (XX.X)		XX/XXXX (XX.X)	
QTc >500ms (H4 and H48/H52)	XXX/XXXX (XX.X)		XXX/XXXX (XX.X)	
Bradycardia: n/N, (%)	XX/XXXX (XX.X)		XX/XXXX (XX.X)	
Change from baseline in haemoglobin at days 3, 7, 28 (stratified for G6PD status)	XXX/XXXX (XX.X)		XXX/XXXX (XX.X)	
Grading of adverse events, n/N, (%)	1-2	3-4	1-2	3-4
Symptoms, n/N (%)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)

Headache	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)
Fatigue	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)
Abdominal pain	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)
Loss of appetite	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)
Nausea	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)
Vomiting	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)
Diarrhoea	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)
Itching	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)
Dizziness	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)
Blurred vision	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)
Sleeping disturbance	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)
Laboratory abnormalities, n/N (%)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)
Creatinine	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)
Total bilirubin	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)
Alkaline phosphatase	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)
Alanyl transferase (ALT)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)
Aspartate transferase (AST)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)

QTc: QT corrected using Bazett's formula. Adverse event grading: grade1: mild; grade 2: moderate; grade 3: severe; grade 4: potentially life-threatening
Incidence of QTc abnormalities (>500 milliseconds) and bradycardia is defined as a subject experiencing these abnormalities at one or more timepoints.

Table 7a Proportions of patients that report a full course of observed TACT or ACT

	AL+Placebo, n/N (%, 95% CI)	A+AQ, n/N (%, 95% CI)	p-value (AL vs AL+AQ)
Completing full course of study drug	XXXX/XXXX (XX.X, XX.X – XX.X)	XXXX/XXXX (XX.X, XX.X – XX.X)	0.XXX

Table 7b. Necessity for retreatment due to vomiting or spitting study drug

	AL+Placebo	**AL+AQ**
Number of participants	XX	XX

Number of total doses	XX	XX
Retreatment events		
Number of participants requiring retreatment	XX	XX
Retreatment events due to vomiting (< 30 min) only†	X(X)	X(X)
Retreatment events due to spitting only†	X(X)	X(X)
Both vomit and spitting*†	X(X)	X(X)
Total number of retreatments†	X(X)	X(X)
Total number of participants vomiting after retreatment (< 60 min), or rescue therapy given	X(X)	X(X)
Number of participants vomiting after retreatment (< 60 min)	X(X)	X(X)
Number of rescue treatments given	X(X)	X(X)

Table 8 QTc-intervals and changes in QTc-intervals over time (relevant time points only will be summarised)

Time-point	AL+Placebo			AL+AQ		
	QTc	∆-QTc	p-value	QTc	∆-QTc	p-value
H0	XXX.X (XX.X)	Baseline	Baseline	XXX.X (XX.X)	Baseline	Baseline
H4	XXX.X (XX.X)	XX.X (XX.X)	0.XXX	XXX.X (XX.X)	XX.X (XX.X)	0.XXX
H24	XXX.X (XX.X)	XX.X (XX.X)	0.XXX	XXX.X (XX.X)	XX.X (XX.X)	0.XXX
H28	XXX.X (XX.X)	XX.X (XX.X)	0.XXX	XXX.X (XX.X)	XX.X (XX.X)	0.XXX
H48	XXX.X (XX.X)	XX.X (XX.X)	0.XXX	XXX.X (XX.X)	XX.X (XX.X)	0.XXX
H52	XXX.X (XX.X)	XX.X (XX.X)	0.XXX	XXX.X (XX.X)	XX.X (XX.X)	0.XXX
H60	XXX.X (XX.X)	XXX.X (XX.X)	0.XXX	XXX.X (XX.X)	XXX.X (XX.X)	0.XXX
H64	XXX.X (XX.X)	XXX.X (XX.X)	0.XXX	XXX.X (XX.X)	XXX.X (XX.X)	0.XXX

3.6 Meta-analysis

Several RCTs have reported on the efficacy of artemether-lumefantrine plus amodiaquine compared with artemether-lumefantrine. Due to declining malaria prevalence in the Greater Mekong subregion recruitment targets in these RCTs were not met, thus individually these RCTs were not powered to show significant differences between the study groups. The design of these studies was very similar to the current study but did not include a placebo.

In addition to reporting the primary outcome results of the current study, we plan to include a meta-analysis of all RCTs reporting on the efficacy of artemether-lumefantrine plus amodiaquine compared with artemether-lumefantrine. The results of the current study will be included in the meta-analysis. This will

allow us to report with greater certainty the efficacy of artemether-lumefantrine plus amodiaquine compared with artemether-lumefantrine.

A systematic review will be conducted to search for relevant RCTs with similar design and analysis approaches to be included in the meta-analysis. The primary outcome of interest is the 42-day efficacy defined as PCR-corrected adequate ACPR. If the reported primary outcome of interest from an RCT includes criteria that are not only the D42-PCR corrected recrudescence-free proportion, then study authors will be contacted to clarify this. Pooled estimates of treatment efficacy based on the D42-PCR corrected recrudescence-free proportion will be presented for each study/site according to the TACT or matching ACT the participant was randomised. A fixed (Common effect) meta-analysis will be conducted and the pooled effect along with the 95% CI will be presented in a meta-analysis forest plot. The I^2 statistic will be used to characterise the percentage of total variation across studies resulting from trial heterogeneity rather than chance, with the p-value of significance included.

If RCTs are identified that report a primary outcome that includes criteria that are not only the D42-PCR corrected recrudescence-free proportion, then an additional meta-analysis using the original published data will be included in the appendices to allow readers to make a clear comparison with the original manuscript.

3.7 Assessing and comparing *P. falciparum* parasite clearance rate, additional parameters of parasite clearance dynamics and fever clearance time of standard ACTs and matching TACTs.

Parasite clearance half-life, proportion of subjects with microscopically detectable *P. falciparum* parasitaemia at Day 3 and fever clearance time (i.e. the time taken for the tympanic temperature to fall below 37.5 ºC in patients who were febrile at inclusion) will be summarized and presented in Table 9 below. In addition, the proportion of subjects with gametocytaemia during and after treatment stratified by the presence of gametocytes at enrolment will be presented in Tables 9 and 10 below.

Table 9. Parasite clearance half-life, microscopy *P. falciparum* positivity and fever clearance time

Arms	**Median Time (hours) to Resolution of Fever** (range) *	**Median Parasite Clearance Half-Life in hours** (range) **	**Median Time (hours) to 50% Parasite Clearance** (range) **	**Median Time (hours) to 90% Parasite Clearance** (range) **	**Parasite Clearance Half-Life >5 hours,** (n/N) % (95% CI) **	**Positive for Parasitaemia on day 3**, (n/N) % (95% CI) ***
AL based overall	XX.X (XX.X-XX.X)	XX.X (XX.X-XX.X)	XX.X (XX.X-XX.X)	XX.X (XX.X-XX.X)	(XX/XX) X(X–X)	(XX/XX) X(X–X)
AL+Placebo	XX.X (XX.X-XX.X)	XX.X (XX.X-XX.X)	XX.X (XX.X-XX.X)	XX.X (XX.X-XX.X)	(XX/XX) X(X–X)	(XX/XX) X(X–X)
AL+AQ	XX.X (XX.X-XX.X)	XX.X (XX.X-XX.X)	XX.X (XX.X-XX.X)	XX.X (XX.X-XX.X)	(XX/XX) X(X–X)	(XX/XX) X(X–X)

Table 10 Gametocytaemia during and after treatment by gametocytes status at enrolment (up to H72 vs after H72)

	Gametocytaemia n (%, 95%CI)	p-value (AL+Placebo vs AL+AQ)

	AL+Placebo	AL+AQ	
Presence of gametocytes at enrolment	(N=XXXX)	(N=XXXX)	
During treatment	XX (XX.X, XX.X-XX.X)	XX (XX.X, XX.X-XX.X)	0.XXX
After treatment	XX (XX.X, XX.X-XX.X)	XX (XX.X, XX.X-XX.X)	0.XXX
Absence of gametocytes at enrolment	(N=XXXX)	(N=XXXX)	
During treatment	XX (XX.X, XX.X-XX.X)	XX (XX.X, XX.X-XX.X)	0.XXX
After treatment	XX (XX.X, XX.X-XX.X)	XX (XX.X, XX.X-XX.X)	0.XXX

3.8 Pharmacokinetic and pharmacodynamic interactions between antimalarials in ACT and TACTs

A detailed analysis plan will be prepared separately.

3.9 Molecular genetic and transcriptomic correlates for artemisinin and partner drug resistance of the infecting *P. falciparum* strains

A detailed analysis plan will be prepared separately.

3.10 In vitro sensitivity of *P. falciparum* to artemisinins and partner drugs according to study sites and genotype

A detailed analysis plan will be prepared separately.

3.11 The selective effect of ACTs versus TACTs on parasites carrying mutations associated with resistance to antimalarial drugs

Recurrent infections with parasites carrying mutations of known functional significance and specimens collected at baseline with parasites carrying mutations of known functional or operational significance (pfkelch13, pfcrt, pfmdr1, pfdhfr, pfdhps, pfplasmepsin2 , partial or complete deletions of pfhrp2 and other current parasite genetic markers associated with resistance or identified over the course of the study) will be summarized as proportions of the total number of samples per site (% and 95% confidence interval) as in table 11.

Table 12 Recurrent infections and specimens collected at baseline with parasites carrying mutations of known functional significance

	AL+Placebo (N=XXXX)	AL+AQ (N=XXXX)	p-value (AL+Placebo vs AL+AQ)
Reinfections, n (%,CI 95%)	XX.X (XX.X-XX.X)	XX.X (XX.X-XX.X)	0.XXX
Pf kelch13, n (%,CI 95%)	XX.X (XX.X-XX.X)	XX.X (XX.X-XX.X)	0.XXX
Pf crt, n (%,CI 95%)	XX (XX.X, XX.X-XX.X)	XX (XX.X, XX.X-XX.X)	0.XXX
Pf mdr1, n (%,CI 95%)	XX.X (XX.X-XX.X)	XX.X (XX.X-XX.X)	0.XXX
Pf dhfr, n (%,CI 95%)	XX.X (XX.X-XX.X)	XX.X (XX.X-XX.X)	0.XXX
Pf dhps, n (%,CI 95%)	XX.X (XX.X-XX.X)	XX.X (XX.X-XX.X)	0.XXX
Pf Plasmepsin 2, n (%,CI 95%)	XX.X (XX.X-XX.X)	XX.X (XX.X-XX.X)	0.XXX
Others, n (%,CI 95%)	XX.X (XX.X-XX.X)	XX.X (XX.X-XX.X)	0.XXX

3.12 Change in hematocrit over time according to geographical location and study arm, stratified for G6PD status

Change in hematocrit on day 1 to 7, 14, 21, 28, 35 and 42 according to geographical location and study arm, stratified for G6PD status will be summarized and analyzed. A detailed analysis plan will be prepared separately.

3.13 Correlation between the host genotype (e.g., CYP2D6, CYP3A4, KCNQ1/LQT1, KCNH2/LQT2, SCN5A/LQT3) and the pharmacokinetics and pharmacodynamics of antimalarials.

A detailed analysis plan will be prepared separately.

3.14 Assess new methods for determination of gametocytaemia, parasite phenotypes and genotypes

A detailed analysis plan will be prepared separately.

3.15 Correlation between specific antibody titres and measures of drug efficacy

A detailed analysis plan will be prepared separately.

3.16 Accuracy of SNPs assessment from dry blood spots versus from whole genome sequencing in leukocyte depleted blood samples

A detailed analysis plan will be prepared separately.

3.17 Candidate markers of resistance identified through genome wide association studies with in vivo or in vitro parasite drug sensitivity phenotypes

A detailed analysis plan will be prepared separately.

3.18 Correlation between qPCR based versus microscopy-based assessments of parasite clearance dynamics

A detailed analysis plan will be prepared separately.

3.19 Correlation of parasite clearance metrics as assessed by microscopy versus digital microscopy

A detailed analysis plan will be prepared separately.

3.20 Comparison of transcriptomic patterns of drug sensitive and resistant parasites before treatment and 6, 12 and 24 hours after start of treatment

A detailed analysis plan will be prepared separately.

3.21 Levels of RNA transcription coding for male or female specific gametocytes at admission up to day 14, stratified by the presence of gametocytes at enrolment

A detailed analysis plan will be prepared separately.