Protocol

This trial protocol has been provided by the authors to give readers additional information about their work.

Protocol for: Maitland K, Kiguli S, Olupot-Olupot P, et al. Immediate transfusion in African children with uncomplicated severe anemia. N Engl J Med 2019;381:407-19. DOI: 10.1056/NEJMoa1900105

This supplement contains the following items:

1. Original protocol, final protocol, summary of changes.
2. Original statistical analysis plan, final statistical analysis plan, summary of changes.

Funders:

Main Sponsor:
Imperial College London

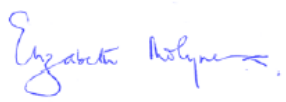
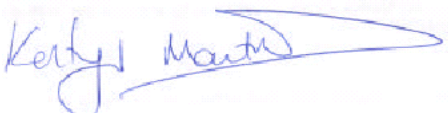
Collaborating groups:

TRACT

TRansfusion and TReatment of severe Anaemia in Afr

Version:	**1.0**
Date:	**20th February 2013**
ISRCTN:	**ISRCTN84086586**
ICREC no:	**13_1_11**

Authorised by:

Name:	**Prof Elizabeth Molyneux**
Role:	**Chair of TSC**
Signature:	
Date:	**1st September 2014**
Name:	**Dr A. Sarah Walker**
Role:	**Trial Statistician**
Signature:	
Date:	**2nd September 2014**
Name:	**Prof Kathryn Maitland**
Role:	**Chief Investigator**
Signature:	
Date:	**2nd September 2014**

Trial Administration

Study Coordination Centre: Clinical Trials Facility, Phyles Maitha; Please direct all queries to the TRACT Trial Administrator at at KEMRI Wellcome Trust Programme (Phyles Maitha; PMaitha@kemri-wellcome.org) Tel: +254715461761

Clinical Queries

All clinical queries will be passed to **Ayub Mpoya (**AMpoya@kemri-wellcome.org**)**, Tel : +254735229037 in the first instance at KEMRI Wellcome Trust Programme who will consult others members of the Clincal Management Group where necessary.

Sponsor

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:

Joint Research Office
Sir Alexander Fleming Building
Imperial College
Exhibition Road
London SW7 2AZ
Tel: 020 7594 1188
Fax: 020 7594 1792

Funder

Medical Research Council (MRC) and Department for International Development (through a concordat with MRC)

This protocol describes the TRACT trial 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 trials 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.

COORDINATING CENTRE

KEMRI Wellcome Trust Programme	Switchboard: Mobile:	+254 (0) 417 522063/522535 +254 (0) 715461761 (Trial manager)
Clinical Trial Facility	Fax:	+254 (0) 417 522390
PO Box 230, Kilifi, Kenya	Email:	TRACT@kemri-wellcome.org

KEMRI WELLCOME TRUST PROGRAMME, KILIFI

TRIAL MANAGEMENT GROUP

Principal Investigator:	Prof Kathryn Maitland MBBS PhD	Mobile: Email:	+254733411022 kathryn.maitland@gmail.com
Trial Administrator	Phyles Maitha Dip.Business.Mgt	Tel: Mobile: Email:	**+254 417525453 (switchboard)** +254715461761 PMaitha@kemri-wellcome.org
Clinical Project Manager:	Ayub Mpoya Dipl.Clin.Med.Chir	Tel: Mobile: Email:	+254 417525453 (switchboard) +254735229037 AMpoya@kemri-wellcome.org
Monitor (Kenya):	Esther Kivaya BSc.Nursing, MSc.Clinical Trial	Tel: Mobile: Email:	+254 417525453 (switchboard) +254 728821026 EKivaya@kemri-wellcome.org
Data Manager:	TBA	Tel: Email:	

GENETIC EPIDEMIOLOGY GROUP

Lead Investigator	Prof Thomas Williams MBBS PhD	Mobile: Email:	+254733411021 tom.n.williams@gmail.com
Coinvestigators	Dr Sophie Uyoga PhD	Mobile: Email:	+254722342686 suyoga@kemri-wellcome.org
Coinvestigators	Alex Macharia MSc	Mobile: Email:	+254721832920 amacharia@kemri-wellcome.org

IMPERIAL COLLEGE

Principal Investigator:	Prof Kathryn Maitland MBBS PhD	Tel: Email:	+44 (0) 207 670 4905 kmaitland@imperial.co.uk

Centre Admininstrator	TBA	Tel: Email:	
Co-investigator	Professor Gary Frost PhD RD	Tel: Mobile: Email:	+44 (0) 20 8383 8037 +44 (0) 7872850308 g.frost@imperial.ac.uk

MRC CTU STAFF

Principal Investigator:	Prof Diana M Gibb, MD MSc	Tel: Email:	+44 (0) 207 670 4905 dmg@ctu.mrc.ac.uk
Trial Statistician:	Dr Sarah Walker, PhD MSc	Tel: Email:	+44 (0) 207 670 4726 asw@ctu.mrc.ac.uk
Statistician:	Elizabeth George, MSc	Tel: Email:	+44 (0) 207 670 4931 Elizabeth.George@ctu.mrc.ac.uk
Clinical Project Manager:	Dr Margaret Thomason, PhD, MSc	Tel: Email:	+44 (0) 207 670 4615 mjth@ctu.mrc.ac.uk
Trial Manager	Ellen-Owen Powell, MSc	Tel: Email	+44 (0) 207 670 4606 ellen.owen-powell @ctu.mrc.ac.uk
Data Manager:	Natalie Young, BSc	Tel: Email:	+44 (0) 207 670 4766 natalie.young@ctu.mrc.ac.uk
Policy & Research Impact Coordinator:	Annabelle South, BSc	Tel: Email:	+44 (0)207 670 4827 A.South@ctu.mrc.ac.uk
Health Economics Co-investigator	Paul Revill, MSc Hons	Tel: Mobile: Email:	+44 (0)1904 651202 +44 (0)7575 907665 paul.revill@york.ac.uk

OTHER TRIAL INVESTIGATORS

UGANDA: Country Principal Investigator Prof Sarah Kiguli			
Department of Paediatrics, Mulago Hospital PO Box 7072 Makerere University, Kampala,			
Principal Investigator:	**Prof Sarah Kiguli** M.B. Ch.B., M.MED	Tel: Mobile: Email:	+ 256 414531875 +256 772588044 skiguliwalube@chs.mak.ac.ug

Study Site Coordinator	Mariam Namutebi BSN	Tel Mobile Email Fax	+256414531875 +25671225547 mariamnamutebi@yahoo.co.uk +256 41 532591
Co-investigators:	Dr Robert Opoka M.B. Ch.B., M.MED	Mobile: Email:	+256 41 531875 opokabob@yahoo.com;
Monitor (Uganda):	Sam Ssenyonjo, BSc N	Tel: Email:	+ 256 0782 964412 senyonjosam@yahoo.com
Uganda Blood Transfusion Service P.O.Box 1772, Kampala, Uganda			
Director	Dr. Dorothy Kyeyune Byabazaire; MBChB; Dip Transfusion Med; Dip Project Planning and Management	Tel: Mobile: Web: Email:	+256 414 259195 +256 772 425293 www.ubts.go.ug kyeyunedorothy@yahoo.com
Mbale Regional Referral Hospital Pallisa Road Zone, P.O Box 921, Mbale, Uganda			
Site Investigator:	Dr Peter Olupot Olupot, MBChB; MPH	Tel: Mobile: Fax: Email:	+256 772 457217; +256 772457217 + 256 45 4435894 polupotolupot@yahoo.com
Study Site Coordinator:	Tonny Sennyondo Nurse	Tel: Mobile: Email:	+ 256 758361604 +256 783620048 tseenyondo@gmail.com
Data Manager:	TBA	Tel: Email:	
Mbale Regional Blood Bank, P.O. Box 284, Mbale, Uganda			
Co_Investigator	Dr. Benjamin Wabwire, MBChB, DTM	Tel: Mobile: Email:	+256 31-2218300/5/10 + 256 77 608302 benjaminwabwire@yahoo.com
Soroti Regional Hospital, P.O Box 289, Soroti.			

Site Investigator:	Dr Charles Engoru, M.B. Ch.B., M.MED	Tel: Mobile: Email:	+256 45 4461382; + 256 772458587 charlesengoru@yahoo.co.uk
Study Site Coordinator::	Denis Amorut	Tel: Mobile: Fax: Email:	+256 45 4461382 + 256 774573911 +256 45 4461382 damoruts@gmail.com
Co Investigators:	TBA		

MALAWI: Country Principal Investigator Dr Yamikani FG Chimalizeni			
College of Medicine, Department of Paediatrics and Child Health Malawi-Liverpool-Wellcome Trust Clinical Research Programme, PO Box 30096, Chichiri, Blantyre 3 Malawi			
Principal investigator:	Dr Yamikani FG Chimalizeni	Tel: Email:	+265 992 233 221 gaveta3@yahoo.com
Co-investigators:	Professor Robert Heyderman FRCP, PhD, DTMH Dr Neil Kennedy MRCPCH, DTMH Dr Macpherson Mallewa MRCPCH, PhD, DTMH	Tel: Email: Tel: Email: Tel: Email:	+265 888 208 044 R.Heyderman@liverpool.ac.uk +265 995 929733 nkennedy@medcol.mw +265 995 835 083 macphers@liverpool.ac.uk
Trial Manager:	TBA	Tel: Email:	+265 **[NNNN]** **[NNNN]**
Malawi Blood Transfusion Service, PO Box 2681, Blantyre, Malawi.			
Co-investigator	Dr Bridon M'baya	Tel: Email:	+265 995 883 117 mbayab@yahoo.com

Other Centres

Liverpool:			
Liverpool School of Tropical Medicine and Hygeine Pembroke Place, Liverpool L3 5QA			
Co-investigators:	Professor Imelda Bates; MBBS, Phd	Tel: Email:	+44 151 705 3115 ibates@liverpool.ac.uk
Co-investigators:	Dr Britta Urban. ; PhD	Tel: Email:	+44 1517053380 burban@liverpool.ac.uk
Amsterdam:			
Emma Children's Hospital AMC (Global Child Health Group) Meibergdreef 9, 1105 AZ Amsterdam			
Co-investigators:	Professor Michael Boele von Hensbroek PhD;	Tel: Email:	+31 20 5666173 mbvh04@gmail.com

ABBREVIATIONS

ARR	Acute transfusion reaction
BTS	Blood transfusion services
CAB	Community Advisory Board
CEA	Cost-effectiveness analyses
CI	Chief Investigator
CRF	Case record form
CRP	C-reactive protein
CTF KWTP	Clinical Trial Facility, KEMRI Wellcome Trust Programme
CTC	Common Toxicity Criteria
CTU	Clinical Trials Unit
DMC	Data Monitoring Committee
ERC	Endpoint Review Committee
GCP	Good Clinical Practice
Hb	Haemoglobin
HTR	Haemolytic transfusion reaction
IBD	Invasive bacterial disease
ICH	International Committee on Harmonisation
IDA	Iron deficiency anaemia
IPT	Intermittent preventative treatment
IRB	Institutional Review Board
IV	Intravenous
LPS	Lipopolysaccharide
MRC	Medical Research Council
MVMM	Multi-vitamin multi-mineral
NTS	Non-Typhoidal Salmonellae
PCT	Pro-calcitonin
PI	Principal Investigator
PTP	Post transfusion purpura
QA	Quality Assurance
QC	Quality Control
QMP	Quality Management Plan
RCT	Randomised controlled trial
REC	Research Ethics Committee
RNI	Recommended Nutritional Intake
RUTF	Ready To Use Foods
SA	Severe anaemia
SAE	Serious adverse events
SAP	Statistical Analysis Plan
SOC	Standard of care
SOP	Standard Operating Procedure
SSA	Sub-Saharan Africa
SNP	Single Nucleotide Polymorphism
SSC	Study Site coordinator
TACO	Transfusion Associated Circulatory Overload
TC	Trial coordinator
TMF	Trial Master File
TMG	Trial Management Group
TMT	Trial Management Team
TSC	Trial Steering Committee
TRALI	Transfusion Related Acute Lung Injury

TTI	Transfusion transmitted infection
U&E	Urea and Electrolytes
WB	Whole Blood
WHO	World Health Organization

GLOSSARY OF TERMS

Coma	Inability to localize a painful stimulus
Impaired perfusion	≥1 of: capillary refill >2 seconds; lower limb temperature gradient; weak radial pulse volume
Intravascular volume depletion	Depletion of circulating volume : **relative** (due to vasodilatation) or **actual** (loss of fluid from intravascular space e.g. burns or blood loss or capillary leak)
Impaired consciousness	Prostration or coma
Profound anaemia	Haemoglobin < 4g/dl
Prostration	Inability to sit unsupported, or to breast feed if <9 months
Respiratory distress	Deep breathing or increased work of breathing
Severe anaemia	Haemoglobin < 6g/dl
Severe and complicated anaemia	Severe anaemia with severe illness or impaired perfusion
Severe illness	Children with impaired consciousness or respiratory distress

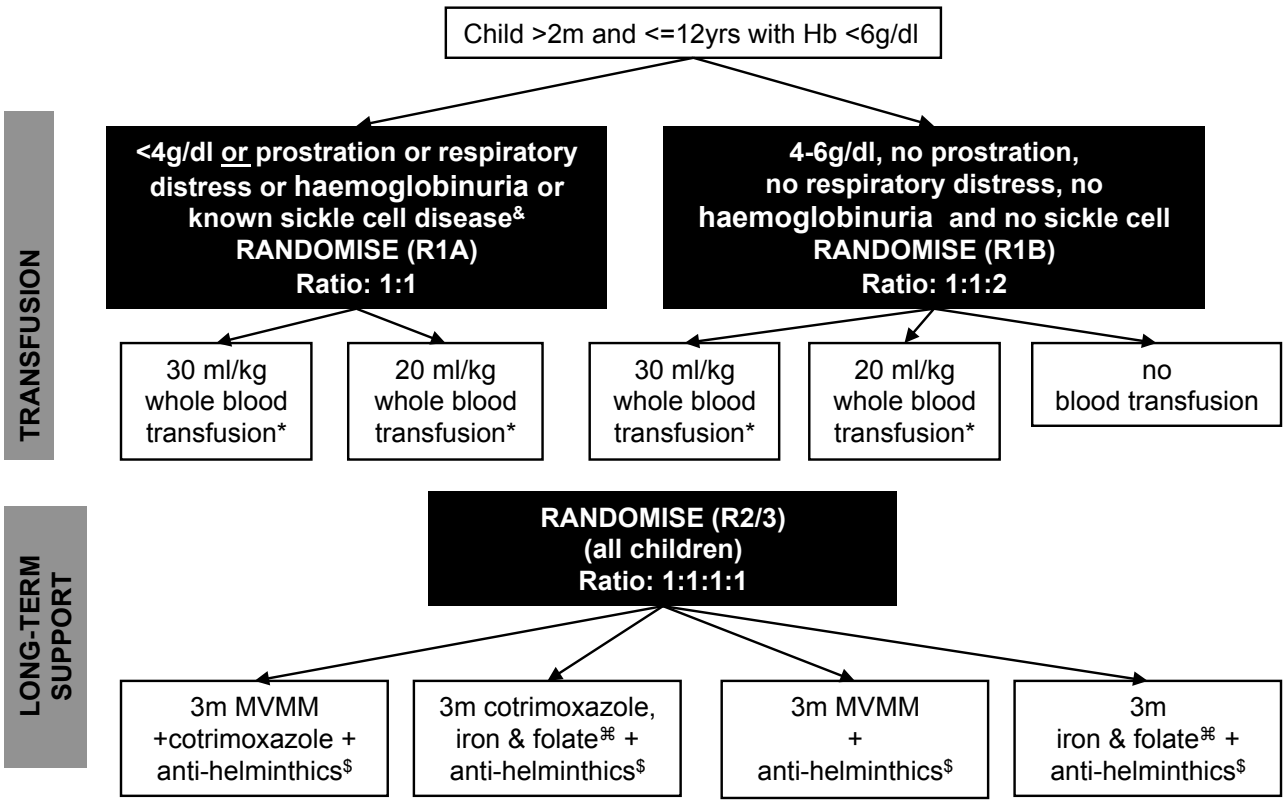
KEYWORDS
Children
Infants
Africa
Malawi
Uganda
Anaemia
Plasmodium falciparum malaria
Sepsis
Randomised controlled trial
Transfusion
Nutrition
Micronutrients
Emergency medicine
Haemoglobinopathies
Glucose 6 Phosphorylase Deficiency

STUDY SUMMARY

SUMMARY INFORMATION TYPE	SUMMARY DETAILS
ACRONYM (or Short Title of Trial)	TRACT
Long Title of Trial	TRansfusion and TReatment of severe Anaemia in African Children: a randomised controlled Trial
Version	1.0
Date	20th February 2013
ISRCTN #	**ISRCTN84086586**
Study Design	A 3x2x2 open-label factorial multi-centre trial, conducted in 4 centres in 2 countries (Malawi and Uganda)
Type of Participants to be Studied	3954 children aged 2 months to 12 years with severe anaemia (SA) (defined as a haemoglobin <6g/dl) on admission to hospital
Interventions to be Compared	The trial will have 3 intervention strategies aimed at to reducing mortality and morbidity in children with SA **R1:** Immediate liberal transfusion (30ml/kg) versus conservative transfusion (20ml/kg) versus no transfusion (last strategy only for children with **uncomplicated SA and Hb 4-6 g/dl**). **R2:**Post-discharge multi-vitamin multi-mineral (MVMM) supplementation (which includes folate and iron) versus routine care (folate and iron) for 3 months. **R3:**Post-discharge cotrimoxazole prophylaxis versus no prophylaxis for 3 months.
Study Hypotheses	Each intervention (R1, R2, R3) will reduce short and longer-term mortality and morbidity following admission to hospital with severe anaemia. Each intervention will be compared with standard of care.
Primary Outcome Measure(s)	Cumulative mortality to 4 weeks for the transfusion strategy comparison, and to 6 months for the nutritional support/antibiotic prophylaxis comparison
Secondary Outcome Measure(s)	• mortality at 48 hours, 4 weeks, 3 months and 6 months (where not the primary outcome); • development of new profound anaemia (Hb<4g/dl) during acute admission or development of severe anaemia (Hb<6g/dl) post discharge; • readmission to hospital; • proportion achieving correction of anaemia (defined by WHO as Hb>9g/dl); • nutrition: changes in weight and MUAC, at 90 day and 180 days • anti-infection: changes in inflammatory markers (C-reactive protein, procalcitonin), incidence of bacterial infections and malaria at 28 days, 90 day and 180 days • Solicited adverse events: suspected transfusion reactions:

	febrile reactions, TRALI (Transfusion Related Acute Lung Injury) (any grade); grade 3-4 toxicity of cotrimoxazole, MVMM or standard iron/folate • Serious adverse events • costs and cost-effectiveness
Randomisation	**R1:** Participants with uncomplicated SA and Hb 4-6 g/dl will be allocated in a 1:1:2 ratio between 30ml/kg versus 20ml/kg versus no transfusion. All other children will be allocated in a 1:1 ratio between 30ml/kg versus 20ml/kg. **R2 and R3**: children will be allocated in a 1:1 ratio between intervention vs control (no intervention). All three randomisations will use a factorial design, ie each randomisation will be balanced by design for allocation to other interventions or not.
Number of Participants to be Studied	3954 children including at least 1950 complicated (<4g/dl or 4-6g/dl with prostration/respiratory distress/known sickle cell disease/dark urine) and no more than 2000 uncomplicated (4-6g/dl without prostration, respiratory distress, known sickle cell disease or dark urine) (minimum 1560)
Duration	Participants will be randomised over 2 years and followed up for 6 months. Tranfusion intervention will be administered in hospital; nutritional and additional cotrimoxale prophylaxis will be administered for 3 months following discharge from hospital by caregivers The overall trial duration is 3 years.
Ancillary Studies/Substudies	▪ Economics and cost-effectiveness ▪ Molecular diagnostics ▪ Haemoglobinopathies ▪ Immunological studies ▪ Enteropathy ▪ Nutritional measure of wellbeing: dietary recall ▪ Micronutrients
Sponsor	Imperial College, London
Funder	Medical Research Council (MRC) UK and Department for International Development, UK (DFID)
Chief Investigator	Prof Kathryn Maitland

TRIAL SCHEMA



& Only applies to a previously established diagnosis of sickle cell disease
* Alternatively 15mls/kg packed cells (for 30mls/kg WB arm); 10 mls/kg packed cells (for 20mls/kg WB arm)
⌘ at treatment doses following WHO recommendations
$ For children > 1 year of age if they have not received antihelminths in previous 6 months- following WHO recommended standard of care
MVMM at usual supplementary dosages

FLOW DIAGRAM

If a child is seen more frequently than indicated, a follow-up form should be completed at each visit.

Hours (h) /Days (d)	**0*h**	**Discharge**	**28d**	**90d**	**180d**
Consent and information sheet	X				
Clinical examination (doctor/doctor visit) [a]	X	X	X		X
Nurse observation/visit, collect medication[b]		X	X	X	X
Vital observations, anthropometry	X		X	X	X
24-hour dietary recall		X	X	X	X
Pill count[c]			X	X	
Laboratory investigations					
Haematology[d]	X		X	X	X
Biochemistry [e]	X				
Lactate/Glucose	X				
Malaria slide + /- RDT	X		X	X	X
Blood culture[f]	X				
HIV testing	X				
Urine dipstick (Multi-stick)	X				
Cross match (for transfusion) (red top)	X				
Stored samples					
Investigations of anaemia aetiology (all)					
Blood Film and Malaria Pigment[g]	X		X	X	X
EDTA (for DNA) human and pathogen[h]	X				X
Plasma [i]	X		X	X	X
Stool [j]	X				
Other investigations (all)					
Donor blood haemoglobin and storage[k]	X				
Investigations of mechanism/response (subgroup only for retrospective analysis)					
Gut Barrier Function (EDX, I I-FABP, IBAP) [l]	X		X		X
Stool Storage[m]	X		X		X
Urine (metabolomics) [n]	X		X		X
PBMCs [o]	X		X		X
Red Cell pellets [p]	X		X		X

*Baseline history and examination at admission: clinician and nursing observations over 48 hours covered in the more comprehensive Table A (below)

a To include baseline history and physical assessment, 24 hour dietary recall prior to illness (at discharge), and daily assessment of solicited adverse events, inter-current illnesses and concomitant medications whilst admitted. Post-discharge assessments will include history of these events since last visit/discharge.

b to include: 24 hour dietary recall, body weight and height (and head circumference for children under 2 years), middle-upper arm circumference (MUAC), and history of illness and symptom checklist. At day 60 the nurse will contact the parent to check status and to enquire about compliance.

c pill count if randomised to cotrimoxazole or MVMM

d Haematology: Admission, Day 28, Day 90 and Day 180 Full haemogram (including haemoglobin, MCV, white cell count, +/-neutrophil, lymphocyte and platelet counts). Hb (by Haemacue) will be measured at additional time points between admission and 48 hours (see table A) or at these time points if haemogram not available.

e Biochemistry: Urea and electrolytes. Liver function tests (AST, ALT and Bilirubin) may be performed if clinically indicated, but are not required by the protocol.

f In all centres except Soroti (which does not have blood culture facilities); repeat culture if children readmitted or clinical sepsis suspected at follow up. All pathogen isolates will be stored for future investigation of antimicrobial resistance.

g Blood film for morphology and malaria pigment
h 1-2ml (EDTA) pellet will be used retrospectively to analyse, in batches, red cell haemoglobinopathies and enzymopathies (including thalassaemia, sickle cell disease and G6PD deficiency). The DNA pellet will also be assayed retrospectively for pathogen diagnosis (largely bacterial, parasite and viral)
i: up to 3ml plasma (store): Changes in inflammatory markers (eg CRP, PCT), cytokines, assays of micronutrients, malaria parasite load (HRP2), gut hormones and gut inflammation and microbial translocation will be assessed retrospectively on stored plasma samples.
j Stool sample for helminths investigation
k Donor blood (EDTA): a blood film will be prepared and plasma saved for potential retrospective investigation eg evidence of adverse effects of storage (storage lesions, cytokine production) and the pellet saved for later preparation of DNA (microbial contamination in a subset incl adverse events or endpoints).

Investigations only in a subset of participants

l, m and n Mbale RRH: Markers of Gut Barrier Dysfunction: Endotoxin/Immunology: Linked plasma urine and stool samples for examine gut barrier dysfunction and alterations of gut microbiome; faecal markers of enteric inflammation (calprotectin, alpha-1 antitrypsin, neopterin).
o and p Mbale RRH: PBMC to be saved, if possible, from the sodium heparin tube taken for plasma storage for future immunologic research; red cell pellets in patients with malaria: for parasite molecular research

Table A: Bedside Observations; Clinical examination and laboratory testing 0-48hours

Procedure	Adm	30m	60m	90m	2hr	4hr	8hr	16hr	24hr	48hr	Daily	Disc*
Source documents												
Clinical notes (doctor)[$]	X		X				X	X	X	X	X	X
Bedside notes (nurse)	X	X	X	X	X	X	X	X	X	X	X	
Haemoglobin (Hb)	X						X	X	X	X	X	X
Lactate	X						X		X			
Glucose	X		X		X		X	X	X			
Prescription Chart	X								X	X	X	X
Transfusion and iv fluids		X	X		X	X	X	X	X	X		X
Anthropometry	X									X		
Documents with data summaries												
CRF	X								X	X		X
Diagnosis[ç]	X											X
Drugs received									X	X		X
Transfusion and iv fluids		X	X		X	X	X		X	X		X
Locator details	X											X
Investigations[&]	X						X	X	X	X	X	X
SAEs							X		X	X		X
24-hour dietary recall												X
Discharge medication[£]												X

* Disc: On Discharge from hospital
[$] Additional reviews by doctors and nurses will be conducted and recorded, where clinically indicated.
[&] Laboratory tests (see Study Flow) and bedside tests including glucose and lactate at time points shown; plus any additional investigation eg Chest-Xray
£ Nutritional and antimicrobial prophylaxis to be issued from the pharmacy
[ç] Working diagnosis at admission and final diagnosis at discharge.

Table of Contents

1. INTRODUCTION

1.1 BACKGROUND

In sub-Saharan Africa (SSA) severe anaemia (SA) in children is a leading cause of hospital admission, a major cause of direct mortality[1] and a key factor in the 800,000 malaria deaths/year[2]. Guidelines developed by the World Health Organization (WHO) encourage the rational use of blood transfusion to preserve this scarce resource and to reduce the risk of transfusion-transmitted infections[3]. However, the evidence base for the paediatric guidelines is weak and the recommendations within these guidelines are confusing - consequently adherence is poor and national transfusion recommendations vary between SSA countries. Outcome of severe anaemia is unsatisfactory with high rates of in-hospital (9-10%)[4] and 6-month (12%) case fatality, and relapse or re-hospitalisation (6%),[5] indicating that the current recommendations and management strategies are not working in practice. Further, the aetiology of severe anaemia is frequently multi-factorial, including potentially treatable co-morbidities such as bacteraemia and multiple vitamin deficiencies - key determinants of outcome[6] that are not addressed in current treatment guidelines. Although the two most recent systematic reviews (both published in 2000) indicated the need for formal evaluation of the restrictive transfusion policy supported by WHO in a controlled trial[4, 7], little progress has been made in the intervening decade. The poor outcomes and recurrent morbidity of children with severe anaemia warrant a definitive trial to establish best transfusion and treatment strategies to prevent both early and delayed mortality and relapse.

1.2 CURRENT MANAGEMENT RECOMMENDATIONS

Transfusion of blood can be a life-saving intervention, and provision of adequate supplies of safe blood for transfusion are an essential undertaking for any health system. Issues of blood safety, adequate supply, equitable access and rational use, however, remain key challenges throughout the world. The pattern of usage of blood in SSA is very different from high-income countries where use is largely elective with supply strictly monitored through specialist transfusion services. In SSA, women and young children are the chief recipients of blood transfusions, accounting for over three-quarters of the blood transfusion requirements, most given as emergency interventions[8]. In order to bridge the major gap between supply and demand, one of the four key goals mandated in a WHO resolution on an integrated strategy of blood safety in 1975 was to 'reduce unnecessary transfusions' - through more effective clinical use of blood and use of simple alternatives to transfusion (crystalloids and colloids) where possible[9]. WHO has subsequently developed and published guidelines for the appropriate use of blood for patient groups suffering the greatest impact of a shortage of supply[10].

1.2.1 PAEDIATRIC TRANSFUSION GUIDELINES FOR CHILDREN IN DEVELOPING COUNTRIES

Current guidelines for paediatric transfusions were developed largely through consensus and based on data from observational studies rather clinical trials evaluating their impact. The pocket book of hospital care for children (guidelines for the management of common illnesses with limited resources, WHO 2005)[3] has two sections that cover transfusion – the Supportive Care chapter (Part 10.5 Management of anaemia) and in the Fever chapter: Part 6.2 Malaria. There are some discrepancies in these guidelines with respect to the threshold haemoglobin or haematocrit at which transfusion is recommended. The Supportive Care Chapter indicates a threshold <6g/dl, whereas in the Fever Chapter/malaria guidelines this is revised to threshold of <5g/dl (see below). No rationale justifying the choice of these two different thresholds is presented.

Current WHO guidelines
Supportive Care: Management of anaemia (page 276-281)
Give a blood transfusion as soon as possible to:
— all children with a haematocrit of ≤12% or Hb of ≤4 g/dl
— less severely anaemic children (haematocrit 13–18%; Hb 4–6 g/dl) with any of the following clinical features (complications):

- clinically detectable dehydration
- shock
- impaired consciousness
- heart failure
- deep and laboured breathing
- very high malaria parasitaemia (>10% of red cells with parasites).

Guidelines for transfusing severe malarial anaemia
Fever chapter: Part 6.2 Malaria (pages 142-143)
Give a blood transfusion as soon as possible to:

- all children with a haematocrit of ≤12% or Hb of ≤4 g/dl
- less severely anaemic children (haematocrit >12–15%; Hb 4–5 g/dl) with any of the above complications.

1.2.2 Definition of Complicated Severe anaemia

The criteria defining complicated SA above are not referenced and some lack scientific justification (including relevant literature). Three of complications warranting immediate transfusion have either concerns related to physiological justification or substantial resource implications: heart failure, dehydration and hyperparasitaemia. First, heart failure (ie 'biventricular' failure or 'overload') is uncommon and best corrected with diuretics and other measures. Only once the patient is stabilized should the decision to cautiously transfuse be reconsidered - this would be standard paediatric practice globally. Second, severe dehydration (loss of intracellular water and electrolytes) should be corrected with crystalloidal solutions and not transfusion (the latter leading to a sluggish, haemoconcentrated circulation). Finally, hyperparasitaemia is very frequent among paediatric admissions in malaria endemic areas and has not been shown to be an independent risk factor for poor outcome. Inclusion of this criteria may result in the substantial overuse of a limited transfusion supplies by a large group with low risk.

The three other criteria (shock, impaired consciousness and deep breathing (a clinical sign of metabolic acidosis)) do have scientific justification for identifying SA subgroups with high immediate risk of mortality. Overall mortality in children with a Hb <4g/dl or SA with life-threatening complications is 15%[4]. Clinical studies in Kenya[11, 12] have shown that profound anaemia (Hb<4g/dl) is independently associated with death (OR=2·5), as is SA (defined in this study as Hb<5g/dl) complicated by reduced consciousness (OR=7·4) or respiratory distress (OR=4·1). Many deaths occur within 48 hours of admission, with 25-50%[13, 14] occurring within 6 hours. In the FEAST trial which enrolled children with shock, a higher case fatality was found in those with anaemia compared to those without anaemia, irrespective of intervention group[15]. In children with uncomplicated SA - a Hb of 4-6g/dl without prostration or respiratory distress - overall case fatality is 4-6%, being lower in parasitaemic children (2-3%)[16] than in those with negative malaria slides (8-10%)[11]. The ratio of complicated to uncomplicated SA is commonly 1:1[17].

1.2.3 Relevant clinical trials of transfusion and Systematic Reviews

A Cochrane review including the only 2 African randomised controlled trials (RCTs) [18, 19] conducted to date (involving 114 and 116 children randomised to blood transfusion or oral haematinics) concluded that there was insufficient information on whether routinely giving blood to clinically stable children with severe anaemia either reduces death or results in a higher haematocrit measured at one month, and indicated the need for a definitive trial[7]. A prospective, randomised, controlled, non-inferiority trial in relatively stable Canadian and European children demonstrated that a restrictive transfusion protocol (with a transfusion threshold <7g/dl) was as safe as a liberal protocol (threshold <9g/dl)[20]. Subsequently, practice guidelines in these countries have been amended to include restrictive transfusion (Hb<7g/dl). It remains to be established whether, as currently advocated by WHO, restrictive guidelines can be safely

applied without detrimental consequences at even lower levels of Hb (4-6g/dl if stable) in African children with no access to the other supportive treatments available in Europe and North America.

A literature review by Brabin and colleagues[4], reporting case fatality in studies of SA from malarious areas in SSA, indicated wide variations in outcome. Mean in-hospital case-fatality rate for severe anaemia (Hb <5 or <6g/dl depending on study definition) was 9% (range 4-39%). Mortality was significantly higher in children with a Hb <5g/dl (pooled RR=1.92 vs >5g/dl, 95% CI 1.7–2.2). Evidence for an increased risk with less severe anaemia was not conclusive: although the risk of death was increased for a Hb <8g/dl, the confidence intervals were wide (below: Fig 4 from Brabin *et al* 2001)[4]. The heterogeneous group of children included and outcomes observed make it difficult to draw specific conclusions. Other studies have addressed this by classifying children into subgroups based on clinical severity and Hb levels. Using this approach, available observational data also indicate no clear association between the receipt of a blood transfusion and in-hospital mortality in children with uncomplicated SA[12]. However, such data are likely to be subject to confounding by indication, because children with a poorer underlying prognosis will be more likely to receive a transfusion. Post-discharge morbidity and mortality are important considerations in this group, but there are few data on the cumulative incidence of poor outcomes in the longer-term.

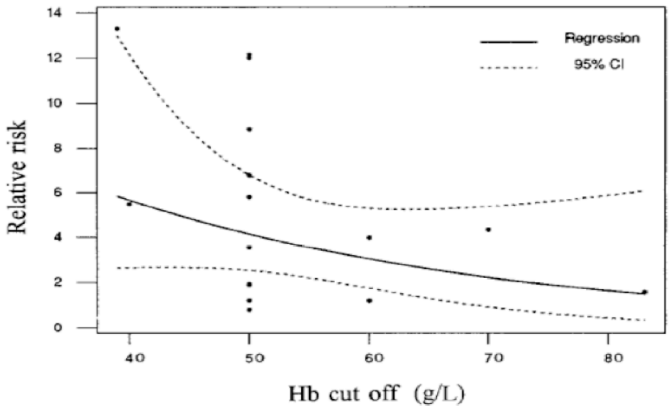


FIGURE 4 Relative risk for child mortality in relation to hemoglobin (Hb) cut-off points. Log $Y = 1.30 - 0.137X$; $R^2 = 13.8\%$; $P = 0.156$. CI, confidence interval.

1.2.4 TRANSFUSION VOLUME AND NEED FOR RE-TRANSFUSION

Current transfusion guidelines are conservative not only in terms of criteria applied for administering a transfusion at all, but also in terms of the volume of blood transfused. Currently, 20ml/kg of whole blood (or 10ml/kg packed cells) are recommended for all levels of anaemia below Hb<6g/dl[3]. Using standard formulae to calculate volume required[21] this under-treats children with profound anaemia by ~30% and this volume may not, therefore, be sufficient to correct anaemia. Larger initial transfusion volumes have not been systematically evaluated. In fact, few data are available on the volumes received. Lackritz reported mean volumes of 26ml/kg whole blood[12] and others following WHO guidelines have shown a modest Hb rise of 2.5-3.3g/dl[11, 12, 22] following initial transfusion with ~25% remaining severely anaemic (<5g/dL)[11]. Anecdotal evidence suggests that multiple, low volume (20ml/kg) transfusions are frequently given, which is wasteful, inefficient and exposes children to additional risks (eg reaction and infection).

Unpublished data from the FEAST trial[15] shows that 1422 (45%) children who were enrolled with signs of shock received a blood transfusion. Overall 322 (23%) of those transfused received 2 or more transfusions, the proportion being greater (212/612, 35%) in those with a Hb <4g/dl at enrolment. Total mean (SD) blood volumes per child were 23.3 (9.5) ml/kg, with those receiving two transfusions receiving a mean (SD) of 36.6 (7.3) ml/kg in total over their 2 transfusions (mostly 2x~20ml/kg). This proportion might be reduced considerably if larger volumes of blood are given initially, an outcome that would also reduce transfusion service time to prepare blood and the risks of transfusion-related morbidity, arguing the need for a trial of initial volumes of transfusion. Follow-up in FEAST was only to 28 days; a key question that will be

addressed in this trial is whether greater initial blood volumes could also reduce longer-term anaemia recurrence. A prospective study of 128 Malawian children aged 3-60 months, transfused according to WHO guidelines, examined transfusion failure (defined as a Hb≤6 g/dl >24hours post-transfusion). Only 104 (81%) received the prescribed volume; of these, 24 (23%) were classified as transfusion failures and 83% of these had a subsequent Hb<4g/dl.[23]

1.2.5 Recent Trends in Transfusion Safety and Malaria Endemicity

The conservative transfusion guidelines were developed to protect scarce resources, avert overuse, and reduce the risk of transfusion-transmissible infections. However, in recent years considerable progress has been made with regard to blood safety in many countries in SSA since the US President's Emergency Plan For AIDS Relief (PEPFAR) began to provide direct support for strengthening transfusion services, improving the supply and safety of transfusion by establishing regional centres to replace hospital-based systems and by providing quality assurance for viral testing[24]. Previously, access to and safety of blood for transfusion would have predicated whether the findings of a trial demonstrating benefit of transfusion in stable children with a Hb 4-6g/dl could be practically implemented, unless new recommendations also considered increasing supply. In some parts of Africa the capacity of transfusion services to provide blood has greatly increased due to year-on-year declines in the intensity of malaria transmission that have led directly to reductions in hospitalisation of children with malaria[25], and indirectly to reduced utilisation of blood transfusion services[17]. However, this is not universal and for the sites involved in the TRACT trial malaria epidemiology has not declined and may have even increased[26] and thus remains a common cause of hospitalisation and transfusion.

1.2.6 Short and Longer term Outcomes in children with severe anaemia

A study in Gambian children showed comparatively better recovery in children with Hb of 4-5g/dL receiving iron treatment than those who received a blood transfusion[19]. In Kenyan children blood transfusion was important in preventing death in children with severe symptomatic malaria anaemia but did not seem to influence faster or superior haemoglobin recovery at one month follow up, with mean haemoglobin at discharge (approximately 3-4 days) being similar in transfused 6.4g/dL [SD: 1.5] and non transfused 6.8 g/dL [SD: 1.6] and remaining similar at follow up (28-35 days) in the transfused (10.2g/dL) and non-transfused (10.0g/dL) groups (P=0.25). The major factor affecting mean haemoglobin concentration at follow-up was concurrent malaria parasitaemia (8.8g/dL compared with a mean of 10.5g/dL in those without parasitaemia, p<0.001)[27], with additional significant effects of both young age (<24 months) and the type of malaria treatment (p=0.03).

Most children are therefore discharged with only partial correction of anaemia. The limited data available indicate a trend towards higher rates of post-discharge mortality, recurrence of anaemia and re-hospitalisation in children with SA than non-SA hospital or community controls. The Malawi case-control study of paediatric SA[5] showed that 17% of cases were readmitted within 6 months of discharge and 6% re-developed SA, compared to re-admission rates of 9-10% and <0.5% respectively in hospital and community controls. In-hospital and 6-month post-discharge mortality was greater in the cases (6% and 8% respectively) than in either the hospital- (0% and 1.6% respectively) or community-controls (0% 6-month mortality). Holzer[18] showed a non-significant increase in deaths at 8 weeks in children with uncomplicated SA randomised to no transfusion (4% vs 2%) and more hospital re-admissions (8%, 4/53) than the transfused group (2%, 1/52). Thus transfusion alone may not achieve optimal outcomes for children with SA, suggesting that exploration of complementary treatments is also essential.

1.2.7 Aetiology and co-morbidities

The causes of anaemia are multi-factorial with several co-factors causally related to mortality risk. To improve poor SA outcomes, it is thus likely that complementary treatment approaches will be necessary, including both immediate (transfusion), and longer term interventions. Malaria still plays an important role

in SA, even if this is less than in previous decades. In Kilifi, Kenya we recently reported that the epidemiological transition in malaria transmission has led to a decline in hospital paediatric admissions (including malaria and SA)[25] and reduced demand for transfusion. However, SA case fatality over this period remained unchanged at 8-10%[17].

In the only comprehensive case-control study of children hospitalized with SA in Africa[6], key associations with SA were bacteraemia (OR=5.3; 95% CI 2.6-10.9), malaria (2.3; 1.6-3.3), hookworm (4.8; 2.0-11.8), HIV infection (2.0; 1.0-3.8), vitamin A deficiency (2.8; 1.3-5.8) and vitamin B12 deficiency (2.2; 1.4-3.6). Neither iron nor folate deficiencies were associated with mortality, and were less prevalent among cases than controls. As iron, folate and anti-helminthics (for any child > 1 year of age that has not received antihelminths in the last 6 months) are already recommended post-SA-discharge[3], the clearest potentially modifiable underlying causes of late anaemia recurrence, which we propose to address in this trial, are nutritional factors and recurrent bacterial infections.

1.2.8 TREATMENT AND PREVENTION OF NUTRITIONAL DEFICIENCIES

WHO treatment guidelines deal specifically with malaria and with folate and iron deficiency that together are widely held as the most important causes of anaemia. Although folate supplementation is recommended, folate deficiency was not found in the Malawian SeVana study[5], in agreement with previous reports[28] and observations that folate supplementation in anemic children with malaria failed to raise haemoglobin concentrations[29]. Unlike folate and iron, Vitamin B12 and Vitamin A supplementation are not recommended in guidelines for the management of severe anemia. In the Malawian SeVana study vitamin B12 deficiency was found in 30.4% of case patients and severe Vitamin A deficiency (less than 10 µg per deciliter) in 32.8% of case patients (versus 14.9% of controls) and was associated with severe anemia[5]. These observations concur with findings among adults in that region[30, 31] and may be explained by the lack of animal products in the diet of Malawian children. However, what is unknown is whether a specific intervention to increase vitamin A and B12 levels would have measurable benefits or whether potential benefits generalise to other SSA cohorts.

Iron supplementation is effective for reduction of iron deficiency and anaemia in iron deficient children. However, a community-based randomized controlled trial in Zanzibar designed to evaluate the impact of zinc and iron plus folic acid supplementation on morbidity and mortality in young children showed that supplementation may also be associated with adverse effects, specifically increased risk of hospitalization (primarily due to malaria and infectious disease), and mortality in malaria-endemic areas[32]. WHO have revised recommendations advising that iron and folic acid should only be targeted to those who are anaemic and at risk of iron deficiency. In addition, they recommended that such children should receive concurrent protection from malaria and other infectious diseases through prevention and effective case management (http://www.who.int/childadolescent-health/-New_Publications/CHILD_HEALTH/WHO_FCH_CAH_06.2.pdf.) As far as we are aware this approach has not been subsequently evaluated in a factorial trial. Moreover, it is not clear whether the currently recommended 'treatment' doses (which assume that the child with severe anaemia are either or both iron or folate deficient) result in better or worse outcome that supplementation (incorporating lower doses of iron and folate).

Also acknowledged in the WHO statement following the Zanzibar study was that these recommendations should not be extrapolated to fortification (or food-based approaches) for delivering iron, where the patterns of iron absorption and metabolism may be substantially different. However in a recent controlled trial reported from Cote D'Ivore[33] which compared fortification of biscuits with 20 mg Fe/day per child 4 times/week to control (biscuit minus iron fortification) found that faecal microbiota were modified by iron fortification compared to control- showing a significant increase in the number of enterobacteria and a decrease in lactobacilli in the iron group at 6 months. In the iron group, there was an increase in the mean faecal calprotectin concentration (a marker of gut inflammation) which correlated with the increase in fecal enterobacteria. Low bioavailabilty and absorption of iron fortificants results in 90% of the iron passing unabsorbed into the colon. Beneficial barrier bacteria, such as lactobacilli, play an important role in the

prevention of colonization by enteric pathogens but do not require iron. In contrast, for most enteric gram-negative bacteria (eg, Salmonella, Shigella, or pathogenic Escherichia coli), iron acquisition plays an essential role in the virulence and colonization of most pathogenic strains[34].

These are important but unstudied consequences of addition the liberal (WHO recommended treatment doses) versus conservative use of iron (at supplementary doses) in malaria endemic populations at high risk of potential iron deficiency.

In children and infants there are several formulae to treat iron deficiency anaemia (IDA) but compliance is often poor as a result of repeated daily dosing, and unpleasant side effects such as the metallic after-taste, staining of the child's teeth (unless the teeth are wiped off immediately) and abdominal discomfort[35]. This has resulted in the development of micronutrient powders (eg Nutromix™ and Sprinkles™) as a novel approach for delivering iron and other micronutrients[36].

1.2.9 Multivitamin Multi-Mineral Powders (MVMM)

The Blantyre SeVana study[5], demonstrating that several micronutrients deficiencies are also aetiologically important in SA, suggests that MVMM could be an attractive and low risk strategy for correcting underlying nutritional-related anaemia. Several cheap MVMM supplements such as Sprinkles Plus™ and Nutrimix™ are widely available. Sprinkles are single-dose daily packets of dry powder containing lipid-encapsulated iron and other micronutrients which are added to any home-prepared food product[36]. A number of studies have demonstrated the efficacy of Sprinkles Plus in treating and preventing anaemia[37, 38]. Aside from iron, the various formulations of MVMM included essential micronutrients such as vitamins A, C and D, folic acid, iodine and zinc in doses that prevent and treat micronutrient deficiencies and improve overall nutritional status. Lipid encapsulation of the iron prevents its interaction with food and masks its taste, and may also reduce gastrointestinal discomfort. The sachets are lightweight and thus are simple to store, transport and distribute. Sprinkles have a long shelf life, even in hot or humid conditions (2 years). Sprinkle formulations were originally developed for infants and young children between 6-24 months of age as limited options exist for the treatment and prevention of micronutrient deficiencies in this age group. However, they have more recently been tested in older populations[39, 40]. Sprinkles or iron/folate supplements are not considered in children < 6 months in whom exclusive breast-feeding is recommended as the main mechanism for supporting full nutritional intake. Specific formulations of MVMM have been developed for pregnant and lactating mothers[41]. These can be given to also help treat breast-feeding infants. Side effects include darkening of the stool, constipation or mild diarrhoea. These maybe transient but if they do not subside caregivers are advised to use half a package of sprinkle formulation at 2 different mealtimes throughout the day.

None of these MVMM been tested against WHO recommended treatments in children hospitalised with severe anaemia for meaningful outcomes such as prevention of SA relapse, readmission or re-transfusion, and longer-term mortality.

1.2.10 Preventing Bacterial Infection: Cotrimoxazole

Cotrimoxazole is a synthetic antibacterial combination (sulphamethoxazole and trimethoprim) that acts by blocking folate metabolism involved in the biosynthesis of nucleic acids and proteins essential to bacteria and some parasites, including *Plasmodium falciparum*. High levels of sulphamethoxazole and trimethoprim are found within granulocytes and there is evidence that cotrimoxazole specifically enhances intracellular bacterial killing[42, 43]. The substantial mortality benefits (allied with extremely low rates of toxicity) associated with cotrimoxazole prophylaxis in HIV-infected children[44] have generally been attributed to reductions in bacterial infections[45, 46]. Of note, these benefits have been observed even in areas of high background resistance[47]. The fact that mortality benefits cannot be attributed solely to pneumonia[45, 46] raises the intriguing possibility that cotrimoxazole may act on a number of different pathways – the most important with regards to SA relapse being enteropathy and intestinal permeability, although any benefits of cotrimoxazole on microbial translocation and/or systemic immune activation, or on reducing

recurrent infections during recovery from SA could also impact longer-term morbidity. Cotrimoxazole has been shown to be effective in preventing malaria in HIV-negative children aged > 5 years [48] and in HIV-exposed (HIV negative children born to HIV-infected mothers) and HIV-infected children[49]; despite high levels of background parasite resistance to sulphamethoxazole.

Cotrimoxazole is cheap, widely available and has an established safety profile in African populations. A 5-day course is currently recommended by WHO for children with uncomplicated severe malnutrition, and cotrimoxazole prophylaxis is also used for children with other immunodeficiencies, especially those with defective neutrophil function. It is plausible, therefore, that cotrimoxazole could also reduce bacterial infections following SA: the question is whether this would impact on SA recurrence.

A recent systematic review found that sulfadoxine-pyrimethamine (which is similar in activity to cotrimoxazole) resulted in a 57% (95%CI 24%–76%) reduction in all-cause mortality[50]. Of interest, the effect of these other drugs used for intermittent preventative treatment (IPT) was similar to artemisinin combination therapies in terms of prevention of malaria. The investigators concluded that a preventative strategy that targeted both malaria and bacterial infections was important and that cotrimoxazole is an obvious candidate for such an approach. This supports the concept that short courses of such drugs may have significant benefits (eg similarly to that of a single azithromycin dose in Ethiopian children[51]).

While cotrimoxazole prophylaxis is widely available and cheap, it is not free and therefore universal use in young children is unlikely to ever be practical. However, demonstrating the value of a 3 month course in high-risk populations such as those with SA would very likely change practice.

1.2.11 RELEVANT STUDIES UNDERWAY OR PLANNED

We note that one randomized, placebo controlled trial of cotrimoxazole prophylaxis amongst HIV-uninfected children with severe malnutrition in currently enrolling in 4 sites in Kenya (http://clinicaltrials.gov/show/NCT00934492). The results are unlikely to be relevant to the majority of children eligible for TRACT trial, since most of these will not be severely malnourished. If WHO does change policy then we would amend the protocol to ensure that children with severe malnutrition, like those who have HIV, will routinely be prescribed cotrimoxazole prophylaxis at discharge. We are unaware of any trial in resource-limited settings addressing mortality as the primary endpoint in children with severe anaemia and were not able to find any planned trials of transfusion or MVMM in children with severe anaemia (ClinicalTrials.gov).

1.3 RATIONALE FOR CURRENT STUDY

In SSA, where infectious diseases and nutritional deficiencies are common, treatment options for the emergency management of children with severe anaemia are very limited. To avert overuse of blood products, the WHO advocate a conservative transfusion policy, reserving blood for children with a haemoglobin (Hb) of <4g/dl (or <6g/dl if accompanied by complications), and providing iron, folate and anti-helminthics (if >1yrs) at discharge. However, within the WHO guidelines these specific recommendations have not been systematically evaluated and they contain inconsistencies and ambiguities[3] resulting in variation in practice across African countries. This is particularly true in the subgroup with 'uncomplicated' severe anaemia (Hb 4-6g/dl without specific complications), where transfusion avoidance is recommended[7]. This large, heterogeneous group includes many children with infectious diseases or other conditions (including sickle cell disease[52]) whose outcome is often worse than their non-anaemic counterparts[4]. The lack of definitive evidence to support these recommendations, together with continuing high rates of re-admission[5] and death[6], suggests that children may well be receiving suboptimal treatment. At a workshop in Kenya in 2008, transfusion service providers, users, and researchers from across Africa called for better evidence to guide the emergency management of children with severe anaemia and to support the WHO policy for withholding blood

transfusions in stable children with a Hb of 4-6 g/dl (www.afsbt.org - Workshops). TRACT was designed as a direct response to this articulated need.

A poor or incomplete response to recommended treatment in children with SA results in relapse, readmission and death. Because severe anaemia is very common, the high 'hidden' morbidity and mortality occurring within the first few weeks after initial diagnosis is likely to contribute importantly to overall under-five mortality[5]. If not adequately addressed, severe anaemia may thus be an obstacle to the achievement of Millennium Development Goal No.4 on child survival in Africa. Combining different strategic approaches to reduce mortality, including more liberal and larger-volume transfusion to increase red cell mass and improve tissue oxygenation, may reduce immediate mortality, subsequent transfusion requirements and morbid and fatal events. It is essential to assess the longevity of any benefits of transfusion and greater transfusion volumes by evaluating post discharge morbidity and mortality, especially where nutrient deficiencies may impair recovery. Addressing these deficiencies by using multi-vitamins and preventing further infections with cotrimoxazole prophylaxis post-discharge might have their greatest impact after the effects of transfusion have waned (by 2-3 months), but could be cheap and low-risk strategies for maintaining risk reductions over the longer-term.

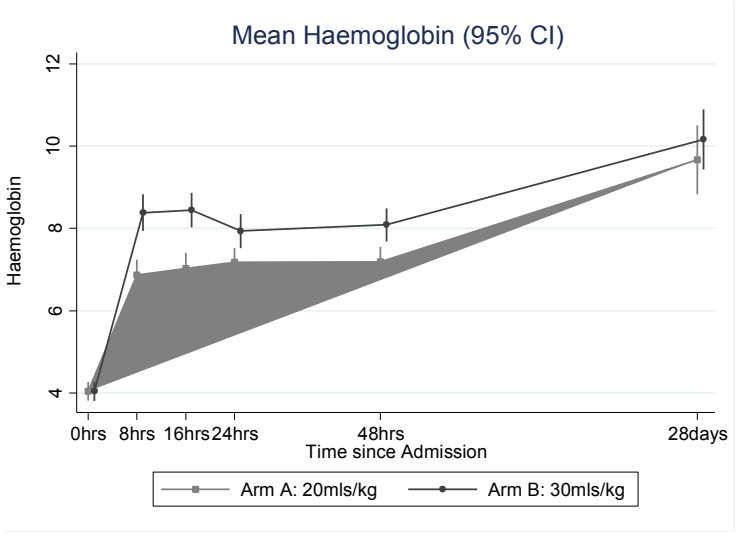
The principal research question is how best to reduce short and long term mortality and morbidity in African children with severe anaemia. We will address this by evaluating three components that could form an integrated treatment package for severe anaemia: transfusion, micro-nutrient supplementation to address underlying nutrient deficiencies and short-term antimicrobial prophylaxis to prevent recurrent infections. Each targets a different mechanism for reducing mortality and morbidity and targets both early and late outcomes: effects are thus expected to be additive. A major reason for including short and long term approaches in the same factorial trial is efficiency: it is more economical and faster to run one large trial than multiple separate trials. Further, rather than leaving these other management aspects to site and clinician preference, a factorial randomisation allows them to be directly controlled and evaluated alongside transfusions.

1.3.1 RISKS

Both MVMM and cotrimoxazole prophylaxis have been widely used in children with minimal risk. Although substantial efforts have been made to ensure the safety of blood, failure to correctly cross-match and/or infected blood have the potential to cause harm. The trial will directly evaluate whether these potential risks are outweighed by improved survival. TRACT teams will work closely with the local blood transfusion services (BTS) to ensure that recommended safety and quality control practices are being maintained. We anticipate developing a blood safety committee for each site, which will meet regularly. Severe adverse event management will follows a dedicated SOP developed for FEAST, that we also plan to use in TRACT.

Blood samples would be required from all study children. However, the volumes of blood required would be minimized wherever possible and be kept well within the maximum locally agreed volumes.

A pilot study, conducted in two sites in Uganda (Oct 2011-Dec 2012) in children fulfilling TRACT eligibility criteria evaluated the safety of a higher volume of whole blood transfusion (30mls/kg: 80 children) compared to the standard volume (20mls/kg: 80 children) (ClinicalTrials.Gov NCT01461590). The study was designed to provide comparative data on safety, and qualitative data on feasibility and operational components of implementation of the study protocol with special reference to the transfusion service. Adherence to volumes of transfusion was excellent. Correction of severe anaemia was superior in the 30mls/kg than the 20mls/kg (see Graph/table below). Nevertheless, this was a small pilot trial: whilst it is encouraging that our hypothesis that a larger initial volume would result in an greater increase in haemoglobin was supported, the small sample size means that it cannot conclusively demonstrate that this leads to improvements in mortality and other long-term benefits, the principal question under investigation in TRACT.



Global test of difference between the arms through to 28 days: p<0.0005						
Time:	0hrs	8hrs	16hrs	24hrs	48hrs	28days
N Arm A:	82	73	76	77	75	32
N Arm B:	78	74	75	74	74	36
P=		<0.0005	<0.0005	0.006	0.003	0.32

Severe adverse events (SAEs) were reported in 7 of the 160 children enrolled. 6 SAEs were deaths, most occurring within <5 hours of admission. All deaths were in the 20ml/kg group. One non-fatal transfusion reaction was reported (in the 30ml/kg arm): a child developed generalised body itching and an urticarial rash shortly after starting the transfusion.

1.3.2 BENEFITS

Extra clinical personnel, regular patient clinical assessment and the basic equipment for continuous patient monitoring will be available during the trial so that if the complications above were to arise they could be detected and treated. Pre-trial training will include sign recognition for these complications and training on treatment. Both these will be covered in detail in the Manual of Operations which will be available on the ward.

1.3.3 FOR THE CHILD

The direct benefits to the child and/or family (outlined in the PIS) include:

- Closer observation during the first 48 hours of admission, which, as a result, allows doctors and nurses to make important changes to the child's treatment during in-hospital admission.
- All routine non-trial medications required by the hospital to treat the child will be made available (when unavailable parents have to resort to sourcing these privately).
- The parents or guardians for the children will be asked to return for follow up at this clinic 28, 90 and 180 days after admission. Reimbursement for transport cost after discharge and for follow up visits plus any treatment costs required during the visits will be made.

1.3.4 FOR THE HOSPITAL

The direct benefits to the hospital include:

- Supporting blood transfusion safety committees : monitoring operational aspects and safety.
- Management of severe uncomplicated anaemia – the 50% control group (receiving no transfusion) should decrease the demand for blood and adherence with WHO restrictive transfusion guidelines.

1.3.5 FOR HEALTH PERSONNEL

The direct benefits to health personnel are mainly professional development of the members of the trial teams and clinical teams for the purposes of running the trial – including basic life support course, clinical trials training, research training.

2 SELECTION OF CENTRES AND CLINICIANS

Four hospitals in two countries will participate:

- **Uganda:** Mulago National Referral Hospital, Kampala; Mbale Regional Referral Hospital; and Soroti Regional Referral Hospital
- **Malawi:** Queen Elizabeth Central Hospital/Wellcome Trust Unit, College of Medicine, University of Malawi, Blantyre

These centres have been chosen on the basis of criteria below.

2.1 CENTRE/INVESTIGATOR INCLUSION CRITERIA

To participate in the trial, investigators and clinical centres must fulfil a set of basic criteria defined below.

2.1.1 CENTRE PIS QUALIFICATIONS & AGREEMENTS

1. The centre PI should be qualified by education, training, and experience to assume responsibility for the proper conduct of the trial at their centre and should provide evidence of such qualifications through an up-to-date curriculum vitae and/or other relevant documentation requested by the Sponsor, the REC or IRB, and/or the regulatory authority(ies).

2. The centre PI should be aware of, and should comply with, the principles of ICH GCP and the applicable regulatory requirements. A record of GCP training should be accessible for all investigators at the centre.

3. The centre PI should permit monitoring and auditing by the Sponsor, and inspection by the appropriate regulatory authority(ies)

4. The centre PI should maintain a delegation log of appropriately-qualified persons to whom the investigator has delegated significant trial-related duties.

5. The centre PI should sign an investigator statement, which verifies that the centre is willing and able to comply with the requirements of the trial.

2.1.2 ADEQUATE RESOURCES

1. The centre PI should be able to demonstrate a potential for recruiting the required number of suitable subjects within the agreed recruitment period

2. The centre PI should have sufficient time to properly conduct and complete the trial within the agreed trial period.

3. The centre PI should have available or appoint an adequate number of qualified staff for the duration of the trial to conduct the trial properly and safely.

4. The centre PI should ensure that all persons assisting with the trial are adequately informed about the protocol, the investigational product(s), and their trial-related duties and functions.

2.1.3 CENTRE ASSESSMENT

Each selected clinical trial centre must provide a completed Investigator Statement, Signature and Delegation of Responsibilities Log, and staff contact details. The Investigator Statement verifies that the

centre is willing, and able to comply with the requirements of the trial. This will be signed by the Principal Investigator at the centre. In addition, and in compliance with the principles of ICH GCP, all centre staff participating in the trial must complete the Signature and Delegation of Responsibilities Log and forward this to the Clinical Trial Facility, KEMRI Wellcome Trust Programme. The CTF KWTP must be notified of any changes to trial personnel and/or their responsibilities. An up-to-date copy of this log must be stored in the Trial Master File (TMF) at the centre and also at the CTF KWTP.

2.2 APPROVAL AND ACTIVATION

On receipt of the above documents at the CTF KWTP, written confirmation will be sent to the PI.

1. The centre should conduct the trial in compliance with the protocol as agreed by the Sponsor and, if required, by the regulatory authority(ies), and approved by the REC and/or IRB.

2. The centre PI or delegate should document and explain any deviation from the approved protocol, and communicate this with the trial team at the CTF KWTP.

A list of activated centres may be obtained from the Trial Administrator.

3 STUDY OBJECTIVES AND DESIGN

3.1 STUDY OBJECTIVES

The primary objective of the trial is to identify effective, safe and acceptable interventions to reduce short and longer-term mortality and morbidity following admission to hospital with severe anaemia in sub-Saharan Africa. There are two hypotheses being tested

1) A liberal rather than a conservative blood transfusion policy will decrease mortality (cumulative to 4 weeks) in children admitted to hospital with severe anaemia (haemoglobin (Hb)<6g/dl).
2) Supplementary multi-vitamin multi-mineral (MVMM) treatments or additional cotrimoxazole prophylaxis or both for 3 months post discharge will reduce rates of readmission, severe anaemia relapse, re-transfusion or death (cumulative to 6 months) compared to current recommendations (iron and folate) and anti-helminths in all if >1 year.

Secondary objectives include

- To identify the most cost-effective interventions to reduce early mortality, and assess their budget impact
- To determine efficacy of long-term support strategies (MVMM and cotrimoxazole prophylaxis) on other markers of nutritional status and causes of death
- To determine the effect of transfusion strategies and long-term support strategies on markers of inflammation and immunological activation and function
- To identify the mechanism of action of the most effective interventions through focussed investigations of human genetic polymorphisms, molecular diagnostics, immunological activation, markers of gut barrier dysfunction, haemotological and nutritional response

3.2 STUDY DESIGN

TRACT is a multicentre randomised controlled trial of 3954 children aged 2 months to 12 years admitted to hospital with a Hb<6g/dl (taken within 2h of admission). Children will be enrolled over 2 years from 2 countries and followed for 6 months. The trial will simultaneously evaluate three ways to reduce short and longer-term mortality (primary endpoint) and morbidity following admission to hospital with severe anaemia in sub-Saharan Africa using a factorial design: (i) liberal rather than conservative blood transfusion; (ii) additional multi-vitamin multi-mineral supplements for 3 months post-discharge; (iii) additional cotrimoxazole prophylaxis for 3 months post-discharge. All randomisations will be open.

3.2.1 PROPOSED TRIAL DESIGN

Randomised controlled factorial trial with a 3x2x2 design.

3.2.2 TRIAL INTERVENTIONS

The trial will have a factorial design with 3 randomisations, each to address one of the potential approaches to reducing mortality and morbidity in children with SA (See **Trial Flow diagram: page *x*)**:

R1: Immediate liberal transfusion (30ml/kg) versus conservative transfusion (20ml/kg) versus no transfusion (last strategy only for children with **uncomplicated SA and a Hb 4-6 g/dl**).

R2: Post-discharge multi-vitamin multi-mineral (MVMM) supplementation for 3 months (which includes folate and iron) and anti-helminthics if >1 years versus routine care (folate and iron at standard treatment doses (varies with age) for 3 months) and anti-helminthics if >1 years.

R3: Post-discharge cotrimoxazole prophylaxis versus no prophylaxis for 3 months.

R1 addresses both conservative aspects of current guidelines: "whether to give blood" in uncomplicated SA (4-6g/dl without complications), and "how much blood to give" in all children with SA. The transfusion and post-discharge interventions will be open-label for reasons of practicality and compliance.

Anti-helminthics will be used following national guidelines (single dose 500mg mebendazole in Uganda, single dose 400 mg albendazole in Malawi).

Potential for interactions between the trial interventions: Because the transfusion, nutritional and antibiotic prophylaxis interventions approach different mechanisms for reducing short and long term mortality/morbidity following SA, we consider that important interactions between randomised groups are unlikely. Further, any interactions that do exist are likely to be quantitative (slightly smaller/larger effects) rather than qualitative (effect on one background, no effect on another).

3.2.3 STUDY NUMBERS

A sample size of 3954 SA cases will be enrolled – to enable multiple comparisons to be made and allowing for a 6% loss to follow-up by 6 months. Since the sample size assumptions are sensitive to the relative contribution of uncomplicated : complicated SA; the uncomplicated SA strata will be capped at 2000 cases, so that at least 1950 of trial participants will have complicated SA – so that the trial retains at least 80% power to address all the key objectives.

3.3 STUDY OUTCOME MEASURES

3.3.1 PRIMARY OUTCOME

Cumulative mortality to 4 weeks for the transfusion strategy comparison, and to 6 months for the nutritional support/antibiotic prophylaxis comparison.

Protection against bias is principally by the use of a completely objective endpoint (mortality). Any patient lost to follow-up before 6 months without withdrawing consent will be traced for vital status.

Cause of death (and other clinical secondary endpoints) will be adjudicated by an Endpoint Review Committee, blinded to randomised allocations: relationship to all possible interventions drugs (transfusion strategy; nutritional support and cotimoxazole) will be solicited to avoid unblinding.

3.3.2 SECONDARY OUTCOMES

Mortality:

- mortality at 48 hours, 28 days, 90 day and 180 days (cumulative) (where not the primary outcome).

Morbidity: endpoints relating to the specific mechanisms of action of each intervention:

- re-admission to hospital;
- proportion achieving correction of anaemia (defined by WHO as Hb>9g/dl) at 48 hours, 28 days, 90 day and 180 days
- development of new profound anaemia (Hb<4g/dl) during acute admission or development of severe anaemia (Hb<6g/dl) post discharge;
- nutrition: changes in weight and MUAC at 90 day and 180 days
- anti-infection: changes in inflammatory markers (CRP,PCT), incidence of bacterial infections and malaria at 28 days, 90 day and 180 days

Solicated adverse events

- suspected transfusion reactions: febrile reactions, Transfusion Related Acute Lung Injury (any grade); grade 3-4 toxicity of cotrimoxazole, MVMM or standard iron/folate
- SAEs

Others

- costs and cost-effectiveness.

A comparison of mortality 90 day and 180 days post-discharge will enable us to identify the maximal impact of enhanced long-term support and any rebound effects.

4 PARTICIPANT ENTRY

4.1 PRE-RANDOMISATION EVALUATIONS

4.1.1 SCREENING PROCEDURE

Each day the clinical team will check with the transfusion services the quantities of blood available for transfusion. Screening, and enrolment, will not be conducted on days when supplies are critical. Numbers of patients enrolled each day will vary from site to site and depend upon blood stocks and requirement by non-study children. Eligible children will be identified by the nurse and clinician on duty and registered in the eligibility screening log. A member of the trial team will then perform a rapid structured assessment of heart rate, oxygen saturation (pulse oximetry), respiratory rate, axillary temperature, blood pressure, markers of shock (capillary refill time, pulse volume and assessment of lower limb temperature) and severity (conscious level and respiratory distress). Children who are potentially eligible with suspected severe anaemia will have a rapid bedside test (Haemocue) to determine haemoglobin level and eligibility. For children with a haemoglobin < 6 g/dl admitting clinicians will enquire about a history of passing dark or red urine in this illness (to determine severity) and whether the child is known to have sickle cell disease. Details of those fulfilling the entry criteria including severity will be entered onto a screening form, while reasons for non-eligibility will be added to the eligibility screening log. Entry criteria will be based on clinical assessment alone. It is anticipated that this process will take approximately 5 minutes.

4.1.2 INCLUSION CRITERIA

- Aged 2 months to 12 years
- Severe anaemia (SA) (Hb<6g/dl) within 2h of admission to hospital
- Carer willing/able to provide consent

4.1.3 EXCLUSION CRITERIA

- Malignancy or other terminal illness
- Children who are exclusively breast fed (thus unable to take nutritional support)
- Chronic renal or liver failure
- Surgery as main reason for admission
- Acute trauma or burns as main reason for admission
- Signs of bi-ventricular heart failure
- Known congenital or valvular heart disease (non-surgically corrected)

See section 5.4 and 5.5 for i) nutritional management of infants below 6-months; ii) management of children with severe malnutrition with regards to MVMM supplementation, and iii) management of children with HIV with regards to cotrimoxazole prophylaxis. These children will not be excluded from a pragmatic trial but are all relatively small but important subgroups in who will either follow standard of care and/or have some pragmatic adjustment in method or timing of delivery of the R2 or R3 randomisations.

4.2 RANDOMISATION AND ENROLMENT PROCEDURE

4.2.1 RANDOMISATION CODES

Randomisation in each part of the factorial would be stratified by centre and the other randomisations in the factorial. Randomisation lists, using variable block sizes, will be generated and kept at the MRC CTU, London. The randomisation envelopes will be prepared before the trial, with one set for complicated SA (A) and one for uncomplicated SA (B). Eligible children will be screened and recruited at the time of hospital admission. At enrolment sealed cards will simultaneously assign interventions R1A/B (according to SA strata), R2 and R3 randomly. The cards for each centre will be numbered consecutively and opened in numerical order. These will contain the actual allocation (transfusion strategy, and long-term intervention(s)) visible only once opened. This system has worked well in the emergency care trial, FEAST. To facilitate protocol adherence, a maximum per site will be agreed upon (for example up to 4 children per day) will be enrolled per site. This approach was very successful with respect to protocol adherence and the quality of data generated in the FEAST trial. It will also put less pressure on the blood banks.

4.2.2 CO-ENROLMENT GUIDELINES

Patients will not ordinarily be permitted to participate in any other clinical intervention trial or research protocol while on the TRACT trial. Participation in other studies that do not involve an intervention may be acceptable, but should be discussed with the TRACT TMG. The TRACT TMG will consider co-enrolment of TRACT participants onto other trials where the interventions do not conflict with the TRACT objectives on a case-by-case basis.

5 TREATMENTS

5.1 STANDARD CASE MANAGEMENT- IN-HOSPITAL

All trial patients will receive standard of care including antibiotics (iv or oral) anti-malarial drugs following national guidelines, based on WHO syndromic patient management[3]. We will collect data on all administered drugs. Antipyretics, anticonvulsants and treatment for hypoglycaemia will be administered according to nationally agreed protocols. If required, maintenance fluids will be run at 3-4 mls/kg per hour irrespective of age until the child can drink and retain oral fluids. At discharge from hospital all children > 1 years will be receive empiric treatment for helminths (500mg mebendazole) or 400mg albendazole in Malawi in accordance to current recommendations (standard of care, SOC) regardless of randomised allocation.

5.2 TRIAL TREATMENTS

5.3 R1: TRANSFUSION STRATEGIES

All children will be assessed at admission and will be divided into 2 groups for randomisation based on:

1/ haemoglobin level and

2/ assessment of clinical severity children or complications (**reduced conscious level; respiratory distress, acute history haemoglobinuria or an established diagnosis of sickle cell disease**)

R1A Complicated Severe Anaemia haemoglobin < 4g/dl OR a haemoglobin < 6g/dl PLUS one or more signs of severity or complications

R1B Uncomplicated severe anaemia: haemoglobin >=4 and < 6g/dl without any of the severity features or complications

5.3.1 TREATMENT ARMS

R1A: Complicated Severe Anaemia

These children will be randomly allocated on a 1:1 basis to receive one of the following:

- **Whole Blood Transfusion** 20mls/kg, alternatively **10mls/kg packed cells;** or
- **Whole Blood Transfusion** 30mls/kg, alternatively **15mls/kg packed cells**

R1B: Uncomplicated Severe Anaemia

These children will be randomly allocated on a 1:1:2 basis to receive one of the following:

- **Whole Blood Transfusion** 20mls/kg alternatively **10mls/kg packed cells**, or
- **Whole Blood Transfusion** 30mls/kg alternatively **15mls/kg packed cells,** or
- **No Transfusion (control, SOC)**

5.3.2 TREATMENT SCHEDULE

A clinician or medical officer will prescribe the blood. Once the randomisation arm has been allocated, for those receiving immediate transfusion the clinician will calculate, using a calculator, the volume of whole blood required (20 or 30mls/kg). If only packed cells are available then the clinician must re-calculate the equivalent volumes of packed cell (10 or 15ml/kg). The request form must specify patient's first and last name, date of birth, desired component (whole blood or packed cells), volume of blood requested, date and time of request. Once the blood has been cross-matched and the blood pack for transfusion is ready for collection, the health worker who collects the donor blood for transfusion will complete the blood collection log. Prior to leaving the laboratory, the health worker will confirm with the lab technologist that the blood has been weighed and the weight recorded on the appropriate log and request form, as well as the volume. This will be repeated on the ward when the blood is received. Transfusions will be administered in gauged blood burettes; an initial aliquot (2ml) will run into a sterile apex tube using an aseptic technique (and ensuring that the tip of the infusion set does not touch anything to prevent contamination) and one drop taken from this to record the haemoglobin of donor blood, a blood film prepared to exam for storage lesions, the rest will be stored (see Study flow). Whole blood will be run over 3-4 hours and packed cells can be administered over 2-3 hours.

Further transfusion management during hospital admission

For all children in the trial an additional, or initial (for SOC control group in R1B only) transfusion (s) will be permitted after 8 hours (at the point of the first reassessment of haemoglobin, Hb) for children who still have either

(i) Profound anaemia Hb <4g/dl, irrespective of other signs of severity
(ii) Severe anaemia 4-6g/dl and one or both *de novo* signs of severity (respiratory distress or impaired consciousness)
(iii) Uncorrected severe anaemia 4-6g/dl in children with acute history haemoglobinuria or known sickle cell disease

However, early sampling of haemoglobin (<8 hours from baseline), and additional transfusion, will be permitted in children randomised to any group in the R1B strata (uncomplicated SA) developing de novo signs of severity.

Transfusion volume

Control (R1B only: randomised to control: no initial transfusion) who meet the above criteria, will receive 20ml/kg whole blood or 10ml/kg packed cells.

Children randomised to initially receive blood (**R1A and R1B)** who subsequently meet above criteria will *follow their randomisation arm* ie will receive either an additional transfusion of 20ml/kg or 30mls/kg of whole blood (or 10ml or 15ml/kg packed cells respectively).

Any child who has already received two transfusions and subsequently fulfils criteria above will receive a maximum of 20mls/kg (or 10ml/kg packed cells) irrespective of randomisation.

Frusemide or other diuretics will be prescribed at the discretion of the attending physician and recorded in the Source documents and CRF, including reasons for prescription.

5.4 R2 MICRONUTRITIONAL SUPPORT

Simultaneously to R1 randomisations, all children entering the trial will also be randomly allocated on a 1:1 basis to receive one of the following:

Micronutritional interventions (R2)

- **MVMM (containing iron, folate and other MVMM) for 3 months post-discharge**
- **Iron and Folate (standard of care) for 3 months post-discharge**

5.4.1 PRODUCT AND TREATMENT SCHEDULE

5.4.2 MULTIVITAMIN MULTIMINERAL (MVMM)

Nutromix™ has been specifically design for children 6-24 months of age with severe anaemia. Other formulations have been developed for older children and adolescents, women of child-bearing age, pregnant women, lactating women (UNICEF and the WHO). The formulation, shown in table below, meets the recommended nutrient intake (RNI) – particularly for vulnerable groups during emergencies[41]. Recommended nutrient intake is defined (RNI) as the daily dietary in−take of a nutrient sufficient to meet the nutrient requirements of nearly all apparently healthy individuals in a specific population group, usually by age and sex (9).

Micronutrients	**Pregnant and lactating women**	**Children (6−59 months)**
Vitamin A µg	800	400
Vitamin D µg	5.0	5.0
Vitamin E mg	15	5.0
Vitamin C mg	55	30
Thiamine (vitamin B1) mg	1.4	0.5
Riboflavin (vitamin B2) mg	1.4	0.5
Niacin (vitamin B3) mg	18.0	6.0
Vitamin B6 mg	1.9	0.5
Vitamin B12 µg	2.6	0.9
Folic acid µg	600	150
Iron mg	27.0b	10*
Zinc mg	10	4.1
Copper mg	1.15	0.56
Selenium µg	30	17
Iodine µg	250	90

Dosage: One sachet to be taken daily by the child and will be prescribed at the time of discharge from hospital.

Guidelines for the use of Nutromix™ Sachets:

1. Open the top of the sachet and empty the entire contents of the sachet in any semi-solid or semi-liquid food, after the food has been prepared and cooled to a temperature acceptable to eat (less than 60^0C)
2. Mix the powder in an amount of food, the child can eat in one meal.
3. The food to which **Nutromix**™ is added should be consumed within 30 minutes, as the micronutrients present in **Nutromix**™ may give the food a dark colour. Even if it turns dark, it is still safe to use.
4. Do not add to juice or water as they will not mix very well.

5.4.3 IRON AND FOLATE

Daily iron syrup or tablets and folate tablets for children < 2 years (25mg iron; 100-400micrograms folate); and >2 years and < 12 years (60mg iron; 400micrograms folate) will be given for 3 months, according to WHO guidelines for the management of severe anaemia.

Special groups: The nutritional supplementation, including MVMM randomisation, will be pragmatic in that all children for whom these supplements should be received according to WHO or national guidelines (e.g. those initially admitted with severe malnutrition) will receive them. Children who < 6 months who are not weaned (fully breast feed) will be excluded from the trial.- The number of infants >2months and <6months are likely to be small (<3% of total trial population).

- **Children with severe malnutrition** Iron-containing supplements are not recommended for severely malnourished children during the first 7 days of acute rehabilitation (WHO guidelines) but can be used effectively and safely after the child's initial nutritional rehabilitation (child is keen to feed and gaining weight). For children with severe malnutrition discharged on ready to use food supplements (RUTF) which contain MVMM, children will essentially ignore the MVMM randomisation on their allocated card, but receive their standard post-discharge supplementation within the RUTF which would be recorded on study CRFs. The number of children with severe malnutrition as their admission diagnosis is expected to be small (<5%).

5.5 R3 SHORT TERM ANTIMICROBIAL PROPHYLAXIS

Children will be randomly allocated on a 1:1 basis to receive one of the following:

Antimicrobial interventions (R3):

- **Cotrimoxazole prophylaxis for 3 months post-discharge**
- **No antibiotic prophylaxis post discharge (control, SOC)**

5.5.1 PRODUCT AND TREATMENT SCHEDULE

5.5.2 COTRIMOXAZOLE

Cotrimoxazole dispersible tablets (240mg: trimethoprim 40 mg/sulphamethoxazole 200mg) and dosing will follow WHO recommendations for prophylaxis in HIV-infected children: age 2 to 6 months: 120 mg; age 6 months to 5 years: 240mg; children >5 year 480mg. The dispersible tablets may be taken with water or mixed with feeds.

Cotrimoxazole will be prescribed from discharge. The cotrimoxazole prophylaxis randomisation will be pragmatic in that all children for whom cotrimoxazole prophylaxis should be prescribed according to WHO or national guidelines (e.g. HIV-infected children) will receive it regardless of randomisation, and no child in whom it is contraindicated (e.g. known GP6D deficiency according to local testing) will receive it. Such children will essentially ignore the cotrimoxazole randomisation on their allocated card; any cotrimoxazole received per guidelines would be recorded on study CRFs. The number of children with these conditions is expected to be small (<5%). HIV-infected children will receive antiretrovirals and will continue in the trial with HIV management and follow-up tailored in collaboration with local HIV clinics.

5.5.3 MEDICATIONS SUPPLIED AFTER DISCHARGE

Throughout the trial, children parents or their carers will be provided with a supply of drugs or nutritional products sufficient to last until their next clinic visit. At each clinic visit they will be requested to return all empty bottles and un-used medication to the follow-up clinic. Drugs will be provided for the trial as scored tablets (cotrimoxazole), iron syrup or tablets (for older children), folate tablets and MVMM sachets. The parents will be instructed that on no account should any drug assigned to a patient be used by anyone else (unless nutritional supplementation is prescribed to a lactating mother with an infant >2months and <6

months who has been not yet weaned). Unused drug must be returned to the site if a patient withdraws from treatment.

All drug dispensed and returned to the site should be documented on a treatment log for each patient. At each site, a named person (research nurse) will be required to maintain complete records of all study medication dispensed. The designated trial nurse will, on receipt of supplies prior to the commencement of the trial, conduct an inventory and complete a receipt. All trial drugs dispensed to participants will be recorded on a Dispensing Log. Inventories will be conducted monthly, and logs returned to KWTP.

5.6 STORAGE CONDITIONS

MVMM and cotrimoxazole to be stored at room temperature and should not to exceed 30°C (86°F). Both can be stored in these conditions for three years as packaged.

5.7 ACCOUNTABILITY

The trial coordinator (TC) at each site will maintain accountability logs for both transfusion interventions and nutritional and antibiotic interventions post discharge. These will kept securely until verified by the external monitor's visit.

5.8 MEASURES OF COMPLIANCE

Lack of patient compliance will not be a major issue for the transfusion interventions, since all children entering this trial will be critically ill and medical personnel will be responsible for administering the interventions. Any non-compliance will likely be a consequence of the intervention itself (e.g. lack of matched blood). To minimise wastage of blood, digital weighing scales will be provided for the blood transfusion laboratories to ensure accurate volumes are being issued and this will also be checked at a clinical level by use of gauged blood burettes.

Cotrimoxazole is used widely as prophylaxis in children with extremely low rates of toxicity and high acceptability. For cotrimoxazole, MVMM and iron/folate we do not anticipate compliance problems in this group with motivated carers following their child's acute admission with severe anaemia, in contrast to the compliance issues often found when these nutritional products are used in community fortification programmes for relatively healthy children. All carers will be asked questions about adherence using a standard questionnaire at clinic visits according to the flow sheet. To ensure compliance, between the day 28 and day 90 clinical visits study coordinators will contact parents, if possible, by phone to check on adherence and enquire about the status of the child.

Any non-compliance due to toxicity would also likely occur if the interventions were incorporated within clinical practice, and is part of a pragmatic strategy being evaluated. The intention-to-treat comparison will therefore incorporate such "strategy non-compliance" that is anticipated in general clinical practice.

6 ASSESSMENT AND FOLLOW-UP

6.1 SUMMARY

Transfusions will be given directly following randomisation and will run over four hours. Subsequently, after 8 hours, all children will receive a transfusion if Hb<4g/dl or if Hb 4-6g/dl with *de novo* signs of severity (impaired consciousness or respiratory distress). MVMM and cotrimoxazole prophylaxis will be given for 3 months post-discharge. Children will be intensively monitored (Table A) on the day of admission by the clinical team, and during any transfusion and then reviewed daily by the study team until discharge, with Hb performed at least 8 hourly in the first 24 hours, and daily thereafter. Locator maps and contact numbers will be obtained to facilitate follow-up. All participants will then be seen at 4 weeks, and 3 and 6 months post-discharge at outpatient clinics attached to each centre for evaluation of morbidity, Hb, and toxicity. Any patient not returning for a study visit will be traced for vital status ascertainment (consent will be sought for this at recruitment).

6.1.1 TRIAL ASSESSMENT SCHEDULE

See Flow diagram (page xi) and Table A (page xii).

6.1.2 BASELINE LABORATORY INVESTIGATIONS

Following consent and randomisation, admission blood samples will be taken for the following investigations: Full blood count, urea and electrolytes (U&E), acid-base status, lactate, glucose, malaria status (malaria slide and Paracheck RDT), blood film, cross match, and if facilities permit blood culture and other clinically indicated investigations (not required by the study protocol). Urine will be taken as soon as possible after admission for Multistick analysis (culture where indicated and facilities permit). Stool samples will be collected as soon as is practical after admission for assessment of helminth infection and stored for subsequent assays. In accordance with national guidelines, HIV testing will be performed after admission procedures are complete and assent given by parents or guardians. Pre- and post-test counselling will be done in accordance to routine practice.

6.1.3 SAMPLE STORAGE

In all children plasma and EDTA (for DNA extraction), and stool, will be stored for subsequent microbiological, parasitological, virological diagnostic assays, genetic studies (including sickle cell and G6PD status), immune activation, micronutrient assays and gut hormones (subset). In a subset of children at Mbale RRH, who have consented to additional research blood samples, red and white cell pellets will also be prepared and stored. Urine samples will be stored on a subset of children. The clinical samples and samples for storage will require no more than 10mls of venous blood, including the additional 7mls of whole blood for research purposes. Any blood taken for research purposes under emergency deferred consent (see Appendix 1 for consent procedures) from children whose parents subsequently refuse consent will be discarded.

6.1.4 DURING ADMISSION

Nurses

All children will be reassessed clinically at 30 mins, 60mins, 90 mins, 2, 4, 8, 16, 24 and 48 hours. At each review conscious level, vital signs (heart rate, oxygen saturation, respiratory rate, axillary temperature, blood pressure) and examinations for adverse events will be recorded. These will be recorded in bedside notes. Nurses will also record details of prescribed drugs, transfusion rate and volume received and all other intravenous fluids. During any transfusion additional bedside monitoring will be conducted and

reported in the source documents. Anthropometric data (weight length or height and MUAC) will collected at admission and prior to discharge.

Brief details of the child's household address will be collected at admission and more detailed locator data will be collected prior to discharge. After discharge the Study Site Coordinator will be responsible for completing the Case Report Forms (CRF) and following up initial data queries and tracing the results of pending laboratory tests.

Doctor

A symptom checklist and targeted physical examination (to evaluate any reported symptoms) will be performed at each clinical assessment will be recorded in the source documents (clinical notes). The doctor will review the child routinely at 1, 8, 16 and 24-hours and daily thereafter. Additional reviews will be done where clinically indicated and recorded in the case notes. At each clinical review the doctor will specifically review the child for solicited adverse events and the doctor will be responsible for documenting and reporting SAEs. Admission and final diagnoses will be recorded in the CRF.

After 48 hours the hospital patient's notes will be used in place of the TRACT source documents; an TRACT trial number sticker will be added on the front page of the patient's hospital notes and a caption **"**TRACT trial patient'': Notes to be reviewed at discharge" written below the sticker. This will enable the study team to track the patient's notes at discharge and collect important patient data. Additional pages in the TRACT trial source documents will be available to report key clinical, laboratory or adverse events during the period prior to discharge.

Additional blood tests

Using handheld monitors haemoglobin will be monitored 8-hourly on day of admission and daily thereafter (Table A). Blood glucose will be monitored at admission, 1, 2, 8, 16 and 24 hours (handheld glucometer) until the child is fully conscious and able to take fluids and/or food orally. Where these do not coincide with the above time points additional glucose measurements will be taken at 30mins and 4 hours (2 hours for packed cell) after the start of any transfusion. Lactate will be recorded at 8 hours and 24 hours. All of these tests will be conducted during any deterioration.

Data Summaries

The CRF will summarise at each time point (Table A) transfusions and IV fluid received, prescribed drugs (including other supportive treatments eg oxygen). Any drugs, intravenous fluids and transfusions received before admission will be captured in the admission CRF. Summaries of routine investigations will be recorded in the CRF and additional investigations and other treatments received during admission in the discharge CRF.

6.1.5 AT DISCHARGE

Before discharge the nurse will conduct the 24-hour dietary recall (detailing dietary intake prior to admission). The doctor will prescribe relevant nutritional supplementation and antibiotic prophylaxis following the randomisation and national guidelines. Prior to discharge children > 1 years of age will be prescribed anti-helminthics, to treat potential helminth or whip worm infection, but only if they have not already been prescribed this in the last 6 months (Page 147, WHO Guidelines For The Management Of Common Illnesses With Limited Resources) (500mg mebendazole in Uganda, 400mg albendazole in Malawi). As per WHO guidelines, children who also have severe malnutrition will only commence iron-containing medications once the child has a good appetite and has started to gain weight (usually in the second week) and is also a usual indicator for discharge for further home management.

Children or their carers will be provided with a supply of drugs sufficient to last until their first clinic visit (day 28). The Parent will receive a follow up invitation on a card. The date will be 28 days after the day of admission. (For children where this falls on a Saturday this will be moved to Friday and for those where it

falls on a Sunday this will be moved to the following Monday and drug supply adjusted accordingly.) Detailed Locator details and contact details will be checked again at discharge. Money for transport will be given by the clinical coordinator or designee.

The clinical coordinator is responsible for ensuring the discharge check-list is complete and for chasing up inpatient notes at discharge. Any relevant information, especially with regard to date of discharge, death, SAE's, treatments, blood transfusions, use of intravenous fluids, oxygen or non-routine treatments or investigations, will be recorded.

6.1.6 FOLLOW UP

A symptom checklist and targeted physical examination will be performed at each at each clinic visit post-discharge. Medical history since last visit including hospital re-admissions, transfusions and grade 3 or 4 adverse events related to nutritional and antibiotic interventions including severity and likely relationship of any adverse events will be documented by a doctor.

At Day 28 adherence to and acceptability of MVMM and/or cotrimoxazole will be queried by carer self-report, and carers will be provided with a supply of drugs sufficient to last for the next 2 months (Day 90 since admission). Blood and other tests and sample storage will be according the schedule outlined in Trial flow (Page xi) requiring a maximum of 4mls heparinised blood (plasma) and 1ml into EDTA (for pathogen diagnostics). At Day 90, adherence/acceptability will again be recorded, but no more supplements/antibiotics will be given. Any participants requiring further care at their 180d visit will transferred into the routine clinics at the centre where the trial is being conducted.

For participants enrolled at Mbale RRH at 28 day and 180 day visits, in specific subgroups of participants, the following will be undertaken: (PMBC/Red Cells/neutrophil function): 6 ml of blood will be taken by venepuncture for the analysis of monocyte and neutrophil function, inflammatory markers, endotoxin concentration known markers of gut barrier dysfunction as well as proteomic and metabolomic analysis. A urine sample will be requested at the same time and stored.

For participants enrolled at Mbale RRH at 28 day, 90 day or 180 day visits, in specific subgroups of participants, the following will be undertaken: (Gut Barrier Function (EDX (1ml), I I-FABP, IBAP on 5 mls of plasma).

6.2 PROCEDURES FOR ASSESSING EFFICACY

6.2.1 CLINICAL EVENTS (ALL PARTICIPANTS)

- Survival status will be recorded at each endpoint (48 hours, 28 days, 90 day and 180 days following admission). Any patient lost to follow-up before 6 months without withdrawing consent will be traced for vital status.
- Other serious adverse events will be reported as and when the doctor becomes aware of them (see SAE section). The details reported will include bedside observations, laboratory data, and additional clinical narrative.
- During the index admission, any child fulfilling criteria for a new or additional transfusion will be recorded
- At all subsequent visits hospital admissions and requirement for transfusion will be solicited.

Cause of death (and other clinical secondary endpoints) will be adjudicated by an Endpoint Review Committee, blinded to randomised allocations: relationship to all possible interventions drugs (transfusion strategy; nutritional support and cotimoxazole) will be solicited to avoid unblinding.

6.2.2 HAEMOTOLOGICAL RECOVERY (ALL PARTICIPANTS)

- Haemoglobin will be recorded at 8 hour periods up to 24 hours, then daily until discharge, then at 28 days, 90 day and 180 days.

6.2.3 PROCEDURES FOR ASSESSING SAFETY (ALL PARTICIPANTS)

The symptom checklist used at each visit will explicitly prompt for symptoms relating to possible drug toxicities. Additional safety blood tests or investigations may be performed to investigate symptoms or monitor emergent laboratory test abnormalities as clinically indicated.

Serious, solicited and grade 3 or 4 adverse events will be reported on the case report form. Adverse events (clinical and laboratory) will be graded according to toxicity/severity grading.

6.2.4 PROCEDURES FOR ASSESSING ADHERENCE (ALL PARTICIPANTS)

Adherence to nutritional and prophylaxis drugs will be assessed in all participants at each visit by pill counts for tablets, and nurse-administered questionnaire to the child's carer, and where appropriate to the child (at the discretion of the nurse or doctor depending on age).

6.3 OTHER ASSESSMENTS

6.3.1 HEALTH ECONOMICS (ALL PARTICIPANTS)

The trial will measure healthcare-related costs in trial participants, starting at randomisation and continuing for the duration of follow-up. Costs incurred by the patients and their families (transport, indirect and companion person's costs) will be obtained by patient reports. Reported transport costs will be confirmed using local information on distance and cost of transport. Information on hospitalisations (number, reason, and duration of stay) will be collected from hospital records, and data on other healthcare resource utilisation (outpatient visits, medications, and procedures) will be collected by abstraction of patient medical notes and by patient interview at the 28, 90 and 180-day visits.

6.3.2 ANTI-INFECTIVE

Changes in inflammatory markers (eg CRP) (all participants) will be measured retrospectively and incidence of bacterial infections and malaria at 28 days, 90 day and 180 days (from blood cultures at all sites except Soroti and using non-culture based molecular diagnostics on stored blood – all sites).

Immune/ Biomarkers (sub-group)

For a subgroup of patients, the following assays will be undertaken on samples stored from admission, day 28 and day 180 of follow-up:

- Whole blood assays measuring oxidative burst, phagocytosis and expression of activation markers will be conducted by flow cytometry (4 colours).
- Remaining blood will be separated into PBMCs and plasma, stored in Liquid Nitrogen and -80C respectively for batch analysis of inflammatory markers (plasma) and additional analysis of immune function (PBMC).
- Whole blood will be used to establish the concentration of endotoxin in blood within 3 hours of admission.
- Stored plasma and urine (-80C) will be used to measure the concentration of known markers of gut barrier dysfunction by ELISA.
- The metabolome and proteome will be determined in a selected group of samples depending on the results of the initial screen of endotoxin and known markers of gut barrier dysfunction.

7 STASTISTICAL CONSIDERATIONS

7.1 SAMPLE SIZE

The sample size calculation is based on the following assumptions:

- 80% power, 2 sided alpha=0.013 to allow for 4 comparisons (see below).
- SA cases are 50% complicated (<4g/dl or 4-6g/dl with prostration/respiratory distress/known sickle cell disease/dark urine) and 50% uncomplicated (4-6g/dl without prostration, respiratory distress, known sickle cell disease or dark urine)[11] (and Dr. Olupot-Olupot personal communication)
- Mortality (cumulative) at 48 hours and 4 weeks is 11% and 16% respectively in complicated SA, and 4% and 9% in uncomplicated SA.
- The cumulative rate of re-admission, severe anaemia relapse and re-transfusion at 6 months is 12.5% in both complicated and uncomplicated SA (in addition to mortality above).
- For the primary comparison of **transfusion vs. no transfusion** in uncomplicated SA at 4 weeks, the minimum clinically relevant difference is a 50% relative reduction (**R1B**): for the other primary comparison of transfusion **volume** (20 vs 30 ml/kg) at 4 weeks **(R1A&B)**, the minimum clinically relevant difference is a 30% relative reduction. The minimum clinically relevant difference is larger for the transfusion vs no transfusion question as provision of safe blood at all will require greater resources than provision of slightly larger vs slightly smaller blood volumes. As the same relative difference translates to a far larger absolute difference at higher event rates, for the primary comparison at 6 months (**R2/3**) the minimum clinically relevant difference is a 5% absolute reduction (see below for control group event rates). Then:

- **(R1B)** comparison of transfusion vs no transfusion (50% reduction from 9% control mortality at 4 weeks) requires 1460 uncomplicated SA cases (1:1 allocation to 30/20ml/kg:no transfusion, 730 in no transfusion group, 365 receiving 20 ml/kg and 365 receiving 30 ml/kg transfusions).
- **(R1A&B)** if the overall ratio of uncomplicated : complicated SAs is 1:1 (ie 50% of each type), then within the subgroup randomised 1:1 to 30 vs 20ml/kg, the ratio will be 1:2 because this comparison excludes the 50% of uncomplicated SA randomised to no transfusion. Overall mortality at 4 weeks in this group will therefore be 13.67% (0.33*9%+0.67*16%). The comparison of 30 vs 20 ml/kg (30% reduction, to 9.57%) requires 2798 SAs, 1399 per group (split 466 uncomplicated, 933 complicated).
- Therefore, comparing required sample sizes for **R1A** and **R1B,** to address both parts of the transfusion question (**R1**) we need slightly more uncomplicated SA children per group from (R1A&B – n=466) than (R1B – n=365), and therefore the total sample size is **3730 cases** (933*2=1866 complicated, 466*4=1864 uncomplicated).
- **(R2/3)** the comparison of multi-vitamins vs standard of care (1:1) and cotrimoxazole prophylaxis vs standard of care (1:1) requires **3162 SA cases**, assuming 50% are complicated and 50% uncomplicated, to detect a 5% absolute reduction from average control mortality of 25% (0.5*21.5%+0.5*28.5%) at 4 weeks).

Thus a sample size of **3730 SA cases** would allow the multiple comparisons above to be made. Assuming a 6% loss to follow-up by 6 months increases this to **3954 SA cases.** As the effect sizes are reasonably large on the relative scale (>30% reduction), inflation factors which adjust for the factorial design are close to 1. However, assumptions are more sensitive to the relative contribution of uncomplicated: complicated SA. Capping the uncomplicated SA strata at 2000 cases (ie recruiting at least 1950 complicated SA) retains at least 80% power to detect the differences above independently of variations in the contributing proportions of complicated SA.

Randomising uncomplicated SA (**R1B**) 1:1:2 between 30ml/kg:20ml/kg:no transfusion provides greater power for the comparison of no transfusion (SOC) versus transfusion in this group because the final randomisation ratio for transfusion: no transfusion is 1:1. In contrast a 1:1:1 randomisation in this strata would produce a 2:1 transfusion:no transfusion ratio which has lower power.

7.2 STATISTICAL ANALYSIS

The analyses will be described in detail in a full Statistical Analysis Plan. This section summarises the main issues.

Each intervention is hypothesised to be superior to standard of care, and therefore the proposed analysis is intention to treat, including all randomised patients. The primary analysis will compare a) transfusion versus no transfusion and b) 20mls/kg vs 30mls/kg in terms of the proportion of children with fatal outcome 28 days after randomisation. Primary outcome analysis will use time-to-event methods (Kaplan-Meier, log-rank test, proportional hazards models) to the time points specified for primary and secondary outcomes, stratified by centre and anaemia severity at baseline. Correction of anaemia will also be analysed using time to event methods.

Pre-specified subgroup analyses will include each of the other randomised allocations (ie exploration of interactions in the factorial design), together with the other randomisation stratification factor (centre) and the anaemia stratification factor (A vs B) for the transfusion randomisation. We will also investigate a priori whether there was any evidence for a different impact of the interventions according to the following categorical variables: previous receipt of a transfusion ever or (at another health centre in this illness); speed (rate) at which the transfusion is administered, fever; malaria; microbiological evidence of sepsis (blood culture or retrospective molecular diagnosis); HIV; known or previously undiagnosed sickle cell disease.

For the cotrimoxazole prophylaxis and MVMM supplementation randomisations the primary analysis will be intention-to-treat based on all randomised participants, as above. However, a secondary analysis will be restricted to patients discharged alive in whom these interventions were neither mandated nor contraindicated (ie excluding HIV-infected children and those with known GP6D deficiency from the cotrimoxazole randomisation, excluding children admitted for severe acute malnutrition from the supplementation randomisation).

Secondary outcome measures will be analysed using time-to-event methods or normal linear regression for continuous variables. The frequency of hospital re-admissions and adverse events will be tabulated by body systems and by randomised groups, and the number of events experienced by each participant will be compared across randomised groups using Fisher's exact test.

For the within-trial analysis, the differential cost of the treatment interventions will be related to their differential outcomes in terms of the primary outcome. The relative cost-effectiveness of the alternative forms of management will then be assessed using standard decision rules and a full stochastic analysis will be undertaken. A cost-utility analysis will also be conducted using a standard approach. The within-trial analysis will be augmented by extrapolation beyond the trial follow-up using decision-analytic modelling. The aim of this analysis will be to predict the implications of any difference in clinical endpoints in the trial for subsequent quality-adjusted survival duration and long-term resource costs. This will inform the question of whether any differences in drug costs between the treatment groups are offset by reduction in other treatment costs or health improvements in the long-term.

7.3 INTERIM REVIEWS

During the trial an independent Data Monitoring Committee would meet to review unblinded data for all three randomised comparisons at least annually. They will review data on enrolment, safety, adherence to randomised strategies, efficacy and safety at regular intervals and in strict confidence. The DMC will determine the frequency of their meetings, which could be more frequent if they think necessary.

A decision to discontinue recruitment, in all patients or in selected subgroups, will be made only if the results are likely to convince the general clinical community and participants in the trial. The DMC will report to the Trial Steering Committee (TSC) and to the Ethics Committee in each country, if, in their view, the data provide proof beyond reasonable doubt that one of the allocated strategies is better than its comparator in terms of the primary outcome. The guiding statistical criterion for "proof beyond reasonable doubt" is the Haybittle-Peto criterion of a difference of at least 3 standard deviations in an interim analysis of a major endpoint. The TSC will then decide whether to amend (including the possibility of dropping one of the transfusion strategies) or stop the trial before the end of the planned follow-up. If a decision is made to continue, the IDMC will advise on the frequency of future reviews of the data on the basis of accrual and event rates. The IDMC will make recommendations to the TRACT Trial Steering Committee as to the continuation of the trial.

7.4 DATA GOVERNANCE

The ownership of the TRACT dataset will lie with the TRACT Trial Steering Committee, who will approve all requests for use of trial data before and after the trial ends (also to be approved by the TRACT Data Monitoring Committee). The TRACT dataset will be held electronically for at least 20 years after the end of the trial in accordance with MRC policy. The TRACT Trial Steering and Data Monitoring Committee Charters will state that proposals to use TRACT data and samples will be welcomed, and supported widely where this does not conflict with existing plans within the Trial Team (e.g. as described in the primary and secondary objectives of the main trial proposal above)

8 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.

8.1 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 in Table 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 necessarily caused by or related to that product.
Adverse Reaction (AR)	Any untoward and unintended response to an investigational medicinal product related to any dose administered.
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 that product.
Serious Adverse Event (SAE) or Serious Adverse Reaction (SAR)* o	Respectively any adverse event, adverse reaction or unexpected adverse reaction that: ▪ Results in death ▪ Is life-threatening ▪ Requires hospitalisation or prolongation of existing hospitalisation (that is not an elective admission) ▪ Results in persistent or significant disability or incapacity

* Suspected Unexpected Serious Adverse Reaction (SUSAR) will not be assessed in this trial as it falls outside the scope of the European Union Clinical Trial Regulations.

Relevant background

Cotrimoxazole and MVMM are licenced medication with known profile of side effects; similarly blood transfusions are commonly used. Adverse event monitoring will therefore focus on serious and causally related events (grade 3 or 4). Clinical or laboratory toxicity will be reported if grade 3 or 4 following the Common Toxicity Criteria (CTC) for Adverse Events (http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_8.5x11.pdf). In the event of an abnormal clinical or laboratory finding by the study clinician, children will receive appropriate treatment according to national or WHO clinical guidelines, including admission for assessment and/or treatment where appropriate. Usual clinical practice for suspected reactions to cotrimoxazole will be followed. For transfusion the solicited adverse events that will be routinely capture and reported include suspected any events Acute transfusion reaction (ARR) Haemolytic transfusion reaction (acute or delayed) (HTR) Transfusion related acute lung injury (TRALI); Post

transfusion purpura (PTP); Transfusion transmitted infection (TTI) and Transfusion associated circulatory overload (TACO) (details covered in Appendix IV) and any events of pulmonary oedema. These will be graded following the CTC.

Any event which does not have a specific grading provided in the CTC should be graded as follows

- 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.

8.1.1 EXEMPTED ADVERSE EVENTS

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, e.g. elective cosmetic surgery, social admissions
- Overdose of medication without signs or symptoms

8.1.2 ANAEMIA-RELATED EVENTS

Pre-specified secondary outcomes in the trial include (lack of) resolution of anaemia and relapse or recurrence of anaemia post-discharge, as these are relatively common clinical outcomes which we hypothesise will be prevented by the interventions under investigation. These events will be detected by the trial team through regular, repeated clinical review of the study patients, documented in the CRF and analysed as specific secondary efficacy endpoints. These anaemia-related events (worsening of anaemia during initial admission, re-admission for anaemia relapse), and their associated signs and symptoms, should therefore not be reported as adverse events, or serious adverse events, to avoid double-counting.

Death should always be reported as a (serious) adverse event, regardless of cause.

8.2 CAUSALITY

The assignment of the 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 Chief Investigators. 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 event occurs 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.

8.3 REPORTING PROCEDURES

At each clinical review the clinician or nurse will check for potential SAEs, grade 3 or 4 AEs and solicited AEs including evidence of pulmonary oedema, suspected TRALI or suspected transfusion-reaction. 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) and all solicited AEs (any grade) will be reported on the CRFs. The reporting procedure is captured within the safety reporting SOP. Any questions concerning adverse event reporting should be directed to the study coordination centre in the first instance. (This is done either via email or by telephone). SAEs will be reviewed immediately by a designated physician (SAE reviewer) in Kilifi and periodically by the Endpoint Review Committee (ERC) who will be blind to study allocation.

SAEs will be reported to the study coordination centre, using an SAE form, which should be completed, scanned and sent electronically to the TRACT study coordination centre within 24 hours. 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). The responsible investigator should sign the causality of the event. Additional information should be sent within 5 days if the reaction has not resolved at the time of reporting.

Local investigators should report all SAEs as required by their Local Research Ethics Committee and/or Research & Development Office. Any questions concerning adverse event reporting should be directed to the Principal Investigator in the first instance.

Contact details for reporting SAEs
Please send SAE forms to:
TRACT@kemri-wellcome.org
Tel: +254715461761 or +254417522063

8.4 LOSS TO FOLLOW-UP

Any child lost to follow-up before 6 months will be traced for vital status. In order to minimise losses to follow-up, locator data (maps and identifiable landmark) and mobile phone numbers will be taken on

discharge and verified at every review. During the 180d study period attempts should be made to contact the patient via phone (if available) and to follow-up with home visits, if at all possible. In the statistical analysis, a patient will be regarded as 'lost to follow-up' if they were not seen in clinic at the 180d visit and were not known to have died.

8.5 TRIAL CLOSURE

The trial will be considered closed after the 180d follow up of the last enrolled child. At the end of the trial, vital status of all participants will be ascertained (inlcuded in the consent).

9 ANCILLARY STUDIES

9.1 HEALTH ECONOMICS

The economics substudy will include cost-effectiveness analyses (CEA) of the trial interventions. This will involve an evidence synthesis and modelling exercise of different treatment strategies, and will build upon previous CEAs in children (eg the FEAST trial). Resource use data will be collected in this trial and will be supplemented by data collected in previous work.

The economic evaluation will be conducted from the health services perspective. Costs will cover the use of medication and laboratory tests as well as hospital, primary care and community health services. Unit costs will be attached to resource use, using the best available estimates of long run marginal opportunity cost, to obtain a cost per patient over the period of follow-up. Routinely available national unit costs will be used where possible with local estimations where necessary. There will also be a budget impact analyses of the consequences of adopting the interventions on the health sector budgets, in each of the countries of the trial.

9.2 MOLECULAR DIAGNOSTICS

A major question that has hindered identification of relevant interventions for this patient population to date is what are the underlying causes of morbidity and mortality in children with SA. Whilst the TRACT randomised comparisons will provide data to support particular mechanisms, stored samples will also provide a valuable resource for the application of new molecular diagnostics methods.

Two molecular methods are being used increasingly in research and clinical practice to identify bacteria and viruses. 16S ribosomal DNA (16S rDNA), common to all species of bacteria, can be detected with a broad-range polymerase chain reaction (PCR); specific quantitative PCR (qPCR) can also be used to quantify the 16S rDNA subunit to measure directly the number of bacteria. However broad range 16S rDNA PCR is subject to artefact from endogenous and exogenous bacterial products[53-55] and therefore without either sequencing the PCR product, or carrying out more sensitive qPCR, there is concern that changes in the qPCR may not be due to circulating organisms. Unfortunately sequencing the 16S rDNA has so far yielded results compatible with environmental contamination rather than recognised gut commensals[56-58]. The use of specific primers renders qPCR less vulnerable to background contaminants than broad-range 16S rDNA PCR, and therefore more sensitive. The disadvantage of qPCR is the need to predict which bacterial or viral species are likely to be relevant.

The goal of this study would be to identify the role of bacteria and viruses in the aetiology of SA in African children. Previous studies have shown comparable results between frozen EDTA plasma and whole blood, and so we would assay standard 16S rDNA PCR, and a panel of 10 qPCR reactions (including Enterobacteriaceae, a panel of anaerobes, Streptococcus pneumoniae, Staphylococcus aureus, group A streptococcus and a range of potential viruses) in plasma samples taken at enrolment, and closest before death (or the corresponding visit week in controls) in all cases and controls.

9.3 HUMAN GENETICS: RED CELL DISORDERS

Human genetic factors are potentially important modifiers of the risk of SA. Some are directly implicated in the pathogenesis including inherited conditions of the red blood cell such as sickle cell disease (HbSS)[59] and G6PD deficiency[60] while others may alter risk more indirectly. Examples of the latter include sickle cell trait (HbAS) and α-thalassaemia, which protect against malaria and thus protect against the evolution of SA and genetic conditions that affect susceptibility to bacterial or viral infections. We anticipate that the contribution of human genetic factors to the risk of SA will be significant. Although it is likely that the birth prevalence of HbSS will be relatively low in most of the populations involved in this study (1-3%) SA is a common presenting symptom of HbSS and children with HbSS will therefore be overrepresented among children presenting with SA [52]. Conversely, in all the populations involved in this study the prevalence of HbAS and α-thalassaemia are likely to exceed 15% and 60% respectively and both are likely to be associated with significant protection.

As described in section 9.2 above, we will extract a sample of DNA from all case patients at the conclusion of the study to investigate the contribution of bacterial and viral infections to the aetiology of SA using molecular methods. We will use aliquots of DNA from this same archive to describe the distribution of HbSS, G6PD deficiency and α-thalassaemia among case patients using PCR. Patients found to be positive for HbSS will be recalled for counselling and for confirmatory testing and if confirmed to be suffering from HbSS they will be encouraged to attend the outpatient clinic for regular treatment.

The DNA samples collected through this study will also be of potential future value for wider studies aimed at investigating the genetic basis for SA. Although such studies are not the focus of the current proposal we would like to retain this archive for such future potential studies that would be the subject of a separate poposal. The possible use of archived samples for future genetic studies, some of which may require transport of samples outside the country, will be explained in the patient information sheet and parents will be asked for specific consent for such potential future use of their children's samples in the consent sheet.

9.4 MARKERS OF NUTRITIONAL WELLBEING

9.4.1 ESTIMATION OF NUTRITIONAL INTAKE

Nutritional intake is fundermentally important to the health of the child and there is a intimate relationship between nutritional intake, nutritional status and infection. Poor nutritional intake is also linked to anaemia, poor gut barrier function and immunity. Nutritional intake can also be used as a surrogate marker of well being. The measurement of nutritional intake is a balance between the complexity of user involvement and engagement of the target group.
Aims:

1. To use a 24-hour multi-pass recall to assess the nutritional intake of children at each assessment point
2. To estimate macro and micro nutritent intake using local food composition tables[61, 62]
3. To use the nutritional intake to inform gut barrier function and immunity studies and to assess its potential use as a variable to inform the treatment of anaemia

Methodology:
All children/parents will be interviewed prior to discharge and at 28, 90 and 180 days for a brief dietary recall. This will be included in the patient information sheet.

Dietary methodology will be based on recent work conducted in Malawi, validating a 24 hour [62]). Data will be collected by the nurse at the same time as collecting other data for the study. The multipass 24-hour recall follows the following algorithm. If relevent, the parent is asked if the child is exclusively breast

feed (if they are the recall is stopped). If not, the parent is asked way is the first thing the child had to eat or drink that day. This is followed by a series of questions what did the child have to eat or drink next until bed time.

1. The list is then read back to the parent to allow for corrections.
2. The nurse then asks to quality the amounts the child has eaten or drank of each food. This can be done with the aid of pictures or household utensils (including different (standarsised) spoon sizes or percentages of a bowl (with a fixed volume~ 200ml).
3. The list is then is read back for any corrections to be made and the recall completed

The inteview should take about 15-20 minutes depending on the complexity of the diet.

Analysis

The record chart will then be analysied using local food tables to calculate 24-hour macro and micro nutritent intake at each study visit. This will be retrospectively analysed by intervention arm stratified by age, clinical and infection status, and markers of nutritional recovery.

9.5 GUT BARRIER FUNCTION

Malaria infection strongly predisposes African children (5-12%) to invasive bacterial disease (IBD) with particularly high mortality in this group (approximately 30%). The leading causes of IBD in *P. falciparum* infection are non-typhoidal salmonellae (NTS), *E. coli* and other enteric organisms with gram-negative organisms becoming increasingly more predominant across the severity spectrum. Endotoxin is a complex lipopolysaccharide (LPS) present in the cell walls of gram-negative bacteria and in substantial quantities in the bowel. We have recently shown that endotoxemia was observed in 28.5% children hospitalized with malaria, most commonly associated with the clinical presentation of severe anaemia and suggested that this may be due to disordered gut barrier function. We also observed that endotoxemia was associated with a depressed inflammatory and anti-inflammatory cytokine response similar to that observed in sepsis. Thus, markers of gut barrier dysfunction may identify children most at risk of invasive bacterial disease, who would benefit from antimicrobial treatment (short and longer term) rather than prophylaxis as evaluated in TRACT.

Aims

1. We aim to determine the extent of gut barrier dysfunction in children with severe anaemia (with and without concurrent malaria) using known markers of gut barrier integrity such as plasma, urine or faeces concentrations of endotoxin, endocap-antibodies, intestinal fatty acid binding protein, intestinal bile binding protein, claudin-3 as well as calprotectin and its relationship with gut hormones.
2. Identify novel biomarkers of gut barrier dysfunction by investigating the metabolome and proteome of plasma and urine in children with severe anaemia with or without endotoxemia on admission.
3. Assess whether there is any evidence that this is modified overtime by the cotrimoxazole prophylaxis (randomised comparison).

Design

Up to 300 children will be recruited, following additional consent (Appendix II) to determine endotoxin levels (EAA, Spectral Diagnostics) immediately and on stored plasma samples, and to assay urine and faeces markers of gut barrier dysfunction at admission, day 28 and day 180. Laboratory data will be interpreted together with the clinical outcome and at the end of the trial by randomised arm. We will use a Millipore human gut hormone panel kit which will allow us to measure Ghrelin, Leptin, GIP, GLP-1, Amylin (active or total), PP, PYY, and Insulin using 25ul of plasma and relate these to other measure of immune activation and gut barrier dysfunction.

9.6 MARKERS OF IMMUNE ACTIVATION/FUNCTION

As above, we observed that 28.5% of children with malaria presented with endotoxaemia most likely due to increased gut barrier dysfunction. Similar to increased susceptibility to NTS in children with malaria, children with endotoxaemia where more likely to present with anaemia. We observed that endotoxaemia in addition to malaria was associated with reduced plasma concentration of pro- and anti-inflammatory cytokines, suggesting that this subgroup of children with malaria is immunologically impaired. Studies in rodent models of malaria demonstrated that malarial was associated with heme oxygenase I induction but impaired oxidative burst in a subset of neutrophils which impaired killing of intra-cellular *Salmonella typhimurium*. By contrast, a recent study in Gambian children showed an increase in heme oxygenease expression due to a promoter polymorphism in children with severe malaria including severe malarial anaemia, however the effect of the polymorphism on increased risk of bacterial co-infection was not investigated. TRACT provides a unique opportunity to analyse the function of innate immune cells in children with severe anaemia (with and without concurrent malaria) at admission and after recovery with respect to cytokine production, oxidative burst and bactericidal activity. In addition, we will be able to establish whether inflammation is causally linked to endotoxaemia (or other markers of gut permeability) and/or increased risk of bacterial co-infection.

Aims
1. Determine neutrophil and monocyte function in children with severe anaemia (with and without concurrent malaria) at admission and after recovery.
2. Determine whether there is an association of neutrophil and monocyte function with endotoxaemia, other markers of gut permeability, or general inflammation and immune activation.
3. Determine whether any aspect of immune function is modified over time by the cotrimoxazole randomisation.

Design:
Neutrophil function and monocyte function using whole blood assays and flow cytometry will be determined in 300 children recruited for the analysis of gut barrier function at admission, day 28 and day 180. Laboratory data will be interpreted together with the clinical outcome, molecular diagnostics (9.2), human genetics (9.3), gut barrier function (9.4) and eventually by randomised arm.

9.7 ANTIMICROBIAL RESISTANCE

S. pneumoniae and Non-typhoidal Salmonella (NTS) have been the commonest bacterial isolates from febrile children under five years of age in hospital-based community-aquired bactermia studies conducted throughout the region. Invasive NTS is a prominent pathogen in children with malaria-associated anaemia. All bacterial isolates from blood cultures (or cerebrospinal fluid) at initial recrutiment and during clinical episodes of sepsis on follow-up will be stored. Antimicrobial resistance profiles and molecular markers of resistance will be compared by cotrimoxazole randomisation at the end of the study

10 QUALITY ASSURANCE AND CONTROL

10.1 RISK ASSESSMENT

The Quality Assurance (QA) and Quality Control (QC) considerations have been based on a formal Risk Assessment, which acknowledges the risks associated with the conduct of the trial and how to address them with QA and QC processes. QA includes all the planned and systematic actions established to ensure the trial is performed and data generated, documented and/or recorded and reported in compliance with the principles of ICH GCP and applicable regulatory requirements. QC includes the operational techniques and activities done within the QA system to verify that the requirements for quality of the trial-related activities are fulfilled. This Risk Assessment has led to the development of a Quality Management Plan (QMP), which will be kept separately.

Safety profiles of blood, cotrimoxazole and MVMM used in the trial are well known and we will look for the side effects of each carefully. Severe adverse events (as defined by Good Clinical Practice guidelines) are secondary outcomes of the trial. The most current Summary of Product Characteristics/ Investigators Brochure will be available at each trial site and any updates will be circulated to sites. MRC CTU compliant SAE data collection and reporting procedures will be adopted, as worked well in FEAST. Clinical staff at sites will be trained by the CI at the initiation visit to recognise expected side effects. However as multi-vitamin multi-mineral supplements and cotrimoxazole are widely used in children the risks of harm are known and are extremely low.

There are also expected inherent hazards of the transfusion intervention – particularly infections in/failure to correctly cross-match transfused blood. These will be minimised by the TRACT team working closely with the national and local blood transfusion services to ensure recommended safety and quality control practices are being achieved and working closely with the blood safety committees at each site.

The trial will be recruiting patients with severe and complicated anaemia with a high mortality. At the start of the trial all sites will receive emergency care training, including triage of those at highest risk. All patient swill be closely monitored so that clinical deteriorations can be identified at the earliest opportunity and appropriate therapy initiated. In general the trial sites in Africa have considerable experience with this population and this will serve minimise the risks to the patients and the trial. A detailed risk assessment will be conducted prior to starting the trial.

10.2 MONITORING AT STUDY COORDINATION CENTRE

Each site will be responsible for its own data entry and local trial management. Data will be entered into the web-enabled trial database directly at the site. The site will retain the original CRF. Data stored on the central database will be checked for missing or unusual values (range checks) and checked for consistency within participants over time. If any such problems are identified, the site will be contacted and asked to verify or correct the entry. Changes will be made on the original CRF and entered into the database at the site. KCTF will also send reminders for any overdue and/or missing data with the regular inconsistency reports of errors.

10.3 MONITORING AT LOCAL SITE

This trial will be monitored according to a Monitoring and Quality Management Plan which will set out the frequency of visits, the degree of source document verification against the case record forms and the requirements for triggered on-site monitoring visits. This plan will also detail the procedures for review and sign-off. The monitoring will adhere to the principles of ICH GCP. It is anticipated that the monitoring will start with 100% source document verification as in the FEAST trial. This will be reviewed for each site once a satisfactory and sustained performance in quality assurance is established.

A detailed site initiation visit with training will be performed at each study site by staff from the KWTP CTF who will be specifically trained for this role. The site initiation visits will include training in the trial procedures, as well as practical training in administration of transfusions and other trial interventions, reporting guidelines for adverse events of study interventions as well as other trial procedures. All staff at sites involved in the trial will be receive formal training in GCP –through a dedicated training programme during site initiation visit, and will also be required to complete an on-line course.

The trial monitoring team will consist of the KWTP-Clinical Trial Facility monitoring team and the MRC CTU who will provide over-site and some visits to maintain the integrity of the monitoring. The Clinical Trial Facility in Kilifi oversees standards and quality of all trials conducted through the KWTP and through its monitoring systems and SOPs is organised to ensure that all sites can be monitored with equal independence and rigor. All monitors will be appropriately qualified and trained.

At each monitoring visit monitors will:

- verify completeness of Trial Master File
- confirm adherence to protocol
- review eligibility verification and consent procedures
- look for missed clinical event reporting
- verify completeness, consistency and accuracy of data being entered on CRFs
- evaluate drug accountability
- provide additional training as needed

The monitors will require access to all patient medical records including, but not limited to, laboratory test results and prescriptions. The investigator (or delegated deputy) should work with the monitor to ensure that any problems detected are resolved.

11 REGULATORY ISSUES

11.1 TRIAL REGISTRATION

This trial has been registered with the International Standard Randomized Clinical Trial Number Register, where it is identified as ISRCTN84086586..

11.2 ETHICS APPROVAL

The Study Coordination Centre has obtained approval from the Imperial College Research Ethics Committee. This trial will be submitted for approval by Research Ethics Committees/Institutional Review Boards in Malawi and Uganda and by all required regulatory authorities in participating countries.

The study will be conducted in accordance with the recommendations for physicians involved in research on human subjects adopted by the 18th World Medical Assembly, Helsinki 1964 and later revisions, the principles of Good Clinical Practice (GCP) as laid down by the ICH topic E6 (Note for Guidance on GCP) and applicable national regulations.

11.3 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 TRACT 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. (See **Appendix I** for Patient Information sheet and consent form). The right of the participant to refuse to participate without giving reasons must be respected.

11.3.1 EMERGENCY VERBAL ASSENT FOLLOWED BY DEFERRED CONSENT

A number of children will present as emergencies where delay in study enrolment, and thus treatment, through a consent procedure would be unacceptable. For the FEAST trial we developed and received ethical approval to use a two stage consent process in this circumstance[63]. 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 treatment allocation, 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 later sign the verbal assent form which will be filed with the consent form. If consent is withdrawn later no data from the subject will be used (Appendix I Template assent form).

11.4 WITHDRAWAL OF PATIENTS AND PROTOCOL TREATMENT DISCONTINUATION

In consenting to the trial, patients are consenting to trial treatment, 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. If a patient chooses to discontinue any part of their trial treatment, they should always be followed up (providing they are willing) and they should be encouraged to not leave the whole trial. 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 from the protocol treatment without giving reasons and without prejudicing further treatment (Appendix III for withdrawal form). 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.

Withdrawal from the transfusion intervention or control arms is unlikely, given that most transfusions are given within the first 12 hours of admission. Severe allergic reaction (toxicity) or TRALI is included as a secondary endpoint and is relevant only to children receiving transfusion. It will not be a reason to withdraw the child from the trial, but further transfusions should be withheld. The child should continue with their cotrimoxazole/MVMM allocation wherever possible.

11.5 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

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 to deffered 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 the policies of the institution where the samples reside at the time (local or central storage).

Patients who stop trial follow-up early will not be replaced, as the total sample size includes adjustment for losses to follow-up.

11.6 CONFIDENTIALITY

Participants' identification data will be required for the registration process. The Study Coordination Centre will preserve the confidentiality of participants taking part in the study, which will comply with requirements for data protection in the countries where the research is being conducted and is registered under the Data Protection Act.

11.7 INDEMNITY

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

11.8 SPONSOR

Imperial College London will act as the main Sponsor for this study and delegates this responsibility to the KWTP, Kilifi and MRC CTU 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.

11.9 FUNDING

The trial is supported by grant funding from Medical Research Council (MRC UK) and of the Department for International Development, UK (DFID) through a concordat with MRC UK. The trial will be coordinated by Imperial College. A written agreement with the site principal investigator and/or the investigator's institution and Imperial College will outline the funding arrangements to sites. The TSC will meet and review the financial aspects of the trial at least 12-monthly and report to the sponsor. Terms of reference will be developed for this activity.

11.10 AUDITS AND INSPECTIONS

The study may be subject to inspection and audit by Imperial College London under their remit as Sponsor and other regulatory bodies to ensure adherence to GCP.

12 TRIAL MANAGEMENT

12.1 SITE TRIAL MANAGEMENT TEAMS

A trial management team will be formed at each site to conduct the day-to-day management of the trial at the site ("Site TMT"). This will include the investigators and trial staff at the site. These groups will meet every one to two weeks and will be chaired by the principal investigator or co-principal investigator at the site. The group will discuss issues related to the progress of the trial at the site, and to ensure that the trial is running well.

There will be a similar trial management team formed to conduct the day-to-day management of the trial at the MRC ("MRC TMT"). This will include the site principal investigator, trial statistician, clinical project manager, trial manager and data manager. The group will meet at least once per month, although may meet more often if required.

12.2 TRIAL MANAGEMENT GROUP

A TRACT Trial Management Group (TMG) will be formed comprising the Chief Investigator; centre Principal Investigators, co-investigators, and Trial Managers, other lead investigators (clinical and non-clinical), members of the MRC Clinical Trials Unit (CTU) and will be responsible for overseeing the progress of the trial. The day-to-day management of the trial will be coordinated through the Kilifi Study Coordination Centre. The TMG will meet approximately once a year in-person and will hold a regular teleconference at approximately monthly intervals at which sites will summarise progress and challenges and bring up for discussion any difficulties, as well as discuss and decide matters of general importance for the trial. This group will be chaired by the Chief Investigator and all decisions regarding the overall running of the trial will be made in this forum with the exception of matters of fundamental importance to the viability of the trial or that require major changes to the protocol. These will be referred to the Trial Steering Committee (TSC). The full details can be found in the TMG Charter.

12.3 TRIAL STEERING COMMITTEE

The trial will be managed by a Trial Steering Committee (TSC) with an independent chairperson (Professor Elizabeth Molyneux OBE), a majority of independent members and one Principal Investigator or key investigator from each of the sites, from Imperial College, and from MRC CTU. Prof Molyneux previously chaired the FEAST TSC.

Each centre would either use their existing Community Advisory Board (CAB) or form a specific patient liaison group who would be responsible for liaising with their independent representatives on the TSC, would feedback concerns and questions from the community, and also hear about the latest developments in the trial and the wider scientific community.

12.4 INDEPENDENT DATA MONITORING COMMITTEE

An independent Data Monitoring Committee (DMC) will be set up to review data on enrolment, safety, adherence to randomised strategies, efficacy and safety at regular intervals and in strict confidence. The Chairman will be Professor Tim Peto. The DMC will report to the TSC and to the Ethics Committee in each country, if, in their view, the data provide proof beyond reasonable doubt that one of the allocated strategies is better than its comparator in terms of the primary outcome. The TSC will then decide whether

to amend (which may include removing one of the intervention arms) or stop the trial before the end of the planned follow-up. If a decision is made to continue, the DMC will advise on the frequency of future reviews of the data on the basis of accrual and event rates. The DMC will make recommendations to the TRACT Trial Steering Committee as to the continuation of the trial.

12.5 ENDPOINT REVIEW COMMITTEE

An Endpoint Review Committee (ERC) will review clinical data (blinded to allocation) and will determine the validity of potential endpoints. The ERC will adjudicate endpoints blinded to randomised allocations: relationship to all possible interventions drugs (transfusion strategy; nutritional support and cotrimoxazole) will be solicited to avoid unblinding. It will have an independent Chair (Dr Jennifer Evans) and will the include Project Leaders from each site as well as other independent clinicians. No member will review endpoints from their own site. Terms of reference for the Endpoint Review Committee will be drawn up.

13 PUBLICATION POLICY

All publications and presentations relating to the study will be authorised by the Trial Management Group. The first publication of the trial results will be in the name of the Trial Management Group, if this does not conflict with the journal's policy. If there are named authors, these will include at least the trial's Chief Investigator, Statistician and Trial Coordinator. Members of the TMG and the Data Monitoring Committee will be listed and contributors will be cited by name if published in a journal where this does not conflict with the journal's policy. Authorship of parallel studies initiated outside of the Trial Management Group will be according to the individuals involved in the project but must acknowledge the contribution of the Trial Management Group and the Study Coordination Centre.

The TRACT TSC is the custodian of the data and specimens generated from the TRACT trial; TRACT trial data are not the property of individual participating investigators or health care facilities where the data were generated.

During the course and following completion of the trial there will be publications, including manuscripts and abstracts for presentation at national and international meetings, as well as the preparation of manuscripts for peer-reviewed publication. In order to avoid disputes regarding authorship, it is important to establish a consensus approach that will provide a framework for all publications derived in full or in part from this clinical trial. The following approach is derived from the Lancet and from the publication policies used in other MRC clinical trials:

- All publications are to be approved by the TMG and TSC before submission for publication. Any publication arising before the end of the trial (not by randomised groups) will also be approved by the DMC in order to ensure that the primary objective of the trial (the randomised comparison) is not compromised. In particular, no analyses by randomised group of any outcome (primary, secondary or other) in either the main trial or associated substudies will be conducted or presented before the end of the trial, other than those for interim review by the DMC. The TMG and TSC will resolve problems of authorship and maintain the quality of publications.

- In line with MRC policy that the results of publicly-funded research should be freely available, manuscripts arising from the trial will, wherever possible, be submitted to peer-reviewed journals which enable Open Access via UK PubMed Central (PMC) within six months of the official date of final publication. All conference presentations will be made available as soon as possible after the event via the TRACT website. All publications will acknowledge the trial's funding sources.

- For all publications, the TMG will nominate a chairperson or approve an individual's request to chair a manuscript writing committee. The chair will usually be the primary or senior author. The chairperson is responsible for identifying fellow authors and for determining with that group the order of authorship that will appear on the manuscript. The TSC will resolve any problems of authorship and maintain the quality of publications.

- The TMG will maintain a list of investigators to be presented in an appendix at the end of the paper. This list will include investigators who contributed to the investigation being reported but who are not members of the writing committee. In principle, substudy reports should include all investigators for the main study, although in some instances where a smaller number of investigators have made any form of contribution, it may be appropriate to abbreviate the listing.

- All headline authors in any publication arising from the main study or sub-studies must have a made a significant academic or project management contribution to the work that is being presented. "Significant" must be defined by a written declaration of exactly what the contribution of any

individual is believed to have been. In addition to fulfilling the criteria based on contribution, additional features that will be considered in selecting an authorship group will include the recruitment of patients who contributed data to any set of analyses contained in the manuscript, and /or the conduct of analyses (laboratory and statistical), leadership and coordination of the project in the absence of a clear academic contribution.

- The data derived from this clinical trial are considered the property of the TRACT Trial Steering Committee. The presentation or publication of any data collected by the participating investigators on patients entered into this trial is under the direct control of the TMG and TSC (and the DMC before the end of the trial). This is true whether the publication or presentation is concerned directly with the results of the trial or is associated with the trial in some other way. However, although individual participating investigators will not have any inherent right to perform analyses or interpretations or to make public presentations or seek publication of any of the data other than under the auspices of and with the approval of the TMG and TSC (and the IDMC before the end of the trial), they will be encouraged to develop sub-studies or propose analyses subject to the approval by the TMG and TSC (and the DMC before the end of the trial). Any requests for access to raw data will be welcomed as long as they are scientifically valid and do not conflict with the integrity of the trial or ongoing analyses by the trial team

- Outcome data by randomised group will not be revealed to the participating investigators until the data collection phase and primary full analysis of the trial has been completed. This policy safeguards against possible bias affecting the data collection. The DMC will be monitoring the outcome results and may recommend that the trial be stopped for safety reasons or if a definitive answer is reached earlier than the scheduled end of the trial.

14 REFERENCES

1. de Benoist B, McLean E, Egli I, Cogswell M: **Worldwide prevalence of anaemia 1993–2005 : WHO global database on anaemia** *WHO report* 2008.
2. Rowe AK, Rowe SY, Snow RW, Korenromp EL, Schellenberg JR, Stein C, Nahlen BL, Bryce J, Black RE, Steketee RW: **The burden of malaria mortality among African children in the year 2000**. *Int J Epidemiol* 2006, **35**(3):691-704.
3. **Hospital Care for Children: guidelines for the management of common illnesses with limited resources.** In. Geneva, Switzerland: World Health Organization; 2005.
4. Brabin BJ, Premji Z, Verhoeff F: **An analysis of anemia and child mortality**. *J Nutr* 2001, **131**(2S-2):636S-645S; discussion 646S-648S.
5. Phiri KS, Calis JC, Faragher B, Nkhoma E, Ng'oma K, Mangochi B, Molyneux ME, van Hensbroek MB: **Long term outcome of severe anaemia in Malawian children**. *PloS one* 2008, **3**(8):e2903.
6. Calis JC, Phiri KS, Faragher EB, Brabin BJ, Bates I, Cuevas LE, de Haan RJ, Phiri AI, Malange P, Khoka M *et al*: **Severe anemia in Malawian children**. *The New England journal of medicine* 2008, **358**(9):888-899.
7. Meremikwu M, Smith HJ: **Blood transfusion for treating malarial anaemia**. *Cochrane database of systematic reviews (Online)* 2000(2):CD001475.
8. Hassell O, Bates I, Maitland K: **Blood Transfusion in Resource-Limited Settings**. In: *Hunter's Tropical Medicine* 9th edn. Edited by Claney N. London: Magill – Maguire – Ryan - Solomon; 2012.
9. **Utilization and supply of human blood and blood products in Twenty-eighth World Health Assembly**. In. Geneva: World Health Organization (WHO). 1975.
10. Berkley JA, Lowe BS, Mwangi I, Williams TN, Bauni E, Mwarumba S, Ngetsa C, Hart A, Maitland K, English M *et al*: **Bacteremia amongst Children Admitted to a Rural Hospital in Kenya.** *N Engl J Med* 2005, **352**(1):39-47.
11. English M, Ahmed M, Ngando C, Berkley J, Ross A: **Blood transfusion for severe anaemia in children in a Kenyan hospital**. *Lancet* 2002, **359**(9305):494-495.
12. Lackritz EM, Campbell CC, Ruebush TK, 2nd, Hightower AW, Wakube W, Steketee RW, Were JB: **Effect of blood transfusion on survival among children in a Kenyan hospital**. *Lancet* 1992, **340**(8818):524-528.
13. Bojang KA, Van Hensbroek MB, Palmer A, Banya WA, Jaffar S, Greenwood BM: **Predictors of mortality in Gambian children with severe malaria anaemia**. *Ann Trop Paediatr* 1997, **17**(4):355-359.
14. Ernest SK, Anunobi NE, Adeniyi A: **Correlates of emergency response interval and mortality from severe anaemia in childhood**. *West Afr J Med* 2002, **21**(3):177-179.
15. Maitland K, Kiguli S, Opoka RO, Engoru C, Olupot-Olupot P, Akech SO, Nyeko R, Mtove G, Reyburn H, Lang T *et al*: **Mortality after fluid bolus in African children with severe infection**. *The New England journal of medicine* 2011, **364**(26):2483-2495.
16. Marsh K, Forster D, Waruiru C, Mwangi I, Winstanley M, Marsh V, Newton C, Winstanley P, Warn P, Peshu N *et al*: **Indicators of life-threatening malaria in African children**. *The New England journal of medicine* 1995, **332**(21):1399-1404.
17. Pedro R, Akech S, Maitland K: **Changing trends in blood transfusion in children and neonates admitted in Kilifi District Hospital, Kenya**. *Malar J* 2010, **9**(1):307.
18. Holzer BR, Egger M, Teuscher T, Koch S, Mboya DM, Smith GD: **Childhood anemia in Africa: to transfuse or not transfuse?** *Acta Trop* 1993, **55**(1-2):47-51.
19. Bojang KA, Palmer A, Boele van Hensbroek M, Banya WA, Greenwood BM: **Management of severe malarial anaemia in Gambian children**. *Trans R Soc Trop Med Hyg* 1997, **91**(5):557-561.

20. Lacroix J, Hebert PC, Hutchison JS, Hume HA, Tucci M, Ducruet T, Gauvin F, Collet JP, Toledano BJ, Robillard P *et al*: **Transfusion strategies for patients in pediatric intensive care units**. *The New England journal of medicine* 2007, **356**(16):1609-1619.
21. Walker RH: **Mathematical calculations in transfusion medicine**. *Clinics in laboratory medicine* 1996, **16**(4):895-906.
22. Cheema B, Molyneux EM, Emmanuel JC, M'Baya B, Esan M, Kamwendo H, Kalilani-Phiri L, Boele van Hensbroek M: **Development and evaluation of a new paediatric blood transfusion protocol for Africa**. *Transfus Med* 2010, **20**(3):140-151.
23. Esan MO, Phiri KS, Molyneux EM, Mukaka M, Cheema B, Boele van Hensbroek M: **High transfusion failure rates in Malawian children with severe anaemia following a standard blood transfusion regimen**. *Br J Haematol* 2011.
24. CDC: **Progress toward strengthening blood transfusion services--14 countries, 2003-2007**. *Mmwr* 2008, **57**(47):1273-1277.
25. O'Meara WP, Bejon P, Mwangi TW, Okiro EA, Peshu N, Snow RW, Newton CR, Marsh K: **Effect of a fall in malaria transmission on morbidity and mortality in Kilifi, Kenya**. *Lancet* 2008, **372**(9649):1555-1562.
26. Okiro EA, Bitira D, Mbabazi G, Mpimbaza A, Alegana VA, Talisuna AO, Snow RW: **Increasing malaria hospital admissions in Uganda between 1999 and 2009**. *BMC medicine* 2011, **9**:37.
27. Akech SO, Hassall O, Pamba A, Idro R, Williams TN, Newton CR, Maitland K: **Survival and haematological recovery of children with severe malaria transfused in accordance to WHO guidelines in Kilifi, Kenya**. *Malar J* 2008, **7**:256.
28. Abdalla SH: **Iron and folate status in Gambian children with malaria**. *Ann Trop Paediatr* 1990, **10**(3):265-272.
29. van Hensbroek MB, Morris-Jones S, Meisner S, Jaffar S, Bayo L, Dackour R, Phillips C, Greenwood BM: **Iron, but not folic acid, combined with effective antimalarial therapy promotes haematological recovery in African children after acute falciparum malaria**. *Trans R Soc Trop Med Hyg* 1995, **89**(6):672-676.
30. van den Broek NR, Letsky EA: **Etiology of anemia in pregnancy in south Malawi**. *The American journal of clinical nutrition* 2000, **72**(1 Suppl):247S-256S.
31. Savage DG, Allen RH, Gangaidzo IT, Levy LM, Gwanzura C, Moyo A, Mudenge B, Kiire C, Mukiibi J, Stabler SP *et al*: **Pancytopenia in Zimbabwe**. *The American journal of the medical sciences* 1999, **317**(1):22-32.
32. Sazawal S, Black RE, Ramsan M, Chwaya HM, Stoltzfus RJ, Dutta A, Dhingra U, Kabole I, Deb S, Othman MK *et al*: **Effects of routine prophylactic supplementation with iron and folic acid on admission to hospital and mortality in preschool children in a high malaria transmission setting: community-based, randomised, placebo-controlled trial**. *Lancet* 2006, **367**(9505):133-143.
33. Zimmermann MB, Chassard C, Rohner F, N'Goran E K, Nindjin C, Dostal A, Utzinger J, Ghattas H, Lacroix C, Hurrell RF: **The effects of iron fortification on the gut microbiota in African children: a randomized controlled trial in Cote d'Ivoire**. *The American journal of clinical nutrition* 2010, **92**(6):1406-1415.
34. Naikare H, Palyada K, Panciera R, Marlow D, Stintzi A: **Major role for FeoB in Campylobacter jejuni ferrous iron acquisition, gut colonization, and intracellular survival**. *Infection and immunity* 2006, **74**(10):5433-5444.
35. Galloway R, McGuire J: **Determinants of compliance with iron supplementation: supplies, side effects, or psychology?** *Soc Sci Med* 1994, **39**(3):381-390.
36. Schauer C, Zlotkin S: **Home fortification with micronutrient sprinkles - A new approach for the prevention and treatment of nutritional anemias**. *Paediatrics & child health* 2003, **8**(2):87-90.
37. Zlotkin S, Arthur P, Antwi KY, Yeung G: **Treatment of anemia with microencapsulated ferrous fumarate plus ascorbic acid supplied as sprinkles to complementary (weaning) foods**. *The American journal of clinical nutrition* 2001, **74**(6):791-795.
38. Zlotkin S, Arthur P, Schauer C, Antwi KY, Yeung G, Piekarz A: **Home-fortification with iron and zinc sprinkles or iron sprinkles alone successfully treats anemia in infants and young children**. *J Nutr* 2003, **133**(4):1075-1080.

39. Suchdev PS, Ruth LJ, Woodruff BA, Mbakaya C, Mandava U, Flores-Ayala R, Jefferds ME, Quick R: **Selling Sprinkles micronutrient powder reduces anemia, iron deficiency, and vitamin A deficiency in young children in Western Kenya: a cluster-randomized controlled trial**. *The American journal of clinical nutrition* 2012, **95**(5):1223-1230.
40. Jefferds ME, Ogange L, Owuor M, Cruz K, Person B, Obure A, Suchdev PS, Ruth LJ: **Formative research exploring acceptability, utilization, and promotion in order to develop a micronutrient powder (Sprinkles) intervention among Luo families in western Kenya**. *Food and nutrition bulletin* 2010, **31**(2 Suppl):S179-185.
41. Suchdev PS: **Multiple micronutrient powders for home (point of use) fortification of foods in pregnant women: a systematic review.** In: *WHO e-Library of Evidence for Nutrition Actions.* World Health Organization; 2011.
42. McDonald PJ, Pruul H: **Phagocyte uptake and transport of azithromycin**. *Eur J Clin Microbiol Infect Dis* 1991, **10**(10):828-833.
43. Lode H: **The pharmacokinetics of azithromycin and their clinical significance**. *Eur J Clin Microbiol Infect Dis* 1991, **10**(10):807-812.
44. Chintu C, Bhat GJ, Walker AS, Mulenga V, Sinyinza F, Lishimpi K, Farrelly L, Kaganson N, Zumla A, Gillespie SH *et al*: **Co-trimoxazole as prophylaxis against opportunistic infections in HIV-infected Zambian children (CHAP): a double-blind randomised placebo-controlled trial**. *Lancet* 2004, **364**(9448):1865-1871.
45. Mulenga V, Ford D, Walker AS, Mwenya D, Mwansa J, Sinyinza F, Lishimpi K, Nunn A, Gillespie S, Zumla A *et al*: **Effect of cotrimoxazole on causes of death, hospital admissions and antibiotic use in HIV-infected children**. *Aids* 2007, **21**(1):77-84.
46. Walker AS, Ford D, Gilks CF, Munderi P, Ssali F, Reid A, Katabira E, Grosskurth H, Mugyenyi P, Hakim J *et al*: **Daily co-trimoxazole prophylaxis in severely immunosuppressed HIV-infected adults in Africa started on combination antiretroviral therapy: an observational analysis of the DART cohort**. *Lancet* 2010, **375**(9722):1278-1286.
47. Mwenya DM, Charalambous BM, Phillips PP, Mwansa JC, Batt SL, Nunn AJ, Walker S, Gibb DM, Gillespie SH: **Impact of cotrimoxazole on carriage and antibiotic resistance of Streptococcus pneumoniae and Haemophilus influenzae in HIV-infected children in Zambia**. *Antimicrobial agents and chemotherapy* 2010, **54**(9):3756-3762.
48. Thera MA, Sehdev PS, Coulibaly D, Traore K, Garba MN, Cissoko Y, Kone A, Guindo A, Dicko A, Beavogui AH *et al*: **Impact of trimethoprim-sulfamethoxazole prophylaxis on falciparum malaria infection and disease**. *The Journal of infectious diseases* 2005, **192**(10):1823-1829.
49. Arinaitwe E, Gasasira A, Verret W, Homsy J, Wanzira H, Kakuru A, Sandison TG, Young S, Tappero JW, Kamya MR *et al*: **The association between malnutrition and the incidence of malaria among young HIV-infected and -uninfected Ugandan children: a prospective study**. *Malar J* 2012, **11**:90.
50. Wilson AL: **A systematic review and meta-analysis of the efficacy and safety of intermittent preventive treatment of malaria in children (IPTc)**. *PloS one* 2011, **6**(2):e16976.
51. Porco TC, Gebre T, Ayele B, House J, Keenan J, Zhou Z, Hong KC, Stoller N, Ray KJ, Emerson P *et al*: **Effect of mass distribution of azithromycin for trachoma control on overall mortality in Ethiopian children: a randomized trial**. *JAMA* 2009, **302**(9):962-968.
52. McAuley CF, Webb C, Makani J, Macharia A, Uyoga S, Opi DH, Ndila C, Ngatia A, Scott JA, Marsh K *et al*: **High mortality from Plasmodium falciparum malaria in children living with sickle cell anemia on the coast of Kenya**. *Blood* 2010, **116**(10):1663-1668.
53. Harris KA, Hartley JC: **Development of broad-range 16S rDNA PCR for use in the routine diagnostic clinical microbiology service**. *J Med Microbiol* 2003, **52**(Pt 8):685-691.
54. Millar BC, Xu J, Moore JE: **Risk assessment models and contamination management: implications for broad-range ribosomal DNA PCR as a diagnostic tool in medical bacteriology**. *J Clin Microbiol* 2002, **40**(5):1575-1580.
55. Ferri E, Novati S, Casiraghi M, Sambri V, Genco F, Gulminetti R, Bandi C: **Plasma levels of bacterial DNA in HIV infection: the limits of quantitative polymerase chain reaction**. *The Journal of infectious diseases* 2010, **202**(1):176-177; author reply 178.

56. Marchetti G, Bellistri GM, Borghi E, Tincati C, Ferramosca S, La Francesca M, Morace G, Gori A, Monforte AD: **Microbial translocation is associated with sustained failure in CD4+ T-cell reconstitution in HIV-infected patients on long-term highly active antiretroviral therapy**. *AIDS* 2008, **22**(15):2035-2038.
57. Merlini E, Bai F, Bellistri GM, Tincati C, d'Arminio Monforte A, Marchetti G: **Evidence for Polymicrobic Flora Translocating in Peripheral Blood of HIV-Infected Patients with Poor Immune Response to Antiretroviral Therapy**. *PloS one* 2011, **6**(4):e18580.
58. Suau A, Bonnet R, Sutren M, Godon JJ, Gibson GR, Collins MD, Dore J: **Direct analysis of genes encoding 16S rRNA from complex communities reveals many novel molecular species within the human gut**. *Appl Environ Microbiol* 1999, **65**(11):4799-4807.
59. Rees DC, Williams TN, Gladwin MT: **Sickle-cell disease**. *Lancet* 2010, **376**(9757):2018-2031.
60. Beutler E: **G6PD deficiency**. *Blood* 1994, **84**(11):3613-3636.
61. Hotz C, Abdelrahman L, Sison C, Moursi M, Loechl C: **A Food Composition Table for Central and Eastern Uganda**. In: *HarvestPlus Technical Monographs.* Washington, USA: International Food Policy Research Institute (IFPRI) and International Center for Tropical Agriculture (CIAT). 2012.
62. Thakwalakwa CM, Kuusipalo HM, Maleta KM, Phuka JC, Ashorn P, Cheung YB: **The validity of a structured interactive 24-hour recall in estimating energy and nutrient intakes in 15-month-old rural Malawian children**. *Maternal & child nutrition* 2012, **8**(3):380-389.
63. Maitland K, Molyneux S, Boga M, Kiguli S, Lang T: **Use of deferred consent for severely ill children in a multi-centre phase III trial**. *Trials* 2011, **12**:90.

15 APPENDIX I TEMPLATE PATIENT INFORMATION SHEETS ,CONSENT AND ASSENT FORM

[Each country to use its own translated Informed Consent for relevant local languages according to local regulatory requirements, on local headed paper for each site]

TRACT (Transfusion and Treatment of Severe Anaemia in African Children)

Information for Parents and Carers

Introduction

We are inviting your child to take part in a research study that is called TRACT. It is being conducted at three hospitals in Uganda – Mulago, Mbale and Soroti hospital and Queen Elizabeth Hospital, Blantyre, Malawi. We will include children between the ages of 2 months to 12 years, and aim to involve nearly 4000 children across these hospitals over the next two years. Before you decide if you want your child to take part, it is important for you to understand why the research is being done and what it will involve. Please take time to read this information sheet carefully or ask someone to read it to you. Please discuss this with the nurses or doctors and ask questions if there is anything that is not clear or if you would like more information. Joining the TRACT study is entirely voluntary. Take time to decide whether or not you wish your child to take part.

B: Study purpose: What is the reason for doing the TRACT study?

Your child has been admitted to hospital because they have severe anaemia. This is a serious illness and a common problem in many children in Africa. We have done a blood test and found the strength of your child's blood (or haemoglobin level) is much lower than normal – this is called severe anaemia. Anaemia often causes your child to feel tired, weak and the nailbed or tongue to look pale (*can demonstrate*). The TRACT study is trying to find the best way to treat this. At the moment we don't know whether a blood transfusion is the best way to treat this and after then how to treat the underlying illnesses which caused your child to have severe anaemia. Some children with severe anaemia may die and some children become sick again in the next 6 months. We need to do this study to find the best treatments for severe anaemia in to order to reduce the chance of these things happening.

What is the TRACT study about?

We want to find out whether or not giving a blood transfusion is the best treatment for your child. For children getting a blood transfusion we also don't know how much blood its best to give, either the standard volume (dose) recommended in the current guidelines or a slightly higher volume (dose).

We also want to find out whether or not giving extra treatments during the first three months after this hospital admission will prevent some children with severe anaemia from dying or becoming sick again.

All children in TRACT over one year of age will get tablets to kill any 'worms' they have inside their stomach when they leave the hospital, and all children will get vitamin medicine to make their blood stronger. On top of this, we will be looking at whether:

1/ A vitamin treatment called Sprinkles containing 15 different chemicals/vitamins taken for 3 months is better than the usual treatment of folate and iron
2/ Whether a single pill containing an antibiotic, cotrimoxazole, taken for 3 months will fight infections and stop children from getting sick.

C: Study Procedures: What will it involve for my child?

What treatments will he/she be given?

The doctors at Mbale/Mulago/Soroti/Queen Elizabeth *(delete as appropriate)* hospital will treat your child according to standard Ugandan/Malawian Ministry of Health guidelines for severe illness and/or severe malaria. For the children in the TRACT study we will be studying

i) Transfusion:

1. If your child has a very low haemoglobin or, if the doctor has checked your child and found features which tell them your child is very sick, then your child will get a blood transfusion. We don't know what the best amount of blood to give so half the children will either receive the standard dose (as currently recommended) and half a slightly higher volume (dose).
2. If the doctor has found your child is not very sick but just has a low haemoglobin then we are not sure whether a blood transfusion is needed. At the moment the recommendations are not to give a transfusion – we want to find out whether giving a transfusion in some children may help them recover faster. Half of the children like this in TRACT will receive no transfusion and half of the children will receive a blood transfusion (either at a standard dose or at a higher dose).

ii) Vitamin treatments
Half the children will get iron and folate for 3 months (the current recommendation) while the other half will get a different vitamin medicine which is sprinkled onto their food every day for 3 months (or if the child is still breast feeding mum will receive this medicine instead). These vitamins are trying to make the child's blood stronger – we don't know which type is best.

iii) Preventing Infection

Often children with severe anaemia come back to hospital with another illness in the 6 months after this current admission – to try to prevent this half the children will get an antibiotic tablet called cotrimoxazole for 3 months while the other half will not.

The decision as to which child gets which treatment will be decided when they join the study by a system based on chance, using a computer, not by any member of the research team. All the medicines used in the study have been widely used in children before and found to be very safe.

Study Procedures

1. Children will be carefully checked over the time they are in hospital – including extra blood tests to check if they still have anaemia. These regular checks that the doctors and nurses do will also help us find out if the child is getting better or not. All children who are found to have a very low haemoglobin during these checks will receive another blood transfusion, or an initial transfusion if they did not get one immediately after they arrived at hospital. If there are any side effects from a blood transfusion we should be able to discover these during these regular checks and treat them promptly.

2. On the day your child is admitted we will be doing blood tests to help us find out what is causing your child's illness, how sick they are and to help us decide what type of blood to transfuse. An extra 5-10mls (one to two teaspoons) will be taken saved for future tests to help us find out why your child became ill. We will also want to take a sample of your child's urine and poo for the same reasons. Some results you will get back during the study - others will be done much later at the end of the study and you may not get the results of some of these tests.

3. When your child is ready to go home we will give you vitamin medicines to make your child's blood stronger and half of the children in the study will receive an antibiotic (cotrimoxazole) – both of these will be taken every day for 3 months (90-days). We will give you enough tablets/medicines to last until your next follow up visit. We will also ask you about the kind of food the child has been eating before they came to hospital, to try to help us find out why they became ill with anaemia in the first place. We will check where you live and take your contact details- to help us find you if you are not able to come back. You can contact us if you have any concerns about your child condition or they have had to go to hospital – our team will call you back if necessary.

4. All children will have to come back after one month, three months and six months. We will check the health of your child at this visit, find out what food they have eaten on the day before they came to the clinic, find out whether they have been ill or to hospital since the last visit and check on whether they have been able to take the treatments and if they are causing any problems. We will give you more medicines at the one month visit to last for the next 2 months. We will check on your child's haemoglobin level and do a malaria test at each of these visits. If they have any illness we will treat these or refer the child if necessary to another specialist. Some of the blood taken at these visits will be stored – approximately 3-5ml (equivalent to half to one teaspoon) to help us understand why your child became sick and how s/he is reacting to the treatments we are giving.

5. In total, 10mls (2-teaspoon full of blood) will be taken from your child at the start of the study and another 5-10mls (1-2teaspoons) when they come for later check up visits, as outlined above. This is a very small amount of blood – which will not harm the health of your child (*Can demonstrate what their child's volume of blood is using pre-prepared drawings – based upon 70ml/kg circulating volume).*

6. Some of the tests to find out what caused your child's illness are needed as part of this research cannot be done in this country at the moment, so part of the samples will be sent to laboratories overseas. This will involve a small portion of the blood that was taken during the study, which we will store. Some of the tests we will do will look at whether your child has a trait or characteristic that they inherited from their parents that will either make them more vulnerable to severe anaemia or help their bodies to fight against diseases that cause anaemia better as a result of these inherited traits. This is called genetic research. Individual names will be removed and will be replaced by codes, so that information cannot be linked to participants. Future research done on these samples will be approved by a national independent expert committee, to ensure that participants' safety and rights are respected.

D: Risks of study participation

There are very few risks to your child being in this study. Both vitamin medicines and the antibiotic cotrimoxazole have been widely used in children with very few problems. The TRACT team is working closely with the local blood transfusion services to make sure that blood used in the study is safe. As with any blood transfusion, there is a small chance that there may be a reaction but we will be monitoring your child very closely during the blood transfusion, and will treat your child quickly if this happens. The same thing will happen if you child has problems with any of the other drugs they take in hospital or at home. If for any reason the doctor thinks that it is not in your child's best interest to be in

the study then they will not be enrolled in the study but will be given their usual treatment. You do not have to pay anything to join the study.

All children will have blood taken as part of this study. Many of the tests that will be done in hospital would also be done if you chose not to join the study. However, we will take a small amount of extra blood at the follow-up visits (see above).

E: Benefits of study participation

Your child will get no direct benefits from this study. However, your child will get close observation during the study, and by taking part your child may help us improve the care of children who have severe anaemia in the future. Regular assessment of your child by doctors and nurses will enable us to make important changes to your child's treatment in hospital, if these are needed. We will help supply routine medical supplies and treatments for your child to the hospital, so that you will not have to buy any treatments. This will mean that there will be no delay to starting treatment for your child. The medical tests we perform during this illness will also be paid for by the study.

You will be asked to bring your child back for follow up visits, and we will pay for your transport from hospital to your home and back to the clinic so you can attend these important visits. During the follow up visits will treat any illnesses we find, or arrange referral to appropriate clinic or hospital.

F: Alternatives to study participation: What will happen if I don't agree to participate?

All participation in research is voluntary. You are free to decide if you want your child to take part or not. Your child will still receive the recommended standard of care treatment if they do not take part. If you do agree to join the study you can change your mind at any time, and can withdraw your child from the research. This will not affect their care now or in the future and not incur any penalties. We hope that if you decide to withdraw later, you would give a reason for your decision. More importantly we hope that you would continue to allow us to provide follow-up care which involves continued regular medical check ups, even if you are no longer taking the study medicines.

G: Compensation

You will not incur any costs from participation in this study. All your travel expenses for attending the visits we invite you too will be paid, based on the cost of public transport to and from your home. As well, when you bring your child for follow up, snacks and drinks will be available and for meals, in a situation where you have to wait for a long time before being attended to.

H: Confidentiality. Who will have access to information about me/my child in this research?

All our research records are stored securely in locked cabinets and password protected computers. Only a few people who are working closely on the study will be able to view information from your child. When we report on the results of the study will not include any private information that will make it possible to identify your child.

I: Study related injury

This research is supported by Imperial College London who holds insurance policies which apply to this study. If you experience harm or injury as a result of taking part in this study, you will be eligible to claim compensation without having to prove that Imperial College is at fault. Some specific treatment and compensation are not included in our insurance policies and if you want more information about this you should discuss it with your doctor.

J: Contacts and questions

Who has allowed this research to take place?

All research conducted in Uganda/Malawi *[delete as appropriate]* is approved by national independent expert committees to make sure the research is conducted properly and that study participants' safety and rights are respected.

What if I have any questions?

You may ask any of our staff questions at any time. You can also contact those who are responsible for the care of your child and this research:
The following will be site dependent:

Professor Sarah Kiguli, Department of Paediatrics, PO Box 7072 Makerere University, Kampala, Uganda.

Tel +256 41 531875; Fax +256 41 532591

Or

Dr Robert Opoka, **MBCHB, MMED** Consultant Paediatrician

opokabob@yahoo.com; Mobile: +254 772 996164; Tel +256 41 531875; Fax +256 41 532591

Or

Dr Peter Olupot Olupot, MBCHB, MPH Mbale Regional Referral Hospital, P.O Box 921, Mbale.
polupotolupot@yahoo.com; Mobile : + 256 (0)772457217/ Tel: (0)392910171/(0)352280584/(0)45 4433193 Fax: +256 45 4435894

Or

Dr Charles Engoru, MBCHB, MMED. Soroti Regional Hospital, P.O Box 289 Soroti.
charlasengoru@yahoo.co.uk; Mobile: +256 772 458587; Fax +256 45 4461382

Or

Dr Yamikani Chimalizeni, Queen Elizabeth Hospital, PO Box 30096, Chichiri Blantyre 3.

Tel Fax

Or

If you have any questions about your rights as a participant, please contact

Professor James Tumwini,
Chairman Faculty of Medicine Research and Ethics Review Committee, School of Medicine, PO Box 7072 Makerere University Kampala, Uganda.
Tel: +256 41 4530020

Or

XXX
Chairman, College of Medicine Ethics Review Committee, Blantyre

If you would like to contact the Chief Investigator, Professor Kathryn Maitland
Please contact
Trial Administrator: Phyles Maitha
Address: KEMRI Wellcome Trust Programme, P.O Box 230-80108, Kilifi, Kenya
E-mail: pmaitha@kemri-wellcome.org
Tel: +254 41 522063
Fax: +254 41 522390

TRIAL Consent Form (To be presented on local-headed paper)

Version 1.0 8th Jan 2013

Child's Initials				Study Number							

TRACT (Transfusion and Treatment of Severe Anaemia in African Children)

Please initial (or mark) box if you agree:

I confirm that I have read/ been read the patient information sheet (version 1.0 dated 21th Jan 2013) for the TRACT study and that I understand what will be required if my child participates in the study. The study has been explained to me and my questions have been answered.	
I understand that my child's participation is voluntary and that I am free to withdraw him or her at any time, without giving any reason, without my medical care or legal rights or my child's medical care or legal rights being affected.	
I understand that sections of any of my child's medical notes may be looked at by responsible individuals involved in the running of the study or from regulatory authorities where it is relevant to my child's participation in this research. I give permission for these individuals to have access to my child's records, but understand that strict confidentiality will be maintained.	
I understand that my child will be given 3 months of treatment after discharge from hospital then followed up for another 3 months. After the study, my child's healthcare will be provided by the national health system.	
I agree to allow blood samples to be taken from my child and for my child's samples to be stored for later testing. I understand that my child and I may not be given the results of tests performed on stored samples.	
I agree to samples being exported overseas for further studies	
I agree for my child to participate in the TRACT study	

Parent/carer's signature (or thumbprint)	Print name	Date (day/month/year) Time

Witness's signature (if thumbprint used above)	Print name	Date (day/month/year) Time

Doctor's signature	Print name	Date (day/month/year) Time

IMPORTANT: one signed original to be kept in TRACT trial file by the researcher, one signed copy to be given to the patient, one signed copy to be kept in the clinic file

Verbal Assent (To be presented on local-headed paper)
Version 1.0 8th Jan 2013

TRACT (Transfusion and Treatment of Severe Anaemia in African Children)

Child's Initials				Male ❍ Female ❍	Date/Year of Birth	D	D	M	M	M	Y	Y	Y	Y	Age (years)		

Date of Form	D	D	M	M	M	2	0	Y	Y

Clinic/Hospital Number								

NOTE: For children who are critically ill and in whom informed consent would lead to significant delay in starting treatment a verbal assent will be obtained by the doctor from the *parent or guardian* after brief discussion with admitting study doctor or nurse.

We advise that this should include the following phrases.

- We are going to provide the treatment for your child that is recommended by the government.
- We want to find out if we can improve on these current recommendations by trying new treatments that we think will work better and we do this by research.
- All research is checked by independent committees to make sure that the potential benefits to individuals outweigh the risks. All participation in research is voluntary, and so you can refuse.
- We would like your child to participate in this research for us to learn the best way to treat severe anaemia.
- Do you agree for your child to take part in this research? You can say no and your child will still receive the same level of care with the governments recommended treatment.

Parent/Guardian assents to research?	**Please circle:** Yes / No

Parent or guardians name	Relationship with child	Time (24 hour clock)			
		H	H	M	M

Doctor or nurses signature	Print name	Date							
		D	D	M	M	M	2	0	Y	Y

Note: Original to be kept in TRACT trial file by the researcher, one signed copy to be given to parent/guardian/carer and one signed copy to be kept in the clinic notes.

16 APPENDIX II TEMPLATE PATIENT INFORMATION SHEETS AND CONSENT FOR GUT BARRIER FUNCTION AND IMMUNE ACTIVATION(MBALE ONLY)

TRACT Study Immune activation and Gut Barrier sub-study - Information for Parents/Guardians

Introduction

We are inviting you to join an extra part of the TRACT study. This extra study is looking at whether the bugs (bacteria) in your gut have made your child ill and whether the medicines you are receiving in TRACT will have an effect on the gut. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully or have someone read it to you, and discuss it with others if you wish. We will give you a copy to keep. Ask the nurses or doctors if there is anything that is not clear or if you would like more information. Providing or not providing and extra volume of blood and a urine and stool samples for storage is an extra part of the TRACT study will not change how you are treated in TRACT. You may decide that you do not wish to take part now or you may wish to withdraw from the study later. This will not influence the care you receive now or in future.

What is the reason for collecting urine and stool (poo) samples in TRACT?

You have already joined the TRACT study, which is looking at whether extra treatments make your child's blood stronger and reduce the risk of dying. We want to understand how these extra treatments may be working. We all have bacteria (bugs) living harmlessly in the gut that keep us well. When we become ill, though, the types of bugs that are found in the gut may change. Sometimes because there is damage to the gut, these bugs can get into the blood and may make your child ill. Some of the extra medicines that people in TRACT are taking may help to repair the gut, and reduce the amount of damage. This may reduce sickness and improve your child's health. We can look at these changes by measuring how well your child is fighting infections in the blood sample we took. We can also use samples of your child's stool (poo) and urine at certain points in the study to measure how damaged their gut is and to look at the types of bugs present in the gut flora. This information might help us to understand how severe anaemia can make your children very sick and how the extra medicines in TRACT are working.

What will happen if I take part?

Joining this extra part of the TRACT study will not make any difference to how you are managed; there will be no changes to your medicines or clinic visits. All children will have blood tests on admission to hospital and a stool sample and urine sample, whether or not they join this extra study. We would like to keep these in storage for later testing. After this, all we want to do in this extra study is to collect an small amount of extra blood, and a urine and stool (poo) sample from your child on 2 different visits to the clinic. We will ask you to collect a small sample of stool at home (on the morning you are due to come to clinic) or while you are in the clinic. This poo sample will be collected into a container using a spatula and the urine sample collected into a tube at the clinic. The nurse will give to you both of these. This is the only extra thing we will ask you to do. There are no additional questionnaires to fill in, and no extra time involved.

What are the risks and benefits of taking part?

There will be no additional risks to collecting urine or stool (poo) samples in TRACT. None of your treatment will change; there will be no extra blood draws or change in medicines. You are encouraged

to wash your hands thoroughly with soap or ash under running water after collecting the poo sample. There are no direct benefits to you of taking part. The main goal of this research study is to gain knowledge that may help us treat children with severe anaemia better in the future.

How long will the study continue?

We will ask you to collect a total of 3 stool and urine samples during your 6 months in the TRACT study. These will be on the day of admission (all children, whether or not they join this extra study), at 28 days, and 190 day (6 months). We will be collecting blood at each clinic in any case to check on your child's response to the treatments- we would like to collect an extra volume (1 teaspoon) to store for extra tests at these same visits - this will not harm your child or make their blood any weaker.

What will happen to the samples that are collected?

All the samples will be frozen until we are ready to do the tests. The tests will tell us how your childs immune system (which fights infections) is working and whether it has been affected by the bugs and the toxins the bugs make that may have come across from your gut into your blood and urine. We will also measure proteins in the stool that tell us how much damage there is in your gut and look at the types of bugs in your gut. These are very specialized techniques – so will need to be sent to a laboratory abroad to do this testing. Once this has finished, any remaining samples will be destroyed and thrown away.

What happens to the information collected in this study?

Information from these tests will be analysed and the results stored on a computer. We will not keep details of your name on the computer. Results will be presented and published so we can understand better how to care for African children with severe anaemia. These tests are just done to find out what is causing anaemia in children in Uganda/Malawi as a whole group, and we do not know what they would mean for an individual child. They will also be done some time after you finish the study visits, so you will not be given the results of these tests.

Benefits and/or compensation

We cannot and do not guarantee or promise that you will receive any benefits from this study. Participants will receive reimbursement for the usual TRACT visits. There will be no additional reimbursement.

Confidentiality

Strict confidentiality will be maintained at all times. Names will not be used for study information and stored stool samples; only the TRACT study number, date of birth and initials will identify these. There is just one list which links this study number to your name (already held by the TRACT study), and this list is safely kept private in a locked cabinet.

How can I join?

After reading this information sheet you will be asked to sign the form below giving consent to participate the next time you see the doctor.

What do I do if I have questions or problems? For questions about this study contact:

Dr Peter Olupot, Mbale Regional Referral Hospital, P.O Box 921, Mbale.

Mobile :+ 256 (0)772457217/ Tel: (0)392910171/(0)352280584/(0)45 4433193 Fax: +256 45 4435894

Before you sign this form, please ask any questions on any aspect of this study that is unclear to you. You may take as much time as necessary to think it over.

TRIAL SubStudy Form (To be presented on local-headed paper)

Version 1.0 8th Jan 2013

Child's Initials				Male ❍ Female ❍	Date/Year of Birth	D	D	M	M	M	Y	Y	Y	Y	Age (years)		

Date of Form	D	D	M	M	M	2	0	Y	Y	Clinic/Hospital Number								

I have read/been read the information sheet for the extract study in the TRACT study. I have understood everything and have had my questions answered satisfactorily. I understand that I may change my mind at any stage and that this will not affect the benefits due to my child.

I agree that samples of blood, urine and stool (poo) from my child may be kept by the TRACT team for studies related to this study, and any other further studies. I understand that these results and samples will not be identified by either my or my child's	
I agree to samples being exported overseas for further studies	

Carer's signature (or thumbprint)	Print name	Date
		D D M M M 2 0 Y Y

Witness's signature (if thumbprint used above)	Print name	Date
		D D M M M 2 0 Y Y

Doctor's or Nurse's signature	Print name	Date
		D D M M M 2 0 Y Y

Note: One signed original to be kept in TRACT trial file by the researcher, one signed copy to be given to guardian, one signed copy to be kept in the clinic notes.

17 APPENDIX III WITHDRAWAL FORM

Please initial (or mark) box if you agree:

I/my child no longer wish to (or cannot) take TRACT study drugs and do not wish to (or cannot) attend further visits. I/my child agree to being contacted in the future (home visits or telephone) and to my/my child's medical records being consulted in future to obtain clinical information for TRACT.	

Need to set up a procedure to follow the child up through visits and medical records and report any trial outcomes on the appropriate form. Inform the child and carer that s/he may still return for follow-up visits only or for further study drugs and follow-up visits at a later date if they change their mind.

I/my child no longer wish to (or cannot) TRACT take study drugs and do not wish to (or cannot) attend further visits. I/my child do not agree to being contacted in the future or to my/my child's medical records being consulted in future to obtain clinical information for	

Discontinue all follow up through medical records. The child and carer must sign a new consent form if s/he decides to rejoin the study at a later date.

Patient or carer's signature (or thumbprint)	Print name	Date (day/month/year)

Child's signature (or thumbprint) where appropriate	Print name	Date (day/month/year)

Witness's signature (if thumbprint used above)	Print name	Date (day/month/year)

Doctor's signature	Print name	Date (day/month/year)

IMPORTANT: One signed original to be given to patient
One signed original to be kept on file by the researcher
One signed original to be kept in the clinic notes

18 APPENDIX IV- TRANSFUSION-RELATED ADVERSE EVENTS

Haemovigilance (defined as the systematic surveillance of adverse reactions and adverse events related to transfusion), aimed at improving safety throughout the transfusion chain from donor to patient will be implemented throughout the trial. We aim to adapt use the guidelines for reporting Serious Adverse Events and Serious Adverse Reactions structure, with some modifications, recommended by UK's Serious Hazards Of Transfusion (SHOT) scheme in the UK.
(http://ww w.mhra.gov.uk/Safetyinformation/Reportingsafetyproblems/Blood/index.htm)

A standard SOP will be developed for investigating suspected serious adverse transfusion-related events, including where and how to report these and allied investigations (some of which may be stored for later analysis) to assist with defining causality of the event (ie the likelihood that a serious adverse reaction in a recipient can be attributed to the blood component transfused). These will include: suspected Acute transfusion reaction (ARR) Haemolytic transfusion reaction (acute or delayed) (HTR) Transfusion related acute lung injury (TRALI); Post transfusion purpura (PTP); Transfusion transmitted infection (TTI) and Transfusion associated circulatory overload (TACO).

Category	Definition	What to report	Where
ARR Suspected acute transfusion reaction	Reactions occurring at any time up to 24 hours following a transfusion of blood or components, *including* cases of acute reactions due to incorrect component being transfused*. **Excludes:** haemolytic reactions, TRALI, TACO or those due to bacterial contamination of the component. * *For simplicity these will be reported under ARR; whereas SHOT reports 'wrong blood to wrong patient' in a separate section*	**Isolated febrile** – a rise in temperature of > 1oC +/- minor rigors and chills (not thought to be due to underlying disease) **Febrile with other symptoms/signs** – rise in temperature of >1oC, with no features of an allergic reaction, but with one or more of myalgia, nausea, change in blood pressure or hypoxia.	CRF CRF
		Minor allergic – Denovo development of skin symptoms +/- rash	CRF
		Anaphylactic/anaphylactoid – hypotension with one or more of: urticaria, rash, dyspnoea, angioedema, stridor, wheeze, pruritus, within 24 hours of transfusion.	SAE
		Severe allergic reaction – Severe allergic reaction with risk to life occurring within 24 hours of transfusion, characterised by bronchospasm causing hypoxia, or angioedema causing respiratory distress. **Hypotension** – a drop in systolic and/or diastolic pressure of >30mm Hg occurring within one hour of completing transfusion, provided all other adverse reactions have been excluded together with underlying conditions that could explain hypotension.	SAE
HRT (acute or delayed)	**Acute** HTRs are defined as fever plus new symptoms / signs of haemolysis within 24 hours of transfusion; confirmed by a fall in Hb, positive DAT and positive crossmatch.		CRF
	Delayed HTRs are defined as		CRF

Haemolytic transfusion reaction	fever and other symptoms / signs of haemolysis more than 24 hours after transfusion; confirmed by one or more of: a fall in Hb PLUS rise in bilirubin, positive DAT and positive crossmatch. (*Simple serological reactions: development of antibody without positive DAT or development of haemolysis are excluded)*		
TRALI Transfusion related acute lung injury	Acute dyspnoea with hypoxia and bilateral pulmonary infiltrates during or within six hours of transfusion, not due to circulatory overload or other likely cause.		SAE
PTP Post transfusion purpura	Thrombocytopenia arising 5 – 12 days following transfusion of red cells, associated (*confirmed if possible with the presence in the patient of alloantibodies directed against the Human Platelet Antigen (HPA) systems*)		SAE
TTI Transfusion-transmitted infection	Include as a TTI if, following investigation, the recipient had evidence of infection post-transfusion, and there was no evidence of infection prior to transfusion and no evidence of an alternative source of infection. **Plus** At least one component received by the infected recipient was shown to contain the infectious pathogen.	Cases of bacterial transmission from blood components, where cultures from the patient's blood match cultures from the component bag and/or from the donor.	SAE
TACO Transfusion associated circulatory overload	Any *four of the following* occurring within **six hours** of transfusion: i) Acute respiratory distress. ii) Severe tachycardia. iii) Increased blood pressure. iv) Acute or worsening pulmonary oedema. v) Evidence of positive fluid balance.		SAE

Funders:

Main Sponsor:
Imperial College London

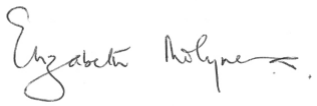
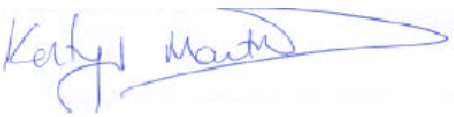
Collaborating groups:

TRACT

TRansfusion and TReatment of severe Anaemia in African Children: a randomised controlled Trial

Version:	**2.0**
Date:	**19th February 2016**
ISRCTN:	**ISRCTN84086586**
ICREC no:	**13_1_11**

Authorised by:

Name:	**Prof Elizabeth Molyneux**
Role:	**Chair of TSC**
Signature:	
Date:	**13.4.2016**
Name:	**Dr A. Sarah Walker**
Role:	**Trial Statistician**
Signature:	
Date:	**13 April 2016**
Name:	**Prof Kathryn Maitland**
Role:	**Chief Investigator**
Signature:	
Date:	**13th April 2016**

Trial Administration

Study Coordination Centre: Clinical Trials Facility, Phyles Maitha; Please direct all queries to the TRACT Trial Administrator at at KEMRI Wellcome Trust Programme (Phyles Maitha; PMaitha@kemri-wellcome.org) Tel: +254715461761

Clinical Queries

All clinical queries will be passed to **Ayub Mpoya (**AMpoya@kemri-wellcome.org**)**, Tel : +254735229037 in the first instance at KEMRI Wellcome Trust Programme who will consult others members of the Clincal Management Group where necessary.

Sponsor

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:

Joint Research Office
Sir Alexander Fleming Building
Imperial College
Exhibition Road
London SW7 2AZ
Tel: 020 7594 1188
Fax: 020 7594 1792

Funder

Medical Research Council (MRC) and Department for International Development (through a concordat with MRC)

This protocol describes the TRACT trial 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 trials 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.

COORDINATING CENTRE

KEMRI Wellcome Trust Programme	Switchboard: Mobile:	+254 (0) 417 522063/522535 +254 (0) 715461761 (Trial manager)
Clinical Trial Facility	Fax:	+254 (0) 417 522390
PO Box 230, Kilifi, Kenya	Email:	TRACT@kemri-wellcome.org

KEMRI WELLCOME TRUST PROGRAMME, KILIFI

TRIAL MANAGEMENT GROUP

Principal Investigator:	Prof Kathryn Maitland MBBS PhD	Mobile: Email:	+254733411022 kathryn.maitland@gmail.com
Trial Administrator	Phyles Maitha Dip.Business.Mgt	Tel: Mobile: Email:	**+254 417525453 (switchboard)** +254715461761 PMaitha@kemri-wellcome.org
Clinical Project Manager:	Ayub Mpoya Dipl.Clin.Med.Chir	Tel: Mobile: Email:	+254 417525453 (switchboard) +254735229037 AMpoya@kemri-wellcome.org
Data Manager:	Abdul Ali BA	Tel: Email:	+254 701040930 AAli@kemri-wellcome.org

GENETIC EPIDEMIOLOGY GROUP

Lead Investigator	Prof Thomas Williams MBBS PhD	Mobile: Email:	+254733411021 tom.n.williams@gmail.com
Coinvestigators	Dr Sophie Uyoga PhD	Mobile: Email:	+254722342686 suyoga@kemri-wellcome.org
Coinvestigators	Alex Macharia MSc	Mobile: Email:	+254721832920 amacharia@kemri-wellcome.org

IMPERIAL COLLEGE

Principal Investigator:	Prof Kathryn Maitland MBBS PhD	Tel: Email:	+44 (0) 207 670 4905 kmaitland@imperial.co.uk
Centre Admininstrator	TBA	Tel: Email:	

Co-investigator	Professor Gary Frost PhD RD	Tel: Mobile: Email:	+44 (0) 20 8383 8037 +44 (0) 7872850308 g.frost@imperial.ac.uk

MRC CTU AND YORK

Principal Investigator:	Prof Diana M Gibb, MD MSc	Tel: Email:	+44 (0) 207 670 4905 diana.gibb@ucl.ac.uk
Trial Statistician:	Dr Sarah Walker, PhD MSc	Tel: Email:	+44 (0) 207 670 4726 rmjlasw@ucl.ac.uk
Statistician	Leanne McCabe, MSc	Tel: Email:	 leanne.mccabe@ucl.ac.uk
Statistician:	Elizabeth George, MSc	Tel: Email:	+44 (0) 207 670 4931 elizabeth.george@ucl.ac.uk
Clinical Project Manager:	Dr Margaret Thomason, PhD, MSc	Tel: Email:	+44 (0) 207 670 4615 m.thomason@ucl.ac.uk
Data Manager:	David Baptiste	Tel: Email:	+44 (0) 207 670 4766 david.baptiste@ucl.ac.uk
Policy & Research Impact Coordinator:	Annabelle South, BSc	Tel: Email:	+44 (0)207 670 4827 a.south@ucl.ac.uk
Health Economics Co-investigator	Paul Revill, MSc Hons	Tel: Mobile: Email:	+44 (0)1904 651202 +44 (0)7575 907665 paul.revill@york.ac.uk

OTHER TRIAL INVESTIGATORS

UGANDA: Country Principal Investigator Prof Sarah Kiguli			
Department of Paediatrics, Mulago Hospital PO Box 7072 Makerere University, Kampala,			
Principal Investigator:	**Prof Sarah Kiguli** M.B. Ch.B., M.MED	Tel: Mobile: Email:	+ 256 414531875 +256 772588044 skiguliwalube@chs.mak.ac.ug
Study Site Coordinator	Musika Cynthia Williams BSc N	Tel Mobile Email	+256414531875 +256 701169400 cmwilly90@gmail.com

		Fax	+256 41 532591
Co-investigators:	Dr Robert Opoka M.B. Ch.B., M.MED	Mobile: Email:	+256 41 531875 opokabob@yahoo.com;
Monitors (Uganda):	Flavia Kyomuhendo BSc N Sarah Nakalanzi BSc N	Tel: Email: Tel: Email:	+ 256 772656622 kyomulady@yahoo.com +256 775277342 snakalanzi@baylor-uganda.org
Uganda Blood Transfusion Service P.O.Box 1772, Kampala, Uganda			
Director	Dr. Dorothy Kyeyune Byabazaire; MBChB; Dip Transfusion Med; Dip Project Planning and Management	Tel: Mobile: Web: Email:	+256 414 259195 +256 772 425293 www.ubts.go.ug kyeyunedorothy@yahoo.com
Mbale Regional Referral Hospital Pallisa Road Zone, P.O Box 921, Mbale, Uganda			
Site Investigator:	Dr Peter Olupot Olupot, MBChB; MPH	Tel: Mobile: Fax: Email:	+256 772 457217; +256 772457217 + 256 45 4435894 polupotolupot@yahoo.com
Study Site Coordinator:	Tonny Sennyondo Nurse	Tel: Mobile: Email:	+ 256 758361604 +256 783620048 tseenyondo@gmail.com
Data Manager:	Macreen Mudoola BSc	Tel: Email:	+256 7022731902 macreen.m@gmail.com
Mbale Regional Blood Bank, P.O. Box 284, Mbale, Uganda			
Co_Investigator	Dr. Benjamin Wabwire, MBChB, DTM	Tel: Mobile: Email:	+256 31-2218300/5/10 + 256 77 608302 benjaminwabwire@yahoo.com
Soroti Regional Hospital, P.O Box 289, Soroti.			
Site Investigator:	Dr Charles Engoru, M.B. Ch.B.,	Tel:	+256 45 4461382;

	M.MED	Mobile: Email:	+ 256 772458587 charlesengoru@yahoo.co.uk
Study Site Coordinator::	Denis Amorut	Tel: Mobile: Fax: Email:	+256 45 4461382 + 256 774573911 +256 45 4461382 damoruts@gmail.com
Co Investigators:	TBA		

MALAWI: Country Principal Investigator Dr Yamikani FG Chimalizeni			
College of Medicine, Department of Paediatrics and Child Health Malawi-Liverpool-Wellcome Trust Clinical Research Programme, PO Box 30096, Chichiri, Blantyre 3, Malawi			
Principal investigator:	Dr Yamikani FG Chimalizeni	Tel: Email:	+265 992 233 221 gaveta3@yahoo.com
Co-investigators:	Professor Robert Heyderman FRCP, PhD, DTMH Dr Neil Kennedy MRCPCH, DTMH Dr Macpherson Mallewa MRCPCH, PhD, DTMH	Tel: Email: Tel: Email: Tel: Email:	+265 888 208 044 R.Heyderman@liverpool.ac.uk +265 995 929733 nkennedy@medcol.mw +265 995 835 083 macphers@liverpool.ac.uk
Trial Manager:	Felistas Kumwenda MSc	Tel: Email:	+265 881132081 fkumwenda@mlw.mw
Monitors (Malawi)	John Chabuka MSc Nkhafwire Mkandawire MPH	Tel: Email: Tel: Email:	+265 999 367 568 jjchabuka@gmail.com + 265 999 910 622 nkhafwire74@gmail.com
Malawi Blood Transfusion Service, PO Box 2681, Blantyre, Malawi.			
Co-investigator	Dr Bridon M'baya	Tel: Email:	+265 995 883 117 mbayab@yahoo.com

Other Centres

Liverpool:			
Liverpool School of Tropical Medicine and Hygeine Pembroke Place, Liverpool L3 5QA			
Co-investigators:	Professor Imelda Bates; MBBS, Phd	Tel: Email:	+44 151 705 3115 ibates@liverpool.ac.uk
Co-investigators:	Dr Britta Urban. ; PhD	Tel: Email:	+44 1517053380 burban@liverpool.ac.uk
Amsterdam:			
Emma Children's Hospital AMC (Global Child Health Group) Meibergdreef 9, 1105 AZ Amsterdam			
Co-investigators:	Professor Michael Boele von Hensbroek PhD;	Tel: Email:	+31 20 5666173 mbvh04@gmail.com

ABBREVIATIONS

ATR	Acute transfusion reaction
BTS	Blood transfusion services
CAB	Community Advisory Board
CEA	Cost-effectiveness analyses
CI	Chief Investigator
CRF	Case record form
CRP	C-reactive protein
CTF KWTP	Clinical Trial Facility, KEMRI Wellcome Trust Programme
CTC	Common Toxicity Criteria
CTU	Clinical Trials Unit
DMC	Data Monitoring Committee
ERC	Endpoint Review Committee
GCP	Good Clinical Practice
Hb	Haemoglobin
HTR	Haemolytic transfusion reaction
IBD	Invasive bacterial disease
ICH	International Committee on Harmonisation
IDA	Iron deficiency anaemia
IPT	Intermittent preventative treatment
IRB	Institutional Review Board
IV	Intravenous
LPS	Lipopolysaccharide
MRC	Medical Research Council
MVMM	Multi-vitamin multi-mineral
NTS	Non-Typhoidal Salmonellae
PCT	Pro-calcitonin
PI	Principal Investigator
PTP	Post transfusion purpura
QA	Quality Assurance
QC	Quality Control
QMP	Quality Management Plan
RCT	Randomised controlled trial
REC	Research Ethics Committee
RNI	Recommended Nutritional Intake
RUTF	Ready To Use Foods
SA	Severe anaemia
SAE	Serious adverse events
SAP	Statistical Analysis Plan
SOC	Standard of care
SOP	Standard Operating Procedure
SSA	Sub-Saharan Africa
SNP	Single Nucleotide Polymorphism
SSC	Study Site coordinator
TACO	Transfusion Associated Circulatory Overload
TC	Trial coordinator
TMF	Trial Master File
TMG	Trial Management Group
TMT	Trial Management Team
TSC	Trial Steering Committee
TRALI	Transfusion Related Acute Lung Injury

TTI	Transfusion transmitted infection
U&E	Urea and Electrolytes
WB	Whole Blood
WHO	World Health Organization

GLOSSARY OF TERMS

Coma	Inability to localize a painful stimulus
Impaired perfusion	≥1 of: capillary refill >2 seconds; lower limb temperature gradient; weak radial pulse volume
Intravascular volume depletion	Depletion of circulating volume : **relative** (due to vasodilatation) or **actual** (loss of fluid from intravascular space e.g. burns or blood loss or capillary leak)
Impaired consciousness	Prostration or coma
Profound anaemia	Haemoglobin < 4g/dl
Prostration	Inability to sit unsupported, or to breast feed if <9 months
Respiratory distress	Deep breathing or increased work of breathing
Severe anaemia	Haemoglobin < 6g/dl
Severe and complicated anaemia	Severe anaemia with severe illness or impaired perfusion
Severe illness	Children with impaired consciousness or respiratory distress

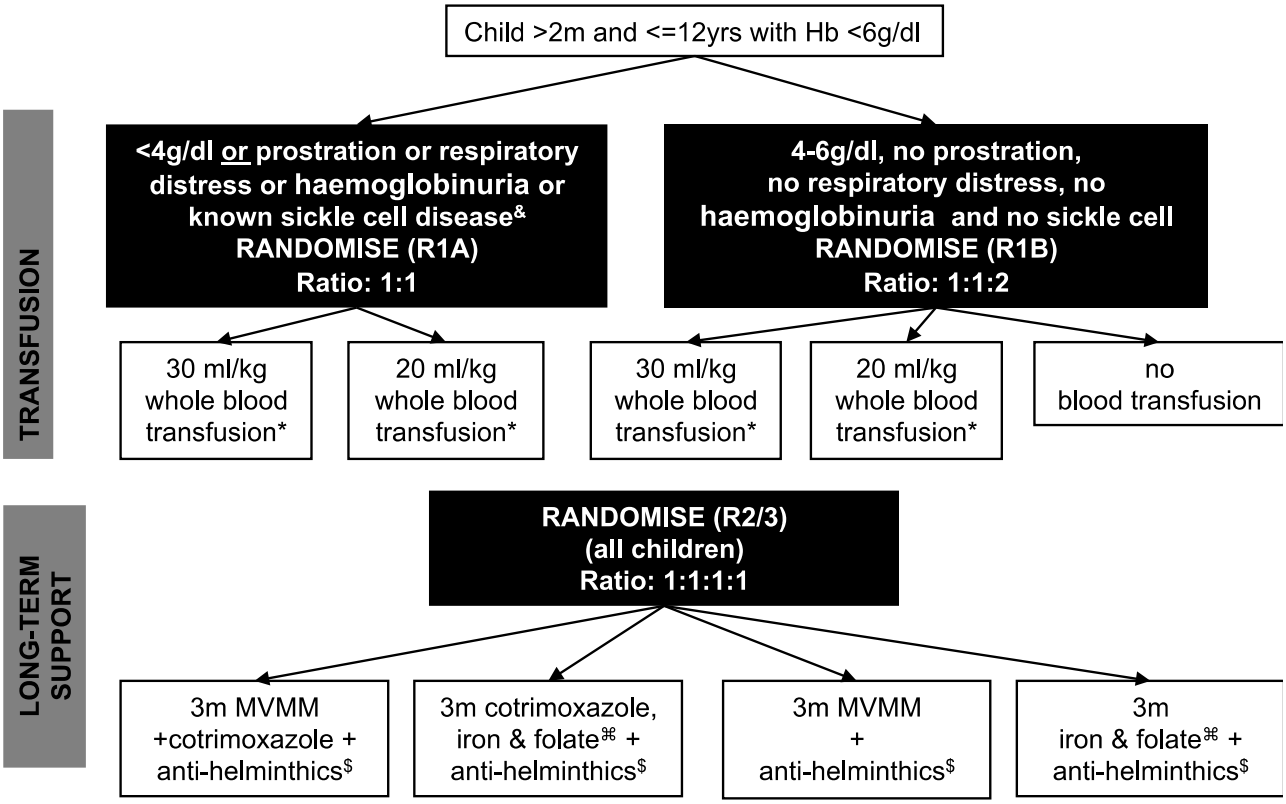
KEYWORDS
Children
Infants
Africa
Malawi
Uganda
Anaemia
Plasmodium falciparum malaria
Sepsis
Randomised controlled trial
Transfusion
Nutrition
Micronutrients
Emergency medicine
Haemoglobinopathies
Glucose 6 Phosphorylase Deficiency

STUDY SUMMARY

SUMMARY INFORMATION TYPE	SUMMARY DETAILS
ACRONYM (or Short Title of Trial)	TRACT
Long Title of Trial	TRansfusion and TReatment of severe Anaemia in African Children: a randomised controlled Trial
Version	1.0
Date	20th February 2013
ISRCTN #	**ISRCTN84086586**
Study Design	A 3x2x2 open-label factorial multi-centre trial, conducted in 4 centres in 2 countries (Malawi and Uganda)
Type of Participants to be Studied	3954 children aged 2 months to 12 years with severe anaemia (SA) (defined as a haemoglobin <6g/dl) during hospital admission
Interventions to be Compared	The trial will have 3 intervention strategies aimed at to reducing mortality and morbidity in children with SA **R1:** Immediate liberal transfusion (30ml/kg) versus conservative transfusion (20ml/kg) versus no transfusion (last strategy only for children with **uncomplicated SA and Hb 4-6 g/dl**). **R2:**Post-discharge multi-vitamin multi-mineral (MVMM) supplementation (which includes folate and iron) versus routine care (folate and iron) for 3 months. **R3:**Post-discharge cotrimoxazole prophylaxis versus no prophylaxis for 3 months.
Study Hypotheses	Each intervention (R1, R2, R3) will reduce short and longer-term mortality and morbidity following admission to hospital with severe anaemia. Each intervention will be compared with standard of care.
Primary Outcome Measure(s)	Cumulative mortality to 4 weeks for the transfusion strategy comparison, and to 6 months for the nutritional support/antibiotic prophylaxis comparison
Secondary Outcome Measure(s)	• mortality at 48 hours, 4 weeks, 3 months and 6 months (where not the primary outcome); • development of new profound anaemia (Hb<4g/dl) during acute admission or development of severe anaemia (Hb<6g/dl) post discharge; • readmission to hospital; • proportion achieving correction of anaemia (defined by WHO as Hb>9g/dl); • nutrition: changes in weight and MUAC, at 90 day and 180 days • anti-infection: changes in inflammatory markers (C-reactive protein, procalcitonin), incidence of bacterial infections and malaria at 28 days, 90 day and 180 days • Solicited adverse events: suspected transfusion reactions:

	febrile reactions, TRALI (Transfusion Related Acute Lung Injury) (any grade); grade 3-4 toxicity of cotrimoxazole, MVMM or standard iron/folate • Serious adverse events • costs and cost-effectiveness
Randomisation	**R1:** Participants with uncomplicated SA and Hb 4-6 g/dl will be allocated in a 1:1:2 ratio between 30ml/kg versus 20ml/kg versus no transfusion. All other children will be allocated in a 1:1 ratio between 30ml/kg versus 20ml/kg. **R2 and R3**: children will be allocated in a 1:1 ratio between intervention vs control (no intervention). All three randomisations will use a factorial design, ie each randomisation will be balanced by design for allocation to other interventions or not.
Number of Participants to be Studied	3954 children including at least 1950 complicated (<4g/dl or 4-6g/dl with prostration/respiratory distress/known sickle cell disease/dark urine) and no more than 2000 uncomplicated (4-6g/dl without prostration, respiratory distress, known sickle cell disease or dark urine) (minimum 1560)
Duration	Participants will be randomised over 2 years and followed up for 6 months. Tranfusion intervention will be administered in hospital; nutritional and additional cotrimoxale prophylaxis will be administered for 3 months following discharge from hospital by caregivers The overall trial duration is 3 years.
Ancillary Studies/Substudies	▪ Economics and cost-effectiveness ▪ Molecular diagnostics ▪ Haemoglobinopathies ▪ Immunological studies ▪ Enteropathy ▪ Nutritional measure of wellbeing: dietary recall ▪ Micronutrients
Sponsor	Imperial College, London
Funder	Medical Research Council (MRC) UK and Department for International Development, UK (DFID)
Chief Investigator	Prof Kathryn Maitland

TRIAL SCHEMA



& Only applies to a previously established diagnosis of sickle cell disease
* Alternatively 15mls/kg packed cells (for 30mls/kg WB arm); 10 mls/kg packed cells (for 20mls/kg WB arm)
⌘ at treatment doses following WHO recommendations
$ For children > 1 year of age if they have not received antihelminths in previous 6 months- following WHO recommended standard of care
MVMM at usual supplementary dosages

FLOW DIAGRAM

If a child is seen more frequently than indicated, a follow-up form should be completed at each visit.

Hours (h) /Days (d)	0*h	Discharge	28d	90d	180d
Consent and information sheet	X				
Clinical examination (doctor/doctor visit) [a]	X	X	X		X
Nurse observation/visit, collect medication[b]		X	X	X	X
Vital observations, anthropometry	X		X	X	X
24-hour dietary recall		X	X	X	X
Pill count[c]			X	X	
Laboratory investigations					
Haematology[d]	X		X	X	X
Biochemistry [e]	X				
Lactate/Glucose	X				
Malaria slide + /- RDT	X		X	X	X
Blood culture[f]	X				
HIV testing	X				
Urine dipstick (Multi-stick)	X				
Cross match (for transfusion) (red top)	X				
Stored samples					
Investigations of anaemia aetiology (all)					
Blood Film and Malaria Pigment[g]	X		X	X	X
EDTA (for DNA) human and pathogen[h]	X				X
Plasma [i]	X		X	X	X
Stool [j]	X				
Other investigations (all)					
Donor blood haemoglobin and storage[k]	X				
Investigations of mechanism/response (subgroup only for retrospective analysis)					
Gut Barrier Function (EDX, I I-FABP, IBAP) [l]	X		X		X
Stool Storage[m]	X		X		X
Urine (metabolomics) [n]	X		X		X
PBMCs [o]	X		X		X
Red Cell pellets [p]	X		X		X

*Baseline history and examination at trial enrolment: clinician and nursing observations over 48 hours covered in the more comprehensive Table A (below)

a To include baseline history and physical assessment, 24 hour dietary recall prior to illness (at discharge), and daily assessment of solicited adverse events, inter-current illnesses and concomitant medications whilst admitted. Post-discharge assessments will include history of these events since last visit/discharge.

b to include: 24 hour dietary recall, body weight and height (and head circumference for children under 2 years), middle-upper arm circumference (MUAC), and history of illness and symptom checklist. At day 60 the nurse will contact the parent to check status and to enquire about compliance.

c pill count if randomised to cotrimoxazole or MVMM

d Haematology: At trial recruitment (D0), Day 28, Day 90 and Day 180 Full haemogram (including haemoglobin, MCV, white cell count, +/-neutrophil, lymphocyte and platelet counts). Hb (by Haemacue) will be measured at additional time points between admission and 48 hours (see table A) or at these time points if haemogram not available.

e Biochemistry: Urea and electrolytes. Liver function tests (AST, ALT and Bilirubin) may be performed if clinically indicated, but are not required by the protocol.

f In all centres except Soroti (which does not have blood culture facilities); repeat culture if children readmitted or clinical sepsis suspected at follow up. All pathogen isolates will be stored for future investigation of antimicrobial resistance.

g Blood film for morphology and malaria pigment
h 1-2ml (EDTA) pellet will be used retrospectively to analyse, in batches, red cell haemoglobinopathies and enzymopathies (including thalassaemia, sickle cell disease and G6PD deficiency). The DNA pellet will also be assayed retrospectively for pathogen diagnosis (largely bacterial, parasite and viral)
i: up to 3ml plasma (store): Changes in inflammatory markers (eg CRP, PCT), cytokines, assays of micronutrients, malaria parasite load (HRP2), gut hormones and gut inflammation and microbial translocation will be assessed retrospectively on stored plasma samples.
j Stool sample for helminths investigation
k Donor blood (EDTA): a blood film will be prepared and plasma saved for potential retrospective investigation eg evidence of adverse effects of storage (storage lesions, cytokine production) and the pellet saved for later preparation of DNA (microbial contamination in a subset incl adverse events or endpoints).

Investigations only in a subset of participants
l, m and n Mbale RRH: Markers of Gut Barrier Dysfunction: Endotoxin/Immunology: Linked plasma urine and stool samples for examine gut barrier dysfunction and alterations of gut microbiome; faecal markers of enteric inflammation (calprotectin, alpha-1 antitrypsin, neopterin).
o and p Mbale RRH: PBMC to be saved, if possible, from the sodium heparin tube taken for plasma storage for future immunologic research; red cell pellets in patients with malaria: for parasite molecular research

Table A: Bedside Observations; Clinical examination and laboratory testing 0-48hours

Procedure	Adm	30m	60m	90m	2hr	4hr	8hr	16hr	24hr	48hr	Daily	Disc*
Source documents												
Clinical notes (doctor)[$]	X		X				X	X	X	X	X	X
Bedside notes (nurse)	X	X	X	X	X	X	X	X	X	X	X	
Haemoglobin (Hb)	X						X	X	X	X	X	X
Lactate	X						X		X			
Glucose	X		X		X		X	X	X			
Prescription Chart	X								X	X	X	X
Transfusion and iv fluids		X	X		X	X	X	X	X	X		X
Anthropometry	X									X		
Documents with data summaries												
CRF	X								X	X		X
Diagnosis[¢]	X											X
Drugs received									X	X		X
Transfusion and iv fluids		X	X		X	X	X		X	X		X
Locator details	X											X
Investigations[&]	X						X	X	X	X	X	X
SAEs							X		X	X		X
24-hour dietary recall												X
Discharge medication[£]												X

* Disc: On Discharge from hospital
[$] Additional reviews by doctors and nurses will be conducted and recorded, where clinically indicated.
[&] Laboratory tests (see Study Flow) and bedside tests including glucose and lactate at time points shown; plus any additional investigation eg Chest-Xray
£ Nutritional and antimicrobial prophylaxis to be issued from the pharmacy
[¢] Working diagnosis at admission and final diagnosis at discharge.

Table of Contents

1. INTRODUCTION

1.1 BACKGROUND

In sub-Saharan Africa (SSA) severe anaemia (SA) in children is a leading cause of hospital admission, a major cause of direct mortality[1] and a key factor in the 800,000 malaria deaths/year[2]. Guidelines developed by the World Health Organization (WHO) encourage the rational use of blood transfusion to preserve this scarce resource and to reduce the risk of transfusion-transmitted infections[3]. However, the evidence base for the paediatric guidelines is weak and the recommendations within these guidelines are confusing - consequently adherence is poor and national transfusion recommendations vary between SSA countries. Outcome of severe anaemia is unsatisfactory with high rates of in-hospital (9-10%)[4] and 6-month (12%) case fatality, and relapse or re-hospitalisation (6%),[5] indicating that the current recommendations and management strategies are not working in practice. Further, the aetiology of severe anaemia is frequently multi-factorial, including potentially treatable co-morbidities such as bacteraemia and multiple vitamin deficiencies - key determinants of outcome[6] that are not addressed in current treatment guidelines. Although the two most recent systematic reviews (both published in 2000) indicated the need for formal evaluation of the restrictive transfusion policy supported by WHO in a controlled trial[4, 7], little progress has been made in the intervening decade. The poor outcomes and recurrent morbidity of children with severe anaemia warrant a definitive trial to establish best transfusion and treatment strategies to prevent both early and delayed mortality and relapse.

1.2 CURRENT MANAGEMENT RECOMMENDATIONS

Transfusion of blood can be a life-saving intervention, and provision of adequate supplies of safe blood for transfusion are an essential undertaking for any health system. Issues of blood safety, adequate supply, equitable access and rational use, however, remain key challenges throughout the world. The pattern of usage of blood in SSA is very different from high-income countries where use is largely elective with supply strictly monitored through specialist transfusion services. In SSA, women and young children are the chief recipients of blood transfusions, accounting for over three-quarters of the blood transfusion requirements, most given as emergency interventions[8]. In order to bridge the major gap between supply and demand, one of the four key goals mandated in a WHO resolution on an integrated strategy of blood safety in 1975 was to 'reduce unnecessary transfusions' - through more effective clinical use of blood and use of simple alternatives to transfusion (crystalloids and colloids) where possible[9]. WHO has subsequently developed and published guidelines for the appropriate use of blood for patient groups suffering the greatest impact of a shortage of supply[10].

1.2.1 PAEDIATRIC TRANSFUSION GUIDELINES FOR CHILDREN IN DEVELOPING COUNTRIES

Current guidelines for paediatric transfusions were developed largely through consensus and based on data from observational studies rather clinical trials evaluating their impact. The pocket book of hospital care for children (guidelines for the management of common illnesses with limited resources, WHO 2005)[3] has two sections that cover transfusion – the Supportive Care chapter (Part 10.5 Management of anaemia) and in the Fever chapter: Part 6.2 Malaria. There are some discrepancies in these guidelines with respect to the threshold haemoglobin or haematocrit at which transfusion is recommended. The Supportive Care Chapter indicates a threshold <6g/dl, whereas in the Fever Chapter/malaria guidelines this is revised to threshold of <5g/dl (see below). No rationale justifying the choice of these two different thresholds is presented.

Current WHO guidelines
Supportive Care: Management of anaemia (page 276-281)
Give a blood transfusion as soon as possible to:
— all children with a haematocrit of ≤12% or Hb of ≤4 g/dl
— less severely anaemic children (haematocrit 13–18%; Hb 4–6 g/dl) with any of the following clinical features (complications):

- clinically detectable dehydration
- shock
- impaired consciousness
- heart failure
- deep and laboured breathing
- very high malaria parasitaemia (>10% of red cells with parasites).

Guidelines for transfusing severe malarial anaemia
Fever chapter: Part 6.2 Malaria (pages 142-143)
Give a blood transfusion as soon as possible to:

- all children with a haematocrit of ≤12% or Hb of ≤4 g/dl
- less severely anaemic children (haematocrit >12–15%; Hb 4–5 g/dl) with any of the above complications.

1.2.2 Definition of Complicated Severe anaemia

The criteria defining complicated SA above are not referenced and some lack scientific justification (including relevant literature). Three of complications warranting immediate transfusion have either concerns related to physiological justification or substantial resource implications: heart failure, dehydration and hyperparasitaemia. First, heart failure (ie 'biventricular' failure or 'overload') is uncommon and best corrected with diuretics and other measures. Only once the patient is stabilized should the decision to cautiously transfuse be reconsidered - this would be standard paediatric practice globally. Second, severe dehydration (loss of intracellular water and electrolytes) should be corrected with crystalloidal solutions and not transfusion (the latter leading to a sluggish, haemoconcentrated circulation). Finally, hyperparasitaemia is very frequent among paediatric admissions in malaria endemic areas and has not been shown to be an independent risk factor for poor outcome. Inclusion of this criteria may result in the substantial overuse of a limited transfusion supplies by a large group with low risk.

The three other criteria (shock, impaired consciousness and deep breathing (a clinical sign of metabolic acidosis)) do have scientific justification for identifying SA subgroups with high immediate risk of mortality. Overall mortality in children with a Hb <4g/dl or SA with life-threatening complications is 15%[4]. Clinical studies in Kenya[11, 12] have shown that profound anaemia (Hb<4g/dl) is independently associated with death (OR=2·5), as is SA (defined in this study as Hb<5g/dl) complicated by reduced consciousness (OR=7·4) or respiratory distress (OR=4·1). Many deaths occur within 48 hours of admission, with 25-50%[13, 14] occurring within 6 hours. In the FEAST trial which enrolled children with shock, a higher case fatality was found in those with anaemia compared to those without anaemia, irrespective of intervention group[15]. In children with uncomplicated SA - a Hb of 4-6g/dl without prostration or respiratory distress - overall case fatality is 4-6%, being lower in parasitaemic children (2-3%)[16] than in those with negative malaria slides (8-10%)[11]. The ratio of complicated to uncomplicated SA is commonly 1:1[17].

1.2.3 Relevant clinical trials of transfusion and Systematic Reviews

A Cochrane review including the only 2 African randomised controlled trials (RCTs) [18, 19] conducted to date (involving 114 and 116 children randomised to blood transfusion or oral haematinics) concluded that there was insufficient information on whether routinely giving blood to clinically stable children with severe anaemia either reduces death or results in a higher haematocrit measured at one month, and indicated the need for a definitive trial[7]. A prospective, randomised, controlled, non-inferiority trial in relatively stable Canadian and European children demonstrated that a restrictive transfusion protocol (with a transfusion threshold <7g/dl) was as safe as a liberal protocol (threshold <9g/dl)[20]. Subsequently, practice guidelines in these countries have been amended to include restrictive transfusion (Hb<7g/dl). It remains to be established whether, as currently advocated by WHO, restrictive guidelines can be safely

applied without detrimental consequences at even lower levels of Hb (4-6g/dl if stable) in African children with no access to the other supportive treatments available in Europe and North America.

A literature review by Brabin and colleagues[4], reporting case fatality in studies of SA from malarious areas in SSA, indicated wide variations in outcome. Mean in-hospital case-fatality rate for severe anaemia (Hb <5 or <6g/dl depending on study definition) was 9% (range 4-39%). Mortality was significantly higher in children with a Hb <5g/dl (pooled RR=1.92 vs >5g/dl, 95% CI 1.7–2.2). Evidence for an increased risk with less severe anaemia was not conclusive: although the risk of death was increased for a Hb <8g/dl, the confidence intervals were wide (below: Fig 4 from Brabin *et al* 2001)[4]. The heterogeneous group of children included and outcomes observed make it difficult to draw specific conclusions. Other studies have addressed this by classifying children into subgroups based on clinical severity and Hb levels. Using this approach, available observational data also indicate no clear association between the receipt of a blood transfusion and in-hospital mortality in children with uncomplicated SA[12]. However, such data are likely to be subject to confounding by indication, because children with a poorer underlying prognosis will be more likely to receive a transfusion. Post-discharge morbidity and mortality are important considerations in this group, but there are few data on the cumulative incidence of poor outcomes in the longer-term.

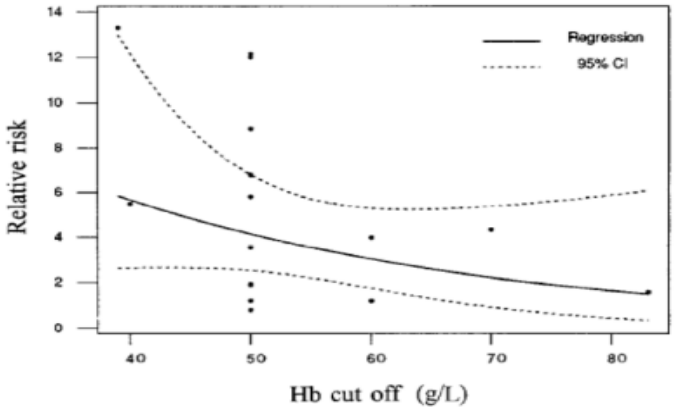


FIGURE 4 Relative risk for child mortality in relation to hemoglobin (Hb) cut-off points. Log $Y = 1.30 - 0.137X$; $R^2 = 13.8\%$; $P = 0.156$. CI, confidence interval.

1.2.4 TRANSFUSION VOLUME AND NEED FOR RE-TRANSFUSION

Current transfusion guidelines are conservative not only in terms of criteria applied for administering a transfusion at all, but also in terms of the volume of blood transfused. Currently, 20ml/kg of whole blood (or 10ml/kg packed cells) are recommended for all levels of anaemia below Hb<6g/dl[3]. Using standard formulae to calculate volume required[21] this under-treats children with profound anaemia by ~30% and this volume may not, therefore, be sufficient to correct anaemia. Larger initial transfusion volumes have not been systematically evaluated. In fact, few data are available on the volumes received. Lackritz reported mean volumes of 26ml/kg whole blood[12] and others following WHO guidelines have shown a modest Hb rise of 2.5-3.3g/dl[11, 12, 22] following initial transfusion with ~25% remaining severely anaemic (<5g/dL)[11]. Anecdotal evidence suggests that multiple, low volume (20ml/kg) transfusions are frequently given, which is wasteful, inefficient and exposes children to additional risks (eg reaction and infection).

Unpublished data from the FEAST trial[15] shows that 1422 (45%) children who were enrolled with signs of shock received a blood transfusion. Overall 322 (23%) of those transfused received 2 or more transfusions, the proportion being greater (212/612, 35%) in those with a Hb <4g/dl at enrolment. Total mean (SD) blood volumes per child were 23.3 (9.5) ml/kg, with those receiving two transfusions receiving a mean (SD) of 36.6 (7.3) ml/kg in total over their 2 transfusions (mostly 2x~20ml/kg). This proportion might be reduced considerably if larger volumes of blood are given initially, an outcome that would also reduce transfusion service time to prepare blood and the risks of transfusion-related morbidity, arguing the need for a trial of initial volumes of transfusion. Follow-up in FEAST was only to 28 days; a key question that will be

addressed in this trial is whether greater initial blood volumes could also reduce longer-term anaemia recurrence. A prospective study of 128 Malawian children aged 3-60 months, transfused according to WHO guidelines, examined transfusion failure (defined as a Hb≤6 g/dl >24hours post-transfusion). Only 104 (81%) received the prescribed volume; of these, 24 (23%) were classified as transfusion failures and 83% of these had a subsequent Hb<4g/dl.[23]

1.2.5 Recent Trends in Transfusion Safety and Malaria Endemicity

The conservative transfusion guidelines were developed to protect scarce resources, avert overuse, and reduce the risk of transfusion-transmissible infections. However, in recent years considerable progress has been made with regard to blood safety in many countries in SSA since the US President's Emergency Plan For AIDS Relief (PEPFAR) began to provide direct support for strengthening transfusion services, improving the supply and safety of transfusion by establishing regional centres to replace hospital-based systems and by providing quality assurance for viral testing[24]. Previously, access to and safety of blood for transfusion would have predicated whether the findings of a trial demonstrating benefit of transfusion in stable children with a Hb 4-6g/dl could be practically implemented, unless new recommendations also considered increasing supply. In some parts of Africa the capacity of transfusion services to provide blood has greatly increased due to year-on-year declines in the intensity of malaria transmission that have led directly to reductions in hospitalisation of children with malaria[25], and indirectly to reduced utilisation of blood transfusion services[17]. However, this is not universal and for the sites involved in the TRACT trial malaria epidemiology has not declined and may have even increased[26] and thus remains a common cause of hospitalisation and transfusion.

1.2.6 Short and Longer term Outcomes in children with severe anaemia

A study in Gambian children showed comparatively better recovery in children with Hb of 4-5g/dL receiving iron treatment than those who received a blood transfusion[19]. In Kenyan children blood transfusion was important in preventing death in children with severe symptomatic malaria anaemia but did not seem to influence faster or superior haemoglobin recovery at one month follow up, with mean haemoglobin at discharge (approximately 3-4 days) being similar in transfused 6.4g/dL [SD: 1.5] and non transfused 6.8 g/dL [SD: 1.6] and remaining similar at follow up (28-35 days) in the transfused (10.2g/dL) and non-transfused (10.0g/dL) groups (P=0.25). The major factor affecting mean haemoglobin concentration at follow-up was concurrent malaria parasitaemia (8.8g/dL compared with a mean of 10.5g/dL in those without parasitaemia, p<0.001)[27], with additional significant effects of both young age (<24 months) and the type of malaria treatment (p=0.03).

Most children are therefore discharged with only partial correction of anaemia. The limited data available indicate a trend towards higher rates of post-discharge mortality, recurrence of anaemia and re-hospitalisation in children with SA than non-SA hospital or community controls. The Malawi case-control study of paediatric SA[5] showed that 17% of cases were readmitted within 6 months of discharge and 6% re-developed SA, compared to re-admission rates of 9-10% and <0.5% respectively in hospital and community controls. In-hospital and 6-month post-discharge mortality was greater in the cases (6% and 8% respectively) than in either the hospital- (0% and 1.6% respectively) or community-controls (0% 6-month mortality). Holzer[18] showed a non-significant increase in deaths at 8 weeks in children with uncomplicated SA randomised to no transfusion (4% vs 2%) and more hospital re-admissions (8%, 4/53) than the transfused group (2%, 1/52). Thus transfusion alone may not achieve optimal outcomes for children with SA, suggesting that exploration of complementary treatments is also essential.

1.2.7 Aetiology and co-morbidities

The causes of anaemia are multi-factorial with several co-factors causally related to mortality risk. To improve poor SA outcomes, it is thus likely that complementary treatment approaches will be necessary, including both immediate (transfusion), and longer term interventions. Malaria still plays an important role

in SA, even if this is less than in previous decades. In Kilifi, Kenya we recently reported that the epidemiological transition in malaria transmission has led to a decline in hospital paediatric admissions (including malaria and SA)[25] and reduced demand for transfusion. However, SA case fatality over this period remained unchanged at 8-10%[17].

In the only comprehensive case-control study of children hospitalized with SA in Africa[6], key associations with SA were bacteraemia (OR=5.3; 95% CI 2.6-10.9), malaria (2.3; 1.6-3.3), hookworm (4.8; 2.0-11.8), HIV infection (2.0; 1.0-3.8), vitamin A deficiency (2.8; 1.3-5.8) and vitamin B12 deficiency (2.2; 1.4-3.6). Neither iron nor folate deficiencies were associated with mortality, and were less prevalent among cases than controls. As iron, folate and anti-helminthics (for any child > 1 year of age that has not received antihelminths in the last 6 months) are already recommended post-SA-discharge[3], the clearest potentially modifiable underlying causes of late anaemia recurrence, which we propose to address in this trial, are nutritional factors and recurrent bacterial infections.

1.2.8 TREATMENT AND PREVENTION OF NUTRITIONAL DEFICIENCIES

WHO treatment guidelines deal specifically with malaria and with folate and iron deficiency that together are widely held as the most important causes of anaemia. Although folate supplementation is recommended, folate deficiency was not found in the Malawian SeVana study[5], in agreement with previous reports[28] and observations that folate supplementation in anemic children with malaria failed to raise haemoglobin concentrations[29]. Unlike folate and iron, Vitamin B12 and Vitamin A supplementation are not recommended in guidelines for the management of severe anemia. In the Malawian SeVana study vitamin B12 deficiency was found in 30.4% of case patients and severe Vitamin A deficiency (less than 10 µg per deciliter) in 32.8% of case patients (versus 14.9% of controls) and was associated with severe anemia[5]. These observations concur with findings among adults in that region[30, 31] and may be explained by the lack of animal products in the diet of Malawian children. However, what is unknown is whether a specific intervention to increase vitamin A and B12 levels would have measurable benefits or whether potential benefits generalise to other SSA cohorts.

Iron supplementation is effective for reduction of iron deficiency and anaemia in iron deficient children. However, a community-based randomized controlled trial in Zanzibar designed to evaluate the impact of zinc and iron plus folic acid supplementation on morbidity and mortality in young children showed that supplementation may also be associated with adverse effects, specifically increased risk of hospitalization (primarily due to malaria and infectious disease), and mortality in malaria-endemic areas[32]. WHO have revised recommendations advising that iron and folic acid should only be targeted to those who are anaemic and at risk of iron deficiency. In addition, they recommended that such children should receive concurrent protection from malaria and other infectious diseases through prevention and effective case management (http://www.who.int/childadolescent-health/-New_Publications/CHILD_HEALTH/WHO_FCH_CAH_06.2.pdf.) As far as we are aware this approach has not been subsequently evaluated in a factorial trial. Moreover, it is not clear whether the currently recommended 'treatment' doses (which assume that the child with severe anaemia are either or both iron or folate deficient) result in better or worse outcome that supplementation (incorporating lower doses of iron and folate).

Also acknowledged in the WHO statement following the Zanzibar study was that these recommendations should not be extrapolated to fortification (or food-based approaches) for delivering iron, where the patterns of iron absorption and metabolism may be substantially different. However in a recent controlled trial reported from Cote D'Ivore[33] which compared fortification of biscuits with 20 mg Fe/day per child 4 times/week to control (biscuit minus iron fortification) found that faecal microbiota were modified by iron fortification compared to control- showing a significant increase in the number of enterobacteria and a decrease in lactobacilli in the iron group at 6 months. In the iron group, there was an increase in the mean faecal calprotectin concentration (a marker of gut inflammation) which correlated with the increase in fecal enterobacteria. Low bioavailabilty and absorption of iron fortificants results in 90% of the iron passing unabsorbed into the colon. Beneficial barrier bacteria, such as lactobacilli, play an important role in the

prevention of colonization by enteric pathogens but do not require iron. In contrast, for most enteric gram-negative bacteria (eg, Salmonella, Shigella, or pathogenic Escherichia coli), iron acquisition plays an essential role in the virulence and colonization of most pathogenic strains[34].

These are important but unstudied consequences of addition the liberal (WHO recommended treatment doses) versus conservative use of iron (at supplementary doses) in malaria endemic populations at high risk of potential iron deficiency.

In children and infants there are several formulae to treat iron deficiency anaemia (IDA) but compliance is often poor as a result of repeated daily dosing, and unpleasant side effects such as the metallic after-taste, staining of the child's teeth (unless the teeth are wiped off immediately) and abdominal discomfort[35]. This has resulted in the development of micronutrient powders (eg Nutromix™ and Sprinkles™) as a novel approach for delivering iron and other micronutrients[36].

1.2.9 Multivitamin Multi-Mineral Powders (MVMM)

The Blantyre SeVana study[5], demonstrating that several micronutrients deficiencies are also aetiologically important in SA, suggests that MVMM could be an attractive and low risk strategy for correcting underlying nutritional-related anaemia. Several cheap MVMM supplements such as Sprinkles Plus™ and Nutrimix™ are widely available. Sprinkles are single-dose daily packets of dry powder containing lipid-encapsulated iron and other micronutrients which are added to any home-prepared food product[36]. A number of studies have demonstrated the efficacy of Sprinkles Plus in treating and preventing anaemia[37, 38]. Aside from iron, the various formulations of MVMM included essential micronutrients such as vitamins A, C and D, folic acid, iodine and zinc in doses that prevent and treat micronutrient deficiencies and improve overall nutritional status. Lipid encapsulation of the iron prevents its interaction with food and masks its taste, and may also reduce gastrointestinal discomfort. The sachets are lightweight and thus are simple to store, transport and distribute. Sprinkles have a long shelf life, even in hot or humid conditions (2 years). Sprinkle formulations were originally developed for infants and young children between 6-24 months of age as limited options exist for the treatment and prevention of micronutrient deficiencies in this age group. However, they have more recently been tested in older populations[39, 40]. Sprinkles or iron/folate supplements are not considered in children < 6 months in whom exclusive breast-feeding is recommended as the main mechanism for supporting full nutritional intake. Specific formulations of MVMM have been developed for pregnant and lactating mothers[41]. These can be given to also help treat breast-feeding infants. Side effects include darkening of the stool, constipation or mild diarrhoea. These maybe transient but if they do not subside caregivers are advised to use half a package of sprinkle formulation at 2 different mealtimes throughout the day.

None of these MVMM been tested against WHO recommended treatments in children hospitalised with severe anaemia for meaningful outcomes such as prevention of SA relapse, readmission or re-transfusion, and longer-term mortality.

1.2.10 Preventing Bacterial Infection: Cotrimoxazole

Cotrimoxazole is a synthetic antibacterial combination (sulphamethoxazole and trimethoprim) that acts by blocking folate metabolism involved in the biosynthesis of nucleic acids and proteins essential to bacteria and some parasites, including *Plasmodium falciparum*. High levels of sulphamethoxazole and trimethoprim are found within granulocytes and there is evidence that cotrimoxazole specifically enhances intracellular bacterial killing[42, 43]. The substantial mortality benefits (allied with extremely low rates of toxicity) associated with cotrimoxazole prophylaxis in HIV-infected children[44] have generally been attributed to reductions in bacterial infections[45, 46]. Of note, these benefits have been observed even in areas of high background resistance[47]. The fact that mortality benefits cannot be attributed solely to pneumonia[45, 46] raises the intriguing possibility that cotrimoxazole may act on a number of different pathways – the most important with regards to SA relapse being enteropathy and intestinal permeability, although any benefits of cotrimoxazole on microbial translocation and/or systemic immune activation, or on reducing

recurrent infections during recovery from SA could also impact longer-term morbidity. Cotrimoxazole has been shown to be effective in preventing malaria in HIV-negative children aged > 5 years [48] and in HIV-exposed (HIV negative children born to HIV-infected mothers) and HIV-infected children[49]; despite high levels of background parasite resistance to sulphamethoxazole.

Cotrimoxazole is cheap, widely available and has an established safety profile in African populations. A 5-day course is currently recommended by WHO for children with uncomplicated severe malnutrition, and cotrimoxazole prophylaxis is also used for children with other immunodeficiencies, especially those with defective neutrophil function. It is plausible, therefore, that cotrimoxazole could also reduce bacterial infections following SA: the question is whether this would impact on SA recurrence.

A recent systematic review found that sulfadoxine-pyrimethamine (which is similar in activity to cotrimoxazole) resulted in a 57% (95%CI 24%–76%) reduction in all-cause mortality[50]. Of interest, the effect of these other drugs used for intermittent preventative treatment (IPT) was similar to artemisinin combination therapies in terms of prevention of malaria. The investigators concluded that a preventative strategy that targeted both malaria and bacterial infections was important and that cotrimoxazole is an obvious candidate for such an approach. This supports the concept that short courses of such drugs may have significant benefits (eg similarly to that of a single azithromycin dose in Ethiopian children[51]).

While cotrimoxazole prophylaxis is widely available and cheap, it is not free and therefore universal use in young children is unlikely to ever be practical. However, demonstrating the value of a 3 month course in high-risk populations such as those with SA would very likely change practice.

1.2.11 RELEVANT STUDIES UNDERWAY OR PLANNED

We note that one randomized, placebo controlled trial of cotrimoxazole prophylaxis amongst HIV-uninfected children with severe malnutrition in currently enrolling in 4 sites in Kenya (http://clinicaltrials.gov/show/NCT00934492). The results are unlikely to be relevant to the majority of children eligible for TRACT trial, since most of these will not be severely malnourished. If WHO does change policy then we would amend the protocol to ensure that children with severe malnutrition, like those who have HIV, will routinely be prescribed cotrimoxazole prophylaxis at discharge. We are unaware of any trial in resource-limited settings addressing mortality as the primary endpoint in children with severe anaemia and were not able to find any planned trials of transfusion or MVMM in children with severe anaemia (ClinicalTrials.gov).

1.3 RATIONALE FOR CURRENT STUDY

In SSA, where infectious diseases and nutritional deficiencies are common, treatment options for the emergency management of children with severe anaemia are very limited. To avert overuse of blood products, the WHO advocate a conservative transfusion policy, reserving blood for children with a haemoglobin (Hb) of <4g/dl (or <6g/dl if accompanied by complications), and providing iron, folate and anti-helminthics (if >1yrs) at discharge. However, within the WHO guidelines these specific recommendations have not been systematically evaluated and they contain inconsistencies and ambiguities[3] resulting in variation in practice across African countries. This is particularly true in the subgroup with 'uncomplicated' severe anaemia (Hb 4-6g/dl without specific complications), where transfusion avoidance is recommended[7]. This large, heterogeneous group includes many children with infectious diseases or other conditions (including sickle cell disease[52]) whose outcome is often worse than their non-anaemic counterparts[4]. The lack of definitive evidence to support these recommendations, together with continuing high rates of re-admission[5] and death[6], suggests that children may well be receiving suboptimal treatment. At a workshop in Kenya in 2008, transfusion service providers, users, and researchers from across Africa called for better evidence to guide the emergency management of children with severe anaemia and to support the WHO policy for withholding blood

transfusions in stable children with a Hb of 4-6 g/dl (www.afsbt.org - Workshops). TRACT was designed as a direct response to this articulated need.

A poor or incomplete response to recommended treatment in children with SA results in relapse, readmission and death. Because severe anaemia is very common, the high 'hidden' morbidity and mortality occurring within the first few weeks after initial diagnosis is likely to contribute importantly to overall under-five mortality[5]. If not adequately addressed, severe anaemia may thus be an obstacle to the achievement of Millennium Development Goal No.4 on child survival in Africa. Combining different strategic approaches to reduce mortality, including more liberal and larger-volume transfusion to increase red cell mass and improve tissue oxygenation, may reduce immediate mortality, subsequent transfusion requirements and morbid and fatal events. It is essential to assess the longevity of any benefits of transfusion and greater transfusion volumes by evaluating post discharge morbidity and mortality, especially where nutrient deficiencies may impair recovery. Addressing these deficiencies by using multi-vitamins and preventing further infections with cotrimoxazole prophylaxis post-discharge might have their greatest impact after the effects of transfusion have waned (by 2-3 months), but could be cheap and low-risk strategies for maintaining risk reductions over the longer-term.

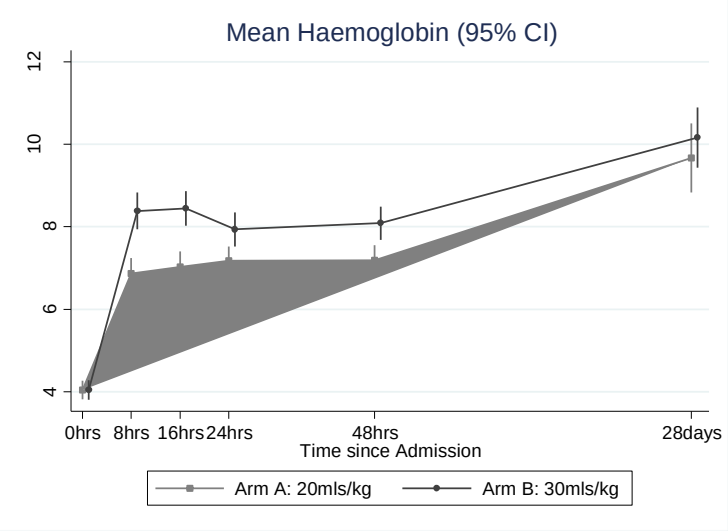
The principal research question is how best to reduce short and long term mortality and morbidity in African children with severe anaemia. We will address this by evaluating three components that could form an integrated treatment package for severe anaemia: transfusion, micro-nutrient supplementation to address underlying nutrient deficiencies and short-term antimicrobial prophylaxis to prevent recurrent infections. Each targets a different mechanism for reducing mortality and morbidity and targets both early and late outcomes: effects are thus expected to be additive. A major reason for including short and long term approaches in the same factorial trial is efficiency: it is more economical and faster to run one large trial than multiple separate trials. Further, rather than leaving these other management aspects to site and clinician preference, a factorial randomisation allows them to be directly controlled and evaluated alongside transfusions.

1.3.1 RISKS

Both MVMM and cotrimoxazole prophylaxis have been widely used in children with minimal risk. Although substantial efforts have been made to ensure the safety of blood, failure to correctly cross-match and/or infected blood have the potential to cause harm. The trial will directly evaluate whether these potential risks are outweighed by improved survival. TRACT teams will work closely with the local blood transfusion services (BTS) to ensure that recommended safety and quality control practices are being maintained. We anticipate developing a blood safety committee for each site, which will meet regularly. Severe adverse event management will follows a dedicated SOP developed for FEAST, that we also plan to use in TRACT.

Blood samples would be required from all study children. However, the volumes of blood required would be minimized wherever possible and be kept well within the maximum locally agreed volumes.

A pilot study, conducted in two sites in Uganda (Oct 2011-Dec 2012) in children fulfilling TRACT eligibility criteria evaluated the safety of a higher volume of whole blood transfusion (30mls/kg: 80 children) compared to the standard volume (20mls/kg: 80 children) (ClinicalTrials.Gov NCT01461590). The study was designed to provide comparative data on safety, and qualitative data on feasibility and operational components of implementation of the study protocol with special reference to the transfusion service. Adherence to volumes of transfusion was excellent. Correction of severe anaemia was superior in the 30mls/kg than the 20mls/kg (see Graph/table below). Nevertheless, this was a small pilot trial: whilst it is encouraging that our hypothesis that a larger initial volume would result in an greater increase in haemoglobin was supported, the small sample size means that it cannot conclusively demonstrate that this leads to improvements in mortality and other long-term benefits, the principal question under investigation in TRACT.



Global test of difference between the arms through to 28 days: p<0.0005						
Time:	0hrs	8hrs	16hrs	24hrs	48hrs	28days
N Arm A:	82	73	76	77	75	32
N Arm B:	78	74	75	74	74	36
P=		<0.0005	<0.0005	0.006	0.003	0.32

Severe adverse events (SAEs) were reported in 7 of the 160 children enrolled. 6 SAEs were deaths, most occurring within <5 hours of admission. All deaths were in the 20ml/kg group. One non-fatal transfusion reaction was reported (in the 30ml/kg arm): a child developed generalised body itching and an urticarial rash shortly after starting the transfusion.

1.3.2 BENEFITS

Extra clinical personnel, regular patient clinical assessment and the basic equipment for continuous patient monitoring will be available during the trial so that if the complications above were to arise they could be detected and treated. Pre-trial training will include sign recognition for these complications and training on treatment. Both these will be covered in detail in the Manual of Operations which will be available on the ward.

1.3.3 FOR THE CHILD

The direct benefits to the child and/or family (outlined in the PIS) include:

- Closer observation during the first 48 hours of admission, which, as a result, allows doctors and nurses to make important changes to the child's treatment during in-hospital admission.
- All routine non-trial medications required by the hospital to treat the child will be made available (when unavailable parents have to resort to sourcing these privately).
- The parents or guardians for the children will be asked to return for follow up at this clinic 28, 90 and 180 days after admission. Reimbursement for transport cost after discharge and for follow up visits plus any treatment costs required during the visits will be made.

1.3.4 FOR THE HOSPITAL

The direct benefits to the hospital include:

- Supporting blood transfusion safety committees : monitoring operational aspects and safety.
- Management of severe uncomplicated anaemia – the 50% control group (receiving no transfusion) should decrease the demand for blood and adherence with WHO restrictive transfusion guidelines.

1.3.5 FOR HEALTH PERSONNEL

The direct benefits to health personnel are mainly professional development of the members of the trial teams and clinical teams for the purposes of running the trial – including basic life support course, clinical trials training, research training.

2 SELECTION OF CENTRES AND CLINICIANS

Four hospitals in two countries will participate:

- **Uganda:** Mulago National Referral Hospital, Kampala; Mbale Regional Referral Hospital; and Soroti Regional Referral Hospital
- **Malawi:** Queen Elizabeth Central Hospital/Wellcome Trust Unit, College of Medicine, University of Malawi, Blantyre

These centres have been chosen on the basis of criteria below.

2.1 CENTRE/INVESTIGATOR INCLUSION CRITERIA

To participate in the trial, investigators and clinical centres must fulfil a set of basic criteria defined below.

2.1.1 CENTRE PIS QUALIFICATIONS & AGREEMENTS

1. The centre PI should be qualified by education, training, and experience to assume responsibility for the proper conduct of the trial at their centre and should provide evidence of such qualifications through an up-to-date curriculum vitae and/or other relevant documentation requested by the Sponsor, the REC or IRB, and/or the regulatory authority(ies).

2. The centre PI should be aware of, and should comply with, the principles of ICH GCP and the applicable regulatory requirements. A record of GCP training should be accessible for all investigators at the centre.

3. The centre PI should permit monitoring and auditing by the Sponsor, and inspection by the appropriate regulatory authority(ies)

4. The centre PI should maintain a delegation log of appropriately-qualified persons to whom the investigator has delegated significant trial-related duties.

5. The centre PI should sign an investigator statement, which verifies that the centre is willing and able to comply with the requirements of the trial.

2.1.2 ADEQUATE RESOURCES

1. The centre PI should be able to demonstrate a potential for recruiting the required number of suitable subjects within the agreed recruitment period

2. The centre PI should have sufficient time to properly conduct and complete the trial within the agreed trial period.

3. The centre PI should have available or appoint an adequate number of qualified staff for the duration of the trial to conduct the trial properly and safely.

4. The centre PI should ensure that all persons assisting with the trial are adequately informed about the protocol, the investigational product(s), and their trial-related duties and functions.

2.1.3 CENTRE ASSESSMENT

Each selected clinical trial centre must provide a completed Investigator Statement, Signature and Delegation of Responsibilities Log, and staff contact details. The Investigator Statement verifies that the

centre is willing, and able to comply with the requirements of the trial. This will be signed by the Principal Investigator at the centre. In addition, and in compliance with the principles of ICH GCP, all centre staff participating in the trial must complete the Signature and Delegation of Responsibilities Log and forward this to the Clinical Trial Facility, KEMRI Wellcome Trust Programme. The CTF KWTP must be notified of any changes to trial personnel and/or their responsibilities. An up-to-date copy of this log must be stored in the Trial Master File (TMF) at the centre and also at the CTF KWTP.

2.2 APPROVAL AND ACTIVATION

On receipt of the above documents at the CTF KWTP, written confirmation will be sent to the PI.

1. The centre should conduct the trial in compliance with the protocol as agreed by the Sponsor and, if required, by the regulatory authority(ies), and approved by the REC and/or IRB.

2. The centre PI or delegate should document and explain any deviation from the approved protocol, and communicate this with the trial team at the CTF KWTP.

A list of activated centres may be obtained from the Trial Administrator.

3 STUDY OBJECTIVES AND DESIGN

3.1 STUDY OBJECTIVES

The primary objective of the trial is to identify effective, safe and acceptable interventions to reduce short and longer-term mortality and morbidity following admission to hospital with severe anaemia in sub-Saharan Africa. There are two hypotheses being tested

1) A liberal rather than a conservative blood transfusion policy will decrease mortality (cumulative to 4 weeks) in children admitted to hospital with severe anaemia (haemoglobin (Hb)<6g/dl).
2) Supplementary multi-vitamin multi-mineral (MVMM) treatments or additional cotrimoxazole prophylaxis or both for 3 months post discharge will reduce rates of readmission, severe anaemia relapse, re-transfusion or death (cumulative to 6 months) compared to current recommendations (iron and folate) and anti-helminths in all if >1 year.

Secondary objectives include

- To identify the most cost-effective interventions to reduce early mortality, and assess their budget impact
- To determine efficacy of long-term support strategies (MVMM and cotrimoxazole prophylaxis) on other markers of nutritional status and causes of death
- To determine the effect of transfusion strategies and long-term support strategies on markers of inflammation and immunological activation and function
- To identify the mechanism of action of the most effective interventions through focussed investigations of human genetic polymorphisms, molecular diagnostics, immunological activation, markers of gut barrier dysfunction, haemotological and nutritional response

3.2 STUDY DESIGN

TRACT is a multicentre randomised controlled trial of 3954 children aged 2 months to 12 years admitted to hospital with a Hb<6g/dl (taken within 2h of trial recruitment). Children will be enrolled over 2 years from 2 countries and followed for 6 months. The trial will simultaneously evaluate three ways to reduce short and longer-term mortality (primary endpoint) and morbidity following admission to hospital with severe anaemia in sub-Saharan Africa using a factorial design: (i) liberal rather than conservative blood transfusion; (ii) additional multi-vitamin multi-mineral supplements for 3 months post-discharge; (iii) additional cotrimoxazole prophylaxis for 3 months post-discharge. All randomisations will be open.

3.2.1 PROPOSED TRIAL DESIGN

Randomised controlled factorial trial with a 3x2x2 design.

3.2.2 TRIAL INTERVENTIONS

The trial will have a factorial design with 3 randomisations, each to address one of the potential approaches to reducing mortality and morbidity in children with SA (See **Trial Flow diagram: page *x*)**:

R1: Immediate liberal transfusion (30ml/kg) versus conservative transfusion (20ml/kg) versus no transfusion (last strategy only for children with **uncomplicated SA and a Hb 4-6 g/dl**).

R2: Post-discharge multi-vitamin multi-mineral (MVMM) supplementation for 3 months (which includes folate and iron) and anti-helminthics if >1 years versus routine care (folate and iron at standard treatment doses (varies with age) for 3 months) and anti-helminthics if >1 years.

R3: Post-discharge cotrimoxazole prophylaxis versus no prophylaxis for 3 months.

R1 addresses both conservative aspects of current guidelines: "whether to give blood" in uncomplicated SA (4-6g/dl without complications), and "how much blood to give" in all children with SA. The transfusion and post-discharge interventions will be open-label for reasons of practicality and compliance.

Anti-helminthics will be used following national guidelines (single dose 500mg mebendazole in Uganda, single dose 400 mg albendazole in Malawi).

Potential for interactions between the trial interventions: Because the transfusion, nutritional and antibiotic prophylaxis interventions approach different mechanisms for reducing short and long term mortality/morbidity following SA, we consider that important interactions between randomised groups are unlikely. Further, any interactions that do exist are likely to be quantitative (slightly smaller/larger effects) rather than qualitative (effect on one background, no effect on another).

3.2.3 STUDY NUMBERS

A sample size of 3954 SA cases will be enrolled – to enable multiple comparisons to be made and allowing for a 6% loss to follow-up by 6 months. Since the sample size assumptions are sensitive to the relative contribution of uncomplicated : complicated SA; the uncomplicated SA strata will be capped at 2000 cases, so that at least 1950 of trial participants will have complicated SA – so that the trial retains at least 80% power to address all the key objectives.

3.3 STUDY OUTCOME MEASURES

3.3.1 PRIMARY OUTCOME

Cumulative mortality to 4 weeks for the transfusion strategy comparison, and to 6 months for the nutritional support/antibiotic prophylaxis comparison.

Protection against bias is principally by the use of a completely objective endpoint (mortality). Any patient lost to follow-up before 6 months without withdrawing consent will be traced for vital status.

Cause of death (and other clinical secondary endpoints) will be adjudicated by an Endpoint Review Committee, blinded to randomised allocations: relationship to all possible interventions drugs (transfusion strategy; nutritional support and cotimoxazole) will be solicited to avoid unblinding.

3.3.2 SECONDARY OUTCOMES

Mortality:

- mortality at 48 hours, 28 days, 90 day and 180 days (cumulative) (where not the primary outcome).

Morbidity: endpoints relating to the specific mechanisms of action of each intervention:

- re-admission to hospital;
- proportion achieving correction of anaemia (defined by WHO as Hb>9g/dl) at 48 hours, 28 days, 90 day and 180 days
- development of new profound anaemia (Hb<4g/dl) during acute admission or development of severe anaemia (Hb<6g/dl) post discharge;
- nutrition: changes in weight and MUAC at 90 day and 180 days
- anti-infection: changes in inflammatory markers (CRP,PCT), incidence of bacterial infections and malaria at 28 days, 90 day and 180 days

Solicated adverse events

- suspected transfusion reactions: febrile reactions, Transfusion Related Acute Lung Injury (any grade); grade 3-4 toxicity of cotrimoxazole, MVMM or standard iron/folate
- SAEs

Others

- costs and cost-effectiveness.

A comparison of mortality 90 day and 180 days post-discharge will enable us to identify the maximal impact of enhanced long-term support and any rebound effects.

4 PARTICIPANT ENTRY

4.1 PRE-RANDOMISATION EVALUATIONS

4.1.1 SCREENING PROCEDURE

Each day the clinical team will check with the transfusion services the quantities of blood available for transfusion. Screening, and enrolment, will not be conducted on days when supplies are critical. Numbers of patients enrolled each day will vary from site to site and depend upon blood stocks and requirement by non-study children. Eligible children will be identified by the nurse and clinician on duty and registered in the eligibility screening log. A member of the trial team will then perform a rapid structured assessment of heart rate, oxygen saturation (pulse oximetry), respiratory rate, axillary temperature, blood pressure, markers of shock (capillary refill time, pulse volume and assessment of lower limb temperature) and severity (conscious level and respiratory distress). Children who are potentially eligible with suspected severe anaemia will have a rapid bedside test (Haemocue) to determine haemoglobin level and eligibility. For children with a haemoglobin < 6 g/dl admitting clinicians will enquire about a history of passing dark or red urine in this illness (to determine severity) and whether the child is known to have sickle cell disease. Details of those fulfilling the entry criteria including severity will be entered onto a screening form, while reasons for non-eligibility will be added to the eligibility screening log. Entry criteria will be based on clinical assessment alone. It is anticipated that this process will take approximately 5 minutes.

4.1.2 INCLUSION CRITERIA

- Aged 2 months to 12 years
- Severe anaemia (SA) (Hb<6g/dl) within a hospital admission and not previously transfused within this admission: if Hb measurement is >24h post-admission, then child should not have been previously transfused in this admission.
- Carer willing/able to provide consent

4.1.3 EXCLUSION CRITERIA

- Malignancy or other terminal illness
- Children who are exclusively breast fed (thus unable to take nutritional support)
- Chronic renal or liver failure
- Surgery as main reason for admission
- Acute trauma or burns as main reason for admission
- Signs of bi-ventricular heart failure
- Known congenital or valvular heart disease (non-surgically corrected)

See section 5.4 and 5.5 for i) nutritional management of infants below 6-months; ii) management of children with severe malnutrition with regards to MVMM supplementation, and iii) management of children with HIV with regards to cotrimoxazole prophylaxis. These children will not be excluded from a pragmatic trial but are all relatively small but important subgroups in who will either follow standard of care and/or have some pragmatic adjustment in method or timing of delivery of the R2 or R3 randomisations.

4.2 RANDOMISATION AND ENROLMENT PROCEDURE

4.2.1 RANDOMISATION CODES

Randomisation in each part of the factorial would be stratified by centre and the other randomisations in the factorial. Randomisation lists, using variable block sizes, will be generated and kept at the MRC CTU, London. The randomisation envelopes will be prepared before the trial, with one set for complicated SA (A) and one for uncomplicated SA (B). Eligible children will be screened and recruited during hospital admission. At enrolment sealed cards will simultaneously assign interventions R1A/B (according to SA strata), R2 and R3 randomly. The cards for each centre will be numbered consecutively and opened in numerical order. These will contain the actual allocation (transfusion strategy, and long-term intervention(s)) visible only once opened. This system has worked well in the emergency care trial, FEAST. To facilitate protocol adherence, a maximum per site will be agreed upon (for example up to 4 children per day) will be enrolled per site. This approach was very successful with respect to protocol adherence and the quality of data generated in the FEAST trial. It will also put less pressure on the blood banks.

4.2.2 CO-ENROLMENT GUIDELINES

Patients will not ordinarily be permitted to participate in any other clinical intervention trial or research protocol while on the TRACT trial. Participation in other studies that do not involve an intervention may be acceptable, but should be discussed with the TRACT TMG. The TRACT TMG will consider co-enrolment of TRACT participants onto other trials where the interventions do not conflict with the TRACT objectives on a case-by-case basis.

5 TREATMENTS

5.1 STANDARD CASE MANAGEMENT- IN-HOSPITAL

All trial patients will receive standard of care including antibiotics (iv or oral) anti-malarial drugs following national guidelines, based on WHO syndromic patient management[3]. We will collect data on all administered drugs. Antipyretics, anticonvulsants and treatment for hypoglycaemia will be administered according to nationally agreed protocols. If required, maintenance fluids will be run at 3-4 mls/kg per hour irrespective of age until the child can drink and retain oral fluids. At discharge from hospital all children > 1 years will be receive empiric treatment for helminths (500mg mebendazole) or 400mg albendazole in Malawi in accordance to current recommendations (standard of care, SOC) regardless of randomised allocation.

5.2 TRIAL TREATMENTS

5.3 R1: TRANSFUSION STRATEGIES

All children will be assessed at the point of trial enrolment and will be divided into 2 groups for randomisation based on:

1/ haemoglobin level and

2/ assessment of clinical severity children or complications (**reduced conscious level; respiratory distress, acute history haemoglobinuria or an established diagnosis of sickle cell disease**)

R1A Complicated Severe Anaemia haemoglobin < 4g/dl OR a haemoglobin < 6g/dl PLUS one or more signs of severity or complications

R1B Uncomplicated severe anaemia: haemoglobin >=4 and < 6g/dl without any of the severity features or complications

5.3.1 TREATMENT ARMS

R1A: Complicated Severe Anaemia

These children will be randomly allocated on a 1:1 basis to receive one of the following:

- **Whole Blood Transfusion** 20mls/kg, alternatively **10mls/kg packed cells;** or
- **Whole Blood Transfusion** 30mls/kg, alternatively **15mls/kg packed cells**

R1B: Uncomplicated Severe Anaemia

These children will be randomly allocated on a 1:1:2 basis to receive one of the following:

- **Whole Blood Transfusion** 20mls/kg alternatively **10mls/kg packed cells**, or
- **Whole Blood Transfusion** 30mls/kg alternatively **15mls/kg packed cells,** or
- **No Transfusion (control, SOC)**

5.3.2 TREATMENT SCHEDULE

A clinician or medical officer will prescribe the blood. Once the randomisation arm has been allocated, for those receiving immediate transfusion the clinician will calculate, using a calculator, the volume of whole blood required (20 or 30mls/kg). If only packed cells are available then the clinician must re-calculate the equivalent volumes of packed cell (10 or 15ml/kg). The request form must specify patient's first and last name, date of birth, desired component (whole blood or packed cells), volume of blood requested, date and time of request. Once the blood has been cross-matched and the blood pack for transfusion is ready for collection, the health worker who collects the donor blood for transfusion will complete the blood collection log. Prior to leaving the laboratory, the health worker will confirm with the lab technologist that the blood has been weighed and the weight recorded on the appropriate log and request form, as well as the volume. This will be repeated on the ward when the blood is received. Transfusions will be administered in gauged blood burettes; an initial aliquot (2ml) will run into a sterile apex tube using an aseptic technique (and ensuring that the tip of the infusion set does not touch anything to prevent contamination) and one drop taken from this to record the haemoglobin of donor blood, a blood film prepared to exam for storage lesions, the rest will be stored (see Study flow). Whole blood will be run over 3-4 hours and packed cells can be administered over 2-3 hours.

Further transfusion management during hospital admission

For all children in the trial an additional, or initial (for SOC control group in R1B only) transfusion (s) will be permitted after 8 hours (at the point of the first reassessment of haemoglobin, Hb) for children who still have either

(i) Profound anaemia Hb <4g/dl, irrespective of other signs of severity
(ii) Severe anaemia 4-6g/dl and one or both *de novo* signs of severity (respiratory distress or impaired consciousness)
(iii) Uncorrected severe anaemia 4-6g/dl in children with acute history haemoglobinuria or known sickle cell disease

However, early sampling of haemoglobin (<8 hours from baseline), and additional transfusion, will be permitted in children randomised to any group in the R1B strata (uncomplicated SA) developing de novo signs of severity.

Transfusion volume

Control (R1B only: randomised to control: no initial transfusion) who meet the above criteria, will receive 20ml/kg whole blood or 10ml/kg packed cells.

Children randomised to initially receive blood (**R1A and R1B)** who subsequently meet above criteria will *follow their randomisation arm* ie will receive either an additional transfusion of 20ml/kg or 30mls/kg of whole blood (or 10ml or 15ml/kg packed cells respectively).

Any child who has already received two transfusions and subsequently fulfils criteria above will receive a maximum of 20mls/kg (or 10ml/kg packed cells) irrespective of randomisation.

Frusemide or other diuretics will be prescribed at the discretion of the attending physician and recorded in the Source documents and CRF, including reasons for prescription.

5.4 R2 MICRONUTRITIONAL SUPPORT

Simultaneously to R1 randomisations, all children entering the trial will also be randomly allocated on a 1:1 basis to receive one of the following:

Micronutritional interventions (R2)

- **MVMM (containing iron, folate and other MVMM) for 3 months post-discharge**
- **Iron and Folate (standard of care) for 3 months post-discharge**

5.4.1 PRODUCT AND TREATMENT SCHEDULE

5.4.2 MULTIVITAMIN MULTIMINERAL (MVMM)

Nutromix™ has been specifically design for children 6-24 months of age with severe anaemia. Other formulations have been developed for older children and adolescents, women of child-bearing age, pregnant women, lactating women (UNICEF and the WHO). The formulation, shown in table below, meets the recommended nutrient intake (RNI) – particularly for vulnerable groups during emergencies[41]. Recommended nutrient intake is defined (RNI) as the daily dietary in−take of a nutrient sufficient to meet the nutrient requirements of nearly all apparently healthy individuals in a specific population group, usually by age and sex (9).

Micronutrients	Pregnant and lactating women	Children (6–59 months)
Vitamin A µg	800	400
Vitamin D µg	5.0	5.0
Vitamin E mg	15	5.0
Vitamin C mg	55	30
Thiamine (vitamin B1) mg	1.4	0.5
Riboflavin (vitamin B2) mg	1.4	0.5
Niacin (vitamin B3) mg	18.0	6.0
Vitamin B6 mg	1.9	0.5
Vitamin B12 µg	2.6	0.9
Folic acid µg	600	150
Iron mg	27.0b	100
Zinc mg	10	4.1
Copper mg	1.15	0.56
Selenium µg	30	17
Iodine µg	250	90

Dosage: One sachet to be taken daily by the child and will be prescribed at the time of discharge from hospital.

Guidelines for the use of Nutromix™ Sachets:

1. Open the top of the sachet and empty the entire contents of the sachet in any semi-solid or semi-liquid food, after the food has been prepared and cooled to a temperature acceptable to eat (less than 60^0C)
2. Mix the powder in an amount of food, the child can eat in one meal.
3. The food to which **Nutromix**™ is added should be consumed within 30 minutes, as the micronutrients present in **Nutromix**™ may give the food a dark colour. Even if it turns dark, it is still safe to use.
4. Do not add to juice or water as they will not mix very well.

5.4.3 IRON AND FOLATE

Daily iron syrup or tablets and folate tablets for children < 2 years (25mg iron; 100-400micrograms folate); and >2 years and < 12 years (60mg iron; 400micrograms folate) will be given for 3 months, according to WHO guidelines for the management of severe anaemia.

Special groups: The nutritional supplementation, including MVMM randomisation, will be pragmatic in that all children for whom these supplements should be received according to WHO or national guidelines (e.g. those initially admitted with severe malnutrition) will receive them. Children who < 6 months who are not weaned (fully breast feed) will be excluded from the trial.- The number of infants >2months and <6months are likely to be small (<3% of total trial population).

- **Children with severe malnutrition** Iron-containing supplements are not recommended for severely malnourished children during the first 7 days of acute rehabilitation (WHO guidelines) but can be used effectively and safely after the child's initial nutritional rehabilitation (child is keen to feed and gaining weight). For children with severe malnutrition discharged on ready to use food supplements (RUTF) which contain MVMM, children will essentially ignore the MVMM randomisation on their allocated card, but receive their standard post-discharge supplementation within the RUTF which would be recorded on study CRFs. The number of children with severe malnutrition as their admission diagnosis is expected to be small (<5%).

5.5 R3 SHORT TERM ANTIMICROBIAL PROPHYLAXIS

Children will be randomly allocated on a 1:1 basis to receive one of the following:

Antimicrobial interventions (R3):

- **Cotrimoxazole prophylaxis for 3 months post-discharge**
- **No antibiotic prophylaxis post discharge (control, SOC)**

5.5.1 PRODUCT AND TREATMENT SCHEDULE

5.5.2 COTRIMOXAZOLE

Cotrimoxazole dispersible tablets (240mg: trimethoprim 40 mg/sulphamethoxazole 200mg) and dosing will follow WHO recommendations for prophylaxis in HIV-infected children: age 2 to 6 months: 120 mg; age 6 months to 5 years: 240mg; children >5 year 480mg. The dispersible tablets may be taken with water or mixed with feeds.

Cotrimoxazole will be prescribed from discharge. The cotrimoxazole prophylaxis randomisation will be pragmatic in that all children for whom cotrimoxazole prophylaxis should be prescribed according to WHO or national guidelines (e.g. HIV-infected children) will receive it regardless of randomisation, and no child in whom it is contraindicated (e.g. known GP6D deficiency according to local testing) will receive it. Such

children will essentially ignore the cotrimoxazole randomisation on their allocated card; any cotrimoxazole received per guidelines would be recorded on study CRFs. The number of children with these conditions is expected to be small (<5%). HIV-infected children will receive antiretrovirals and will continue in the trial with HIV management and follow-up tailored in collaboration with local HIV clinics.

5.5.3 MEDICATIONS SUPPLIED AFTER DISCHARGE

Throughout the trial, children parents or their carers will be provided with a supply of drugs or nutritional products sufficient to last until their next clinic visit. At each clinic visit they will be requested to return all empty bottles and un-used medication to the follow-up clinic. Drugs will be provided for the trial as scored tablets (cotrimoxazole), iron syrup or tablets (for older children), folate tablets and MVMM sachets. The parents will be instructed that on no account should any drug assigned to a patient be used by anyone else (unless nutritional supplementation is prescribed to a lactating mother with an infant >2months and <6 months who has been not yet weaned). Unused drug must be returned to the site if a patient withdraws from treatment.

All drug dispensed and returned to the site should be documented on a treatment log for each patient. At each site, a named person (research nurse) will be required to maintain complete records of all study medication dispensed. The designated trial nurse will, on receipt of supplies prior to the commencement of the trial, conduct an inventory and complete a receipt. All trial drugs dispensed to participants will be recorded on a Dispensing Log. Inventories will be conducted monthly, and logs returned to KWTP.

5.6 STORAGE CONDITIONS

MVMM and cotrimoxazole to be stored at room temperature and should not to exceed 30°C (86°F). Both can be stored in these conditions for three years as packaged.

5.7 ACCOUNTABILITY

The trial coordinator (TC) at each site will maintain accountability logs for both transfusion interventions and nutritional and antibiotic interventions post discharge. These will kept securely until verified by the external monitor's visit.

5.8 MEASURES OF COMPLIANCE

Lack of patient compliance will not be a major issue for the transfusion interventions, since all children entering this trial will be critically ill and medical personnel will be responsible for administering the interventions. Any non-compliance will likely be a consequence of the intervention itself (e.g. lack of matched blood). To minimise wastage of blood, digital weighing scales will be provided for the blood transfusion laboratories to ensure accurate volumes are being issued and this will also be checked at a clinical level by use of gauged blood burettes.

Cotrimoxazole is used widely as prophylaxis in children with extremely low rates of toxicity and high acceptability. For cotrimoxazole, MVMM and iron/folate we do not anticipate compliance problems in this group with motivated carers following their child's acute admission with severe anaemia, in contrast to the compliance issues often found when these nutritional products are used in community fortification programmes for relatively healthy children. All carers will be asked questions about adherence using a standard questionnaire at clinic visits according to the flow sheet. To ensure compliance, between the day 28 and day 90 clinical visits study coordinators will contact parents, if possible, by phone to check on adherence and enquire about the status of the child.

Any non-compliance due to toxicity would also likely occur if the interventions were incorporated within clinical practice, and is part of a pragmatic strategy being evaluated. The intention-to-treat comparison will therefore incorporate such "strategy non-compliance" that is anticipated in general clinical practice.

6 ASSESSMENT AND FOLLOW-UP

6.1 SUMMARY

Transfusions will be given directly following randomisation and will run over four hours. Subsequently, after 8 hours, all children will receive a transfusion if Hb<4g/dl or if Hb 4-6g/dl with *de novo* signs of severity (impaired consciousness or respiratory distress). MVMM and cotrimoxazole prophylaxis will be given for 3 months post-discharge. Children will be intensively monitored (Table A) during admission by the clinical team, and during any transfusion and then reviewed daily by the study team until discharge, with Hb performed at least 8 hourly in the first 24 hours following recruitment, and daily thereafter. Locator maps and contact numbers will be obtained to facilitate follow-up. All participants will then be seen at 4 weeks, and 3 and 6 months post-discharge at outpatient clinics attached to each centre for evaluation of morbidity, Hb, and toxicity. Any patient not returning for a study visit will be traced for vital status ascertainment (consent will be sought for this at recruitment).

6.1.1 TRIAL ASSESSMENT SCHEDULE

See Flow diagram (page xi) and Table A (page xii).

6.1.2 BASELINE LABORATORY INVESTIGATIONS

Following consent and randomisation, blood samples will be taken for the following investigations: Full blood count, urea and electrolytes (U&E), acid-base status, lactate, glucose, malaria status (malaria slide and Paracheck RDT), blood film, cross match, and if facilities permit blood culture and other clinically indicated investigations (not required by the study protocol). Urine will be taken as soon as possible after trial enrolment for Multistick analysis (culture where indicated and facilities permit). Stool samples will be collected as soon as is practical after trial enrolment for assessment of helminth infection and stored for subsequent assays. In accordance with national guidelines, HIV testing will be performed after admission procedures are complete and assent given by parents or guardians. Pre- and post-test counselling will be done in accordance to routine practice.

6.1.3 SAMPLE STORAGE

In all children plasma and EDTA (for DNA extraction), and stool, will be stored for subsequent microbiological, parasitological, virological diagnostic assays, genetic studies (including sickle cell and G6PD status), immune activation, micronutrient assays and gut hormones (subset). In a subset of children at Mbale RRH, who have consented to additional research blood samples, red and white cell pellets will also be prepared and stored. Urine samples will be stored on a subset of children. The clinical samples and samples for storage will require no more than 10mls of venous blood, including the additional 7mls of whole blood for research purposes. Any blood taken for research purposes under emergency deferred consent (see Appendix 1 for consent procedures) from children whose parents subsequently refuse consent will be discarded.

6.1.4 DURING ADMISSION

Nurses

All children will be reassessed clinically at 30 mins, 60mins, 90 mins, 2, 4, 8, 16, 24 and 48 hours following study recruitment. At each review conscious level, vital signs (heart rate, oxygen saturation, respiratory rate, axillary temperature, blood pressure) and examinations for adverse events will be recorded. These will be recorded in bedside notes. Nurses will also record details of prescribed drugs, transfusion rate and volume received and all other intravenous fluids. During any transfusion additional bedside monitoring will

be conducted and reported in the source documents. Anthropometric data (weight length or height and MUAC) will collected at admission and prior to discharge.

Brief details of the child's household address will be collected at admission and more detailed locator data will be collected prior to discharge. After discharge the Study Site Coordinator will be responsible for completing the Case Report Forms (CRF) and following up initial data queries and tracing the results of pending laboratory tests.

Doctor

A symptom checklist and targeted physical examination (to evaluate any reported symptoms) will be performed at each clinical assessment will be recorded in the source documents (clinical notes). The doctor will review the child routinely at 1, 8, 16 and 24-hours following trial enrolment and daily thereafter. Additional reviews will be done where clinically indicated and recorded in the case notes. At each clinical review the doctor will specifically review the child for solicited adverse events and the doctor will be responsible for documenting and reporting SAEs. Admission and final diagnoses will be recorded in the CRF.

After 48 hours (post-enrolment) the hospital patient's notes will be used in place of the TRACT source documents; an TRACT trial number sticker will be added on the front page of the patient's hospital notes and a caption **"**TRACT trial patient'': Notes to be reviewed at discharge" written below the sticker. This will enable the study team to track the patient's notes at discharge and collect important patient data. Additional pages in the TRACT trial source documents will be available to report key clinical, laboratory or adverse events during the period prior to discharge.

Additional blood tests

Using handheld monitors haemoglobin will be monitored 8-hourly on day of recruitment and daily thereafter (Table A). Blood glucose will be monitored at admission, 1, 2, 8, 16 and 24 hours (handheld glucometer) until the child is fully conscious and able to take fluids and/or food orally. Where these do not coincide with the above time points additional glucose measurements will be taken at 30mins and 4 hours (2 hours for packed cell) after the start of any transfusion. Lactate will be recorded at 8 hours and 24 hours. All of these tests will be conducted during any deterioration.

Data Summaries

The CRF will summarise at each time point (Table A) transfusions and IV fluid received, prescribed drugs (including other supportive treatments eg oxygen). Any drugs, intravenous fluids and transfusions received before admission will be captured in the admission CRF. Summaries of routine investigations will be recorded in the CRF and additional investigations and other treatments received during admission in the discharge CRF.

6.1.5 AT DISCHARGE

Before discharge the nurse will conduct the 24-hour dietary recall (detailing dietary intake prior to admission). The doctor will prescribe relevant nutritional supplementation and antibiotic prophylaxis following the randomisation and national guidelines. Prior to discharge children > 1 years of age will be prescribed anti-helminthics, to treat potential helminth or whip worm infection, but only if they have not already been prescribed this in the last 6 months (Page 147, WHO Guidelines For The Management Of Common Illnesses With Limited Resources) (500mg mebendazole in Uganda, 400mg albendazole in Malawi). As per WHO guidelines, children who also have severe malnutrition will only commence iron-containing medications once the child has a good appetite and has started to gain weight (usually in the second week) and is also a usual indicator for discharge for further home management.

Children or their carers will be provided with a supply of drugs sufficient to last until their first clinic visit (day 28). The Parent will receive a follow up invitation on a card. The date will be 28 days after the day of

recruitment. (For children where this falls on a Saturday this will be moved to Friday and for those where it falls on a Sunday this will be moved to the following Monday and drug supply adjusted accordingly.) Detailed Locator details and contact details will be checked again at discharge. Money for transport will be given by the clinical coordinator or designee.

The clinical coordinator is responsible for ensuring the discharge check-list is complete and for chasing up inpatient notes at discharge. Any relevant information, especially with regard to date of discharge, death, SAE's, treatments, blood transfusions, use of intravenous fluids, oxygen or non-routine treatments or investigations, will be recorded.

6.1.6 FOLLOW UP

A symptom checklist and targeted physical examination will be performed at each at each clinic visit post-discharge. Medical history since last visit including hospital re-admissions, transfusions and grade 3 or 4 adverse events related to nutritional and antibiotic interventions including severity and likely relationship of any adverse events will be documented by a doctor.

At Day 28 adherence to and acceptability of MVMM and/or cotrimoxazole will be queried by carer self-report, and carers will be provided with a supply of drugs sufficient to last for the next 2 months (Day 90 since admission). Blood and other tests and sample storage will be according the schedule outlined in Trial flow (Page xi) requiring a maximum of 4mls heparinised blood (plasma) and 1ml into EDTA (for pathogen diagnostics). At Day 90, adherence/acceptability will again be recorded, but no more supplements/antibiotics will be given. Any participants requiring further care at their 180d visit will transferred into the routine clinics at the centre where the trial is being conducted.

For participants enrolled at Mbale RRH at 28 day and 180 day visits, in specific subgroups of participants, the following will be undertaken: (PMBC/Red Cells/neutrophil function): 6 ml of blood will be taken by venepuncture for the analysis of monocyte and neutrophil function, inflammatory markers, endotoxin concentration known markers of gut barrier dysfunction as well as proteomic and metabolomic analysis. A urine sample will be requested at the same time and stored.

For participants enrolled at Mbale RRH at 28 day, 90 day or 180 day visits, in specific subgroups of participants, the following will be undertaken: (Gut Barrier Function (EDX (1ml), I I-FABP, IBAP on 5 mls of plasma).

6.2 PROCEDURES FOR ASSESSING EFFICACY

6.2.1 CLINICAL EVENTS (ALL PARTICIPANTS)

- Survival status will be recorded at each endpoint (48 hours, 28 days, 90 day and 180 days following admission). Any patient lost to follow-up before 6 months without withdrawing consent will be traced for vital status.
- Other serious adverse events will be reported as and when the doctor becomes aware of them (see SAE section). The details reported will include bedside observations, laboratory data, and additional clinical narrative.
- During the index admission, any child fulfilling criteria for a new or additional transfusion will be recorded
- At all subsequent visits hospital admissions and requirement for transfusion will be solicited.

Cause of death (and other clinical secondary endpoints) will be adjudicated by an Endpoint Review Committee, blinded to randomised allocations: relationship to all possible interventions drugs (transfusion strategy; nutritional support and cotimoxazole) will be solicited to avoid unblinding.

6.2.2 HAEMOTOLOGICAL RECOVERY (ALL PARTICIPANTS)

- Haemoglobin will be recorded at 8 hour periods up to 24 hours, then daily until discharge, then at 28 days, 90 day and 180 days.

6.2.3 PROCEDURES FOR ASSESSING SAFETY (ALL PARTICIPANTS)

The symptom checklist used at each visit will explicitly prompt for symptoms relating to possible drug toxicities. Additional safety blood tests or investigations may be performed to investigate symptoms or monitor emergent laboratory test abnormalities as clinically indicated.

Serious, solicited and grade 3 or 4 adverse events will be reported on the case report form. Adverse events (clinical and laboratory) will be graded according to toxicity/severity grading.

6.2.4 PROCEDURES FOR ASSESSING ADHERENCE (ALL PARTICIPANTS)

Adherence to nutritional and prophylaxis drugs will be assessed in all participants at each visit by pill counts for tablets, and nurse-administered questionnaire to the child's carer, and where appropriate to the child (at the discretion of the nurse or doctor depending on age).

6.3 OTHER ASSESSMENTS

6.3.1 HEALTH ECONOMICS (ALL PARTICIPANTS)

The trial will measure healthcare-related costs in trial participants, starting at randomisation and continuing for the duration of follow-up. Costs incurred by the patients and their families (transport, indirect and companion person's costs) will be obtained by patient reports. Reported transport costs will be confirmed using local information on distance and cost of transport. Information on hospitalisations (number, reason, and duration of stay) will be collected from hospital records, and data on other healthcare resource utilisation (outpatient visits, medications, and procedures) will be collected by abstraction of patient medical notes and by patient interview at the 28, 90 and 180-day visits.

6.3.2 ANTI-INFECTIVE

Changes in inflammatory markers (eg CRP) (all participants) will be measured retrospectively and incidence of bacterial infections and malaria at 28 days, 90 day and 180 days (from blood cultures at all sites except Soroti and using non-culture based molecular diagnostics on stored blood – all sites).

Immune/ Biomarkers (sub-group)

For a subgroup of patients, the following assays will be undertaken on samples stored from admission, day 28 and day 180 of follow-up:

- Whole blood assays measuring oxidative burst, phagocytosis and expression of activation markers will be conducted by flow cytometry (4 colours).
- Remaining blood will be separated into PBMCs and plasma, stored in Liquid Nitrogen and -80C respectively for batch analysis of inflammatory markers (plasma) and additional analysis of immune function (PBMC).
- Whole blood will be used to establish the concentration of endotoxin in blood within 3 hours of admission.
- Stored plasma and urine (-80C) will be used to measure the concentration of known markers of gut barrier dysfunction by ELISA.
- The metabolome and proteome will be determined in a selected group of samples depending on the results of the initial screen of endotoxin and known markers of gut barrier dysfunction.

7 STASTISTICAL CONSIDERATIONS

7.1 SAMPLE SIZE

The sample size calculation is based on the following assumptions:

- 80% power, 2 sided alpha=0.013 to allow for 4 comparisons (see below).
- SA cases are 50% complicated (<4g/dl or 4-6g/dl with prostration/respiratory distress/known sickle cell disease/dark urine) and 50% uncomplicated (4-6g/dl without prostration, respiratory distress, known sickle cell disease or dark urine)[11] (and Dr. Olupot-Olupot personal communication)
- Mortality (cumulative) at 48 hours and 4 weeks is 11% and 16% respectively in complicated SA, and 4% and 9% in uncomplicated SA.
- The cumulative rate of re-admission, severe anaemia relapse and re-transfusion at 6 months is 12.5% in both complicated and uncomplicated SA (in addition to mortality above).
- For the primary comparison of **transfusion vs. no transfusion** in uncomplicated SA at 4 weeks, the minimum clinically relevant difference is a 50% relative reduction (**R1B**): for the other primary comparison of transfusion **volume** (20 vs 30 ml/kg) at 4 weeks **(R1A&B)**, the minimum clinically relevant difference is a 30% relative reduction. The minimum clinically relevant difference is larger for the transfusion vs no transfusion question as provision of safe blood at all will require greater resources than provision of slightly larger vs slightly smaller blood volumes. As the same relative difference translates to a far larger absolute difference at higher event rates, for the primary comparison at 6 months (**R2/3**) the minimum clinically relevant difference is a 5% absolute reduction (see below for control group event rates). Then:

- **(R1B)** comparison of transfusion vs no transfusion (50% reduction from 9% control mortality at 4 weeks) requires 1460 uncomplicated SA cases (1:1 allocation to 30/20ml/kg:no transfusion, 730 in no transfusion group, 365 receiving 20 ml/kg and 365 receiving 30 ml/kg transfusions).
- **(R1A&B)** if the overall ratio of uncomplicated : complicated SAs is 1:1 (ie 50% of each type), then within the subgroup randomised 1:1 to 30 vs 20ml/kg, the ratio will be 1:2 because this comparison excludes the 50% of uncomplicated SA randomised to no transfusion. Overall mortality at 4 weeks in this group will therefore be 13.67% (0.33*9%+0.67*16%). The comparison of 30 vs 20 ml/kg (30% reduction, to 9.57%) requires 2798 SAs, 1399 per group (split 466 uncomplicated, 933 complicated).
- Therefore, comparing required sample sizes for **R1A** and **R1B,** to address both parts of the transfusion question (**R1**) we need slightly more uncomplicated SA children per group from (R1A&B – n=466) than (R1B – n=365), and therefore the total sample size is **3730 cases** (933*2=1866 complicated, 466*4=1864 uncomplicated).
- **(R2/3)** the comparison of multi-vitamins vs standard of care (1:1) and cotrimoxazole prophylaxis vs standard of care (1:1) requires **3162 SA cases**, assuming 50% are complicated and 50% uncomplicated, to detect a 5% absolute reduction from average control mortality of 25% (0.5*21.5%+0.5*28.5%) at 4 weeks).

Thus a sample size of **3730 SA cases** would allow the multiple comparisons above to be made. Assuming a 6% loss to follow-up by 6 months increases this to **3954 SA cases.** As the effect sizes are reasonably large on the relative scale (>30% reduction), inflation factors which adjust for the factorial design are close to 1. However, assumptions are more sensitive to the relative contribution of uncomplicated: complicated SA. Capping the uncomplicated SA strata at 2000 cases (ie recruiting at least 1950 complicated SA) retains at least 80% power to detect the differences above independently of variations in the contributing proportions of complicated SA.

Randomising uncomplicated SA (**R1B**) 1:1:2 between 30ml/kg:20ml/kg:no transfusion provides greater power for the comparison of no transfusion (SOC) versus transfusion in this group because the final randomisation ratio for transfusion: no transfusion is 1:1. In contrast a 1:1:1 randomisation in this strata would produce a 2:1 transfusion:no transfusion ratio which has lower power.

7.2 STATISTICAL ANALYSIS

The analyses will be described in detail in a full Statistical Analysis Plan. This section summarises the main issues.

Each intervention is hypothesised to be superior to standard of care, and therefore the proposed analysis is intention to treat, including all randomised patients. The primary analysis will compare a) transfusion versus no transfusion and b) 20mls/kg vs 30mls/kg in terms of the proportion of children with fatal outcome 28 days after randomisation. Primary outcome analysis will use time-to-event methods (Kaplan-Meier, log-rank test, proportional hazards models) to the time points specified for primary and secondary outcomes, stratified by centre and anaemia severity at baseline. Correction of anaemia will also be analysed using time to event methods.

Pre-specified subgroup analyses will include each of the other randomised allocations (ie exploration of interactions in the factorial design), together with the other randomisation stratification factor (centre) and the anaemia stratification factor (A vs B) for the transfusion randomisation. We will also investigate a priori whether there was any evidence for a different impact of the interventions according to the following categorical variables: previous receipt of a transfusion ever or (at another health centre in this illness); speed (rate) at which the transfusion is administered, fever; malaria; microbiological evidence of sepsis (blood culture or retrospective molecular diagnosis); HIV; known or previously undiagnosed sickle cell disease.

For the cotrimoxazole prophylaxis and MVMM supplementation randomisations the primary analysis will be intention-to-treat based on all randomised participants, as above. However, a secondary analysis will be restricted to patients alive at the minimum of discharge or initial prescription of cotrimoxazole or MVMM (where these TRACT medications are started during admission) in whom these interventions were neither mandated nor contraindicated (ie excluding HIV-infected children and those with known GP6D deficiency from the cotrimoxazole randomisation, excluding children admitted for severe acute malnutrition from the supplementation randomisation).

Secondary outcome measures will be analysed using time-to-event methods or normal linear regression for continuous variables. The frequency of hospital re-admissions and adverse events will be tabulated by body systems and by randomised groups, and the number of events experienced by each participant will be compared across randomised groups using Fisher's exact test.

For the within-trial analysis, the differential cost of the treatment interventions will be related to their differential outcomes in terms of the primary outcome. The relative cost-effectiveness of the alternative forms of management will then be assessed using standard decision rules and a full stochastic analysis will be undertaken. A cost-utility analysis will also be conducted using a standard approach. The within-trial analysis will be augmented by extrapolation beyond the trial follow-up using decision-analytic modelling. The aim of this analysis will be to predict the implications of any difference in clinical endpoints in the trial for subsequent quality-adjusted survival duration and long-term resource costs. This will inform the question of whether any differences in drug costs between the treatment groups are offset by reduction in other treatment costs or health improvements in the long-term.

7.3 INTERIM REVIEWS

During the trial an independent Data Monitoring Committee would meet to review unblinded data for all three randomised comparisons at least annually. They will review data on enrolment, safety, adherence to randomised strategies, efficacy and safety at regular intervals and in strict confidence. The DMC will determine the frequency of their meetings, which could be more frequent if they think necessary.

A decision to discontinue recruitment, in all patients or in selected subgroups, will be made only if the results are likely to convince the general clinical community and participants in the trial. The DMC will report to the Trial Steering Committee (TSC) and to the Ethics Committee in each country, if, in their view, the data provide proof beyond reasonable doubt that one of the allocated strategies is better than its comparator in terms of the primary outcome. The guiding statistical criterion for "proof beyond reasonable doubt" is the Haybittle-Peto criterion of a difference of at least 3 standard deviations in an interim analysis of a major endpoint. The TSC will then decide whether to amend (including the possibility of dropping one of the transfusion strategies) or stop the trial before the end of the planned follow-up. If a decision is made to continue, the IDMC will advise on the frequency of future reviews of the data on the basis of accrual and event rates. The IDMC will make recommendations to the TRACT Trial Steering Committee as to the continuation of the trial.

7.4 DATA GOVERNANCE

The ownership of the TRACT dataset will lie with the TRACT Trial Steering Committee, who will approve all requests for use of trial data before and after the trial ends (also to be approved by the TRACT Data Monitoring Committee). The TRACT dataset will be held electronically for at least 20 years after the end of the trial in accordance with MRC policy. The TRACT Trial Steering and Data Monitoring Committee Charters will state that proposals to use TRACT data and samples will be welcomed, and supported widely where this does not conflict with existing plans within the Trial Team (e.g. as described in the primary and secondary objectives of the main trial proposal above)

8 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.

8.1 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 in Table 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 necessarily caused by or related to that product.
Adverse Reaction (AR)	Any untoward and unintended response to an investigational medicinal product related to any dose administered.
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 that product.
Serious Adverse Event (SAE) or Serious Adverse Reaction (SAR)* o	Respectively any adverse event, adverse reaction or unexpected adverse reaction that: ▪ Results in death ▪ Is life-threatening ▪ Requires hospitalisation or prolongation of existing hospitalisation (that is not an elective admission) ▪ Results in persistent or significant disability or incapacity

* Suspected Unexpected Serious Adverse Reaction (SUSAR) will not be assessed in this trial as it falls outside the scope of the European Union Clinical Trial Regulations.

Relevant background
Cotrimoxazole and MVMM are licenced medication with known profile of side effects; similarly blood transfusions are commonly used. Adverse event monitoring will therefore focus on serious and causally related events (grade 3 or 4). Clinical or laboratory toxicity will be reported if grade 3 or 4 following the Common Toxicity Criteria (CTC) for Adverse Events (http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_8.5x11.pdf). In the event of an abnormal clinical or laboratory finding by the study clinician, children will receive appropriate treatment according to national or WHO clinical guidelines, including admission for assessment and/or treatment where appropriate. Usual clinical practice for suspected reactions to cotrimoxazole will be followed. For transfusion the solicited adverse events that will be routinely capture and reported include suspected any events Acute transfusion reaction (ATR) Haemolytic transfusion reaction (acute or delayed) (HTR) Transfusion related acute lung injury (TRALI); Post

transfusion purpura (PTP); Transfusion transmitted infection (TTI) and Transfusion associated circulatory overload (TACO) (details covered in Appendix IV) and any events of pulmonary oedema. These will be graded following the CTC.

Any event which does not have a specific grading provided in the CTC should be graded as follows

- 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.

8.1.1 EXEMPTED ADVERSE EVENTS

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, e.g. elective cosmetic surgery, social admissions
- Overdose of medication without signs or symptoms

8.1.2 ANAEMIA-RELATED EVENTS

Pre-specified secondary outcomes in the trial include (lack of) resolution of anaemia and relapse or recurrence of anaemia post-discharge, as these are relatively common clinical outcomes which we hypothesise will be prevented by the interventions under investigation. These events will be detected by the trial team through regular, repeated clinical review of the study patients, documented in the CRF and analysed as specific secondary efficacy endpoints. These anaemia-related events (worsening of anaemia during initial admission, re-admission for anaemia relapse), and their associated signs and symptoms, should therefore not be reported as adverse events, or serious adverse events, to avoid double-counting.

Death should always be reported as a (serious) adverse event, regardless of cause.

8.2 CAUSALITY

The assignment of the 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 Chief Investigators. 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 event occurs 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.

8.3 REPORTING PROCEDURES

At each clinical review the clinician or nurse will check for potential SAEs, grade 3 or 4 AEs and solicited AEs including evidence of pulmonary oedema, suspected TRALI or suspected transfusion-reaction. 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) and all solicited AEs (any grade) will be reported on the CRFs. The reporting procedure is captured within the safety reporting SOP. Any questions concerning adverse event reporting should be directed to the study coordination centre in the first instance. (This is done either via email or by telephone). SAEs will be reviewed immediately by a designated physician (SAE reviewer) in Kilifi and periodically by the Endpoint Review Committee (ERC) who will be blind to study allocation.

SAEs will be reported to the study coordination centre, using an SAE form, which should be completed, scanned and sent electronically to the TRACT study coordination centre within 24 hours. 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). The responsible investigator should sign the causality of the event. Additional information should be sent within 5 days if the reaction has not resolved at the time of reporting.

Local investigators should report all SAEs as required by their Local Research Ethics Committee and/or Research & Development Office. Any questions concerning adverse event reporting should be directed to the Principal Investigator in the first instance.

Contact details for reporting SAEs
Please send SAE forms to:
TRACT@kemri-wellcome.org
Tel: +254715461761 or +254417522063

8.4 LOSS TO FOLLOW-UP

Any child lost to follow-up before 6 months will be traced for vital status. In order to minimise losses to follow-up, locator data (maps and identifiable landmark) and mobile phone numbers will be taken on

discharge and verified at every review. During the 180d study period attempts should be made to contact the patient via phone (if available) and to follow-up with home visits, if at all possible. In the statistical analysis, a patient will be regarded as 'lost to follow-up' if they were not seen in clinic at the 180d visit and were not known to have died.

8.5 TRIAL CLOSURE

The trial will be considered closed after the 180d follow up of the last enrolled child. At the end of the trial, vital status of all participants will be ascertained (inlcuded in the consent).

9 ANCILLARY STUDIES

9.1 HEALTH ECONOMICS

The economics substudy will include cost-effectiveness analyses (CEA) of the trial interventions. This will involve an evidence synthesis and modelling exercise of different treatment strategies, and will build upon previous CEAs in children (eg the FEAST trial). Resource use data will be collected in this trial and will be supplemented by data collected in previous work.

The economic evaluation will be conducted from the health services perspective. Costs will cover the use of medication and laboratory tests as well as hospital, primary care and community health services. Unit costs will be attached to resource use, using the best available estimates of long run marginal opportunity cost, to obtain a cost per patient over the period of follow-up. Routinely available national unit costs will be used where possible with local estimations where necessary. There will also be a budget impact analyses of the consequences of adopting the interventions on the health sector budgets, in each of the countries of the trial.

9.2 MOLECULAR DIAGNOSTICS

A major question that has hindered identification of relevant interventions for this patient population to date is what are the underlying causes of morbidity and mortality in children with SA. Whilst the TRACT randomised comparisons will provide data to support particular mechanisms, stored samples will also provide a valuable resource for the application of new molecular diagnostics methods.

Two molecular methods are being used increasingly in research and clinical practice to identify bacteria and viruses. 16S ribosomal DNA (16S rDNA), common to all species of bacteria, can be detected with a broad-range polymerase chain reaction (PCR); specific quantitative PCR (qPCR) can also be used to quantify the 16S rDNA subunit to measure directly the number of bacteria. However broad range 16S rDNA PCR is subject to artefact from endogenous and exogenous bacterial products[53-55] and therefore without either sequencing the PCR product, or carrying out more sensitive qPCR, there is concern that changes in the qPCR may not be due to circulating organisms. Unfortunately sequencing the 16S rDNA has so far yielded results compatible with environmental contamination rather than recognised gut commensals[56-58]. The use of specific primers renders qPCR less vulnerable to background contaminants than broad-range 16S rDNA PCR, and therefore more sensitive. The disadvantage of qPCR is the need to predict which bacterial or viral species are likely to be relevant.

The goal of this study would be to identify the role of bacteria and viruses in the aetiology of SA in African children. Previous studies have shown comparable results between frozen EDTA plasma and whole blood, and so we would assay standard 16S rDNA PCR, and a panel of 10 qPCR reactions (including Enterobacteriaceae, a panel of anaerobes, Streptococcus pneumoniae, Staphylococcus aureus, group A streptococcus and a range of potential viruses) in plasma samples taken at enrolment, and closest before death (or the corresponding visit week in controls) in all cases and controls.

9.3 HUMAN GENETICS: RED CELL DISORDERS

Human genetic factors are potentially important modifiers of the risk of SA. Some are directly implicated in the pathogenesis including inherited conditions of the red blood cell such as sickle cell disease (HbSS)[59] and G6PD deficiency[60] while others may alter risk more indirectly. Examples of the latter include sickle cell trait (HbAS) and α-thalassaemia, which protect against malaria and thus protect against the evolution of SA and genetic conditions that affect susceptibility to bacterial or viral infections. We anticipate that the contribution of human genetic factors to the risk of SA will be significant. Although it is likely that the birth prevalence of HbSS will be relatively low in most of the populations involved in this study (1-3%) SA is a common presenting symptom of HbSS and children with HbSS will therefore be overrepresented among children presenting with SA [52]. Conversely, in all the populations involved in this study the prevalence of HbAS and α-thalassaemia are likely to exceed 15% and 60% respectively and both are likely to be associated with significant protection.

As described in section 9.2 above, we will extract a sample of DNA from all case patients at the conclusion of the study to investigate the contribution of bacterial and viral infections to the aetiology of SA using molecular methods. We will use aliquots of DNA from this same archive to describe the distribution of HbSS, G6PD deficiency and α-thalassaemia among case patients using PCR. Patients found to be positive for HbSS will be recalled for counselling and for confirmatory testing and if confirmed to be suffering from HbSS they will be encouraged to attend the outpatient clinic for regular treatment.

The DNA samples collected through this study will also be of potential future value for wider studies aimed at investigating the genetic basis for SA. Although such studies are not the focus of the current proposal we would like to retain this archive for such future potential studies that would be the subject of a separate poposal. The possible use of archived samples for future genetic studies, some of which may require transport of samples outside the country, will be explained in the patient information sheet and parents will be asked for specific consent for such potential future use of their children's samples in the consent sheet.

9.4 MARKERS OF NUTRITIONAL WELLBEING

9.4.1 ESTIMATION OF NUTRITIONAL INTAKE

Nutritional intake is fundermentally important to the health of the child and there is a intimate relationship between nutritional intake, nutritional status and infection. Poor nutritional intake is also linked to anaemia, poor gut barrier function and immunity. Nutritional intake can also be used as a surrogate marker of well being. The measurement of nutritional intake is a balance between the complexity of user involvement and engagement of the target group.

Aims:

1. To use a 24-hour multi-pass recall to assess the nutritional intake of children at each assessment point
2. To estimate macro and micro nutritent intake using local food composition tables[61, 62]
3. To use the nutritional intake to inform gut barrier function and immunity studies and to assess its potential use as a variable to inform the treatment of anaemia

Methodology:

All children/parents will be interviewed prior to discharge and at 28, 90 and 180 days for a brief dietary recall. This will be included in the patient information sheet.

Dietary methodology will be based on recent work conducted in Malawi, validating a 24 hour [62]). Data will be collected by the nurse at the same time as collecting other data for the study. The multipass 24-hour recall follows the following algorithm. If relevent, the parent is asked if the child is exclusively breast

feed (if they are the recall is stopped). If not, the parent is asked way is the first thing the child had to eat or drink that day. This is followed by a series of questions what did the child have to eat or drink next until bed time.

1. The list is then read back to the parent to allow for corrections.
2. The nurse then asks to quality the amounts the child has eaten or drank of each food. This can be done with the aid of pictures or household utensils (including different (standarsised) spoon sizes or percentages of a bowl (with a fixed volume~ 200ml).
3. The list is then is read back for any corrections to be made and the recall completed

The inteview should take about 15-20 minutes depending on the complexity of the diet.

Analysis
The record chart will then be analysied using local food tables to calculate 24-hour macro and micro nutritent intake at each study visit. This will be retrospectively analysed by intervention arm stratified by age, clinical and infection status, and markers of nutritional recovery.

9.5 GUT BARRIER FUNCTION

Malaria infection strongly predisposes African children (5-12%) to invasive bacterial disease (IBD) with particularly high mortality in this group (approximately 30%). The leading causes of IBD in *P. falciparum* infection are non-typhoidal salmonellae (NTS), *E. coli* and other enteric organisms with gram-negative organisms becoming increasingly more predominant across the severity spectrum. Endotoxin is a complex lipopolysaccharide (LPS) present in the cell walls of gram-negative bacteria and in substantial quantities in the bowel. We have recently shown that endotoxemia was observed in 28.5% children hospitalized with malaria, most commonly associated with the clinical presentation of severe anaemia and suggested that this may be due to disordered gut barrier function. We also observed that endotoxemia was associated with a depressed inflammatory and anti-inflammatory cytokine response similar to that observed in sepsis. Thus, markers of gut barrier dysfunction may identify children most at risk of invasive bacterial disease, who would benefit from antimicrobial treatment (short and longer term) rather than prophylaxis as evaluated in TRACT.

Aims
1. We aim to determine the extent of gut barrier dysfunction in children with severe anaemia (with and without concurrent malaria) using known markers of gut barrier integrity such as plasma, urine or faeces concentrations of endotoxin, endocap-antibodies, intestinal fatty acid binding protein, intestinal bile binding protein, claudin-3 as well as calprotectin and its relationship with gut hormones.
2. Identify novel biomarkers of gut barrier dysfunction by investigating the metabolome and proteome of plasma and urine in children with severe anaemia with or without endotoxemia on admission.
3. Assess whether there is any evidence that this is modified overtime by the cotrimoxazole prophylaxis (randomised comparison).

Design
Up to 300 children will be recruited, following additional consent (Appendix II) to determine endotoxin levels (EAA, Spectral Diagnostics) immediately and on stored plasma samples, and to assay urine and faeces markers of gut barrier dysfunction at admission, day 28 and day 180. Laboratory data will be interpreted together with the clinical outcome and at the end of the trial by randomised arm. We will use a Millipore human gut hormone panel kit which will allow us to measure Ghrelin, Leptin, GIP, GLP-1, Amylin (active or total), PP, PYY, and Insulin using 25ul of plasma and relate these to other measure of immune activation and gut barrier dysfunction.

9.6 MARKERS OF IMMUNE ACTIVATION/FUNCTION

As above, we observed that 28.5% of children with malaria presented with endotoxaemia most likely due to increased gut barrier dysfunction. Similar to increased susceptibility to NTS in children with malaria, children with endotoxaemia where more likely to present with anaemia. We observed that endotoxaemia in addition to malaria was associated with reduced plasma concentration of pro- and anti-inflammatory cytokines, suggesting that this subgroup of children with malaria is immunologically impaired. Studies in rodent models of malaria demonstrated that malarial was associated with heme oxygenase I induction but impaired oxidative burst in a subset of neutrophils which impaired killing of intra-cellular *Salmonella typhimurium*. By contrast, a recent study in Gambian children showed an increase in heme oxygenease expression due to a promoter polymorphism in children with severe malaria including severe malarial anaemia, however the effect of the polymorphism on increased risk of bacterial co-infection was not investigated. TRACT provides a unique opportunity to analyse the function of innate immune cells in children with severe anaemia (with and without concurrent malaria) at admission and after recovery with respect to cytokine production, oxidative burst and bactericidal activity. In addition, we will be able to establish whether inflammation is causally linked to endotoxaemia (or other markers of gut permeability) and/or increased risk of bacterial co-infection.

Aims
1. Determine neutrophil and monocyte function in children with severe anaemia (with and without concurrent malaria) at admission and after recovery.
2. Determine whether there is an association of neutrophil and monocyte function with endotoxaemia, other markers of gut permeability, or general inflammation and immune activation.
3. Determine whether any aspect of immune function is modified over time by the cotrimoxazole randomisation.

Design:
Neutrophil function and monocyte function using whole blood assays and flow cytometry will be determined in 300 children recruited for the analysis of gut barrier function at admission, day 28 and day 180. Laboratory data will be interpreted together with the clinical outcome, molecular diagnostics (9.2), human genetics (9.3), gut barrier function (9.4) and eventually by randomised arm.

9.7 ANTIMICROBIAL RESISTANCE

S. pneumoniae and Non-typhoidal Salmonella (NTS) have been the commonest bacterial isolates from febrile children under five years of age in hospital-based community-aquired bactermia studies conducted throughout the region. Invasive NTS is a prominent pathogen in children with malaria-associated anaemia. All bacterial isolates from blood cultures (or cerebrospinal fluid) at initial recrutiment and during clinical episodes of sepsis on follow-up will be stored. Antimicrobial resistance profiles and molecular markers of resistance will be compared by cotrimoxazole randomisation at the end of the study

10 QUALITY ASSURANCE AND CONTROL

10.1 RISK ASSESSMENT

The Quality Assurance (QA) and Quality Control (QC) considerations have been based on a formal Risk Assessment, which acknowledges the risks associated with the conduct of the trial and how to address them with QA and QC processes. QA includes all the planned and systematic actions established to ensure the trial is performed and data generated, documented and/or recorded and reported in compliance with the principles of ICH GCP and applicable regulatory requirements. QC includes the operational techniques and activities done within the QA system to verify that the requirements for quality of the trial-related activities are fulfilled. This Risk Assessment has led to the development of a Quality Management Plan (QMP), which will be kept separately.

Safety profiles of blood, cotrimoxazole and MVMM used in the trial are well known and we will look for the side effects of each carefully. Severe adverse events (as defined by Good Clinical Practice guidelines) are secondary outcomes of the trial. The most current Summary of Product Characteristics/ Investigators Brochure will be available at each trial site and any updates will be circulated to sites. MRC CTU compliant SAE data collection and reporting procedures will be adopted, as worked well in FEAST. Clinical staff at sites will be trained by the CI at the initiation visit to recognise expected side effects. However as multi-vitamin multi-mineral supplements and cotrimoxazole are widely used in children the risks of harm are known and are extremely low.

There are also expected inherent hazards of the transfusion intervention – particularly infections in/failure to correctly cross-match transfused blood. These will be minimised by the TRACT team working closely with the national and local blood transfusion services to ensure recommended safety and quality control practices are being achieved and working closely with the blood safety committees at each site.

The trial will be recruiting patients with severe and complicated anaemia with a high mortality. At the start of the trial all sites will receive emergency care training, including triage of those at highest risk. All patient swill be closely monitored so that clinical deteriorations can be identified at the earliest opportunity and appropriate therapy initiated. In general the trial sites in Africa have considerable experience with this population and this will serve minimise the risks to the patients and the trial. A detailed risk assessment will be conducted prior to starting the trial.

10.2 MONITORING AT STUDY COORDINATION CENTRE

Each site will be responsible for its own data entry and local trial management. Data will be entered into the web-enabled trial database directly at the site. The site will retain the original CRF. Data stored on the central database will be checked for missing or unusual values (range checks) and checked for consistency within participants over time. If any such problems are identified, the site will be contacted and asked to verify or correct the entry. Changes will be made on the original CRF and entered into the database at the site. KCTF will also send reminders for any overdue and/or missing data with the regular inconsistency reports of errors.

10.3 MONITORING AT LOCAL SITE

This trial will be monitored according to a Monitoring and Quality Management Plan which will set out the frequency of visits, the degree of source document verification against the case record forms and the requirements for triggered on-site monitoring visits. This plan will also detail the procedures for review and sign-off. The monitoring will adhere to the principles of ICH GCP. It is anticipated that the monitoring will start with 100% source document verification as in the FEAST trial. This will be reviewed for each site once a satisfactory and sustained performance in quality assurance is established.

A detailed site initiation visit with training will be performed at each study site by staff from the KWTP CTF who will be specifically trained for this role. The site initiation visits will include training in the trial procedures, as well as practical training in administration of transfusions and other trial interventions, reporting guidelines for adverse events of study interventions as well as other trial procedures. All staff at sites involved in the trial will be receive formal training in GCP –through a dedicated training programme during site initiation visit, and will also be required to complete an on-line course.

The trial monitoring team will consist of the KWTP-Clinical Trial Facility monitoring team and the MRC CTU who will provide over-site and some visits to maintain the integrity of the monitoring. The Clinical Trial Facility in Kilifi oversees standards and quality of all trials conducted through the KWTP and through its monitoring systems and SOPs is organised to ensure that all sites can be monitored with equal independence and rigor. All monitors will be appropriately qualified and trained.

At each monitoring visit monitors will:

- verify completeness of Trial Master File
- confirm adherence to protocol
- review eligibility verification and consent procedures
- look for missed clinical event reporting
- verify completeness, consistency and accuracy of data being entered on CRFs
- evaluate drug accountability
- provide additional training as needed

The monitors will require access to all patient medical records including, but not limited to, laboratory test results and prescriptions. The investigator (or delegated deputy) should work with the monitor to ensure that any problems detected are resolved.

11 REGULATORY ISSUES

11.1 TRIAL REGISTRATION

This trial has been registered with the International Standard Randomized Clinical Trial Number Register, where it is identified as ISRCTN84086586..

11.2 ETHICS APPROVAL

The Study Coordination Centre has obtained approval from the Imperial College Research Ethics Committee. This trial will be submitted for approval by Research Ethics Committees/Institutional Review Boards in Malawi and Uganda and by all required regulatory authorities in participating countries.

The study will be conducted in accordance with the recommendations for physicians involved in research on human subjects adopted by the 18th World Medical Assembly, Helsinki 1964 and later revisions, the principles of Good Clinical Practice (GCP) as laid down by the ICH topic E6 (Note for Guidance on GCP) and applicable national regulations.

11.3 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 TRACT 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. (See **Appendix I** for Patient Information sheet and consent form). The right of the participant to refuse to participate without giving reasons must be respected.

11.3.1 EMERGENCY VERBAL ASSENT FOLLOWED BY DEFERRED CONSENT

A number of children will present as emergencies where delay in study enrolment, and thus treatment, through a consent procedure would be unacceptable. For the FEAST trial we developed and received ethical approval to use a two stage consent process in this circumstance[63]. 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 treatment allocation, 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 later sign the verbal assent form which will be filed with the consent form. If consent is withdrawn later no data from the subject will be used (Appendix I Template assent form).

11.4 WITHDRAWAL OF PATIENTS AND PROTOCOL TREATMENT DISCONTINUATION

In consenting to the trial, patients are consenting to trial treatment, 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. If a patient chooses to discontinue any part of their trial treatment, they should always be followed up (providing they are willing) and they should be encouraged to not leave the whole trial. 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 from the protocol treatment without giving reasons and without prejudicing further treatment (Appendix III for withdrawal form). 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.

Withdrawal from the transfusion intervention or control arms is unlikely, given that most transfusions are given within the first 12 hours of admission. Severe allergic reaction (toxicity) or TRALI is included as a secondary endpoint and is relevant only to children receiving transfusion. It will not be a reason to withdraw the child from the trial, but further transfusions should be withheld. The child should continue with their cotrimoxazole/MVMM allocation wherever possible.

11.5 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

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 to deffered 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 the policies of the institution where the samples reside at the time (local or central storage).

Patients who stop trial follow-up early will not be replaced, as the total sample size includes adjustment for losses to follow-up.

11.6 CONFIDENTIALITY

Participants' identification data will be required for the registration process. The Study Coordination Centre will preserve the confidentiality of participants taking part in the study, which will comply with requirements for data protection in the countries where the research is being conducted and is registered under the Data Protection Act.

11.7 INDEMNITY

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

11.8 SPONSOR

Imperial College London will act as the main Sponsor for this study and delegates this responsibility to the KWTP, Kilifi and MRC CTU 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.

11.9 FUNDING

The trial is supported by grant funding from Medical Research Council (MRC UK) and of the Department for International Development, UK (DFID) through a concordat with MRC UK. The trial will be coordinated by Imperial College. A written agreement with the site principal investigator and/or the investigator's institution and Imperial College will outline the funding arrangements to sites. The TSC will meet and review the financial aspects of the trial at least 12-monthly and report to the sponsor. Terms of reference will be developed for this activity.

11.10 AUDITS AND INSPECTIONS

The study may be subject to inspection and audit by Imperial College London under their remit as Sponsor and other regulatory bodies to ensure adherence to GCP.

12 TRIAL MANAGEMENT

12.1 SITE TRIAL MANAGEMENT TEAMS

A trial management team will be formed at each site to conduct the day-to-day management of the trial at the site ("Site TMT"). This will include the investigators and trial staff at the site. These groups will meet every one to two weeks and will be chaired by the principal investigator or co-principal investigator at the site. The group will discuss issues related to the progress of the trial at the site, and to ensure that the trial is running well.

There will be a similar trial management team formed to conduct the day-to-day management of the trial at the MRC ("MRC TMT"). This will include the site principal investigator, trial statistician, clinical project manager, trial manager and data manager. The group will meet at least once per month, although may meet more often if required.

12.2 TRIAL MANAGEMENT GROUP

A TRACT Trial Management Group (TMG) will be formed comprising the Chief Investigator; centre Principal Investigators, co-investigators, and Trial Managers, other lead investigators (clinical and non-clinical), members of the MRC Clinical Trials Unit (CTU) and will be responsible for overseeing the progress of the trial. The day-to-day management of the trial will be coordinated through the Kilifi Study Coordination Centre. The TMG will meet approximately once a year in-person and will hold a regular teleconference at approximately monthly intervals at which sites will summarise progress and challenges and bring up for discussion any difficulties, as well as discuss and decide matters of general importance for the trial. This group will be chaired by the Chief Investigator and all decisions regarding the overall running of the trial will be made in this forum with the exception of matters of fundamental importance to the viability of the trial or that require major changes to the protocol. These will be referred to the Trial Steering Committee (TSC). The full details can be found in the TMG Charter.

12.3 TRIAL STEERING COMMITTEE

The trial will be managed by a Trial Steering Committee (TSC) with an independent chairperson (Professor Elizabeth Molyneux OBE), a majority of independent members and one Principal Investigator or key investigator from each of the sites, from Imperial College, and from MRC CTU. Prof Molyneux previously chaired the FEAST TSC.

Each centre would either use their existing Community Advisory Board (CAB) or form a specific patient liaison group who would be responsible for liaising with their independent representatives on the TSC, would feedback concerns and questions from the community, and also hear about the latest developments in the trial and the wider scientific community.

12.4 INDEPENDENT DATA MONITORING COMMITTEE

An independent Data Monitoring Committee (DMC) will be set up to review data on enrolment, safety, adherence to randomised strategies, efficacy and safety at regular intervals and in strict confidence. The Chairman will be Professor Tim Peto. The DMC will report to the TSC and to the Ethics Committee in each country, if, in their view, the data provide proof beyond reasonable doubt that one of the allocated strategies is better than its comparator in terms of the primary outcome. The TSC will then decide whether

to amend (which may include removing one of the intervention arms) or stop the trial before the end of the planned follow-up. If a decision is made to continue, the DMC will advise on the frequency of future reviews of the data on the basis of accrual and event rates. The DMC will make recommendations to the TRACT Trial Steering Committee as to the continuation of the trial.

12.5 ENDPOINT REVIEW COMMITTEE

An Endpoint Review Committee (ERC) will review clinical data (blinded to allocation) and will determine the validity of potential endpoints. The ERC will adjudicate endpoints blinded to randomised allocations: relationship to all possible interventions drugs (transfusion strategy; nutritional support and cotrimoxazole) will be solicited to avoid unblinding. It will have an independent Chair (Dr Jennifer Evans) and will the include Project Leaders from each site as well as other independent clinicians. No member will review endpoints from their own site. Terms of reference for the Endpoint Review Committee will be drawn up.

13 PUBLICATION POLICY

All publications and presentations relating to the study will be authorised by the Trial Management Group. The first publication of the trial results will be in the name of the Trial Management Group, if this does not conflict with the journal's policy. If there are named authors, these will include at least the trial's Chief Investigator, Statistician and Trial Coordinator. Members of the TMG and the Data Monitoring Committee will be listed and contributors will be cited by name if published in a journal where this does not conflict with the journal's policy. Authorship of parallel studies initiated outside of the Trial Management Group will be according to the individuals involved in the project but must acknowledge the contribution of the Trial Management Group and the Study Coordination Centre.

The TRACT TSC is the custodian of the data and specimens generated from the TRACT trial; TRACT trial data are not the property of individual participating investigators or health care facilities where the data were generated.

During the course and following completion of the trial there will be publications, including manuscripts and abstracts for presentation at national and international meetings, as well as the preparation of manuscripts for peer-reviewed publication. In order to avoid disputes regarding authorship, it is important to establish a consensus approach that will provide a framework for all publications derived in full or in part from this clinical trial. The following approach is derived from the Lancet and from the publication policies used in other MRC clinical trials:

- All publications are to be approved by the TMG and TSC before submission for publication. Any publication arising before the end of the trial (not by randomised groups) will also be approved by the DMC in order to ensure that the primary objective of the trial (the randomised comparison) is not compromised. In particular, no analyses by randomised group of any outcome (primary, secondary or other) in either the main trial or associated substudies will be conducted or presented before the end of the trial, other than those for interim review by the DMC. The TMG and TSC will resolve problems of authorship and maintain the quality of publications.

- In line with MRC policy that the results of publicly-funded research should be freely available, manuscripts arising from the trial will, wherever possible, be submitted to peer-reviewed journals which enable Open Access via UK PubMed Central (PMC) within six months of the official date of final publication. All conference presentations will be made available as soon as possible after the event via the TRACT website. All publications will acknowledge the trial's funding sources.

- For all publications, the TMG will nominate a chairperson or approve an individual's request to chair a manuscript writing committee. The chair will usually be the primary or senior author. The chairperson is responsible for identifying fellow authors and for determining with that group the order of authorship that will appear on the manuscript. The TSC will resolve any problems of authorship and maintain the quality of publications.

- The TMG will maintain a list of investigators to be presented in an appendix at the end of the paper. This list will include investigators who contributed to the investigation being reported but who are not members of the writing committee. In principle, substudy reports should include all investigators for the main study, although in some instances where a smaller number of investigators have made any form of contribution, it may be appropriate to abbreviate the listing.

- All headline authors in any publication arising from the main study or sub-studies must have a made a significant academic or project management contribution to the work that is being presented. "Significant" must be defined by a written declaration of exactly what the contribution of any

individual is believed to have been. In addition to fulfilling the criteria based on contribution, additional features that will be considered in selecting an authorship group will include the recruitment of patients who contributed data to any set of analyses contained in the manuscript, and /or the conduct of analyses (laboratory and statistical), leadership and coordination of the project in the absence of a clear academic contribution.

- The data derived from this clinical trial are considered the property of the TRACT Trial Steering Committee. The presentation or publication of any data collected by the participating investigators on patients entered into this trial is under the direct control of the TMG and TSC (and the DMC before the end of the trial). This is true whether the publication or presentation is concerned directly with the results of the trial or is associated with the trial in some other way. However, although individual participating investigators will not have any inherent right to perform analyses or interpretations or to make public presentations or seek publication of any of the data other than under the auspices of and with the approval of the TMG and TSC (and the IDMC before the end of the trial), they will be encouraged to develop sub-studies or propose analyses subject to the approval by the TMG and TSC (and the DMC before the end of the trial). Any requests for access to raw data will be welcomed as long as they are scientifically valid and do not conflict with the integrity of the trial or ongoing analyses by the trial team

- Outcome data by randomised group will not be revealed to the participating investigators until the data collection phase and primary full analysis of the trial has been completed. This policy safeguards against possible bias affecting the data collection. The DMC will be monitoring the outcome results and may recommend that the trial be stopped for safety reasons or if a definitive answer is reached earlier than the scheduled end of the trial.

14 REFERENCES

1. de Benoist B, McLean E, Egli I, Cogswell M: **Worldwide prevalence of anaemia 1993–2005 : WHO global database on anaemia** *WHO report* 2008.
2. Rowe AK, Rowe SY, Snow RW, Korenromp EL, Schellenberg JR, Stein C, Nahlen BL, Bryce J, Black RE, Steketee RW: **The burden of malaria mortality among African children in the year 2000**. *Int J Epidemiol* 2006, **35**(3):691-704.
3. **Hospital Care for Children: guidelines for the management of common illnesses with limited resources.** In. Geneva, Switzerland: World Health Organization; 2005.
4. Brabin BJ, Premji Z, Verhoeff F: **An analysis of anemia and child mortality**. *J Nutr* 2001, **131**(2S-2):636S-645S; discussion 646S-648S.
5. Phiri KS, Calis JC, Faragher B, Nkhoma E, Ng'oma K, Mangochi B, Molyneux ME, van Hensbroek MB: **Long term outcome of severe anaemia in Malawian children**. *PloS one* 2008, **3**(8):e2903.
6. Calis JC, Phiri KS, Faragher EB, Brabin BJ, Bates I, Cuevas LE, de Haan RJ, Phiri AI, Malange P, Khoka M *et al*: **Severe anemia in Malawian children**. *The New England journal of medicine* 2008, **358**(9):888-899.
7. Meremikwu M, Smith HJ: **Blood transfusion for treating malarial anaemia**. *Cochrane database of systematic reviews (Online)* 2000(2):CD001475.
8. Hassell O, Bates I, Maitland K: **Blood Transfusion in Resource-Limited Settings**. In: *Hunter's Tropical Medicine* 9th edn. Edited by Claney N. London: Magill – Maguire – Ryan - Solomon; 2012.
9. **Utilization and supply of human blood and blood products in Twenty-eighth World Health Assembly**. In. Geneva: World Health Organization (WHO). 1975.
10. Berkley JA, Lowe BS, Mwangi I, Williams TN, Bauni E, Mwarumba S, Ngetsa C, Hart A, Maitland K, English M *et al*: **Bacteremia amongst Children Admitted to a Rural Hospital in Kenya.** *N Engl J Med* 2005, **352**(1):39-47.
11. English M, Ahmed M, Ngando C, Berkley J, Ross A: **Blood transfusion for severe anaemia in children in a Kenyan hospital**. *Lancet* 2002, **359**(9305):494-495.
12. Lackritz EM, Campbell CC, Ruebush TK, 2nd, Hightower AW, Wakube W, Steketee RW, Were JB: **Effect of blood transfusion on survival among children in a Kenyan hospital**. *Lancet* 1992, **340**(8818):524-528.
13. Bojang KA, Van Hensbroek MB, Palmer A, Banya WA, Jaffar S, Greenwood BM: **Predictors of mortality in Gambian children with severe malaria anaemia**. *Ann Trop Paediatr* 1997, **17**(4):355-359.
14. Ernest SK, Anunobi NE, Adeniyi A: **Correlates of emergency response interval and mortality from severe anaemia in childhood**. *West Afr J Med* 2002, **21**(3):177-179.
15. Maitland K, Kiguli S, Opoka RO, Engoru C, Olupot-Olupot P, Akech SO, Nyeko R, Mtove G, Reyburn H, Lang T *et al*: **Mortality after fluid bolus in African children with severe infection**. *The New England journal of medicine* 2011, **364**(26):2483-2495.
16. Marsh K, Forster D, Waruiru C, Mwangi I, Winstanley M, Marsh V, Newton C, Winstanley P, Warn P, Peshu N *et al*: **Indicators of life-threatening malaria in African children**. *The New England journal of medicine* 1995, **332**(21):1399-1404.
17. Pedro R, Akech S, Maitland K: **Changing trends in blood transfusion in children and neonates admitted in Kilifi District Hospital, Kenya**. *Malar J* 2010, **9**(1):307.
18. Holzer BR, Egger M, Teuscher T, Koch S, Mboya DM, Smith GD: **Childhood anemia in Africa: to transfuse or not transfuse?** *Acta Trop* 1993, **55**(1-2):47-51.
19. Bojang KA, Palmer A, Boele van Hensbroek M, Banya WA, Greenwood BM: **Management of severe malarial anaemia in Gambian children**. *Trans R Soc Trop Med Hyg* 1997, **91**(5):557-561.

20. Lacroix J, Hebert PC, Hutchison JS, Hume HA, Tucci M, Ducruet T, Gauvin F, Collet JP, Toledano BJ, Robillard P *et al*: **Transfusion strategies for patients in pediatric intensive care units**. *The New England journal of medicine* 2007, **356**(16):1609-1619.
21. Walker RH: **Mathematical calculations in transfusion medicine**. *Clinics in laboratory medicine* 1996, **16**(4):895-906.
22. Cheema B, Molyneux EM, Emmanuel JC, M'Baya B, Esan M, Kamwendo H, Kalilani-Phiri L, Boele van Hensbroek M: **Development and evaluation of a new paediatric blood transfusion protocol for Africa**. *Transfus Med* 2010, **20**(3):140-151.
23. Esan MO, Phiri KS, Molyneux EM, Mukaka M, Cheema B, Boele van Hensbroek M: **High transfusion failure rates in Malawian children with severe anaemia following a standard blood transfusion regimen**. *Br J Haematol* 2011.
24. CDC: **Progress toward strengthening blood transfusion services--14 countries, 2003-2007**. *Mmwr* 2008, **57**(47):1273-1277.
25. O'Meara WP, Bejon P, Mwangi TW, Okiro EA, Peshu N, Snow RW, Newton CR, Marsh K: **Effect of a fall in malaria transmission on morbidity and mortality in Kilifi, Kenya**. *Lancet* 2008, **372**(9649):1555-1562.
26. Okiro EA, Bitira D, Mbabazi G, Mpimbaza A, Alegana VA, Talisuna AO, Snow RW: **Increasing malaria hospital admissions in Uganda between 1999 and 2009**. *BMC medicine* 2011, **9**:37.
27. Akech SO, Hassall O, Pamba A, Idro R, Williams TN, Newton CR, Maitland K: **Survival and haematological recovery of children with severe malaria transfused in accordance to WHO guidelines in Kilifi, Kenya**. *Malar J* 2008, **7**:256.
28. Abdalla SH: **Iron and folate status in Gambian children with malaria**. *Ann Trop Paediatr* 1990, **10**(3):265-272.
29. van Hensbroek MB, Morris-Jones S, Meisner S, Jaffar S, Bayo L, Dackour R, Phillips C, Greenwood BM: **Iron, but not folic acid, combined with effective antimalarial therapy promotes haematological recovery in African children after acute falciparum malaria**. *Trans R Soc Trop Med Hyg* 1995, **89**(6):672-676.
30. van den Broek NR, Letsky EA: **Etiology of anemia in pregnancy in south Malawi**. *The American journal of clinical nutrition* 2000, **72**(1 Suppl):247S-256S.
31. Savage DG, Allen RH, Gangaidzo IT, Levy LM, Gwanzura C, Moyo A, Mudenge B, Kiire C, Mukiibi J, Stabler SP *et al*: **Pancytopenia in Zimbabwe**. *The American journal of the medical sciences* 1999, **317**(1):22-32.
32. Sazawal S, Black RE, Ramsan M, Chwaya HM, Stoltzfus RJ, Dutta A, Dhingra U, Kabole I, Deb S, Othman MK *et al*: **Effects of routine prophylactic supplementation with iron and folic acid on admission to hospital and mortality in preschool children in a high malaria transmission setting: community-based, randomised, placebo-controlled trial**. *Lancet* 2006, **367**(9505):133-143.
33. Zimmermann MB, Chassard C, Rohner F, N'Goran E K, Nindjin C, Dostal A, Utzinger J, Ghattas H, Lacroix C, Hurrell RF: **The effects of iron fortification on the gut microbiota in African children: a randomized controlled trial in Cote d'Ivoire**. *The American journal of clinical nutrition* 2010, **92**(6):1406-1415.
34. Naikare H, Palyada K, Panciera R, Marlow D, Stintzi A: **Major role for FeoB in Campylobacter jejuni ferrous iron acquisition, gut colonization, and intracellular survival**. *Infection and immunity* 2006, **74**(10):5433-5444.
35. Galloway R, McGuire J: **Determinants of compliance with iron supplementation: supplies, side effects, or psychology?** *Soc Sci Med* 1994, **39**(3):381-390.
36. Schauer C, Zlotkin S: **Home fortification with micronutrient sprinkles - A new approach for the prevention and treatment of nutritional anemias**. *Paediatrics & child health* 2003, **8**(2):87-90.
37. Zlotkin S, Arthur P, Antwi KY, Yeung G: **Treatment of anemia with microencapsulated ferrous fumarate plus ascorbic acid supplied as sprinkles to complementary (weaning) foods**. *The American journal of clinical nutrition* 2001, **74**(6):791-795.
38. Zlotkin S, Arthur P, Schauer C, Antwi KY, Yeung G, Piekarz A: **Home-fortification with iron and zinc sprinkles or iron sprinkles alone successfully treats anemia in infants and young children**. *J Nutr* 2003, **133**(4):1075-1080.

39. Suchdev PS, Ruth LJ, Woodruff BA, Mbakaya C, Mandava U, Flores-Ayala R, Jefferds ME, Quick R: **Selling Sprinkles micronutrient powder reduces anemia, iron deficiency, and vitamin A deficiency in young children in Western Kenya: a cluster-randomized controlled trial**. *The American journal of clinical nutrition* 2012, **95**(5):1223-1230.
40. Jefferds ME, Ogange L, Owuor M, Cruz K, Person B, Obure A, Suchdev PS, Ruth LJ: **Formative research exploring acceptability, utilization, and promotion in order to develop a micronutrient powder (Sprinkles) intervention among Luo families in western Kenya**. *Food and nutrition bulletin* 2010, **31**(2 Suppl):S179-185.
41. Suchdev PS: **Multiple micronutrient powders for home (point of use) fortification of foods in pregnant women: a systematic review.** In: *WHO e-Library of Evidence for Nutrition Actions.* World Health Organization; 2011.
42. McDonald PJ, Pruul H: **Phagocyte uptake and transport of azithromycin**. *Eur J Clin Microbiol Infect Dis* 1991, **10**(10):828-833.
43. Lode H: **The pharmacokinetics of azithromycin and their clinical significance**. *Eur J Clin Microbiol Infect Dis* 1991, **10**(10):807-812.
44. Chintu C, Bhat GJ, Walker AS, Mulenga V, Sinyinza F, Lishimpi K, Farrelly L, Kaganson N, Zumla A, Gillespie SH *et al*: **Co-trimoxazole as prophylaxis against opportunistic infections in HIV-infected Zambian children (CHAP): a double-blind randomised placebo-controlled trial**. *Lancet* 2004, **364**(9448):1865-1871.
45. Mulenga V, Ford D, Walker AS, Mwenya D, Mwansa J, Sinyinza F, Lishimpi K, Nunn A, Gillespie S, Zumla A *et al*: **Effect of cotrimoxazole on causes of death, hospital admissions and antibiotic use in HIV-infected children**. *Aids* 2007, **21**(1):77-84.
46. Walker AS, Ford D, Gilks CF, Munderi P, Ssali F, Reid A, Katabira E, Grosskurth H, Mugyenyi P, Hakim J *et al*: **Daily co-trimoxazole prophylaxis in severely immunosuppressed HIV-infected adults in Africa started on combination antiretroviral therapy: an observational analysis of the DART cohort**. *Lancet* 2010, **375**(9722):1278-1286.
47. Mwenya DM, Charalambous BM, Phillips PP, Mwansa JC, Batt SL, Nunn AJ, Walker S, Gibb DM, Gillespie SH: **Impact of cotrimoxazole on carriage and antibiotic resistance of Streptococcus pneumoniae and Haemophilus influenzae in HIV-infected children in Zambia**. *Antimicrobial agents and chemotherapy* 2010, **54**(9):3756-3762.
48. Thera MA, Sehdev PS, Coulibaly D, Traore K, Garba MN, Cissoko Y, Kone A, Guindo A, Dicko A, Beavogui AH *et al*: **Impact of trimethoprim-sulfamethoxazole prophylaxis on falciparum malaria infection and disease**. *The Journal of infectious diseases* 2005, **192**(10):1823-1829.
49. Arinaitwe E, Gasasira A, Verret W, Homsy J, Wanzira H, Kakuru A, Sandison TG, Young S, Tappero JW, Kamya MR *et al*: **The association between malnutrition and the incidence of malaria among young HIV-infected and -uninfected Ugandan children: a prospective study**. *Malar J* 2012, **11**:90.
50. Wilson AL: **A systematic review and meta-analysis of the efficacy and safety of intermittent preventive treatment of malaria in children (IPTc)**. *PloS one* 2011, **6**(2):e16976.
51. Porco TC, Gebre T, Ayele B, House J, Keenan J, Zhou Z, Hong KC, Stoller N, Ray KJ, Emerson P *et al*: **Effect of mass distribution of azithromycin for trachoma control on overall mortality in Ethiopian children: a randomized trial**. *JAMA* 2009, **302**(9):962-968.
52. McAuley CF, Webb C, Makani J, Macharia A, Uyoga S, Opi DH, Ndila C, Ngatia A, Scott JA, Marsh K *et al*: **High mortality from Plasmodium falciparum malaria in children living with sickle cell anemia on the coast of Kenya**. *Blood* 2010, **116**(10):1663-1668.
53. Harris KA, Hartley JC: **Development of broad-range 16S rDNA PCR for use in the routine diagnostic clinical microbiology service**. *J Med Microbiol* 2003, **52**(Pt 8):685-691.
54. Millar BC, Xu J, Moore JE: **Risk assessment models and contamination management: implications for broad-range ribosomal DNA PCR as a diagnostic tool in medical bacteriology**. *J Clin Microbiol* 2002, **40**(5):1575-1580.
55. Ferri E, Novati S, Casiraghi M, Sambri V, Genco F, Gulminetti R, Bandi C: **Plasma levels of bacterial DNA in HIV infection: the limits of quantitative polymerase chain reaction**. *The Journal of infectious diseases* 2010, **202**(1):176-177; author reply 178.

56. Marchetti G, Bellistri GM, Borghi E, Tincati C, Ferramosca S, La Francesca M, Morace G, Gori A, Monforte AD: **Microbial translocation is associated with sustained failure in CD4+ T-cell reconstitution in HIV-infected patients on long-term highly active antiretroviral therapy**. *AIDS* 2008, **22**(15):2035-2038.
57. Merlini E, Bai F, Bellistri GM, Tincati C, d'Arminio Monforte A, Marchetti G: **Evidence for Polymicrobic Flora Translocating in Peripheral Blood of HIV-Infected Patients with Poor Immune Response to Antiretroviral Therapy**. *PloS one* 2011, **6**(4):e18580.
58. Suau A, Bonnet R, Sutren M, Godon JJ, Gibson GR, Collins MD, Dore J: **Direct analysis of genes encoding 16S rRNA from complex communities reveals many novel molecular species within the human gut**. *Appl Environ Microbiol* 1999, **65**(11):4799-4807.
59. Rees DC, Williams TN, Gladwin MT: **Sickle-cell disease**. *Lancet* 2010, **376**(9757):2018-2031.
60. Beutler E: **G6PD deficiency**. *Blood* 1994, **84**(11):3613-3636.
61. Hotz C, Abdelrahman L, Sison C, Moursi M, Loechl C: **A Food Composition Table for Central and Eastern Uganda**. In: *HarvestPlus Technical Monographs.* Washington, USA: International Food Policy Research Institute (IFPRI) and International Center for Tropical Agriculture (CIAT). 2012.
62. Thakwalakwa CM, Kuusipalo HM, Maleta KM, Phuka JC, Ashorn P, Cheung YB: **The validity of a structured interactive 24-hour recall in estimating energy and nutrient intakes in 15-month-old rural Malawian children**. *Maternal & child nutrition* 2012, **8**(3):380-389.
63. Maitland K, Molyneux S, Boga M, Kiguli S, Lang T: **Use of deferred consent for severely ill children in a multi-centre phase III trial**. *Trials* 2011, **12**:90.

15 APPENDIX I TEMPLATE PATIENT INFORMATION SHEETS ,CONSENT AND ASSENT FORM

[Each country to use its own translated Informed Consent for relevant local languages according to local regulatory requirements, on local headed paper for each site]

TRACT (Transfusion and Treatment of Severe Anaemia in African Children)
Information for Parents and Carers

Introduction

We are inviting your child to take part in a research study that is called TRACT. It is being conducted at three hospitals in Uganda – Mulago, Mbale and Soroti hospital and Queen Elizabeth Hospital, Blantyre, Malawi. We will include children between the ages of 2 months to 12 years, and aim to involve nearly 4000 children across these hospitals over the next two years. Before you decide if you want your child to take part, it is important for you to understand why the research is being done and what it will involve. Please take time to read this information sheet carefully or ask someone to read it to you. Please discuss this with the nurses or doctors and ask questions if there is anything that is not clear or if you would like more information. Joining the TRACT study is entirely voluntary. Take time to decide whether or not you wish your child to take part.

B: Study purpose: What is the reason for doing the TRACT study?

Your child has been admitted to hospital because they have severe anaemia. This is a serious illness and a common problem in many children in Africa. We have done a blood test and found the strength of your child's blood (or haemoglobin level) is much lower than normal – this is called severe anaemia. Anaemia often causes your child to feel tired, weak and the nailbed or tongue to look pale (*can demonstrate*). The TRACT study is trying to find the best way to treat this. At the moment we don't know whether a blood transfusion is the best way to treat this and after then how to treat the underlying illnesses which caused your child to have severe anaemia. Some children with severe anaemia may die and some children become sick again in the next 6 months. We need to do this study to find the best treatments for severe anaemia in to order to reduce the chance of these things happening.

What is the TRACT study about?

We want to find out whether or not giving a blood transfusion is the best treatment for your child. For children getting a blood transfusion we also don't know how much blood its best to give, either the standard volume (dose) recommended in the current guidelines or a slightly higher volume (dose).

We also want to find out whether or not giving extra treatments during the first three months after this hospital admission will prevent some children with severe anaemia from dying or becoming sick again.

All children in TRACT over one year of age will get tablets to kill any 'worms' they have inside their stomach when they leave the hospital, and all children will get vitamin medicine to make their blood stronger. On top of this, we will be looking at whether:

1/ A vitamin treatment called Sprinkles containing 15 different chemicals/vitamins taken for 3 months is better than the usual treatment of folate and iron
2/ Whether a single pill containing an antibiotic, cotrimoxazole, taken for 3 months will fight infections and stop children from getting sick.

C: Study Procedures: What will it involve for my child?

What treatments will he/she be given?

The doctors at Mbale/Mulago/Soroti/Queen Elizabeth *(delete as appropriate)* hospital will treat your child according to standard Ugandan/Malawian Ministry of Health guidelines for severe illness and/or severe malaria. For the children in the TRACT study we will be studying

i) Transfusion:

1. If your child has a very low haemoglobin or, if the doctor has checked your child and found features which tell them your child is very sick, then your child will get a blood transfusion. We don't know what the best amount of blood to give so half the children will either receive the standard dose (as currently recommended) and half a slightly higher volume (dose).
2. If the doctor has found your child is not very sick but just has a low haemoglobin then we are not sure whether a blood transfusion is needed. At the moment the recommendations are not to give a transfusion – we want to find out whether giving a transfusion in some children may help them recover faster. Half of the children like this in TRACT will receive no transfusion and half of the children will receive a blood transfusion (either at a standard dose or at a higher dose).

ii) Vitamin treatments
Half the children will get iron and folate for 3 months (the current recommendation) while the other half will get a different vitamin medicine which is sprinkled onto their food every day for 3 months. These vitamins are trying to make the child's blood stronger – we don't know which type is best.

iii) Preventing Infection

Often children with severe anaemia come back to hospital with another illness in the 6 months after this current admission – to try to prevent this half the children will get an antibiotic tablet called cotrimoxazole for 3 months while the other half will not.

The decision as to which child gets which treatment will be decided when they join the study by a system based on chance, using a computer, not by any member of the research team. All the medicines used in the study have been widely used in children before and found to be very safe.

Study Procedures

1. Children will be carefully checked over the time they are in hospital – including extra blood tests to check if they still have anaemia. These regular checks that the doctors and nurses do will also help us find out if the child is getting better or not. All children who are found to have a very low haemoglobin during these checks will receive another blood transfusion, or an initial transfusion if they did not get one immediately after they arrived at hospital. If there are any side effects from a blood transfusion we should be able to discover these during these regular checks and treat them promptly.

2. On the day your child is admitted we will be doing blood tests to help us find out what is causing your child's illness, how sick they are and to help us decide what type of blood to transfuse. An extra 5-10mls (one to two teaspoons) will be taken saved for future tests to help us find out why your child became ill. We will also want to take a sample of your child's urine and poo for the same reasons. Some results you will get back during the study - others will be done much later at the end of the study and you may not get the results of some of these tests.

3. When your child is ready to go home we will give you vitamin medicines to make your child's blood stronger and half of the children in the study will receive an antibiotic (cotrimoxazole) – both of these will be taken every day for 3 months (90-days). We will give you enough tablets/medicines to last until your next follow up visit. We will also ask you about the kind of food the child has been eating before they came to hospital, to try to help us find out why they became ill with anaemia in the first place. We will check where you live and take your contact details- to help us find you if you are not able to come back. You can contact us if you have any concerns about your child condition or they have had to go to hospital – our team will call you back if necessary.

4. All children will have to come back after one month, three months and six months. We will check the health of your child at this visit, find out what food they have eaten on the day before they came to the clinic, find out whether they have been ill or to hospital since the last visit and check on whether they have been able to take the treatments and if they are causing any problems. We will give you more medicines at the one month visit to last for the next 2 months. We will check on your child's haemoglobin level and do a malaria test at each of these visits. If they have any illness we will treat these or refer the child if necessary to another specialist. Some of the blood taken at these visits will be stored – approximately 3-5ml (equivalent to half to one teaspoon) to help us understand why your child became sick and how s/he is reacting to the treatments we are giving.

5. In total, 10mls (2-teaspoon full of blood) will be taken from your child at the start of the study and another 5-10mls (1-2teaspoons) when they come for later check up visits, as outlined above. This is a very small amount of blood – which will not harm the health of your child (*Can demonstrate what their child's volume of blood is using pre-prepared drawings – based upon 70ml/kg circulating volume).*

6. Some of the tests to find out what caused your child's illness are needed as part of this research cannot be done in this country at the moment, so part of the samples will be sent to laboratories overseas. This will involve a small portion of the blood that was taken during the study, which we will store. Some of the tests we will do will look at whether your child has a trait or characteristic that they inherited from their parents that will either make them more vulnerable to severe anaemia or help their bodies to fight against diseases that cause anaemia better as a result of these inherited traits. This is called genetic research. Individual names will be removed and will be replaced by codes, so that information cannot be linked to participants. Future research done on these samples will be approved by a national independent expert committee, to ensure that participants' safety and rights are respected.

D: Risks of study participation

There are very few risks to your child being in this study. Both vitamin medicines and the antibiotic cotrimoxazole have been widely used in children with very few problems. The TRACT team is working closely with the local blood transfusion services to make sure that blood used in the study is safe. As with any blood transfusion, there is a small chance that there may be a reaction but we will be monitoring your child very closely during the blood transfusion, and will treat your child quickly if this happens. The same thing will happen if you child has problems with any of the other drugs they take in hospital or at home. If for any reason the doctor thinks that it is not in your child's best interest to be in

the study then they will not be enrolled in the study but will be given their usual treatment. You do not have to pay anything to join the study.

All children will have blood taken as part of this study. Many of the tests that will be done in hospital would also be done if you chose not to join the study. However, we will take a small amount of extra blood at the follow-up visits (see above).

E: Benefits of study participation

Your child will get no direct benefits from this study. However, your child will get close observation during the study, and by taking part your child may help us improve the care of children who have severe anaemia in the future. Regular assessment of your child by doctors and nurses will enable us to make important changes to your child's treatment in hospital, if these are needed. We will help supply routine medical supplies and treatments for your child to the hospital, so that you will not have to buy any treatments. This will mean that there will be no delay to starting treatment for your child. The medical tests we perform during this illness will also be paid for by the study.

You will be asked to bring your child back for follow up visits, and we will pay for your transport from hospital to your home and back to the clinic so you can attend these important visits. During the follow up visits will treat any illnesses we find, or arrange referral to appropriate clinic or hospital.

F: Alternatives to study participation: What will happen if I don't agree to participate?

All participation in research is voluntary. You are free to decide if you want your child to take part or not. Your child will still receive the recommended standard of care treatment if they do not take part. If you do agree to join the study you can change your mind at any time, and can withdraw your child from the research. This will not affect their care now or in the future and not incur any penalties. We hope that if you decide to withdraw later, you would give a reason for your decision. More importantly we hope that you would continue to allow us to provide follow-up care which involves continued regular medical check ups, even if you are no longer taking the study medicines.

G: Compensation

You will not incur any costs from participation in this study. All your travel expenses for attending the visits we invite you too will be paid, based on the cost of public transport to and from your home. As well, when you bring your child for follow up, snacks and drinks will be available and for meals, in a situation where you have to wait for a long time before being attended to.

H: Confidentiality. Who will have access to information about me/my child in this research?

All our research records are stored securely in locked cabinets and password protected computers. Only a few people who are working closely on the study will be able to view information from your child. When we report on the results of the study will not include any private information that will make it possible to identify your child.

I: Study related injury

This research is supported by Imperial College London who holds insurance policies which apply to this study. If you experience harm or injury as a result of taking part in this study, you will be eligible to claim compensation without having to prove that Imperial College is at fault. Some specific treatment and compensation are not included in our insurance policies and if you want more information about this you should discuss it with your doctor.

J: Contacts and questions

Who has allowed this research to take place?
All research conducted in Uganda/Malawi *[delete as appropriate]* is approved by national independent expert committees to make sure the research is conducted properly and that study participants' safety and rights are respected.

What if I have any questions?

You may ask any of our staff questions at any time. You can also contact those who are responsible for the care of your child and this research:
The following will be site dependent:

Professor Sarah Kiguli, Department of Paediatrics, PO Box 7072 Makerere University, Kampala, Uganda.

Tel +256 41 531875; Fax +256 41 532591

Or

Dr Robert Opoka, **MBCHB, MMED** Consultant Paediatrician

opokabob@yahoo.com; Mobile: +254 772 996164; Tel +256 41 531875; Fax +256 41 532591

Or

Dr Peter Olupot Olupot, MBCHB, MPH Mbale Regional Referral Hospital, P.O Box 921, Mbale.
polupotolupot@yahoo.com; Mobile : + 256 (0)772457217/ Tel: (0)392910171/(0)352280584/(0)45 4433193 Fax: +256 45 4435894

Or

Dr Charles Engoru, MBCHB, MMED. Soroti Regional Hospital, P.O Box 289 Soroti.
charlasengoru@yahoo.co.uk; Mobile: +256 772 458587; Fax +256 45 4461382

Or

Dr Yamikani Chimalizeni, Queen Elizabeth Hospital, PO Box 30096, Chichiri Blantyre 3.

Tel Fax

Or
If you have any questions about your rights as a participant, please contact

Professor James Tumwini,
Chairman Faculty of Medicine Research and Ethics Review Committee, School of Medicine, PO Box 7072 Makerere University Kampala, Uganda.
Tel: +256 41 4530020
Or
XXX
Chairman, College of Medicine Ethics Review Committee, Blantyre

If you would like to contact the Chief Investigator, Professor Kathryn Maitland
Please contact
Trial Administrator: Phyles Maitha
Address: KEMRI Wellcome Trust Programme, P.O Box 230-80108, Kilifi, Kenya
E-mail: pmaitha@kemri-wellcome.org
Tel: +254 41 522063
Fax: +254 41 522390

TRIAL Consent Form (To be presented on local-headed paper)

Version 1.0 8th Jan 2013

Child's Initials				Study Number							

TRACT (Transfusion and Treatment of Severe Anaemia in African Children)

Please initial (or mark) box if you agree:

I confirm that I have read/ been read the patient information sheet (version 1.0 dated 21th Jan 2013) for the TRACT study and that I understand what will be required if my child participates in the study. The study has been explained to me and my questions have been answered.	
I understand that my child's participation is voluntary and that I am free to withdraw him or her at any time, without giving any reason, without my medical care or legal rights or my child's medical care or legal rights being affected.	
I understand that sections of any of my child's medical notes may be looked at by responsible individuals involved in the running of the study or from regulatory authorities where it is relevant to my child's participation in this research. I give permission for these individuals to have access to my child's records, but understand that strict confidentiality will be maintained.	
I understand that my child will be given 3 months of treatment after discharge from hospital then followed up for another 3 months. After the study, my child's healthcare will be provided by the national health system.	
I agree to allow blood samples to be taken from my child and for my child's samples to be stored for later testing. I understand that my child and I may not be given the results of tests performed on stored samples.	
I agree to samples being exported overseas for further studies	
I agree for my child to participate in the TRACT study	

Parent/carer's signature (or thumbprint)	Print name	Date (day/month/year) Time

Witness's signature (if thumbprint used above)	Print name	Date (day/month/year) Time

Doctor's signature	Print name	Date (day/month/year) Time

IMPORTANT: one signed original to be kept in TRACT trial file by the researcher, one signed copy to be given to the patient, one signed copy to be kept in the clinic file

Verbal Assent (To be presented on local-headed paper)
Version 1.0 8th Jan 2013

TRACT (Transfusion and Treatment of Severe Anaemia in African Children)

Child's Initials				Male ❍ Female ❍	Date/Year of Birth	D	D	M	M	M	Y	Y	Y	Y	Age (years)		

Date of Form	D	D	M	M	M	2	0	Y	Y

Clinic/Hospital Number								

NOTE: For children who are critically ill and in whom informed consent would lead to significant delay in starting treatment a verbal assent will be obtained by the doctor from the *parent or guardian* after brief discussion with admitting study doctor or nurse.

We advise that this should include the following phrases.

- We are going to provide the treatment for your child that is recommended by the government.
- We want to find out if we can improve on these current recommendations by trying new treatments that we think will work better and we do this by research.
- All research is checked by independent committees to make sure that the potential benefits to individuals outweigh the risks. All participation in research is voluntary, and so you can refuse.
- We would like your child to participate in this research for us to learn the best way to treat severe anaemia.
- Do you agree for your child to take part in this research? You can say no and your child will still receive the same level of care with the governments recommended treatment.

Parent/Guardian assents to research?	**Please circle:** Yes / No

Parent or guardians name	Relationship with child	Time (24 hour clock)			
		H	H	M	M

Doctor or nurses signature	Print name	Date								
		D	D	M	M	M	2	0	Y	Y

Note: Original to be kept in TRACT trial file by the researcher, one signed copy to be given to parent/guardian/carer and one signed copy to be kept in the clinic notes.

16 APPENDIX II TEMPLATE PATIENT INFORMATION SHEETS AND CONSENT FOR GUT BARRIER FUNCTION AND IMMUNE ACTIVATION(MBALE ONLY)

TRACT Study Immune activation and Gut Barrier sub-study - Information for Parents/Guardians

Introduction

We are inviting you to join an extra part of the TRACT study. This extra study is looking at whether the bugs (bacteria) in your gut have made your child ill and whether the medicines you are receiving in TRACT will have an effect on the gut. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully or have someone read it to you, and discuss it with others if you wish. We will give you a copy to keep. Ask the nurses or doctors if there is anything that is not clear or if you would like more information. Providing or not providing and extra volume of blood and a urine and stool samples for storage is an extra part of the TRACT study will not change how you are treated in TRACT. You may decide that you do not wish to take part now or you may wish to withdraw from the study later. This will not influence the care you receive now or in future.

What is the reason for collecting urine and stool (poo) samples in TRACT?

You have already joined the TRACT study, which is looking at whether extra treatments make your child's blood stronger and reduce the risk of dying. We want to understand how these extra treatments may be working. We all have bacteria (bugs) living harmlessly in the gut that keep us well. When we become ill, though, the types of bugs that are found in the gut may change. Sometimes because there is damage to the gut, these bugs can get into the blood and may make your child ill. Some of the extra medicines that people in TRACT are taking may help to repair the gut, and reduce the amount of damage. This may reduce sickness and improve your child's health. We can look at these changes by measuring how well your child is fighting infections in the blood sample we took. We can also use samples of your child's stool (poo) and urine at certain points in the study to measure how damaged their gut is and to look at the types of bugs present in the gut flora. This information might help us to understand how severe anaemia can make your children very sick and how the extra medicines in TRACT are working.

What will happen if I take part?

Joining this extra part of the TRACT study will not make any difference to how you are managed; there will be no changes to your medicines or clinic visits. All children will have blood tests on admission to hospital and a stool sample and urine sample, whether or not they join this extra study. We would like to keep these in storage for later testing. After this, all we want to do in this extra study is to collect an small amount of extra blood, and a urine and stool (poo) sample from your child on 2 different visits to the clinic. We will ask you to collect a small sample of stool at home (on the morning you are due to come to clinic) or while you are in the clinic. This poo sample will be collected into a container using a spatula and the urine sample collected into a tube at the clinic. The nurse will give to you both of these. This is the only extra thing we will ask you to do. There are no additional questionnaires to fill in, and no extra time involved.

What are the risks and benefits of taking part?

There will be no additional risks to collecting urine or stool (poo) samples in TRACT. None of your treatment will change; there will be no extra blood draws or change in medicines. You are encouraged

to wash your hands thoroughly with soap or ash under running water after collecting the poo sample. There are no direct benefits to you of taking part. The main goal of this research study is to gain knowledge that may help us treat children with severe anaemia better in the future.

How long will the study continue?

We will ask you to collect a total of 3 stool and urine samples during your 6 months in the TRACT study. These will be on the day of admission (all children, whether or not they join this extra study), at 28 days, and 190 day (6 months). We will be collecting blood at each clinic in any case to check on your child's response to the treatments- we would like to collect an extra volume (1 teaspoon) to store for extra tests at these same visits - this will not harm your child or make their blood any weaker.

What will happen to the samples that are collected?

All the samples will be frozen until we are ready to do the tests. The tests will tell us how your childs immune system (which fights infections) is working and whether it has been affected by the bugs and the toxins the bugs make that may have come across from your gut into your blood and urine. We will also measure proteins in the stool that tell us how much damage there is in your gut and look at the types of bugs in your gut. These are very specialized techniques – so will need to be sent to a laboratory abroad to do this testing. Once this has finished, any remaining samples will be destroyed and thrown away.

What happens to the information collected in this study?

Information from these tests will be analysed and the results stored on a computer. We will not keep details of your name on the computer. Results will be presented and published so we can understand better how to care for African children with severe anaemia. These tests are just done to find out what is causing anaemia in children in Uganda/Malawi as a whole group, and we do not know what they would mean for an individual child. They will also be done some time after you finish the study visits, so you will not be given the results of these tests.

Benefits and/or compensation

We cannot and do not guarantee or promise that you will receive any benefits from this study. Participants will receive reimbursement for the usual TRACT visits. There will be no additional reimbursement.

Confidentiality

Strict confidentiality will be maintained at all times. Names will not be used for study information and stored stool samples; only the TRACT study number, date of birth and initials will identify these. There is just one list which links this study number to your name (already held by the TRACT study), and this list is safely kept private in a locked cabinet.

How can I join?

After reading this information sheet you will be asked to sign the form below giving consent to participate the next time you see the doctor.

What do I do if I have questions or problems? For questions about this study contact:

Dr Peter Olupot, Mbale Regional Referral Hospital, P.O Box 921, Mbale.

Mobile :+ 256 (0)772457217/ Tel: (0)392910171/(0)352280584/(0)45 4433193 Fax: +256 45 4435894

Before you sign this form, please ask any questions on any aspect of this study that is unclear to you. You may take as much time as necessary to think it over.

TRIAL SubStudy Form (To be presented on local-headed paper)

Version 1.0 8th Jan 2013

Child's Initials				Male ❍ Female ❍	Date/Year of Birth	D	D	M	M	M	Y	Y	Y	Y	Age (years)		

Date of Form	D	D	M	M	M	2	0	Y	Y

Clinic/Hospital Number								

I have read/been read the information sheet for the extract study in the TRACT study. I have understood everything and have had my questions answered satisfactorily. I understand that I may change my mind at any stage and that this will not affect the benefits due to my child.

I agree that samples of blood, urine and stool (poo) from my child may be kept by the TRACT team for studies related to this study, and any other further studies. I understand that these results and samples will not be identified by either my or my child's	
I agree to samples being exported overseas for further studies	

Carer's signature (or thumbprint)	Print name	Date
		D D M M M 2 0 Y Y

Witness's signature (if thumbprint used above)	Print name	Date
		D D M M M 2 0 Y Y

Doctor's or Nurse's signature	Print name	Date
		D D M M M 2 0 Y Y

Note: One signed original to be kept in TRACT trial file by the researcher, one signed copy to be given to guardian, one signed copy to be kept in the clinic notes.

17 APPENDIX III WITHDRAWAL FORM

Please initial (or mark) box if you agree:

I/my child no longer wish to (or cannot) take TRACT study drugs and do not wish to (or cannot) attend further visits. I/my child agree to being contacted in the future (home visits or telephone) and to my/my child's medical records being consulted in future to obtain clinical information for TRACT.	

Need to set up a procedure to follow the child up through visits and medical records and report any trial outcomes on the appropriate form. Inform the child and carer that s/he may still return for follow-up visits only or for further study drugs and follow-up visits at a later date if they change their mind.

I/my child no longer wish to (or cannot) TRACT take study drugs and do not wish to (or cannot) attend further visits. I/my child do not agree to being contacted in the future or to my/my child's medical records being consulted in future to obtain clinical information for	

Discontinue all follow up through medical records. The child and carer must sign a new consent form if s/he decides to rejoin the study at a later date.

Patient or carer's signature (or thumbprint)	Print name	Date (day/month/year)

Child's signature (or thumbprint) where appropriate	Print name	Date (day/month/year)

Witness's signature (if thumbprint used above)	Print name	Date (day/month/year)

Doctor's signature	Print name	Date (day/month/year)

IMPORTANT: One signed original to be given to patient
One signed original to be kept on file by the researcher
One signed original to be kept in the clinic notes

18 APPENDIX IV- TRANSFUSION-RELATED ADVERSE EVENTS

Haemovigilance (defined as the systematic surveillance of adverse reactions and adverse events related to transfusion), aimed at improving safety throughout the transfusion chain from donor to patient will be implemented throughout the trial. We aim to adapt use the guidelines for reporting Serious Adverse Events and Serious Adverse Reactions structure, with some modifications, recommended by UK's Serious Hazards Of Transfusion (SHOT) scheme in the UK.
(http://ww w.mhra.gov.uk/Safetyinformation/Reportingsafetyproblems/Blood/index.htm)

A standard SOP will be developed for investigating suspected serious adverse transfusion-related events, including where and how to report these and allied investigations (some of which may be stored for later analysis) to assist with defining causality of the event (ie the likelihood that a serious adverse reaction in a recipient can be attributed to the blood component transfused). These will include: suspected Acute transfusion reaction (ATR) Haemolytic transfusion reaction (acute or delayed) (HTR) Transfusion related acute lung injury (TRALI); Post transfusion purpura (PTP); Transfusion transmitted infection (TTI) and Transfusion associated circulatory overload (TACO).

Category	Definition	What to report	Where
ATR Suspected acute transfusion reaction	Reactions occurring at any time up to 24 hours following a transfusion of blood or components, *including* cases of acute reactions due to incorrect component being transfused*. **Excludes:** haemolytic reactions, TRALI, TACO or those due to bacterial contamination of the component. * *For simplicity these will be reported under ATR; whereas SHOT reports 'wrong blood to wrong patient' in a separate section*	**Isolated febrile** – a rise in temperature of > 1$_{o}$C +/- minor rigors and chills (not thought to be due to underlying disease) **Febrile with other symptoms/signs** – rise in temperature of >1$_{o}$C, with no features of an allergic reaction, but with one or more of myalgia, nausea, change in blood pressure or hypoxia.	CRF CRF
		Minor allergic – Denovo development of skin symptoms +/- rash	CRF
		Anaphylactic/anaphylactoid – hypotension with one or more of: urticaria, rash, dyspnoea, angioedema, stridor, wheeze, pruritus, within 24 hours of transfusion.	SAE
		Severe allergic reaction – Severe allergic reaction with risk to life occurring within 24 hours of transfusion, characterised by bronchospasm causing hypoxia, or angioedema causing respiratory distress. **Hypotension** – a drop in systolic and/or diastolic pressure of >20mm Hg occurring within one hour of completing transfusion, provided all other adverse reactions have been excluded together with underlying conditions that could explain hypotension.	SAE
HTR (acute or delayed)	**Acute** HTRs are defined as fever plus new symptoms / signs of haemolysis within 24 hours of transfusion; confirmed by a fall in Hb, positive DAT and positive crossmatch.		CRF
	Delayed HTRs are defined as		CRF

Haemolytic transfusion reaction	fever and other symptoms / signs of haemolysis more than 24 hours after transfusion; confirmed by one or more of: a fall in Hb PLUS rise in bilirubin, positive DAT and positive crossmatch. (*Simple serological reactions: development of antibody without positive DAT or development of haemolysis are excluded)*		
TRALI Transfusion related acute lung injury	Acute dyspnoea with hypoxia and bilateral pulmonary infiltrates during or within six hours of transfusion, not due to circulatory overload or other likely cause.		SAE
PTP Post transfusion purpura	Thrombocytopenia arising 5 – 12 days following transfusion of red cells, associated (*confirmed if possible with the presence in the patient of alloantibodies directed against the Human Platelet Antigen (HPA) systems*)		SAE
TTI Transfusion-transmitted infection	Include as a TTI if, following investigation, the recipient had evidence of infection post-transfusion, and there was no evidence of infection prior to transfusion and no evidence of an alternative source of infection. **Plus** At least one component received by the infected recipient was shown to contain the infectious pathogen.	Cases of bacterial transmission from blood components, where cultures from the patient's blood match cultures from the component bag and/or from the donor.	SAE
TACO Transfusion associated circulatory overload	Any *four of the following* occurring within **six hours** of transfusion: i) Acute respiratory distress. ii) Severe tachycardia. iii) Increased blood pressure. iv) Acute or worsening pulmonary oedema. v) Evidence of positive fluid balance.		SAE

TRACT Protocol versions and Revision History

Version	Author	Date	Reason for Revision
1.0	K Maitland	20th February 2013	Protocol version 1.0
2.0	K Maitland	19th February 2016	1) Updated email addresses of MRC CTU staff and new staff/trial team members (Page i-iv) 2) Eligibility criteria Enrolment permitted in the first 24 hours of admission (in children not transfused during admission) rather than at hospital admission. (Pages vii in Trial summary; 13, 16-18)

TRACT Statistical Analysis Plan

Version Number and Date: Final 1.0 04April2016

Supersedes version: Draft 0.4 10March2016

Author	Position	Signature	Date
Leanne McCabe	Delegated Statistician		06/04/16
Approved by			
Professor A. Sarah Walker	Trial Statistician		6/4/2016
Professor Kathryn Maitland	Chief Investigator		6th April 2016

Revision History

Version	Author	Date	Reason for Revision
Draft 0.1			Protocol version 1.0
Draft 0.2	Leanne McCabe	26Jan2016	Leanne McCabe first draft
Draft 0.3	Leanne McCabe	19Feb2016	Incorporated Sarah Walker's comments on first draft
Draft 0.4	Leanne McCabe	10March2016	Incorporated IDMC's comments on second draft
Final 0.1	Leanne McCabe	04April2016	Incorporated TSC's comments on third draft

Contents

1 Trial design

1.1 Design and outline

TRACT is a randomised controlled factorial trial with a 3x2x2 design enrolling 3954 children aged 2 months to 12 years from four sites in two sub-Saharan countries (Uganda and Malawi). The trial is designed to evaluate three ways to reduce longer term mortality and morbidity in children who have been admitted to hospital with severe anaemia (haemoglobin (Hb)<6g/dl). There are two strata within the trial, TRACT A and TRACT B, which children are allocated to according to the severity of their anaemia.

The three randomisations are:

R1A – children with <4g/dl or prostration or respiratory distress or haemoglobinuria or known sickle cell disease: immediate liberal transfusion (30mls/kg) vs immediate conservative transfusion (20mls/kg).

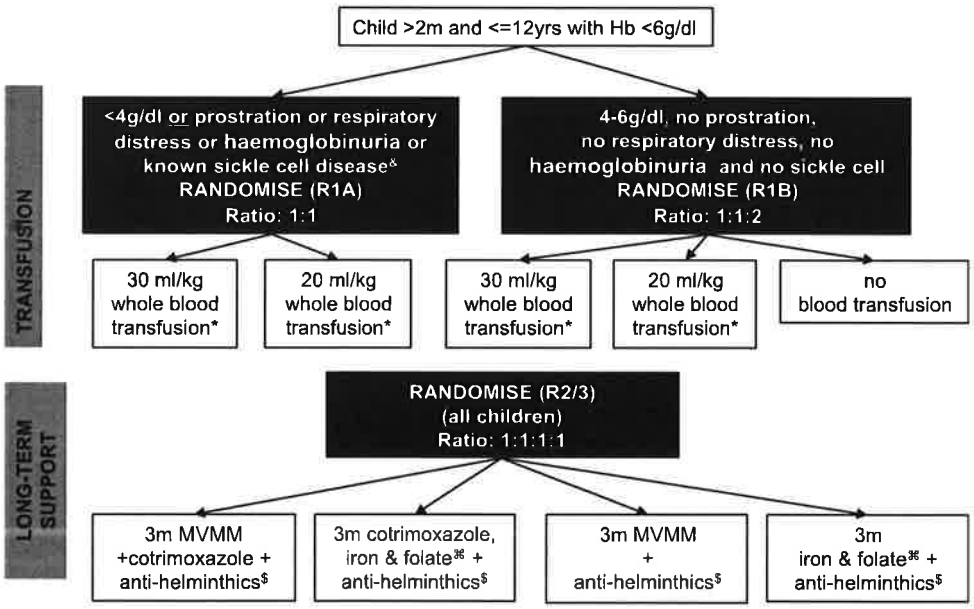
R1B – children with 4-6g/dl, no prostration, no respiratory distress, no haemoglobinuria and no sickle cell disease: immediate liberal transfusion vs immediate conservative transfusion vs no transfusion.

R2 – all children: multi-vitamin multi-mineral (MVMM) supplementation (including iron and folate) vs routine care of iron and folate for three months.

R3 – all children: cotrimoxazole prophylaxis for three months vs no prophylaxis.

Additionally, all children will receive anti-helminthics if >1 years.

The trial design is summarised in the trial scheme below.



& Only applies to a previously established diagnosis of sickle cell disease
* Alternatively 15mls/kg packed cells (for 30mls/kg WB arm); 10 mls/kg packed cells (for 20mls/kg WB arm)
⌘ at treatment doses following WHO recommendations
$ For children > 1 year of age if they have not received antihelminths in previous 6 months- following WHO recommended standard of care
MVMM at usual supplementary dosages

1.2 Population

Eligibility to the trial is based on the child meeting all of the inclusion criteria and none of the exclusion criteria.

Inclusion criteria:

- aged 2 months to 12 years;
- severe anaemia (Hb<6g/dl) at admission to hospital;
- carer willing/able to provide consent.

Exclusion criteria:

- malignancy or other terminal illness;
- children who are exclusively breast fed;
- chronic renal or liver failure;
- surgery as man reason for admission;
- acute trauma or burns as main reason for admission;
- signs of bi-ventricular heart failure;
- known congenital or valvular heart disease (non-surgically corrected).

Primary analysis will be intention to treat on all randomised children.

The protocol prespecified a secondary analysis of the nutritional support and antibiotic prophylaxis randomisations to be performed including only those children who were discharged alive in which the treatment was neither required or contraindicated due to other medical conditions, that is excluding children who were admitted for severe acute malnutrition in the supplementation randomisation and children with HIV or GP6D deficiency in the prophylaxis randomisation.

However, a substantial minority of children (32% as of January 2016) start cotrimoxazole and MVMM during admission so this secondary analysis will be modified to include all children alive at a minimum of discharge or randomisation plus 5 days, rather than only alive at discharge, in which the treatment was neither required nor contraindicated due to other medical conditions.

2 Outcome measures

Cause of death and suspected transfusion reactions will be adjudicated by an Endpoint Review Committee (ERC) blinded to randomised allocations. Relationship to all possible interventions and drugs will be solicited to avoid unblinding.

2.1 Primary outcome

Cumulative mortality to 28 days for the transfusion strategy comparison and to 180 days for the nutritional support and antibiotic prophylaxis comparisons

2.2 Secondary outcomes

Mortality:

- cumulative mortality at 48 hours, 28 days, 90 days and 180 days (where not the primary outcome).

Morbidity:

- re-admission to hospital;
- proportion achieving correction of anaemia (defined by WHO as Hb>9g/dl) at 48 hours, 28 days, 90 days and 180 days;
- development of new profound anaemia (Hb<4g/dl) during acute admission or development of severe anaemia (Hb<6g/dl) post discharge;
- nutrition: changes in weight and mid-upper arm circumference (MUAC) at 90 days and 180 days;
- anti-infection: changes in inflammatory markers (C-reactive protein, procalcitonin), incidence of bacterial infections and malaria at 28 days, 90 days and 180 days.

Solicited adverse events:

- suspected transfusion reactions: febrile reactions, Transfusion Related Acute Lung Injury (TRALI) (any grade), grade 3-4 toxicity of cotrimoxazole, MVMM or iron and folate
- serious adverse events.

Others:

- costs and cost-effectiveness (this SAP will not cover this analysis).

3 Derivation of data to be analysed

Time

Time will be from randomisation 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 clinical evaluation form or their first blood test taken within 48 hours of admission as appropriate.

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. However, the number of children above this age is expected to be small (3% as of January 2016). Children older than 10 years will be excluded from analysis using weight-for-age z-scores.

The charts only have reference values for MUAC-for-age for children aged between 3 months and 5 years. The number of children aged 2 months is expected to be very small (one child as of January 2016). Children aged below 3 months will be excluded from the analysis using MUAC-for-age z-scores. For children aged above 5 years z-scores will be calculated from values provided by Jay Berkeley (unpublished data, personal communication).

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, for analyses at 90 days censoring will occur on day 90 and for analyses at 180 days censoring will occur on day 183 as this corresponds to 6 months.

Definition of visit schedule

Analyses of measurements at a given point in follow up (Hb, weight and MUAC) will use the closest available measurement to that time point in evenly spaced windows. For the day 28 visit there will be a window of ± 21 days, for the day 90 visit there will be a window of ± 30 days and for the day 180 visit there will be a window of ± 60 days.

Blood volume

Blood volumes of transfusions will be reported as whole blood equivalent. For a child receiving whole blood, either direct or using transfer bags, this will be as recorded on forms. For children receiving packed or settled cells, the recorded volume will be doubled before analysis. Haematocrit of the transfused blood will not be taken into account when converting blood volumes.

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.

Truncation

All Hb, weight-for-age and MUAC-for-age values during the trial will be visually inspected. Outliers four standard deviations beyond the mean at each time point will be set to missing, after querying with site. Values larger than the 99^{th} percentile or smaller than the 1^{st} percentile across all time points will be compared to other values in the child's records. Any large deviations, greater than two standard deviations of values at that timepoint, from prior or subsequent measurements, where both exist, will be truncated to the 99^{th} percentile. For weight-for-age and MUAC-for-age, any truncated values will be back transformed to the original scale.

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.

4 Statistical analysis

Analyses comparing transfusion strategies will be stratified by site and anaemia severity stratum and will compare 20mls/kg vs 30mls/kg (A and B), and transfusion vs no transfusion (B only, pooling

20mls/kg vs 30mls/kg). Analyses comparing MVMM supplementation or cotrimoxazole prophylaxis randomisations will also be stratified by site and stratum. Log-rank tests will be performed stratified and unstratified and where inference is similar standard unstratified results will be presented.

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

4.1 Recruitment

- **Recruitment tabulated by site and anaemia strata, n(% of recruitment per site)**
- Screened, but not randomised: n overall , n (%) by category consent not given, Hb>6g/dl, child <2 months or >12 years, at least one exclusion criteria met
- Eligibility: number and reasons for any children randomised in error and excluded or ineligible children included in the analysis

4.2 Baseline characteristics

The following baseline characteristics will be summarised by the specified statistics and presented by stratum. Variables will be presented by randomisation 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.

- **Sex: n(%) male, female**
- **Age at admission (months): median (IQR)**
- **Haemoglobin at admission (g/dl): median (IQR)**
- **Weight (kg), MUAC (cm),** weight-for-age z-score, MUAC-for-age z-score**: median (IQR)**
- **Heart rate (bpm), axillary temperature (˚C), systolic blood pressure (mmHg), diastolic blood pressure (mmHg), oxygen saturation (%), respiratory rate (bpm), capillary refill time (s): median (IQR)**
- **Temperature gradient, weak pulse: n(%) yes, no**

Severity criteria

- **Severity features: impaired consciousness (prostration or unconsciousness), respiratory distress, haemoglobinuria, sickle cell anaemia, HB<4g/dl: n(%) yes, no**
- **Number of severity features: n(%) one feature, two features, three features, four features, five features**

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
- Fits lasting more than 30 minutes: n(% of those with fits) yes, no, don't know
- Haemoglobinuria: n(%) yes, no, don't know; median (IQR) length (days)

Treatment in this illness

- Admitted for over 24 hours into another hospital, had 2 or more doses of IV or IM quinine/artesunate, 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
- **Blood transfusion in this illness: n(%) yes, no, don't know; n(% of those with yes) 1, 2, 3+**

Past clinical history

- Known to have HIV, two or more hospital admissions in the last year, 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
- **Blood transfusion ever: n(%) yes, no; n(% of those with yes) 1, 2, 3-4, 5+**

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

Coma

- Coma: n(%) yes, no, not assessed
- Eyes: n(%) not directed, directed
- Motor: n(%) no response, withdraws, localises pain
- Verbal: n(%) no response, moan only, meaningful cry

Ward tests at admission

- HIV test result: n(%) positive, negative, invalid or not done
- Lactate (mmol/l), glucose (mmol/l): median (IQR)

Working diagnosis

- Severe malaria – all types, sepsis/septicaemia, LRTI – all types, URTI – all types, other chest syndrome, profound anaemia, malnutrition, osteomyelitis/pyogenic arthritis, developmental delay, cerebral palsy, recurrent haemoglobinuria, encephalopathy, schistosomiasis, sickle cell anaemia, sickle cell crisis, meningitis – all types, HIV/AIDS, tuberculosis – all types, hepatitis, gastroenteritis, urinary tract infection, pyrexia of unknown original, dark urine syndrome, helminth infection, other: n(%)
- Summary of other working diagnosis where indicated

Presentation

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

Admission blood test results

- **Malaria: n(%) positive, negative, not known. A child is positive if either the RDT or blood film provides a positive result, negative if all tests that have been done return a negative result and not known otherwise.**
- 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)

4.3 Description of follow-up

The following will be tabulated by transfusion randomisation and anaemia severity stratum. 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 28 days, 90 days, 180 days** and overall: **n(%)**
- **Child status at 28 days, 90 days, 180 days** and overall: **n(%) visit done, died, lost to follow up, missed visit.**

Completeness of Hb records

- **Records considered complete, defined as a non-missing entry or died before the time point, at 48 hours, 28 days, 90 days and 180 days: n(%)**
- **Record status at 48 hours, 28 days, 90 days, 180 days: n(%) Hb recorded, form entered – no Hb recorded, died, LTFU/absconded, no form entered**

4.4 Adherence to treatment

The following will be tabulated by randomisation. For transfusion adherence this will be no transfusion vs 20mls/kg vs 30mls/kg, for MVMM adherence this will be MVMM vs iron and folate and for cotrimoxazole this will be cotrimoxazole vs no cotrimoxazole.

Transfusion strategy

- **Children receiving a transfusion: n(%)**
- **Volume of first transfusion per child (mls/kg, whole blood equivalent): median, range; n(%) by volume categories of <17mls/kg, 17-23mls/kg, >23 - <27mls/kg, 27-33mls/kg, >33mls/kg**
- **Number of transfusions per child: median (IQR), maximum; n(% of those receiving a transfusion) 1 transfusion, 2 transfusions, 3+ transfusions**
- **Time until first transfusion (hours): median (IQR)**
- **First transfusion within 4 hours: n(% of those receiving a transfusion)**
- **For those randomised to receive no transfusion only: n(% those who received a transfusion) first transfusion following recorded de novo severity signs, first transfusion with no recorded de novo severity signs, first transfusion after 48 hours, no bedside obs form on the database, not compliant with protocol**
- **Volume per transfusion overall (mls/kg, whole blood equivalent): median, range; n(%) by volume categories of <17mls/kg, 17-23mls/kg, >23 - <27mls/kg, 27-33mls/kg, >33mls/kg**
- **Adherent transfusions: n(%) overall, correct amount first transfusion, correct amount second transfusion, correct amount further transfusions, stopped due to transfusion reaction, death during transfusion. Denominator is number of transfusions in that randomisation, apart from for the correct amount categories where it is the number of first, second or further transfusions as appropriate**
- **Non-adherent transfusions: n(%) overall, too little first transfusion, too much first transfusion, too little second transfusion, too much second transfusion, too little further transfusions, too much further transfusions, other reasons. Denominator is number of transfusions in that randomisation, apart from for the correct amount categories where it is the number of first, second or further transfusions as appropriate**
- For each antibiotic prescribed during admission: n(%) ever received; median (IQR) range prescription length

Nutritional supplementation

- **MVMM supplementation at discharge or initial prescription: n(%) receiving MVMM, receiving plumpynut, receiving iron and folate, died or absconded before discharge/prescription, discharge or medication form not entered**

- **MVMM supplementation post discharge: n(%) receiving MVMM, receiving plumpynut, receiving iron and folate, stopped treatment at earlier visit, stopping treatment at this visit, died or LTFU, missed visit; missed doses: n(%) any; yesterday, 2-7 days ago, 8-14 days ago, 15-28 days ago. Denominator is those enrolled long enough to be eligible for a visit at that time point, have died, are known to be LTFU or who have completed their visit early**

Cotrimoxazole prophylaxis

- **Cotrimoxazole prophylaxis at discharge or initial prescription: n(%) receiving cotrimoxazole, not receiving cotrimoxazole – contraindicated, not receiving cotrimoxazole – other reason, died or absconded before discharge/prescription, discharge or medication form not entered**
- **Cotrimoxazole prophylaxis post discharge: n(%) receiving cotrimoxazole, stopped treatment earlier, stopping treatment at this visit, died or LTFU, missed visit; missed doses: n(%) any; yesterday, 2-7 days ago, 8-14 days ago, 15-28 days ago. Denominator is those enrolled long enough to be eligible for a visit at that time point, have died, are known to be LTFU or who have completed their visit early**

4.5 Efficacy analyses

4.5.1 Primary outcome

- **Cumulative mortality to 4 weeks for the transfusion strategy comparison and to 6 months for the MVMM supplementation and cotrimoxazole prophylaxis comparisons**

Mortality at the specified time points will be analysed using time to event methods with hazard ratios and 95% confidence intervals calculated using stratified Cox proportional hazard models. Kaplan-Meier curves will be plotted and log-rank tests, stratified and unstratified, will be performed.

As a sensitivity analysis, flexible survival models will be used to estimate absolute survival difference over time, unadjusted and adjusted.

4.5.2 Secondary outcomes

- **Cumulative mortality at 48 hours, 28 days, 90 days and 180 days where not the primary outcome**

The transfusion strategy will be analysed at all time points, whereas the supplementation and prophylaxis randomisations will only be analysed at the time points post discharge.

Mortality at the specified time points will be analysed using time to event methods with hazard ratios and 95% confidence intervals calculated using stratified Cox proportional hazard models. Kaplan-Meier curves will be plotted and log-rank tests, stratified and unstratified, will be performed.

- **Readmission to hospital**

Frequency of hospital readmissions will be tabulated by body systems and **by randomised group. All treatment comparisons will be analysed.**

First readmission will also be analysed using competing risks analysis with death post discharge 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. Children only become at risk from discharge.

- **Development of new profound anaemia (Hb<4g/dl) during acute admission**

Acute admission is defined as from 8 hours after admission until discharge and children only become at risk after having at least one Hb measurement above 4g/dl. Only the transfusion randomisation will be analysed.

Analysis will be using competing risks analysis with death and discharge as competing risks. Subhazard ratios and 95% confidence intervals will be calculated using cause-specific hazards regression and cumulative incidence curves will be plotted.

- **Development of severe anaemia (Hb<6g/dl) post discharge**

Analysis will be performed on all treatment comparisons.

The proportion (%) of those developing severe anaemia will be tabulated by time point and treatment arm as will the mean (SD) Hb level at each time point based on observed data only with no imputation in the primary analysis.

The difference in proportions between treatment arms at each of the visits will be estimated using poisson regression using a robust variance estimator to obtain rate ratios and 95% confidence intervals. Generalised estimating equations (GEEs) with an independent correlation structure will be used to provide an overall test of difference in the proportion with severe anaemia post discharge between the arms.

- **Proportion achieving correction of anaemia (Hb>9g/dl)**

Those achieving correction of anaemia prior to discharge will be analysed using a competing risks analysis with discharge or death. Subhazard ratios and 95% confidence intervals will be calculated using cause-specific hazards regression and cumulative incidence curves will be plotted. Correction prior to discharge will only be analysed for the transfusion strategies.

Post discharge, the proportion (%) of children achieving correction of anaemia will be tabulated by time point and by randomisation. The difference in proportions between treatment arms at each of the visits will be estimated using poisson regression using a robust variance estimator to obtain rate ratios and 95% confidence intervals. **This analysis will be performed for all treatment comparisons and as observed initially.**

The change in Hb from baseline to all times of observed Hb measurements (8 hours, 16 hours, 24 hours 48 hours, 28 days, 90 days, 180 days) will be analysed using GEEs with an independent correlation structure and adjusted for baseline Hb. Global tests of difference will be performed to compare 20mls/kg vs 30mls/kg in TRACT A and transfusion vs no transfusion in TRACT B.

Imputation using chained estimating equations within each strata and randomisation and including all Hb values, age, mls/kg received and pack type will be considered as a sensitivity analysis.

- **Changes in weight, weight-for-age, MUAC and MUAC-for-age at 90 days and 180 days**

Analysis will be performed in all randomisations.

Normal linear regression adjusted for absolute baseline values will be used to calculate mean changes from baseline and 95% confidence intervals to specified time point and mean difference and 95% confidence intervals between the treatment arms at the time points for weight, MUAC and their z-scores. A global test of difference between treatment arms across the time points will also be conducted using GEEs with an independent correlation structure and adjusting for baseline values as above.

- Changes in inflammatory markers (CRP, PCT), incidence of bacterial infections and malaria at 28 days, 90 days and 180 days

Changes in inflammatory markers will be measured retrospectively. Analysis of changes in inflammatory markers will be performed using normal linear regression adjusted for baseline values as above. Mean changes and 95% confidence intervals from baseline to specified time point and mean difference and confidence intervals between the treatment arms at the time points will be calculated. A global test of difference between treatment arms across the time points will also be conducted using GEEs with an independent correlation structure and adjusting for baseline values as above.

- Incidence of bacterial infections and malaria

Incidence of bacterial infections and malaria will be taken from blood cultures or molecular diagnostics retrospectively. As incidence is expected to be low and dates of onset are unlikely to be known precisely, as these events will primarily be ascertained at follow-up visits, these will be analysed using poisson regression to calculate rate ratios and 95% confidence intervals over all follow up time.

4.6 Safety analyses

4.6.1 Secondary outcomes

- **Suspected transfusion reactions: febrile reactions, TRALI (any grade); grade 3-4 toxicity of cotrimoxazole, MVMM or standard iron and folate**

Number of suspected transfusion reactions will be tabulated by transfusion randomisation and the number of reactions compared across the randomised the groups using a chi-squared test or Fisher's exact test if values are small.

Grade3-4 toxicities will also be tabulated by randomisation.

- **Serious adverse events**

The number (%) of children ever having an SAE will be tabulated and compared across randomised groups with a chi-squared test. Relationship of serious adverse events to transfusion, transfusion volume, MVMM and to cotrimoxazole will be tabulated across randomised groups and by body systems**. The number of children having SAEs (% 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 randomisation group.

SAEs considered to be definitely, probably or possibly related to an intervention or to transfusion volume by the ERC will also be listed by the appropriate randomisation. All SAEs and all causes of death will be tabulated by transfusion randomisation.

4.7 Subgroup analyses

Subgroup analyses pre-specified in the protocol include:

- each of the other randomisations to investigate interactions in the factorial design
- the centre stratification for all randomisations
- anaemia severity stratification for the transfusion randomisations
- previous transfusion ever
- previous transfusion at another health centre during this illness
- rate of transfusion
- fever
- malaria
- HIV
- known or previously undiagnosed sickle cell disease
- microbiological evidence of sepsis.

Further subgroup analyses not included in the protocol will also examine any potential difference in the impact of treatments according to haemoglobinuria, blood pack type (whole blood vs packed and settled cells), donor Hb, donor haematocrit and malnutrition(baseline weight-for-age z-score and MUAC).

Subgroup analyses will be based on tests of interaction. Continuous factors will be grouped into terciles. Fractional polynomial models will also be used to investigate interactions between randomised group and continuous factors (Royston and Sauerbrei, Stat. Med 2014).

Additional analyses will investigate the impact of donor haematocrit, donor Hb, blood pack type, age of donor blood, actual ml/kg received (whole blood equivalent), age of the child and baseline values of Hb, weight-for-age and MUAC-for-age z-scores on outcomes.

TRACT Statistical Analysis Plan

Version Number and Date: Final 2.0 24 July 2017

Supersedes version: Draft 1.1 6 June 2017

Author	Position	Signature	Date
Elizabeth George	Delegated Statistician	ECGeorge	29/8/2017
Approved by			
Professor A. Sarah Walker	Trial Statistician	ASarahW	29/8/2017
Professor Kathryn Maitland	Chief Investigator		**1st Sept 2017**

Revision History

Version	Author	Date	Reason for Revision
Draft 0.1			Protocol version 1.0
Draft 0.2	Leanne McCabe	26Jan2016	Leanne McCabe first draft
Draft 0.3	Leanne McCabe	19Feb2016	Incorporated Sarah Walker's comments on first draft
Draft 0.4	Leanne McCabe	10March2016	Incorporated IDMC's comments on second draft
Final 1.0	Leanne McCabe	04April2016	Incorporated TSC's comments on third draft
Draft 1.1	Elizabeth George	06June2017	Clarification of population detail, additional subgroup analyses and additional analyses.
Final 2.0	Elizabeth George	24July 2017	Incorporated Kath Maitland's comments on draft 1.1

Contents

1 Trial design

1.1 Design and outline

TRACT is a randomised controlled factorial trial with a 3x2x2 design enrolling 3954 children aged 2 months to 12 years from four sites in two sub-Saharan countries (Uganda and Malawi). The trial is designed to evaluate three ways to reduce longer term mortality and morbidity in children who have been admitted to hospital with severe anaemia (haemoglobin (Hb)<6g/dl). There are two strata within the trial, TRACT A and TRACT B, which children are allocated to according to the severity of their anaemia.

The three randomisations are:

R1A – children with <4g/dl or prostration or respiratory distress or haemoglobinuria or known sickle cell disease: immediate liberal transfusion (30mls/kg) vs immediate conservative transfusion (20mls/kg).

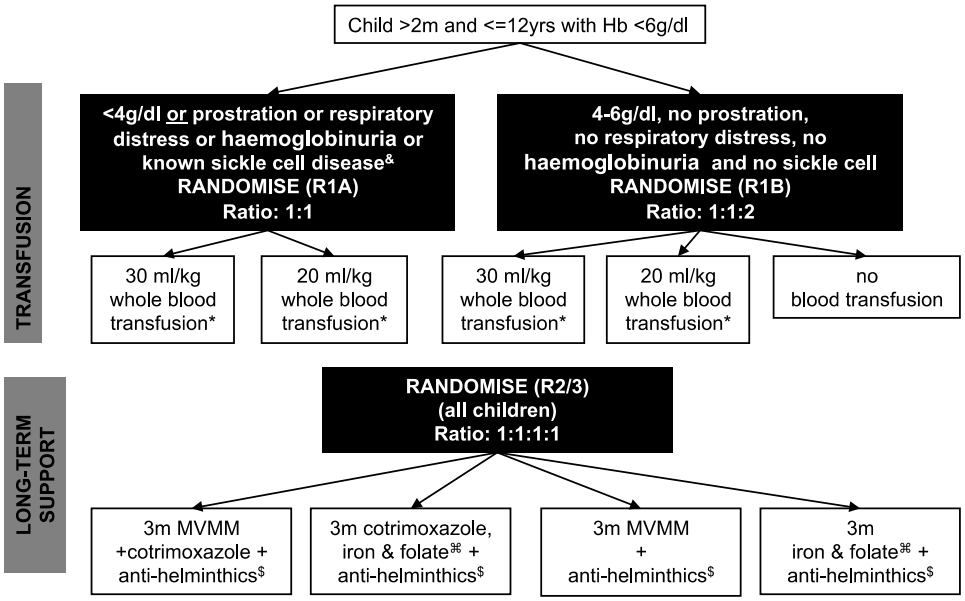
R1B – children with 4-6g/dl, no prostration, no respiratory distress, no haemoglobinuria and no sickle cell disease: immediate liberal transfusion vs immediate conservative transfusion vs no transfusion.

R2 – all children: multi-vitamin multi-mineral (MVMM) supplementation (including iron and folate) vs routine care of iron and folate for three months.

R3 – all children: cotrimoxazole prophylaxis for three months vs no prophylaxis.

Additionally, all children will receive anti-helminthics if >1 years.

The trial design is summarised in the trial scheme below.



& Only applies to a previously established diagnosis of sickle cell disease
* Alternatively 15mls/kg packed cells (for 30mls/kg WB arm); 10 mls/kg packed cells (for 20mls/kg WB arm)
⌘ at treatment doses following WHO recommendations
$ For children > 1 year of age if they have not received antihelminths in previous 6 months- following WHO recommended standard of care
MVMM at usual supplementary dosages

1.2 Population

Eligibility to the trial is based on the child meeting all of the inclusion criteria and none of the exclusion criteria.

Inclusion criteria:

- aged 2 months to 12 years;
- severe anaemia (Hb<6g/dl) at admission to hospital;
- carer willing/able to provide consent.

Exclusion criteria:

- malignancy or other terminal illness;
- children who are exclusively breast fed;
- chronic renal or liver failure;
- surgery as man reason for admission;
- acute trauma or burns as main reason for admission;
- signs of bi-ventricular heart failure;
- known congenital or valvular heart disease (non-surgically corrected).

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.

The protocol pre-specified a secondary analysis of the nutritional support and antibiotic prophylaxis randomisations to be performed including only those children who were discharged alive in which the treatment was neither required or contraindicated due to other medical conditions, that is excluding children who were admitted for severe acute malnutrition in the supplementation randomisation and children with HIV or known GP6D deficiency in the prophylaxis randomisation.

However, a substantial minority of children (32% as of January 2016) started cotrimoxazole and MVMM during admission so this secondary analysis will be modified to include all children alive at a minimum of discharge or randomisation plus 5 days, rather than only alive at discharge, in which the treatment was neither required nor contraindicated due to other medical conditions.

2 Outcome measures

Cause of death and suspected transfusion reactions will be adjudicated by an Endpoint Review Committee (ERC) blinded to randomised allocations. Relationship to all possible interventions and drugs will be solicited to avoid unblinding.

2.1 Primary outcome

Cumulative mortality to 28 days for the transfusion strategy comparison and to 180 days for the nutritional support and antibiotic prophylaxis comparisons

2.2 Secondary outcomes

Mortality:

- cumulative mortality at 48 hours, 28 days, 90 days and 180 days (where not the primary outcome).

Morbidity:

- re-admission to hospital;
- proportion achieving correction of anaemia (defined by WHO as Hb>9g/dl) at 48 hours, 28 days, 90 days and 180 days;
- development of new profound anaemia (Hb<4g/dl) during acute admission or development of severe anaemia (Hb<6g/dl) post discharge;
- nutrition: changes in weight and mid-upper arm circumference (MUAC) at 90 days and 180 days;
- anti-infection: changes in inflammatory markers (C-reactive protein, procalcitonin), incidence of bacterial infections and malaria at 28 days, 90 days and 180 days.

Solicited adverse events:

- suspected transfusion reactions: febrile reactions, Transfusion Related Acute Lung Injury (TRALI) (any grade), grade 3-4 toxicity of cotrimoxazole, MVMM or iron and folate
- serious adverse events.

Others:

- costs and cost-effectiveness (this SAP will not cover this analysis).

3 Derivation of data to be analysed

Time

Time will be from randomisation 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 clinical evaluation form or their first blood test taken within 48 hours of admission as appropriate.

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. However, the number of children above this age is expected to be small (3% as of January 2016). Children older than 10 years will be excluded from analysis using weight-for-age z-scores.

The charts only have reference values for MUAC-for-age for children aged between 3 months and 5 years. The number of children aged 2 months is expected to be very small (one child as of January 2016). Children aged below 3 months will be excluded from the analysis using MUAC-for-age z-

scores. For children aged above 5 years z-scores will be calculated from values provided by Jay Berkeley (unpublished data, personal communication).

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, for analyses at 90 days censoring will occur on day 90 and for analyses at 180 days censoring will occur on day 183 as this corresponds to 6 months.

Definition of visit schedule

Analyses of measurements at a given point in follow up (Hb, weight and MUAC) will use the closest available measurement to that time point in evenly spaced windows. For the day 28 visit there will be a window of ± 21 days, for the day 90 visit there will be a window of ± 30 days and for the day 180 visit there will be a window of ± 60 days.

Blood volume

Blood volumes of transfusions will be reported as whole blood equivalent. For a child receiving whole blood, either direct or using transfer bags, this will be as recorded on forms. For children receiving packed or settled cells, the recorded volume will be doubled before analysis. Haematocrit of the transfused blood will not be taken into account when converting blood volumes.

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.

Truncation

All Hb, weight-for-age and MUAC-for-age values during the trial will be visually inspected. Outliers four standard deviations beyond the mean at each time point will be set to missing, after querying with site. Values larger than the 99^{th} percentile or smaller than the 1^{st} percentile across all time points will be compared to other values in the child's records. Any large deviations, greater than two standard deviations of values at that timepoint, from prior or subsequent measurements, where both exist, will be truncated to the 99^{th} percentile. For weight-for-age and MUAC-for-age, any truncated values will be back transformed to the original scale.

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.

4 Statistical analysis

Analyses comparing transfusion strategies will be stratified by site and anaemia severity stratum and will compare 20mls/kg vs 30mls/kg (A and B), and transfusion vs no transfusion (B only, pooling 20mls/kg vs 30mls/kg). Analyses comparing MVMM supplementation or cotrimoxazole prophylaxis randomisations will also be stratified by site and stratum. Log-rank tests will be performed stratified and unstratified and where inference is similar standard unstratified results will be presented.

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

4.1 Recruitment

- **Recruitment tabulated by site and anaemia strata, n(% of recruitment per site)**
- Screened, but not randomised: n overall , n (%) by category consent not given, Hb>6g/dl, child <2 months or >12 years, at least one exclusion criteria met
- Eligibility: number and reasons for any children randomised in error and excluded or ineligible children included in the analysis

4.2 Baseline characteristics

The following baseline characteristics will be summarised by the specified statistics and presented by stratum. Variables will be presented by randomisation if there is a difference between randomised groups of p≤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.

- **Sex: n(%) male, female**
- **Age at admission (months): median (IQR)**
- **Haemoglobin at admission (g/dl): median (IQR)**
- **Weight (kg), MUAC (cm),** weight-for-age z-score, MUAC-for-age z-score**: median (IQR)**
- **Heart rate (bpm), axillary temperature (˚C), systolic blood pressure (mmHg), diastolic blood pressure (mmHg), oxygen saturation (%), respiratory rate (bpm), capillary refill time (s): median (IQR)**
- **Temperature gradient, weak pulse: n(%) yes, no**

Severity criteria

- **Severity features: impaired consciousness (prostration or unconsciousness), respiratory distress, haemoglobinuria, known sickle cell anaemia, HB<4g/dl: n(%) yes, no**
- **Number of severity features: n(%) one feature, two features, three features, four features, five features**

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
- Fits lasting more than 30 minutes: n(% of those with fits) yes, no, don't know

- Haemoglobinuria: n(%) yes, no, don't know; median (IQR) length (days)

Treatment in this illness

- Admitted for over 24 hours into another hospital, had 2 or more doses of IV or IM quinine/artesunate, 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
- **Blood transfusion in this illness: n(%) yes, no, don't know; n(% of those with yes) 1, 2, 3+**

Past clinical history

- Known to have HIV, two or more hospital admissions in the last year, 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
- **Blood transfusion ever: n(%) yes, no; n(% of those with yes) 1, 2, 3-4, 5+**

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

Coma

- Coma: n(%) yes, no, not assessed
- Eyes: n(%) not directed, directed
- Motor: n(%) no response, withdraws, localises pain
- Verbal: n(%) no response, moan only, meaningful cry

Ward tests at admission

- HIV test result: n(%) positive, negative, invalid or not done
- Lactate (mmol/l), glucose (mmol/l): median (IQR)

Working diagnosis

- Severe malaria – all types, sepsis/septicaemia, LRTI – all types, URTI – all types, other chest syndrome, profound anaemia, malnutrition, osteomyelitis/pyogenic arthritis, developmental delay, cerebral palsy, recurrent haemoglobinuria, encephalopathy, schistosomiasis, sickle cell anaemia, sickle cell crisis, meningitis – all types, HIV/AIDS, tuberculosis – all types, hepatitis, gastroenteritis, urinary tract infection, pyrexia of unknown original, dark urine syndrome, helminth infection, other: n(%)
- Summary of other working diagnosis where indicated

Presentation

- Healthcare facility first presented to: n(%) this hospital, level II, level III, level IV, other district hospital, private hospital
- Time to randomisation since presented at other facility, time to randomisation 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: n(%) positive, negative, not known. A child is positive if either the RDT or blood film provides a positive result, negative if all tests that have been done return a negative result and not known otherwise.**
- 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(%)

4.3 Description of follow-up

The following will be tabulated by transfusion randomisation and anaemia severity stratum. 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 28 days, 90 days, 180 days** and overall**: n(%)**
- **Child status at 28 days, 90 days, 180 days** and overall**: n(%) visit done, died, lost to follow up, missed visit.**

Completeness of Hb records

- **Records considered complete, defined as a non-missing entry or died before the time point, at 48 hours, 28 days, 90 days and 180 days: n(%)**
- **Record status at 48 hours, 28 days, 90 days, 180 days: n(%) Hb recorded, form entered – no Hb recorded, died, LTFU/absconded, no form entered**

4.4 Adherence to treatment

The following will be tabulated by randomisation. For transfusion adherence this will be no transfusion vs 20mls/kg vs 30mls/kg, for MVMM adherence this will be MVMM vs iron and folate and for cotrimoxazole this will be cotrimoxazole vs no cotrimoxazole.

Transfusion strategy

- **Children receiving a transfusion: n(%)**
- **Volume of first transfusion per child (mls/kg, whole blood equivalent): median, range; n(%) by volume categories of <17mls/kg, 17-23mls/kg, >23 - <27mls/kg, 27-33mls/kg, >33mls/kg**
- **Number of transfusions per child: median (IQR), maximum; n(% of those receiving a transfusion) 1 transfusion, 2 transfusions, 3+ transfusions**
- **Time until first transfusion (hours): median (IQR)**
- **First transfusion within 4 hours: n(% of those receiving a transfusion)**
- **For those randomised to receive no transfusion only: n(% those who received a transfusion) first transfusion following recorded de novo severity signs, first transfusion with no recorded de novo severity signs, first transfusion after 48 hours, no bedside obs form on the database, not compliant with protocol**
- **Volume per transfusion overall (mls/kg, whole blood equivalent): median, range; n(%) by volume categories of <17mls/kg, 17-23mls/kg, >23 - <27mls/kg, 27-33mls/kg, >33mls/kg**
- **Adherent transfusions: n(%) overall, correct amount first transfusion, correct amount second transfusion, correct amount further transfusions, stopped due to transfusion reaction, death during transfusion. Denominator is number of transfusions in that randomisation, apart from for the correct amount categories where it is the number of first, second or further transfusions as appropriate**
- **Non-adherent transfusions: n(%) overall, too little first transfusion, too much first transfusion, too little second transfusion, too much second transfusion, too little further transfusions, too much further transfusions, other reasons. Denominator is number of transfusions in that randomisation, apart from for the correct amount categories where it is the number of first, second or further transfusions as appropriate**
- For each antibiotic prescribed during admission: n(%) ever received; median (IQR) range prescription length

Nutritional supplementation

- **MVMM supplementation at discharge or initial prescription: n(%) receiving MVMM, receiving plumpynut, receiving iron and folate, died or absconded before discharge/prescription, discharge or medication form not entered**
- **MVMM supplementation post discharge: n(%) receiving MVMM, receiving plumpynut, receiving iron and folate, stopped treatment at earlier visit, stopping treatment at this visit, died or LTFU, missed visit; missed doses: n(%) any; yesterday, 2-7 days ago, 8-14 days ago, 15-28 days ago. Denominator is those enrolled long enough to be eligible for a visit at that time point, have died, are known to be LTFU or who have completed their visit early**
- **MVMM supplementation post discharge: any missed doses n(%)by Cotrimoxazole randomisation arm**

Cotrimoxazole prophylaxis

- **Cotrimoxazole prophylaxis at discharge or initial prescription: n(%) receiving cotrimoxazole, not receiving cotrimoxazole – contraindicated, not receiving cotrimoxazole – other reason, died or absconded before discharge/prescription, discharge or medication form not entered**
- **Cotrimoxazole prophylaxis post discharge: n(%) receiving cotrimoxazole, stopped treatment earlier, stopping treatment at this visit, died or LTFU, missed visit; missed doses: n(%) any; yesterday, 2-7 days ago, 8-14 days ago, 15-28 days ago. Denominator is those enrolled long enough to be eligible for a visit at that time point, have died, are known to be LTFU or who have completed their visit early**
- **Cotrimoxazole prophylaxis post discharge: any missed doses n(%) by MVMM randomisation arm**

4.5 Efficacy analyses

4.5.1 Primary outcome

- **Cumulative mortality to 4 weeks for the transfusion strategy comparison and to 6 months for the MVMM supplementation and cotrimoxazole prophylaxis comparisons**

Mortality at the specified time points will be analysed using time to event methods with hazard ratios and 95% confidence intervals calculated using stratified Cox proportional hazard models. Kaplan-Meier curves will be plotted and log-rank tests, stratified and unstratified, will be performed.

As a sensitivity analysis, flexible survival models will be used to estimate absolute survival difference over time, unadjusted and adjusted.

4.5.2 Secondary outcomes

- **Cumulative mortality at 48 hours, 28 days, 90 days and 180 days where not the primary outcome**

The transfusion strategy will be analysed at all time points, whereas the supplementation and prophylaxis randomisations will only be analysed at the time points post discharge.

Mortality at the specified time points will be analysed using time to event methods with hazard ratios and 95% confidence intervals calculated using stratified Cox proportional hazard models.

Kaplan-Meier curves will be plotted and log-rank tests, stratified and unstratified, will be performed.

- **Readmission to hospital**

Frequency of hospital readmissions will be tabulated by body systems and **by randomised group. All treatment comparisons will be analysed.**

First readmission will also be analysed using competing risks analysis with death post discharge 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. Children only become at risk from discharge.

- **Development of new profound anaemia (Hb<4g/dl) during acute admission**

Acute admission is defined as from 8 hours after admission until discharge and children only become at risk after having at least one Hb measurement above 4g/dl. Only the transfusion randomisation will be analysed.

Analysis will be using competing risks analysis with death and discharge as competing risks. Subhazard ratios and 95% confidence intervals will be calculated using cause-specific hazards regression and cumulative incidence curves will be plotted.

- **Development of severe anaemia (Hb<6g/dl) post discharge**

Analysis will be performed on all treatment comparisons.

The proportion (%) of those developing severe anaemia will be tabulated by time point and treatment arm as will the mean (SD) Hb level at each time point based on observed data only with no imputation in the primary analysis.

The difference in proportions between treatment arms at each of the visits will be estimated using poisson regression using a robust variance estimator to obtain rate ratios and 95% confidence intervals. Generalised estimating equations (GEEs) with an independent correlation structure will be used to provide an overall test of difference in the proportion with severe anaemia post discharge between the arms.

- **Proportion achieving correction of anaemia (Hb>9g/dl)**

Those achieving correction of anaemia prior to discharge will be analysed using a competing risks analysis with discharge or death. Subhazard ratios and 95% confidence intervals will be calculated using cause-specific hazards regression and cumulative incidence curves will be plotted. Correction prior to discharge will only be analysed for the transfusion strategies.

Post discharge, the proportion (%) of children achieving correction of anaemia will be tabulated by time point and by randomisation. The difference in proportions between treatment arms at each of the visits will be estimated using poisson regression using a robust variance estimator to obtain rate ratios and 95% confidence intervals. **This analysis will be performed for all treatment comparisons and as observed initially.**

The change in Hb from baseline to all times of observed Hb measurements (8 hours, 16 hours, 24 hours 48 hours, 28 days, 90 days, 180 days) will be analysed using GEEs with an independent correlation structure and adjusted for baseline Hb. Global tests of difference will be performed to compare 20mls/kg vs 30mls/kg in TRACT A and transfusion vs no transfusion in TRACT B.

Imputation using chained estimating equations within each strata and randomisation and including all Hb values, age, mls/kg received and pack type will be considered as a sensitivity analysis.

- **Changes in weight, weight-for-age, MUAC and MUAC-for-age at 90 days and 180 days**

Analysis will be performed in all randomisations.

Normal linear regression adjusted for absolute baseline values will be used to calculate mean changes from baseline and 95% confidence intervals to specified time point and mean difference and 95% confidence intervals between the treatment arms at the time points for weight, MUAC and their z-scores**.** A global test of difference between treatment arms across the time points will also be conducted using GEEs with an independent correlation structure and adjusting for baseline values as above.

- Changes in inflammatory markers (CRP, PCT), incidence of bacterial infections and malaria at 28 days, 90 days and 180 days

Changes in inflammatory markers will be measured retrospectively. Analysis of changes in inflammatory markers will be performed using normal linear regression adjusted for baseline values as above. Mean changes and 95% confidence intervals from baseline to specified time point and mean difference and confidence intervals between the treatment arms at the time points will be calculated. A global test of difference between treatment arms across the time points will also be conducted using GEEs with an independent correlation structure and adjusting for baseline values as above.

- Incidence of bacterial infections and malaria

Incidence of bacterial infections and malaria will be taken from blood cultures or molecular diagnostics retrospectively. As incidence is expected to be low and dates of onset are unlikely to be known precisely, as these events will primarily be ascertained at follow-up visits, these will be analysed using poisson regression to calculate rate ratios and 95% confidence intervals over all follow up time.

4.6 Safety analyses

4.6.1 Secondary outcomes

- **Suspected transfusion reactions: febrile reactions, TRALI (any grade); grade 3-4 toxicity of cotrimoxazole, MVMM or standard iron and folate**

Number of suspected transfusion reactions will be tabulated by transfusion randomisation and the number of reactions compared across the randomised the groups using a chi-squared test or Fisher's exact test if values are small.

Grade3-4 toxicities will also be tabulated by randomisation.

- **Serious adverse events**

The number (%) of children ever having an SAE will be tabulated and compared across randomised groups with a chi-squared test. Relationship of serious adverse events to transfusion, transfusion volume, MVMM and to cotrimoxazole will be tabulated across randomised groups and by body systems**. The number of children having SAEs (% 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 randomisation group.**

SAEs considered to be definitely, probably or possibly related to an intervention or to transfusion volume by the ERC will also be listed by the appropriate randomisation. All SAEs and all causes of death will be tabulated by transfusion randomisation.

4.7 Subgroup analyses

Subgroup analyses pre-specified in the protocol:

Subgroup	Comparison			
	20mls/kg vs 30mls/kg	Transfusion vs no transfusion	MVMM vs Iron and Folate	Cotrimoxazole vs none
Volume transfused			✓	✓
MVMM randomisation	✓	✓		✓
Cotrimoxazole randomisation	✓	✓	✓	
Centre	✓	✓	✓	✓
Anaemia severity stratification (TRACT A vs B)	✓	✓	✓	✓
Previous transfusion ever	✓	✓	✓	✓
Previous transfusion at another health centre during this illness	✓	✓	✓	✓
Rate of transfusion	✓	✓		
Fever (Temperature >37.5°C)	✓	✓	✓	✓
Malaria	✓	✓	✓	✓
HIV	✓	✓	✓	✓
Known or previously undiagnosed sickle cell disease	✓	✓	✓	✓
Microbiological evidence of sepsis	✓	✓	✓	✓

Further pre-specified subgroup analyses not included in the protocol will also examine any potential difference in the impact of treatments according to:

Subgroup	Comparison			
	20mls/kg vs 30mls/kg	Transfusion vs no transfusion	MVMM vs Iron and Folate	Cotrimoxazole vs none
Haemoglobinurea	✓	✓	✓	✓
Blood pack type (whole blood vs pack and settled cells)	✓	✓		
Donor haemoglobin	✓	✓		
Donor haematocrit	✓	✓		
Malnutrition*	✓	✓	✓	✓
Dehydration (sunken eyes or decreased skin turgor)	✓	✓		
Shock (one of weak pulse volume, temperature gradient or capillary refill time ≥2s)	✓	✓	✓	✓
Hypothermia (<36°C)	✓	✓	✓	✓

* **Severe malnutrition (SAM):** SAM defined as one or more of mid-upper arm circumference (MUAC) < 11·0cm (children aged 2 to 6 months) or MUAC < 11·5cm (children aged 6 months to 59 months) or WHZ < -3 (or WAZ if height not recorded) or presence of kwashiorkor at any age. **Undernutrition:** defined as one or more of as mid-upper arm circumference (MUAC) ≥ 11·0cm – 11.9cm (children aged 2 to 6 months) or MUAC ≥ 11·5cm – 12.4 (children aged 6 months to 59 months) or WHZ-3 to -2 (or WAZ if height not recorded) at any age. **Well nourished:** None of the above

Subgroup analyses will be based on tests of interaction. Continuous factors will be grouped into terciles. Fractional polynomial models will also be used to investigate interactions between randomised group and continuous factors (Royston and Sauerbrei, Stat. Med 2014).

4.8 Additional analyses

Additional analyses will investigate the impact of donor haematocrit, donor Hb, donor inherited blood disorders (G6PD, alpha thalassaemia, sickle cell trait), blood pack type, age of donor blood, actual ml/kg received (whole blood equivalent), child's iron status, child's inherited blood disorders (G6PD, alpha thalassaemia, sickle cell trait), age of the child and baseline values of Hb, weight-for-age and MUAC-for-age z-scores on outcomes.

TRACT SAP Revision History – further details.

Version	Author	Date	Reason for Revision
Draft 0.1			Protocol version 1.0
Draft 0.2	Leanne McCabe	26Jan2016	Leanne McCabe first draft
Draft 0.3	Leanne McCabe	19Feb2016	Incorporated Sarah Walker's comments on first draft: to clarify handling of free text fields, continuous measures, definition of malaria, to add further detail on included analysis methods and sensitivity analyses.
Draft 0.4	Leanne McCabe	10March2016	Incorporated IDMC's comment on second draft: clarifying population for secondary analysis of nutritional support and cotrimoxazole randomisation as all children alive at minimum of discharge or 5 days of admission.
Final 1.0	Leanne McCabe	04April2016	Incorporated TSC's comments on third draft: clarification of blood volume calculation and an additional subgroup analysis regarding malnutrition.
Draft 1.1	Elizabeth George	06June2017	Clarification of population detail, additional subgroup analyses and additional analyses.
Final 2.0	Elizabeth George	24July 2017	Incorporated Kath Maitland's comments on draft 1.1: describing microbiology at admission, further description of missed doses of MVMM or iron and folate and cotrimixazole, and additional analyses of inherited blood disorders and iron status.