TABS-PKPD (ISRCTN49726849) Statistical Analysis Plan

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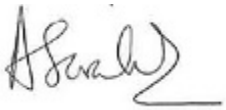
Sarah Walker	Trial Statistician		29 May 2021

Revision History

Version	Author	Date	Reason for Revision
0.1			Protocol version 1.0
0.2	Roisin Connon	30.4.2021	Roisin Connon first draft
0.3	Roisin Connon	6.5.2021	Incorporated Elizabeth George's (EG) comments on v0.2
0.4	Roisin Connon	7.5.2021	Incorporated Kath Maitland's comments on v0.3
1.0	Roisin Connon	20.5.2021	Incorporated Sarah Walker's comments on v0.3 and further comments from EG on v0.4

Contents

1. BACKGROUND

Malaria remains the commonest cause of hospital admission in much of sub-Saharan Africa, and plays a substantial role in under 5-year mortality. Children with severe malaria and bacterial co-infection have substantially higher rates of in-hospital and post-discharge mortality. An estimated one third of all severe malaria deaths in African children are attributable to bacterial co-infection. Current guidance and evidence for treating co-infection in children is lacking, and there is no consensus on the dosage or length of treatment required. The indiscriminate use of antibiotics is both financially costly and may perpetuate the rise of antibiotic resistance. Establishing which children with malaria are at greatest risk of bacteraemia is critical to pragmatically inform a policy for targeted antibiotic therapy that could substantially reduce malaria-associated mortality while minimising the risks of excess antibiotic prescribing.

Full details of the background to the trial are presented in the protocol.

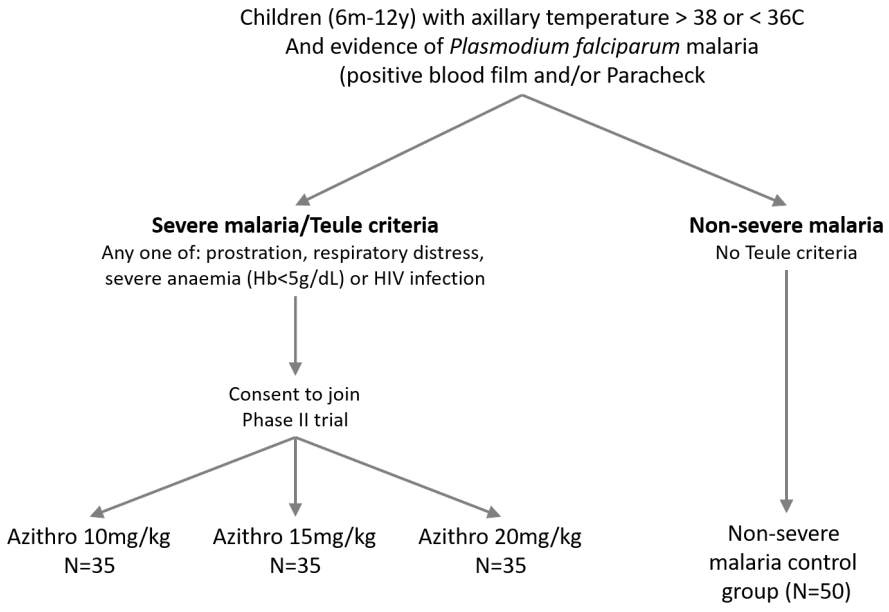
2. DESIGN

The study consists of a Phase I/II trial comparing three doses of azithromycin. 105 children with severe malaria and high risk of bacterial co-infection will be randomised 1:1:1 to 10, 15 and 20 mg/kg (prescribed for feasibility by weight-bands) taken orally over 5 days. 50 children with malaria but not meeting the criteria for high risk of bacterial co-infection will be enrolled in a non-randomised control group.

The study will be conducted in Mbale Regional Referral Hospital (MRRH), Eastern Uganda.

Randomisation lists will be generated and kept at the MRC CTU at UCL, London. The randomisation envelopes will be prepared before the trial at the Clinical trials facility, KWTRP, Kilifi. These will contain the actual allocation visible only once opened.

2.1 Study flow diagram



2.2 Outcome measures

The co-primary outcomes are:

- Change in C-reactive protein (CRP), a putative marker of sepsis, at 72 hours (continuous)
- Microbiological cure (7-day) (binary), alone and as a composite with 7-day survival

Secondary outcomes are:

- Mortality at 48-hours and longer-term survival (to day-28 and to day-90).
- Length of hospital stay (days)
- Re-admission to hospital by 90 days
- Serious adverse events, all grade 3/4 adverse events, adverse events that are definitely, probably or possibly related to azithromycin (any grade) and adverse events of any grade leading to change in azithromycin (including temporary or permanent discontinuation).
- The population pharmacokinetics of azithromycin and their relation (combined with pathogen susceptibility) with treatment outcome (pharmacodynamics).

2.3 Sample size

A formal sample size was not calculated. Phase II trials are not usually designed to compare superiority of different interventions. The aim of this trial is to generate pilot efficacy data on the optimal azithromycin dose for children with severe malaria, which will inform the design of a later larger Phase III trial. The numbers required to address the trial objectives are therefore balanced against the exposure of children in these settings to a therapeutic intervention (dose) for which there are limited data to date.

The overall sample size for the trial will be 105 children randomised 1:1:1 to receive 10, 15 or 20mg/kg azithromycin (based on weight-bands). This is sufficient for the PKPD sampling and modelling to determine an optimal dose in children with severe malaria using the primary outcomes. This is under the assumption that 20% of enrolled children (meeting 'Teule' severity criteria) will have bacteraemia and that 80% of these infections will be caused by non-typhoidal Salmonellae or other enteric gram-negative organisms.

2.4 Selection of patients

Children will be considered eligible for enrolment in this trial if they fulfil all the inclusion criteria and none of the exclusion criteria.

Patient inclusion criteria

Children aged 6 months to 12 years at admission to hospital with Plasmodium falciparum malaria (on either blood film or ParacheckTM rapid diagnostic test) and all of the following:

i) Axillary temperature >38°C or <36°C
ii) [randomised trial only] 'Teule' severity criteria[1]: any one of the following: prostration; respiratory distress; severe anaemia (haemoglobin<5g/dL) or HIV infection
iii) Parents willing/able to provide consent

Patient exclusion criteria

The following exclusion criteria will be used for the randomised trial:

i) Major contraindications to azithromycin, eg strong existing clinical diagnosis of QT-prolongation.
ii) Concomitant use of interacting drugs: drugs that may cause QT-prolongation or drugs that may cause a pharmacokinetic interaction with azithromycin, like strong CYP3A/P-GP inducers and concomitantly administered antacids.

Since the control group will not receive azithromycin the criteria above do not apply to enrolment as a control.

3. DATA

Full details of data management procedures are provided in the Data Management Plan for the trial, which forms part of the Trial Master File.

3.1 CRF forms and variables

Full details of data collection and timing are described in the trial protocol (version 1.2, 8th October 2018). A copy of the CRFs are presented in the Trial Master File. Details of the variables are presented within the metadata which forms part of the Trial Master File.

3.2 Management of datasets

- For all analyses, datasets of all data stored in the database will be extracted from Open Clinica by the statistician. This will act as the frozen dataset. It is the responsibility of the statistician to accurately record the date of freezing and ensure all data is retrieved.
- For interim analyses, new data can continue to be entered onto the Open Clinica database. If any outstanding data queries are resolved during the analysis that relate to data in the frozen dataset (e.g. problems that are found during analysis or amended CRFs that are data entered post-freeze), the data should be changed at the start of the set of analysis programs using an auditable statistical program, separate from all other programs (by the Trial/Delegated Statistician). The main Open Clinica database will be amended in parallel at sites.

For the final analysis the Trial Statistician will be responsible for defining when the data are clean and ready for database lock in the Data Management Plan.

3.3 Data verification

Data verification, consistency and range checks, as well as checks for missing data will be performed prior to analysis. Details of these checks can be found in the Statistical Master File. All variables will be examined for unusual, outlying, unlabelled or inconsistent values.

Any problems with trial data will be queried with the Trial Managers, Data Managers, or statisticians, as appropriate. For interim analyses, if possible, data queries will be resolved and amended, although it is accepted that due to administrative reasons and data availability a small number of problems will continue to exist.

4. DERIVATION OF DATA TO BE ANALYSED

Definition of time of enrolment

For time-to-event analyses, time will be calculated from randomisation for children in the randomised trial. For children in the control group time will be calculated from time of admission.

Definition of baseline

Baseline values for all measurements will be those recorded on the screening form, the consent form, clinical evaluation form (taken at admission), or lab results from samples taken at day 0, as appropriate.

Definition of censoring

For time-to-event analyses children will censored at the earlier of 90 days post-enrolment or date last known alive.

Continuous measures

Normality of all continuous measures and their change from baseline will be assessed using the Shapiro-Wilk test. Box-Cox transformations of the original absolute measurements will be used in the case of gross ($p<0.0001$) deviations.

Definition of visit schedule

Analyses of measurements at a given point in follow up will use the closest available measurement to that time point in evenly spaced windows. If there are two measurements that are equally close to the timepoint, the earliest measurement will be used.

Free text

Several fields are free text for other conditions. These will be categorised based on self-evident corrections, e.g. spelling. Adverse events and hospitalisations will be coded consistently (e.g. anaemia and malaria will be equivalent to malaria and anaemia) in consultation with the Chief Investigator.

Standardisation of anthropometry

Weight and MUAC will be standardised for age and z-scores calculated using WHO Reference 2007 Charts.

The WHO charts only have reference values for weight-for-age for children up to 10 years, which does not cover the full age range of children included in the trial. Children older than 10 years will be excluded from analysis using weight-for-age z-scores.

The WHO charts have reference values for MUAC-for-age for children aged between 3 months and 5 years. For children aged above 5 years z-scores will be calculated from reference data published in Mramba 2017[2].

Missing data

Interim analyses will be based on observed data only. For the final report, analysis of primary outcomes will be based on observed data only, unless the endpoint has missing data for >10% of children. In that case multiple imputation by chained equations will be conducted separately for each randomised group. The microbiological cure outcome will be imputed using logistic regression, and CRP will be imputed using normal linear regression. The imputation models will include all available CRP measurements and microbiology results (categorised as gram-positive, gram-negative, or no pathogen isolated). Age will also be included unless the model fails to converge. Analyses of secondary outcomes will be based on observed data only.

5. STATISTICAL ANALYSIS

Primary analysis will be intention to treat. Children for whom assent was given but subsequent full consent refused will be excluded. Children where assent was given but then absconded (so full consent was not obtained) will be included.

All statistical tests will use a significance level of 0.05. Confidence intervals will be presented at the 95% level.

All analysis will be included in the final report, and all except for the items listed in italics will be included in reports for the Data Monitoring Committee (DMC).

5.1 Recruitment

- Number of cases and controls enrolled
- Eligibility: number and reasons for any children randomised in error and excluded or ineligible children included in the analysis

5.2 Baseline characteristics

The following baseline characteristics will be summarised by the specified statistics. These will be presented for participants in the randomised trial and the control group. Variables will be presented by randomised arm if there is a difference between randomised groups of $p \leq 0.05$, used as a flagging device for imbalance and expected for 1 in 20 characteristics by chance, with p-values from Kruskal-Wallis tests, chi-squared tests or Fisher's exact test if cell values are small.

- Age at admission (months), axillary temperature (°C): median (IQR)
- Severity criteria: impaired consciousness, increased work of breathing, severe anaemia (Hb < 5g/dL) , known HIV: n (%) yes, no
- Weight-for-age z-score, height-for-age z-score, MUAC-for-age z-score, heart rate (bpm), systolic blood pressure (mmHg), diastolic blood pressure (mmHg), respiration rate (breaths per minute), oxygen saturation (%), capillary refill time (seconds), Blantyre Coma Score (total score), haemoglobin (g/dL), lactate (mmol/L), blood glucose (mmol/L): median (IQR)
- Weak radial pulse volume, temperature gradient: n (%) yes, no
- HIV test result: n (%) previously tested positive, previously tested negative, tested positive at screening, tested negative at screening, invalid test at screening, test not done at screening

Clinical history of this illness

- *History of fever, history of cough, increased work of breathing, vomiting, diarrhoea, bloody diarrhoea, haemoglobinuria, seizures, seizures lasting more than 30 minutes, inability to sit upright unsupported: n (%) yes, no, don't know*
- *Duration of fever, days since start of haemoglobinuria: median (IQR) days*

Treatment in this illness

- Admitted for over 24 hours into another hospital, received oral antimalarials in the last week, received a blood transfusion, received oral antibiotics in last week: n (%) yes, no, don't know
- Number of doses of IV or IM quinine/artesunate: median (IQR)

Past history before this illness

- *Two or more hospital admissions in the last year, previously received a blood transfusion, epilepsy, on regular anticonvulsants, able to sit without support, able to walk without help: n (%) yes, no, don't know*
- *Number of occasions of blood transfusions: n (%) in categories 1, 2, 3-4, 5+*

Family details

- *Number of siblings: median (IQR)*
- *Father's ethnic group, mothers ethnic group: n (%) in categories*
- *Parents still alive: n (%) both alive, one alive, both died*
- *Homestead where child lives: n (%) urban, semi-urban, rural*
- *Sleeps under mosquito net: n (%) yes, no, don't know*

Clinical examination

- *Indrawing, deep breathing, sunken eyes, decreased skin turgor, cold hands or feet only, liver size >2cm below costal margin, jaundice, very severe wasting, generalised lymphadenopathy, flaky paint dermatitis, oral candidiasis: n (%) yes, no, not assessed*
- *Crackles: n (%) unilateral, bilateral, none, not assessed*
- *Splenomegaly: n (%) not palpable, enlarged, gross*
- *Signs of kwashiorkor: n (%) none, pretibial, hands/legs, generalised*

Neurological

- *Fitting currently, neck stiffness, bulging fontanelle (infants only), abnormal motor posturing: n (%) yes, no, not assessed*
- *Pupil symmetry: n (%) equal, unequal*

Working diagnosis

- *Severe malaria, sepsis/septicaemia, LRTI, URTI, other chest syndrome, severe anaemia (Hb <6g/dL), malnutrition, developmental delay/cerebral palsy, recurrent haemoglobinuria, encephalopathy, haemoglobinuria, sickle cell anaemia, sickle cell crisis, meningitis, tuberculosis, hepatitis, gastroenteritis, urinary tract infection, pyrexia of unknown origin, HIV/AIDS: n (%) yes*

- *Summary of other working diagnoses where indicated*

Presentation

- *Healthcare facility first presented to: n (%) this hospital, level II, level III, level IV, other district hospital, private hospital*
- *Time to enrolment since presented at other facility, time to enrolment since referred from other facility: median (IQR)*

Admission blood test results and admission microbiology

- Malaria RDT, malaria blood film: n (%) positive, negative, invalid, not done
- Malaria pigment: n (%) yes, no, not done
- Malaria species: *P. falciparum, P. ovale, P. malariae, P. vivax*: n (% of those with malaria)
- Parasite count per 200 WBC, parasite count per 500 RBC: median (IQR)
- From the FBC: WBC (10^9/L), RBC (10^{12}/L), haematocrit (%), MCV (fL), MCH (Picograms), MCHC (g/dL), platelets (10^9/L), lymphocytes (10^9/L), neutrophils (10^9/L), granulocytes (10^9/L), monocytes (10^9/L): median (IQR)
- Clinical Chemistry: *Sodium (mmol/L), potassium (mmol/L), urea/BUN (mg/dL), creatinine (mg/dL), albumin (g/dL), AST (U/L), ALT (U/L), bilirubin (mg/dL),* CRP (mg/dL), *PCT (ng/ml)*: median (IQR)
- Pathogens isolated: n (%) yes, no
- List of pathogens: n (%)

5.3 Description of follow-up

The following will be tabulated by randomised group for cases, and will also be presented for the control arm unless otherwise indicated. Denominators will include those who at the time of the data extract have been enrolled long enough ago for that visit to have occurred or to have completed follow up, including those who have been confirmed lost to follow up.

Completeness of follow up visits

- Visits considered complete, defined as attended or died before the visit took place, at 7 days, 28 days, and 90 days: n (%)
- Child status at 7 days, 28 days and 90 days: n (%) visit done, died, withdrawn, lost to follow up (LTFU)/absconded, missed visit.

Completeness of data for primary outcomes [cases only]

- CRP at day 0 and day 3 considered complete, defined as non-missing entry or died before the time point: n (% of expected) complete
- CRP record status at day 0: n (%) CRP recorded, no CRP recorded, sample not yet processed
- CRP record status at day 3: n (%) CRP recorded, no CRP recorded, sample not yet processed, withdrawn/LTFU/absconded, died
- Microbiology at day 0 and day 7 considered complete, defined as non-missing entry or died before the time point: n (% of expected) complete
- Microbiology record status at day 0: n (%) microbiology recorded, form entered - no microbiology recorded, no form entered

- Microbiology record status at day 7: microbiology recorded, form entered - no microbiology recorded, no form entered, withdrawn/LTFU/absconded, died

5.4 Treatment details

The following will be presented for cases only, and will be tabulated by randomised group.

- Dose of azithromycin prescribed (mg/kg): median (IQR)
- Correctly prescribed dose using weight-band dosing: n (%)
- Number of children who stopped azithromycin early: n (%)
- Number of azithromycin doses received: median (IQR)
- Number of children needing at least one re-dose: n (%)

5.5 Efficacy analyses

5.5.1 Primary outcomes

These analyses will include cases only and tests will compare the 10mg/kg arm with the 15mg/kg and 20mg/kg individually.

- Change in CRP at 72 hours

Normal linear regression adjusted for baseline values will be used to calculate mean change in CRP from day 0 to day 3 and 95% confidence intervals, and to test for differences between the randomised arms.

- Microbiological cure (7-day) (binary), alone and as a composite with 7-day survival

The analysis of microbiological cure will include children where a pathogen was isolated from the day 0 sample (in either BACTEC or CSF samples, if taken). These children will be defined as microbiologically cured if no pathogen was found in the day 7 samples.

Analysis of the composite measure of microbiological cure and day 7 survival will include all children in the randomised trial. A binary outcome will be used, with a positive outcome defined as alive and having no pathogen at 7 days and a negative outcome of died before 7 days or having a pathogen isolated in a day 7 sample.

For both measures the difference in proportions between randomised arms will be estimated using logistic regression to obtain odds ratios and 95% confidence intervals.

5.5.2 Secondary outcomes

The following analyses will be presented for cases and controls. Tests for differences between randomised arms will compare the 10mg/kg arm to the 15mg/kg and 20mg/kg arms individually.

- Mortality at 48 hours, day 7, day 28 and day 90

Mortality will be analysed at the specified timepoints using time-to-event methods, with hazard ratios and 95% confidence intervals calculated using a Cox proportional hazards

model. Time will be measured from enrolment. Children who were lost to follow up or withdrew before the timepoint will be censored at the date last seen.

Kaplan-Meier curves will be plotted and log-rank tests will be performed to test for difference between randomised arms.

- Length of hospital stay (days)

The median and IQR length of stay in days will be tabulated by group. Competing risks methods will be used to analyse time from enrolment to discharge from hospital, with in-hospital death treated as a competing risk. Subhazard ratios and 95% confidence intervals will be calculated using cause-specific hazards regression and cumulative incidence curves will be plotted.

- Readmission to hospital by 90 days

The number and proportion of children readmitted and the total number of readmissions within 90 days of enrolment will be tabulated by group. Time from discharge to first readmission will be analysed using competing risks analysis with death as a competing risk. Subhazard ratios and 95% confidence intervals will be calculated using cause-specific hazards regression and cumulative incidence curves will be plotted.

5.6 Safety analyses

5.6.1 Secondary outcomes

The following analyses will be presented for cases and controls. Tests for differences between randomised arms will compare the 10mg/kg arm to the 15mg/kg and 20mg/kg individually.

- Serious adverse events (SAEs)
- All grade 3/4 adverse events
- Adverse events that are definitely, probably or possibly related to azithromycin (any grade)
- Adverse events of any grade leading to change in azithromycin (including temporary or permanent discontinuation).

The number and proportion of children ever having an adverse event will be tabulated for each of the categories above and compared across randomised arms with a chi-squared test. SAEs considered to be definitely, probably or possibly related to azithromycin or the dose of azithromycin will also be listed by randomised arm.

The number of children having a serious adverse event (% of all children) and number of events per child will also be tabulated by SAE criteria (fatal, life threatening, cause or prolonged hospitalisation, persistent or significant disability, other) and randomised arm.

All causes of death will be tabulated by randomised arm.

5.7 Other analyses

Completeness of data for pharmacokinetic/pharmacodynamics (PKPD) analysis

The following will be tabulated by randomised arm.

- Completeness of azithromycin dose times for days 0-4: n (%)
- Completeness of blood samples at each timepoint (day 0 at 1, 2, and 8 hours after drug intake; day 2 before dose, and 4 and 18 hours after intake; and day 7): n (%)

Analysis of PKPD will be performed separately and is not covered by this SAP.

6. DISSEMINATION OF RESULTS

Details of the publication policy are described in the trial protocol (version 1.2, 8th October 2018).

7. REFERENCES

1. Nadjm B, Amos B, Mtove G, Ostermann J, Chonya S, Wangai H, Kimera J, Msuya W, Mtei F, Dekker D *et al*: **WHO guidelines for antimicrobial treatment in children admitted to hospital in an area of intense Plasmodium falciparum transmission: prospective study**. *BMJ* 2010, **340**:c1350.
2. Mramba L, Ngari M, Mwangome M, Muchai L, Bauni E, Walker AS, Gibb DM, Fegan G, Berkley JA: **A growth reference for mid upper arm circumference for age among school age children and adolescents, and validation for mortality: growth curve construction and longitudinal cohort study**. *BMJ* 2017, **358**:j3423.