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Sevuparin as a potential adjunctive therapy in children with severe malaria:

Phase I Safety and Dose finding Trial

(SEVUSMART trial)

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Imperial College London is the main research Sponsor for this study. For further information regarding the sponsorship conditions, please contact the Head of Regulatory Compliance at:

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This protocol describes the SEVUSMAART study and provides information about procedures for entering participants. The protocol should not be used as a guide for the treatment of other participants; every care was taken in its drafting, but corrections or amendments may be necessary. These will be circulated to investigators in the study, but centres entering participants for the first time are advised to contact the trial centre to confirm they have the most recent version. Problems relating to this trial should be referred, in the first instance, to the study coordination centre.

This trial will adhere to the principles outlined in the International Conference on Harmonisation Good Clinical Practice (ICH GCP) guidelines. It will be conducted in compliance with the protocol, the Data Protection Act and other regulatory requirements as appropriate.

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2 SUMMARY OF THE TRIAL

Summary Information	Summary Details
ACRONYM	SEVUSMART
Long Title of Trial	SEVUparin as a potential Adjunctive Treatment in children with severe malaria
Version	2.1
Date	28th February 2023
PACTR number:	**202007890194806**.
ICREC	**18IC4513**
Study Design	Phase I Safety and Dose Finding Trial: where dose-toxicity will be assessed using the Continual Reassessment Method (CRM) to adapt or inform subsequent doses for each child entering the trial
Participants to be Studied	20 children aged 3 months to 12 years hospitalised with malaria (*Plasmodium falciparum* positive malaria slide) plus lactate >2 mmol/L
Setting	Kilifi County Hospital/KEMRIWTRP High Dependency Unit and Nchelenge Hospital, Luapula Province, Zambia
Interventions to be studied	Sevuparin given as three infusions at 0, 8 and 16 hours after enrolment. Initially two cohorts of 2 participants will receive a dose of 1.5 mg/kg/dose with the plan to escalate to a cohort of 2 participants receiving 3mg/kg/dose and a cohort of 2 participants receiving 6.0 mg/kg/dose. Using the CRM each subsequent patient would then be assigned the largest dose between 1.5 and 6.0 mg/kg/dose (maximum) with an estimated risk of toxicity below the target toxicity level, designated as the maximum tolerated dose (MTD).
Study Hypotheses	Sevuparin given in addition to antimalarial treatment, early in the course of admission (<24 hours), blocks merozoite invasion, prevents cytoadherence and transiently de-sequesters infected erythrocytes (which cause microcirculatory impairment) and could thus result in improvements in outcomes from severe malaria for the subgroups at greatest risk and during the period of greatest risk (first day of hospitalisation).
Primary Outcome Measure	Activated partial thromboplastin time (APTT)>2.5x upper limit of normal (ULN) (Common Toxicity Criteria grade 3) 1h post any Sevuparin dose
Secondary Outcome Measure(s)	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 ▪ APTT 24h post enrolment (absolute level and grade) ▪ Development of abnormalities of coagulation indices of 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
No of Participants	20 children
Duration	18 months

Sponsor	**Imperial College, London**

Funder	**Wellcome**
Trial Manager	**Emmanuel Oguda**
Chief Investigator	**Kathryn Maitland**
MRC CTU Project Leader	**Sarah Walker**

2.1 Lay Summary:

SEVUparin in Severe Malaria as a potential Adjunctive Treatment

What is the problem/background?
Even on the best antimalarial treatments (injectable artesunate) many African children with severe malaria have poor outcomes with most deaths occurring early in the course of hospital admission (<24hours). One of the bad things that happen in severe malaria is that red blood cells that are infected with malaria parasites that stick to the very deep parts of our blood vessels. This occurs throughout the body and therefore the blood flow is poor tissues which leads to a build-up of body acids (called lactate). Up to the present there have been no treatments available to prevent or reverse stop red cells sticking to the blood vessels when they have malaria parasites in them.

The SMAART: Severe Malaria in African children: A Research and Trials consortium have identified one key innovation which could improve this. A novel new drug candidate called sevuparin, has been identified and safely tested in adults with malaria in Thailand. Sevuparin acts by preventing malaria parasites getting into red cells in the first place (this means that the malaria parasite cannot survive); it also prevents red cells with infected with malaria parasites from sticking to the blood vessels and also able to 'unstick' red cells with infected with malaria parasites that are already sticking to the blood vessel and causing poor blood flow.

Given its potential to be transformative in improving current outcomes from severe malaria, a large group of specialist doctors in severe malaria research and clinical trials suggested that sevuparin should be tested in children with severe malaria. They have all helped to design this clinical trial, which will be conducted on the high dependency ward in Kilifi, Kenya.

What questions are we trying to answer?
Study design: Phase I Safety and Dose Finding to assess the optimal dose of sevuparin given as a supportive therapy in severe malaria alongside of the usual antimalarial treatments to determine toxic level, adverse events and to identify the highest dose with an acceptable safety profile
Study Population: 20 children hospitalised with severe malaria.

Sample Size: This is a Phase I trial designed to test safety and Dose Finding Trial of sevuparin as a supportive therapy in severe malaria; there is therefore no formal sample size calculation.

Where is the study taking place, how many people does it involve and how are they selected?
The study will be carried out at Kilifi County Hospital, Kenya and Nchelenge Hospital, Luapula Province, Zambia and plans to enroll 20 children. Children enrolled in the study will be aged 3 months to 12 years and presenting to Kilifi County Hospital with severe malaria evidence of malaria (rapid diagnostic test positive or positive malaria slide). The carers of these children will be approached for consent and enrolment in the study. The study drug will be given on the first day of admission only alongside of usual treatments given for children with malaria.

What does the study involve for those who are in it?

- Parental information and consent/parental assent (for emergencies when the child is too sick for full consent).
- Admission to the high dependency ward at Kilifi County Hospital or paediatric ward at Nchelenge
- Hospital, Luapula Province, Zambia
- The sevuparin will be given as 3 infusions on the first day at study enrolment (0 hours), 8 and 16 hours following this. All other treatments for severe malaria will follow the usual guidelines

- Two groups of 2 participants will receive a dose of 1.5 mg/kg/dose with the plan to escalate to a cohort of 2 participants receiving 3mg/kg/dose and a cohort of 2 participants receiving 6.0 mg/kg/dose. The model will then inform each subsequent patient what dose they would then be assigned (between 1.5 and the maximum dose of 6.0 mg/kg/dose)
- All children will have regular (4 hourly clinical and vital sign) monitoring during the period of admission to the high dependency ward and twice daily thereafter until discharge and will be followed up to day 28. Routine blood tests will be done at admission, 8 and 24 hours and at follow up. Additional samples will be taken to check clotting at 0, 1-hour post sevuparin infusion (i.e., 1, 9 and 17 hours) and lactate (the body acid that builds up in severe malaria) will be check regularly at admission, 4, 8 16 and 24 hours.
- The microcirculation, using a small soft probe placed on the gum of a children will be checked three times during admission.

What are the benefits and risks/costs of the study for those involved?

Benefits: Close monitoring of all children on the high dependency unit during admission and follow up to day 28 after discharge. Hospital bills for participants older than 5 years will be covered by the study (covering the costs for standard treatment for severe malaria and related complications).

Risks: One potential risk of sevuparin, as it is heparin-like drug is that it increases one of the blood clotting markers. This was seen in the study in Thailand but was short lived (i.e., only happened for a few hours only) and did not cause any side effects. We are carefully monitoring this blood clotting marker, and this will help us make decisions about whether the child receives the next dose and as weare using a continuous reassessment method to monitor toxicity this will inform the dosages for the children enrolled in the trial.

How will the study benefit society?

The study aims to improve the outcome from severe malaria. It will generate robust feasibility and safety data to support a future larger phase II trial with the aim of improving management of a vulnerable population of children and improving outcomes.

When does the study start and finish? Once we have received ethical approval.

Enrolment will be over 12-15 months and study will end after 2 years.

ABBREVIATIONS/GLOSSARY

ACT	Artemisinin combination therapy
AE	Adverse event
APTT	Activated partial thromboplastin time
AT	Antithrombin
BCS	Blantyre Coma Scale
CI	Confidence interval
CM	Cerebral Malaria
CRF	Case Record Form
CRM	Continuous Reassessment Method
CTC	Common Toxicity Criteria
DIC	Disseminated intravascular coagulation
DLT	Dose limiting toxicity
DMC	Data Monitoring Committee
ECG	Electrocardiogram
ED50	Median effective dose (effective dose for 50% of people receiving the drug)
GMP	Good Manufacturing Practice
HDU	High Dependency Unit
HRP2	*P. falciparum* Histidine Rich Protein 2
IV	Intravenous
KCH	Kilifi County Hospital
KEMRI	Kenya Medical Research Institute
KWTRP	Kilifi Wellcome Trust Research Programme
ICREC	Imperial College Research Ethics Committee
MRC CTU	Medical Research Council Clinical Trials Unit
MTD	Maximal tolerated dose
OR	Odds ratio
PI	Principal Investigator
PK	Pharmacokinetic
PKPD	Pharmacokinetic-pharmacodynamic
PPB	Pharmacy and Poisons Board
RDT	Rapid diagnostic test
SAE	Serious adverse event
SDF	Side stream-dark field
SERU	Scientific and Ethics Review Unit
SMAART	Severe Malaria in African children: A Research and Trials consortium
SOP	Standard Operating Procedures
TAT	Thrombin-AT III
TF	Tissue factor
TM	Thrombomodulin
ULN	Upper limit of normal

3 ABSTRACT

3.1 Abstract

Severe Malaria In African Children: A Research and Trials Consortium (SMAART) was funded in 2018 by a collaboration award in science from the Wellcome Trust. Owing the continued high burden of malaria and poor outcomes of severe malaria, even on the best antimalarial, artesunate, the major objective of SMAART is to conduct better research faster across a collaborative platform to identify interventions to optimise the whole treatment pathway for children with severe malaria, and thus to achieve a step-change in improving their outcomes in the current era.

A novel new drug candidate for adjunctive treatment of severe malaria, sevuparin, has been identified that can blocks merozoite invasion, prevent cytoadherence and transiently de-sequesters infected erythrocytes in humans with uncomplicated *P. falciparum* malaria. If given, in addition to antimalarial treatment, early in the course of admission (<24 hours) this could result in improvements in outcomes from severe malaria for the subgroups at greatest risk and during the period of greatest risk (first day of hospitalisation). Sevuparin has been shown to be safe and well tolerated in adults with only some mild effects on activated partial thromboplastin time (APTT) at higher doses given over longer periods of time (3 days), which are not clinically relevant to the time of greatest risk.

In this Phase I trial dose-finding paediatric study, we aim to use only 3 doses given at admission (0 hours), and 8 and 16 hours later, and measure APTT 1 hour after each dose (to assess maximum toxicity based on adult data). The Phase I trial is designed to obtain data on safety, dosing, feasibility and potentially lactate clearance of sevuparin given as an adjuvant therapy in severe malaria in children. We aim to study 20 children admitted to hospital with severe malaria which will allow sufficient data on safety to be generated across a range of doses to identify the maximum tolerated dose (MTD) using the Continual Reassessment Method (CRM), which adapts or informs subsequent doses for each child entering the trial based on the data from previously enrolled children. The maximum tolerated dose (MTD) will be identified based on the dose-toxicity model being updated by each previous patient's APTT results using standard methods.

The results of the Phase I trial will identify the final dosage to be tested in a Phase II trial in terms of both efficacy and safety outcomes. The primary endpoint for the future Phase II trial (lactate clearance) reflects the primary hypothesis that sevuparin improves microcirculatory flow by reversing and preventing parasite sequestration. The Phase I trial will also assess whether this endpoint, lactate clearance, is realistic; thus, lactate will be collected along with other test results at baseline 4 hours and 8 hours, as well as other time points.

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5 INTRODUCTION/BACKGROUND

5.1 Background

Over the last decade there has been an unprecedented rise in funding for malaria control activities, including the scale-up in long-lasting insecticidal bed nets and introduction and access to effective artemisinin-combination treatments (ACT). This has resulted in the disease retreating from large parts of the globe, yet malaria remains stubbornly unyielding in sub-Saharan Africa and in some parts of Asia [1]. In some African countries, e.g., Nigeria, DRC, Uganda, Mozambique, the pattern of transmission has not changed appreciably, despite implementation of early treatment and control strategies and they continue to contribute most to the global disease burden [2]. Many of the African countries most afflicted by malaria are amongst the poorest on the continent with weak health services infrastructure. Severe malaria remains a major public health problem in Africa and a chief factor in child mortality; particularly in countries experiencing high transmission. In 2016 it was estimated that there were over 445,000 deaths from malaria with the vast majority of these deaths in African children [3, 4]. Whilst the AQUAMAT trial has provided definitive evidence for optimal antimalarial treatment in severe malaria [5], progress on improving outcome through supportive treatments has been very slow [6, 7].

5.2 Treatment of severe malaria

The multi-centre AQUAMAT trial conducted in 11 centres in 9 countries in Africa compared quinine and artesunate in 5425 children hospitalised with severe malaria. The primary endpoint, in-hospital mortality, in the intention to treat analysis occurred in 297/2713 (10.9%) children receiving quinine treatment compared to 230/2712 (8.5%) children receiving artesunate - translating to a relative reduction in mortality of 22.5% (95% CI 8.1-36.9) with artesunate (p=0.002) [5]. However, outside the context of a clinical trial, overall in-patient mortality for severe malaria remains unacceptably high (~10%), and unlikely to improve without wider implementation of pre-referral artemisinin [8] and better supportive treatments [6, 9]. As demonstrated in Table 1 below, case fatalities in AQUAMAT were even higher within large subgroups of patients presenting with one or more of the 3 key prognostic markers (coma, acidosis, or a high blood urea nitrogen [10]). The evidence base to guide best management of these and other complications is lacking. To date none of 33 clinical trials of adjunctive (supportive) treatments conducted globally since a seminal severe malaria trial in 1980 have shown benefit [6]. Over 60% of these trials involved children and 15 were specifically directed to the sub-group with cerebral malaria. The majority were single-centre Phase I or II trials involving few participants, and a reasonable number were stopped prematurely because they showed harm.

5.3 Severe Malaria in African children A Research and Trials Consortium (SMAART)

Severe Malaria In African Children: A Research and Trials Consortium (SMAART) was funded in 2018 by a collaboration award in science from the Wellcome Trust. The major objective of the collaborative award was to identify which interventions could optimise the whole treatment pathway for children with severe malaria from the hospital 'gateway' to survival 6-months post-discharge, and hence achieve a step-change in improving their outcomes in the current era. A 2-day inception meeting of the SMAART consortium brought together a multidisciplinary group of scientists encompassing

decades of clinical and academic experience, with track records of successful high-quality research in low-income settings in severe malaria. The group considered the high priority areas of research and key targets for intervention. One high risk group identified by the group was children presenting with acidosis (increased base excess); in this sub-group mortality remained at 15%, and acidosis was one of the three key factors predicting poor outcome [10].

Table 1 High priority risk factors for severe malaria and recent trials

Admission feature or complication	**Frequency**	**AQUAMAT in-hospital Mortality* (Artesunate-arm)**	
Coma	32-35%	18%	
Metabolic acidosis (base excess<-8 or lactate>5mmol/L)	43-44%	15%	
Renal impairment (Urea/BUN > 20 mmol/L)	24%	22%	
Hypoglycaemia (blood glucose <3 mmol/L)	10%	15% (DOI 10.1186/1471-2334-10-334)	
Convulsions	30-32%	14%	
Invasive bacterial co-infection	5.5%	24% (DOI: 10.1186/1741-7015-12-31)	
Blackwater Fever (region specific)	14-21%	Day-28 mortality 12% (DOI:10.1093/cid/cix003)	
Recent or ongoing trials	**Frequency**	**Mortality**	**Trial:** results expected
Shock (mortality = no-bolus arm)	12%	8.5%	**FEAST:** 10.1056/NEJMoa1101549
Severe anaemia	29-30%	10%	**TRACT:** July 2018 ISRCTN84086586
Hypoxaemia (<90%)	15%-17%	14%-30%	**COAST:** Late 2020 ISRCTN15622505

* Data from AQUAMAT unless indicated where mortality figures are for quinine-treated children

In order to identify supportive therapies which could target acidosis, the following section summarises the current understanding of the pathophysiology of severe malaria and likely aetiology of 'malaria' acidosis.

5.4 Pathophysiology of severe malaria and metabolic acidosis

During the course of infection, ring stage parasitaemia or infected erythrocytes in children with *P. falciparum* is amplified. Unlike other malarias species, *Plasmodium falciparum* has the unique ability to cause cytoadherence, a phenomenon called sequestration, of late stage parasitised infected erythrocytes in the deep vascular beds [11]. The pathophysiological process is mediated by excessive sequestration of *P. falciparum* infected erythrocytes [12], rosetting [13] and decreased deformability of non-parasitised red cells [14]. Whilst this can occur during a non-severe infection, autopsy studies have shown that there is intense sequestration of parasitized erythrocytes in vital organs in children who have died from malaria (i.e., had severe malaria), but sequestration varies between organs, and even varies even within an organ, with some vessels completely blocked while other proximate vessels remain patent [14, 15]. Whether sequestration causes mechanical obstruction and impaired tissue perfusion or is damaging in other ways (active parasite metabolism, release of toxins, cytokine induction) [16] is not known.

One marker associated with poor outcome is a raised lactate, which is generally considered to be directly linked to the degree of impaired perfusion [17]. Evidence to support this include the fact that improvements in lactate concentration over the first 24 hours of admission were strongly prognostic for survival in adults with severe malaria [18]. Moreover, faster clearance of plasma lactate was predictive of the treatment effect on mortality of artemether compared to quinine [19]. Artemether results in a rapid killing of ring stage parasites, preventing their further maturation and sequestration in the microcirculation and this is thought to be a main contributor to the improvement in case fatality. In the fluid resuscitation (FEAST) trial that included a large number of children with severe malaria and sepsis, a sub-analysis showed that severe lactataemia (>5 mmol/L) was strongly associated with mortality (Odds Ratio (OR) 6.96; 95% CI 3.52, 13.76, p<0.001) and that failure to clear lactate at 8 hours was strongly associated with death at 72h (OR 4.62; 95% CI 2.7, 8.0; p < 0.001) [20].

5.5 Adjuvant or supporting treatments aiming at improving the microcirculation

The demonstration in autopsy studies of microvascular obstruction by heavy parasites burdens [21] and that an overall measure of parasite biomass (*Plasmodium falciparum* histidine-rich protein2 (pfHRP2), a protein released on sequestration by infected erythrocytes) correlates with worse outcomes [22] suggest that adjuvant therapies which can reverse sequestration and reduce overall biomass (by preventing merozoite invasion) early in the course of the disease may lead to substantial improvements when the risk of fatal outcome is highest. Moreover, the time of development of a merozoite into an adhesive infected erythrocyte that sequesters and blocks the micro-vasculature is ~18-20 hours which is the same time period (first day of hospitalization) when the majority of paediatric deaths from severe malaria in Africa occur.

In studies conducted in Indonesia, it was hypothesized that heparin could inhibit *P. falciparum* sequestration and merozoite invasion since heparin binds to heparan sulphate binding structure of *Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP1), the Duffy-binding like domain 1α (DBL1α), known as a vital contributor to sequestration of infected erythrocytes [23, 24]. Clinical trials showed that heparin, as an adjunctive therapy to antimalarial drugs, had variable results, with some showing reduced mortality in children with severe *P. falciparum* malaria [25, 26], others showing no clinical improvements when given as a low dose [27] and others demonstrating potentially severe effects on coagulations in simian studies in *P. knowlesei* malaria [28, 29] at the same dosage as those showing benefit in children with severe malaria. As a result, heparin was not subsequently adopted into clinical practice owing to the substantial concern over haemostatic side effects. Subsequent investigation has shown that that the inhibitory effect of heparin on *P. falciparum* sequestration and merozoite invasion (which also is mediated through the heparan sulphate binding site of PfEMP) is independent of its anti-coagulant activity [30, 31]. The next step was to develop a heparin compound that was devoid of its therapeutic limiting effects on coagulation.

5.5.1 SEVUPARIN

The drug sevuparin was developed from heparin because its heparan sulfate binding is nearly identical; thus, the rationale was that sevuparin would act as a decoy receptor during malaria infection [32]. Sevuparin, akin to other heparins, is a poly-disperse chemical, encompassing a range of polysaccharide chain lengths with molecular weights of 3.6–9.6 kDa. Sevuparin is negatively charged and derived from

heparin through chemical depolymerization. In sevuparin, the specific pentasaccharide involved in high-affinity binding to antithrombin III has been deleted. Thus, since sevuparin has no specific binding sequence for antithrombin (AT) which is the main contributor to prolonged coagulation, it has no direct effect on factor Xa or on thrombin, and its effect on activated partial thromboplastin time (APTT) is markedly reduced compared to that of standard dose heparin [33]. For example, for it to have the same effect (ED50) on APTT prolongation (measured as ED50), sevuparin would need to be given in doses five times higher compared to low molecular weight heparin and 35 times higher compared to full length heparin.

Like heparin, sevuparin binds to the heparin sulphate binding structure of Plasmodium falciparum erythrocyte membrane protein 1 (PfEMP1), which is the key receptor for sequestration of infected erythrocytes [34, 35]. Preclinical investigations have demonstrated that sevuparin blocks *P. falciparum* merozoite invasion into fresh erythrocytes *in vitro*, and both disrupts and blocks the binding of infected erythrocytes to uninfected erythrocytes (rosetting) and binding to vascular endothelial cells (also known as cytoadherence) *in vitro*. *In vivo* studies also demonstrated that sevuparin led to de-sequestering of schizonts both rats and in non-human primate preclinical studies [25-27] and to disruption of rosetting in a dose-dependent manner [34].

Phase I studies in healthy volunteers and a clinical study of adults with sevuparin have been conducted in patients with mild *P. falciparum* malaria have been conducted in which it was proposed that sevuparin would act as a decoy receptor during malaria infection to block merozoite invasion as an adjunctive therapeutic approach to preclude the early expansion of an infection and reduce the sequestered biomass [36].

In the Phase I dose escalation trial, sevuparin was found to be safe and well-tolerated with minimal effects on APTT at doses of 1.5 mg/kg and 3mg/kg every six hours but a dose of 6.0 mg/kg led to higher APTT values. The Data Monitoring Committee (DMC) therefore recommended that the study should continue to the Phase II with a dose of 3.0 mg/kg sevuparin every 6h, given the potentially increased risk of adverse events at higher doses [36].

In the Phase II trial, sevuparin was administered as an intravenous infusion over 5 minutes in addition to standard care i.e., a fixed dose combination of 1000 mg atovaquone and 400 mg proguanil once daily for three days, in subjects with uncomplicated *P. falciparum* malaria (experimental arm), as compared to atovaquone/proguanil treatment alone (control). There were minimal and non-clinically relevant changes in anti-Xa- and prothrombin-times, and the international normalized ratios associated to the sevuparin dose [36] (see Supplementary Tables S1 and S2 from the manuscript). Increases in APTT were dose-dependent and appeared to follow the time-concentration curve for each sevuparin infusion. The 6-hour interval between the infusions allowed for nearly full reversibility of APTT levels after each dose, and no accumulative effects were seen over the course of the 12 consecutive infusions (see Supplementary Tables S3 and S4 from the manuscript) [36]. Thrombocytopenia occurred in one subject but was noted to be present before the initiation of sevuparin treatment. There was no incident of bleeding in any of the participants nor were there any other serious adverse events or adverse events.

Sevuparin was therefore judged to be safe and well tolerated in adults with mild malaria. It led to a reduction in numbers of ring-stage infected erythrocytes after a single sevuparin infusion and resulted

in a transient appearance of mature parasite infected erythrocytes (schizonts appearing in the circulation, indicating these had been released after desequestration). These both occurred within one hour after the first sevuparin injection. Thus, these studies indicate that a novel new drug candidate for adjunctive treatment of severe malaria had been identified that blocks merozoite invasion and transiently de-sequesters infected erythrocytes in humans with uncomplicated *P. falciparum* malaria.

5.6 What is known about coagulation in children with severe malaria?

5.6.1.A General Background

Normal haemostasis is a complex balance between pro- and anti-thrombotic pathways [37]. Most reactions take place on endothelial or platelet cell surfaces and involve soluble coagulation factors and natural inhibitors. The 'intrinsic' and 'extrinsic' pathways of the coagulation cascade are no longer thought of as separate processes that occur in the plasma. Instead, these reactions occur in concert on membrane surfaces provided by endothelial cells, platelets, monocytes and neutrophils [38]. The recruitment and tethering of these cellular components is a vital step in the initiation of the procoagulant pathways. Endothelial injury, whether due to trauma, inflammation or infection causes activation of three main pro-coagulant pathways: the coagulation cascade, platelet reactions and vasoconstriction. In severe *P. falciparum* malaria, adhesion molecule upregulation has been demonstrated [39, 40] and thrombomodulin levels (TM) have been reported to be high [40, 41] suggesting that any coagulation activation seen might be due to endothelial dysfunction. Few detailed studies exist of coagulation abnormalities in severe malaria; frank disseminated intravascular coagulation (DIC) is rare despite thrombocytopenia being common [42-44]. Early studies of the mechanisms involved in the activation of the coagulation cascade in severe *falciparum* malaria in Thai adults, many of whom had multiple vital organ dysfunction, suggested activation of the intrinsic pathway of the clotting cascade and complement system including reduction in the concentration of plasma antithrombin III (AT III) concentrations, elevation in thrombin-AT III (TAT) complexes [45, 46], and reductions in factor XII and prekallikrein activities. Protein-C activity was also shown to be reduced [46]. Subsequent studies have also shown that *P. falciparum* malaria is associated with procoagulant activity but not with clinical evidence of thromboembolism. Plasma levels of TM have been used to assess the participation of the vascular endothelium in human *falciparum* malaria. Studies in adults have shown that elevated plasma levels of TM correlate directly with the levels of parasitaemia, TNF alpha, elastase and TAT [41, 47]; and that the low plasma levels of Protein-C and protein-C inhibitor-1 and increased TAT concentrations present in almost all patients correlated with severity and parasitaemia [41, 47]. Together these data suggest that there is endothelial activation and a shift towards a pro-coagulant state in *P. falciparum* malaria, both of which can be reversed after anti-malarial treatment [47].

5.6.1.B Paediatric Studies

Studies of coagulation in African children with severe malaria are relatively few but clinical evidence of DIC is rare [48]. In paediatric cerebral malaria (CM), autopsy studies have shown that fibrin degradation products are raised [49], indicating a pro-coagulant state. These studies also showed a consistent staining for tissue factor (TF) in the endothelial cells and TF was also shown to be

upregulated in the brain postmortem studies in paediatric CM [50]. However, the latter study also showed that TF was also found in postmortem samples from parasitaemic children whose underlying illness was non-malarial [50]. Moreover, when comparing functional coagulation assays in African children with CM to children with mild malaria, no marked differences were found [51].

A more recent and comprehensive case-control study of coagulation compared a range of indices in children with true cerebral malaria (defined by a malarial retinopathy) compared with children with other forms of severe malaria, mild malaria and healthy controls. Compared to healthy controls (n=19), TAT, a sensitive marker of thrombin generation, was increased in children with retinopathy-positive CM (n=66) ($P<0.001$) and levels were greater than those in children with uncomplicated malaria (n=30) ($P<0.01$). In the retinopathy-positive CM group, TAT levels were higher in 16 fatalities than in those children who survived. Prothrombin times were mildly and similarly prolonged in both CM children and children with uncomplicated malaria compared to healthy controls [52]. APTT levels were similar to the controls in all malaria groups [52], indicating activation of coagulation through TF activation rather than increased factor XII [46].

6 RATIONALE

A novel new drug candidate for adjunctive treatment of severe malaria, sevuparin, has been identified that can block merozoite invasion, prevent cytoadherence and transiently de-sequester infected erythrocytes in adults with uncomplicated *P. falciparum* malaria. If given, in addition to antimalarial treatment, early in the course of admission (<24 hours) this could result in improvements in the outcome from severe malaria for the subgroups at greatest risk and during the period of greatest risk (first day of hospitalisation). Sevuparin has been shown to be safe and well tolerated with only some mild effects on APTT levels at higher doses given over a longer period of time (3 days), which is not clinically relevant to the time period of greatest risk (first day of hospitalization). In this Phase I trial dose-finding paediatric study, we aim to use only 3 doses given at admission (0 hours), and then 8 and 16 hours subsequently, and will measure the key toxicity of interest, APTT, 1 hour after each dose (to assess maximum toxicity). The normal ranges of APTT in children have been shown to be the same those in as adults [53] so this study will learn from and build upon what has already been published on sevuparin in adults with malaria. A large comprehensive study of coagulation abnormalities in African children with severe malaria, mild malaria and healthy controls demonstrated that there are no derangements in APTT in children with severe malaria compared to mild malaria and healthy controls [52], providing reassurance that the comparison of APTT levels with normal ranges in this study is clinically meaningful in terms of identifying a maximal tolerated dose (MTD). Finally, if sevuparin resulted, in a future Phase III trial, in increased disability-free survival, then this may be a future suitable candidate to use in pre-referral management alongside parenteral or artesunate.

The design of this dose finding study uses the Continual Reassessment Method (CRM). This adaptive dose-finding study design is increasingly embraced by clinical trialists [54] as a more efficient method for identifying an “optimal” dose using as small a number of participants as possible, in contrast with heuristic methods such as, for example, comparing three arbitrarily chosen doses. The CRM ‘learns’ (i.e., reassesses risk/toxicity) after each patient is entered into the trial and proposes a subsequent dose for the next child entered in a way that provides the most information about doses closest to the

MTD. The CRM and has been shown to incur fewer toxicity events overall in identifying the MTD, and to estimate the MTD more accurately as compared the standard Phase I dose escalation designs [55]. In terms of safety, the study will be run using a 'live' Data Monitoring Committee (DMC) review of toxicity events. The DMC will review data after the first 2 'cohorts' of 2 children each have been enrolled to the lowest dose (1.5 mg/kg/dose), and then after each Dose Limiting Toxicity (DLT) event.

7 TRIAL DESIGN

7.1 Null Hypothesis

We hypothesize that sevuparin, a de-polymerised heparan sulphate mimetic, will not improve microcirculatory flow by reversing and preventing parasite sequestration when given to children with severe malaria and will not improve overall outcome.

7.2 General Objectives

The primary objective of this trial is to conduct a dose-finding study of intravenous sevuparin given in 3 doses over the first 18 hours from enrolment (within 24h of hospital admission), defining toxicity events as any APTT >2.5 upper limit of normal (ULN) (grade 3 toxicity) 1 hour after each dose to identify the maximum tolerated dose (MTD). The initial dosage (1.5 mg/kg) and the *a priori* dose-toxicity curve are based upon the results of the adult trial (where a dose of 1.5 mg/kg was associated with minimal risk of toxicity) and experimental evidence of dose-dependent efficacy i.e. inhibition of merozoite invasion and reversal of cytoadherence of infected erythrocytes [36]. Almost all adults enrolled in this trial experienced grade 2 toxicity after one or more sevuparin doses, but APTT rapidly normalized, hence the choice of grade 3 toxicity to define the MTD in this dose-finding trial.

The results of this Phase I trial will identify the final dosage selected for a subsequent Phase II that will include both efficacy and safety outcomes. The Phase II trial will be conducted by the SMAART consortium and plans to use change in lactate at 8 hours as its primary endpoint; likely secondary endpoints include neurological sequelae, day-28 and day-180 mortality, length of initial hospitalisation, re-admission to hospital, grade 3/4 and serious adverse events.

The primary endpoint for the future Phase II trial reflects the primary hypothesis that Sevuparin improves microcirculatory flow by reversing and preventing parasite sequestration. Data collected in the Phase I trial will also assess whether lactate clearance at 8 hours is a realistic and feasible primary endpoint for a subsequent Phase II trial.

7.3 Specific Objectives

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.

The secondary objective will be to assess whether lactate clearance at 8 hours is a realistic and feasible primary endpoint for a subsequent Phase II trial.

8 LOCATION AND STUDY DESIGN

Site: High dependency ward in Kilifi County Hospital, Kenya or the paediatric ward at Nchelenge Hospital, Luapula Province, Zambia

Study design: Phase I trial

Study population: 20 children hospitalised with severe malaria

8.1 Inclusion criteria

1. Aged between 3 months and 12 years admitted to the paediatric wards within the last 24h
2. Current evidence of *P. falciparum* malaria (slide 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

8.2 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).

9 PROCEDURES

9.1 Sample Size determination

This is a Phase I trial designed to obtain data on safety, dosing, feasibility, and lactate clearance of sevuparin given as an adjuvant therapy in severe malaria. We aim to study 20 children since this will allow sufficient data on safety to be generated across a range of doses to identify the maximum tolerated dose (MTD) from a more informed model relating dose to toxicity events (denoted the 'dose-toxicity' curve) than that available *a priori* based on published data from adult studies. After each patient is enrolled, the dose-toxicity curve will be updated based on levels of APTT taken over three time points (1h post each infusion), defining a toxicity event as APTT >2.5xULN at any time point (grade 3 following the Common Toxicity Criteria (CTC)). This enables the MTD to be estimated more rapidly using the Continuous Reassessment Method (CRM): once determined, subsequent participants will be allocated to this MTD to provide the most accurate estimate of future toxicity event rates until we reach the sample size of 20 children. However, the CRM method will continue to use information from all these children; for example, if a number of children receiving the originally identified MTD experience toxicity events, the dose would again be lowered, and future children would receive this lower dose.

9.2 Sampling Procedures

Eligible children will have been admitted to the High Dependency Unit in Kilifi so children can be closely monitored. Once eligibility is verified, the parents can be approached for consent and, if they agree to participate, they will receive their allocated treatment dosage.

The blood tests taken at admission and during the trial include standard of care and research bloods (see Table 2). Admission and serial assessment of full blood count, admission point of care clinical chemistry including PH, blood cultures and repeated assessment of malaria parasitaemia are part of the standard clinical tests. Additional to this will be serial assessment of lactate, measurements of coagulation (by iSTAT *(*Kaolin ACT) and laboratory based APTT) and samples will be stored in Kilifi for future pharmacokinetic (PK) tests, plasma HRP2 tests and malaria parasites(research). The reliability of Point of care ACT measurements (including ISAT) have previously assessed against gold standard and have shown that the coefficients of variation of POC PT and whole blood were between 2% and 3.6%, indicating that POC assessments are reliable and able to support on-site decision-making for patients in acute and intensive care [56].

Management and outcome data will be collected (clinical parameters and recovery, developmental assessment, number of transfusions, use of drugs (specifically anticonvulsants, paracetamol, and antibiotics), date of discharge or in-hospital death. Contact and locator data will be recorded so that children can be followed at day 7 and day 28.

9.3 Study Procedures

All children admitted with suspected malaria and either coma or respiratory distress (defining severe malaria for this Phase I study) will be screened for study inclusion by the paediatric triage/admission team.

9.4 Consent process

Once eligibility has been confirmed, authorized trial staff will approach parents/guardians to invite their child to take part in the trial. An information sheet will be provided to the parent/guardian in their usual language. The sheet will be read aloud to those who are unable to read. The doctor/nurse will check that the information has been fully understood and parents/guardians will be encouraged to ask questions they may have about their child's participation.

Where possible, prospective written informed consent will be sought from parents/guardians by asking them to sign the Consent Form. If parents/guardians are unable to sign, a thumbprint will be taken in lieu of a signature. A copy of the Consent Form will be given to the parent/guardian, the original placed in the patient's medical notes, and a copy kept in the Investigator Site File.

If it is considered that the full consent process would significantly delay enrolment, and consequently be detrimental to the child's health, then emergency verbal assent, used in the FEAST, TRACT and COAST trials [57-59], will be sought from parents/guardians by the admitting medical team. Following verbal assent, written informed consent will be sought from the parent as soon as possible once the child's clinical condition has stabilized.

9.5 Trial Treatments

We aim to study 20 children. All children will receive standard care including parenteral artesunate and sevuparin given as three infusions at 0, 8 and 16 hours after enrolment. The initial participants (two 'cohorts' of 2 children each, i.e., 4 children in total) will receive a dose of 1.5 mg/kg/dose with theplan to escalate up to a cohort of 2 children receiving 3mg/kg/dose and a cohort of two children receiving 6.0 mg/kg/dose (maximum). In order to determine whether, and the rate of escalation, to a higher dosage for subsequent patients after the first 4 children we will use a design called the Continuous Reassessment Method (CRM). This starts with an estimated dose-toxicity curve, reflecting the probability of experiencing a toxicity event (here APTT>2.5xULN 1h after any of the three doses) to the dose received. In this method of dose finding, the dose-toxicity curve is fitted to the data after each child is enrolled, based on their observed dose and toxicity outcome. After the first 8 children, each subsequent patient would be assigned the largest dose with an estimated risk of toxicity below the target toxicity level, designated as the maximum tolerated dose (MTD). The dose limiting toxicity (grade 3 APTT, >2.5xULN) *a priori* dose-toxicity curve and chosen target toxicity rate (15%) used in this trial have been based upon data shared by the investigators from the adult sevuparin studies in Thailand [36]. In particular, almost all adults enrolled in this trial experienced grade 2 toxicity after one or more sevuparin doses, but APTT rapidly normalized, hence the choice of grade 3 toxicity to define the MTD in this dose-finding trial. APTT 24h post-enrolment is a key secondary safety endpoint to confirm rapid normalization.

Any child with APTT>2.5xULN 1h after a dose of sevuparin will immediately discontinue trial treatment (and be counted as a toxicity event). They will continue to be followed according to the trial schedule (on-study, off-study-drug), to confirm resolution of APTT and to record clinical outcomes.

9.5.1. A Sevuparin

The drug product, sevuparin 150 mg/mL solution for intravenous (IV) infusion, is formulated in a 0.015M phosphate buffer at a pH of 7.0. It requires storage in a refrigerator at 2-8° and to be protectedfrom light. The non-preserved sterile solution needs to be dispensed (5.4 mL) in a glass vial sealed witha rubber stopper and covered with a tear-off aluminum cap. The solution for administration will be prepared in a syringe and will be kept refrigerated and used within 24 hours. One vial will only be usedfor one subject.

The drug product is produced in compliance with current Good Manufacturing Practice (GMP). The same material and compositions are being used by the Sponsor in a currently ongoing clinical trial in Sickle Cell Disease (see below). We will obtain the product from MODUS, Sweden.

Sevuparin is currently in clinical phase II for treatment of acute painful Vaso-Occlusive Crisis in hospitalized Sickle Cell Disease patients (TVOC01 study - clinicaltrials.gov/ct2/show/NCT02515838). This study is expected to complete enrolment in 2018. Children 12 – 16 years of age are included in this study (as are adults), but younger children are not eligible. It is not clear what dose is being used in eligible adolescents (or adults).

9.5.2 ASSESSMENTS OF MICROCIRCULATION

The sublingual microcirculation will be assessed at the Kilifi site only using sidestream-dark field (SDF) imaging (CytoCam, Braedius Medical BV®). SDF imaging technology is a validated real-time visualization of the microcirculation using a 2ms pulsed green light emitted at a 530nm wavelength for optimal optical absorption by the haemoglobin in red blood cells, independent of oxygenation

state. It is safe, non- invasive and can be performed rapidly at the patients' bedside.

A sterilised disposable lens is used to prevent contact between the instrument and patient and therefore to prevent any transmission of infection. Two trained individuals will collect this data to limit inter-user variability. Images will be recorded from the proximal, mid, and distal portions in each half of the sublingual mucosa and averaged. Where possible the microcirculation will be assessed at time 0 (prior to sevuparin dose), at 8-9 hours (before and after infusion) and again at 17-18 hours (after final dose). The image acquisition and analysis used for assessment of the microcirculation will be in line with the 2007 consensus agreement. This will include a Capillary Network Analysis for vessel densities (total and perfused), the proportion of perfused vessel and the average perfusion speed indicator.

9.5.3 Assessments of Pharmakokinetics of Sevuparin

An aliquot of plasma from the samples taken during the trial will allow pharmacokinetic-pharmacodynamic (PKPD) modelling of the relationships between drug levels and longitudinal APTT and plasma lactate levels. The evaluation of the PK data will focus on the association between the sparsely sampled sevuparin concentrations and the APTT levels, plasma lactate levels and renal function. These assays will be contracted to Accelera (Nerviano (MI), Italy) since they have validated methodology for Sevupain measurement and the data sent to MORU, Bangkok who will undertake tbe PK modelling at the end of the clinical study.

9.6 Clinical Endpoints

9.6.1 Primary Endpoint

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

9.6.2 Secondary Endpoints

9.6.2.A 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

9.6.2.B Safety Endpoints

- APTT 24h post enrolment (absolute level and grade)
- Development of abnormalities 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-existing 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).

9.7 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. Professor Timothy Peto (Oxford) has agreed to be the Chairman is the DMC, other members will include an independent statistician and 2 pediatricians with relevant DMC or clinical trial experience. They will meet by regular teleconference. 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.

10 CLINICAL MANAGEMENT

Members of the clinical team and study team will all receive pre- and peri-trial training on the management of severe malaria. A manual of operations for the trial will be available for the study team, to anticipate and troubleshoot any potential issues.

Children will initially receive parenteral antimalarial treatment (artesunate), followed by on day 3 (or when the child can safely take and retain oral feeds and fluids) an oral course of artemisinin combination therapy (ACT). All trial patients will receive intravenous antibiotics. Intravenous maintenance fluids will be given at a rate of 4ml/kg/hour until the child is able to drink and retain oral fluids. Antipyretics, anticonvulsants and treatment for hypoglycaemia and other treatments will be given as clinically required and will be administered according to nationally agreed protocols. Children with Hb <4 g/dl (or Hb <6 g/dl and respiratory distress) will be transfused with 20mls/kg of whole blood as soon as blood is available. In the absence of blood standard care as per local treatment guidelines will be followed.

Table 2: Clinical monitoring

Table 2: Clinical monitoring

	Assessment Time										
Procedure	**Adm**	**1h**	**2h**	**4h**	**8h**	**9h**	**17h**	**24h**	**At least bi- daily until discharge**	**Day 7**	**Day 28**
Clinical assessment	X	X	X	X	X			X	X	X	X
ECG continuous to 24 hours	X							X			
APTT and coagulation tests (ACT) (1.7ml)	X	X 1 hr post dose				X		X 1 hr post dose			
Microperfusion[&] (Kilifi)	x					x					
Lactate (point of care)	X	X				X		X			
* Standard clinical test non-research (FBC, POC chemisty Istat)	X							X		X (Hb or FBC only)	
Malaria slides	X					X		X	36,48 (and 72 hr)	X	X
Stored red cells (Kilifi) & admission plasma **	X					X					X
Samples for PK studies		X				X					
Neuro-developmental exam/questionnaire	X										X

[&] Kilifi Site only

* Standard- non research clinical tests full blood count, clinical chemistry POC IStat (electrolytes, Ph BUN) will be done at admission and at 24 hours and Day 7 (Full blood count only).

Other laboratory test: venous blood gases (including base excess) will be done at 0, 9 and 24 hours

Malaria slide and morphology will be done at 0, 9, 17, 24, 36, 48 hours (and 72 hours if in hospital); at follow up (Day 7 and 28)

**For quantitative plasma HRP2 assessment, biomarkers and parasite morphology (Kilifi site only)

Clinical Assessment: Vital signs by bedside patient monitor (including temperature, heart rate, respiratory rate, blood pressure, oxygen saturation, conscious level). These will continue twice-daily after 24 hours until the child is discharged from HDU. All children will be managed on the HDU until conscious and able to take and retain oral fluids/food. During this time children will have 4 hours vital signs conducted. During the period of sevuparin administration (0-24 hours) children with have continuous electrocardiogram (ECG) monitoring for safety. However, it is notable that the trial in Thai adults showed no signs of QT prolongation [36]. Once discharged from HDU children will be reviewed daily until discharge and followed up at day 7 and day 28. On admission and Day 28 children will be assess by an adapted Kilifi Developmental Index to assess developmental status and clinically for neurodevelopmental sequelae **(see Table 2).**

Non-compliance is limited by the intervention being administered by clinical teams during admission. Any child who develops APTT>2.5xULN (grade 3 toxicity) will not receive further doses of sevuparin but will continue to be followed up. Children lost to follow-up before day 28 will be traced for vital status (permission requested within consent) using locator data and multiple contact phone numbers recorded before discharge.

Additional laboratory tests

At 1- and 8- hours and Day 28 plasma and red cell pellets will be used measure of the level Plasmodium falciparum Histidine Rich Protein (plasma); presence of rosetting and detailed microscopic examination of the infected red-cell morphology to stage the maturity of the parasite.

11 SAFETY REPORTING

The principles of ICH GCP require that both investigators and sponsors follow specific procedures when notifying and reporting adverse events or reactions in clinical trials.

DEFINITIONS

The definitions of the EU Directive 2001/20/EC Article 2 based on the principles of ICH GCP apply to this trial protocol. These definitions are given below.

TABLE	DEFINITION
Adverse Event (AE)	Any untoward medical occurrence in a patient or clinical trial subject to whom a medicinal product has been administered including occurrences that are not necessarilycaused by or related to that product.
Adverse Reaction (AR)	Any untoward and unintended response to an investigational medicinal product related to any doseadministered.
Unexpected Adverse Reaction (UAR)	An adverse reaction, the nature or severity of which is not consistent with the information about the medicinal product in question set out in the Summary of Product Characteristics (SPC) or Investigator Brochure (IB) for thatproduct.
Serious Adverse Event (SAE) or Serious Adverse Reaction (SAR) orSuspected Unexpected Serious Adverse Reaction (SUSAR)***	Respectively any adverse event, adverse reaction or unexpected adverse reaction that: ▪ Results in death ▪ Is life-threatening* ▪ Requires hospitalisation or prolongation of existing hospitalisation** ▪ Results in persistent or significant disability or incapacity ▪ Consists of a congenital anomaly or birth defect ▪ Is another important medical condition

*The term life-threatening in the definition of a serious event refers to an event in which the patient is at risk of death at the time of the event

**Hospitalisation is defined as an inpatient admission, regardless of length of stay, even if the hospitalisation is a precautionary measure for continued observation. Hospitalisations for a pre-existing condition (including

elective procedures that have not worsened) do not constitute an SAE.

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). Any conditions not explicitly included should be graded according to the following definitions, which have been adapted from Directive 2001/20/EC of the European Parliament (Clinical Trials Directive) [61] and ICH-GCP guidelines (E6(R1), 1996) for grading of the severity of the events.

- Grade 1 Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
- Grade 2 Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living.
- Grade 3 Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self care activities of daily living.
- Grade 4 Life-threatening consequences; urgent intervention indicated.
- Grade 5 Death related to AE.

11.1.1 Exempted Adverse Events

All patients eligible for SEVUSMAART are critically ill and due to the complexity of their condition are at increased risk of experiencing AEs. Many of these events are expected as a result of the patient's underlying severe malaria and are not related to participation in the trial. Consequently, AEs occurring as a result of the patient's medical condition will not be reported eg episode of hypoglycaemia or seizures. Pre-existing conditions do not qualify as AEs unless they worsen in a way that is not expected for the patient's medical condition at admission but should be documented in the patient's medical notes.

Other than this, Adverse Events include:

- An exacerbation of a pre-existing illness
- An increase in frequency or intensity of a pre-existing episodic event or condition
- A condition (even though it may have been present prior to the start of the trial) detected after trial drug administration
- Continuous persistent disease or a symptom present at baseline that worsens following administration of the study treatment

Adverse Events do not include:

- Medical or surgical procedures; the condition that leads to the procedure is the adverse event
- Pre-existing disease or a condition present before treatment that does not worsen
- Hospitalisations where no untoward or unintended response has occurred, eg, elective cosmetic surgery, social admissions
- Overdose of medication without signs or symptoms

CAUSALITY

The assignment of causality should be made by the investigator responsible for the care of the participant using the definitions in the table below. If any doubt about the causality exists the local investigator should inform the study coordination centre who will notify the Principal Investigator. Other clinicians may be asked to advise in some cases.

In the case of discrepant views on causality between the investigator and others, all parties will discuss the case.

Relationship	Description
Unrelated	There is no evidence of any causal relationship
Unlikely	There is little evidence to suggest there is a causal relationship (e.g. the event did not occur within a reasonable time after administration of the trial medication). There is another reasonable explanation for the event (e.g., the participant's clinical condition, other concomitant treatment).
Possible	There is some evidence to suggest a causal relationship (e.g., because the eventoccurs within a reasonable time after administration of the trial medication). However, the influence of other factors may have contributed to the event (e.g.,the participant's clinical condition, other concomitant treatments).
Probable	There is evidence to suggest a causal relationship and the influence of other factors is unlikely.
Definitely	There is clear evidence to suggest a causal relationship and other possible contributing factors can be ruled out.
Not assessable	There is insufficient or incomplete evidence to make a clinical judgement of the causal relationship.

11.2 Reporting Procedures

At each clinical review the clinician or nurse will check for potential SAEs and grade 3 or 4 AEs, and any potentially intervention-related AEs (e.g., coagulation test results). All serious adverse events will be reported in the case report form (CRF) and on SAE forms. All grade 3 or 4 events (regardless of causality) will be reported on the CRFs. Coagulation adverse events will be identified directly from the laboratory values reported on the laboratory test result CRF. The reporting procedure is captured within the safety reporting Standard Operating Procedure (SOP). Any questions concerning adverse event reporting should be directed to the study coordination centre in the first instance.

SAEs will be reported to the study coordinator, using an SAE form. The SAE form asks for nature of event, date of onset, severity, corrective therapies given, outcome and causality (i.e., unrelated, unlikely, possible, probably, definitely) to all medical interventions received. The responsible investigator should sign the causality of the event. Additional information should be sent within 5 daysif the reaction has not resolved at the time of reporting. The SAEs will be reported by the study PI to ethics and regulatory bodies within 24 hours(electronically) and 5 days for the full report.

12 DATA MANAGEMENT

12.1 Data Storage

All clinical and laboratory data will be recorded in the CRF and stored with a unique serial number identifier. Data will be entered onto Open Clinica. All data will be regularly backed up and backup copies stored both on and off site. Paper records will be archived in locked cabinets at Kilifi County Hospital, Kenya. These cabinets will have limited access with prior authorisation. All data will be partially- anonymized prior to presentation or publication of any results. Archive documents will be sent for long term storage (10 years) at an appropriate facility according to Kenya Medical Research Institute (KEMRI)policies.

12.2 Data Management and Statistical Analysis

Clinical data will be summarized using means and medians where appropriate for continuous data depending on the distribution. Primary and secondary endpoints will be described using means or medians or proportions. Analyses will follow intention-to-treat. As this is a Phase I trial no subgroup analyses are planned.

12.3 Intellectual Property

Any Intellectual property rights that arise from the work will be safeguarded according to the KEMRI IPR Policy of 2015 and the Industrial Property Act of 2001, sections 32, 58 and 80. The scientific and intellectual contributions of all persons involved in the research will be appropriately acknowledged in all publications and presentations arising from the work.

12.4 Time Frame/Duration of the Project:

a) Study enrolment – 15 months total, 1st September 2022 – 31st December 2023
b) Data analysis – 1st February 2024 – 1st May 2024 – 3 months
c) Report preparation – 1st May 2024 – 1st July 2024 – 2 months

13 ETHICAL CONSIDERATION:

Ethical approval will be sought from KEMRI Scientific and Ethics Review Unit (SERU) and from Imperial College Research Ethics Committee (ICREC), who is the sponsor of the study.

13.1 Human subjects

13.1.1 RISKS

"First, do no harm." The study will be performed in patients who may potentially benefit from the treatment. The risks of cannula insertion and blood drawing include pain, infection at the site of the cannula and thrombophlebitis. These will be minimised by careful technique according to a standard SOP, cannula site inspection and replacement or removal where necessary. No more than 1ml/kg of blood will be drawn for research at any one time. The trial will be recruiting patients with severe illness and likely a high mortality rate. At the start of the trial, the site will receive appropriate training on the use of Sevuparin and will have 2 dedicated clinicians. Sevuaprin can lead to minimal and non-clinically relevant changes in APPT (grade II toxicity) In the Phase I dose escalation trial, sevuparin was found to be safe and well-tolerated with minimal effects on APTT at doses of 1.5 mg/kg and 3mg/kg every six hours but a dose of 6.0 mg/kg led to higher APTT values. The Data Monitoring Committee (DMC) therefore recommended that the study should continue to the Phase II with a dose of 3.0 mg/kg sevuparin every 6h, given the potentially increased risk of adverse events at higher doses.

Risk will be minimised by the trial design/methods (see page 23) whereby the initial participants (two 'cohorts' of 2 children each, i.e. 4 children in total) will receive a dose of 1.5 mg/kg/dose with the plan to escalate up to a cohort of 2 children receiving 3mg/kg/dose and a cohort of two children receiving 6.0 mg/kg/dose (maximum). In order to determine whether, and the rate of escalation, to a higher dosage for subsequent patients after the first 4 children we will use a design called the Continuous Reassessment Method (CRM). This starts with an estimated dose-toxicity curve, reflecting the probability of experiencing a toxicity event (here APTT>2.5xULN 1h after any of the three doses) to the dose received. In this method of dose finding, the dose-toxicity curve is fitted to the data after each child is enrolled, based on their observed dose and toxicity outcome. After the first 8 children, each subsequent patient would be assigned the largest dose with an estimated risk of toxicity below the target toxicity level, designated as the maximum tolerated dose (MTD).

13.1.2 BENEFITS

All patients will be closely monitored so that clinical deteriorations can be identified at the earliest opportunity and appropriate therapy initiated. In general, the high dependency ward at Kilifi County Hospital has considerable experience with this population and this will serve to minimise the risks to the patients and the trial. Prior to the start, the dedicated study teams will undergo detailed training on general management of severe malaria and its complications and receive very detailed training on the use of sevuparin. We believe this will afford all children enrolled in the trial with a higher quality of care.

All routine non-trial medications required by the hospital to treat the child will be made available.

Hospital bills for participants older than 5 years will be covered by the study (covering the costs for standard treatment for severe malaria and related complications).

The parents or guardians for the children will be asked to return for a follow up clinic visit at day 7 and day 28 and thus will be offered continuing care for concurrent illness, including any investigations or blood tests that are clinically indicated.

13.2 Patient Consent

Prospective written, informed consent will be sought from parents or guardians of children who are considered to be sufficiently stable. Parents or guardians will be given an information sheet in their usual language containing details of the SEVUSMAART study. These will be translated into to local languages, and then back translated (to ensure details are correct) prior to piloting before the initiation of the trial. The sheet will be read aloud to those who are unable to read. Parents and guardians will be encouraged to ask questions about the trial prior to signing the consent form. Consent will include permission for the collection of data and for aetiological investigations. The rights of the participant to refuse to participate without giving reasons must be respected. A number of children will present as emergencies where delay in study enrolment, and thus treatment, will not be practical or indeed humane. We will use a modified form of deferred consent that we developed, received ethical approval for, and used in the FEAST trial. It proposes to use a 'two-stage' consent process in this circumstance [57]. Verbal assent will be sought from parents or guardians by the admitting medical team, if it is considered that the full consent process would significantly delay enrolment, and consequently could be detrimental to the child's health. Full consent will be sought once the child's clinical condition has been stabilized. Caregivers will be provided with a brief verbal description of the trial and will be given the opportunity to "opt out" of clinical research. The clinician will then sign the verbal assent form, which will be filed with the consent form. A social science study of the consent processes used in FEAST found this to be acceptable to parents and health-care workers [62]. As in the FEAST trial, if following an assent process a child died prior to full written consent, full consent would not be sought. This was process of emergency consent was approved (by multiple ethics research committees) for FEAST and has been subsequently approved for use in a transfusion trial in Uganda and Blantyre (TRACT) [58].

13.3 WITHDRAWAL OF PATIENTS and Protocol Treatment Discontinuation

In consenting to the trial, patients are consenting to trial treatment (sevuparin), data collection and follow-up. If a carer wishes to withdraw their child from trial treatment, the investigator will explain the importance and benefits of follow-up, and the value of allowing routine clinical data to be used for trial purposes. Patients/parents are free to discontinue any part of their trial treatment or discontinue from follow up. After the participant has entered the trial, the clinician remains free to give alternative treatment to that specified in the protocol at any stage if he/she feels it is in the participant's best interest, but the reasons for doing so should be recorded. In these cases, the participants remain within the study for the purposes of follow-up and data analysis. All carers and participants are free to withdraw at any time the protocol treatment without giving reasons and

without prejudicing further treatment. If they do not wish to remain on trial follow-up, however, their decision must be respected, and the patient will be withdrawn from the trial completely.

13.4 Protocol Treatment Discontinuation

An individual patient may stop treatment early or be stopped early for any of the following reasons:

- Unacceptable toxicity or adverse event
- Intercurrent illness that prevents further treatment
- Any change in the patient's condition that justifies the discontinuation of treatment in the clinician's opinion
- Withdrawal of consent for treatment by the patient or parent.

Participation in the trial is entirely voluntary, and parents, carers or older children may choose to discontinue the trial treatment at any time without penalty or loss of benefits to which they are otherwise entitled. Although not required to give a reason for discontinuing their trial treatment, a reasonable effort should be made to establish this reason while fully respecting the patient's rights.

Patients should remain in the trial for the purpose of follow-up wherever possible (unless the patient withdraws their consent for follow-up). If a patient withdraws from the trial, the medical data collected during their previous consented participation in the trial will be kept and used in analysis. This will also apply to parents/carers who withdraw from the trial after assent, that have not completed the deferred consent process. Consent for future use of stored samples already collected can be refused when leaving the trial early (but this should be discouraged and should follow a discussion). If consent for future use of stored samples already collected is refused, then all such samples will be destroyed following Kenya Medical Research Institute (KEMRI) policies.

13.5 Community Engagement Strategy and feedback

Community engagement will be through regular meetings with the community involving KEMRI-Community Representatives and County Health teams. At these meetings, information and feedback will be given and received. Information arising from the study will be fed back through hospital-wide meetings. This is a Phase I trial of an emergency intervention where our engagement has been at a scientific rather than public/community level. If a larger platform trial arose from this study, we aim to develop a dedicated and informed engagement strategy as part of this future trial. In general, we plan to feed into existing community engagement mechanisms. We aim to build general community awareness of research processes at the local hospital, and support community representative inputs into decisions around research design, consent procedures, patient information and trial conduct.

13.6 Compensation

Families will not incur any costs from participation in this study. All children who are enrolled in the study travel expenses for attending the visits on Day 7 and Day 28 will be paid, based on the cost of public transport to and from the participant's home and compensation for out-of-pocket expenses using standard rates (KES 350). At follow up visits snacks and drinks will be provided for children enrolled in the trial.

13.7 Confidentiality

Participant's identification data will be required for the registration process. All clinical and laboratory data will be recorded in the CRF and stored with a unique serial number identifier. Information will only be made available to those caring for the child and those directly involved with the study. Data will be entered onto Open Clinica (FDA approved, web-based application). All data will be regularly backed up and backup copies stored both on and off site. Paper records will be archived in locked cabinets. These cabinets will have limited access with prior authorisation. All data will be partially-anonymized prior to presentation or publication of any results. All clinical data will be held confidentially, and personal identifiers will be removed before analysis of the data and presentation of the results.

13.8 Data Sharing

After completion of the study, requests for data access from researchers outside the study team will be considered by a subgroup of the Centre Scientific Committee (Data Governance Committee), and where indicated, requestors will be asked to develop scientific protocols for approval of secondary analyses. The potential to share data will be included in the participant Information and Consent Form.

13.9 Indemnity

Imperial College London holds negligent harm and non-negligent harm insurance policies, which apply to this study.

13.10 Sponsor

Imperial College London will act as the main Sponsor for this study and delegates this responsibility to the KWTRP, Kilifi and MRC CTU at UCL to oversee the implementation of the study by ensuring that arrangements are put into place for adequate management, monitoring, analysis and reporting of the trial.

EXPECTED APPLICATION OF THE RESULTS

The results of the Phase I trial will identify the final dosage selected for the Phase II that will include both efficacy and safety outcomes. The primary endpoint for the future Phase II reflects the primary hypothesis that sevuparin improves microcirculatory flow by reversing and preventing parasite sequestration. Data collected in the Phase I trial will assess whether the planned primary endpoint for the Phase II trial, lactate clearance at 8 hours, is realistic and feasible.

TIMEFRAME

Total duration of 15 months:
Start Date 1st September 2022
Recruitment over 15 Months (3 malaria seasons)
Data Analysis and report writing 2 months

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15 ROLE OF INVESTIGATORS

Name	Study design and development	Professional and Community engagement	Clinical care	Data and sample collection	Endpoint review	Clinical data Analysis	Report writing
Kathryn Maitland	⊙		⊙	⊙			⊙
Mainga Hamaluba	⊙	⊙	⊙	⊙			⊙
Mike Chaponda Nchafatso Obonyo			⊙ ⊙	⊙ ⊙			⊙ ⊙
Emmanual Odugu		⊙		⊙	⊙		⊙
Tom Williams	⊙		⊙	⊙			⊙
Walker, George and Gibb	⊙				⊙	⊙	⊙
Andrew Turnbull	⊙		⊙	⊙			⊙
Day, Dorndorp	⊙					⊙	⊙

Appendices

1) Patient information sheets, consent forms, assent form, withdrawal form