TACT-CV

STATISTICAL ANALYSIS PLAN

A multi-centre, open-label randomised trial to assess the efficacy, safety and tolerability of the Triple ACT

artemether lumefantrine+amodiaquine (AL+AQ) compared to the ACT artemether-lumefantrine (AL)

in uncomplicated falciparum malaria in Cambodia and Vietnam

Version 1.4
Date 28th June 2020

WRITTEN BY:

MAVUTO MUKAKA 

DATE: 28th JUNE 2020

PRINT NAME

STATISTICIAN

REVIEWED AND APPROVED BY:

THOMAS PETO 

DATE: 28TH JUNE 2020

PRINT NAME

PROJECT COORDINATOR

ARJEN DONDORP 

DATE: 28TH JUNE 2020

PRINT NAME

PRINCIPAL INVESTIGATOR

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1. Analysis Considerations

1.1 General Analysis Approach

The main analytic strategy for the primary outcome will be the intention-to-treat (ITT) principle. Patients will be analysed according to the arm of randomisation irrespective of the treatment that was actually given. Kaplan Meier 1st event analyses will be the main approach to estimate efficacy. In addition, efficacy using proportions will also be presented to allow comparison with previous malaria trials. The 95% confidence intervals for efficacy estimates will be presented, along with risk differences, and hazard ratios as appropriate. Statistical significance will be declared at the 5% level. The analyses will be based on the number of participants that have actually been recruited by the trial end or trial closure.

There is a fortuitous inverse correlation between parasite susceptibility to lumefantrine (which is included in the ACT, and hence also in the Triple ACT) and amodiaquine (which is included only in the Triple ACT). Amodiaquine was chosen as the third drug partly because of this inverse correlation, and partly because its anti-malarial effects persist about as long as those of lumefantrine. In patients treated with both these drugs, therefore, neither will persist for much longer than the other; this could help avoid or delay the emergence of resistance.

Hence, in addition to the randomised comparison, a well-documented case series reliably treated with Triple ACT (artemether-lumefantrine plus amodiaquine) is of interest. Likewise, a well-documented case series assessing the efficacy in various locations in the late 2010s of the ACT is also of interest, as artemether-lumefantrine has not previously been widely used in areas where artemisinin resistance is common. The planned analyses will assess the efficacy of each regimen separately against PCR-corrected recrudescence (particularly for artemisinin-resistant *Pfkelch13* mutants), in addition to the randomised comparison of the two regimens to assess the effects on *Pf* recrudescence of adding amodiaquine.

The assessment of Triple ACT against ACT will directly compare the two regimens (to provide generalizable evidence on the adverse events of adding the third drug). On the other hand, future treatment recommendations depend on the individual efficacy estimates of each regimen independently.

These analyses will be followed by the per protocol (PP) analysis. In this analysis only those that adhered to the protocol with respect to the primary outcome will be included for analysis of the primary outcome. All inferences will be based on the ITT analyses, but the PP will be attached in an appendix to be consistent with reporting guidelines for antimalarial efficacy studies (WHO 2009) - as randomization is potentially compromised by PP analysis.

Key secondary endpoints such as parasite clearance parameters (e.g. half-lives), safety and tolerability data will be analysed by ITT approach as for the primary study 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 parasite half-life data. In the event of some subjects being withdrawn at baseline and not receiving any does of study drugs will be removed from the ITT analyses as there was no intention to treat these patients. These will

also be followed by the per protocol analyses. All other secondary analyses will follow the per protocol approach. 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 performed using Stata 15 or higher, StataCorp, 4905 Lakeway Drive College Station, Texas 77845 USA

1.2 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.

1.3 Data cleaning and verification

All data will be cleaned and verified prior to statistical analysis. The study site will be visited by the Monitor periodically at times agreed on with the Investigator. At the time of each monitoring visit, the 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 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 statistician for analysis. The statistician will cross-check that the available data for analysis is clean. Any data cleaning queries will need to be resolved before statistical analyses.

1.4 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 robust backup media.

1.5 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 amount of queries if clean data is provided to the trial statistician. 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 15 or higher. Statistical programs and output logs will be kept for all analysis and made available upon request.

1.6 Interim analyses

We plan to have two interim analyses on clinical, laboratory and electrocardiographic data to assess the safety of the novel TACT (AL+AQ). The interim reports will be reviewed by DSMB. The interim analyses will be performed after the first 60 patients and after 180 patients. After this, safety analysis will be performed based on recommendations by the DSMB. No stopping rules both statistical as well as 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 study 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 4 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 they exist.

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

2.1. Study objectives and endpoints

2.1.1. Primary objective

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

2.1.2. Primary endpoint

The Primary endpoint is the 42-day PCR corrected efficacy defined as adequate clinical and parasitological response (ACPR) by Kaplan Meier 1st event analyses.

(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 efficacy of the TACT (artemether-lumefantrine+amodiaquine) versus ACT (artemether-lumefantrine) as defined by the 42-day PCR according to site/geographical region

  - *42-day PCR corrected efficacy defined as adequate clinical and parasitological response (ACPR) according to site/geographical region*

- To assess and compare *P. falciparum* parasite clearance rates of standard AL and matching AL+AQ.
  - *Parasite clearance half-life assessed by microscopy as primary parameter to determine parasite clearance*
  - *Additional parameters of parasite clearance dynamics*
- To assess and compare fever clearance rates of standard AL and matching AL+AQ.
  - *Fever clearance time (i.e. the time taken for the tympanic temperature to fall below 37.5˚C and remain there for at least 24 hours)*
- To compare the safety and tolerability of AL+AQ versus standard AL
  - *Incidence of adverse events and serious adverse events by study arms within the first 42 days.*
  - *Incidence of adverse events concerning markers of hepatic or renal toxicity such as billirubin, ALT, AST, Alkaline Phosphatase and creatinine*
  - *Proportion of patients that reports completing a full course of observed AL+AQ or AL without withdrawal of consent or exclusion from study because of drug related serious adverse event*
- To compare changes in the electrocardiogram (such as prolongation of the QTc-interval) in patients treated with AL+AQ versus standard AL
  - *Incidence of prolongation of the QTc-interval above 500 ms or >60ms above baseline values.*
  - *Prolongation of QTc-interval compared to baseline at timepoint H4, H24, H28, H48, H52, H60, H64 and between these timepoints*
- To assess the spread of genetic markers of artemisinin (such as *Kelch13* mutations) and partner drug resistance and identify additional genetic determinants of drug resistance.
  - *Prevalence of Kelch13 mutations of known functional significance*
  - *Prevalence/incidence of other genetic markers of antimalarial drug resistance such as MDR1 copy number and MDR1 mutations*
- 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
  - *Correlation between SNPs measured in dry blood spots and whole genome sequencing in leukocyte depleted blood samples*
  - *Genome wide association with in vivo/in vitro sensitivity parasite phenotype*
- 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
  - *Transcriptomic patterns at t=0 and t=6h after start of treatment comparing sensitive and resistant parasites*

- To compare clearance dynamics estimated with quantitative PCR measurements of parasite loads versus microscopy
    - *Correlation between qPCR based versus microscopy-based assessments of parasite clearance dynamics up to day 14*
- To measure and compare the incidence and duration of gametocyte carriage in patients with antimalarial sensitive and resistant malaria before and after treatment with TACT(AL+AQ) and matching ACT (AL)
    - *Proportion of patients with gametocytemia before, during and after treatment with TACT (AL+AQ) or ACT (AL), assessed at admission, up to day 14, stratified by presence of gametocytes at enrolment*
- 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*
- To compare *ex vivo* 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 assess pharmacokinetic and pharmacodynamic interactions between AL and AL+AQ
    - *Pharmacokinetic profiles and interactions of artemisinin-derivatives and partner drugs (C_{max} and AUC) in 20 ACT treated and 20 AL+AQ treated patients of both study arms in Vietnam*
    - *Day 7 drug levels of partner drugs in association with treatment efficacy and treatment arm*
- To obtain additional safety data (in particular incidence and rate of hemolysis) on the deployment of single low dose primaquine, stratified according to 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*
- To obtain additional data on the effect of the host genotype on the pharmacokinetics and pharmacodynamics of antimalarials
    - *Correlation between the host genotype and the pharmacokinetics and pharmacodynamics of antimalarials*
- To compare clearance dynamics estimated with plasma HRP2 levels of parasite loads versus microscopy
    - *Correlation between HRP2 based versus microscopy based assessments of parasite clearance dynamics*
- To obtain data on the place of residence, work, recent travel history and mobile phone usage in order to improve the understanding of locations of malaria transmission and possible routes spread of malaria and artemisinin resistance.
    - *Data on the place of residence, work, recent travel history and mobile phone use*

- To obtain data and GPS mapping on a select group of participants and their peers relating to their understanding of the behaviours and risk factors associated with malaria infection in order to improve understanding of local malaria transmission.
    - *Data and GPS mapping for a select group of participants and their peers in relation to behaviours and risk factors associated with malaria infection*

2.2. Study design

Brief Description

This is an Open-label Randomised Trial comparing standard AL treatment with matching triple artemisinin-based combination therapies (AL+AQ), evaluating efficacy in sites experiencing ACT failure and safety, tolerability and artemisinin and partner drug resistance in all sites. The study sites are shown in figure 1 below. Patients will be randomized to artemether-lumefantrine +/- amodiaquine.

Figure 1 Study sites

There are 3 centres of recruitment in 2 different countries (Cambodia and Vietnam). 200 patients will be recruited from each of three centres: western Cambodia, north-eastern Cambodia, and from two sites in southern Vietnam (combined as one centre, enrolling patients under competitive recruitment).

2..2.1 Determination of sample size

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

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

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

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 42-day endpoint will be summarised in a CONSORT flow diagram, figure 2, below.

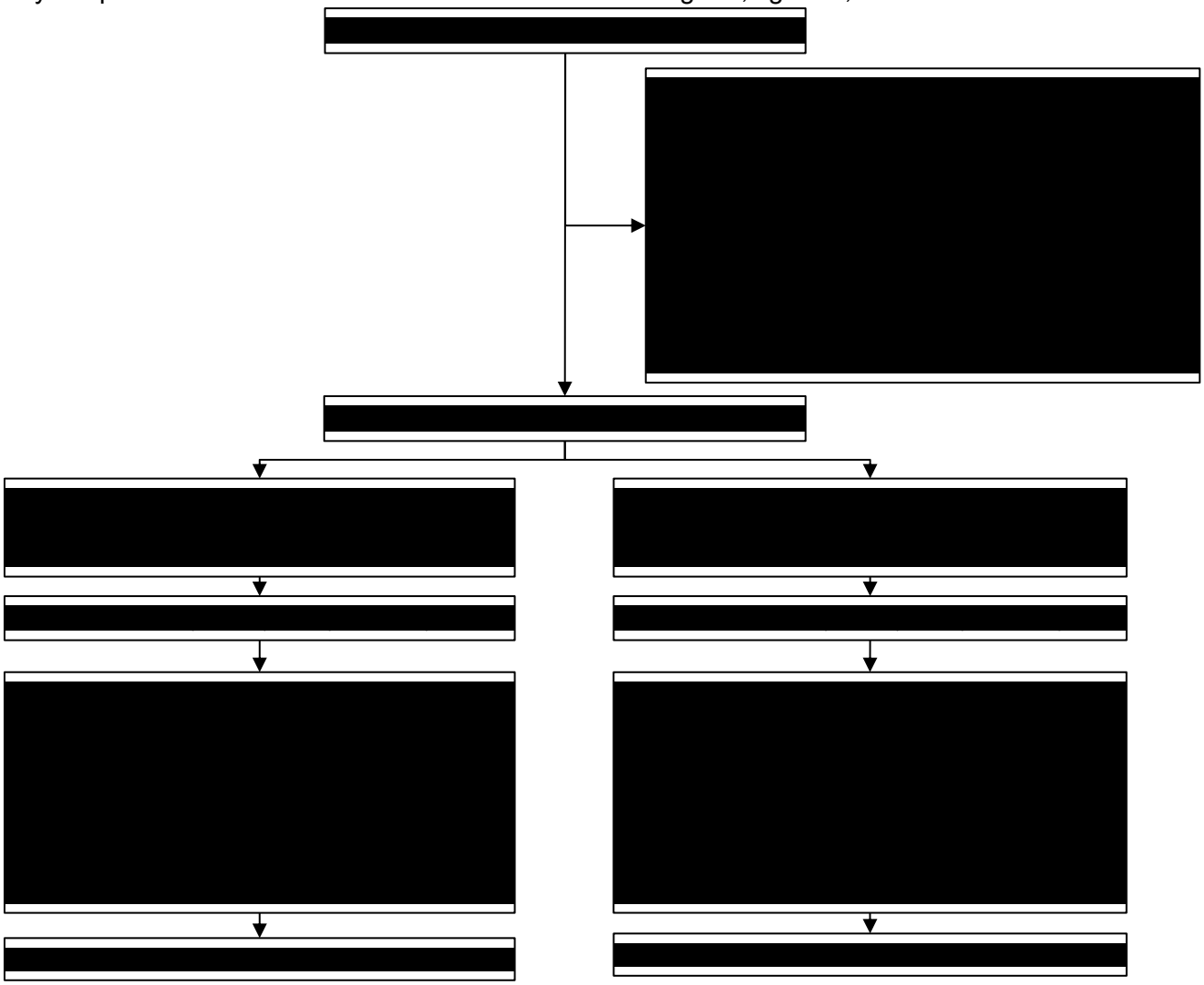

Figure 2 Consort Trial Profile by Arms
ITT Kaplan-Meier analyses, by follow-up week, of time to Pf recrudescence as a first event (The K-M analyses are to week 6, censored at the first recurrence if it is not a Pf recrudescence.)

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, respiratory rate will be summarized using median and interquartile rage. Continuous variables such as weight, height, systolic and diastolic blood pressure, QTc-intervals, haemoglobin will be summarized using mean ± standard deviation. Parasitaemia at baseline will be described as geometric mean and range. Categorical variables such as sex, presence of fever and gametocytaemia at baseline will be summarized using frequencies and percentages.

Table 1. Baseline Characteristics of the Patients

Characteristics	Artemether-Lumefantrine (N=XXX)	Artemether-Lumefantrine +Amodiaquine (N=XXX)	Total (N=XXX)
Age (years), med (IQR)	XX.X (XX.X-XX.X)	XX.X (XX.X-XX.X)	XX.X (XX.X-XX.X)
Male sex, n (%)	XX (XX.X)	XX (XX.X)	XX (XX.X)
Baseline tympanic temperature >37·5°C, n (%)	XX (XX.X)	XX (XX.X)	XX (XX.X)
Weight (kg), mean (SD)	XX.X (XX.X)	XX.X (XX.X)	XX.X (XX.X)
Height (cm) mean (SD)	XX.X (XX.X)	XX.X (XX.X)	XX.X (XX.X)
Heart rate (beats/minute), med (IQR)	XX.X (XX.X-XX.X)	XX.X (XX.X-XX.X)	XX.X (XX.X-XX.X)
Respiratory rate (breaths/minute), med (IQR)	XX.X (XX.X-XX.X)	XX.X (XX.X-XX.X)	XX.X (XX.X-XX.X)
Systolic blood pressure (mmHg) , mean (SD)	XX.X (XX.X)	XX.X (XX.X)	XX.X (XX.X)
Diasystolic blood pressure (mmHg) , mean (SD)	XX.X (XX.X)	XX.X (XX.X)	XX.X (XX.X)
QTcB interval (miliseconds) , mean (SD)	XX.X (XX.X)	XX.X (XX.X)	XX.X (XX.X)
Hb (g/dL) , mean (SD)	XX.X (XX.X)	XX.X (XX.X)	XX.X (XX.X)
Parasites/µL, geometric mean (range)	XX.X (XX.X-XX.X)	XX.X (XX.X-XX.X)	XX.X (XX.X-XX.X)
Gametocytemia present at baseline, n/N (%)	XX (XX.X)	XX (XX.X)	XX (XX.X)

3.3 Day 42 Efficacy assessments of the ACT (AL) and matching TACT (AL+AQ)

Primary objective

To assess and compare by ITT analyses the efficacies of the ACT and Triple ACT in achieving the primary endpoint of durable cure of the original infection. Failure is pre-defined in terms of PCR-corrected 42-day adequate clinical and parasitological response. This objective is equivalent to avoiding PCR-confirmed *P. falciparum* recrudescence.

Primary endpoint

42-day PCR-corrected *Pf* recrudescence: K-M 1st event analyses, censored at re-infection

Handling of missing data

In the ITT Kaplan-Meier/survival analysis, participants who are lost to follow up, or who have Pf or Pv reinfections or inconclusive PCR correction, will be censored or treated as competing risks, as appropriate, from the moment of occurrence of one of these events. Protocol deviations/violations (such as those that may switch treatment due to severe malaria, or those who may get rescue therapy) that are followed up to the end of the study will be analysed as having completed follow up in the ITT, or censored at time of their last follow up.

In the ITT analyses where efficacy will be presented as a proportions, the outcomes for day 42 for the patients that had definitive follow up shortly after day 42 will be included according to their status (success or recrudescent infection) as described in (Stepniewska & White 2006). This is because it is anticipated that some participants will be lost to follow-up (i.e. not seen during the 42-day follow-up visit) but that follow up can be performed shortly thereafter. In the standard operating procedures (SOPs) it is arranged that, whenever possible, participants will be seen as soon as possible after day 42 to ensure they have no malaria parasites. If not seen by day 90 no further effort will be made to see these participants. Based on a recommendation from Stepniewska and White 2006, these patients who were lost to follow-up but were followed up subsequently should not be censored from efficacy assessments if they have remained well and are PCR negative, as it is unlikely that a recrudescent malaria infection would have occurred during the missed appointment period which then rapidly and symptomless self-cured. Thus, such patients will be included as successes and will be clearly identified and documented when presenting the results to be easily distinguished from those that were followed up on day 42 as scheduled. If a participant is seen after day 42 with a recrudescent infection, this will be reported along with the interval period in which it emerged (time since last seen to time detected) will be reported to estimate the onset of recrudescence.

In per protocol analyses, for the reporting of proportions, it will be considered that participants not seen at day 42 have not completed follow up. Missing follow up visits between D7 and D42 will not cause participants to be censored if follow up at D42 is complete, and these participants will not be removed from per protocol analyses. 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, Pf or Pv reinfections and inconclusive PCR correction) will be excluded from the analysis.

Similar analyses will be performed for the 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 ACT (AL) with matching TACT (AL+AQ) will be presented as outlined in tables 2 and 3 below.

Table 2 Comparison of Day 42 PCR corrected Efficacy using survival methods of AL vs AL+AQ, overall and by site

	PCR corrected ACPR at day 42, Survival percentage (efficacy), %		Hazard ratio (95% CI)	p-value
	AL (N=XXX)	AL+AQ (N=XXX)		
Overall (all sites - pooled)	XX.X	XX.X	XX.X (XX.X - XX.X)	X.XXX
By sites				
Cambodia, west	XX.X	XX.X	XX.X (XX.X - XX.X)	X.XXX
Cambodia, east	XX.X	XX.X	XX.X (XX.X - XX.X)	X.XXX
Vietnam	XX.X	XX.X	XX.X (XX.X - XX.X)	X.XXX

Table 3 Comparison of Day 42 PCR corrected efficacy as proportions of AL vs AL+AQ, overall, and by site

	PCR corrected ACPR at day 42, n/N, (%)		Risk difference (95% CI)	p-value
	AL (N=XXX)	AL+AQ (N=XXX)		
Overall	XX/XXX (XX.X)	XX/XXX (XX.X)	XX.X (XX.X - XX.X)	X.XXX
By sites				
Cambodia, west	XX/XXX (XX.X)	XX/XXX (XX.X)	XX.X (XX.X - XX.X)	X.XXX
Cambodia, east	XX/XXX (XX.X)	XX/XXX (XX.X)	XX.X (XX.X - XX.X)	X.XXX
Vietnam	XX/XXX (XX.X)	XX/XXX (XX.X)	XX.X (XX.X - XX.X)	X.XXX

Table 3b PCR-confirmed weekly numbers with Pf recrudescence, and with new malarial re-infection without Pf recrudescence

	Recrudescence of P. falciparum		Re-infection by a new P. falciparum		Re-infection by P. vivax only	
Day * tested	ACT (AL)	Triple ACT (AL+AQ)	ACT (AL)	Triple ACT (AL+AQ)	ACT (AL)	Triple ACT (AL+AQ)
Day 7	x	x	x	x	x	x
Day 14	x	x	x	x	x	x
Day 21	x	x	x	x	x	x
Day 28	x	x	x	x	x	x
Day 35	x	x	x	x	x	x
Day 42	x	x	x	x	x	x
Total	x	x	x	x	x	x

* Rounded to nearest multiple of 7, with tests just after Day 42 rounded to Day 42

Note to be added to state falciparum infections not due to artemisinin-resistant Pf

3.4 **Fever clearance and parasite clearance during treatment, by treatment allocation**

Early parasite clearance rates should be dominated by the effects of the artemisinin rather than the partner drugs. Hence, little effect on them is expected from the random allocation to Triple ACT rather than ACT. Similar analyses, split by *Pfkelch13* mutation status (determined for all trial participants) rather than trial treatment, will be given as Table 5 and plotted as Figure 3.

Table 4 Fever clearance and parasite clearance by allocated treatment

	ACT (AL) N=XXX	**Triple ACT (AL+AQ) N=XXX**	**Both groups N=XXX**	**P-value (2-sided)**
Hours to fever clearance, mean (SD) (baseline to start of first 24h period <37.5C; temperature was recorded every 6 hours)				
Western Cambodia	XX.X (X.X)	XX.X (X.X)	XX.X (X.X)	X.XXX
Eastern Cambodia	XX.X (X.X)	XX.X (X.X)	XX.X (X.X)	X.XXX
Vietnam	XX.X (X.X)	XX.X (X.X)	XX.X (X.X)	X.XXX
All 3 study sites	**XX.X (X.X)**	**XX.X (X.X)**	**XX.X (X.X)**	**X.XXX**
Day 3 blood smear still positive, n/N (%) (taking prior clearance or discharge as negative)				
Western Cambodia	XX/XXX (XX)	XX/XXX (XX)	XX/XXX (XX)	X.XXX
Eastern Cambodia	XX/XXX (XX)	XX/XXX (XX)	XX/XXX (XX)	X.XXX
Vietnam	XX/XXX (XX)	XX/XXX (XX)	XX/XXX (XX)	X.XXX
All 3 study sites	**XX/XXX (XX)**	**XX/XXX (XX)**	**XX/XXX (XX)**	**X.XXX**
Parasite clearance half-life longer than 5 hours, n/N (%) (excluding patients with initial count insufficient to estimate half-life)				
Western Cambodia	XX/XXX (XX)	XX/XXX (XX)	XX/XXX (XX)	X.XXX
Eastern Cambodia	XX/XXX (XX)	XX/XXX (XX)	XX/XXX (XX)	X.XXX
Vietnam	XX/XXX (XX)	XX/XXX (XX)	XX/XXX (XX)	X.XXX
All 3 study sites	**XX/XXX (XX)**	**XX/XXX (XX)**	**XX/XXX (XX)**	**X.XXX**
Parasite clearance half-life in hours, mean (SD) (excluding patients with initial count insufficient to estimate half-life)				
Western Cambodia	X.X (X.X)	X.X (X.X)	X.X (X.X)	X.XXX
Eastern Cambodia	X.X (X.X)	X.X (X.X)	X.X (X.X)	X.XXX
Vietnam	X.X (X.X)	X.X (X.X)	X.X (X.X)	X.XXX
All 3 study sites	**X.X (X.X)**	**X.X (X.X)**	**X.X (X.X)**	**X.XXX**

Table 5 Fever clearance and parasite clearance by *Pfkelch13* status

	Relevant *Pfkelch13* mutation detected?			
	Yes (N=XXX)	**No (wild type) (N=XXX)**	**Difference, Yes minus No**	**P-value (2-sided)**
Hours to fever clearance, mean (SD) (baseline to start of first 24h period <37.5C; temperature was recorded every 6 hours)				
Western Cambodia	XX.X (X.X)	XX.X (X.X)	XX.X (X.X)	X.XXX
Eastern Cambodia	XX.X (X.X)	XX.X (X.X)	XX.X (X.X)	X.XXX
Vietnam	XX.X (X.X)	XX.X (X.X)	XX.X (X.X)	X.XXX
All 3 study sites	**XX.X (X.X)**	**XX.X (X.X)**	**XX.X (X.X)**	**X.XXX**
Day 3 blood-smear still positive, n/N (%) (taking prior clearance or discharge as negative)				
Western Cambodia	X.X (X.X)	X.X (X.X)	X.X (X.X)	X.XXX
Eastern Cambodia	X.X (X.X)	X.X (X.X)	X.X (X.X)	X.XXX
Vietnam	X.X (X.X)	X.X (X.X)	X.X (X.X)	X.XXX
All 3 study sites	**X.X (X.X)**	**X.X (X.X)**	**X.X (X.X)**	**X.XXX**
Parasite clearance half-life longer than 5 hours, n/N (%) (excluding patients with initial count insufficient to estimate half-life)				
Western Cambodia	XX/XXX (XX)	XX/XXX (XX)	XX/XXX (XX)	X.XXX
Eastern Cambodia	XX/XXX (XX)	XX/XXX (XX)	XX/XXX (XX)	X.XXX
Vietnam	XX/XXX (XX)	XX/XXX (XX)	XX/XXX (XX)	X.XXX
All 3 study sites	**XX/XXX (XX)**	**XX/XXX (XX)**	**XX/XXX (XX)**	**X.XXX**
Parasite clearance half-life in hours, mean (SD) (excluding patients with initial count insufficient to estimate half-life)				
Western Cambodia	XX/XXX (XX)	XX/XXX (XX)	XX/XXX (XX)	X.XXX
Eastern Cambodia	XX/XXX (XX)	XX/XXX (XX)	XX/XXX (XX)	X.XXX
Vietnam	XX/XXX (XX)	XX/XXX (XX)	XX/XXX (XX)	X.XXX
All 3 study sites	**XX/XXX (XX)**	**XX/XXX (XX)**	**XX/XXX (XX)**	**X.XXX**

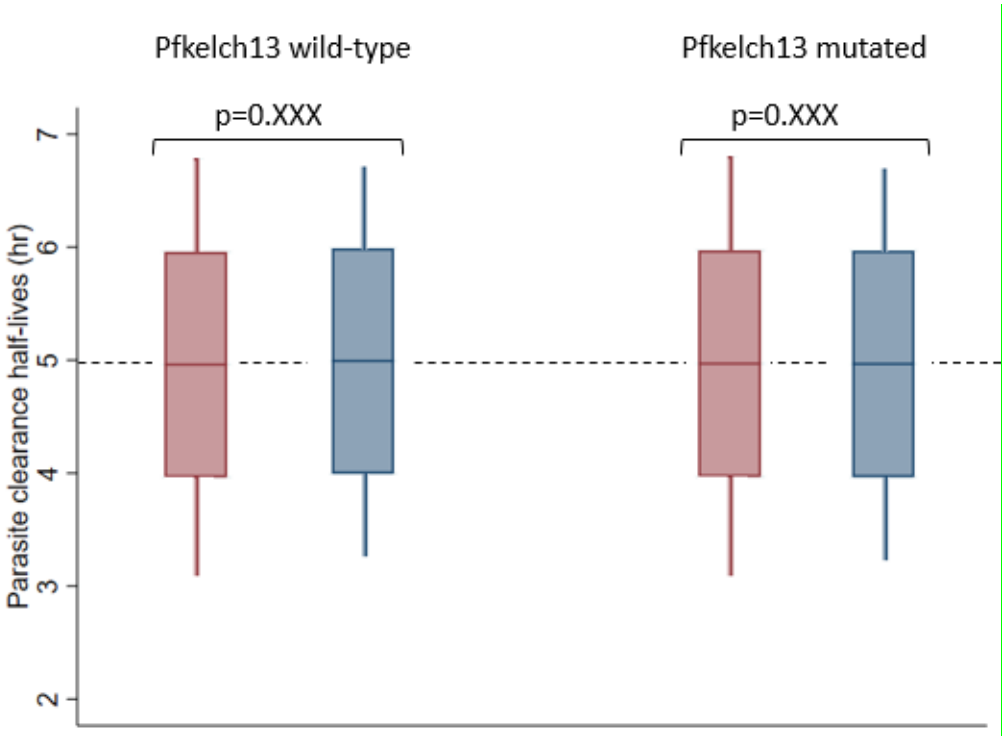

Figure 3 Parasite clearance half-life by *PfKelch13* mutation status and treatment

3.5 Safety assessments and tolerability (Incidence of adverse events and serious adverse events by study arms within the first 42 days, incidence of adverse events concerning markers of hepatic or renal toxicity such as billirubin, ALT, AST, Alkaline Phosphatase and creatinine; and QTc Prolongation)

Safety analyses will be based on the whole population that get administered the study drug. That is, the safety and tolerability data will be pooled from all the sites that received the same antimalarial treatment.

The safety and tolerability of AL+AQ versus AL 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 renal toxicity (creatinine) and hepatic toxicity (such as billirubin, ALT, AST, Alkaline Phosphatase) and markers of bone marrow toxicity (Haemoglobin, White blood cell count and differentiation, platelet counts), using the Fisher's exact test.

Safety data will be presented in tabular and/or graphical format and summarized descriptively. Any clinically relevant abnormalities or values of potential clinically concern will be described. Patients will be analysed 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 Division of AIDS table for grading the severity of ADULT AND PEDIATRIC adverse Events Version 2.0, November, 2014. The relevant DAIDS table will be appended to this document. The safety and tolerability summaries will be presented in table 5. A previous randomised comparison of these two regimens (R van der Pluijm et al, 2020 https://doi.org/10.1016/S0140-6736(20)30560-2), which involved a

total of 575 patients, reported an increased incidence of bradycardia and possible increases in vomiting and in creatinine.

3.6 Comparison of changes in the electrocardiogram (such as prolongation of the QTcB-interval) in patients treated with TACT versus standard ACT

Incidence of prolongations of the QTc-interval>60 miliseconds compared to baseline or >500 miliseconds and bradycardia (heart rate<54 beats/minute) at least once will be summarized in table 5. Absolute changes in the QTc-interval will be summarized as in tables 7 and 8, and will be graphically displayed by arm as in figure 5 (only QTcB). Comparisons on the changes in the QTcB-interval will be made using the unpaired t-test for each specific time point.

Table 5 Incidence of adverse events within first 42 days by arm

	AL		AL+AQ		p-value
Number of subjects	XX		XX		
Vomiting/number of treatments, n/N, (%)	X/XX (X.X)		X/XX (X.X)		X.XXX
Serious adverse events (SAEs) , n/N, (%)	XX		XX		X.XXX
Possible, probable or definite drug related SAEs, n/N, (%)	X/XX (X.X)		X/XX (X.X)		X.XXX
QTc >60ms above baseline (H64)	X/XX (X.X)		X/XX (X.X)		X.XXX
QTc >500ms (H64)	X/XX (X.X)		X/XX (X.X)		X.XXX
Bradycardia (<=54 bpm) (H64)	X/XX (X.X)		X/XX (X.X)		X.XXX
Grading of adverse events, n/N, (%)	1-2	3-4	1-2	3-4	
Symptoms					
Headache	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X.XXX
Fatigue	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X.XXX
Abdominal pain	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X.XXX
Loss of appetite	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X.XXX
Nausea	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X.XXX
Vomiting	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X.XXX
Diarrhea	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X.XXX
Itching	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X.XXX
Dizziness	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X.XXX
Blurred vision	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X.XXX
Sleeping disturbance	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X.XXX

Total	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X.XXX
Laboratory abnormalities					
Creatinine	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X.XXX
Total bilirubin	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X.XXX
Alkaline phosphatase	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X.XXX
Alanyl transfarase (ALT)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X.XXX
Aspartate transfarase (AST)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X.XXX
Hemoglobin decrease	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X.XXX
Leukocytopenia	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X.XXX
Neutrocytopenia	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X.XXX
Lymphocytopenia	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X.XXX
Thrombocytopenia	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X/XX (X.X)	X.XXX

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 (>60 miliseconds above baseline or >500 miliseconds) and bradycardia is defined as a subjects experience these abnormalities at one or more timepoints.

The frequency and proportions of patients that report a full course of observed TACT or ACT without withdrawal of consent or exclusion from the study because of drug related serious adverse events will be summarized for the whole study population. 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 6.

Table 6 Proportions of patients that report a full course of observed TACT or ACT

	AL, n/N (%, 95% CI)	A+AQ, n/N (%, 95% CI)
Completing full course of study drug		

Table 7 QTcB interval and change in it since study entry, by time and allocated treatment

	ACT (AL)			Triple ACT (AL+AQ)		
	QTcB (ms)	Change in QTcB	p-value	QTcB (ms)	Change in QTcB	p-value
Hour 0	XXX (XX)	-	-	XXX (XX)	-	-
Hour 4	XXX (XX)	XX.X (X.X)	X.XXX	XXX (XX)	XX.X (X.X)	X.XXX
Hour 24	XXX (XX)	XX.X (X.X)	X.XXX	XXX (XX)	XX.X (X.X)	X.XXX
Hour 28	XXX (XX)	XX.X (X.X)	X.XXX	XXX (XX)	XX.X (X.X)	X.XXX
Hour 48	XXX (XX)	XX.X (X.X)	X.XXX	XXX (XX)	XX.X (X.X)	X.XXX
Hour 52	XXX (XX)	XX.X (X.X)	X.XXX	XXX (XX)	XX.X (X.X)	X.XXX
Hour 60	XXX (XX)	XX.X (X.X)	X.XXX	XXX (XX)	XX.X (X.X)	X.XXX
Hour 64	XXX (XX)	XX.X (X.X)	X.XXX	XXX (XX)	XX.X (X.X)	X.XXX
Day 7	XXX (XX)	XX.X (X.X)	X.XXX	XXX (XX)	XX.X (X.X)	X.XXX
Day 28	XXX (XX)	XX.X (X.X)	X.XXX	XXX (XX)	XX.X (X.X)	X.XXX

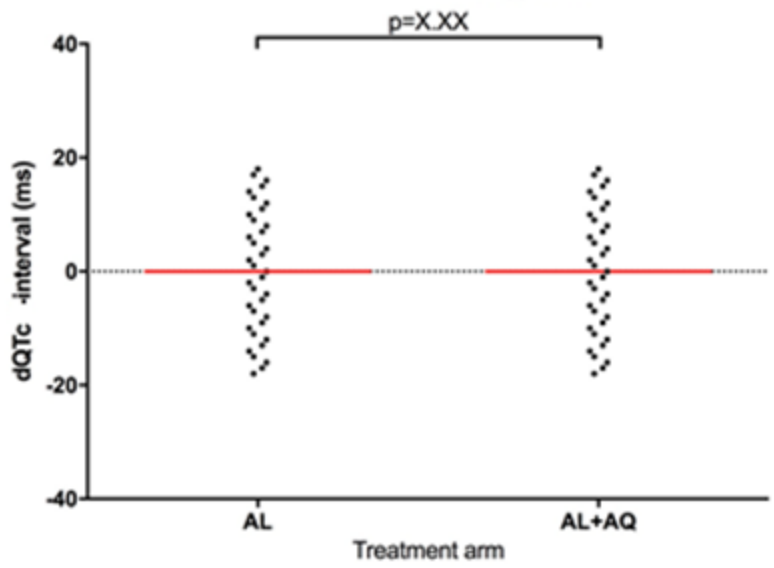

Figure 5 Changes in QTc-intervals at hour 64 from baseline by treatment arm

Table 8: Heart rate and change in it since study entry, by time and allocated treatment

	ACT (AL)			Triple ACT (AL+AQ)		
	Heart rate (bpm)	Change in heart rate	p-value	Heart rate (bpm)	Change in heart rate	p-value
Hour 0	XX (XX)	-	-	XX (XX)	-	-
Hour 4	XX (XX)	X.X (X.X)	X.XXX	XX (XX)	X.X (X.X)	X.XXX
Hour 24	XX (XX)	X.X (X.X)	X.XXX	XX (XX)	X.X (X.X)	X.XXX
Hour 28	XX (XX)	X.X (X.X)	X.XXX	XX (XX)	X.X (X.X)	X.XXX
Hour 48	XX (XX)	X.X (X.X)	X.XXX	XX (XX)	X.X (X.X)	X.XXX
Hour 52	XX (XX)	X.X (X.X)	X.XXX	XX (XX)	X.X (X.X)	X.XXX
Hour 60	XX (XX)	X.X (X.X)	X.XXX	XX (XX)	X.X (X.X)	X.XXX
Hour 64	XX (XX)	X.X (X.X)	X.XXX	XX (XX)	X.X (X.X)	X.XXX
Day 7	XX (XX)	X.X (X.X)	X.XXX	XX (XX)	X.X (X.X)	X.XXX
Day 28	XX (XX)	X.X (X.X)	X.XXX	XX (XX)	X.X (X.X)	X.XXX

3.7 Genetic markers of artemisinin (particularly *Pfkelch13*) and partner drug resistance

All parasite samples collected at baseline and at the times of any recurrences within the first 6 weeks after recruitment will be genotyped for known markers of resistance to artemisins and to the other study drugs. This information will be used to help interpret parasite clearance rates, and to help investigate the reasons for any recurrences during the first 6 weeks after baseline.

Table 8 Molecular resistance markers in baseline *P. falciparum* specimens, by study site

	Number studied, N	Wild-type Kelch13 n (%)	C580Y Kelch13 n (%)	Other Kelch13 n (%)	Plasmepsin 2/3 amplified n (%)	MDR1 amplified n (%)
Western Cambodia	XXX	xxx (xx)	xxx (xx)	xxx (xx)	xxx (xx)	xxx (xx)
Eastern Cambodia	XXX	xxx (xx)	xxx (xx)	xxx (xx)	xxx (xx)	xxx (xx)
Vietnam	XXX	xxx (xx)	xxx (xx)	xxx (xx)	xxx (xx)	xxx (xx)
All three study sites	XXX	xxx (xx)	xxx (xx)	xxx (xx)	xxx (xx)	xxx (xx)

3.8 Representability/accuracy of parasite genome sequencing from dry blood spots for the genome sequencing results from leukocyte depleted blood samples

A detailed analysis plan will be prepared separately.

3.9 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

A detailed analysis plan for the analysis of the transcriptome will be prepared separately.

3.10 Comparison of clearance dynamics estimated with quantitative PCR measurements of parasite loads versus microscopy

At each time point the level of agreement (if absolute values will be of interest) or correlation (if relative relationship will be of interest) will be assessed between qPCR and microscopy parasite load measurements. If the agreement or correlation (whichever will be appropriate) will show a consistent relationship/agreement at all time points, the data may be pooled across all time points. If it will be deemed appropriate/ feasible, half-lives achieved by the two methods will be compared. A detailed analysis plan will be prepared separately.

3.11 Measurement and comparison the incidence and duration of gametocyte carriage in patients with antimalarial sensitive and resistant malaria before and after treatment with primaquine in addition to the ACT (AL) or matching TACT (AL+AQ)

The baseline proportion of participants with patent gametocytaemia (gametocyte densities above the microscopy level of detection) will be summarized and reported by site. To measure and compare the incidence and duration of gametocyte carriage in patients with antimalarial sensitive and resistant malaria before and after treatment with primaquine in addition to TACT (AL+AQ) or matching ACT (AL). Proportion of patients with gametocytaemia before, during and after treatment with primaquine in addition to TACT (AL+AQ) or matching ACT (AL), assessed at admission, up to day 14. The patients will be categorized into four gametocytaemia status and summarized as presented in the tables 10a and 10b below. McNemar's test will be used to assess whether there will be a significant difference in proportions of gametocytaemia between admission and either day 7 or day 14.

Table 10a Proportion of patients with gametocytaemia at admission and day 7

Country, site	AL				AL+AQ			
	Admission	Day 7	n (%)	p-value	Admission	Day 7	n (%)	p-value
Cambodia, east	Yes	Yes	XX (XX.X)	X.XXX	Yes	Yes	XX (XX.X)	X.XXX
	Yes	No	XX (XX.X)		Yes	No	XX (XX.X)	
	No	Yes	XX (XX.X)		No	Yes	XX (XX.X)	
	No	No	XX (XX.X)		No	No	XX (XX.X)	
Cambodia, west	Yes	Yes	XX (XX.X)	X.XXX	Yes	Yes	XX (XX.X)	X.XXX
	Yes	No	XX (XX.X)		Yes	No	XX (XX.X)	
	No	Yes	XX (XX.X)		No	Yes	XX (XX.X)	
	No	No	XX (XX.X)		No	No	XX (XX.X)	
Vietnam	Yes	Yes	XX (XX.X)	X.XXX	Yes	Yes	XX (XX.X)	X.XXX
	Yes	No	XX (XX.X)		Yes	No	XX (XX.X)	
	No	Yes	XX (XX.X)		No	Yes	XX (XX.X)	
	No	No	XX (XX.X)		No	No	XX (XX.X)	

Table 10b Proportion of patients with gametocytaemia at admission and day 14

Country, site	AL				AL+AQ			
	Admission	Day 14	n (%)	p-value	Admission	Day 14	n (%)	p-value
Cambodia, east	Yes	Yes	XX (XX.X)	X.XXX	Yes	Yes	XX (XX.X)	X.XXX
	Yes	No	XX (XX.X)		Yes	No	XX (XX.X)	
	No	Yes	XX (XX.X)		No	Yes	XX (XX.X)	
	No	No	XX (XX.X)		No	No	XX (XX.X)	
Cambodia, west	Yes	Yes	XX (XX.X)	X.XXX	Yes	Yes	XX (XX.X)	X.XXX
	Yes	No	XX (XX.X)		Yes	No	XX (XX.X)	
	No	Yes	XX (XX.X)		No	Yes	XX (XX.X)	
	No	No	XX (XX.X)		No	No	XX (XX.X)	
Vietnam	Yes	Yes	XX (XX.X)	X.XXX	Yes	Yes	XX (XX.X)	X.XXX
	Yes	No	XX (XX.X)		Yes	No	XX (XX.X)	
	No	Yes	XX (XX.X)		No	Yes	XX (XX.X)	
	No	No	XX (XX.X)		No	No	XX (XX.X)	

Time to gametocyte appearance will be summarised in a Kaplan-Meier Plot for patients who will have no gametocytaemina at admission but develop gametocytemia during follow-up. Similarly, time to gametocyte disappearance will be summarised in a Kaplan-Meier Plot for patients who will have gametocytaemina either at admission or during follow-up.

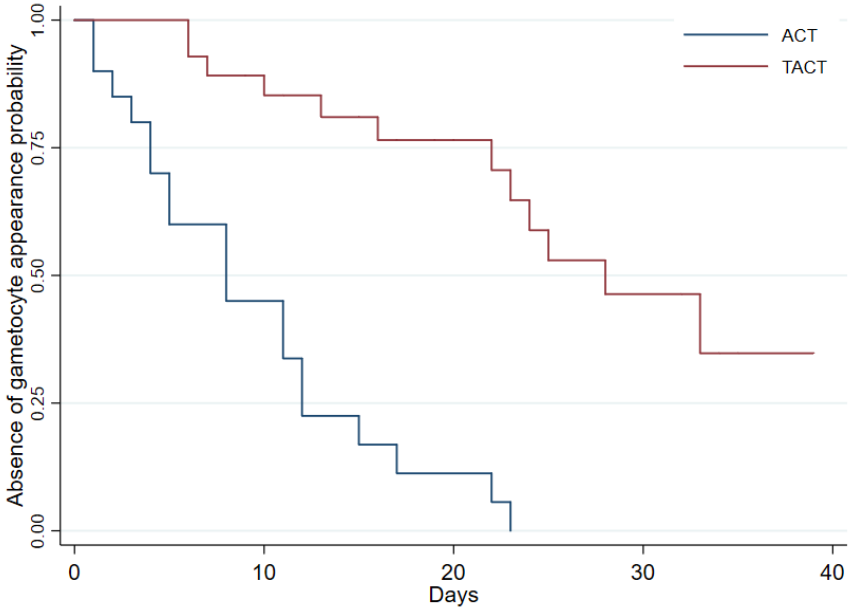

Figure 6 Kaplan-Meier Plot on gametocyte appearance in patients who have no gametocytaemina at admission

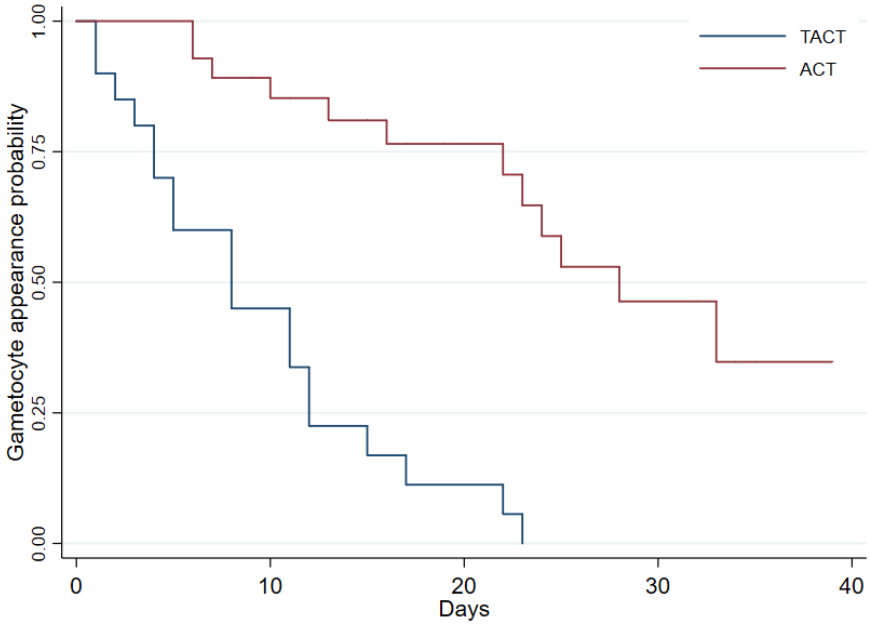

Figure 7 Kaplan-Meier Plot on gametocyte disappearance in patients who have gametocytaemina at admission

For those with gametoctytaemia at admission, the duration of patent gametocytemia will be summarized using median and interquartilerange (IQR) and/or range in table 10. Mann-Whitney U test will be used to test whether the median duration of patent gametocytemia differ according to

treatment given in each site. We will also assess whether there is correlation between the duration of patent gametocytemia and the parasite clearance half-life.

Table 11 Duration of gametocyte carriage in patients with gametoctytaemia at admission

Country, site	Duration of gametocyte carriage		p-value
	AL	AL+AQ	
Cambodia, east	XX.X (XX.X – XX.X)	XX.X (XX.X – XX.X)	X.XXX
Cambodia, west	XX.X (XX.X – XX.X)	XX.X (XX.X – XX.X)	X.XXX
Vietnam	XX.X (XX.X – XX.X)	XX.X (XX.X – XX.X)	X.XXX

The proportion and duration of gametocyte carriage in patients with antimalarial sensitive and resistant malaria before and after treatment with primaquine will be summarized.

3.12 DNA and RNA measurement methods for quantification of male and female gametocytes.

Samples will be obtained at baseline, hour 24 and hour 48. A detailed analysis plan for the analysis of the transcriptome and genome will be prepared separately.

3.13 *ex vivo* susceptibility profiles of *P. falciparum* isolates across geographic regions

Parasite half-lives (IC50 or other related parameters) of the drugs will be used to assess drug susceptibility. The drug susceptibility half-life cut-off points will be determined from a statistical model that will be developed based on previous data. A statistical approach will be developed from previous drug susceptibility data to infer the characteristics of the component distributions and their relative contribution to the composite mixture. The model may be either unimodal, bimodal or multimodal depending on prevalence of resistance from the different areas. The geometric mean (IQR) parasite half-lives consistent with the sub-distributions will be reported together with the 95% confidence intervals to determine the cut-off point from the model. The half-lives of parasites in the individual data will be used to obtain an estimate of the likelihood of a resistant infection depending of an individual which will depend on the population prevalence of resistance in that area as determined by the model

3.14 Pharmacokinetic profiles of ACT (AL) and Triple ACT (AL+AQ) components

A sub-study of multiple blood samples from each of about 40 patients in Vietnam, half given Triple ACT and half given standard ACT will be undertaken. Standard pharmacokinetic parameters of lumefantrine and amodiaquine during the first 24 hours will be estimated from a non-compartment analysis, including as C_{max}, T_{max}, $T_{1/2}$ and area under the curve (AUC). Results will be presented as below, to check whether amodiaquine affects artemether or lumefantrine pharmacokinetics.

Table 12 Pharmacokinetic parameters of the individual drugs artemether and lumefantrine when given in combination with amodiaquine (Triple ACT) and when given without amodiaquine

	ACT (AL)	**Triple ACT (AL+AQ)**	**p-value**
Artemether (geometric mean, 95%CI)			
C_{max} (ng/ml)	x.xx (xx.x - xx.x)	x.xx (xx.x - xx.x)	x.xxx
T_{max} (hours, h)	x.xx (xx.x - xx.x)	x.xx (xx.x - xx.x)	x.xxx
AUC (h × ng/ml)	x.xx (xx.x - xx.x)	x.xx (xx.x - xx.x)	x.xxx
Lumefantrine (geometric mean, 95%CI)			
C_{max} (ng/ml)	x.xx (xx.x - xx.x)	x.xx (xx.x - xx.x)	x.xxx
T_{max} (hours, h)	x.xx (xx.x - xx.x)	x.xx (xx.x - xx.x)	x.xxx
AUC (h × ng/ml)	x.xx (xx.x - xx.x)	x.xx (xx.x - xx.x)	x.xxx
Amodiaquine (geometric mean, 95%CI)			
C_{max} (ng/ml)	NA	x.xx (xx.x - xx.x)	NA
T_{max} (hours, h)	NA	x.xx (xx.x - xx.x)	NA
AUC (h × ng/ml)	NA	x.xx (xx.x - xx.x)	NA

Table 13 Pharmacokinetic parameters of lumefantrine on day 7 when given in combination with amodiaquine (Triple ACT) and when given without amodiaquine in all patients from Cambodia and Vietnam. Also amodiaquine levels in all patients allocated to amodiaquine (Triple ACT).

	ACT (AL)	Triple ACT (AL+AQ)	p-value
Lumefantrine (geometric mean, 95%CI)			
C_{max} (ng/ml)	x.xx (xx.x - xx.x)	x.xx (xx.x - xx.x)	x.xxx
T_{max} (hours, h)	x.xx (xx.x - xx.x)	x.xx (xx.x - xx.x)	x.xxx
AUC (h × ng/ml)	x.xx (xx.x - xx.x)	x.xx (xx.x - xx.x)	x.xxx
Amodiaquine (geometric mean, 95%CI)			
C_{max} (ng/ml)	NA	x.xx (xx.x - xx.x)	NA
T_{max} (hours, h)	NA	x.xx (xx.x - xx.x)	NA
AUC (h × ng/ml)	NA	x.xx (xx.x - xx.x)	NA

3.15 Change in haematocrit according to geographical location and study arm and stratified according to 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.