# Vascular Function Intervention Trial in sickle cell disease: V-FIT

## Development of a ready-to-use nutraceutical food for patients with sickle cell disease (SCD): Testing of vascular support components

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**Protocol authorised by:**

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### Sponsor

London School of Hygiene & Tropical Medicine is the main research sponsor for this study. For further information regarding the sponsorship conditions, please contact Clinical Trials QA Manager

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The Wellcome Trust, UK

This protocol describes the V-FIT study and provides information about procedures for entering participants. The protocol should not be used as a guide for the treatment of other participants; every care was taken in its drafting, but corrections or amendments may be necessary. Problems relating to this trial should be referred, in the first instance, to the Trial coordinator.

This trial will adhere to the principles outlined in the International Conference on Harmonisation Good Clinical Practice (ICH GCP) guidelines, protocol and all applicable local regulations.

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## GLOSSARY OF ABBREVIATIONS

| | |
|---|---|
| MUHAS | Muhimbili University of Health & Allied Sciences |
| MNH | Muhimbili National Hospital |
| RUSF | Ready-to-use-supplementary food |
| L-Arg | L-Arginine |
| L-Cit | L-Citrulline |
| CQ | Chloroquine |
| FMD | Flow Mediated Dilatation |
| TNEC | Tanzanian National Ethics Committee |
| TFDA | Tanzania Food and Drug Authority |
| | |

## KEYWORDS

Sickle cell disease
Tanzania
Ready-to-use-supplementary foods
Chloroquine
Children
Vascular function
Growth
Nitric oxide

## STUDY SUMMARY

**TITLE** **Development of a ready-to-use nutraceutical food for patients with sickle cell disease (SCD): Testing of vascular support components**

**DESIGN** Double-blinded, two-treatment, randomised, crossover clinical trial of a food based, 'nutraceutical' intervention.

**AIMS** To develop a safe, deliverable, cost-effective intervention to improve the long-term health of sickle cell disease (SCD) patients in low-income countries.

**OUTCOME MEASURES** The primary endpoints on which the trial is powered are growth velocities (height, weight and fat-free mass), plasma amino acid profiles and nitric oxide dependent endothelial function assessed by flow-mediated dilatation.

**POPULATION** The study will enrol 120 children from within the Wellcome Trust supported hospital-based Muhimbili Sickle Cohort (MSC) (N≈2,000) at Muhimbili National Hospital, Dar-es-Salaam, Tanzania

**ELIGIBILITY** Children will be eligible if they are enrolled in the MSC, resident in urban Dar-es-Salaam and aged 8-11 years at enrolment.

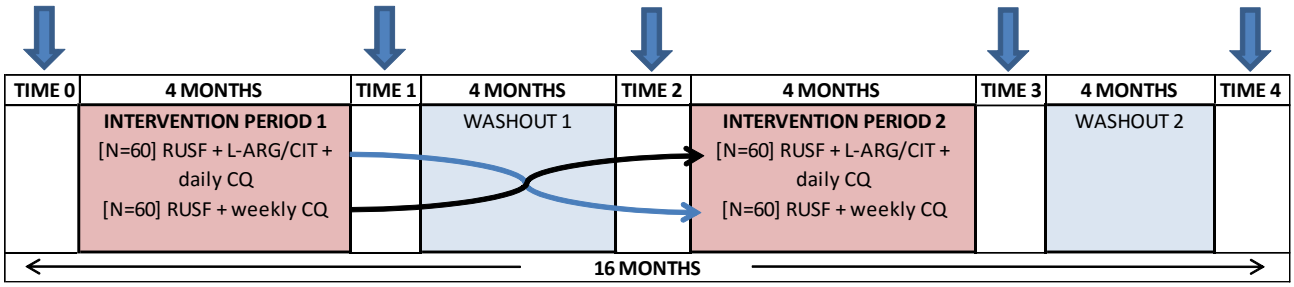
**TREATMENT** Treatments are assigned in random order consisting of:

**(A)** 4-months of twice-daily lipid-based, ready-to-use supplementary food (RUSF), comprehensively fortified with vitamins and minerals (1 x RDA) and delivered with weekly chloroquine and daily placebo; or,

**(B)** the same RUSF additionally fortified with arginine (L-Arg) and citrulline (L-Cit) amino acids and delivered with daily chloroquine.

**DURATION** Follow-up over 16 months.

## REFERENCE STUDY DESIGN DIAGRAM



# 1. INTRODUCTION

## 1.1 BACKGROUND

*Prevalence, morbidity, mortality and health burden of SCD in the African setting*

The homozygous inheritance of sickle haemoglobin (HbSS), is the most severe and predominant type of SCD. An estimated 7,800 children with HbSS are born annually in Tanzania, the 4th highest globally [1]. Current estimates suggest survival rates to adulthood of 20-50% in African settings [2], compared to up to 94% survival in the USA [3, 4] and up to 99% in the UK [5]. Improvements in public health in Africa are thought to be increasing the survival of infants [6] and the introduction of penicillin prophylaxis and pneumococcal vaccination, especially in the context of neonatal screening, will further decrease mortality in early life [2]. This gain will result in increased numbers requiring chronic care and place additional burden on limited health-care resources. It is estimated that 6 million people will be living with SCD in Africa [1]. In the near- to medium-term the costs and complexity of follow-up for interventions such as hydroxyurea (HU) therapy and prophylactic blood transfusions will make them difficult to implement outside of some tertiary level centres.

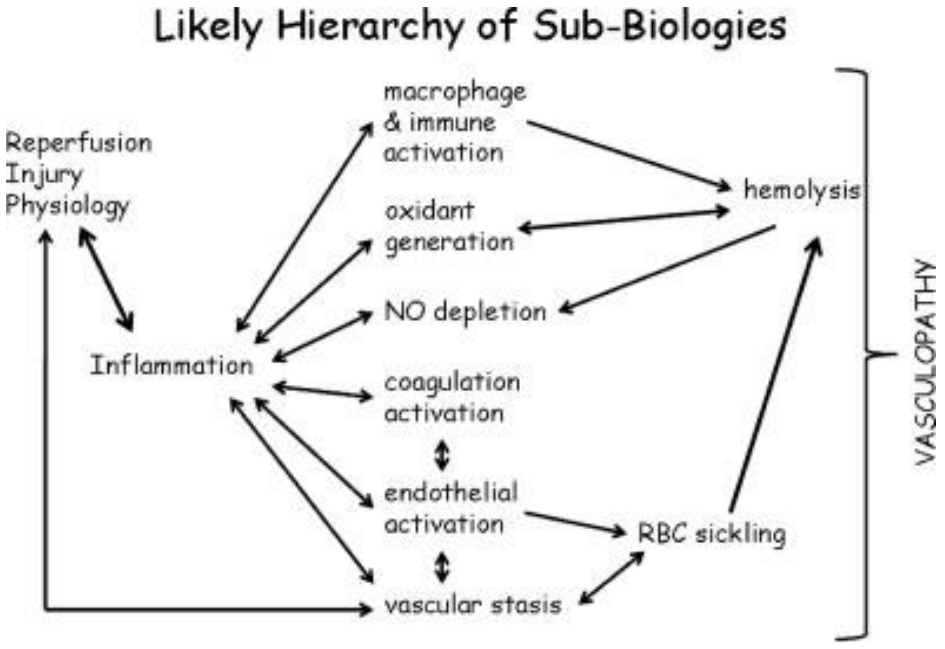
*Malnutrition in SCD*

Reduced growth has frequently been observed in SCD [7-9]. Energy and nutrient supplies are limited or perturbed in SCD from reduced dietary intake [10, 11], elevated metabolic rate [12-14], increased nutrient degradation and disturbed metabolic pathways [13, 15, 16].

*Vascular dysfunction in SCD*

In those that survive the first five years of life vasculopathy is an important cause of subsequent morbidity and mortality [17, 18]. A spectrum of endothelial dysfunction or "vasculopathy" has been documented that includes abnormal tone, responsiveness, structure and an activated and adhesive state of the vessels [19]. The many roles of the endothelium may be disrupted in SCD by several pathways (**Figure 1**).

***Figure 1. Probable hierarchies of implicated sub-biologies in SCD vasculopathy***
*(taken from reference 19).*



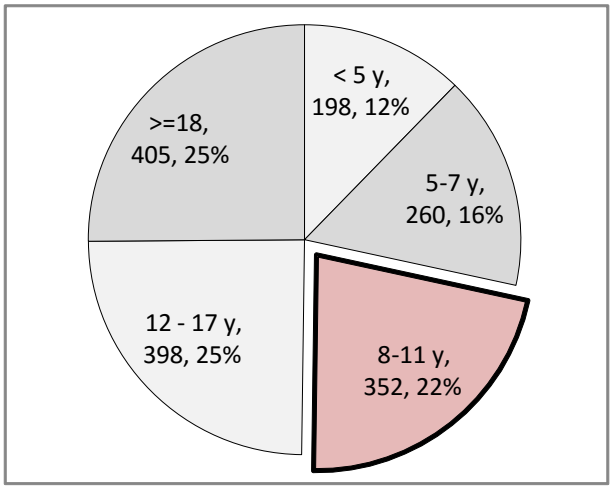
Polymerisation of sickle haemoglobin under hypoxic conditions causes sickling of red cells. This is thought to be the initiating cause of interacting downstream processes, causing vascular stasis and ischaemic reperfusion injury, leading to inflammation and activation of the endothelium. Intravascular haemolysis causes elevated oxidant stress, further endothelial and immune cell activation and decreased nitric oxide (NO) bioavailability. Plasma arginase is released from ruptured erythrocytes, platelets [20] and possibly from liver damage, resulting in abnormally high plasma levels [21]. Arginase degrades plasma arginine, the sole substrate of endothelial nitric oxide synthase (eNOS), thus limiting NO production. Ornithine is the degradation product from arginase activity and is a competitive inhibitor of arginine uptake by endothelial cells, thus the arginine:ornithine ratio may be critical to eNOS activity. Low plasma arginine is common in SCD [22], and is further decreased during vaso-occlusive episodes (VOC) and acute chest syndrome (ACS) [23]. Low plasma ratios of arginine to ornithine and high asymmetric di-methylated arginine (ADMA), an endogenous inhibitor of eNOS, are observed in SCD and are associated with increased pulmonary artery pressure and death in adult SCD patients [21, 24-26]. Reduced NO bioavailability is implicated as a key component in the pathophysiology of a range of severe morbidities in SCD and other disorders including malaria and cardiovascular disease [27-32].

We propose that sub-optimal nutrition contributes to the processes involved in vascular pathology including haemolysis, NO bioavailability, vascular stasis, reperfusion injury, oxidative stress and hypoxia. This forms the basis for our proposed nutraceutical intervention for which the **detailed supporting case and justification of dosages and choice of assessment methods** is provided in **Appendix 1**.

*The Muhimbili Sickle Cohort (MSC)*

The Muhimbili Sickle Cohort was started in March 2004 with a Wellcome Trust clinical PhD fellowship awarded to Dr Julie Makani. As of May 2011 there were 1,613 enrolled SCD patients (HbSS) regularly attending clinic at Muhimbili National Hospital (MNH) (**Figure 2**) and resident in urban Dar-es-Salaam, with 118 documented deaths and 234 patients lost to follow-up. Newborn screening is not yet available; hence this represents a survivor cohort. Patients in the target 8-11y age group are prescribed daily folate. Clinical and laboratory data are collected, and samples stored from all routine clinic visits and during hospitalization. We believe this may be the world's largest single site SCD cohort. In the absence of newborn screening, the mortality rate is highest in the youngest age group 7.4/100 PYO [95% CI 4.8-11] in the under 5-year age group and 1.4/100 PYO [95% CI 1.1-1.9] in the 5-19 year olds, (overall rate is 2.0/100 PYO [95% CI 1.5-2.9]) [33].

***Figure 2. Age structure of 1613 HbSS enrolled patients currently attending MNH clinics on 1st May 2011*** (highlighting shows target age group for this trial).



The MSC differs significantly from cohorts based in the USA, UK or Jamaica due to the near 100% of patients with HbSS, compared to the HbSC observed in diaspora populations of a West African origin (with its milder clinical course and lower mortality rate). Additionally a very high proportion of Tanzanian SCD patients (>90%) (Makani et al. unpublished data) have the Central African Republic (Bantu) haplotype associated with more severe disease and end-organ damage [34] compared to the Senegal, Benin or Arab haplotypes. This more severe sub-type of SCD strengthens the need for intervention and offers an appropriate location for this trial.

*Malnutrition in MSC*

The growth charts in Appendix 2 illustrate the profound growth retardation of patients in the MSC. From around 2 years old, median heights and weights are close to the 5$^{th}$ centile of the UK 1990 standards, with the greatest difference in teenage years (partly an artefact of delayed puberty). Local non-sickle controls are also stunted and underweight but even using these controls SCD predicts stunting (OR=1.82 [95%CI 1.43/2.32] P<0.001) and wasting (1.66 [1.21/2.28] P=0.002 respectively)[35]. Unsurprisingly, wasting was a predictor of hospital admissions: increasing 13% for each Z-score decrease in BMI [35]. Within the MSC, preliminary data indicate a mean age at Tanner Stage 2, when children normally experience increased growth rates, of 13.8y in boys and 14.1y in girls, and a mean age at menarche of 16y (N=54) (Jacob & Cox preliminary data). These data have determined our choice of the 8-11y age group, to avoid complications of puberty especially in interpreting growth effects, whilst being old enough for SCD to have initiated effects on the vascular system.

*Micronutrient status in the MSC*

Steady state concentrations of vitamins B and C and markers of iron status were measured in SCD and non-SCD controls as part of our previous WT-funded project grant (WT 80025). Except for folate (patients receive 1 or 5mg/d) the cohort exhibits high levels of deficiency according to standard cut-offs (58% for vitamin C, 57% for vitamin $B_6$ and 45% for $B_{12}$). Markers of iron status suggest a lower prevalence of iron deficiency in the MSC compared to local controls: transferrin saturation <16% = 25% [N=835] *vs* 47%[N=79] in controls. Moreover, our data suggest that higher iron status (but not iron overload) assessed by transferrin saturation is associated with lower daytime and nocturnal haemoglobin oxygen saturations [36]. Transferrin saturation is not associated with haemoglobin concentrations in SCD cases, compared to a strong correlation in the non-SCA controls, whilst adequate iron status assessed by the F-index (ratio of soluble transferrin receptor to logged ferritin), is paradoxically associated with greatly increased odds of having averaged steady state haemoglobin concentrations in the lowest septile (RR=5.45 [2.71/10.96] P<0.001). Hence iron will not be included in the fortificants of the current intervention.

*Amino acid status in MSC*

In a small pilot nested-case-control study, we assessed plasma amino acids in stored steady-state plasma samples from 11 SCD patients who had died (age at death 20.9±7.4y) compared to 12 matched survivors. In confirmation of results from adult patients in the USA [21], we found significantly lower ratios of arginine to ornithine (0.51±0.14 *vs* 0.68±0.17, p=0.014) in the patients who died [37]. Furthermore, plasma arginine and arginine:ornithine ratios were significantly lower in our SCD patients compared to non-sickle children from Dar-es-Salaam 37±13 *vs* 120±10µmol/L and 0.60±0.18 *vs* 1.35±0.49 (Mwaikambo & Granger personal communication). Indeed plasma arginine was comparable to those observed in children with cerebral malaria (40µmol/L) [38].

## 1.2 RATIONALE FOR CURRENT STUDY

### 1.2.1 Study rationale

Sickle cell disease (SCD) is the world's most common single gene disorder affecting 300,000 births annually. In high-income countries, interventions and comprehensive care packages have greatly reduced mortality in children and young adults, though survivors still suffer significant disability and long-term organ damage. In sub-Saharan Africa, home to >70% of SCD patients, limited data suggests mortality is decreasing but morbidity and disability rates remain many fold higher. In low-income settings, the relative cost and complexity of standard therapies (routine blood transfusion and HU, for the prevention of complications may limit their widespread implementation. Alternative and complementary, logistically simple, safe and cost effective Interventions are required.

The intervention is designed to target:

i. the moderate to severe growth retardation commonly observed in children with SCD especially in low income countries;
ii. endothelial dysregulation secondary to low NO bioavailability, inflammation and oxidant stress, hypothesised to underlie much of the clinical pathology in SCD, including risk of stroke, priapism, acute chest syndrome, vaso-occlusive episodes and eventual chronic end-organ damage in later life.

If successful then larger studies of efficacy and effectiveness would be needed to assess long-term endpoints of hospitalization, stroke, and mortality. Existing evidence suggests that the proposed intervention also has the potential to increase the efficacy of HU therapy.

The successful development of an affordable ready-to-use 'nutraceutical' food with proven efficacy in growth promotion and vascular health could represent a major step forward for SCD patients in low-income countries.

### 1.2.2 Research questions

**1)** Can provision of a comprehensively-fortified ready-to-use supplementary food (RUSF) reverse the growth retardation frequent in Tanzanian children with SCD?

**2)** Can further supplementation with the NO substrates arginine and citrulline plus the addition of daily chloroquine (an arginase inhibitor) ameliorate vascular pathology by improving NO-dependent endothelial function, assessed by flow mediated dilatation?

### 1.2.3 Study hypotheses

This study will test the following hypotheses:

1. That the provision of energy, protein and micronutrients within a ready to use supplementary food will increase linear growth, weight gain and proportion of fat-free mass children with SCD.
2. That the provision of supplementary L-arginine and L-citrulline within the matrix of a twice-daily RUSF plus daily chloroquine (CQ) for 4 months, compared to a standard RUSF and weekly anti-malarial prophylaxis CQ to children with SCA will:
    a. Increase plasma arginine concentrations and the ratio of plasma arginine: orninthine.
    b. Decrease or not alter plasma ADMA concentrations
    c. Improve NO-dependent vascular function as detected by an increase in maximum flow mediated dilatation ($FMD^{max}$)
3. That the provision of daily CQ at a dosage of 2-3mg base/kg/day for 4 months to children with SCA will:
    a. Decrease the activity of plasma arginase through competitive inhibition

b. Decrease levels of plasma inflammatory markers

# 2. STUDY OBJECTIVES

The aims of this trial are to determine the effects of an RUSF on growth in a non-screened population of African children with SCD and to determine whether an RUSF fortified with L-arginine and L-citrulline, delivered with daily CQ, compared to the standard RUSF can increase NO bioavailability and consequent improved vascular endothelial function.

The primary and secondary objectives are to detect effects on the selected endpoints as detailed below (Section3.2 Study outcome measures).

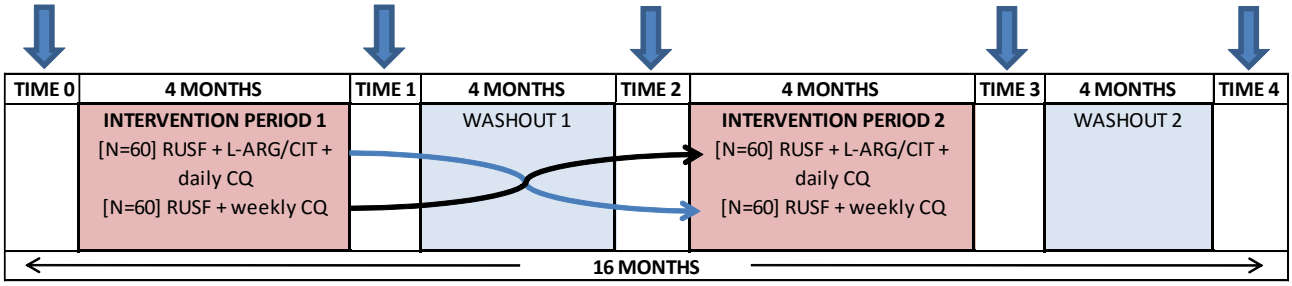
# 3. STUDY DESIGN

The study comprises a random-ordered, double-blinded crossover clinical trial of two interventions with 4-month washout periods – see Fig 3 below. A crossover design has been selected on the grounds of increased power and because we deemed the use of a control group receiving no RUSF to be potentially ethically questionable. The proposed design allows growth trajectories on RUSF to be compared to the adjacent 4 month periods without RUSF.

**Fig 3 Study Design.**

**PRIMARY ENDPOINTS**

1. **Plasma amino acids, arginase will be assessed at time points 0,1 & 3**
2. **Vascular function (flow mediated dilatation) will be assessed at time points 0, 1, 2 & 3**
3. **Growth (SCD-specific height-for age & BMI Z-scores) will be assessed at time points 0,1,2,3 & 4**




120 children with confirmed SCD and enrolled in the MSC will be enrolled

## 3.1 SAMPLE SIZE JUSTIFICATION

The sample size of 120 has more than 99% power to detect a small, but potentially clinically relevant effect size (Δ) of 1.25 unit change in $FMD_{max}$ between the treatment arms, thus even if we allow for 33% drop outs or incomplete data at any of the time points, we will still have more than 95% power. Similarly a sample size of 120 has more than 99% power to detect a 20% difference in growth rates for height and weight.

## 3.2 STUDY OUTCOME MEASURES

Primary endpoints:

1. ***Plasma amino acids and arginase levels and activity (Time points 0,1,2&3):*** Plasma for complete amino acid profiles including ADMA (by ion-exchange elution Biochrom-30, Biochrom, UK) and arginase analyses [39] will be separated and frozen immediately.

2. ***Assessment of vascular function (Time points 0,1,2&3):*** In a temperature controlled room, we will assess NO-dependent endothelial function by measuring brachial arterial dilatation with ultrasound (Siemens Accuson P50) in response to hyperaemia (5 min at 200 mmHg) induced after release of transient blood pressure cuff occlusion (Hokanson SC5), a technique known as flow mediated dilatation (FMD). Automated B-mode image edge detection will be used to calculate the maximum FMD percentage ($FMD_{max}$) as a change from resting baseline (Brachial Tools, Medical Imaging Applications) using a stereotactic holder with micrometer movement for the 12Mz probe [40, 41]. Endothelial-independent responses to 25µg sub-lingual glyceryl-trinitrate (GTN) will also be measured. All measurements will be performed by a single operator trained by the Halcox group (see appendices for justification of choice of FMD and detailed FMD protocol).

3. ***Anthropometry (Time points 0,1,2,3 & 4):*** Height, weight, lean body mass (LBM) and fat mass (FM) (Tanita BC418 segmental bio-impedance analyser) will be measured independently by two rigorously trained and cross-validated SP with real-time data-entry checks for acceptable ranges between readings. Equipment will be checked weekly and calibrated as necessary.

Secondary endpoints:

1. ***Haemoglobin concentration (Time points 0, 1, 2, 3 & 4):*** Full blood counts (FBC) of EDTA whole blood will be conducted (Pentra 60, Horiba ABX).

2. ***Markers of inflammation and vascular activation*** [42-44] ***(Time points 0, 1, 2 & 3):*** Concentrations of soluble adhesion molecules (VCAM-1, VEGF-1, TNF-α & e-selectin, tissue factor and IL-6) will be measured in frozen (-80$^{o}$C) plasma aliquots (ELISA kits). C-reactive protein concentrations will be measured in frozen serum samples (Architect C8000, Abbott Diagnostics) and leukocyte counts from FBC.

3. ***Markers of haemolysis (Time points 0, 1, 2 & 3):*** plasma-Hb will be measured in frozen serum aliquots (ELISA, Bethyl Labs) and unconjugated bilirubin and lactate dehydrogenase in fresh serum (Architect C8000, Abbott Diagnostics).

4. ***Estimated Glomerular filtration rate (eGFR)(Time points 0, 1, 2, 3 & 4):*** Serum createnine will be measured in fresh serum samples at all time points and used to estimate GFR using the Schwartz equation for children, or a modified version in which measured muscle mass is used in place of the estimated value in the k constant.

5. ***Frequency of VOC-painful episodes***: study personnel will administer detailed questionnaires at bi-weekly home visits and by telephone in the intervening weeks to assess the frequency of all sickle and non-sickle associated morbidity and health seeking behaviour, with a focus on painful episodes. Participatory research will be used to determine the likely application and optimal formatting of pain diaries to be completed by patients and families in addition to the standard questionnaire.

## 3.2 RISKS AND BENEFITS

### 3.2.1 Potential benefits

Participation in the trial should lead to benefits for all children as a result of increased contacts with health professionals both at the study clinic visits and through the weekly home-based visits by study personnel and increased rates of early detection and management of SCD-related events. It is likely that the process of monitoring painful episodes at home will lead to improved management within the home and increased care-seeking behaviour.

Both RUSF interventions are expected to increase growth and improve malnutrition in study participants (primary endpoint) and improve micronutrient status. Both the RUSF and RUSFv contain additional folate thus replacing the need for folate supplementation. Both RUSF interventions may increase steady-state haemoglobin levels (a secondary endpoint), both of which are likely to be associated with increased well-being and quality of life. Lower steady state haemoglobin level is associated with an increased risk of hospitalisation and mortality in the MSC and wasting with an increased risk of hospitalization [35]. The RUSFv may improve vascular function, the downstream effects of which could result in a reduction in the number and or severity of vaso-occlusive painful episodes, assessed as a secondary endpoint.

#### 3.2.2 Potential risks

There are minimal safety concerns associated with these interventions.

The level of **micronutrient fortification** is conservative at 1xRDA. Additional iron is not being included in the fortificants. In the absence of tolerable upper intake levels for amino acids, an observed safe level for long term use of L-arginine has been estimated at 20g/day for healthy adults, although few side-effects have been reported at much higher doses [45]. Whilst this is not available for L-citrulline, we can find no reports of side effects or toxicity associated with its use. No side effects were reported in a pilot phase II trial of L-citrulline in 5 SCD adolescent subjects (approx 0.1g/kg/day). Similarly, no toxicity or negative effects have been reported in randomised trials of L-citrulline infusions in children undergoing heart surgery [46, 47], or 4 weeks oral supplementation (6g/day) in healthy adults [48]. Mild diarrhoea and intestinal disturbance has been proposed as a possible side effect of supplementation with these amino acids, from increased local NO production. However, a recent review of available studies concluded that gastro-intestinal side effects were only associated with single large doses of 9g or more [49].

**Chloroquine**. As with all drugs there are risks of experiencing side effects, adverse reactions, drug interactions and risk of overdose. The daily dose of chloroquine we will be giving is approximately half that recommended in BNF treatment guidelines for use in children with rheumatoid arthritis, whilst the weekly dose is as per current Tanzanian National Malaria Control Programme and British National Formulary (BNF) guidelines for use as a prophylactic. Long term-use of CQ has been associated with rare incidence of associated retinopathy and yearly ophthalmologic examinations are recommended by some authorities. Considering the short duration of the daily chloroquine and lower dosage, such outcomes are considered extremely unlikely. The risk of accidental over-dose is limited with both of our tested interventions due to the limited supply of CQ provided at any one time (please see 6.1).

# 4. PARTICIPANT ENTRY

## 4.1 PRE-RANDOMISATION OR PRE-REGISTRATION EVALUATIONS

Pre-enrolment trial procedures include the following:

Patient eligibility will initially be screened based on data available from the MSC database and hospital records (place of residence, age, SCD disease status, normal previous steady state liver and renal function tests and known illnesses – eg HIV). Parents of eligible children will be contacted by telephone or at scheduled routine outpatient clinics and these details confirmed and the presence of any known exclusion criteria checked (e.g. use of hydroxyurea) and if appropriate, informed about the trial and asked if they would consider for their child to participate. On receipt of this verbal consent, we will make a study appointment at which full informed written consent will be sought, and if obtained, baseline examinations conducted.

### 4.2 INCLUSION CRITERIA

- Aged 8-11 years old at enrolment and resident within urban Dar-es-Salaam
- Enrolled in MSC and attending routine MNH sickle clinics
- HbSS phenotype confirmed by electrophoresis and HPLC

### 5.3 EXCLUSION CRITERIA

- >95$^{th}$ percentile for body mass index (BMI) for age using British 1990 growth standards
- Receiving HU therapy or significant other long-term drug therapy
- Diagnosis with clinically significant non-SCD related disease including:
    - stage III or above HIV – or receiving ART therapy regardless of AIDS stage
    - Tuberculosis infection
- Blood transfusion within previous 30 days
- Previously diagnosed clinical pulmonary hypertension or cardiac dysfunction or clinical signs of pulmonary hypertension (loud pulmonary second heart sound) or heart failure (displaced apex beat, high jugular venous pressure, enlarged liver, peripheral oedema)
- Low visual acuity at baseline (<6/9 using a modified (for Tanzania) Snellen chart or previously diagnosed chronic eye disorder likely to suggest retinopathy or macular degeneration
- Significant hepatic/renal dysfunction assessed by clinical chemistry panel at baseline
- Epilepsy, psoriasis or currently taking any drugs listed as interacting with chloroquine

### 4.4 WITHDRAWAL CRITERIA

This trial is overseen by a Data Safety Monitoring Board (DSMB) operating under a charter. The DSMB will monitor adverse events (AE's) and severe adverse events (SAE's) and advise the trial sponsor on whether the trial should be stopped or modified at any time due to safety or for implementation reasons. No interim analyses of the primary endpoints are planned. Participants who suffer SAE will be thoroughly investigated and the Trial Steering Committee (TSC), sponsor and statistician will be consulted. In the event of an SAE resulting in a death, the statistician will undertake an interim analysis of the number of AE by treatment within 1 month. At present, in the absence of an SAE occurring, the proposed strategy is to analyse the number of AE's by treatment at 4 monthly intervals.

Patients may withdraw at any stage without giving a reason and their clinical care will not be affected. If study participants wish to withdraw their consent from the study at any time, they will be asked if they are willing to give permission for archived samples to be used for study analyses. Written consent will be required in order for this to occur. If consent is not provided, archived samples will be destroyed and data will not be used in analyses of study outcomes, but may be used in comparisons of baseline data between groups of patients, screened, randomised, completed and drop-outs.

## 5. RANDOMISATION AND ENROLMENT PROCEDURE

### 5.1 RANDOMISATION & BLINDING

Study sample ID numbers will be generated in advance and block randomised (in blocks of 12) to one of the four treatment codes for each of the two treatment periods. Participants will be assigned the next sequential study ID at enrolment. The **allocation code** (representing the 4 shapes used on the RUSF packets) indicating the contents (RUSFv *vs.* RUSF) will be generated by the RUSF manufacturer and known only to the producer, the DSMB and the study pharmacist. The same allocation code will be used by the study pharmacist when making up and dispensing participant CQ syrup bottles each week (see section 6.X). Packages of RUSFv and RUSF will be delivered by the producer in lots designated by allocation code. The manufactuer of the CQ and placebo syrups will deliver these in 5L containers, manufactured as single lots to the study site. The trial

pharmacist will make up the CQ and placebo bottles for the trial participants using the study ID randomisation list, allocation code and weight category information collected at baseline. Participants will be recruited to sequential study IDs after having signed consent forms and completed baseline assessments. Participants will be asked on exit from the study if they could guess their treatments.

### 5.2 UNBLINDING

Reporting of SAEs and SUSARs will not require un-blinding of the research staff managing the day to day activities of the trial. Un-blinded data will only be available to the DSMB.

## 6. TREATMENTS

### 6.1 TREATMENT ARMS

Both interventions will consist of twice-daily RUSF in single portion packs, comprehensively fortified with vitamins and minerals at approximately 1xRDA (except for folate [1mg/day] and iron [not included in the fortificants]) providing 500kcal/d (**Table 1**). The simple RUSF will be given with placebo base syrup on 6/7 days and chloroquine (malaviron, Wallace Manufacturing Chemists, UK) every 7$^{th}$ day to match the anti-malarial action of chloroquine in the vascular arm and as per Tanzanian guidelines. The enhanced 'vascular-RUSF' (RUSFv) will be additionally fortified with L-arginine and L-citrulline depending on subject weight (<or≥ 25kg, median weight is 24kg in this age range) to achieve mean intakes of 0.2g L-Arg and 0.1g L-Cit/kg/day and maximum intakes of 0.33/0.165g/kg/d. The RUSFv will be given with daily chloroquine syrup to achieve a maximum dose of 3mg base/kg/day (**Figure 4**).

**Table 1. Detailed amino acid and micronutrient composition of RUSF intervention**

| | Daily dose | RUSF (placebo) | RUSFv (low, <25kg) | RUSFv (high, >25kg) |
|---|---|---|---|---|
| **Calories total** | Kcal | 500 | 500 | 500 |
| **Proteins** | g | 13 | 13 | 13 |
| **Arginine** | g | 0 | 5 | 7,5 |
| **Citrulline** | g | 0 | 2,5 | 3,75 |
| **Vitamin A /** Retinol | µg | 600 | 600 | 600 |
| **Vitamin B1 /** Thiamine | mg | 1,1 | 1,1 | 1,1 |
| **Vitamin B2 /** Riboflavin | mg | 1 | 1 | 1 |
| **Vitamin B3 /** Niacin | mg | 16 | 16 | 16 |
| **Vitamin B5 /** Pant. Acid | mg | 5 | 5 | 5 |
| **Vitamin B6 /** Pyridoxine | mg | 1,2 | 1,2 | 1,2 |
| **Vitamin B8 /** Biotin | µg | 25 | 25 | 25 |
| **Vitamin B9 /** Folate | µg | 1000 | 1000 | 1000 |
| **Vitamin B12 /** Cobalamin | µg | 2,4 | 2,4 | 2,4 |
| **Vitamin C /** Ascorbate | mg | 45 | 45 | 45 |
| **Vitamin D /** Calciferol | µg | 15 | 15 | 15 |
| **Vitamin E /** Tocopherol | mg | 11 | 11 | 11 |
| **Vitamin K** | µg | 55 | 55 | 55 |
| **Phosphor** | mg | 1250 | 1250 | 1250 |
| **Calcium** | mg | 1300 | 1300 | 1300 |
| **Potassium** | mg | 4,5 | 4,5 | 4,5 |
| **Iodine** | µg | 120 | 120 | 120 |
| **Iron** | mg | 0 | 0 | 0 |

| | | | | |
|---|---|---|---|---|
| **Magnesium** | mg | 240 | 240 | 240 |
| **Selenium** | µg | 40 | 40 | 40 |
| **Copper** | µg | 700 | 700 | 700 |
| **Zinc** | mg | 11,2 | 11,2 | 11,2 |
| **Sodium** | g | low | low | low |

The RUSF will be manufactured to GMP standards, by Nutriset, France, with batch certificates and following recommended International Code of practice for foods for infants and children of the Codex Alimentarius Standard CC/RCP 21-1979 and ISO22000 standards.

The chloroquine syrup and placebo base syrup will be purchased from Wallace Manufacturing Chemists, UK, manufactured to GMP standards.

The RUSF product is being provided by Nutriset at a flat-rate research cost of 3 Euros/Kg. The product will be delivered to the research site in 2 shipments and will be stored in a dedicated room at room temperature, with stock monitoring and tracking protocols, with access to remove stock restricted to named staff.

***Figure 4. RUSF fortification formulation per sachet pack (to be consumed twice daily)***

| | **< 25 Kg [expected range 15-24.9 kg]** | | **> =25 kg [expected range 25-40 kg]** | |
|---|---|---|---|---|
| | **RUSF** | **RUSFv** | **RUSF** | **RUSFv** |
| **Chloroquine** | 150 mg weekly* | 50 mg daily† | 225 mg weekly* | 75 mg daily† |
| **L-Arginine** | -- | 2.5 g | -- | 3.75 g |
| **L-Citrulline** | -- | 1.25 g | -- | 1.875g |
| **Folate** | 0.5 mg | 0.5 mg | 0.5 mg | 0.5 mg |
| **Energy kcal** | 250 | 250 | 250 | 250 |

*All formulations will be fortified to achieve daily intakes of approx 1x Reference Nutrient Intake (WHO/ UK) or Recommended Daily Allowance (USDA), whichever is the higher of the following vitamins and minerals: Vitamins A, B1, B2, B6, B12, C, D, E, K, biotin, pantothenic acid, niacin, potassium, calcium, phosphorus (excluding phytate), magnesium, zinc, copper, selenium, iodine and manganese.*

*No additional iron will be included in the fortificant mix.*

**BNF for children 2007 p390, chloroquine for malarial prophylaxis*

*†BNF for children 2007 p573, chloroquine use in juvenile active rheumatoid arthritis/systemic & discoid lupus erythromatosus*

*All added mineral salts and vitamins are on the advisory list of mineral salts and vitamin compounds for use in foods for infants and children of the Codex Alimentarius Standard CAC/GL10-1979.*

***Dosage of L-arginine and L-citrulline***

The final targeted dosages of the amino acids (0.2g/kg/d for L-arginine plus 0.1g/kg/d for L-citrulline) were chosen on the basis of three criteria: 1) safety; 2) evidence of efficacy; and 3) limitations on the amounts that can be incorporated into the RUSF, whist maintaining sufficiently high energy-density, taste and textural properties.

The observed safe level (OSL) for L-arginine is 20g/day in healthy adults (please see below). This is equivalent to 0.33g/kg/d assuming an average adult weight of 60kg. Doses of 0.1-0.2g/kg/day of L-arginine [50, 51] & L- citrulline [52] have been utilized previously in SCD subjects with no adverse effects or toxicities reported and resulted in significant increases in plasma arginine concentrations. Three months' L-arginine

supplementation (0.1-0.2g/kg/d) increased plasma arginine over time from baseline (50.1+/-17.0 µMol/L, N=8) to a near 100% increase at 12 weeks [50].

A lower dose of L-citrulline compared to L-arginine is proposed based on: i) doubled or greater AUC plasma arginine responses compared to L-arginine [53]; ii) relative expense of L-citrulline compared to L-arginine; and iii) less evidence available to determine observed safe levels.

***Packaging of the interventions***

The daily RUSF rations will come in 2 chains of 7 packets. Each chain of 7 will be numbered 1-7 and labelled with the corresponding day of the week with arrows printed pointing from 1 to 7 (**Figure 5a & b**). The chloroquine and placebo base syrup will be provided as amber coloured translucent plastic bottles of 2 different sizes (400ml & 100ml) with safety dispenser caps and appropriate labelling to each participant. **In the weekly CQ intervention**, the larger bottle will contain placebo base syrup enough to provide 2 weeks, plus 2 extra days (14 daily doses) of supply and the smaller bottle will contain 2 weeks supply of the the CQ syrup (2 daily doses). Participants will receive instructions to take a daily dose from the small bottle containing teh active CQ syrup on the same day every week (Sundays) and doses from the large bottle the remaining days. In the **daily CQ intervention**, participants will receive the same two bottles with instructions to take doses from the small bottle on the sundays and doses from the other bottle the remaining days, but both bottles will contain chloroquine syrup, diluted to the appropriate daily dose using the placebo syrup.

**Figure 5a Individual packets showing randomisation code options**

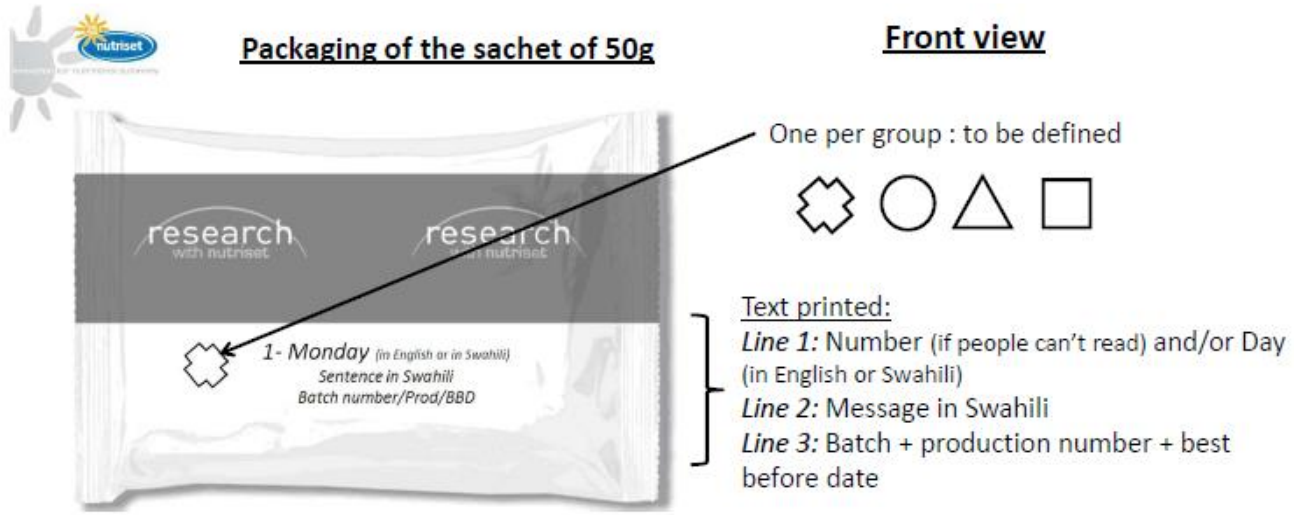
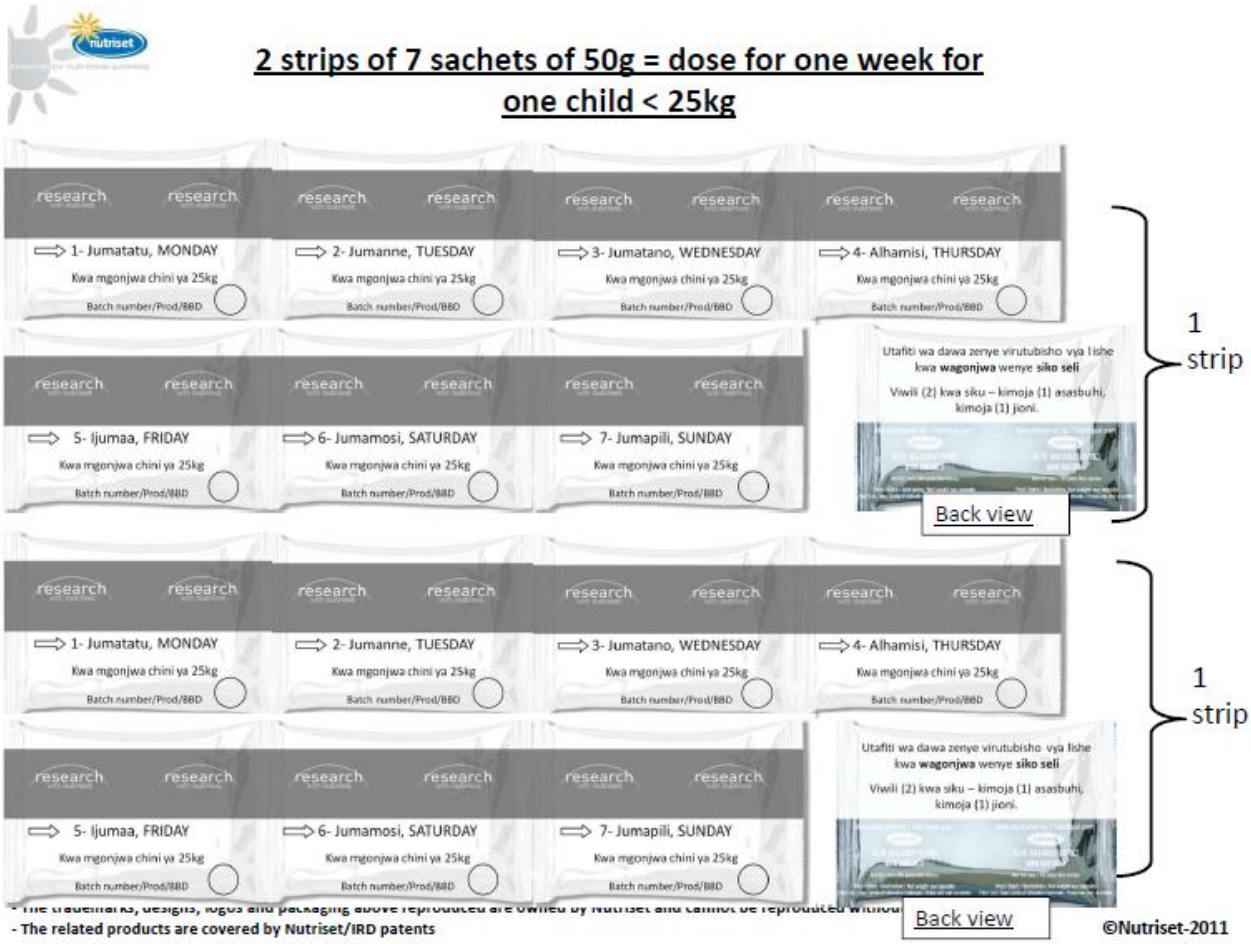


**Fig 5b Proposed Final RUSF packaging in strips with Swahili labelling**




## 6.2 DOSE MODIFICATIONS FOR TOXICITY

Not applicable

## 6.3 PREMEDICATION

No drugs are to be prescribed as part of the study protocol.

## 6.4 INTERACTION WITH OTHER DRUGS

Of the list of drugs with reported possible interactions with chlororquine (BNF 2011), none are expected to be used during the current study. Please see **Appendix 2** for a summary of these.

## 6.5 DISPENSING AND ACCOUNTABILITY

The RUSF, chloroquine and placebo syrups will be delivered by the manufacturer to the research site. Thereafter, the RUSF & chloroquine will be delivered to the dedicated trial storage area. Only designated persons will have access to the RUSF & chloroquine and protocols will be in place to monitor stocks and distribution of the supplements to patient's families, to be done during home visits by a study field worker.

# 7. SAFETY REPORTING

### 7.1 DEFINITIONS

**Adverse Event (AE):** any untoward medical occurrence in a patient or clinical trial subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment. *An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an investigational medicinal product (IMP), whether or not considered related to the IMP.*

**Adverse Reaction (AR):** all untoward and unintended responses to an IMP related to any dose administered. *All AEs judged by either the reporting investigator or the sponsor as having reasonable causal relationship to a medicinal product qualify as adverse reactions. The expression reasonable causal relationship means to convey in general that there is evidence or argument to suggest a causal relationship.*

**Unexpected Adverse Reaction:** an AR, the nature or severity of which is not consistent with the applicable product information (e.g. investigator's brochure for an unapproved investigational product or summary of product characteristics (SmPC) for an authorised product*). When the outcome of the adverse reaction is not consistent with the applicable product information this adverse reaction should be considered as unexpected. Side effects documented in the SmPC which occur in a more severe form than anticipated are also considered to be unexpected.*

**Serious Adverse Event (SAE)** or **Serious Adverse Reaction:** any untoward medical occurrence or effect that at any dose

- **Results in death**
- **Is life-threatening** – *refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe*
- **Requires hospitalisation, or prolongation of existing inpatients' hospitalisation**
- **Results in persistent or significant disability or incapacity**
- **Is a congenital anomaly or birth defect**

Medical judgement should be exercised in deciding whether an AE/AR is serious in other situations. Important AE/ARs that are not immediately life-threatening or do not result in death or hospitalisation but may jeopardise the subject or may require intervention to prevent one of the other outcomes listed in the definition above, should also be considered serious.

**Suspected Unexpected Serious Adverse Reaction (SUSAR):** any suspected adverse reaction related to an IMP that is both unexpected and serious.

### 7.2 CAUSALITY

Most adverse events that will occur in this study are likely to be as a result of ongoing sickle cell disease, such as vaso-occlusive painful episodes (please see **Appendix 4**). For the purposes of this study, an expected SAE is an adverse event that is serious, expected and likely related to the subject's underlying disease process. For this study, AEs listed in Table 2 are expected SAEs. Recording these will be particularly important in this trial as the intervention may change the pattern of complications.

Adverse drug reactions, whether they are serious or not, will be expected treatment-related toxicities due to choroquine (please see **Appendix 2**). The assignment of the causality will 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 trial coordinator will notify the Chief Investigator. Chloroquine manufacturing pharmaceutical companies and/or 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. In the event that no agreement is made, the **Tanzanian Food & Drug Authority** will be informed of both points of view.

| 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. |

## 7.3 REPORTING PROCEDURES

All AE's will be reported. Depending on the nature of the event the reporting procedures below will be followed according to a modified version of London School of Hygiene & Tropical Medicine (LSHTM) standard Operating Procedures (SOP) for reporting of AE's and SAE's (SOP LSHTM/SOP/010a V2), taking into account local reporting requirements. Any questions concerning adverse event reporting will be directed to the Trial Coordinator, Dr Beatrice Kamala in the first instance. A flowchart is given in **appendix 5** to aid in the reporting procedures.

### 7.3.1 Non serious Adverse Reactions (ARs)/Adverse Events (AEs)

All such events, whether expected or not, will be recorded in the appropriate AE CRF and entered into the research database within one week.

### 7.3.2 Serious Adverse Reactions (SARs)/Serious Adverse Events (SAEs)

All fatal serious SAEs, SARs and SUSARs will be reported on the day trial staff are aware of the event (within 24h). All SAEs, regardless of outcome will be reported to the local regulatory authority, the Tanzanian Food & Drug Authority, (TFDA) and the Tanzanian national ethics Committee (TNEC) within 14 days. The SAE form asks for nature of event, date of onset, severity, outcome and causality (i.e. unrelated, unlikely, possible, probably, definitely). The responsible investigator Dr Julie Makani or Prof C Newton or designated medically qualified study personnel will 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.

### SAEs

An SAE form should be completed and sent to the CI & Prof F Kirkham (see contacts below) for all SAEs within 24 hours. However, hospitalisations for elective treatment of a pre-existing condition do not need reporting as SAEs.

### SUSARs

In the case of serious, unexpected and related adverse events, trial staff will:

Complete the SAE case report form & send it immediately (within 24 hours as scanned e-mail attachment), signed and dated to Dr Cox & Prof Kirkham together with relevant treatment forms and anonymised copies of all relevant investigations.

The CI, **S Cox** and **Prof F. Kirkham** will be responsible for reporting all fatal or life threatening SUSARs occurring during the study to the **Tanzanian Food & Drug Authority**, **Tanzanian National Ethics Committee** within 24h of notification and within 7 days to **LSHTM & MUHAS ethics committees.** Non-fatal or life-threatening will be notified to the above authorities within 14 days. All investigators will be informed of all SUSARs occurring throughout the study. SAE's will be similarly reported.

**Contact details for reporting SAEs and SUSARs**
**Please send SAE forms to: f.kirkham@ich.ucl.ac.uk & Sharon.cox@LSHTM.AC.UK**
**Tel: +44 0208 743 2980/ +255 755 406115**

# 8. ASSESSMENT AND FOLLOW-UP

Participants will be followed up for a total of 16 months from enrolment. All participants will be seen at 4 clinic research-specific visits, 4 months apart (please see **Figure 3**) at which a clinical history and examination including detailed anthropometry, temperature, respiratory rate and blood pressure will be conducted. A 5 ml blood sample will also be taken and assessment of flow mediated dilatation will take place (please see **section 3.2** for description of assessments and **appendix 3 for a schedule of events table**. In addition, participants will be seen throughout the study period at bi-weekly home visits by a study field worker, who will deliver the intervention (during the intervention periods). At these visits parents/guardians will be asked to assist in the completion of a short questionnaire concerning adherence to the intervention, report any illnesses, sickle ad non-sickle related and health seeking behaviour and return the home diaries monitoring the incidence, duration and severity of painful episodes, to be completed by children with parental assistance. In the intervening weeks, an adapted version of the same questionnaire will be administered by telephone. In addition subsets of children will be seen clinic visits at 1, 2 and 3 months after enrolment (40 children per time point) during the first intervention period for interim assessments of FMD to provide data to determine of FMD responses are limited to short term effects and for additional safety monitoring through collection of a 2ml blood sample for full blood picture and clinical chemistry assessment of liver and kidney function.

## 8.1 LOSS TO FOLLOW-UP

If participants are not found during weekly home visits, families will be contacted by telephone immediately and a new appointment made. If participant families cannot be contacted by phone a further home visits at usual time the following week will be conducted. If participants fail to attend a clinic visit they will be telephoned the same or following day and another appointment scheduled. Participants who are unable to be traced or contacted for 4 weeks or more will be deemed loss to follow-up.

## 8.2 DATA SECURITY & TRIAL CLOSURE

### 8.2.1 Data security

Brachial FMD scans will be backed up onto an external hard drive at the end of each day. A further copy of the scans will be uploaded onto the main server. The FMD scans will be analysed offline on the computer they were recorded on. These completed analyses will also be backed up daily onto an external hard drive and the main server)

Off-site data back-up of the MSC server is conducted nightly with encrypted data sent to a server hosted by Uhurunet.com over a secure connection.

Paper records will be securely stored in the MSC office. Informed consent documents will be electronically scanned and stored on the MSC server linked to the study and MSC database. The three computers to hold study data (the desktop recording and used for offline analysis of the echo data, the laptop for the study coordinator and the PI laptop) will be encrypted and have software loaded allowing remote deletion of all stored data on the hard-drives, in the event that the computer is stolen.

#### 8.2.2 Trial close out

At the end of the trial, whether stopped early or at the end of recruitment procedures will be followed to ensure the following are completed:

- All investigational products (RUSF & chloroquine) are accounted for and returned (RUSF to Nutriset) or destroyed;
- GCP documents are complete and archived;
- Trial documents including participant log, randomisation codes, product batch numbers, CRFs, signed consent documents are archived;
- A trial report is produced, capturing all the key data and stating where key documents are archived;
- Trial results and the trial report are disseminated to all the appropriate parties, including but not limited to: the regulatory authority (TFDA), the ethical committees (Tanzanian NEC, MUHAS & LSHTM), Tanzanian MoH and Muhimbili National Hospital, Tanzanian Sickle Cell Foundation, the study participants and the trial sponsor (LSHTM).

Data and all appropriate documentation will be securely stored for a minimum of 5 years after the completion of the study, including the follow-up period.

# 9. STATISTICS AND DATA ANALYSIS

### 9.1 DATA ANALYSIS PLAN

The primary analyses will be by intention to treat.

We will use random-effects models to compare the effects of supplementation with RUSF-v and RUSF on: (i) the ratios of plasma arginine:ornithine and arginine:ADMA; (ii) the concentrations and enzyme activity of plasma arginase; and (iii) $FMD_{max}$. Weights and heights will be converted to internal Z-scores (generated from data of all HbSS children in this age group) and mixed effect models used to compare rates of growth during periods when receiving RUSF with washout periods. The large sample size and repeated assessments of FMD has been planned to allow:

- high precision in estimating outcomes;
- power to test for effect modification on $FMD_{max}$ by high vs low FMD, ADMA concentrations, arterial diameter and peak blood flow at baseline;
- formal testing for a carry-over effect (not expected) by testing for an interaction between treatment and order of assignment.

Further analyses will test for associations between proximal markers of vascular dysfunction and $FMD_{max}$ and incidence/duration/severity of VOC painful episodes during the supplementation periods. Painful episodes will be fitted in a negative binomial model relating the number, total days, or total severity score of self-reported painful episodes to age, sex, baseline Hb and fetal haemoglobin percentage (HbF%).

### 9.2 SAMPLE SIZE

The sample size of 120 has more than 99% power to detect a small, but potentially clinically relevant effect size (Δ) of 1.25 unit change in $FMD_{max}$ between the treatment arms, thus even if we allow for 33% drop outs or incomplete data at any of the time points, we will still have more than 95% power. Similarly a sample size of 120 has more than 99% power to detect a 20% difference in growth rates for height and weight.

Sample size calculations were conducted using the following data (see **Fig 7** for worked example).

1. $FMD_{max}$: The within-individual standard deviation (SD) for repeated measures over 3 months of $FMD_{max}$, using the same protocol, in 42 healthy British adults, is 1.04 units [41]. However, as HbSS children have a fluctuating disease state, we have taken a conservative approach and doubled this to 2.08. $FMD_{max}$ in 18 British HbSS children with obstructive sleep apnoea, was 7.71 (SD) 6.27 (Kirkham, unpublished data) compared to 6.3 (SD) 5.4 in 31 British HIV+ children treated with protease inhibitor ARVs, who had significantly lower $FMD_{max}$ (and greater variance) compared to HIV positive children not treated with protease inhibitor drugs [40]. Taking a conservative approach we used the largest inter-individual SD of 6.27.
2. *Growth rates*. The mean within-individual growth rate in 181 Tanzanian HbSS children aged 8-11y at first measurement, with a minimum of 8 months' follow-up and 3 measurements is 0.377+/-0.283cm/month. The size of the SD compared to the mean (SD/mean = 0.75) is greater compared to Gambian children of the same age, collected under strict quality control conditions (SD 0.177/0.425 = 0.41). This is likely a function of greater inaccuracy in single measurements conducted in a busy clinic, and genuine increased within-individual variance due to SCD status. In Gambians, an increase of 0.5 SD translates to a 20% increase in growth rate, which is a clinically significant effect likely to be observed in the duration of RUSF supplementation in this study. Between-individual variation (SD) in growth in the HbSS children was 0.234cm/month. Hence we based our calculations on an effect size of 0.5 x within-individual SD in Tanzanian HbSS children for height and weight growth rates.

***Figure 7. Worked example of sample size calculation***.

1. **$FMD_{max}$**

Within individual variance **($\sigma_w^2$)** = $SD^2/2 = 2.08^2/2 =$ **2.163**

Between individual variance **($\sigma_B^2$)** = $SD^2/2 = 6.27^2/2 =$ **19.66**

**Sample size at 90% power and 5% significance for two-sided test is estimated from the following formula:**

$N = 1+R \times [(1.96+1.28)/(\Delta/\sigma_w)]^2$

R=reliability of measurements = $\sigma_B^2/(\sigma_w^2 + \sigma_B^2)$.

**R**=19.66/(2.163 + 19.66) = 19.66/21.82= **0.9009**

$N = 1+R \times [(1.96+1.28)/(\Delta/\sigma_w)]^2$

$N = 1+0.9009 \times [3.24/0.85]^2$

**N**=1.9009 x 14.52 = 27.6 rounded up to an equal number for randomisation = **28**.

An interaction effect between two orthogonal binary variables both with equally sized groups, requires four times the sample size required for the same sized main effect.

**2. Growth**

**Sample size at 90% power and 5% significance and one sided test (height) two sided test (weight) is estimated from the same formula giving a sample size of N=16 for growth in height and N=20 for weight.**

### 9.3 INTERIM ANALYSES

No interim analyses of the study endpoints are planned. The DSMB will monitor safety data, rates of enrolment, randomisation and trial conduct. At present the plan is to analyse the number of adverse events in each arm of the trial at 4 monthly intervals, unless there is a serious adverse event resulting in a death or as otherwise deemed necessary by the DSMB, in which case the number of adverse events in each arm of the trial will be assessed within 1 month.

# 10. MONITORING

## 10.1 RISK ASSESSMENT

Based on a review of available data concerning these and similar interventions in SCD and non-SCD populations, this study is considered to have a low risk. As such monitoring is as outlined below.

## 10.2 INTERNAL MONITORING AT STUDY SITE

OpenClinica (https://community.openclinica.com/) will be used to manage trial data. OpenClinica is an opensource clinical trials data management systems that supports both electronic data capture (EDC) and

paper CRFs. OpenClinica is 21 CFR part 11 compliant and is designed to meet the ICH harmonised tripartite guideline. To ensure data quality and completeness, range and consistency checks will be built into OpenClinica and paper-based CRFs.

Real-time data collection into electronic CRFs will be utilized using study-dedicated computers networked to the MSC intranet and server, in the cases of: (i) patient registration at clinic-study visits, to confirm the identity and details of the patients from the trial-specific patient demographic table and record trial visits within the MSC outpatient register; (ii) demographic, clinical and anthropometric data at clinic study visits and; (iii) ECG and ultrasound data FMD data (CRF & direct recordings from the ultrasound and ECG equipment). Paper forms will be available in the event of breakdowns in the network. The advantage of real-time data entry is that the quality of the data being collected can be assessed immediately, with event specific instructions built-in and multiple "what if" options. For example, the accuracy of the anthropometric data is paramount and therefore observations must be made by two independent observers, which if not within a set limit of agreement, will result in a trigger for both observers to repeat the measurements, until agreement is reached. Range and consistency checks can be built-in, which can also look up data from previous visits to check for anomalies.
To ensure continuity of study routines and data collection in the event of a system / network failure, paper CRFs will be used as a backup. This will be identical to the electronic CRFs in OpenClinica allowing the seamless transfer of data from paper to OpenCLinica once the system is back online

Paper records will still be used for home-visits. This is because the risk of theft of handhelds or netbooks is considered too high in these circumstances and the reliability/security of the mobile internet networks not good enough to ensure real-time, secure data transmission to the study-server. Paper records will also be used for recording informed consent and these will then be scanned and also saved as electronic files.

Daily and weekly monitoring will be conducted by the trial coordinator and internal monitor (MWP Data coordinator) for quality assessment and control to ensure completeness and accuracy of paper and online CRFs with immediate follow-up of missing or extreme data points. Source document verification checks will also be conducted for the online CRFs by comparing to what is in the actual current database compared to separately stored original and corrected CRFs.

## 10.3 EXTERNAL MONITORING AT STUDY SITE

Independent Trial monitoring will be conducted by **KEMRI-Kilifi Clinical Trials Facility**. A total of 5-6 site visits by the monitoring team are planned: pre-study visit, site initiation, 2 monitoring visits at 2 and 9 months post start of enrolment and a trial-close out visit. At the site initiation visit final procedures will be agreed between the TSC & DSMB for protocol violations, any interim analyses by the DSMB, and definitions, monitoring and reporting procedures for adverse events. The independent trial monitoring team will check on all study procedures including: appropriate randomised recruitment and blinding, adherence to protocols, and amendments, compliance with GCP and applicable regulatory requirements, completeness and accuracy of data forms, appropriate storage of confidential information, and appropriate labelling and storage of serum samples. The following data will be validated from source documents:

- Eligibility and signed consent
- Random sample of anthropometric data
- Random sample of FMD data
- a random sample of reported laboratory results (haematology full blood pictures, clinical chemistry safety data, plasma amino acid concentrations)

Reports of each site visit for monitoring will be sent to the TSC & DSMB. The DSMB will have access to unblinded data and will inform the TSC & sponsor if the trial should be stopped early for any reason.

# 11. REGULATORY ISSUES

## 11.1 CTA

Local regulatory approval for this trial is being sought from the **Tanzanian Food & Drug Authority** as per Tanzanian national requirements

## 11.2 ETHICS APPROVAL

Ethical approval has been obtained from the LSHTM Research Ethics Committee, as well as from the Tanzanian National Ethics Committee (TNEC) as per requirements for research studies in Tanzania. Ethical approval has also been obtained from MUHAS Research & Publications Committee as required for research conducted by MUHAS. The TSC will require copies of the ethics approval letters (LSHTM, TNEC & MUHAS) before enrolling participants into the study. 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.

## 11.3 CONSENT

Consent to enter the study must be sought from the parent or guardian of the child to be enrolled only after a full explanation has been given, an information leaflet offered in Kiswahili and English and time allowed for consideration. Assent from participating children will also be sought. Signed participant consent must be obtained (unless an individual is unable to write, in which case a thumb print is acceptable). The right of the parents or guardians to refuse to participate without giving reasons must be respected. All participants are free to withdraw at any time from the protocol treatment without giving reasons and without prejudicing further treatment. Please see **Appendix 6** for the Patient Information Sheet and Informed consent form.

## 11.4 CONFIDENTIALITY

Participants' identification data will be required for the registration process. Thereafter CRFs will not use participant's identification data and the study database will contain anonymised data linked to the MSC ID and further personal identifiers.

## 11.5 INDEMNITY

London School of Hygiene & Tropical Medicine holds Public Liability ("negligent harm") and Clinical Trial ("non-negligent harm") insurance policies which apply to this trial.

## 11.6 SPONSOR

London School of Hygiene & Tropical Medicine will act as the main sponsor for this study. Delegated responsibilities will be assigned locally.

## 11.7 FUNDING

The Wellcome Trust, UK is funding this study.

Study participants will receive a contribution towards travel expenses on attending each of the 5 study visits at the research office in Muhimbili National Hospital [TSH 5,000 ≈ £2.00]

No payments are being made to the investigators, beyond the standard LSHTM salary of the chief Investigator, Dr Sharon Cox for the duration of the research grant as detailed in the grant application, award letter and GRN budget loading form.

### 11.8 AUDITS AND INSPECTIONS

The trial will be audited and monitored by KEMRI-Kilifi Clinical Trials facility, who will report to the TSC DSMB & Trial sponsor in order to ensure adherence to GCP policies as approved by the study sponsor the London School of Hygiene & Tropical Medicine. The trial may also be subject to audit by the Tanzanian Food and Drug Authority with whom the trial will be registered locally.

Personal medical data may be reviewed by appropriately authorised individuals as part of monitoring and/or audit of the trial but such information will be treated as strictly confidential and will in no circumstances be made publicly available. Monitoring visits are scheduled as in Section 10.3.

# 12. TRIAL MANAGEMENT

### 12.1 TRIAL MANAGEMENT GROUP

The Trial Management Group (TMG) consists of the CI and co-investigators as listed above on p3.

### 12.2 THE TRIAL STEERING COMMITTEE

The Trial Steering Committee (TSC) includes one independent and locally experienced member, Dr Saidi Kapiga, Head of Mwanza Interventions Trials Unit, Tanzania (Chair), The chief investigator (Dr Sharon Cox), Co-investigator, Professor Fenella Kirkham and the trial statistician (to be appointed) will make up the remaining members of the TSC.

### 12.3 DATA SAFETY MONITORING BOARD

The DSMB consists of 4 independent experienced and suitably qualified members as per LSHTM guidelines (LSHTM/SOP/033 FINAL v2.0) and will operate under a charter. Dr Trudie Lang, (University of Oxford) is the chair. The other members include Professor Esther Mwaikambo, (Pediatrician, Herbert Kariuki University, Tanzania), Dr Ramadhani Noor (African Academy of Public Health, Tanzania) and a statistician (Dr Prabin Dahal, University of Oxford

The role of the DSMB includes the review of the implementation and progress of the study. It provides initial, regular, and closing advice on safety-related issues to the investigators and sponsor. Its advice is based on the interpretation of study data with reference to the study protocol. The DSMB will meet before the initiation of the study (pre-initiation review) and 4-monthly thereafter. They will review the Protocol and Report and Analysis Plan (RAP). Other unscheduled meetings may be required. Meetings may be face to face or via teleconference. Meetings must be documented and minutes made available to the sponsors. The DSMB may, if deemed necessary, convene a meeting with, or request further information from the Chief Investigator or sponsor at any stage of the study.

The DSMB may recommend to the sponsor to suspend the enrolment to the trial and/or interventions based on their review of safety data arising in this trial or other relevant trials of similar interventions.

The DSMB will be informed of:

- All SAEs. SAEs judged to be related or fatal will be sent to the DSMB within 24 hours.
- SAE summary tables will be in advance of DSMB meetings (DSMB will have the allocation code to allow unblinding).
- All withdrawals of study subjects by the CI or the parent(s)/guardian(s) of a subject due to adverse events.
- New information that may affect adversely the safety of the subjects or the conduct of the study.
- All subsequent protocol amendments, informed consent changes or revisions of other documents originally submitted for review.

# 13. PUBLICATION POLICY

All publications and presentations relating to the study will be authorised by the TMG in advance. Members of the TSC and the DSMB will be listed and acknowledged. 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 TMG members.

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# APPENDIX 1. DETAILED RATIONALE FOR INTERVENTION COMPONENTS, DOSAGE & CHOICE OF VASCULAR FUNCTION ASSESMENT

***Endothelial dysfunction occurs in SCA, including in children***

Endothelial function assessed in 16 HbSS adult patients and 15 HbAA controls, using a variety of techniques, demonstrated increased resting wall shear stress, decreased NO-mediated vasodilatory responses assessed by FMD, and an absence of vasoconstriction in response to inhalation of 100% oxygen compared to controls [54]. Although endothelium-dependent microvascular dilatation (assessed by measuring forearm blood flow (FBF) response to acetylcholine using venous occlusion plethysmography) appeared to be increased, this was no longer apparent after adjusting for increased baseline blood flow [54]. These findings suggest inappropriately low NO release under basal conditions and a reduced ability of the conduit vessels to adjust appropriately to the rheologic condition. This situation could potentially explain an increased likelihood of precipitating interactions between HbSS erythrocytes and the dysfunctional endothelium, and therefore vaso-occlusive events. FMD was decreased and inflammatory markers and soluble adhesion molecules increased in 10 young adult SCD patients compared to matched controls and FMD further decreased during sickle cell crisis [55]. In 21 HbSS children, mean age 10 years, mean FMD maximum response (4 mins ischaemia of ipsilateral upper arm) was 5.6% +/- 0.2%, significantly lower compared to Afro-Caribbean control children (8.0%+/-0.2%) P=0.008. No differences between the groups were observed for non NO-mediated response to glyceryltrinitrate [56].

***Justification for intervention with L-arginine (L-arg) & L-citrulline (L-cit): Metabolism, pharmacokinetics, nitric-oxide synthesis and vascular function***

L-arg, a semi-essential basic amino acid, is the sole substrate for eNOS. Endogenous arginine synthesis is insufficient in growing children [57] and deficiency is observed in malnourished children (Jahoor Am J Clin Nut 07). Even in the USA, it is estimated that 25% of adults consume sub-optimal intakes of less than 2.6g/d arginine [58]. The best dietary sources of arginine are soy protein, seeds, nuts, meats and seafood; foods not readily available in low-income African settings. L-cit is a non-protein amino acid, which can be synthesized in the gut from glutamine and proline, and importantly, is a precursor for arginine synthesis in the gut, kidneys and elsewhere, including endothelial cells [59].

Oral supplementation with L-arg and L-cit greatly increases plasma arginine levels, with low urinary excretion, even at very high doses [60]. L-cit has higher bioavailability and longer half-life compared to L-arg, resulting in greater plasma arginine levels and area under the curve compared to similar doses of L-arg [53, 60], probably due to lower first pass metabolism in the gut and liver [61]. In conditions of inflammation and injury, particularly to liver parenchymal cells, arginase I is released into the plasma. Haemolysis releases arginase from erythrocytes. These conditions can result in arginine deficiency [62, 63].

Limited arginine supply uncouples eNOS, producing reactive oxygen species (ROS), thus generating a positive feedback loop of oxidative stress, increased haemolysis, release of arginase and further endothelial dysfunction including increased adherence of platelets and leukocytes. Plasma haemoglobin (p-Hb), which is increased in SCD, is a potent scavenger of NO [64] and is associated with endothelial dysfunction in animal models of acute haemolysis [65], although the level of contribution of p-Hb in SCD to NO bioavailability has recently been questioned [66].

***Arginine and arginine metabolites in vascular function in non-SCA populations***

High plasma concentrations of the methylated arginine metabolite, asymmetric dimethylated arginine (ADMA), an endogenous inhibitor of eNOS, and low ratios of arginine to ADMA predict cardiovascular risk and disease progression in a number of conditions associated with endothelial dysfunction including type 2 diabetes [67], peripheral arterial disease [68], malaria [69] and chronic heart failure [70] and in the general population [71, 72]. Increased ADMA concentrations are the only significant predictor of lower FMD measurements in healthy black European men compared to matched whites [73].

ADMA is thought to be produced in response to breakdown of arginine-containing proteins during inflammation and is not cleared by the kidney efficiently, but is degraded by dimethylarginine demethylaminohydrolases (DDAH) expressed in the vascular endothelium and cardiomyocytes. In an atherosclerotic mouse model, over expression of DDAH decreased levels of ADMA and improved vascular function [74], whilst polymorphisms in DDAH have been associated with arterial disease severity in diabetes [75], suggesting that ADMA is causally associated with vascular dysfunction.

Global arginine bioavailability ratio (arginine:ornithine+citrulline), independently of ADMA, predicted major adverse cardiovascular events in a community based follow up of a healthy older population [76].

***Clinical trials of L-arginine or L-citrulline in relation to vascular function and disease***

Clinical trials of short-term (parenteral and oral, 1-7 days) and longer-term supplemention with L-arg, and a few with L-cit, have shown significant effects on measures of vascular function and associated markers. Most have been short-term studies in subjects with disease states including diabetes [77, 78] malaria [79], stable coronary heart disease or hypercholesterolaemia [80, 81]. In a randomised, cross-over clinical trial in 20 healthy volunteers with ADMA concentrations in the highest quartile of the normal range , oral L-cit and L-arg supplementation for 1 week (L-cit max 6g/day [approx. 0.1g/kg/day], L-arg max 3.2 g/day [0.05g/kg/day]), significantly increased plasma arginine concentrations and the ratio of plasma arginine to ADMA [53]. L-citrulline supplementation increased the area-under the curve (AUC) 2-3 times more than similar doses of L-arginine [53]. The max dose of L-citrulline also significantly increased markers of NO production and NO bioactivity (urinary nitrate and cGMP) [53]. None of the treatments in this study resulted in significant changes in $FMD_{60}$, (measured at 60 seconds peak reactive hyperaemia). However, when data from all the treatments were combined a significant correlation was observed between an increase in arginine:ADMA ratio and increased FMD. As these were healthy volunteers, FMD at baseline was normal (6.9% +/-1%) and therefore effects may have been limited to those with vascular function at the lower end of the range or those with the lowest arginine:ADMA ratios, which was not formally tested [53]. A recent meta-analysis assessing the effect of short term L-arginine supplementation on endothelial function assessed by FMD, in a range of population groups, concluded that it was effective when baseline FMD measures were low (<7%) [82]. The one long-term trial of L-arg (3g/day, 6 months) [83] in adults with intermittent claudication, showed increased plasma arginine but failed to show an effect on the primary outcome of absolute claudication distance (ACD) whilst FMD decreased in the L-arg arm. This is in contrast to similar short-term trials in patients with the same disease condition in which ACD and FMD improved [84] [85]. In the trial by Wilson and colleagues [83] functional capacity improved in both arms from baseline. Tolerance to long-term L-arg supplementation was suggested as the possible cause. Alternatively, arginase activity could be up-regulated, resulting in increased ornithine available for synthesis of polyamines and proline, which may encourage vascular remodelling, increasing stiffness and ability to respond to vasodilatory stimuli [86]. Hence we propose to measure FMD in a subset of patients at intermediate time points in the supplementary period, whilst this further justifies the strategy of combining a potential arginase inhibitor with L-arg supplementation.

***Arginine and arginine metabolites in SCD***

In American adult SCD patients, low ratios of plasma arginine to ornithine were associated with high plasma arginase activity, increased tricuspid rejurgitant jet velocities (TRV - a predictor of pulmonary hypertension (PH) in this population) and death [21]. Low ratios of L-arg:ADMA have also been observed in SCD [87, 88] and strongly associated with TRV and death [24, 25]].

In mice models of SCA, arginine supplementation lowers oxidant stress markers, increases NO metabolites [89] decreases, p-Hb and increases microvascular function [90]. There are limited published studies of oral L-arg or L-cit supplementation in SCD. The addition of single dose L-arg to patients stabilised on hydroxyurea (HU), resulted in increases in metabolite markers of NO production for all patients, supporting the hypothesis that HU induces NOS as well as increasing HbF levels, but is dependent on available arginine [91]. Five days of oral supplementation (0.1g/kg/day in 3 divided doses) in 10 SCD patients with PH diagnosed by echocardiography resulted in a significant decrease in TRV and increased plasma arginine [51]. In the one long-term (12 week), open label-phase I-II trial of L-arg in SCD, (0.1-0.2g/kg/d in 3 divided doses) *vs.* sildenafil in

adults stabilised on HU therapy, L-arg had no effect on TRV or on 6 minute walk distances (n=11), which improved in the sildenafil group (n=13) [50], although the use of sildenafil may be limited by increased pain. In addition, this study is seriously compromised by its small sample and significant imbalances in baseline characteristics, with patients in the sildenafil group having significantly worse TRV at baseline. An even smaller study of 4 weeks L-citrulline (0.1g/kg/day, 2 divided doses) in 5 SCD adolescent patients, resulted in significantly increased plasma arginine and decreased white cell counts compared to baseline. Patient self-assessment of well-being using a visual analogue scale also increased [52]. A definitive trial is required.

***Dosage of L-arginine and L-citrulline***

The final targeted dosages of the amino acids (0.2g/kg/d for L-arginine plus 0.1g/kg/d for L-citrulline) were chosen on the basis of three criteria: 1) safety; 2) evidence of efficacy; and 3) limitations on the amounts that can be incorporated into the RUSF, whist maintaining sufficiently high energy-density, taste and textural properties.

The observed safe level (OSL) for L-arginine is 20g/day in healthy adults (please see below). This is equivalent to 0.33g/kg/d assuming an average adult weight of 60kg. Doses of 0.1-0.2g/kg/day of L-arginine [50, 51] & L- citrulline [52] have been utilized previously in SCD subjects with no adverse effects or toxicities reported and resulted in significant increases in plasma arginine concentrations. Three months' L-arginine supplementation (0.1-0.2g/kg/d) increased plasma arginine over time from baseline (50.1+/-17.0 µMol/L, N=8) to a near 100% increase at 12 weeks [50].

A lower dose of L-citrulline compared to L-arginine is proposed based on: i) doubled or greater AUC plasma arginine responses compared to L-arginine [53]; ii) relative expense of L-citrulline compared to L-arginine; and iii) less evidence available to determine observed safe levels.

***Justification for use of chloroquine: inhibition of arginase-I and anti-inflammatory properties***

Chloroquine (CQ) is used as an anti-inflammatory drug in rheumatoid arthritis and lupus as well as an anti-malarial. CQ has been shown *in vitro*, to competitively inhibit arginase, at physiological pH and micromolar levels [92]. Thus in addition to anti-inflammatory effects through immune-modulation, CQ may increase NO bioavailability, by competitive inhibition of arginase in the plasma, endothelium and potentially in the liver.

In rheumatoid arthritis, daily doses in adults of 4mg CQ base/kg are used to achieve plasma CQ concentrations of around 1 µMolar, although there are reports of large inter-individual variability in steady state values [93], whilst 10-14-fold higher CQ concentrations occur in red cells and leukocytes [94]. Allowing for plasma protein binding compared to the *in-vitro* model using 10% serum, we estimate that plasma concentrations of 1 µMolar will achieve a meaningful reduction in plasma arginase activity. We do not know what CQ concentration may be reached in the vascular endothelium and smooth muscle cells and therefore what levels of arginase inhibition.

Evidence from *in-vitro* and animal models supports the hypothesis that inhibition of arginase can increase NO bioavailability and vascular function: firstly, blood vessel arginase activity is associated with *in vitro* levels of NO production [95]; secondly, pre-treatment of rats with the synthetic arginase inhibitor $N_w$-hydroxy-nor-L-arginine (nor-NOHA) reduces liver ischaemic reperfusion injury by preventing the rapid drop in L-arg and reduces liver necrosis [63]; thirdly, nor-NOHA treatment prevents the development of hypertension and improves aortic endothelial function via a NO-dependent mechanism in pre-hypertensive and young spontaneously hypertensive rats [96], and significantly reduces blood pressure and improves endothelial function in already hypertensive rats to levels comparable to normal rats [97, 98].

Weekly CQ as an anti-malarial is currently recommended by Tanzanian government guidelines. However, due to possible resistance to CQ, alternative anti-malarial prophylactics are being discussed (although there are no obvious choices for affordable, safe, effective and life-long use in this setting). It is also importnatn to consider the very different malaria transmission levels within a country like Tanzania, with urban Dar-es-Salaam experiencing low transmission levels, particularly in SCD patients [99]. In the light of this on-going policy debate it is potentially important to evaluate possible additional benefits of CQ use (we plan to assess levels of CQ resistance as part of separate research project using archived samples).

***Justification of flow mediated dilatation to assess vascular function***

The principle vascular research question is whether the RUSFv intervention will improve the bioavailability of NO in the arterial wall. The gold standard method of addressing this question is to analyse basal vascular tone and vasomotor responses to endothelium-dependent stimuli by venous occlusion plethysmography with and without intra-arterial administration of the NO inhibitor L-NG monomethyl arginine. This approach would require multiple arterial punctures with prolonged invasive analysis of forearm vascular function, which would not be clinically or ethically appropriate in a paediatric study of this size.

A more suitable alternative approach is non-invasive assessment of flow-mediated dilatation (FMD) of the brachial artery. This technique uses well validated methodology to measure the vasodilatation of the conduit artery supplying the forearm in response to a reactive hyperaemic stimulus. Local inhibiton of nitric oxide synthase with LNMMA at doses that did not affect systemic haemodynamics have shown that FMD response is predominantly, if not entirely NO-dependent [100-104]. Other non-invasive techniques for assessing endothelial function are less reproducible in children [105] or only partially nitric oxide dependent [106]. EndoPAT has been proposed as a potential means of assessing endothelial function in children with sickle cell disease [107]. However, it has not been fully validated in children and requires a new set of finger probes for each study at a substantial cost.

Non-invasive assessment of reactive hyperaemic response could also be an informative outcome measure. This can be measured using venous occlusion plethysmography, but can also be determined during the FMD protocol by measuring brachial artery blood flow at baseline and throughout reactive hyperaemia using pulse wave Doppler. We will consider this response as a secondary vascular outcome as it is only partially NO-dependent and previous studies of short-term L-arginine supplementation in other conditions have not consistently affected the magnitude of reactive hyperaemia [108, 109].

# APPENDIX 2. EXAMPLE LIST OF EXPECTED TOXICITIES & POTENTIAL DRUG INTERACTIONS

| Toxicity* | Chloroquine* | Notes for Chloroquine | L-Arginine & L-Citrulline supplemented RUSF | Notes L-Arg & L-Cit |
|---|---|---|---|---|
| General | | | | |
| Headache | ✓ | | | |
| Haematopoeitic | | | | |
| bone marrow depression, including aplastic anaemia, agranulocytosis, thrombocytopenia, neutropenia | ✓ | rare | | |
| Hepatic | | | | |
| Changes in liver function, hepatitis and abnormal liver function tests | ✓ | rare | | |
| Hypersensitivity | | | | |
| Allergic and anaphylactic reactions | ✓ | rare | | |
| urticaria | ✓ | rare | | |
| Angiodema | ✓ | Rare | | |
| Gastrointestinal | | | | |
| Abdominal cramps | ✓ | | ✓ | Sometimes reported at high doses >9g/kg/day |
| Vomiting, | ✓ | | | |
| Diarrhoea | ✓ | | ✓ | Sometimes reported at high doses >9g/kg/day |
| Nausea | ✓ | | | |
| Cardiovascular | | | | |
| Cardiomyopathy | ✓ | Reported rarely at long term therapy at high doses | | |
| Cardiac dysrhythmias | ✓ | Can occur at high doses | | |
| hypotension | ✓ | | | |
| Central Nervous system | ✓ | | | |
| Seizures | ✓ | | | |
| Convulsions | ✓ | Reported rarely may result from cerebral malaria – give injections of phenobarbitol | | |
| Psychiatric disorders – anxiety, confusion, hallucinations, delirium | ✓ | | | |
| Eye disorders | | | | |

| | | | | |
|---|---|---|---|---|
| Transient blurred vision & reversible corneal opacity | ✓ | | | |
| Retinopathy | ✓ | | | |
| Irreversible retinal damage | ✓ | Rarely reported at long-term high dosage | | |
| Macular defects of colour vision, optic atrophy, scotomas, field defects, blindness & pigmented deposits, difficulty in focussing & diplopia | ✓ | | | |
| Muscular | | | | |
| neuropthy | ✓ | | | |
| myopathy | ✓ | | | |
| Skin | | | | |
| skin eruptions, pruritis, depigmentation, loss of hair, exacerbation of psoriasis & photosensitivity | ✓ | | | |
| Pigmentation of nails & mucosae | ✓ | Rarely reported at long-term high dosage | | |
| Erythema multiforme, Stevens-Johnson syndrome, toxic epidermal necrolysis, exfoliative dermatitis and similar desquamation-type events | ✓ | Rarely reported | | |

* taken from the Summary of product characteristics (SPC) CQ-sulphate – Nivaquine (http://www.medicines.org.uk/EMC/medicine/2272/SPC/Nivaquine/) accessed 23/09/2011.

**Concomittant use of CQ should be avoided with the following drugs: (taken from British National Formulary 61, March 2011)**

- **Amiodarone** (anti-arrhythymic drug) – increased risk of ventricular arrhythmia
- **Moxifloxacin** (antibiotic) - increased risk of ventricular arrhythmia
- **Droperidol** (antipsychotic) - increased risk of ventricular arrhythmia
- **Antiepileptics** - possible increased risk of convulsions
- **Mefloquine** (antimalarial) - increased risk of convulsions.

**Other known drug interactions**

- **Antacids** reduce absorption of CQ
- **Digoxin** – CQ increases plasma concentrations of digoxin
- **Ciclosporin** (immune-suppressant) - CQ increases plasma concentrations of ciclosporin and therefore potential toxicity
- **Cimetedine** – (ulcer healing) affects metabolism of CQ resulting in increased plasma concentrations
- **Lanthanum** (renal failure) reduces absorption of CQ
- **Laronidase** (enzyme replacement for mucopolysaccharidosis) – CQ possibly inhibits effects of laronidase (manufacturers recommendation)
- **Histamine** (anti-inflammatory) – antimalarials to be avoided, recommended by manufacturer of histamine
- **Neostigmine & Pyridostigmine** (parasympathomimetics) CQ potential to increases symptoms of myasthenia gravis and therefore reduce effects of these drugs

# APPENDIX 3. SUMMARY OF INVESTIGATIONS, TREATMENT AND ASSESSMENTS

Table A1: Investigations and assessments for all study participants at clinic-study visits.

| **Exam** | | **Month of follow up** | | | |
|---|---|---|---|---|---|
| | Pre-treatment | 4 | 8 | 12 | 16 |
| Informed consent | X | X | X | X | X |
| History, physical exam | X | X | X | X | X |
| Anthropometry | X | X | X | X | X |
| Brachial FMD by ultrasound | X | X | X | X | X |
| 5ml blood sample – FBC, clinical chemistry panel and serum/plasma archiving | X | X | X | X | X |

Please see draft V-FIT CRFs#1, 2 & 3

All participants will also have data collected in V-FIT CRF#-4 home visits at bi-weekly home visits and telephone interviews in the intervening weeks by a study field worker.

# APPENDIX 4. EXPECTED ADVERSE EVENTS IN THIS STUDY

**Table A4a: Expected Adverse Events**

| Expected AE's | Expected AE's | Expected AE's |
|---|---|---|
| Abnormal haematology/clinical chemistry tests† | Hemiplegia | Pulmonary embolism |
| Acute chest syndrome | Hepatosplenomegaly | Pulmonary hypertension |
| Anaemia | HIV-infection | Pyelonephritis |
| Aplastic crisis/anaemia | Hyperplastic bone marrow | Renal insufficiency/albuminuria |
| Avascular necrosis of femoral head | Hyposthenuria | Reticulocytosis (10%–20%) |
| Avascular necrosis of hip/shoulder | Hypoxaemia ($SpO_2$<96%) | Retinal haemorrhage |
| Bone infarction | Infection, pneumococcal | Retinopathy* |
| Bruising | Infiltrates on chest x-ray | Rhabdomyolysis |
| Cardiomegaly | Jaundice | Sepsis |
| Cholecystitis, hepatic sequestration | Leukocytosis | Seizures* |
| Cranial nerve palsy | LRTI | Silent cerebral infarct |
| Dactylitis | Macular defects* | Skin disorders pruritis, depigmentation* |
| Decreased kidney function | Malaria | Skin ulcers |
| Decreased lung function | Meningitis | Splenic sequestration |
| Delayed growth | Overt Stroke | TB infection |
| Fever | Pain, joint | Transient Ischaemic attack |
| Haematuria | Pain, long bone | Urinary tract infection |
| Haemolysis | Pain, severe abdominal | URTI |
| Hair loss | Priapism | Urticaria* |
| Headache | Psychiatric disorders* | Wind** |

† Significant deviations from normal steady-state values for sickle children aged 8-11 when free of symptoms (See Appendix 4b)
*AE that could be associated to CQ intervention
**AE that could be associated with the RUSFv intervention

**For reporting purposes AE's will be categorised according to the following headings (Table 4b) with more information given as available.**

**Table 4b.**

| ADVERSE EVENT | code | ADVERSE EVENT | code | ADVERSE EVENT | code |
|---|---|---|---|---|---|
| Acute Abdomen/Obstruction/Helminthiasis | 1 | Fever with source | 12 | Priapism | 23 |
| Acute anaemia | 2 | Fever without source | 13 | Psychiatric (all types) | 24 |
| Acute chest syndrome | 3 | Gastro-enteritis | 14 | Sepsis | 25 |
| Asthma | 4 | Hepatitis | 15 | Seizures | 26 |
| Cardiac/pulmonary problems (non-congenital) | 5 | Leg ulcers | 16 | Splenic sequestration | 27 |
| Cellulitis/Pyomiositis/abscess | 6 | LRTI | 17 | Stroke | 28 |
| Dactylitis | 7 | Malaria | 18 | TB infection - all types | 29 |
| Encephalopathy- unknown | 8 | Meningitis | 19 | URTI | 30 |
| Epilepsy | 9 | New avascular necrosis | 20 | Urinary tract infetction | 31 |
| Eye/vision problems | 10 | Osteomyelitis | 21 | Vaso-occlusive pain | 32 |
| Febrile convulsions | 11 | Pneumonia | 22 | OTHER | 33 |

**Table A4c: Grading of Laboratory Adverse Events**

| *LABORATORY EVENTS* | | | | |
|---|---|---|---|---|
| **PARAMETER** | **GRADE 1 MILD** | **GRADE 2 MODERATE** | **GRADE 3 SEVERE** | **GRADE 4 POTENTIALLY LIFE-THREATENING** |
| **HAEMATOLOGY** | ULN = Upper Limits of Normal<br>(steady state values in Tanzanian SCA children aged 8-11.9y = mean +2 x SD)* | | | |
| Hemoglobin | any decrease ≥ 1.0 g/dL from baseline | any decrease ≥ 2.0 g/dL from baseline | any decrease ≥ 2.5 g/dL from baseline | any decrease ≥ 3 g/dL **OR** < 4g/dl |
| WBC $10^3/mm^3$ Elevated | 1.1– 1.3 x ULN | 1.4 – 1.8 x ULN | 1.9 – 2.5 x ULN | > 2.5 x ULN |
| WBC $10^3/mm^3$ -Decreased | 0.9-1.0 x LLN | 0.75-0.9 x LLN | 0.5-0.75 x LLN | <0.5 x LLN |
| Neutrophil % | 1.1– 1.3 x ULN | 1.4 – 1.8 x ULN | 1.9 – 2.5 x ULN | > 2.5 x ULN |
| Lymphocyte % | 1.1– 1.3 x ULN | 1.4 – 1.8 x ULN | 1.9 – 2.5 x ULN | > 2.5 x ULN |
| Platelets—Decreased | 1.1– 1.3 x ULN | 1.4 – 1.8 x ULN | 1.9 – 2.5 x ULN | > 2.5 x ULN |
| Platelets—Elevated | 0.9-1.0 x LLN | 0.75-0.9 x LLN | 0.5-0.75 x LLN | <0.5 x LLN |
| **CHEMISTRIES** | | | | |
| Creatinine | 1.1 – 1.3 x ULN | 1.4 – 1.8 x ULN | 1.9 – 2.5 x ULN | > 2.5 x ULN |
| AST | 1.1 – 3.0 x ULN | 3.1 – 6.0 x ULN | 6.1 – 10.0 x ULN | > 10.0 x ULN |
| ALP | 1.1 – 3.0 x ULN | 3.1 – 6.0 x ULN | 6.1 – 10.0 x ULN | > 10.0 x ULN |
| Bilirubin total | 1.1 – 3.0 x ULN | 3.1 – 6.0 x ULN | 6.1 – 10.0 x ULN | > 10.0 x ULN |
| Bilirubin conjugated | 1.1– 3.0 x ULN | 3.1 – 6.0 x ULN | 6.1 – 10.0 x ULN | > 10.0 x ULN |
| Bilrubin non-conjugated | 1.1 – 3.0 x ULN | 3.1 – 6.0 x ULN | 6.1 – 10.0 x ULN | > 10.0 x ULN |
| LDH | 1.1 – 3.0 x ULN | 3.1 – 6.0 x ULN | 6.1 – 10.0 x ULN | > 10.0 x ULN |

*Normal steady state reference ranges for clinical chemistry in Tanzanian children with SCD aged 8-11.9 years.

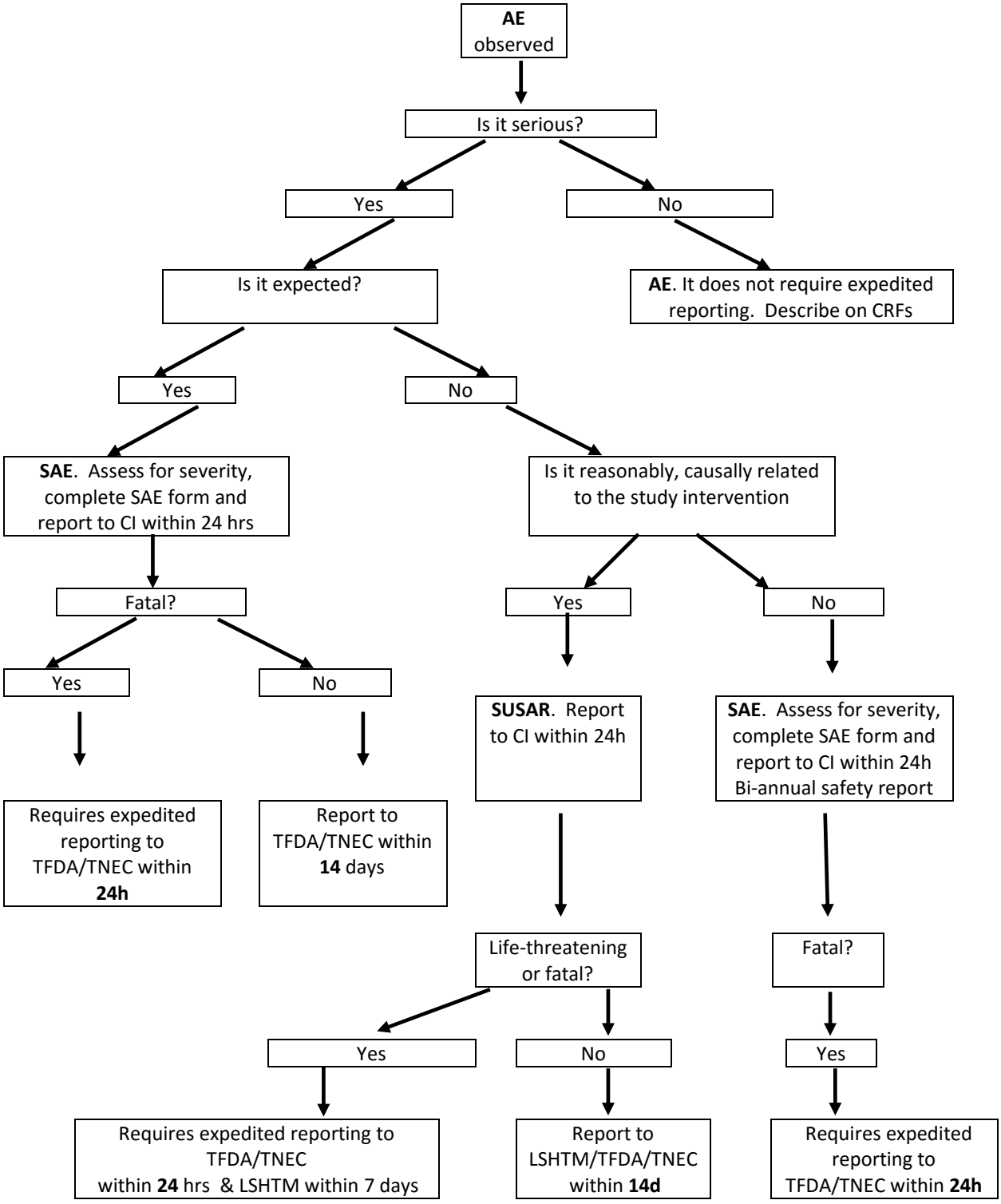
| | **Mean (SD)** | **ULN (Mean + 2SD)** | **LLN (Mean – 2SD)** |
|---|---|---|---|
| WBC $10^3/mm^3$* Females | 13.9 (4.25) | 22.4 | 5.4 |
| Males | 14.6 (4.06) | 22.7 | 6.5 |
| Neutrophils %* | 44.0 (8.7) | 61.0 | |
| Lymphocyte %* Females | 45.0 (10.5) | 66.0 | |
| Males | 43.8 (10.1) | 64.0 | |
| Platelets $10^3/mm^3$* | 447 (176) | 799 | 94 |
| Createnine^ | 36.8 (10.0) | 56.8 | |
| Aspartate transaminase (IU)† | 42 (9) | 60 | |
| Alkaline phosphatise (IU)† | 173 (41) | 255 | |
| Lactate dehydrogenase (IU)† | 523 (110) | 743 | |
| Total Bilirubin (µmol/L)† | 52 (28) | 108 | |
| Conjugated bilirubin (µmol/L)† | 13 (3) | 19 | |
| Non-conjugated bilirubin (µmol/L)† | 40 (29) | 98 | |

**Based on measurements in 602 Tanzanian children with SCD with an average of 3 measurements each, aged 8-11.9Y at time of analysis whilst in steady state defined as as no malaria parasitaemia, recorded temperature <37.5°C, reported pain or hospitalization within 30 days of assessment (before or after).*

*^Based on measurements in 512 children with SCD with an average of <2 measurements each, aged 8-11.9Yat time of analysis whilst in steady state, defined as above*

*†Based on averaged multiple steady state measurements in 35 children Tanzanian SCD aged 8-11.9Y at the time of assessment and at steady state as defined above*

# APPENDIX 5. FLOWCHART FOR SAFETY REPORTING




*TFDA Tanzania Food and Drug authority = local regulatory authority for clinical trials*
*TNEC: Tanzania National Ethics Committee*

# APPENDIX 6. PATIENT INFORMATION AND INFORMED CONSENT FORM

**[V1.1 15.05.2012]**

MUHIMBILI UNIVERSITY OF HEALTH & ALLIED SCIENCES

**P.O Box 65001 Dar es Salaam. Tanzania**

**INFORMED CONSENT FORM**

| Site name | Site number | Serial number (site specific) | Study ID | | | | |
|---|---|---|---|---|---|---|---|
| **MUHAS** | **N/A** | | | | | | |

**STUDY TITLE**

***Vascular Function Intervention Trial (V-FIT) in Sickle cell disease***

**THE RESEARCHERS**

This research is being conducted in collaboration with investigators from the UK and the researchers in the Muhimbili Sickle Cohort study in which you have already agreed to be enrolled in (please see details below).

**PURPOSE OF STUDY**

From the results of the research we have conducted so far, we have evidence to suggest that a food supplement **might** have beneficial effects on sickle cell disease. We have found that many children with SCD are malnourished and therefore are often short and thin compared to Tanzanian children of the same age without SCD. Poor growth is associated with poor health outcomes in many diseases including in SCD. We have also seen that blood levels of some nutritional factors are much lower in individuals with severe compared to mild SCD disease. Levels of these factors are thought to be important in maintaining the function of the vessels that carry our blood. We know that if these vessels do not function well in SCD, it can contribute to some of the symptoms and long-term effects of SCD. Also, we think that the chloroquine we have been prescribing as an anti-malarial prophylaxis may also have beneficial effects on blood vessel function, but we are not sure how much is needed for this effect.

Food supplements are being used in Tanzania to treat and prevent malnutrition. We do not know if a similar food supplement, containing necessary vitamins and minerals, will improve growth and other outcomes in children with SCD, such as reducing the severity of the anemia (lack of blood). We would also like to find out if adding higher levels of particular nutritional factors to the same food supplement and giving lower but more frequent (daily) amounts of chloroquine can improve the function of blood vessels in SCD. To find out, we need to compare the growth and blood vessel function of children after periods of no food supplement and normal compared when taking a standard food supplement and when taking the food supplement with the extra nutritional factors added and the different chloroquine dosing from normal.

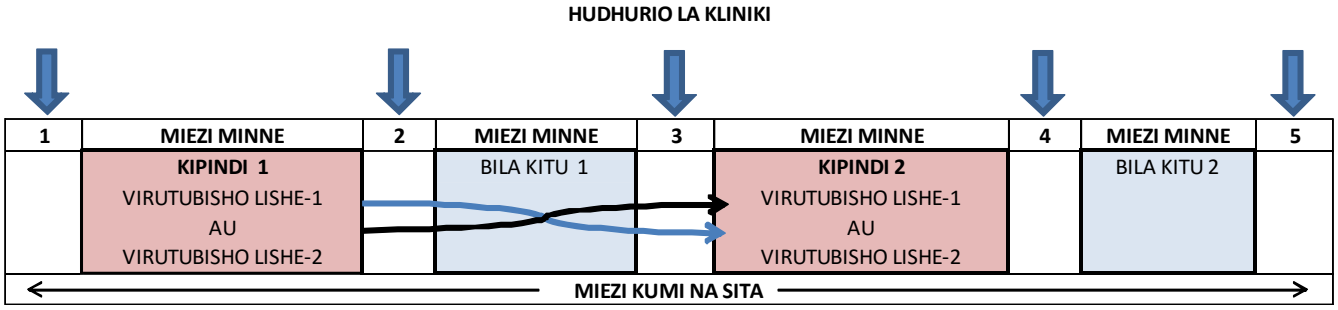
We think that many SCD children are experiencing more episodes of pain and illness than we are able to see by only collecting information during admissions to Muhimbili National Hospital. We also think that the food supplements may affect painful crises experienced by SCD children. To find this out we need to be able to measure these accurately. Therefore we want to collect detailed information on how often children experience painful crises and fever by asking families to help us in recording this information and by telephoning and visiting children and families at home.

**WHO WILL BE IN THE STUDY**

We are inviting children aged between 8-11 years who are already participating in the Muhimbili Sickle Cohort and who are permanently resident in urban Dar-es-Salaam to be included in this study. Children with pre-existing conditions, like epilepsy or using some kinds of drugs will also not be included in this study.

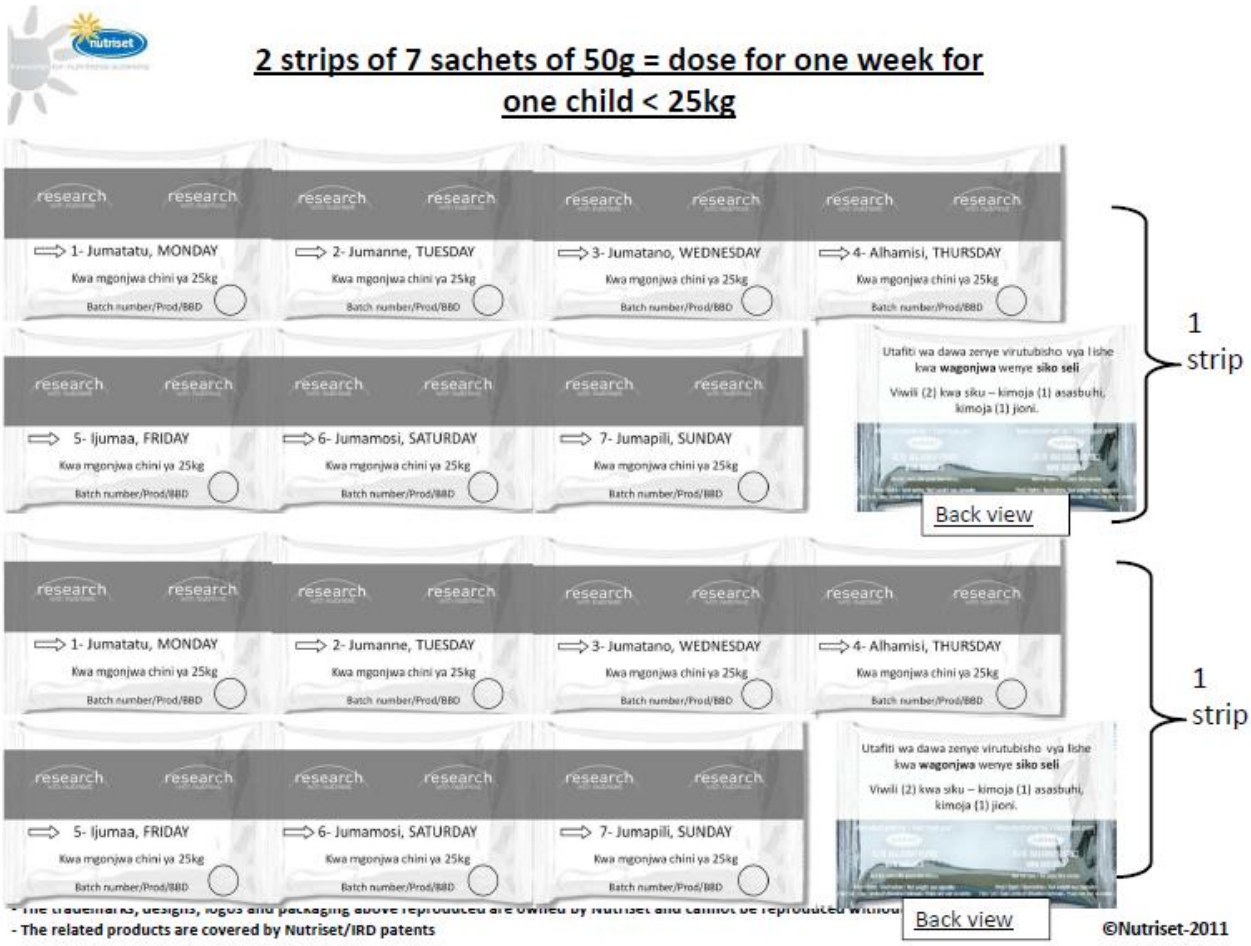
**WHAT PARTICIPATION INVOLVES**

To answer all these questions, children will receive one of the two food supplements at the beginning of the study for 4 months, followed by 4 months with no food supplement, another 4 month period with the different food supplement and a final 4 months with no food supplement, for a total study period of 16 months. Neither the investigators, or you or your child will know which supplement is which, until after the study is completed. All children enrolled in this study will receive home visits by a study worker every 2 weeks throughout the study period, who will deliver the food supplement and chloroquine syrup. In the intervening weeks the field worker will telephone you.




If you agree to participate in this study your **child will be randomly allocated**, to receive either the standard food supplement or the enhanced food supplement first.

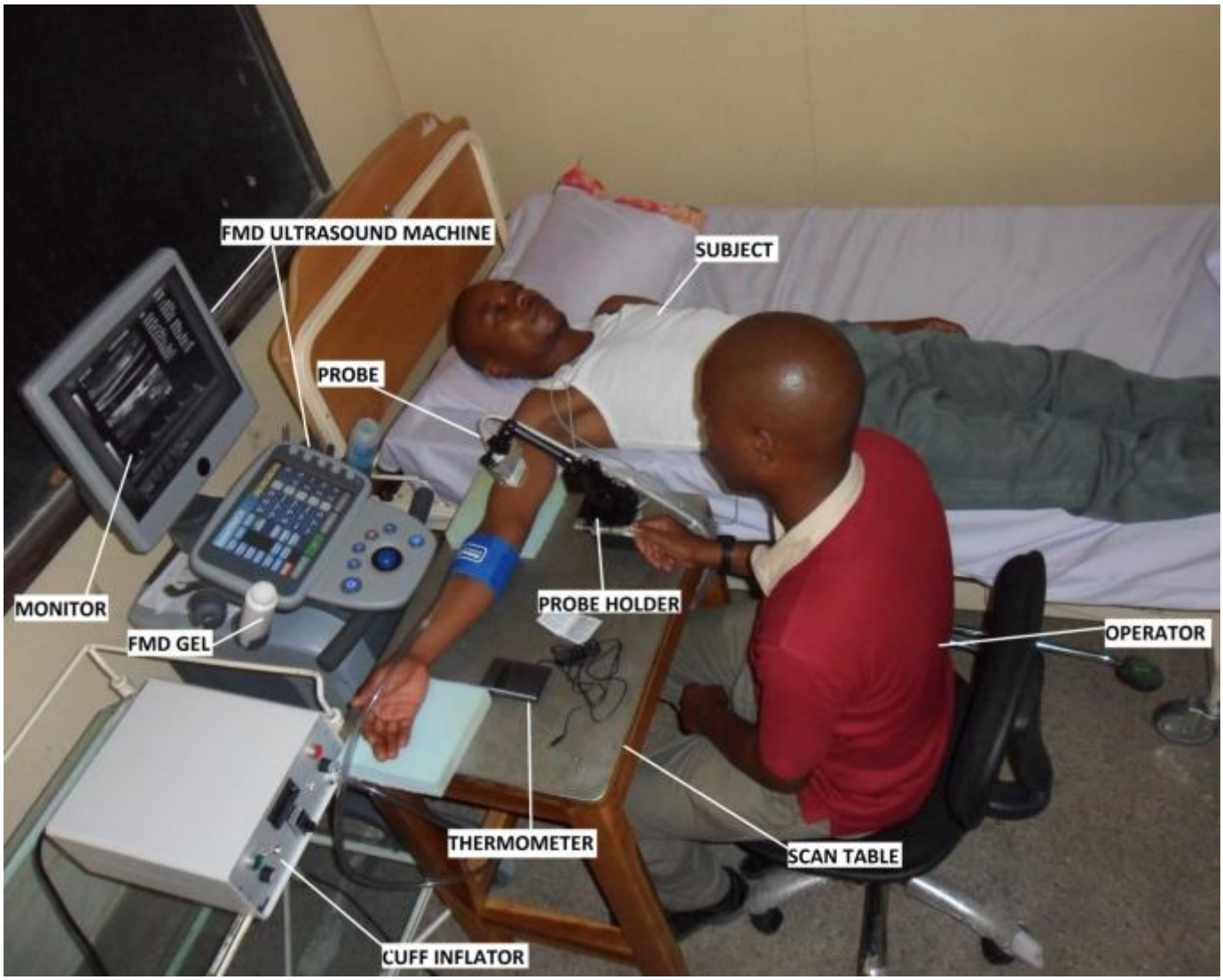
- During the study period, children should not take additional vitamin and mineral supplements unless recommended by a Doctor, who should be informed if your child is taking one of the food supplements at the time.
- During the periods when children are NOT receiving the food supplement, children should take their normal folate supplements. Folate is not required when children are receiving the food supplements as the required folate is included within the food supplement already.




**Procedures at clinic visits**

Children enrolled in this study will have **5 study-specific clinic visits**; at the **start** of the study then **4**, **8 12 & 16 months** later at the end of the study. These will replace your normal sickle clinic visits, so that extra visits are not required. At each of these study visits the following tests or measurements will be conducted.

- **Blood samples** will be collected (5ml - teaspoon) as at normal sickle clinics.
- Children's **height and weight** will be measured plus their amount of **fat and muscle**, measured using a new piece of equipment on which your child stands on the machine and holds the handles for 1 minute.
- Blood vessel function will be measured at only the first 4 visits using a machine that uses sound to take pictures of the inside of our bodies – the same as we use to check on the babies of women who are pregnant and also similar to what we use when we have measured the speed of blood travelling inside blood vessels in the brain which we call "TCD". Your child may already have had TCD done as a part of previous SCD research, and which is done routinely in SCD centres in other parts of the world. We will measure the function of a large blood vessel in the arm and to do this, we will take pictures during the inflation and release of a pressure cuff, just like we use to measure blood pressure when your child comes to SCD clinics. The only difference is that the cuff will remain inflated for a bit longer (5 minutes) than normal. Finally we will also take pictures of the blood vessel before and 5 minutes after giving a small dose of drug (glyceryl-trinitrate) dissolved under the tongue. This drug is completely safe and is used in larger amounts to improve blood vessel and heart function in patients with damaged hearts and blood vessels.




**Home visits and parents involvement in data collection**

- Families will be asked to complete a simple picture diary recording when children experience a painful episode, its duration and a ranking of how severe the pain as evaluated by the child and what pain medication, if any, was used.

You can discuss any difficulties or worries you may be experiencing in the study during home visits and we will try to ensure you see the same study worker each time.

The home visits are an important part of this study and **your participation is crucial**. We think we can learn a lot about SCD by hearing about **your experiences** as parents, outside of the formal hospital environment.

**How will the blood samples collected at study clinics be used?**

We will collect only one blood sample at the study clinic visits. The normal tests that are done at the routine sickle clinics will be performed (full blood picture) and malaria test if required. The rest of the sample will be used to measure the specific factors involved in blood vessel function. Some of these measurements will be done in the sickle cell laboratory in Muhimbili and some will be need to be done in laboratories in the UK. Any left-over sample will be stored long-term for possible future studies related to increasing our understanding of sickle cell disease. This is the same as is happening to samples collected during normal sickle clinics.

**BENEFITS OF THE STUDY**

There will be no direct or financial benefits to participation in this study. Your participation will mean that we will find out if a food supplement and daily compared to weekly chloroquine is of significant benefit to patients with SCD. Although this is a food supplement and chloroquine is already used in SCD patients, it is still very important that we have proof that it is of benefit, safe and is acceptable to families.

**POTENTIAL RISKS OF THE STUDY**

- There is a very small risk of children having an allergic reaction to the food supplements. We will minimize this risk by giving a small test amount of the supplement while at the study clinic.
- The volume of blood collected is small and only slightly more than is normally collected at the sickle clinics and will not affect the health of your child.
- Some children may find the pressure cuff uncomfortable while it is inflated and there is a possible risk that your child may experience some pain after its release, although we have never observed this to happen. We will minimize this risk be ensuring that your child is warm and we will ask you and your child to remain resting in a comfortable place we will provide, for an hour after completing the blood vessel function measurement to make sure that no symptoms are experienced or are managed with appropriate pain relief.
- As with any drug, there is a small risk that your child may experience some adverse effects associated with the use of chloroquine, either the weekly or daily doses. The majority of such effects are short-lived and non-serious, but we will be monitoring all these events and ask you to report any symptoms or concerns during the weekly clinic visits or to call the SCD patient hotline.

**FREEDOM TO PARTICIPATE IN THE STUDY**

We would like to stress that your **participation** in this study is **strictly voluntary**. It is your decision. Should you decide not to participate; it will **NOT** affect the treatment or management that you will receive from the hospital, or your participation in the ongoing cohort. In addition, you are **free to withdraw** your participation in this study (and also if you wish, the ongoing cohort study) **at any point** and would be effective immediately. If it is your wish for us to destroy any stored samples we have, we will do so. Any such decision will be respected and will not influence the quality of health care we will give you or your child.

**CONFIDENTIALITY**

All the information that is obtained from your child and family will be confidential and in addition to using your current SCD no, a new study number will also be given. Only the principal researcher or somebody authorized by him or her will be able to link personal information back to study participants.

**RESEARCHERS**

Dr Sharon Cox (London School of Hygiene & Tropical Medicine, UK)
Dr Julie Makani (Muhimbili University of Health & Allied Sciences, Tanzania)
Professor Charles Newton (UCL-Institute of Child Health, UK & KEMRI-Kilifi, Kenya)
Professor Fenella Kirkham (UCL-Institute of Child Health, UK)
Professor Andrew Prentice (London School of Hygiene & Tropical Medicine, UK)
Professor Julian Halcox (University of Cardiff, UK)

Please feel free to ask any questions about the information you have just been given or anything else to do with SCD and the care of your child. We are happy to provide you with more detailed written information concerning the composition of the different food supplements and the chloroquine doses at your request (available in English).

There is more information in the form of leaflets and published papers that are available for you to learn more about SCD. THE SICKLE CELL FOUNDATION OF TANZANIA also has independent information on sickle cell disease and they would be pleased to hear from you any worries or questions you may have (please ask for contact details.)

Please feel free to take this home with you and you can contact us or ask us during your next visit for more information.

For the study, we will ask you to sign this paper to confirm that you have received this information and that you consent to participate in this study.

In case there is any further information that you require with regard to the study please ask to speak to Dr J Makani, Department of Haematology and Blood Transfusion, Muhimbili University of Health & Allied Sciences (MUHAS). Tel: 0754 381551 or any of the other investigators. If you ever have questions about your rights as a participant, you may call the chairman of MUHAS Research and Publications Committee, P. O. Box 65001. Tel: 2150302.

MUHIMBILI UNIVERSITY OF HEALTH & ALLIED SCIENCES

**P.O Box 65001 Dar es Salaam. Tanzania**

**INFORMED CONSENT FORM**

| Site name | Site number | Serial number (site specific) | Study ID | | | | |
|---|---|---|---|---|---|---|---|
| **MUHAS** | **NA** | | | | | | |

## *Vascular Function Intervention Trial (V-FIT) in Sickle cell disease*

**Informed consent for participants:**

I have read or I have been read the attached information regarding the SCD study in English / Swahili (please circle one), a language I speak fluently. I have also had an opportunity to discuss the study and ask questions to the investigators and I am satisfied that I understand what the study involves and my questions answered.

I agree to or allow my child (listed below) to take part in this study:

(1)_____

**Patients/ Parent / Guardian's**
**Signature or thumb print _____ Date_____**

**Parent / Guardian's Name: _____**
*(Please print name)*

**Witness**
**Signature (if caretaker cannot read)_____Date_____**

**Witness' Name: _____**
*(Please print name)*

I certify that the above was explained verbally to the parent/guardian, and that s/he understands the nature and the purpose of the study and consents to the participation in the study of the above patients.

I have given them the opportunity to ask questions which have been answered satisfactorily.

**Research Officer**
**Signature _____ Date_____**

**Research Officer Name: _____**

MUHIMBILI UNIVERSITY OF HEALTH & ALLIED SCIENCES

**P.O Box 65001 Dar es Salaam. Tanzania**

**INFORMED <u>ASSENT</u> FORM**

| Site name | Site number | Serial number (site specific) | Participant number | | | | |
|---|---|---|---|---|---|---|---|
| **MUHAS** | **N/A** | | | | | | |

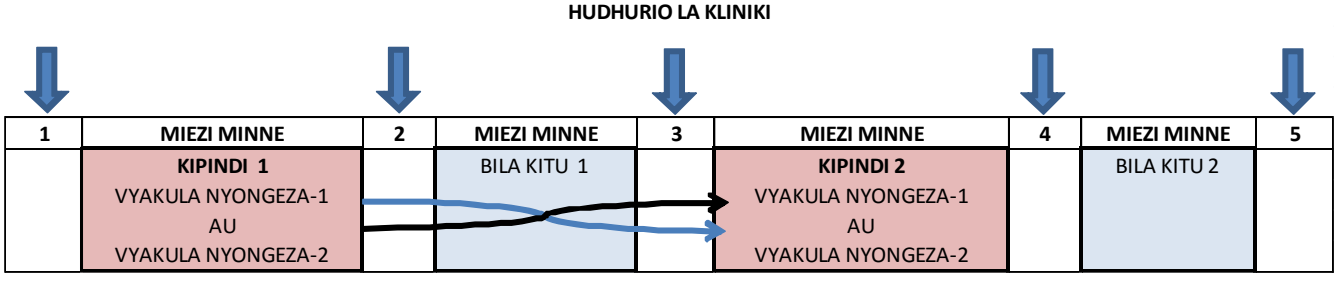
**STUDY TITLE:** ***Vascular function trial (V-FIT) in Sickle Cell Disease***

**THE STUDY**

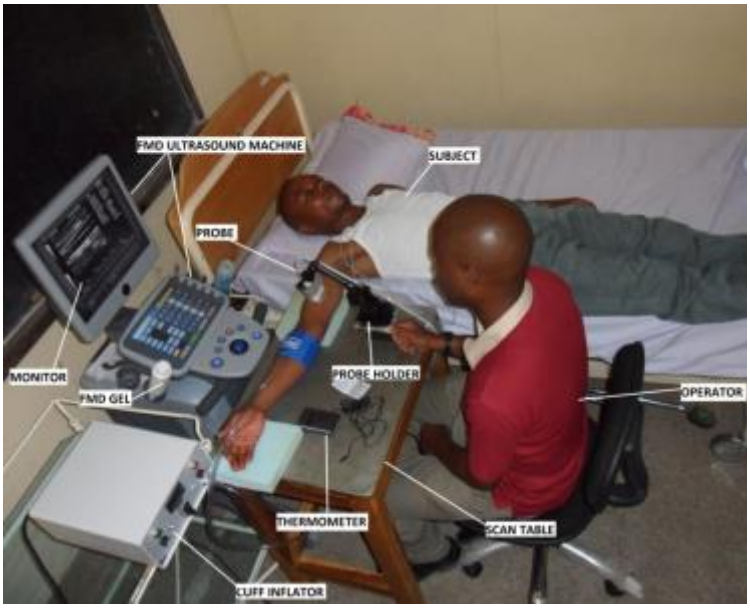
We do not know if a food supplement, or a "super-food supplement" with extra ingredients, can improve growth and reduce the effects of SCD in children like you with SCD. Children with SCD are often recommended to take chloroquine (CQ) pill or syrup once a week to protect them from getting malaria. We do not know if taking a smaller amount of CQ every day instead of once a week, can have more of a good effect in SCD; other than stopping you from getting malaria. To find out answers to these questions, we need to compare how fast children grow (changes in height, weight over time) and to take pictures of the vessels that carry blood, when children are not taking any food supplement, and when children are taking a normal, or a super-food supplement.

If you take part in this study you will be given one of the food supplements and daily chloroquine syrup. When that one has finished, and you have had some time without it, you will be given the other, different, food supplement. You should eat two packets of the food supplement every day, one in the mornings and one in the afternoon or evening. You can eat them straight from the packet and they are especially for you, and not to be shared with your friends or family, as they don't have SCD. You will not know which is the normal and which is the super-food supplement and nor will your family, or us – until the study is finished. When you start and finish taking each food supplement, we will see you in a special study clinic and we will measure your height and weight, like we do normally. We will also take pictures of a blood vessel in your arm with a special machine that can "see" through your skin! This is like when a pregnant woman gets a picture of the baby in their tummy before the baby is born. This does not hurt at all and does not harm the baby or the mother. During the study you will not have to come to the normal SCD clinic as well, as we will be seeing lots of you at the special study clinic instead. All children taking part in this study will be visited every other week by someone from the study, who will deliver the food supplement and check how you are by asking your parents some questions.

**HUDHURIO LA KLINIKI**



**Musa having a picture of his blood vessel taken by Dr Selemani (V-FIT Dr)**




**Food supplement – one dose**


**Food supplement for each morning and afternoon of the week**


We think that many SCD children are experiencing more episodes of pain and illness than we know about. We plan to find out by visiting children and families at home once a week – and *asking* them! We will ask for your help and your families also by filling out a simple daily diary with pictures and stickers which records how you have felt each day. We hope this will be fun for you to do.

**Please feel free to take this home with you. For the study, we will ask you to sign to confirm that you understand and want to be in this study. You can speak to Dr J Makani: Tel: 0754 38151.**

MUHIMBILI UNIVERSITY OF HEALTH & ALLIED SCIENCES

**P.O Box 65001 Dar es Salaam. Tanzania**

**INFORMED ASSENT FORM**

| Site name | Site number | Serial number (site specific) | Participant number | | | | |
|---|---|---|---|---|---|---|---|
| **MUHAS** | **NA** | | | | | | |

## *Vascular Function Intervention Trial (V-FIT) in Sickle cell disease*

**Informed ASSENT for participating children:**
I have read or I have been read the attached information regarding the SCD study in English / Swahili (please circle one), a language I understand. I have also had an opportunity to ask questions and am willing to participate.

I agree to take part in this study:

**Patients**
**Signature or thumb print _____ Date_____**

**Patients Name: _____ Age _____**
*(Please print name)*

**Parent/Caretaker**

I confirm that my child has had the study explained, has had the opportunity to ask questions and understands what will be involved for him/her and is willing to take part.

**Signature or thumbprint _____ Date_____**

**Witness' Name: _____**
*(Please print name)*

I certify that the above was explained verbally to the parent/guardian, and that s/he understands the nature and the purpose of the study and consents to the participation in the study of the above child.

I have given them the opportunity to ask questions which have been answered satisfactorily.

**Research Officer**
**Signature _____ Date_____**

**Research Officer Name: _____**
*(Please print name)*

**CHUO KIKUU CHA TIBA NA SAYANSI MUHIMBILI**
**P.0.BOX 65001, DAR-ES-SALAAM.TANZANIA**

**FOMU YA TAARIFA**

| Jina la mahali | Namba ya mahali | Namba ya safu(mahali halisi) | Kitambulisho cha Utafiti | | | |
|---|---|---|---|---|---|---|
| **MUHAS** | **N/A** | | | | | |

**JINA LA UTAFITI:**

***Vascular Function Intervention Trial (V-FIT) in Sickle cell disease***

***Majaribio Ya Muingiliano Wa Ufanyaji Kazi Wa Mishipa Ya Damu (V-FIT) Katika Ugonjwa Wa Siko Seli.***

**WATAFITI**

Utafiti huu unaendeshwa kwa ushirikiano kati ya watafiti kutoka Uingereza na watafiti kutoka kitengo cha utafiti wa ugonjwa wa siko seli Muhimbili ambapo wewe ulikubali kuwa mshiriki. (Kwa maelezo ya kina tafadhali angalia maelezo yafuatayo hapo chini)

**MADHUMUNI YA UTAFITI**

Kutokana na matokeo ya utafiti tulioufanya hadi sasa tuna ushahidi wa kutosha kushauri kwamba vyakula nyongeza vinaweza kuleta manufaa katika ugonjwa wa siko seli.Tumegundua kuwa watoto wengi wenye ugonjwa wa siko seli wana utapia mlo na mara nyingi huwa wafupi na wembamba ukilinganisha na watoto wa Tanzania wenye umri kama huo wasiokuwa na ugonjwa wa siko seli.Ukuaji duni unaambatana na matokeo mabaya ya afya katika magonjwa mengi yakiwemo na magonjwa ya siko seli.Tumeona pia kuwa wingiwa virutubisho lishe katika damu ni pungufu sana kwa watu wenye ugonjwa wa siko seli ambao huumwa sana au mara kwa mara ukilinganisha na wale wenye kuumwa kidogo au mara chache u. Wingi wa virutubisho lishe unadhaniwa kuwa muhimu katika kuwezesha mishipa ya damu kumudu vyema kazi ya kusafirisha damu.Tunafahamu kuwa iwapo mishipa hiyo haitafanya kazi ipasavyo,inaweza kuchangia kwa baadhi ya dalili na matokeo ya muda mrefu ya ugonjwa wa siko seli.Pia tunadhani kuwa dawa ya klorokwini tuliyokuwa tukiwapa wagonjwa kama tiba ya malaria inaweza kuwa na matokeo ya kufaa katika ufanyaji kazi wa mishipa ya damu,Lakini hatuna hakika kiasi gani kinahitajika kwa ili kupata matokeo hayo.

Virutubisho lishe hutumika nchini Tanzania kutibu na kuzuia utapiamlo.Hatuna hakika kama Virutubisho lishekama hivi vyenye vitamin na madini vitasaidia kuongeza ukuaji na matokeo mengine kwa watoto wenye siko seli,kama vile kupunguza tatizo la upungufu mkubwa wa damuTunapenda pia kufahamu ikiwa kuongeza zaidi aina fulani kwenye hivi hivi virutubisho lishe na kutoa kidogo lakini mara nyingi kwa siku dawa ya klorokwini kinaweza kuboresha ufanyaji kazi wa mishipa ya damu kwa wagonjwa wa siko seli.Ili kufahamu,inabidi kulinganisha ukuaji na ufanyaji kazi wa mishipa ya damu kwa watoto baada ya muda wa kutowapa Virutubisho lishena vile vya kawaida ukilinganisha na wakati wanapotumia Virutubisho lishecha kawaida na wakati wa kutumia Virutubisho lisheviivyoongezewaa virutubisho na dozi tofauti ya klorokwini kutoka ile ya kawaida.

Tunadhani kuwa watoto wengi wenye ugonjwa wa siko seli wanapatwa na na maumivu makali pamoja na kuumwa mara nyingi zaidi kulinganisha na taarifa ambazo huwa tunakusanya wanapolazwa katika Hospitali ya Taifa ya Muhimbili.Pia tunadhani kuwa Virutubisho lishe vinaweza kuwa na uhusiano na maumivu makali

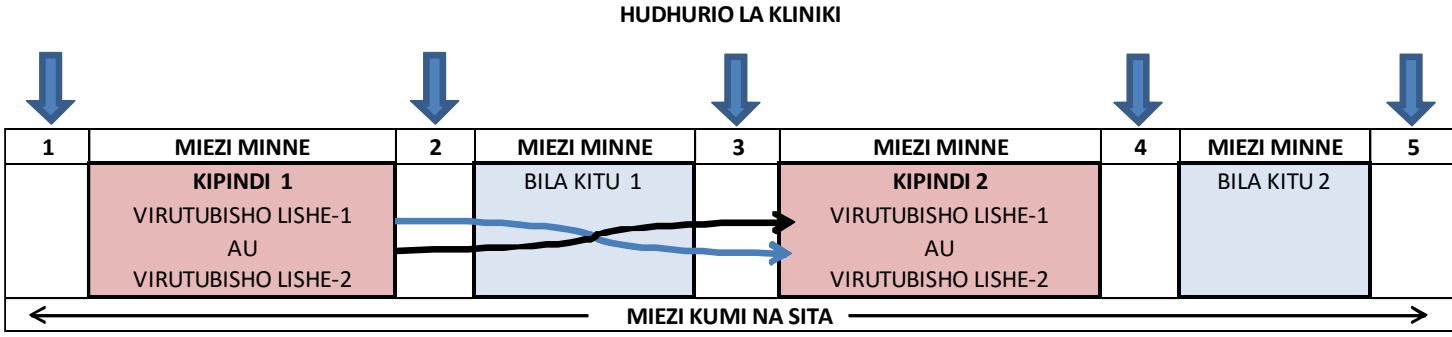
yanayowapata watoto wenye ugonjwa wa siko seli. Kudhihirisha hayo inabidi tuweze kupima kiusahihi.Hivyo basi tunataka kukusanya taarifa sahihi juu ya idadi ya matukio ya maumivu na homa, kwa kuomba familia zao zitusaidie kurekodi matukio haya pindi yatokeapo kwa mtoto pamoja na kukubali kupigiwa simu, na kuwatembelea watoto na familia zao majumbani.

**NANI ATAHUSIKA KATIKA UTAFITI**

Tunawakaribisha watoto wenye umri kati ya miaka 8 hadi 11 ambao tayari wanashiriki katika utafiti wa siko seli Muhimbili na wawe ni wakazi wa kudumu wa vitongoji vya Dar-es-salaam kushiriki katika utafiti huu.Watoto watakaoonekana wana utapiamlo mkali hawatashirikishwa kwenye utafiti ila watapewa rufaa kwenda katika kitengo kinachojihusisha na kutibu utapimlo katika Hospitali ya Taifa Muhimbili kwa matibabu.Watoto wenye matatizo mengine ya kiafya kama vile kifafa , kifua kikuu nakadhalika au wale ambao wanatumia aina fulani ya dawa hawatashirikishwa katika utafiti huu.

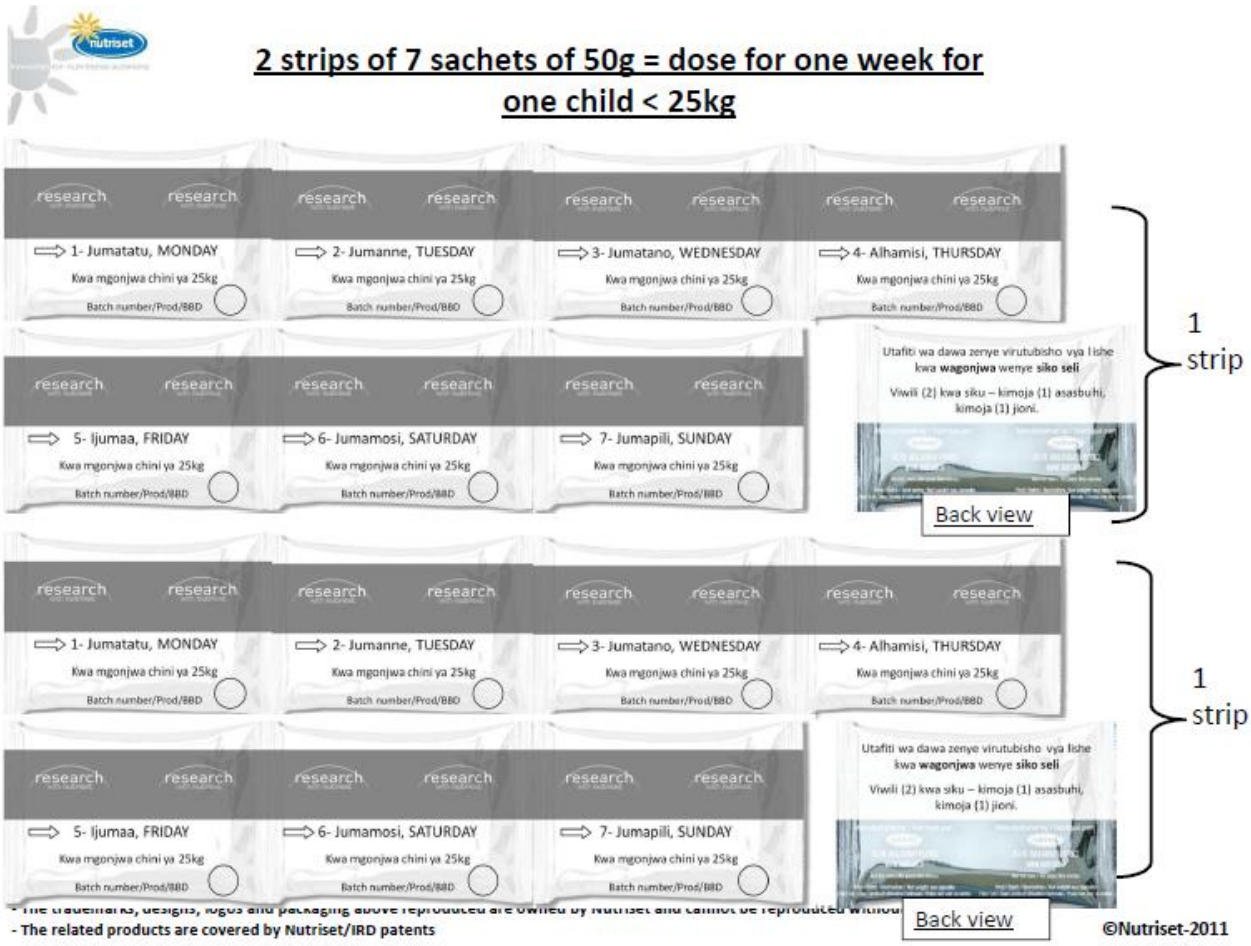
**NINI KITAHUSIKA KATIKA USHIRIKI**

Kujibu maswali yote haya, watoto watapewa moja kati ya aina mbili ya vyakula nyongeza mwanzo wa utafiti kwa kipindi cha miezi 4, ikifuatiwa na kipindi cha miezi 4 ambacho hawatapewa vyakula nyongeza. Halafu itafuatiwa na kipindi kingine cha miezi 4 ambapo watapewa aina tofauti ya vyakula nyongeza na mwisho kipindi kingine cha miezi 4 bila kupewa vyakula nyongeza, kwahiyo zoezi zima la utafiti litachukua kipindi cha miezi miezi 16.Si mchunguzi, au wewe na mtoto wako watakaojua ni aina hipi ya chakula nyongeza mtoto anapewa baada ya kumalizika kwa utafiti. Watoto wote watakaoshiriki kwenye utafiti huu watatembelewa majumbani na mfanyakazi wa utafiti mara moja kila baada ya wiki 2 kwa kipindi chote cha utafiti. Ambaye atawapatia vyakula nyongeza na dawa ya maji ya klorokwini, katika wiki ya kati mfanyakazi wa utafiti atawapigia simu.




Ikiwa utakubali kushiriki katika utafiti huu mtoto atapangwa katika moja ya makundi mawili kwa njia ya bahati nasibu itakayofanyika kwa kutumia bahasha zilizofungwa kabisa, , kisha kila kundi litapewa ama vyakula nyongeza maalumu au vyakula nyongeza vya mwanzo.

- Katika kipindi cha utafiti, watoto hawataruhusiwa kutumia vitamin wala madini ya nyongeza ila tu kwa kuidhinishwa na daktari, ambaye atakuwa na taarifa kuwa mtoto wako anatumia vyakula nyongeza vinavyotolewa katika utafiti huu..
- Kwa wakati ule ambao watoto watakuwa hawapewi vyakula nyongeza vya utafiti watoto wataruhusiwa kuendelea kutumia dawa yao ya Folic Acid kama kawaida. , Lakini kwa kile kipindi watakachokuwa wanapewa vyakula nyongeza hawatahitajika kutumia dawa zao za Folic acid kwani tayari vyakula nyongeza watavyokuwa wanapewa vinayo folic acid.



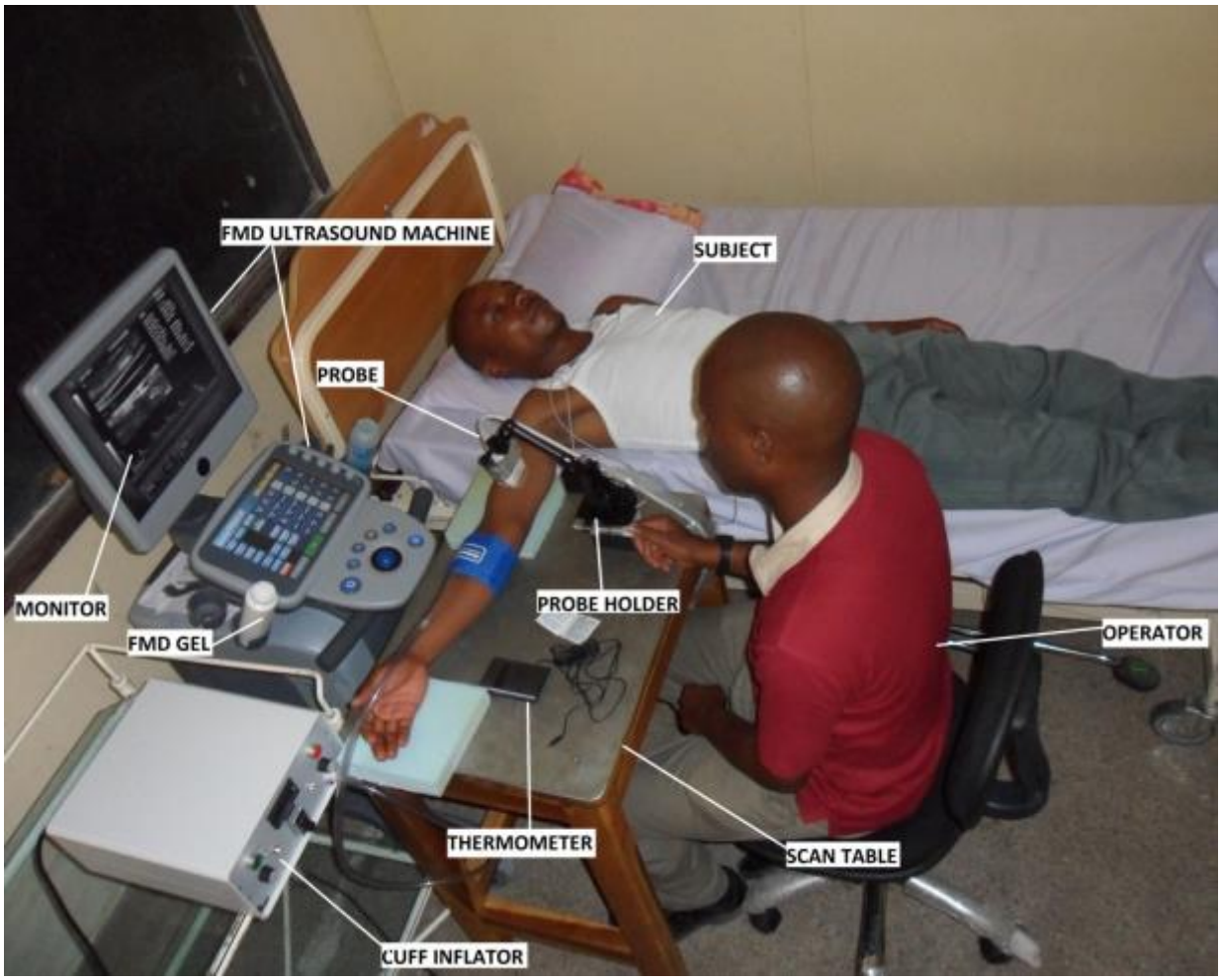

(mchoro wa kungara utatumika katika maelezo)

**Taratibu za kuhudhuria kliniki**

Watoto watakaoshiriki katika utafiti huu watatkiwa kuhudhuria kliniki maalum 5 za utafiti kama ifuatavyo; yakwanza wakati wa kuanza halafu baada ya **miezi 4, 8, 12 & 16.**.Hizi zitakuwa badala ya kliniki zako za siko seli za kawaida,kwa hiyo mtoto hatahitaji tena ya ziada .Kila unapohudhuria kliniki hizi maalum kwa ajili ya utafiti huu,vipimo vifuatavyo vitachukuliwa.

- **Kipimo cha damu.** Mtoto atachukuliwa kiasi kidogo cha damu mls 5 au ujazo wa kijiko cha chai kama ambavyo huchukuliwa ahudhuriapo kliniki yake ya kawaida..
- **Urefu na uzito** wa watoto vitapimwa, halafu pia watapimwawingi wa **mafuta na misuli** kwa kutumia mashine maalum ambapo mwanao atasimama kwenye mashine hiyo na kushika mikono yake kwenye mikono ya mashine kwa dakika moja.
- Ufanyaji kazi wa mishipa ya damu utapimwa katika mahudhurio manne ya kwanza kwa kutumia mashine maalum ambayo inatumia sauti kwa kupiga picha za ndani ya miili yetu-sawa na na mashine tunazotumia tunavyomchunguza mtoto ndani ya matumbo ya mama waja wazito (ultra sound) au sawasawa na mashine tunayotumia wakati tunavyopima spidi ya damu inavyosafiri ndani ya mishipa ya damu katika kichwa ambayo tunaita "TCD"Mtoto wako pengine atakuwa ameshafanyiwa kipimo cha TCD kama sehemu ya utafiti wa ugonjwa wa siko seli,kipimo hiki huwa kinafanywa mara kwa mara katika sehemu zingine duniani zinazojihusisha na matibabu ya ugonjwa wa siko seli. Tutapima ufanyaji kazi wa mshipa mkubwa wa damu katika mkono kwa kupiga picha wakati mshipa umekazwa na wakiti umelegezwa kama tufanyavyo wakati wa kupima shinikizo la damu (BP) kila uhudhuriapoe kliniki ya siko seli.Tofauti iliyopo ni kipimo hiki kitachukua muda kidogo zaidi kulinganishwa na wakati wa kupima shinikizo la damu lakini

hautazidi dakika 5.Mwisho tutachukua picha za mishipa ya damu kabla na baada ya dakika 5 baada ya kupewa dozi kidogo ya dawa iitwayo glyceryl-trinitrate ambayo mtoto atawekewa chini ya ulimi. Dawa hiyo ni salama kabisa na hutumika kwa kiasi kikubwa kuimarisha utendaji kazi wa mishipa ya damu na mapigo ya moyo kwa wagonjwa wenye matatizo ya moyo na mishipa ya damu.




(michoro ya kungara na picha za chumba na watoto wanaofanyiwa zoezi hilo)

**Kutembelea majumbani na ushiriki wa wazazi katika kukusanya takwimu.**

- Familia zitaombwa kujaza kumbukumbu za matukio ya maumivu katika kitabu maalum ambacho kitakuwa na michoro ili kurahisha ujazaji.Katika kitabu hiki watajaza pia kiasi cha maumivu au ukali pamaja na matibabu yeyote ikiwa alitibiwa.

Kama utapata tatizo, ugumu au wasiwasi wowote kutokana na kushiriki katika utafiti huu, utamjulisha mtafiti atakayekuwa anakutembelea nyumbani.Tutajitahidi kuhakikisha kuwa unatembelewa na mtafiti huyohuyo kila wiki.

Matembezi ya nyumbani ya kila wiki ni sehemu muhimu katika utafiti hu na **ushiriki wako ni wa maana**.Tunadhani tunaweza kujifunza mengi kuhusu siko seli kwa kupata uzoefu wako kama mzazi,nje ya mazingira ya kawaida ya hospitali.

**Je sampuli za damu zilizochukuliwa kutoka kliniki za utafiti zitatumikaje?**

Tutachukua sampuli moja tu ya damu kutoka kwenye kliniki za utafiti.Vipimo vya kawaida ambavyo huwa vinavyofanyika kwenye kliniki za siko seli vitafanyika kama vilefull blood picture na vipimo vya malaria kama vitahitajika.Sampuli itakayobaki itatumika kupima viashiria maalum vinavyohusiana na utendaji kazi wa mishipa ya damu. Baadhi ya vipimo vitafanyika kwenye maabara ya siko seli,hapa Muhimbili na vingine vitatakiwa kufanyika katika maabara za uingereza.Sampuli zozote zitakazobakia zitahifadhiwa kwa muda mrefu kwa ajili ya tafiti zingine za baadae zinazosabiana na ongezeko la uelewa wa ugonjwa wa siko seli.Hii ni sawa na inavyotokea kwa sampuli zinazokusanywa katika kliniki za kawaida za siko seli.

**FAIDA YA UTAFITI**

Hakutakuwa na faida ya moja kwa moja au ya kifedha kwa washiriki wa utafiti huu.Ushiriki wako utatusaidia kujua iwapo vyakula nyongeza pamoja na utoaji wa kila siku wa dawa klorokwini kulinnganishwa na utoaji wa klorokwini kwa wiki una faida pekee kwa wagonjwa wa siko seli.Ingawa tunatumia vyakula nyongeza na klorokwini ambayo tayari ilishatumika kwa wagonjwa wa siko seli,bado ni muhimu sana tuwe ushahidi na uhakika kwamba matumizi haya ni yenye manufaa, salama na yanakubalika na familia.

**ATHARI MUHIMU ZA UTAFITI**

- Utafiti huu unaweza kuwa na athari i ndogo sana kwa watoto kama vile kupata aleji itokanayo na vyakula nyongeza.Tutajitahidi kupunguza athari hiyo kwa kuwapatia kwanza kiasi kidogo cha vyakula nyongeza kama majaribia hapa kwenye kliniki
- Kiasi cha damu itakayochukuliwa ni ndogo na haiwezi kuleta athari zozote kwa mtoto kwani ni kiasi kidogo zaidi ya damu itolewayokwenye kliniki za siko Watoto wengine wanaweza kijisikia vibaya kutokanwa na kukazwa mkono wakati wa kufanya kipimo maalum cha kuangalia utendaji kazi wa mishipa ya damu hali hii pia inaweza kutokea baada kipimo ya kulegezwa ingawa hatujawahi kushuduia hili likitokea. .Ili kupunguza athari hii tutahakikisha mtoto wako anakuwa mtulivu wakati wa kipimo na baada ya kipimo tutakuomba wewe na mtoto wako kupumzika sehemu salama tutakayoiandaa kwa saa moja baada ya kumaliza kufanyiwa kipimo cha kuangalia ufanyaji kazi wa mshipa wa damu ili kuhakikisha kuwa hakuna madhara yaliyotokea au kama kuna madhara yametokea basi tuweze kuyatibia.
- Kama kawaida ya dawa yeyote ile huwa na athari ndogo hivyo basi mtoto wako anaweza akapata athari hizo kutokana na matumizi ya klorokwini kila siku au kila wiki**.**Hata hivyo nyingi ya athari hizo ni za muda mfupi na hazina madhara, lakini tutafuatilia matukio yote hayo kwa ukaribu na pia tunakuomba utupe taarifa ya tatizo lolote litakalotokea kwa mtoto wako au kama kuna jambo lolote unalohitaji ufafanuzi wakati wa kuhudhuria kliniki za kila wiki au piga simu kwenye namba za simu za wagonjwa wa siko seli.

**UHURU WA KUSHIRIKI KATIKA UTAFITI**

Tungependa kusisitiza kuwa **ushiriki** wako katika utafiti huu ni wa **hiari kabisa**. Ni uamuzi wako.Ukiamua kutokushiriki; **kamwe** haitaathiri matibabu au uangalizi utakaoupata kutoka hapa hospitali au ushiriki wako katika utafiti unaoendelea, kwa kuongezea uko huru kujitoa kwenye

ushiriki katika utafiti huu(na pia ukipenda hata katika utafiti unaoendelea) wakati wowote ule utakapojisikia kufanya hivyo na sisi tutakuondoa kwenye utafiti mara moja.Ikiwa pia utapendelea tuharibu sampuli yeyote iliyohifadhiwa tuliyo nayo tutafanya hivyo.Maamuzi yeyote ya namna hiyo yataheshimika na hayataathiri ubora wa huduma za afya tutakazokupa wewe na mtoto wako.

**USIRI**

Taarifa zote zilizopatikana kutoka kwa mtoto wako na familia yako zitakuwa za siri na kwa kuongezea pamoja kutumia namba yako ya sasa ya siko seli, tutakupatia pia namba nyingine mpya kwa ajili ya utafiti huu..Ni Mtafiti Mkuu tu au mtu atakayeidhinishwa na yeye ndiye ataweza kuunganisha taarifa binafsi kwenda kwa washiriki wa utafiti.

**WATAFITI**

Dr. Sharon Cox (London School of Hygiene &Tropical Medicine, UK)
Dr. Julie Makani (Muhimbili University of Health &Allied Scieences.Tanzania)
Professor Charles Newton (UCL-Institute of Child Health, UK&KEMRI-Kilifi, Kenya)
Professor Fenella Kirkham (UCL-Institute of Child Health,UK)
Professor Andrew Prentice (London School of Hygiene &Tropical Medicine, UK)
Professor Julian Halcox (University of Cardiff, UK)

**Tafadhali jisikie huru kuuliza maswali yeyote kuhusu taarifa ambazo tumekupatia hivi au jambo lolote linalohusiana na ugonjwa wa Siko Seli na matunzo/huduma ya mtoto wako.** tutafurahi ya kukupatia taarifa zaidi iliyoandikwa, kuhusiana na aina tofauti za virutubisho vilivyopo kwenye vyakula nyongeza pamoja na dawa ya klorokwini kama utahitaji (inapatikana kwa kiingereza)

Kuna taarifa zaidi katika mfumo wa majarida, vipeperushi na machapisho ambazo zipo kwa ajili yako ili usome zaidi kuhusu ugonjwa wa siko seli. Taasisi ya THE SICKLE CELL FOUNDATION IN TANZANIA pia inazo taarifa kuhusiana na ugonjwa wa siko seli na wangefurahi kusikia kutoka kwako juu ya wasiwasi wowote au maswali ambayo unayo(tafadhali ulizia namna ya kuwasiliana)

Tafadhali jisikie huru kuchukua taarifa hizi kwenda nazo nyumbani na unaweza kuwasiliana nasi au kutuliza maswali zaidi wakati utakapokuja tena kliniki..

Kwa ajili ya utafiti tutakuomba uweke saini yako katika karatasi hii ili kuhakikisha kuwa umepokea taarifa hizi na kwamba umekubali kwa hiari kushiriki katika utafiti huu.

Kama kuna taarifa yeyote ambayo utahitaji kuhusu taratibu ya utafiti tafadhali omba ili uonane na Dr.J Makani, Idara ya Magonjwa ya damu katika Chuo Kikuu Kishiriki cha Sayansi na Tiba Muhimbili (MUHAS).Simu +255 0754 381551 au watafiti wengine.Iwapo una maswali juu ya haki yako kama mshiriki, unaweza kuwasiliana na Mwenyekiti wa Kamati ya Utafiti na Mawasiliano ya Chuo kikuu cha muhimbili (MUHAS) P.O.BOX 65001 Simu 2150302 Dar-es-salaam

**CHUO KIKUU CHA TIBA NA SAYANSI MUHIMBILI**
**P.0.BOX 65001, DAR-ES-SALAAM.TANZANIA**

**FOMU YA TAARIFA**

| Jina la mahali | Namba ya mahali | Namba ya safu(mahali halisi) | Kitambulisho cha Utafiti | | | |
|---|---|---|---|---|---|---|
| **MUHAS** | **N/A** | | | | | |

## *Majaribio Ya Muingiliano Wa Ufanyaji Kazi Wa Mishipa Ya Damu (V-FIT) Katika Ugonjwa Wa Siko Seli.*

**Taarifa ya idhini kwa washiriki:**
Nimesoma/nimesomewa taarifa iliyoambatanishwa kuhusu utafiti wa ugonjwa wa siko seli kwa lugha ya Kiswahili, lugha ninayoizungumza kiufasaha.pia nimepata nafasi ya kujadili na kuuliza maswali kwa wachunguzi na nimeridhika kwamba nimeelewa utafiti huu unahusu nini na kujibiwa maswali yangu

Nimekubali/kumruhusu mtoto wangu (aliyetajwa hapo chini) kushiriki katika utafiti huu

(1)……..

Mgonjwa/Mzazi/Mlezi
Sahihi au dole gumba……..Tarehe…….

Jina la Mzazi/Mlezi……
(herufi kubwa tafadhali)

Shahidi
Sahihi (kama mdhaminiwa hajui kusoma)……Tarehe…….

Jina la shahidi……
(herufi kubwa tafadhali)

Ninathibitisha kwamba yaliyoandikwa hapo juu yameelezwa kwa mdomo kwa mzazi/mlezi na kwamba ameelewa nia na madhumuni ya utafiti na kutoa idhini kwa washiriki katika utafiti kwa wagonjwa waliotajwa hapo juu.

Nimewapa nafasi ya kuuliza maswali ambayo yamejibiwa kwa kuridhisha.

Afisa wa Utafiti
Sahihi……Tarehe……

Jina la Afisa wa Utafiti……

**CHUO KIKUU CHA TIBA NA SAYANSI MUHIMBILI**
**P.0.BOX 65001, DAR-ES-SALAAM.TANZANIA**

**FOMU YA TAARIFA (ASSENT)**

| Jina la mahali | Namba ya mahali | Namba ya safu(mahali halisi) | Kitambulisho cha Utafiti | | | |
|---|---|---|---|---|---|---|
| **MUHAS** | **N/A** | | | | | |

**JINA LA UTAFITI:**

***Vascular Function Intervention Trial (V-FIT) in Sickle cell disease***

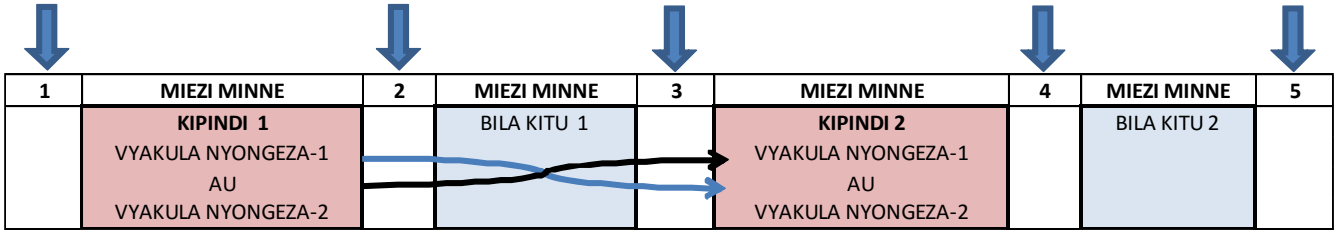
***Majaribio Ya Muingiliano Wa Ufanyaji Kazi Wa Mishipa Ya Damu (V-FIT) Katika Ugonjwa Wa Siko Seli.***

**UTAFITI**

Hatujui kuwa Vyakula nyongeza au " vyakula nyongeza bora" ambavyo vina virutubisho lishe zaidi vinaweza kuboresha ukuaji na kupunguza matokeo ya ugonjwa wa siko seli kwa watoto kama wewe mwenye ugonjwa wa siko seli.Watoto wenye siko seli Tanzania wanashauriwa kutumia kidonge cha klorokwini au klorokwini ya maji mara moja kwa wiki ili kuwazuia wasipate malaria. Hatujui kama iwapo watatumia kiasi kidogo cha cha klorokwini kila siku badala ya wiki moja inaweza ikaleta matokeo mazuri katika ugonjwa wa siko seli zaidi ya kukuzuia kupata malaria. Kupata majibu kutoka maswali hayo, inatubidi kulinganisha ni haraka namna gani watoto wanavyokua (mabadiliko ya urefu, uzito kupita mda)na kupiga picha ya mishipa ya damu wakati watoto hawatumii vyakula nyongeza na wakati watoto wanatumia vyakula nyongeza bora au vya kawaida.

Kama utashiriki katika utafiti huu, utapewa mojawapo ya vyakula nyongeza pamoja na dawa ya maji ya klorokwini. Utakapomaliza kutumia vyakula nyongeza hivi utakuwa na kipindi kingine fulani ambacho utakaa bila kupewa vyakula nyongeza. Kisha utapewa aina nyingine tofauti ya vyakula nyongeza. Itabidi ule pakiti mbili za vyakula nyongeza kila siku, moja asubuhi na moja mchana au jioni.Unaweza kula moja kwa moja kutoka kwenye paketi na vyakula hivi viko maalumu kwa ajili yako, na siyo kwa kuwapa rafiki zako au mwanafamilia, kwani wao hawana siko seli. Wewe sisi au mzazi wako tatuwezi kujua ni aina ipi ya vyakula nyongeza (kati ya ile ya kawaida au vyakula vyongeza bora) utakayokuwa unatumia mpaka mwisho wa utafiti. Utakapoanza na kumaliza kutumia kila vyakula nyongeza tutakuona katika cliniki maalumu ya utafiti na tutakupima urefu na uzito kama kawaida tunavyofanya.Pia tutapiga picha za mishipa ya damu kwenye mkono wako kwa kutumia mashine maalumu ambayo inaweza kuona ndani ya ngozi yako. Mashine hii ni sawa na ile ambayo hutumika mama mja mzito anapopigwa picha ya mtoto wake ndani ya tumbo kabla mtoto wake hajazaliwa. Kipimo hiki hakiumizi kabisa wala hakiwezi kumdhuru mtoto au mama.Wakati wa utafiti huu hutahitajika kuhudhuria kliniki yako ya kawaida ya siko seli, kwani badala yake tutakuwa tunawaona wengi wenu katika kliniki maalumu ya utafiti. Watoto wote wanaoshiriki katika utafiti huu watatembelewa kila baada ya wiki moja kupita na mfanyakazi kutoka katika utafiti, ambaye atatoa vyakula nyongeza na kuchunguza jinsi afya yako ilivyo na kukuuliza maswali kadhaa.

**HUDHURIO LA KLINIKI**



**Musa having a picture of his blood vessel taken by Dr Selemani (V-FIT Dr)**


**Food supplement – one dose**


**Food supplement for each morning and afternoon of the week**


Tunadhani kuwa watoto wengi wenye ugonjwa wa siko seli wanapata taabu sana ya maumivu ya mara kwa mara kuliko tunavyojua. Tuna mpango wa kulichunguza jambo hili kwa njia ya kuwatembelea watoto na familia zao majumbani mara moja kwa wiki-na kuwahoji.Tutaomba msaada wako na wa familia yako pia kwa kujaza kitabu maalum ambacho ni rahisi kwani kina maelekezo ya picha vibandiko ambamo utawekarekodi za maendeleo yako ya namna unavyojisikia kila siku.Tuna imani utafurahia kujaza kitabu hiki.

**Tafadhali jisikie huru na chukua hii nyumbani. Kwa ajili ya utafiti tutakuomba utie saini kutuhakikishia kuwa umeelewa na ungependa kushiriki kwenye utafiti. Unaweza kuongea na Dr Makani: Tel: 0754 381551.**

**CHUO KIKUU CHA TIBA NA SAYANSI MUHIMBILI**
**P.0.BOX 65001, DAR-ES-SALAAM.TANZANIA**

**FOMU YA TAARIFA (ASSENT)**

| Jina la mahali | Namba ya mahali | Namba ya safu(mahali halisi) | Kitambulisho cha Utafiti | | | |
|---|---|---|---|---|---|---|
| **MUHAS** | **N/A** | | | | | |

***Majaribio Ya Muingiliano Wa Ufanyaji Kazi Wa Mishipa Ya Damu (V-FIT) Katika Ugonjwa Wa Siko Seli.***

**Taarifa ya idhini kwa washiriki:**
Nimesoma/nimesomewa taarifa iliyoambatanishwa kuhusu utafiti wa ugonjwa wa siko seli kwa lugha ya Kiswahili, lugha ninayoizungumza kiufasaha.pia nimepata nafasi ya kujadili na kuuliza maswali kwa wachunguzi na nimeridhika kwamba nimeelewa utafiti huu unahusu nini na kujibiwa maswali yangu

Nimekubali/kumruhusu mtoto wangu (aliyetajwa hapo chini) kushiriki katika utafiti huu

(1)……

Mgonjwa/Mzazi/Mlezi
Sahihi au dole gumba……..Tarehe…….

Jina la Mzazi/Mlezi……
(herufi kubwa tafadhali)

Shahidi
Sahihi (kama mdhaminiwa hajui kusoma)……Tarehe……

Jina la shahidi……
(herufi kubwa tafadhali)

Ninathibitisha kwamba yaliyoandikwa hapo juu yameelezwa kwa mdomo kwa mzazi/mlezi na kwamba ameelewa nia na madhumuni ya utafiti na kutoa idhini kwa washiriki katika utafiti kwa wagonjwa waliotajwa hapo juu.

Nimewapa nafasi ya kuuliza maswali ambayo yamejibiwa kwa kuridhisha.

Afisa wa Utafiti
Sahihi……Tarehe……

Jina la Afisa wa Utafiti……