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SEVUparin as a potential Adjunctive Treatment in children with severe malaria

(SEVUSMART trial)

Statistical Analysis Plan			
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1) Trial Design

Trial Outline

The objective of this trial is to identify the maximum tolerated dose (MTD) of intravenous sevuparin as an adjunctive therapy in children with severe malaria given as three infusions at 0, 8 and 16 hours using the Continual Reassessment Method (CRM) to adapt or inform subsequent doses for each child entering the trial, based on a toxicity event defined as any APTT >2.5 upper limit of normal (ULN) 1 hour after each dose, and updating the dose-toxicity model using the previous patients' APTT results.

Trial Population

Inclusion criteria

1. Aged between 3 months and 12 years admitted to the paediatric wards within the last 24h
2. Current or recent evidence of *P. falciparum* malaria (slide or rapid diagnostic test (RDT) positive)
3. Clinical evidence of severe malaria: impaired consciousness (coma (inability to localize painful stimulus) or prostration (inability to sit unsupported for those above 6 months)) or deep breathing
4. Lactate > 2 mmol/L
5. Guardian or parent willing and able to provide consent

Exclusion criteria

1. Clinical evidence or a history of a bleeding/coagulation disorder
2. A comorbidity which clinician believes has a significant risk of poor outcome e.g. malignancy, end-stage renal failure, major cardiac condition
3. Thrombocytopenia (platelet count <25 $x10^9$/L).

2) Interim reviews

The DMC will review data after the first 2 'cohorts' of 2 children each have been enrolled to the lowest dose, and then after each Dose Limiting Toxicity event. The continual reassessment method uses data from each enrolled participant to update the dose-toxicity curve and then suggests an escalation of dose to the largest dose with risk of estimated risk of toxicity below the target toxicity level if appropriate.

3) Endpoints

Primary Endpoint

- APTT>2.5xULN 1h post any sevuparin dose (grade 3 following the CTC)

Secondary Endpoints

Efficacy

- Change in lactate from 0 to 8 hours
- Presence of mature infected erythrocytes on the blood films at 8 and 24 hours
- Parasite clearance time
- Change in sublingual microcirculation over time

Safety Endpoints

- APTT 24h post enrolment (absolute level and grade)
- Development of abnormalites of coagulation indices (prothrombin) (Grade 2 and above)
- Neurological sequelae through day 28
- Mortality through day 28
- Serious adverse events through day 28
- Grade 3/4 adverse events through day 28

APTT 24h post enrolment will be used as an assessment of normalization 8h after the final sevuparin dose. Both absolute levels and grade will be considered.

De-novo evidence of neurological sequelae will be ascertained using a modified Kilifi Developmental Index [60] assessed at admission (to identify pre-exisiting conditions) and follow up (which we have adapted to use for the COAST trial) [59].

Serious adverse events will use the standardized definitions (see section 11 below). SAEs will be independently reviewed in real-time by the DMC.

Adverse events will be graded following the Common Toxicity Criteria v5.0 (https://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_5x7.pdf).

4) Derivation of data to be analysed

Time
Time will be from enrolment for primary analysis and secondary analysis.

Definition of baseline
Baseline values for all measurements will be those recorded at screening either on the screening form, the baseline clinical evaluation form or their first blood test taken within 48 hours of admission as appropriate.

Definition of visit schedule
Analyses of measurements at a given point in follow up (clinical symptoms and neurological sequelae assessments) 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.

Definition of censoring
Children lost to clinic follow up will be censored on the date they were last known alive including data from contact tracing visits. For analyses concerning events at specific time points, surviving children will be censored at that time point. That is for analyses at 28 days, censoring will occur on day 28.

5) Statistical Analysis

Clinical data will be summarised using means and medians where appropriate for continuous data depending on the distribution. Analyses will follow intention-to-treat. Primary and secondary endpoints will be described using means or medians or proportions.

As this is a Phase I trial no subgroup analyses are planned.

All analysis will be included in the final report, but only analysis in bold below will be included in the DMC report.

Baseline characteristics

- **Sex: n(%) male, female**
- **Age at admission (months): median (IQR)**
- **Weight (kg): median (IQR)**
- **Heart rate (bpm), axillary temperature (˚C), systolic blood pressure (mmHg), diastolic blood pressure (mmHg), oxygen saturation (%), respiratory rate (brpm), capillary refill time (s): median (IQR)**
- **Blantyre Coma Score (BCS): n (%) each value of score (0 to 5)**

- **Temperature gradient, weak pulse: n(%) yes, no**

Clinical history of this illness

- History of fever within 14 days, history of fever more than 14 days, history of cough, increased work of breathing, vomiting, inability to sit up right unsupported, diarrhoea, fits in this illness: n(%) yes, no, don't know
- Bloody diarrhoea: n(% of those with diarrhoea), yes, no, don't know
- **Seizures in this illness: n (%) yes, no, don't know**
- **Seizures lasting more than 30 minutes: n(%) yes, no, don't know**
- Haemoglobinuria: n(%) yes, no, don't know; median (IQR) length (days)
- Inability to sit upright unsupported (prostrate): n(%), yes, no

Treatment in this illness

- Admitted for over 24 hours into another hospital, received oral anti-malarials in last week, received oral antibiotics in last week, received traditional medicine in last week: n(%) yes, no, don't know
- N**umber of doses of IV or IM quinine/artesunate received before enrolment: median (IQR) or tabulation depending on numbers**

Past clinical history

- Two or more hospital admissions in the last year, previously received a blood transfusion (ever), received anti-helminths in last 6 months, has epilepsy, able to sit unsupported before this illness, able to walk without help before this illness: n(%) yes, no, don't know
- On regular anticonvulsants (n % of those with epilepsy) yes, no, don't know.

Child's family

- Number of siblings: median (IQR), range
- Any siblings with sickle cell disease: n(% of those with siblings) yes, no, don't know
- Father's ethnic group, mother's ethnic group
- Mother attended secondary school, child sleeps under a bed net/mosquito net: n(%) yes, no, don't know
- Parents alive: n(%) both alive, one alive, both dead
- Child's homestead: n(%) urban, semi-urban, rural

Clinical examination

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

Neurological

- Inability to sit up right unsupported, fitting currently, neck stiffness: n(%) yes, no, not assessed
- Bulging fontanelle (infants only) : n(% infants) yes, no, not assessed
- Pupil symmetry: n(%) equal, unequal

Ward tests at admission

- HIV test result: n(%) previously positive, previously negative, tested positive today, tested negative today, tested today invalid
- **Lactate (mmol/l), glucose (mmol/l): median (IQR)**
- **Glucose given: n (%) yes, no,**
- **Blood gas: pH, bicarb, PCO_2, Base deficit (ecf), base deficit (b): median (IQR)**

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), range
- Distance from other facility (estimated km): median (IQR), range

Admission blood test results and admission microbiology

- **Malaria RDT test: n(%) positive, negative, invalid or not done**
- **Malaria blood film: n(%) positive, negative, invalid or not done**
- Malaria pigment: n(%) yes, no
- Malaria species: n(% those with malaria) *P. falciparum*, *P. malariae*, *P. ovale*, *P. vivax*
- Parasite count per 200 WBC, parasite count per 500 RBC: median (IQR)
- WBC, RBC, Hb from FBC, haematocrit, MCV, MCH, MCHC, platelets, lymphocytes, neutrophils, granulocytes, monocytes, reticulocyte count: median (IQR)
- Sodium, potassium, urea/BUN, creatinine, albumin, AST, ALT, bilirubin: median (IQR)
- Pathogens isolated: n(% samples tested) yes, no.
- List of pathogens: n(%)

Description of follow-up

Denominator in each case is those who have been enrolled long enough ago for that visit to have occurred or to have completed follow up as appropriate, including those who have been lost to follow up.

Completion of follow up visits

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

Co**mpleteness of neurological assessment records**

- **Records considered complete, defined as a non-missing entry or died before the time point at 28 days: n(%)**

Completeness of safety and efficacy data at 8 hours and 24 hours

- **Records considered complete, defined as a non-missing entry or died before the time point: n(%)**

Adherence to treatment

Number received infusion at 0 hours: N (%)
Number received infusion at 8 hours: N (%)
Number received infusion at 16 hours: N (%)

Primary analysis

Number of APTT>2.5xULN 1h post any sevuparin dose (grade 3 following the CTC) at each dose level: N(%)

Secondary analysis

Efficacy

Change in lactate from 0 to 8 hours: mean (sd) overall and by each dose level
Presence of mature infected erythrocytes on the blood films at 8 and 24 hours: N (%) overall and by each dose level
Parasite clearance time: mean (sd) hrs overall and by each dose level
Change in sublingual microcirculation over time: mean (sd) proportion of perfused vessels; mean (sd) average perfusion speed indicator overall and by each dose level calculated using a Capillary Network Analysis.

Safety Endpoints

APTT 24h post enrolment (absolute level and grade): mean (sd) and N (%) with each grade, overall and by each dose level.
Development of abnormalites of coagulation indices (prothrombin) (Grade 2 and above): N (%) overall and by each dose level
Neurological sequelae present at day 28: N(%)
Died by day 28: N(%)
Serious adverse events by day 28: N(%)
Grade 3/4 adverse events by day 28: N(%)