Zinc for Infection Prevention in Sickle Cell Anemia (ZIPS)

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SYNOPSIS

DESIGN: A randomized double-blinded placebo-controlled trial of zinc to reduce the incidence of severe or invasive infections in Ugandan children with sickle cell anemia (SCA)

POPULATION: Children with confirmed SCA between 1.00 and 4.99 years of age, living in the malaria endemic area of mid-eastern Uganda, who attend the Nalufenya Sickle Cell Clinic, Jinja Regional Referral Hospital whose caretakers consent to study participation.

SAMPLE SIZE: 250 children, randomized to receive either zinc (n=125) or placebo (n=125)

TREATMENT: Study treatment will be zinc (10 mg zinc sulphate) or placebo, administered once a day in tablet form.

ENDPOINTS: To determine if zinc reduces incidence of severe or invasive infections compared to placebo in Ugandan children 1.00-4.99 of age with SCA

DURATION: The primary study endpoint will be evaluated after twelve (12) months of study treatment (zinc or placebo).

ABSTRACT

BACKGROUND: Approximately 240,000 children, accounting for 75% of all children with sickle cell anemia (SCA) are born in sub-Saharan Africa annually. Infection is a major cause of illness and death in these children. Interventions to reduce the incidence and severity of infections are needed urgently. Zinc deficiency leads to impaired immunity and an increased risk of infection, and a high proportion of adults and children with SCA are zinc deficient. Zinc supplementation has decreased infection in otherwise healthy children <5 years of age, and in adults and adolescents with SCA, but there is no data on the effectiveness of zinc for prevention of infection in children <5 years of age with SCA.

GAP: This study will define whether zinc supplementation can decrease incidence of severe or invasive infections in Ugandan children with SCA.

HYPOTHESIS: Zinc supplementation will lead to a lower incidence of severe or invasive infections than placebo in Ugandan children with SCA.

METHODS: The study will be a randomized, placebo-controlled, double blind clinical trial in which 250 Ugandan children 1.00-4.99 years of age with SCA will receive zinc (10 mg oral dispersible tablet daily) or placebo (identical to zinc in appearance) for 12 months. The primary study outcome will be incidence of severe or invasive infections. Secondary outcomes will include incidence of all clinical infections, confirmed bacterial infections (by culture or PCR), incidence of vaso-occlusive crisis (VOC), change in height-for-age z-score, and incidence of zinc-related adverse events.

IMPACT: If this trial shows a reduction in severe or invasive infection incidence, it would be the basis for a multi-site pre-post intervention clinical trial to assess real-world safety and efficacy of zinc in African children with SCA. Since zinc is safe, inexpensive, and easy to administer, this trial has the potential to improve the health of hundreds of thousands of African children with SCA through reduction of infection-related morbidity and mortality.

LIST OF ABBREVIATIONS

ZIPS = Zinc for Infection Prevention in Sickle cell anemia

Hb = Hemoglobin

HbF = Fetal hemoglobin

SCA = Sickle cell anemia

SCD = Sickle cell disease

WHO = World Health Organization

US FDA = United States Food and Drug Administration

1.0 INTRODUCTION

1.1 Background and Rationale

Globally, 75% of all children with sickle cell anemia (SCA) are born in sub-Saharan Africa, approximately 240,000 children annually[1]. Yet few research studies on interventions to prevent complications of SCA have been conducted in African children. Infection is a major cause of morbidity and mortality in African children with SCA. In our recently concluded hydroxyurea in SCA study, 37% of children with SCA had clinically severe infections (pneumonia, sepsis, bacteremia, or malaria) over the 12 months of study, and 2 of the 3 deaths in the study were attributed to sepsis. Prevention of infection could therefore significantly decrease morbidity and mortality in African children with SCA.

Zinc supplementation may provide a simple, safe and low-cost way to prevent infection in children with SCA. Zinc is important in development of T and B cell immune responses[2], and through its effects on these responses may help to prevent or reduce infection. In multiple studies in healthy children < 5 years of age, zinc supplementation reduced incidence of diarrhea and pneumonia with minimal side effects[3]. Zinc deficiency is common in adults and children with SCA, due to increased release of zinc during bone degradation in vaso-occlusive crises (VOC)[4] and subsequent loss of zinc in the urine[5]. These losses are frequently not compensated because local diets that are consumed in many regions where SCA is most prevalent tend to be poor in zinc. In clinical trials in adults or adolescents with SCA, zinc supplementation decreased incidence of infection by 47-95% compared to placebo or to a prior period without zinc supplementation[6-8]. However, to date no zinc supplementation trials for prevention of infection have been conducted in children <5 years of age with SCA. Zinc may also have other beneficial effects in SCA, including decreased VOC, due to its effects on red cell hydration[6-8], and improved growth[9].

1.2 Sickle Cell Anemia (SCA) is a Major Health Problem in Sub-Saharan Africa

SCA is an inherited hemoglobinopathy characterized by chronic hemolytic anemia and vascular occlusion. Africa is the most highly affected continent with approximately 240,000 infants born with SCA annually, contributing to 5% of deaths in children under 5 years of age [10,11]. SCA is a major public health problem in Uganda: approximately 15,000 Ugandan children are born with SCA annually [12], and it has been estimated that as many as 70-80% of children with SCA in Uganda die before the age of 2 years [10], though accurate data on deaths in children with SCA in Uganda are very difficult to obtain, because many may die before receiving a diagnosis.

1.3 Infection Causes Substantial Morbidity and Mortality in African Children with SCA

Infections in children with SCA are a major cause of hospitalization and mortality. The risk of infectious complications in SCA is most evident in low- and middle-income countries (LMICs), where access to care and treatment options are limited. Pneumococcal prophylaxis and pneumococcal vaccination have decreased invasive pneumococcal disease in SCA, but infection remains a common cause of significant illness in African children with SCA. For example, in the NOHARM study of hydroxyurea we recently conducted at the Mulago Hospital Sickle Cell Clinic (MHSCC), children with SCA had an average of 0.7 infections/child/year classified as “severe or invasive” infections (see Appendix I for infections that met this criterion). In addition, two of the three deaths that occurred in the first year of study were attributed to sepsis.

1.4 Zinc Deficiency is Common in Ugandan Children

Multiple studies report inadequate zinc intake by Ugandan children[13,14], with estimates of up to as many as 98% of children younger than five years of age in one sample not consuming the Recommended Nutrient Intake (RNI) prescribed by the World Health Organization[13]. Based largely on unrefined cereals and legumes, the Ugandan diet is rich in phytate, the storage compound of phosphorous in plants that is a potent inhibitor of zinc absorption. The burden of zinc deficiency in Ugandan children is thus assumed high, although the difficulty of measuring a biomarker for zinc in the blood has hindered accurate assessment of the prevalence of zinc deficiency in Uganda and much of the world. Zinc is a common contaminant in the field, making collection of blood into metal-free equipment a prerequisite of accurate assessment [15]. Studies by Dr. Ware and colleagues show that renal dysfunction, in the form of glomerular hyperfiltration, starts in infancy, suggesting that zinc loss in urine likely also starts to occur during this time period, and that young children with SCA will be risk through dietary deficiency combined with renal zinc loss[16].

As part of a recent community-based survey aimed to determine the blood concentration of 13 heavy metals in healthy Ugandan children, we measured zinc concentration in whole blood samples collected with contaminant-free equipment from 100 children 6-59 months living in the Katanga neighborhood adjacent to Mulago Hospital in Kampala. No child was acutely ill at the time of assessment. We found the median whole blood zinc concentration to be 354 µg/dL (5^{th}p, 95^{th}p: 213, 551 µg/dL), well below the median of a European pediatric reference population (462 µg/dL; 5^{th}p, 95^{th}p: 317, 580 µg/dL) [17]. Twenty-nine percent of the Ugandan children in our sample fell below the 5^{th} percentile of the reference population (317 µg/dL). Thus, we estimate that nearly one in three healthy Ugandan children living in the recruitment area for the current study is zinc-deficient.

An earlier study found that zinc deficiency was common in Ugandan children with SCA [18], and this would be expected given the prevalence of dietary zinc deficiency in Ugandan children, combined with the increased urinary loss of zinc in children with SCA [19]. However, the benefits of zinc supplementation may be seen in SCA even in children without lowered plasma levels of zinc. For example, Fung et al found that growth in children with SCA improved with zinc treatment even in some children with normal zinc levels, and that zinc levels did not increase in the children supplemented with zinc despite improved growth[9]. This may be because release of free zinc into plasma often occurs during hemolysis, which is constantly present in children with SCA, and so plasma zinc levels may be "normal" when whole body zinc levels are low. In the largest study of zinc supplementation in adolescents and adults for prevention of infection, done by Gupta et al in India, zinc levels were not tested, and incidence of infection decreased by 47% in the zinc supplemented group[7]. For all of these reasons, we believe that children with SCA should be enrolled in this study regardless of baseline plasma or whole blood zinc levels. We will obtain plasma at enrollment and at the end of treatment for each child, and test zinc levels at study end (testing must be done in a specialized lab in the US, so the samples will need to be sent as a group rather than testing at the time obtained), so that we can determine if children with low levels have the greatest benefit from zinc supplementation.

1.5 Zinc May Be a Cost-Effective Way to Decrease Infection and Improve the Health of African Children with SCA

Studies from older children and adults with SCA, and from children without SCA, show that zinc supplementation can decrease risk of infection. Zinc supplementation is inexpensive, has minimal side effects, and has been used successfully in children in LMIC in other contexts to prevent infection[3]. There is also evidence from studies in adolescents and young adults that zinc may decrease vaso-occlusive crises (VOC)[6-8] and improve growth[9]. There are few side effects if zinc is taken correctly and at the correct dose, and overdosing is extremely rare in the formulations provided. In addition, zinc is generic and inexpensive. Zinc supplements are already available in Uganda, and retail for $0.02 per tablet. A year of zinc for a child with SCA would cost $7.30, affordable even to low-income families in Uganda. The annual cost of zinc supplementation for all children with SCA in Uganda at these prices would be $880,000, and with bulk purchase by the Ministry of Health, the cost could be considerably lower. Zinc supplementation would thus be cost-effective and sustainable in Uganda.

Clinical trial results in adults often differ from those in children, and only a placebo-controlled RCT can determine efficacy and the level of efficacy of zinc supplementation for prevention of infection in young children with SCA. Since there are no data on the efficacy of zinc supplementation in children with SCA <5 years of age, there is clinical equipoise for the trial. Given the evidence of zinc deficiency in children with SCA, the safety and cost-effectiveness of zinc as an intervention, and the efficacy of zinc in prevention of infection in older children and adults with SCA*, a randomized controlled clinical trial of zinc supplementation in Ugandan children could lead to an intervention that transforms the health of African children with SCA.*

1.6 Proposal

To assess the effects of zinc supplementation on infection in African children with SCA, and to evaluate any adverse events of zinc in this population, we propose a randomized, double-blind, placebo-controlled clinical trial, the **"Zinc for Infection Prevention in Sickle cell anemia" (ZIPS) trial**. Study participants will be recruited from Nalufenya Sickle Cell Clinic (NSCC) at Jinja Regional Referral Hospital in Uganda, which follows more than 3,500 children with SCA.

2.0 SPECIFIC AIMS

The **specific aim** of the study is to determine if zinc supplementation reduces severe or invasive infections in Ugandan children 1.00-4.99 years of age with SCA.

The study will also assess **four secondary outcomes** that may be affected by zinc supplementation in children: 1) incidence of all clinically diagnosed infections; 2) incidence of culture or PCR-confirmed bacterial infections, 3) incidence of vaso-occlusive crises (VOC), and 4) change in height-for-age z-score. We hypothesize that children with SCA who receive zinc supplementation will have less clinical infections, less culture or PCR-confirmed bacterial infections, less VOC and better growth than children who receive placebo.

The primary study goal is assessment of severe or invasive infection, and the study is powered for that outcome. However, information on differences of confirmed bacterial infection, VOC, and growth will provide important preliminary data for future studies. Finally, the study will assess the **frequency of**

adverse events with zinc, with particular attention to nausea and vomiting, the most common side effects seen with zinc supplementation.

3.0 CLINICAL SITE SELECTION

A benefit of pursuing this intervention in Uganda is the presence of an existing specialized sickle cell clinic at NSCC in the Children's Ward at Jinja Regional Referral Hospital. NSCC serves a region of high malaria transmission intensity along the shores of Lake Victoria. The clinic has over 3500 registered SCA children and is run by a senior consultant Pediatrician assisted by medical officers, nurses and counselors. Nalufenya Childrens Ward, part of Jinja Regional Referral Hospital, has been the site of several epidemiologic and clinical studies of children, including the ongoing severe malaria studies on neurodevelopmental impairment and post-discharge malaria chemoprophylaxis and Dr Opoka are PI's respectively. This project represents an opportunity to leverage and build on the clinical infrastructure in place at Jinja Regional Referral Hospital to study an intervention with potentially high impact findings that could change the practice of sickle cell disease care in Uganda. The goal is for this to serve as a model for other LMIC. The study team has extensive experience in studies of infectious diseases (John, Opoka), sickle cell disease (Ware, Hume) and micronutrient deficiency (Cusick) in children in LMIC. The investigators of this study were the ones that conducted the Novel use of Hydroxyurea in an African Region with Malaria (NOHARM) trial (NCT01976416) for which Dr. John was PI. Results from the NOHARM study provided evidence that is being used to make hydroxyurea available for use in children with SCA in Uganda[20].

4.0 STUDY DESIGN

4.1 Design

Randomized, double-blind, placebo-controlled clinical trial.

4.2 Study Population

Children with SCA age 1.00-4.99 years who attend NSCC.

4.3 Inclusion Criteria

1. Documented sickle cell anemia (HbSS supported by hemoglobin electrophoresis)
2. Age range of 1.00-4.99 years, inclusive, at the time of enrollment
3. Weight at least 5.0 kg at the time of enrollment
4. Willingness to comply with all study-related treatments, evaluations, and follow-up

4.4 Exclusion Criteria

1. Known other chronic medical condition (e.g., HIV, malignancy, active clinical tuberculosis)
2. Severe malnutrition determined by impaired growth parameters as defined by WHO (weight for length/height or height for age z-score <-3, using WHO growth standards)

4.5 Patient Eligibility

We will recruit and enroll study participants at the NSCC. The clinic database provides estimates of >1000 children in the target age group of the study (12-59 months of age). Parents of children who fall within the age range will be asked when the child is seen in clinic if they are interested in enrolling their child in the study. We anticipate a minimum of 30 children seen per week for medication refill or other non-illness related problems will be within the study age range. Those who are interested will be provided with information about the study, asked if they have any questions, and if they are interested will be asked the screening inclusion and exclusion criteria questions. If they meet inclusion criteria and do not meet exclusion criteria, they will be eligible for enrollment. Those who are eligible and wish to enroll in the study will be scheduled for a follow-up visit, where they will go through the full informed consent process, get baseline labs, and be given initial study medication and instructions for follow-up.

4.6 Sample Size

A total 250 children, randomized to receive either zinc (n=125) or placebo (n=125)

4.7 Consenting

Written informed consent will be obtained from the parent or guardian by trained medical staff at the NSCC. The original informed consent form will be retained at the study site; a copy will be put in the subject's records. A copy of the consent form will also be provided to the parent or guardian.

4.8 Randomization and Blinding

Block randomization will be used. The study pharmacist will have identical appearing zinc or placebo tablets and will provide the appropriate medication to the child. Children will be randomized (in blocks of 8) into treatment groups by order of entry in the study, based on a pre-determined blinded randomization list created and managed by an Indiana University study data manager. Treatment group will be provided to the study pharmacist, who will know only that the child is randomized to group A or B, but will not know which group is zinc or placebo. The designation of A or B on packets will be removed by the pharmacist prior to providing study drug to the child, so the medication will appear identical to study staff and parents/children. The child's study identification number will be recorded and treatment group may only be determined by comparing the child's study id to the blinded list, which only the IU data manager will have access to until the study is completed or stopping rules are reached.

4.9 Study Endpoints

To determine if zinc reduces incidence of severe or invasive infections more than placebo in Ugandan children 1.00-4.99 of age with SCA.

4.10 Primary Outcome

To assess for the incidence of infections, we will aim to evaluate all illnesses in children during the study period, we will request parents or guardians of children in the ZIPS study to bring their child in to the NSCC or after hours to emergency room at Nalufenya Children's Ward, Jinja Regional Referral Hospital

for any illness. Reimbursement will be provided for transportation, and all medical care will be provided at no cost to the study participant. With this approach in the NOHARM study, children used the MHSCC almost exclusively when they had illness, so we have high confidence that >90% of all illness episodes in this cohort will be taken care of by our study team. We will also record at scheduled visits on month 1, 3, 6, 9 and 12 visits any visits to outside clinics, and illnesses that occurred during those visits. All children will be evaluated for clinical evidence of infection by taking clinical history and exam, and diagnostic work up. Children with a measured axillary temperature of ≥37.5C will have blood obtained for a malaria smear and a blood culture for a measured fever of ≥38C. Children with history of fever or temperature ≥37.5˚C and age-specific tachypnea and cough will have a chest radiograph obtained.

Severe or invasive infections will include: abscesses, bacteremia, cellulitis, diarrhea, dysentery, malaria, meningitis/encephalitis, osteomyelitis, pharyngitis/tonsillitis, pneumonia/acute chest syndrome, sepsis, acute sinusitis. Infections will be defined as outlined in Appendix I (Definitions for Severe and Invasive Infections). We understand that the list is not comprehensive and that clinicians may differ on what constitutes a severe infection, but this list includes the most common infections seen in the NOHARM study that were considered to affect child well-being significantly. Other common infections in this age group (e.g., acute upper respiratory infection (URI), otitis media, conjunctivitis, tinea capitis, tinea corporis) will be recorded, and included (along with the severe and invasive infections) in the category of "clinical infections". Viral infections with a well-defined clinical picture (e.g., measles, varicella) will be defined by clinical signs and symptoms.

A codebook with specific definitions for each infection, based on WHO or similar criteria, will be created, and clinicians will have to check off criteria for the infection in order to assign the diagnosis of that infection to the child. Children who have fever with no defined source or clinical syndrome consistent with a specific infectious organisms or clinical syndrome will have a diagnosis of "fever, no defined source", and will not be included as "clinical infections" because fever could be non-infectious and because a "clinical infection" should conform to a clear clinical diagnosis or have PCR or culture confirmation of infection. Children with culture or PCR confirmed bacterial infections will be a subset of the children with clinical infections, and children with "fever, no source" as their initial diagnosis who are culture or PCR positive for an organism consistent with their clinical syndrome will have the diagnosis corrected to infection with the bacteria or virus confirmed by culture/PCR.

Culture or PCR-confirmed bacterial infections are a secondary objective in this aim. In many severe infections, including pneumonia, sepsis, osteomyelitis, sinusitis, and meningitis, blood cultures are often negative, and they are treated clinically. Other infections that are less severe in children with SCA, such as diarrhea, are typically viral in etiology, yet of clinical significance to these children and are known to be reduced with zinc supplementation. Malaria, which can cause severe disease in children with SCA is also not a bacterial infection. For these reasons, while we acknowledge that severe or invasive infection is a less specific outcome than proven bacterial infection (even with the rigorous definitions we will have in place for severe or invasive infection), we believe it is the right primary outcome for this aim.

For children with pneumonia/ALRI, the multiplex PCR test we use will determine, in addition to infection with *M. pneumoniae* and *C. pneumoniae*, infection with *B. pertussis*, and 17 viruses that commonly cause respiratory infections (see Testing Methods section below). This will provide additional epidemiologic information about viral infections causing febrile or URI syndromes in this population.

4.11 Secondary Outcomes

1) Clinical infection: as described above: 2) Culture confirmed bacterial infection (e.g., bacteremia, urinary tract infection, tonsillitis, abscess, osteomyelitis, meningitis) or PCR-confirmed infection with *Chlamydophila pneumoniae* or *Mycoplasma pneumoniae* (from nasopharyngeal swab) in children with pneumonia/acute chest syndrome. Pneumonia (acute lower respiratory infection, ALRI) will be defined as: history of fever or measured axillary temperature ≥37.5˚C, with age-specific tachypnea, cough, and an infiltrate and/or effusion on chest x-ray consistent with pneumonia (see Appendix II for full definition).
2) *Vaso-occlusive crises (VOC):* pain with the requirement for oral morphine or IM diclofenac, per SCC guidelines.
3) *Change in height for age z-score*, from enrollment to 12 months follow up, calculated using WHO standards.
4) *Serious adverse events*: 1) death; 2) a life-threatening event; or 3) hospitalization > 7 days.
5) *Adverse events*: based on CTCAE criteria, with modifications for SCA, as in the NOHARM study, will be assessed at the 1, 3, 6, 9 and 12-month follow-up visits, and at all sick visits.

5.0 STUDY TREATMENT

5.1 Dosing and Administration

Dispersible zinc sulfate tablet (10 mg) or identical placebo tablet, manufactured by Laboratoires Pharmaceutiques Rodael, France, and imported from India by Uganda Health Marketing Group. Tablet regimens of 30 tablets will be packaged and distributed in air tight containers to prevent moisture contamination. These tablets are currently available in Uganda, are certified as manufactured under good manufacturing process (GMP). The use of tablets currently available on the market will allow for easy implementation of the intervention if successful. Each tablet is designed to dissolve completely in a small amount of water or breast milk in <3 minutes, removing the need for small children to swallow a pill. Caregivers will be instructed to place the pill in 5-10 mL of clean water or breastmilk, stir gently, and wait three minutes until the pill is completely dissolved. Both active tablets and placebo will have the same flavoring added to enhance palatability.

5.2 Side Effects

The primary side effect of zinc is vomiting, and it is infrequent. For children who do have problems with vomiting, parents will be told to give the zinc with food, as this practice can decrease vomiting. Long term, zinc can decrease copper levels. We will check copper levels from samples at baseline and 12 months, to see if copper levels are affected in children in the ZIPS study.

5.3 Study Discontinuation and Stopping Rules

Data from this study will be reviewed in real-time by the Principal Investigator and Co-Investigator team members. Data will be reviewed periodically with an external DSMB. ZIPS will be discontinued if at any time the DSMB or study team feels that it is in the best interests of study subjects to do so.

6.0 STUDY ASSESSMENTS AND STUDY PROCEDURES

In addition to zinc or placebo prescribed at fixed doses as described above, procedures to be performed as part of the data collection and analysis for the ZIPS Study are detailed in this section and summarized in the Study Evaluations SOP.

6.1 Baseline and Follow Up Visit Sample Collection and Clinical and Lab Assessment

At baseline, children will have a history and physical exam performed, relevant history related to SCA obtained, including prior history of stroke, transfusions, and hospitalizations. Age, sex, height, weight and geographical area where the child lives will be recorded. At baseline and 12 month visits, 7 ml of blood will be obtained for a CBC with differential and reticulocyte count, and hemoglobin electrophoresis, and plasma, whole blood and filter paper dried blood spots samples obtained and stored. 5 ml of blood will be drawn at the 6 month visit for plasma, whole blood and filter paper dried blood spot samples. Plasma, blood and filter paper samples will be stored because some testing cannot be done immediately but only when all samples are collected, and also because new literature often shows novel factors that may relate to risk of infection or micronutrient deficiency, so we may test for these in future studies. We will assess genetic and immune risk factors, including variants of sickle cell disease and factors such as heme oxygenase-1 polymorphisms, that may affect the clinical course of sickle cell disease or response to infection, and will also store samples to look for novel genetic factors discovered in the course of the study that may relate to risk of infection, micronutrient deficiency or adverse effects from sickle cell disease.

Socio-economic status and dietary history will be assessed by a previously validated tool. An instrument for dietary history developed and validated in other child health studies in this area will be used to assess child diet, and this will be reassessed at 12-month follow-up. The instrument includes assessment of foods rich in zinc and assessment of pica. Other variables assessed will include other medications taken that are not standard MHSCC administered medications, and HIV infection (children will be tested unless parents elect not to have testing).

Urine samples will be collected in zinc-free containers at enrollment and 12-month follow-up in 100 randomly selected children to test for urine zinc levels, to assess degree of urinary zinc loss in study children at baseline and after 12 months of zinc or placebo treatment. This data will provide valuable new information about the degree of zinc lost in the urine in African children <5 years of age. Stool samples will also be collected from children who are able to provide them, for future microbiome testing.

6.2 Follow Up Clinical and Lab Assessments and Treatment for Acute Illness

Children in the study will have follow up visits at 1, 3, 6, 9 and 12 months. The visits will allow replenishment of zinc or placebo tablet supply, assess adherence (by pill counts), and evaluation of adverse events. They will also allow interim measurements of height and weight. Parents or guardians will be asked to bring their children to the NSCC for any illness. All care during the study will provided without cost, and with reimbursement for transportation. A full list of study evaluations and testing is provided in the Study Evaluations SOP.

Children with illness will be evaluated clinically and all illnesses recorded on a sick visit case report form (CRF). Children will be managed according to the SCC treatment protocols developed at the Mulago

National Referral Hospital Sickle Cell Clinic. Definitions will be provided for the most common infections, which will be listed on the CRF, and any other infections will be entered as free text diagnosis. Children with pneumonia/acute lower respiratory tract infection (ALRI) will have a swab collected for later testing by nasopharyngeal swab PCR for *Mycoplasma pneumoniae, Chlamydophila pneumoniae, Bordetella pertussis* and 17 viral upper respiratory infection (URI) pathogens. We are not able to do testing in real time, but treatment with a macrolide antibiotic is part of standard of care for children with pneumonia/ALRI. For this study children who have clinical signs of pneumonia (see Appendix I) will receive a chest x-ray to confirm the diagnosis of pneumonia. Other labs will be collected as needed for clinical diagnosis, and a blood sample collected and stored for future infectious pathogen or inflammatory response testing.

6.3 Standard Infection Prevention and Other Preventive Care in Children with SCA

Current standard of care for infection prevention per the Mulago Hospital Sickle Cell Clinic (MHSCC) guidelines for Ugandan children under 5 years of age with SCA includes immunization with pneumococcal 13-valent conjugate vaccine in infancy, penicillin prophylaxis until age 5 years to prevent pneumococcal infection, and sulfadoxine-pyrimethamine monthly prophylaxis to prevent malaria. Children also receive daily folic acid to decrease risk of anemia. We will provide any vaccinations, medications or supplements that a child requires and has not received. To these standards, we will add provision of an insecticide-treated bednet for all children, to further decrease risk of malaria.

Hydroxyurea therapy. We recently completed the blinded phase of the NOHARM study, a placebo-controlled study of hydroxyurea safety and efficacy in children with SCA at the MHSCC, which is in Kampala, Uganda. The data suggest that hydroxyurea therapy was both safe and effective in these children[20], but they live in an area of much lower malaria transmission than the children at Jinja Regional Referral Hospital. There are currently no national guidelines on hydroxyurea therapy, and Mulago Hospital Sickle Cell Clinic guidelines provide an outline of children who may benefit from therapy (e.g., children who have had stroke, or have had more than 3 pain crises requiring hospitalization), but recommend that any decision should be made individually by a sickle cell clinic clinician.
For this reason, for the ZIPS study, until national guidelines on hydroxyurea are available, the Nalufenya Sickle Cell Clinic (NSCC) clinicians will decide which children should receive hydroxyurea therapy, based on clinical history, and will monitor the child for adverse events if placed on hydroxyurea therapy. If hydroxyurea is prescribed by clinic physicians but cannot be afforded by the child's family during the study, the study will facilitate covering hydroxurea costs for the duration of the study. If hydroxyurea is given, it will be assessed as a covariate in analyses.

6.4 Testing Methods

Plasma and urine zinc testing and plasma copper testing. At study completion, plasma zinc and copper levels and urine zinc levels will be tested in the baseline and 12 month samples at the Wright Lab at Mount Sinai Hospital, New York, New York or a similarly certified lab for zinc testing. Blood and urine samples will be collected using a tight trace metal specific sample collection protocol to minimize contamination.

Hemoglobin electrophoresis will be done using standard capillary electrophoresis techniques on fresh samples or thawed samples stored for <6 months.

PCR testing of NP swabs for C. pneumonaie, M. pneumoniae, B pertussis and 17 viral URI pathogens. Nasopharyngeal (NP) swab specimens will be analyzed using the FilmArray® Respiratory Panel (RP; BioFire Diagnostics, Salt Lake City, UT) by collaborators at the Indiana University Health Pathology Laboratory (IUHPL) in Indianapolis, IN.

Microscopy testing for malaria. Microscopy for *Plasmodium* species by thick and thin smear, with parasite quantification, will be performed as previously described[21], with a minimum of two independent readings.

7.0 DATA MANAGEMENT

7.1 Introduction

Clinical trial management systems (CTMS) utilizing standard database programs are widely utilized in clinical research centers across the US and internationally. These systems provide a platform for electronic protocol management (including regulatory affairs and clinical quality assurance monitoring), billing compliance, data management/EDC functionality, as well as biospecimen and registry/repository management. The study will use a database to enter, clean and store data.

All study personnel will be trained in the study protocols. The clinical staff shall record the data from the patients into study forms. Laboratory results shall also be recorded into study forms. All study forms will be brought to the data room and entered into databases. All laboratory results will be entered into similar databases. These relational databases allow merging of data from the numerous separate data tables. Each study participant shall have a file that will contain all the study related source documents for the child. These files and other data sheets will be kept in file cabinets in the data room. The data room will be accessible by study personnel only. The data room will be locked securely any time staff members are not present. Databases will be kept on password-protected computers accessible only to authorized study personnel.

7.2 Data Security and Validation

The study database will be password protected, backed up in real-time, and allows for audit trail review. The data management team, after the appropriate documentation will assign credentials and security privileges based upon the individual roles and specifications appropriate to the protocol. These privileges also impact the information that is viewable by the user(s), which allows for securing of protected health information (PHI) as well as ensuring that protocol de-identification or blinding procedures are followed from a data standpoint.

7.3 Protocol Deviations

In the event that an unanticipated or unintended alteration/action occurs, different from what is expected from this study in regards to the protocol, consent documents, or study addenda, it is deemed a protocol deviation. A protocol deviation log will be maintained within the study database and at the study site. Protocol deviations will be filed with the ethical review boards.

8.0 SAFETY ASSESSMENT AND REPORTING

8.1 Adverse Event Reporting Requirements

All adverse events (AE) and serious adverse events (SAE) will be collected and reported to the Medical and Data Coordinating Center at IU using the correct data collection forms. AE reporting will utilize the Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0 available since 2009, where all AE are categorized by organ system and graded by severity. A list of expected and potentially serious AE's, as well as exceptions to the CTCAE's is included in Appendix II (Tables 1 and 2).

SAE reporting will use standard definitions (any life-threatening event hospitalization or death) however, since hospitalization is common in children with SCA, we will use hospital stay of more than 7 days to define hospitalization-related SAE in this study. This is based on the knowledge that children who are admitted with sickle cell-related conditions (such as anemia requiring transfusion, acute chest syndrome, and stroke) have an average length of stay of approximately 7 days. A SAE for this study will therefore be defined as: 1) hospitalization for more than 7 days; 2) any other life-threatening event* (based on clinician judgement); or 3) death. All SAEs will be followed until resolution or stabilization.

All SAEs will be reported via email to the in-country principal investigator or an assigned representative within 48 hours of the staff becoming aware of it, using an SAE form, which should be completed, scanned and sent electronically. The SAE form asks for nature of event, date of onset, severity, corrective therapies given, outcome and causality (i.e. unrelated, unlikely, possible, probably, definitely). The responsible study clinician should assign the causality of the event. Additional information will be sent within 14 additional days (full SAE report) if the condition has not resolved at the time of e-mail notification.

SAEs will be reported to the primary site IRB (Makerere University School of Medicine Research Ethics Committee) within 7 days of the team being aware of the SAE. Complied SAE logs will be sent to the Uganda National Drug Authority (NDA) in quarterly reports, and to the study IRBs and DSMB annually or as requested by those organizations.

*Life-threatening in the definition of a serious adverse event or serious adverse reaction 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.

9.0 STATISTICAL ANALYSIS

9.1 Sample Size and Power Calculations

We will assess reduction in incidence of severe or invasive infections, with or without culture or PCR confirmation. Sample size is based on incidence of severe or invasive infections, using a baseline rate of 0.71 infections/child/year, derived from data of children from an earlier clinical trial conducted at MHSCC (the NOHARM study). Power calculations assume an alpha of 0.025 for a one-sided test or 0.05 for a two-sided test. With an incidence of 0.71 severe or invasive infections/year in the placebo group, a sample size of 250 children (with a 10% loss to follow-up) will have 80% power to detect a decrease of ≥40% in severe or invasive infection incidence over the 12-month study period. This decrease is smaller than the 47-88% reduction in clinical infection incidence in adolescents and adults in previous studies[6-8], so the study sample size should allow us to detect the expected effects of zinc on infection incidence.

9.2 Proposed Analysis

We hypothesize that the incidence of infection in the zinc supplemented group will be ≥40% lower than that of the placebo group. Incidence of clinical infection will be compared using Poisson or negative binomial regression analysis. Other factors that are potentially associated with the risk of infection will be included in the regression models as covariates. Analysis will be implemented using SAS PROC GENMOD or NLMIXED as appropriate. Similar analyses will be conducted for incidence of culture or PCR-confirmed bacterial infections, VOC, SAE and AE. Frequency of infection, culture or PCR-confirmed bacterial infection, VOC, AE and SAE will also be compared using χ^2 analyses.

We will conduct one interim analysis, for examination of the efficacy and futility of the trial. The interim analysis will be conducted at the time when we accumulate half of the expected outcome events. In the one-year follow-up period, we expect to see a total of 142 episodes of severe or invasive infections between the two trial arms. We plan to perform the interim analysis when we have 71 episodes of severe or invasive infections. We follow a symmetric two-sided group sequential design with 80% power and total 5% Type I error rate. Using the O'Brian-Fleming bounds, we determine the p values of spending function for the interim analysis to be 0.006. We will reject the null hypothesis when the p value of the interim analysis is less than 0.006.

10.0 HUMAN SUBJECTS

10.1 Protection of Human Subjects

This protocol, the informed consent document, patient recruitment brochures, and any subsequent modifications will be reviewed and approved by the local Institutional Review Board responsible for review and approval of the study (Makerere University School of Medicine Research Ethics Committee), as well as the Indiana University IRB. Compliance with GCP guidelines for the conduct and monitoring of this clinical trial will occur through observation of the ethical and regulatory requirements presented in ICH E6, Good Clinical Practice: Consolidated Guideline. The Investigators will comply with all clinical trial disclosure and registration regulations. Approval of the local Ethics Committee will be received prior to initiation of the study, along with IRB approval by Indiana University.

10.2 Informed Consent Process

Written informed consent will be obtained from the parent or guardian of subjects since they are below the legal age for consent or assent. A notation that written informed consent was obtained will be made on the subject's case report form. The original informed consent form will be retained at the study site; a copy will be put in the subject's records. A copy of the consent form will also be provided to the parent or guardian.

10.3 Confidentiality

All ZIPS laboratory specimens, evaluation forms, reports, and other records will be identified by a coded number to maintain subject confidentiality. All records will be kept in a locked file cabinet. Databases accessible to study personnel other than the PIs and data clerks and managers will include coded numbers only. Identifying information will not be released by the ZIPS data center without written permission of the subject/parent/guardian, except as necessary for monitoring. Clinical information can be shared by the site investigators and staff for patient care reasons, e.g., local consultations.

10.4 Data and Safety Monitoring Board (DSMB) and Stopping Rules

The DSMB will consist of 4 individuals, Drs. Ezekiel Mupere (pediatrician, nutrition expert), Peter Olupot Olupot (pediatrician, malaria and sickle cell research expert), Seethal Jacob (pediatrician, clinical sickle cell care and research expert) and John Connett (biostatistician). The DSMB will meet every 6 months via Skype or other teleconference. The DSMB will review the data and provide guidance on study conduct and continuance. DSMB members will be independent and look at the trial from an ethical point of view of the participant safety, future patients and society in general. It is the responsibility of the DSMB to prevent patients being exposed to any excess risks by recommending to the study team and sponsors for trial suspension or termination early if SAEs appear to differ between study groups. The trial statistician will be invited to attend part of the DSMB meeting to present the most current data from the trial. This will be blinded, unless the DSMB specifically requests an unblinded analysis. If an unblinded analysis is requested by the DSMB, it will be provided to them and not to study personnel. Each DSMB member will be expected to serve for the duration of the trial. If a member is unable to continue participation, the reason will be documented and a replacement will be selected by the sponsor.

Stopping rules will be created for SAEs and for efficacy. Serious adverse events (SAEs) will be compiled and reviewed internally by the safety officer of the DSMB. Full SAE information will also be provided to the DSMB for review during the 6-monthly board meetings. Final stopping rules for SAEs will be developed by Dr. Tu (study biostatistician) in conjunction with the DSMB. Our proposed stopping rules for the DSMB are that the the study statistician, who will be unblinded to the treatment assignment, will calculate the SAE rates in the cumulated sample, as well as in the two treatment groups. Summary results will be reported back to the DSMB in a blinded fashion (e.g., treatment "A" versus treatment "B"). The statistician will calculate the 95% confidence interval for the control group's rate of SAEs and compare the intervention SAE rate against the confidence limits. If the SAE rate of the intervention group falls outside of the 95% confidence limits of the control group rate, which means there is a strong chance that the two treatment groups have different SAE rates, the study statistician will bring the issue of early stopping for safety reasons to the DSMB. The DSMB will then make an informed decision on whether to recommend that the trial be stopped early on the basis of SAEs. This safety stopping rule will not affect the power of the efficacy analysis.

Similarly, stopping rules for efficacy will be based on whether the interim analysis provides strong evidence in support of the intervention efficacy. As described in the plan for interim analysis, if the p value for the comparison of severe infection rates was less than 0.006, the study statistician will bring the issue of early stopping for efficacy reasons to the DSMB. The DSMB will then make an informed decision on whether to recommend that the trial be stopped early based on evidence of efficacy.

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12.0 APPENDICES

APPENDIX I: Definitions for severe or invasive infections in ZIPS study

Infection	Definition
Abscess	Opaque, fluid-filled/fluctuant collection on skin (with purulent discharge if drained)
Bacteremia	Children with a positive blood culture with a true pathogen (e.g., *S. aureus, S. pneumoniae*, *Salmonella*, other Gram negative infections)
Cellulitis	Area of reddened, warm skin in a child with a history of fever or measured axillary temperature of ≥37.5ºC
Diarrhea	More than 3 loose stools in a 24-hour period
Dysentery	Fever with bloody stools
Malaria	Measured fever (axillary temperature ≥37.5˚C) or fever by history and *Plasmodium* species infection on blood smear
Meningitis/Encephalitis	Fever with 1) nuchal rigidity or altered mental status and 2) CSF with >5 WBC or with positive CSF culture for meningitis-associated organisms (e.g., *S. pneumoniae*, *N. meningiditis*, *H. influenzae*)
Osteomyelitis	Fever with bone pain, redness of skin over bone and x-ray findings consistent with osteomyelitis
Pharnyngitis/Tonsillitis	Inflamed, erythematous pharynx and/or tonsils, with pharyngeal or tonsillar exudate
Pneumonia/Acute Chest Syndrome (ACS)	**Pneumonia:** history of fever or measured axillary temperature ≥37.5˚C, with age-specific tachypnea, cough, and an infiltrate and/or effusion on chest x-ray consistent with pneumonia* **Acute chest syndrome:** signs of pneumonia above plus chest pain and/or tenderness
Sepsis	Meets modified criteria for SIRS/sepsis in International pediatric sepsis consensus guidelines (2 or more of the following criteria, one of which must be abnormal temperature: T≥38.5˚C, age-specific tachycardia, age-specific tachypnea, age-specific leukopenia). Modified to remove leukocytosis because, per NOHARM study data, >80% of children with SCA at Mulago Hospital will have age-specific leukocytosis at baseline, which is an IPSC criterion for SIRS/sepsis. Since SIRS in a child with SCA is always suspected to be due to infection, we will use the term sepsis.
Sinusitis (acute)	Congestion, nasal discharge or cough for more than 10 days without improvement; or symptoms of congestion with purulent nasal discharge for more than 3 days
Urinary tract infection	Symptoms (fever with urinary frequency, burning or new incontinence after prior toilet training) plus urinalysis positive for LE or nitrite OR clean catch urine culture with >100,000 colonies of a single pathogen

* Any child with a standard clinical diagnosis of pneumonia (clinical signs above) will be treated for pneumonia regardless of CXR findings, as this is Mulago Hospital Sickle Cell Clinic protocol. Chest x-rays will be read by on call radiologist for acute clinical care, and also saved for reading by second radiologist. Specific criteria will be assessed by both radiologists, and only children who meet criteria from the WHO Radiology Working Group for pneumonia will be given a final diagnosis of pneumonia (Cherian T et al, Bulletin of WHO, 2005;83:353-359). Children who do not meet radiographic criteria will be given a final diagnosis of "respiratory infection" and not included in primary category of "severe or invasive infections" that constitutes the primary study endpoint. They will be considered for the secondary endpoint of "all clinical infections".

APPENDIX II. Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0 and Exceptions to CTCAE

Table 1. Sickle Cell Disease Symptoms and Associated Conditions

Acute chest syndrome	Decreased lung function	Hypertension	Renal papillary necrosis
Adenotonsillar disease	Delayed growth/puberty	Hypocalcemia	Reticulocytopenia
Albuminuria	Depression	Hyposthenuria	Reticulocytosis
Amenorrhea	Dizziness	Nephropathy	Retinopathy
Anemia (severe)	Electrolyte imbalance	Osteomyelitis	Retinal hemorrhage
Aplastic crisis	Elevated urinary urobilinogen	Pain, back	Rhabdomyolysis
Arthralgia	Elevated serum transaminases	Pain, chest	Seizure
Avascular necrosis of hip/shoulder	Elevated TCD velocities	Pain, joint	Septicemia
Bacteremia	Fever	Pain, long bone	Silent organ infarction
Bone infarction	Empyema	Pain, severe abdominal	Skin ulcer
Cardiac arrythmia	Hand-foot syndrome/dactylitis	Pain, sternal or rib	Splenic sequestration
Cardiomegaly	Headache	Priapism	Splenomegaly
Cerebrovascular accident	Hematuria	Proteinuria	Stroke
Cholecystitis	Hemiplegia	Pneumonia	Transient Ischemic Attack (TIA)
Cholelithiasis	Hemolysis	Pulmonary embolism	Transfusion, unanticipated
Cognitive dysfunction	Hepatic sequestration	Pulmonary hypertension	Vaso-occlusive pain
Constipation	Hepatomegaly	Pulmonary infiltrate on chest x-ray	
Cranial nerve palsy	Hospitalization >24 hours	Pyelonephritis	
Death	Hyperbilirubinemia	Renal failure	
Decreased renal function	Hypersplenism	Renal insufficiency	

Table 2. Laboratory Exceptions to the CTCAE List (version 4.0, Guidelines)

Parameter	Grade 2	Grade 3	Grade 4
Hemoglobin (gm/dL)	5.0 – 6.0	4.0 – 4.9	< 4.0
Total WBC (x 10^9/L)	1.0 – 1.999	0.5 – 0.999	< 0.5
ANC (x 10^9/L)	0.5 – 0.999	0.2 – 0.499	< 0.2
Platelets (x 10^9/L)	50 – 79	20 – 49	< 20
Total Bilirubin (mg/dL)	5.0 – 10.0	10.1 – 20.0	> 20.0
AST (IU/L)	150 – 300	301 – 1000	> 1000
ALT (IU/L)	150 - 300	301 – 1000	> 1000
Creatinine (mg/dL)	2Xf baseline serum creatinine and value ≥1.0	1.6 – 2.0	> 2.0
ARC (x 10^9/L) and Hb < 7.0 gm/dL	50-80	10-49	< 10