



## STUDY PROTOCOL

**REVISED** **A novel nano-iron supplement to safely combat iron deficiency and anaemia in young children: The IHAT-GUT double-blind, randomised, placebo-controlled trial protocol [version 2; peer review: 2 approved]**

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### Abstract

**Background:** Iron deficiency and its associated anaemia (IDA) are the leading forms of micronutrient malnutrition worldwide. Here we describe the rationale and design of the first clinical trial evaluating the efficacy and safety of an innovative nano iron supplement, iron hydroxide adipate tartrate (IHAT), for the treatment of IDA in young children (IHAT-GUT trial). Oral iron is often ineffective due to poor absorption and/or gastrointestinal adverse effects. IHAT is novel since it is effectively absorbed whilst remaining nanoparticulate in the gut, therefore should enable supplementation with fewer symptoms.

**Methods:** IHAT-GUT is a three-arm, double-blind, randomised, placebo-controlled phase II trial conducted in Gambian children 6-35 months of age. The intervention consists of a 12-week supplementation with either IHAT, ferrous sulphate (both at doses bioequivalent to 12.5 mg  $\text{Fe}$ /day) or placebo. The trial aims to include 705 children with IDA who will be randomly assigned (1:1:1) to each arm. The primary objectives are to test non-inferiority of IHAT in relation to ferrous sulphate at treating IDA, and to test superiority of IHAT in relation to ferrous sulphate and non-inferiority in relation to placebo in terms of diarrhoea incidence and prevalence. Secondary objectives are mechanistic assessments, to test whether IHAT reduces the burden of enteric pathogens, morbidity, and intestinal inflammation, and that it does not cause detrimental changes to the gut microbiome, particularly in relation to *Lactobacillaceae*, *Bifidobacteriaceae* and *Enterobacteriaceae*.

### Open Peer Review

Approval Status

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1. **Toby Richards**, University College London, London, UK

2. **Robert C. Hider** , King's College London, London, UK

Any reports and responses or comments on the article can be found at the end of the article.

**Discussion:** This trial will test the hypothesis that supplementation with IHAT eliminates iron deficiency and improves haemoglobin levels without inducing gastrointestinal adverse effects. If shown to be the case, this would open the possibility for further testing and use of IHAT as a novel iron source for micronutrient intervention strategies in resource-poor countries, with the ultimate aim to help reduce the IDA global burden.

**Registration:** This trial is registered at clinicaltrials.gov ([NCT02941081](https://clinicaltrials.gov/ct2/show/study/NCT02941081)).

### Keywords

iron supplementation, children, IHAT, microbiome, iron deficiency, IDA, anaemia, diarrhoea

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**Competing interests:** D.I.A.P. is one of the inventors of the IHAT iron supplementation technology, for which she could receive future awards to inventors through the MRC Awards to Inventor scheme. Notwithstanding, the authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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**REVISED Amendments from Version 1**

The revisions to the manuscript (version 2) are below:

- Highlighted the novelty in the abstract and Introduction
- Added the specific effects of IDA in children to the Introduction
- Added the additional subheadings suggested throughout
- Added sickle cell and thalassaemia separately in the exclusion criteria
- Noted that we have developed standardised protocols that are being used by trial staff for conducting all trial procedures. We also added that these methods can be made available upon request. (Follow up section)
- In the DSMB section we added the comment that there have been so far no interim analysis requested and that the current recommendation is for the study to continue as planned.
- We feel that we justified sufficiently in the manuscript how iron deficiency will be assessed (in the Outcomes section) and why we use the 1g/dL rise in Hb as an indication of efficacy (Discussion)

In relation to the complexity of the primary endpoints, we do appreciate this point and assure the reviewer that we thought very carefully about this and had several discussions with the several independent review boards that have approved this protocol. We felt that maintaining the hypothesis-driven study design with the analysis of the 4 primary study endpoints is appropriate taking into consideration the stage of development of IHAT and the fact that at the end of this trial we must be able to make a 'go/no-go' decision to move to the next stages of trials in resource-poor settings. The 'go' decision to continue development of IHAT in these settings will only be justified if we determine in this trial if IHAT can help correct iron deficiency anaemia without promoting diarrhoea, which is the largest limitation of current supplements. This is why we maintained the current 4 primary endpoints.

**See referee reports**

## Introduction

Iron deficiency anaemia (IDA) remains the most common forms of micronutrient malnutrition in the world today. Globally, IDA is estimated to affect 1.24 billion people, the majority of whom are children and women from resource-poor countries, and is responsible for an estimated loss of 35 million disability-adjusted life years (DALYs) (1.5% of total DALYs)<sup>1-3</sup>. IDA is estimated to cause more years lived with disability (YLD) than all other micronutrient deficiencies, haemoglobinopathies and haemolytic anaemias combined, and is the leading contributor to YLD in most low-income countries<sup>1</sup>. Most sub-Saharan Africa countries have an anaemia prevalence above 40% in young children and pregnant women, a severe public health problem according to the World Health Organization (WHO)<sup>4</sup>. Iron deficiency is frequently exacerbated by concomitant parasitic and bacterial enteric infections and, together, these account for the majority of anaemia cases in developing countries<sup>5-7</sup>. The effects of anaemia on child cognition are also well recognised with several trials finding a combined 1.73 lower IQ points per 1 g/dL decrease in haemoglobin<sup>8</sup>. Even mild iron deficiency in the absence of anaemia appears to impair intellectual development in young children and is lowering national IQs, whilst overt IDA is associated with increased risk of serious morbidity, poor motor and mental development in children and impaired immunity.

## The problem

Iron supplementation with simple ferrous salts is cheap and widely available, but constitutes a non-physiological approach to providing iron that has been associated with significant side-effects and adverse events<sup>9-15</sup>. Data and meta-analysis from trials involving nearly 10,000 young children, mainly from resource-poor countries, have consistently shown that conventional oral iron supplements used to treat IDA are associated with increased infection, including bloody diarrhoea<sup>10,13,16,17</sup>, detrimental changes to the gut microbiome and gut inflammation<sup>17-20</sup>. Therefore, in countries with poor infection control, iron supplementation in young children could further increase the burden from enteric infection and environmental enteropathy (i.e. persistent gut damage and inflammation that leads to malabsorption), which is a major cause of growth failure in children in resource-poor environments and may later exacerbate the risk of IDA<sup>21,22</sup>. Consequently, children living in areas where enteric infection is endemic remain without an effective and safe cure for ID and IDA, and, as such, it is perhaps not surprising that, despite considerable investment and effort, we have been unable to reduce the burden of this disease in young children in sub-Saharan Africa<sup>23</sup>.

## Rationale and aims

Since 2005, we have been developing an engineered analogue of natural food iron as an alternative iron supplement. Iron hydroxide adipate tartrate or IHAT, is completely different from other iron compounds currently used in supplementation or home fortification strategies since it defies the established dogma that iron absorption requires ionic solubilised iron to be taken up by the duodenal enterocytes. The novelty of IHAT, is precisely that it is not a soluble compound nor does it require solubilisation in the stomach prior to uptake by the enterocytes as it is taken up as whole nanoparticles by endocytosis<sup>24,25</sup>, similarly to that proposed for dietary plant ferritin<sup>26-28</sup>. This means that the unabsorbed fraction of the compound that transits to the lower gut, which is usually at least 70% of all ingested oral iron, irrespective of the form, will remain nanoparticulate and, therefore not soluble, and as such should not be available to promote significant pathogen growth and tissue inflammation<sup>29,30</sup>. A crucial aspect of the IHAT nanostructure is that once it has entered the enterocyte it is sufficiently labile to break down effectively inside lysosomes/endosomes and deliver its iron because the native iron oxo-hydroxide structure (i.e. ferrihydrite) in IHAT has been purposely destabilised with the incorporation of dietary tartaric and adipic acids<sup>24,25,31</sup>, much in the same way as what occurs in the ferritin iron core due to interactions with the amino-acid residues in the protein shell<sup>32</sup>. Our pre-clinical and early-clinical data indicates that IHAT is effectively absorbed in humans, corrects IDA in animal models, is not redox reactive and does not have a detrimental impact on the gut microbiome<sup>24,29-31,33</sup>.

## Aims

The main purpose of the IHAT-GUT study is to determine whether supplementation with IHAT safely corrects IDA in young children compared to the present standard of care. We hypothesise that 12-weeks supplementation with IHAT will correct iron deficiency and improve haemoglobin levels in

young children without causing diarrhoea or inducing intestinal inflammation and detrimental changes in the gut microbiome.

Here we summarise the protocol for the IHAT-GUT study, the first clinical study to assess the efficacy and safety of IHAT in anaemic children living in resource-poor rural areas of The Gambia.

## Protocol

### Hypothesis and objectives

We will test the hypothesis that supplementation with IHAT eliminates iron deficiency and improves haemoglobin (Hb) levels in young children without increasing diarrhoea or promoting negative changes in the gut microbiome or inducing gut inflammation.

The IHAT-GUT study has four primary combined objectives to test efficacy and safety of IHAT. The primary objectives in terms of efficacy for this trial are to test non-inferiority of IHAT in relation to ferrous sulphate at correcting ID and improving Hb levels after 12 weeks of supplementation. The primary objectives for safety are to test superiority of IHAT in relation to ferrous sulphate and non-inferiority in relation to placebo based on moderate-severe diarrhoea incidence and prevalence.

Secondary objectives of the IHAT-GUT trial are to test whether IHAT is non-detrimental with respect to enteric pathogen burden, does not increase morbidity, does not decrease abundances of *Lactobacillaceae* and *Bifidobacteriaceae* relative to *Enterobacteriaceae*, and does not cause intestinal inflammation.

### Study design and setting

**Design.** The IHAT-GUT trial is a three-arm, parallel, randomised, placebo-controlled, double-blind study with iron supplementation in young children with mild to moderate iron deficient anaemia. Ferrous sulphate ( $\text{FeSO}_4$ ), the current standard of care for iron supplementation, is used as the active comparator at the conventional daily dose of 12.5mg iron<sup>34</sup>.

Children are randomised (1:1:1) to IHAT,  $\text{FeSO}_4$  or placebo, and each arm includes an intervention period of 12 weeks. The daily iron dose is 12.5 mg elemental iron bioequivalent.

**Trial governance.** The trial is being conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki, and that are consistent with the International Conference on Harmonisation (ICH) requirements for Good Clinical Practice (GCP), and the applicable regulatory requirements. The study is sponsored by the London School of Hygiene and Tropical Medicine (LSHTM) and is conducted at the Medical Research Council (MRC) Unit The Gambia at LSHTM (MRCG).

**Setting.** The study population in IHAT-GUT are children under the age of 3 years living in the north bank rural communities in the Upper River Region (URR) of The Gambia in West Africa. The URR has an approximate population of 200,000, and only

one major town, Basse, and is otherwise typical of rural sub-Saharan Africa. The temperature varies between 15°C and 40°C, but can reach as high as 46°C in the north bank. There are distinctive dry (November to May) and wet (June to October; mean annual rainfall = 876 mm) seasons. According to the most recent Gambia Demographic and Health Survey<sup>35</sup>, the URR has the highest under-5 mortality rate in the country (92 deaths per 1000 livebirths), the highest percentage of severely malnourished children (7–11%), and the highest prevalence of malaria and anaemia in children under 5 years (4.5% and 82.5%, respectively). Severe anaemia in children is highly prevalent in these communities, with 22.4% of children under 5 years having haemoglobin below 8 g/dl<sup>35</sup>. Diarrhoeal diseases are also common<sup>36,37</sup>. Therefore, there is a clear clinical need for safe and effective iron supplementation strategies in these communities.

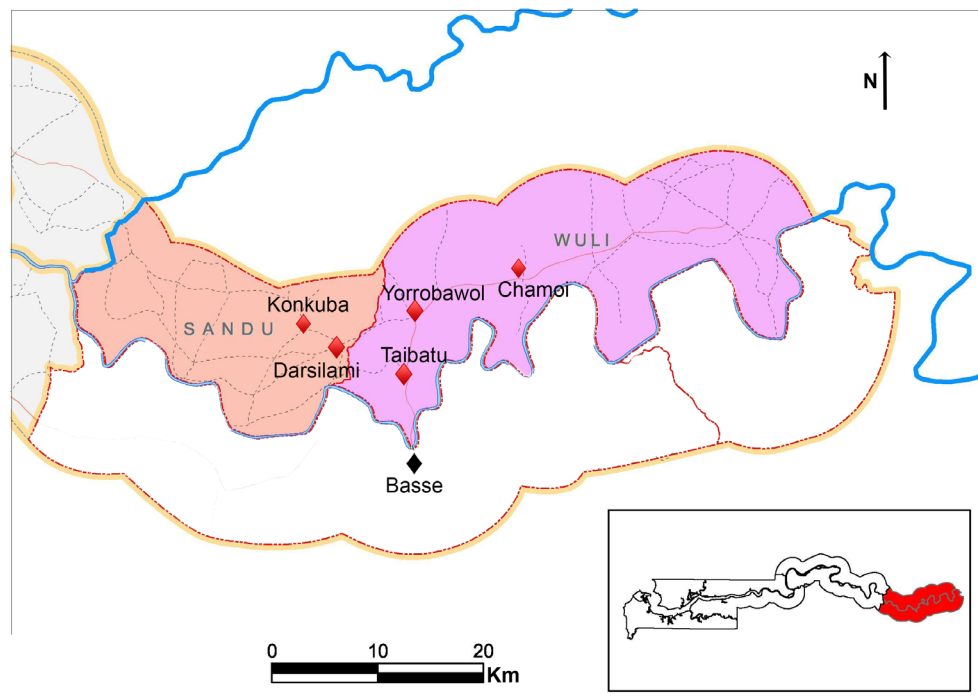
The study area includes 45 villages in the Wuli and Sandu districts ([Figure 1](#)), situated on the north bank of the river Gambia, approximately 400 km east of the capital Banjul, with a population of approximately 2800 children aged 6–35 months. All communities have access to borehole tap water at central places. Study specimen samples are collected at one of the study health clinics: Yorrobawol health center, Darsilami community health post, Konkuba community health post, Taibatu health post and Chamoi Health Center ([Figure 1](#)). Samples are transported in cold boxes to the study laboratory in Basse for sample processing and analysis, and from there to the Keneba and Fajara laboratories, or to external laboratories for further analysis.

### Study participants

Participants are young children and the study protocol is explained to their parents orally by the field staff. An impartial witness is present during consent for all illiterate parents and also signs the consent form next to the parent's thumb print. No children start any study specific procedure without full, written informed consent being first obtained. A copy of the study information sheet and signed consent form is provided to those consented ([Supplementary File 1](#)). Parents are free to withdraw their consent at any time. Participants must meet all of the inclusion criteria and none of the exclusion criteria to be eligible for the trial.

**Inclusion criteria:** Apparently healthy; 6–35 months old; free of malaria; with IDA defined as  $7 \leq \text{Hb} < 11$  g/dl and ferritin  $< 30 \mu\text{g/l}$ <sup>38</sup>; resident in the study area (and planning to remain in the study area for the duration of the trial); able and willing to comply with the study protocol; informed consent given by parent.

**Exclusion criteria:** Congenital anomalies/birth defects (except minor external congenital malformation); severe malnutrition (z-scores for length/height-for-age (HAZ), weight-for-age (WAZ), weight-for-length/height (WHZ)  $< -3$  standard deviations (SD); shock syndrome; chronic conditions; sickle cell and thalassaemia; currently participating in another study; currently taking iron supplements/multiple micronutrient supplements;



**Figure 1. Map of Upper River Region in The Gambia, with location of study clinical facilities.** Study samples are collected at one of the clinical facilities, Yorrobawol health center, Darsilami community health post, Konkuba community health post, Taibatu health post and Chamoi Health Center, and transported to the study laboratory in Basse for sample processing and analysis, and from there to other laboratories for further analysis. Map adapted from the Atlas of the Gambia 2004.

currently experiencing moderate-severe diarrhoea, defined as those diarrhoea episodes where (i) the child passes more than five loose or watery stools per day, (ii) there is blood in the stool (dysentery), or (iii) the child shows signs of clinical dehydration (assessed by the study nurse based on physical signs such as little or no urination, sunken eyes, and skin that lacks its normal elasticity).

### Recruitment and screening

Prospective participants are identified through the enumeration data collected by the study field team in the 45 study villages. The communities and regional health teams in the study area have been sensitised to the study. The field team visits the parents of all young children identified as prospective participants to explain the study and answer any questions they may have, those interested in taking part in the study are then invited to attend a screening visit at one of the study health facilities. At screening, the child is physically examined by a study nurse or clinician; those potentially eligible to take part in the study have their height and weight measured and a finger prick blood sample collected for Hb measurement and rapid diagnostic testing (RDT) for malaria. Children with Hb <7 g/dl or those severely malnourished cannot be enrolled and are referred to the regional health centre for treatment according to national guidelines. Malaria-positive children (positive RDT and confirmation by blood film) are not enrolled and will be treated according to national guidelines. If HAZ, WAZ, WHZ are all above -3 SD,  $7 \leq \text{Hb} < 11$  g/dl and the RDT is negative,

then a small venous blood sample is collected by the study nurse to send to the laboratory for confirmation of Hb levels and determination of serum ferritin. If  $7 \leq \text{Hb} < 11$  g/dl and serum ferritin < 30 ng/ml, the child is invited to a pre-enrolment day back at the clinic (Day 0), which is 4–5 weeks after the screening visit, for a finger prick to confirm absence of malaria by RDT and haemoglobin concentration.

### Randomisation and blinding

Randomisation is based on a stratified block design to achieve group balance in terms of age and baseline haemoglobin concentration. Based on the assessments at the pre-enrolment day (Day 0), each child is categorised into two Hb classes (below or equal to/above the median Hb for the recruited cohort) and also according to age into 3 classes (6–11 months, 12–23 months and 24–35 months). This divides children into 6 different strata and in each strata the children are randomly assigned to one of the three study treatment arms (1:1:1 ratio) using R version 3.4.3 and a block randomisation approach with fixed block size determined by age and Hb levels.

Randomisation is performed by the study statistician who remains blinded to the treatment codes. Participants, parents and the entire study team are blinded as to which intervention/treatment arm participants belong to. Each child has a unique intervention code which is also their study ID/randomisation number, this means that the study team is unaware of which children belong to the same treatment arm. The child study ID is the same



code that is used to label the capsules for the study treatment they receive (see below). If emergency unblinding is required, only the particular study subject in question will be unblinded, since each participant has a unique treatment code. Following investigation by the Data and Safety Monitoring Board (DSMB) of an emergency unblinding case, and only at the request of the DSMB, we may also unblind a whole treatment arm or the entire study. In all cases, someone independent from the trial team will perform the unblinding.

### Interventions

In each arm, children receive a daily dose of either IHAT,  $\text{FeSO}_4$  or placebo for 12 weeks. Children in the ferrous sulphate arm receive 12.5 mg elemental iron equivalent daily, as  $\text{FeSO}_4 \cdot \text{H}_2\text{O}$ , in line with WHO recommendations<sup>34</sup> and Gambian national guidelines for the 6–35 months age group. Children in the IHAT arm receive 20 mg elemental iron equivalent daily, which is the bioequivalent dose considering the bioavailability of IHAT relative to  $\text{FeSO}_4$ . Children in the placebo group receive ca. 30 mg pharmaceutical grade sucrose daily. All three materials are formulated with 6 mg of a food colourant for colour matching and each daily dose is contained in a powder-filled easy-open capsule. Capsules for each treatment are packed in medicine bottles, each bottle containing enough capsules for one child for the entire study duration, and these bottles are individually labelled with the child's unique study ID, which is their randomisation code, as described above. All capsules appear identical and the powders inside them do not have any apparent visual differences in texture or colour. Every day the field worker visits each child and provides them with the daily dose of their allocated treatment. For each child, the field worker opens one of their allocated capsules and adds all its contents to a small amount of a local juice drink, in a disposable plastic cup, immediately before administration directly into the child's mouth. There are no apparent differences in the colour, turbidity or taste of the powder and juice mixes. Before administration, the mother is encouraged to feed the child so that the supplements are not ingested on an empty stomach.

Besides the study interventions mentioned here, children do not receive any other concomitant interventions or supplements during the 12-week intervention period, but all children are followed up weekly by the study clinical team, and any clinical conditions are treated following national guidelines.

### Follow-up study visits

A flow chart of the study participant timeline is presented in [Figure 2](#). On study Day 1, i.e. the first day of supplementation, each child is invited back to the study clinic and a photo is taken (with consent) for a study ID card that also contains the child's randomisation/study ID number. At this visit, demographic and immunisation data are collected and the study morbidity questionnaire is completed ([Table 1](#)). A venous blood sample and a stool sample are also collected (study baseline samples). After blood collection, the field staff give the child their allocated study arm supplement.

Every day over the following 12 weeks, the field staff visits the child at home in order to administer the allocated study

supplement and to check on the child's health status. They complete the study morbidity questionnaire three times per week, which includes questions regarding fever, diarrhoea, vomiting, malaria symptoms, other illness, hospitalisation, appetite and medication. If a child is found unwell they are referred to the study nurse or clinician for evaluation and treatment. These check-ups continue 4 weeks post-supplementation to follow-up on adverse events.

Each week during the study supplementation period, children are invited back to the study clinic for a check-up by the study nurse and a finger prick to determine their malaria and Hb status. Children found with a positive RDT at any time during the supplementation period are further tested with a blood film and treated according to The Gambian national guidelines if malaria is confirmed. Any child where Hb falls below 7 g/dl during the supplementation period, discontinues the study supplementation and is offered standard-of-care oral iron treatment according to the national guidelines. These children continue to be followed up by the clinical team at the weekly clinics and will not be excluded from the data analysis but considered as treatment failures.

On study days 15 and 85, the child visits the study clinic and stool and venous blood samples are collected. Immunisation data is also recorded on these occasions. Height and weight is re-measured on study Day 85.

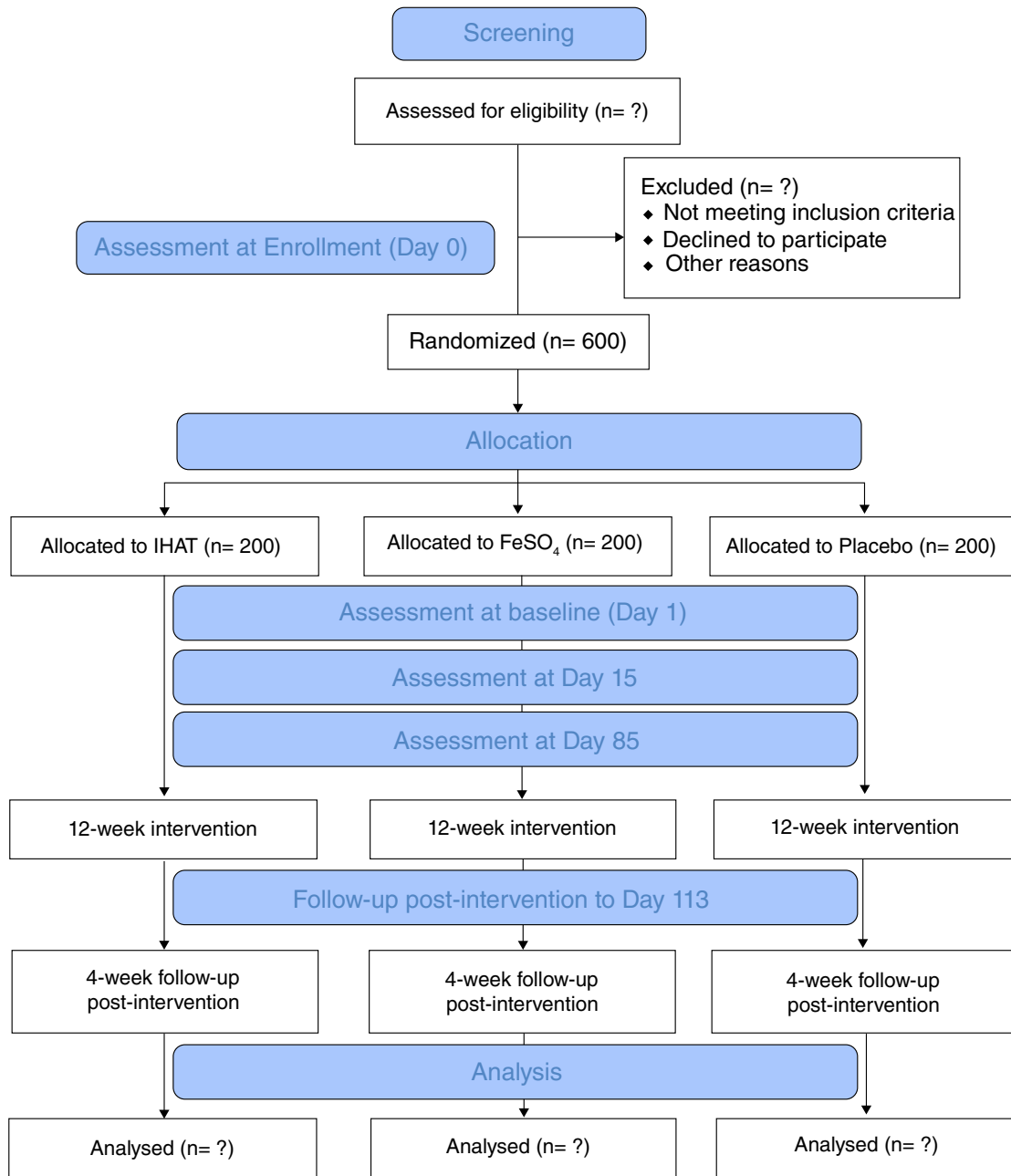
Venous blood samples are collected in S-Monovette® blood collection tubes either before the first meal of the day or, when this is not possible, at least 1 hour after the last meal. All blood samples are kept cool before transport to the Basse laboratory for processing. Stool samples are collected at home in toilet pots provided by the study team and lined with a disposable plastic liner at study days 1, 15 and 85. Stool samples can be collected within 7 days of the study time-point when it becomes difficult to collect the sample on that exact day, i.e. if the child travels or is not able to pass stool. The stool samples are aliquoted by the field staff as soon as possible after collection into a sterile Sarstedt stool collection tube and an OMNIgene® GUT sample collection kit (DNA Genotek).

At the end of the study follow-up period (i.e. Day 113), the children in any arm who still have anaemia ( $\text{Hb} < 11 \text{ g/dl}$ ) are provided with standard-of-care iron supplementation for three consecutive months as per Gambian national and WHO guidelines.

Standardised protocols have been developed and are being used by all trial staff for conducting all field, laboratory and data procedures employed in this study. All deviations to these procedures is promptly reported following the study sponsor procedure and ICH-GCP guidelines. These methods can be made available upon request.

### Outcomes

All participants are assessed at three different time-points: at baseline (Day 1 of supplementation), and at 15 and 85 days after start of supplementation. The Day 15 time point will provide



**Figure 2. Consort 2010 flow diagram.**

an indication of acute compound-related effects and the Day 85 time-point will be indicative of chronic (i.e. longer term) compound-related effects, for all the ‘safety’ outcomes.

The primary efficacy outcome of the trial is the proportion of children in each arm who resolve iron deficiency and either achieve a normal Hb or show an increase of at least 1 g/dl after 12 weeks of supplementation (i.e. at study Day 85). The primary safety outcome is the burden of moderate-severe diarrhoea. Therefore, there are four primary endpoints of the trial: (1) iron

deficiency at Day 85; (2) Hb levels at Day 85; (3) ‘incidence density’ of moderate-severe diarrhoea over the 12 weeks (i.e. the number of new moderate-severe diarrhoea episodes per child over the 12 weeks supplementation period); (4) ‘period prevalence’ of moderate-severe diarrhoea over the 12 weeks supplementation period (i.e. the proportion of children with at least one episode of moderate-severe diarrhoea in this period). To assess iron deficiency, we will use regression-adjusted ferritin concentration below 12 µg/l, where ferritin values are adjusted for inflammation using the regression model recommended

**Table 1. Overview of the study morbidity questionnaire.**

Category	Questions
Diarrhoea	Any diarrhoea? If yes, duration (days) If yes, number of stools per day If yes, is there blood/mucus in stool? Is the child urinating less? Is the child lethargic or unconscious? Is the child restless and irritable? Does the child have sunken eyes? Is the child drinking poorly? Is the child thirsty, drinking eagerly? Does the skin pinch go back slowly?
Fever	Axillary temperature History of fever? If yes, number of days
Cough	Cough? If yes, duration of cough (days)
Vomiting	Has the child been vomiting since last visit? If yes, number of days
Appetite	How is your child's appetite? If decreased, number of days If decreased, describe
Medication	Has the child been or is on any medication since last visit? If yes, please state the medication
Other	Difficulty breathing? Convulsion? Has the child had any other illness since last visit? If yes, describe

by the Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) group<sup>6</sup>.

Iron deficiency and Hb levels at Day 85 will be used to assess non-inferiority of IHAT relative to FeSO<sub>4</sub> in terms of efficacy at treating IDA. We will determine the proportion of children in each arm who resolve iron deficiency and either achieve a normal Hb ( $\geq 11$  g/dl) or an increase in Hb of at least 1 g/dl after 12 weeks of iron supplementation.

'Incidence density' and 'period prevalence' of moderate-severe diarrhoea will both be used to assess superiority of IHAT relative to FeSO<sub>4</sub> and non-inferiority relative to placebo for the diarrhoea outcome (i.e. safety or tolerability). Moderate-severe diarrhoea is defined as those diarrhoea episodes where: (i) the child passes more than five loose or liquid stools per day, (ii) there is blood or mucus in the stool (dysentery), or (iii) the child shows signs of clinical dehydration (assessed by the study nurse based on physical signs such as little or no urination, sunken eyes, and skin that lacks its normal elasticity).

Secondary endpoints are faecal microbiome diversity and profile (particularly in terms of abundance of *Enterobacteriaceae*, *Bifidobacteriaceae*, *Lactobacillaceae*), abundance of enteric

pathogens, faecal calprotectin (marker of gut inflammation), hospitalisation and morbidity (data collected three times per week using the study questionnaire), malaria infection (data collected every week with RDT), treatment failures (i.e. the number of children who have to discontinue study supplementation because their Hb falls below 7 g/dl), the proportion of days a child has diarrhoea over the intervention period ('longitudinal prevalence' of diarrhoea), the proportion of days a child has moderate-severe diarrhoea over the intervention period ('longitudinal prevalence' of moderate-severe diarrhoea), 'incidence density' of bloody diarrhoea (i.e. the number of bloody diarrhoea episodes per child-month of observation), markers of systemic inflammation (serum C-reactive protein (CRP) and alpha 1-acid glycoprotein (AGP)), and systemic markers of iron status (hepcidin, soluble transferrin receptor (sTfR), transferrin saturation and circulating non-transferrin bound iron - NTBI).

#### Laboratory evaluations

Unless otherwise stated, all laboratory evaluations are carried out at the laboratories of the MRCG.

**Blood samples.** In the venous blood samples collected on study Days 1, 15 and 85, the following parameters are assessed: full haematology panel (using a Medonic™ Haematology Analyzer



M-Series, Boule Diagnostics AB); serum ferritin, sTfR, serum iron and total iron binding capacity (for calculating transferrin saturation), CRP, AGP (using a fully automated biochemistry analyser Cobas Integra 400 plus); serum hepcidin (using the DRG® Hepcidin 25 (bioactive) HS ELISA); serum NTBI (using a fluorescent beads assay<sup>39</sup>, at an external laboratory in the U.K.). In the weekly finger prick blood samples, Hb is determined using a HemoCue® Hb 301 system and RDT is performed using the SD Bioline Malaria Ag *Pf* test (Standard Diagnostics, Inc.), these evaluations are conducted at the field study clinics.

**Stool samples.** Stool samples collected in the OMNIgene® GUT tube contain a DNA stabilising agent that ensures that samples can be kept at ambient temperature for 60 days. Total stool DNA is extracted from these samples using the Mo Bio PowerLyzer® PowerSoil® DNA Isolation Kit (Qiagen) within 6 weeks of sample collection. Stool DNA will be used for microbiome 16S rRNA sequencing (Illumina MiSeq platform), which will be conducted at an external laboratory in the U.K., and also for targeted qPCR of enteric pathogens.

In the stool samples collected in the Sarstedt stool collection tube the following parameters are assessed: calprotectin (using the Bühlmann fCAL® ELISA) and helminth egg count (using the Kato-Katz method<sup>40</sup>).

#### Data collection and data management

A password-protected study database has been established using the [Research Electronic Data Capture \(REDCap\)](#) secure web-based application based in PHP and MySQL. Access to the database is only given to members of the study team and the trial monitors and the database is managed by a dedicated data manager. All field data is captured in electronic case report forms (eCRF) and entered directly into handheld tablets (Samsung Galaxy Tab A (2016) SM-T580) using the Android 7.0 software and the REDCap mobile app. Data is synced daily into the database via secure Wi-Fi network.

The following data is recorded: date of informed consent, personal data (ID, initials, date of birth), socioeconomic data, height and weight, information on health status and regarding participation in other studies, date and time of all venous and finger prick blood collections, date and time of all faecal sample collections, date and time of supplement administration, data on morbidity, adverse events, lab results.

#### Sample size

The hypotheses being tested in this study are:

1. Non-inferiority of IHAT compared to ferrous sulphate for correction of iron deficiency and either achieving a normal Hb or an increase of at least 1 g/dl after 12 weeks of iron supplementation. A non-inferiority margin of 0.1 is used. The null hypothesis is therefore the probability in the IHAT arm minus the response probability in the ferrous sulphate arm is less than or equal to -0.1.

2. Superiority of IHAT compared to ferrous sulphate in terms of incidence density of moderate-severe diarrhoea. Here the null hypothesis is that the mean number of new episodes in the IHAT arm is greater than or equal to the mean number in the ferrous sulphate arm.
3. Superiority of IHAT compared to ferrous sulphate in terms of prevalence of moderate-severe diarrhoea. Here the null hypothesis is that the prevalence of diarrhoea in the IHAT arm is greater than or equal to the prevalence in the ferrous sulphate arm.
4. Non-inferiority of IHAT compared to placebo in terms of prevalence of moderate-severe diarrhoea. Here the null hypothesis is that the prevalence of diarrhoea in the placebo arm minus the prevalence in the IHAT arm is less than or equal to -0.1.

Because this is a preliminary study, with any significant results being retested in a subsequent pivotal study, we do not adjust for multiple testing. Each hypothesis is to be tested at a 10% one-sided type I error rate.

A sample size of 600 subjects completed equally randomised between the three arms, provides high power for each of the four hypotheses:

1. An 89% power to test non-inferiority on the IDA response probability, with a non-inferiority margin of 0.1 (0.583 on the odds ratio scale) assuming that the true response probability is 0.3 on IHAT and ferrous sulphate<sup>10,13,41</sup> arms.
2. A 90% power to test superiority for the incidence density of moderate-severe diarrhoea outcome assuming IHAT provides a 20% reduction in the mean for ferrous sulphate (i.e. from 1.28 episodes per child over the 12 weeks supplementation period to 1.02; calculations based on unpublished data for studies with iron supplements in rural Gambia).
3. A 90% power to test reduction in prevalence of moderate-severe diarrhoea from 25% in the ferrous sulphate arm to 15% in the IHAT arm.
4. A 93% power to test non-inferiority (0.1 non-inferiority margin, 0.583 on the odds ratio scale) of IHAT against placebo for prevalence of moderate-severe diarrhoea when both have 15% prevalence. For these calculations, we used published diarrhoea period prevalence data from studies with iron supplementation<sup>13,17</sup>.

For the secondary outcomes, the trial (n=200 per arm) will have over 85% power to detect significant differences between all the arms in terms of enterobacteria<sup>17</sup>, NTBI<sup>39</sup> and calprotectin<sup>17</sup>.

To account for a non-completion rate of ca. 15%, the total number of children we plan to enrol in the study is n=705.

## Statistical analysis

A full statistical analysis plan will be finalised prior to database lock and before breaking the randomisation code. We summarise briefly the planned statistical analysis here.

For the IDA response and diarrhoea prevalence outcomes, we will fit a logistic regression, adjusting for the strata created by age and Hb level groups prior to enrolment. For non-inferiority comparisons, the odds ratio for effect of IHAT relative to the relevant comparator arm will be estimated, and if the lower 90% one-sided confidence interval is above 0.583 (equivalent to the 10% non-inferiority margin above), non-inferiority will be declared for IHAT. For superiority comparisons, the data will be analysed in a similar way but superiority will be declared if the one-sided p-value for the Wald test of the effect of IHAT is less than 0.1.

For the diarrhoea incidence endpoint, a Poisson regression with treatment arm as predictor will be fitted to the data adjusting for the strata.

For the primary non-inferiority hypothesis, we will conduct per-protocol and intention-to-treat analyses and for the superiority hypotheses we will conduct intention-to-treat analyses.

The secondary endpoints will be tested using linear regression with the treatment arm, age and Hb level as covariates.

Multiple imputation will be used to account for missing data if there are missing covariates in more than 5% of participants. If there is substantial difference in the loss-to-follow-up rate in the different arms we will consider applying simple sensitivity analyses to investigate the robustness of results.

## Ethics statement

This study is being conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki, and that are consistent with the ICH GCP requirements, and in keeping with local regulatory requirements.

Scientific advice on the study protocol has been given by the UK Medicines and Healthcare products Regulatory Agency (MHRA 1400, 21/12/2016). The study protocol and any subsequent amendments have been reviewed and approved by The Gambia Government/MRC Joint Ethics Committee (reference SCC1489). Clinical Trials Authorisation has been granted by the Medicines Control Agency, The Gambia (HP373/347/16/MJK(80)).

This paper is written following the SPIRIT 2013 guidelines<sup>42</sup> (Supplementary File 2). The trial is registered in ClinicalTrials.gov, identifier: NCT02941081 (registration date: 21 Oct 2016; <https://clinicaltrials.gov/ct2/show/NCT02941081>).

## Data and safety monitoring

An independent local safety monitor (LSM) and an independent Data and Safety Monitoring Board (DSMB) have been appointed to monitor quality control of the data, progress of recruitment and safety aspects of the IHAT-GUT trial, including

the regular review of all adverse events. The LSM reviews adverse events monthly and the DSMB reviews adverse events quarterly during the study. Any serious adverse events are reported in real time to both the LSM and the DSMB.

No interim analysis will be conducted unless requested by the DSMB in response to adverse events. By August 2018, no interim analysis had been requested by the DSMB and the most recent recommendation was for the study to continue as planned.

## Trial monitoring

Trial oversight is provided by the Sponsor through the Clinical Trials Department at MRCG. An independent trial monitor regularly reviews good clinical practice (GCP) compliance, quality of data collection, sample analysis and the progress of the trial.

## Informed consent

All field workers taking part in the recruitment of participants have received GCP and informed consent training prior to study start. These field workers have also been trained on translating the contents of the information sheet into the different local languages. The field staff explain details of the study to illiterate parents in a language they understand, in the presence of an impartial literate witness, and in a room which ensures adequate privacy. The literate parents are allowed to read the information sheet in their own time. Parents are given enough time to ask questions and decide if they want their child to participate. No child starts any study specific procedure before informed consent is obtained. Participants will not get any remuneration for taking part in the study but will have access to free medical care for the duration of the study. Participants are protected in accordance with the study Sponsor Clinical Trial/Non Negligent Harm Insurance and Medical Malpractice Insurance.

## Confidentiality

Each participant is allocated an individual identification (ID) number, which is used to label all samples collected for the study and on the eCRF during the course of the study. All data is linked-anonymised and the linkage to the ID is not possible without a lookup table, which is held only by the data manager and designated data staff during the course of the study. Once data collection is complete, analysis will be performed on an anonymised copy of the dataset. At all stages, staff/collaborators responsible for sample analysis will be blinded as to the subject's identification. Together, these processes will ensure complete confidentiality of the data gathered and impartiality of data analysis.

## Dissemination and data access

Results of the study will be submitted for publication in relevant peer-reviewed open-access journals and key findings presented at international scientific meetings. The main findings will be disseminated to the National Nutrition Agency and the Ministry of Health in The Gambia. If this study suggests a possible benefit of IHAT above ferrous sulphate, contact will be made with key international organizations (e.g. WHO, UNICEF)

and funding bodies to plan future pivotal trials. Any request for use of study data will have to be approved by the study Sponsor and the Ethics Committee. Data will only be made available in an anonymous format to external users. Data sharing will be in agreement with the Sponsor policy on research data sharing and with the Bill & Melinda Gates Foundation Global Access requirements.

### Study status

Screening for enrollment started in November 2017 and by August 2018, 645 subjects have been successfully recruited and randomised. The first patient first visit (Day 1) was on January 8, 2018. Field data collection will be completed in December 2018. Analysis, and submission for publication, of primary endpoint results will be completed by March 2019. The current protocol version is SCC1489 V5.0, 21 May 2018.

## Discussion

### Rationale for enrolling young children as the study population group

Young children living in resource-poor rural areas in sub-Saharan Africa, where there is high burden from diarrhoeal diseases, are one of the main population groups in need of safer forms of iron supplementation. Randomised-controlled clinical trials conducted in nearly 10,000 young children living in developing countries have consistently shown that current forms of iron supplements are associated with increased infection, including bloody diarrhoea<sup>10,13,16,17</sup>, and detrimental changes to the gut microbiome and gut inflammation<sup>17,18,43</sup>, further increasing the burden from enteric infection and environmental enteropathy (i.e. persistent gut damage and inflammation that leads to malabsorption), which is a major cause of growth failure in children in resource-poor environments<sup>21,22</sup>. Furthermore, it appears that these effects are much more relevant in those resource-poor countries where enteric infection risk is higher and in the pre-school age group, since in older South-African children, with a low enteropathogen burden, iron supplementation does not appear to significantly affect the dominant bacterial groups in the colon or to increase gut inflammation<sup>44</sup>.

If IHAT shows a benefit in the proposed trial, where it will be tested in the population group that is most sensitive to the gastrointestinal adverse effects of iron supplementation, then we believe it can be effective in any other population group. However, the reverse is not true, if IHAT was tested and found to be effective in adults or older children, it would not mean it would work in the young children group, since these have the most immature guts where the microbiome is not stable and where the mucosa is more susceptible<sup>45–48</sup>.

The health of all children in our study is monitored daily, as are all adverse events, including all diarrhoea episodes, malaria and other co-infections. Thus, ethically we are not subjecting children to any unnecessary risk or, in fact, any added risk beyond that expected with current iron supplements when used as per national guidelines.

### Rational for inclusion of a placebo group

Due to the possible detrimental effect of iron supplements in pre-school age children, particularly those living in resource-poor

settings<sup>10,13,16–18,43</sup>, there is now a strong rationale for a ‘no iron’ (placebo) control group thus leading to ethical equipoise. Furthermore, in these settings, there have been numerous randomised-controlled trials with iron supplementation in young children that have failed to show a significant decrease in anaemia prevalence or a clinically relevant increase in haemoglobin after intervention with iron supplements<sup>10,13,41</sup>, as such the active control or standard treatment does not have proven efficacy in this setting. Furthermore, in The Gambia there is no national screening program or mandatory iron supplementation or home fortification policy for children, even though the prevalence of anaemia in this age group is extremely high<sup>35</sup>.

Therefore, inclusion of a placebo arm in this trial adheres to the guidelines for non-inferiority trials as stipulated in the ICH guidance *Choice of Control Group and Related Issues in Clinical Trials (ICH E10)* stating that “where there is no serious harm, such as death or irreversible morbidity in the study population, it is generally considered ethical to ask patients to participate in a placebo-controlled trials, even if they may experience discomfort as a result, provided the setting is non-coercive and patients are fully informed about available therapies and the consequences of delaying treatment”<sup>49</sup>.

### Rational for treatment effect sizes

Because many trials conducted in children from resource-poor countries with ferrous sulphate or ferrous fumarate supplementation have still failed to provide anaemia resolution in interventions shorter than 6 months, we considered that an increase of 1 g/dl in haemoglobin after 3 months is an indication of treatment efficacy. We have based our assay sensitivity on data published with relevant iron supplement studies in children available in the literature<sup>10,13,41</sup>. Nonetheless, we plan to also use the data collected in this study to conduct an exploratory analysis for assay sensitivity, using the comparisons between the active control and the placebo arms to determine the true effect size of iron supplementation in this population group. This is important since assay sensitivity is an essential property of a non-inferiority trial, allowing us to determine if both iron supplements are effective or if neither was effective as per FDA guidelines<sup>50</sup>. These data will be very useful so that adequate treatment effect sizes can be used to power any future studies, in particular a pivotal trial, both for ferrous sulphate and IHAT supplementation effects.

Additionally, efficacy comparisons with the placebo group will tell us if iron supplements are preventing haemoglobin from decreasing further and preventing severe anaemia as children grow, which we consider to be a positive effect, even when overall anaemia prevalence may not decrease for this age group due to the high demands for iron during fast growth.

In terms of safety, the placebo arm will also allow us to rule-out any negative impact of IHAT on diarrhoea episodes, the gut microbiome and infection risk.

### Mitigation of risk of malaria and co-infections

There are risks associated with a large intake of iron supplements, especially in areas of malaria endemicity. The dose of iron given daily in the reference arm (12.5 mg) is the

recommended by WHO for this age group in non-malarious areas or malaria-endemic areas where it should be implemented in conjunction with measures to prevent, diagnose and treat malaria and co-infections<sup>34</sup>. The iron dose in the IHAT arm (20 mg) is the bioequivalent dose, i.e. the same absolute amount of iron should be absorbed as in the ferrous sulphate arm, and because IHAT should be safer to the gut, the unabsorbed fraction should not cause detrimental effects, such as infectious diarrhoea. This dose (20 mg Fe) is still less than the new WHO recommendation for children in the 24–35 month age group (i.e. 30 mg,<sup>34</sup>).

In any case, in this study with the daily visits by the field staff and the weekly visits to the study clinics, all children are closely monitored and at the first sign of infection or malaria they are referred to the study nurse or clinician for clinical assessment and treatment as per clinical guidelines.

## Conclusions

This study will provide the first clinical trial data for IHAT, an innovative nano-iron supplement, and will enable us to obtain high-quality data for its safety and efficacy in correcting iron deficiency and improving anaemia in sub-Saharan African children, the population group most in need of a better oral iron supplement.

The trial will also contribute to our better understanding of iron deficiency and anaemia treatment effect sizes with the current gold-standard of iron supplementation, and to our understanding of how iron supplementation and iron deficiency impact on enteric infection and diarrhoea risk.

If IHAT is successful in this trial, these data will provide the evidence needed to encourage further development so that IHAT can be implemented as a novel iron source for use in micronutrient intervention strategies aimed at children and women living in resource-poor countries and, hence, help to reduce the global burden of IDA. We envisage that a future phase III pragmatic trial would test efficacy and safety of IHAT, used

as part of multi-micronutrient supplements, in children and pregnant women across multiple countries.

## Data availability

No data is associated with this article.

## Grant information

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## Supplementary material

Supplementary File 1: Participant information sheet and consent form.

[Click here to access the data.](#)

Supplementary File 2: Completed SPIRIT checklist.

[Click here to access the data.](#)

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## Open Peer Review

Current Peer Review Status:  

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### Version 2

Reviewer Report 15 October 2018

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**Toby Richards**

Division of Surgery and Interventional Science, University College London, London, UK

No further comments.

**Competing Interests:** No competing interests were disclosed.

**I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.**

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### Version 1

Reviewer Report 04 October 2018

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**Robert C. Hider** 

Division of Pharmaceutical Science, King's College London, London, UK

This article is very well planned and well resourced. The project is highly relevant to treatment of anemia. To provide a FeSO<sub>4</sub> substitute of equivalent activity is an important advance. This is not glamorous work but the possible replacement of FeSO<sub>4</sub> by an alternate iron source is extremely important, as compliance with FeSO<sub>4</sub> can be poor.

A relatively new development in this type of work is to study any changes induced in the gut flora.

The results from this part of the proposed study will certainly be interesting, but may be difficult to interpret.

The clinical trial includes a placebo as well as a FeSO<sub>4</sub> control. This is important, as many such trials include one or the other, but not both. The direct comparison of the three different conditions will facilitate the interpretation of the clinical data.

**Is the rationale for, and objectives of, the study clearly described?**

Yes

**Is the study design appropriate for the research question?**

Yes

**Are sufficient details of the methods provided to allow replication by others?**

Yes

**Are the datasets clearly presented in a useable and accessible format?**

Yes

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Medicinal chemistry

**I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.**

Reviewer Report 01 October 2018

<https://doi.org/10.21956/gatesopenres.13950.r26671>

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**Toby Richards**

Division of Surgery and Interventional Science, University College London, London, UK

Many thanks for asking me to review this trial protocol. This trial offers a potential breakthrough to one of the biggest diseases worldwide.

Iron deficiency is the most common nutritional disease worldwide. A disease that impacts people from countries of all GDP. Iron deficiency is the commonest cause of anaemia and associated with 8 years of living with disability. Iron deficiency and anaemia are so common they are hidden in plain sight.

In low income countries, the situation is worsened due to poor diet (low in meat, which has good iron absorption) and concurrent infections (which reduce iron absorption). Iron deficiency leads to

childhood growth retardation and reduced cognitive development. In adults' iron deficiency impacts mental and physical performance. In pregnancy the situation worsens with a higher risk of intrauterine growth retardation and death to both mother and baby at childbirth.

A key problem to iron deficiency is lack of available good therapies. First line is dietary modification and then iron tablets. Iron tablets are essentially iron salts. The iron is poorly absorbed (only 5%) the remainder sits in the gut causing local toxicity, side effects and potentially exacerbating infection. An alternative is an iron infusion. This is simply not available or possible in many populations let alone in those countries who cannot afford healthcare.

There is little funding to trials with simple and cheap solutions and no new oral iron preparations in the last decade. The Gates Foundation are to be applauded to support this work.

### Main Comments

Overall, this manuscript would benefit from being shortened and simplified. The message that this is a novel oral iron with a unique absorption pathway that may impact anaemia management without GI side effects does not come through. The authors should not under estimate the novelty of the invention of a nano-iron and how this works

For a full protocol it can be difficult as there is so much information. The main issue is complexity of the primary endpoint. It is very unusual to have 4 primary endpoints. This leads to a lot of confusion in the hypothesis, aim(s) objectives and methods. Ideally this should be one key primary endpoint or in this case one efficacy and one safety endpoint. It is not a Multiarm multistage or adaptive trial so randomising to 3 groups with 4 primary endpoints is difficult. Perhaps, broach this early on and explain (I suspect) the funder's and sponsors requirement for the additional endpoints, then refer to efficacy and safety endpoints moving forwards.

### Abstract

Please simplify and shorten and explain what IHAT is and why it is relevant:

Oral iron is often ineffective as poorly absorbed and associate with gut side effects. IHAT is a novel nano-iron preparation with high efficacy and >50% absorption therefore overcoming these issues enabling lower dosage than conventional therapy with fewer side effects.

Is this a Phase 2a? efficacy trial to test non-inferiority of IHAT to treat anaemia compared to FeSo<sub>4</sub> and safety of IHAT in this population.

Remove: Assessments are conducted at baseline, Day 15, and Day 85 of the supplementation period.

Secondary objectives are to assess the superiority of IHAT to ferrous sulphate and non-inferiority to placebo in terms of side effects of diarrhoea.

Secondary endpoints could be referred to mechanistic endpoints. Further, mechanistic assessments, to test whether IHAT reduces the burden of enteric pathogens, morbidity, and intestinal inflammation, and that it does not cause detrimental changes to the gut microbiome, particularly in relation to *Lactobacillaceae*, *Bifidobacteriaceae* and *Enterobacteriaceae*.

### Manuscript

Many readers will not know how iron is absorbed in the body so the authors are encouraged to simplify and explain the basic physiology of iron absorption, the problem of oral iron compounds and therefore the novel properties of IHAT.

### **Introduction**

The authors provide an overview of ID and Anaemia. However, data on YLD and DALY are from adults not specific to children. They then talk about data in children. Please add a sentence on how ID and anaemia impact childhood development and illness to bring these two points closer together.

Also perhaps explain the difference between ID and Anaemia or simply refer to the problem as IDA throughout

Before rationale and aims, introduce a further sub heading 'the problem' to highlight how normal iron supplements do not work due to poor absorption and GI side effect. This is a key point to why IHAT is needed and many readers simply will not know that of 200mg FeSo<sub>4</sub> less than 5% is actually absorbed.

In rationale, please reduce the finer details of the compound and increase the background on earlier preclinical studies. What have you shown so far?

Please list the aim (correct hb) and objectives (infection / side effects) as a separate subheading for clarity (as above comments).

### **Protocol**

Hypothesis is clear.

Remove comparator from this paragraph as that is a method.

Normally there is one primary endpoint then a series of secondary endpoints. It is confusing with 4 objectives. Please clarify through the manuscript the aim and objectives, primary and secondary endpoints.

Remove details of any follow up study as not relevant to this protocol?

### **Study Design & Setting**

Please use sub headings to the design and sponsorship etc. then Setting to highlight the population (move details on transport of samples to methods not setting)

### **Criteria**

Why exclude previous / current iron supplements – as if still have IDA then this is failure of therapy.

Is haemoglobinopathy excluded?

Childhood blood testing can be difficult – was this a criteria for inclusion i.e. a child must be well and able to give a venous blood sample?

### **Intervention**



Conducting, clinical trials in this setting is very difficult. There is clearly a labour intensive methodology. Has this been validated (i.e. are these standardised methods in this setting) please provide a reference.

The trial preparations have been manufactured to enable concealment and therefor blinding of the children. Are there blinded and unblinded assessors?

How is concealment bias ensured if there are weekly hb testing - are these results kept blinded also do all children attend or only some - will there be reporting bias as only the sicker children return for repeat testing?

Will children and healthcare workers be asked if they have an ideal of what intervention was given to ensure lack of bias? It may be clear that a child's health is improving and this may in turn lead to higher compliance compared to a group where the intervention is causing side effects.

### **Outcomes**

Resolving iron deficiency and anaemia correction are two separate endpoints. What is the definition of resolving iron deficiency and also for correcting anaemia. A patient can have no anaemia but be iron deficient and vice versa.

It seems the endpoint is Hb correction but hb rise  $> 1\text{g/dl}$  is not the same as an absolute correction of anaemia. Should this endpoint be change in Hb?

Again, having 4 primary endpoints is unusual and difficult to comprehend in light of the aim and objectives. Also the primary endpoint normally reflects the previous data, principle question and denominator of the power calculation. I suspect the funder/regulatory authority had input to this but I urge a more simplified primary question.

If 705 to be consented and enrolled please use this number throughout for enrolment to the trial.

### **Discussion**

As many of the patients have been enrolled, can the authors please highlight any interim analysis and also (I assume) the DSMC have approved continuation.

Overall this is a superb study but I urge the authors to simplify to two questions – does IHAT work and is it safe?

### **Is the rationale for, and objectives of, the study clearly described?**

Partly

### **Is the study design appropriate for the research question?**

Yes

### **Are sufficient details of the methods provided to allow replication by others?**

No

### **Are the datasets clearly presented in a useable and accessible format?**

Not applicable

**Competing Interests:** No competing interests were disclosed.

**I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.**

Author Response 06 Oct 2018

**Dora Pereira**, University of Cambridge, Cambridge, UK

Many thanks for your valuable comments and for reviewing this protocol. We hope we have address your reservations in the revised manuscript.

Here are some specific responses to some your comments.

- *The authors should not under estimate the novelty of the invention of a nano-iron and how this works*

We have tried to highlight better the novelty of IHAT and why it should work better than current oral iron.

- *The main issue is complexity of the primary endpoint. It is very unusual to have 4 primary endpoints.*

In relation to the complexity of the primary endpoints, we do appreciate this point and assure the reviewer that we thought very carefully about this and had several discussions with the several independent review boards that have approved this protocol. We felt that maintaining the hypothesis-driven study design with the analysis of the 4 primary study endpoints is appropriate taking into consideration the stage of development of IHAT and the fact that at the end of this trial we must be able to make a 'go/no-go' decision to move to the next stages of trials in resource-poor settings. The 'go' decision to continue development of IHAT in these settings will only be justified if we determine in this trial if IHAT can help correct iron deficiency anaemia without promoting diarrhoea, which is the largest limitation of current supplements. This is why we maintained the current 4 primary endpoints.

- *Is this a Phase 2a? efficacy trial to test non-inferiority of IHAT to treat anaemia compared to FeSo4 and safety of IHAT in this population.*

This is a Phase II where we test both efficacy and safety as primary endpoint. We have added this information to the abstract.

- Secondary endpoints could be referred to mechanistic endpoints. Further, mechanistic assessments, to test whether IHAT reduces the burden of enteric pathogens, morbidity, and intestinal inflammation, and that it does not cause

detrimental changes to the gut microbiome, particularly in relation to *Lactobacillaceae*, *Bifidobacteriaceae* and *Enterobacteriaceae*.

Thank you for this suggestion, we have included this.

*- Many readers will not know how iron is absorbed in the body so the authors are encouraged to simplify and explain the basic physiology of iron absorption, the problem of oral iron compounds and therefore the novel properties of IHAT.*

The protocol is already quite long and we don't have much room to explain iron absorption and metabolism. We do highlight better in the introduction what is unique about IHAT in relation to how current oral iron is absorbed. So this should help the reader to understand the novelty.

#### **- Introduction**

*The authors provide an overview of ID and Anaemia. However, data on YLD and DALY are from adults not specific to children. They then talk about data in children. Please add a sentence on how ID and anaemia impact childhood development and illness to bring these two points closer together.*

*Also perhaps explain the difference between ID and Anaemia or simply refer to the problem as IDA throughout*

Thank you, we have now done this in the revised version.

- *Adding more sub-headings for clarity.*

This has been done, thank you.

- **Protocol** *Remove comparator from this paragraph as that is a method.*

We moved this paragraph to the design.

#### **- Criteria**

*Why exclude previous / current iron supplements – as if still have IDA then this is failure of therapy.*

They are excluded because treatment failure is a secondary outcome of the trial and we did not want to have children currently taking iron supplements acting as confounders. But in practice, we did not have to exclude any child due to them taking iron supplements currently since none of the ~ 1500 children we screened had been screened for anaemia or was taking iron supplements in the last year.

*- Is haemoglobinopathy excluded?*

Yes we had included these in the 'chronic conditions' criteria but now we have separated them.

- *Childhood blood testing can be difficult – was this a criteria for inclusion i.e. a child must be well and able to give a venous blood sample?*

Yes, these criteria are part of our inclusion (ability to follow the protocol which includes the blood sampling). Our field nurses are very experienced and we have less than 5% of children where we have been unable to collect venous blood in one of the study visits.

*- Conducting, clinical trials in this setting is very difficult. There is clearly a labour intensive methodology. Has this been validated (i.e. are these standardised methods in this setting) please provide a reference.*

Agreed! There were no existing validated procedures for our field procedures or for many of the data and lab procedures. We spend the 1 year implementation period of the trial designing new standardised protocols for all trial procedures (lab, field and data) and have currently 25 standardised study specific procedures that we are using in IHAT-GUT and that we can make available to other researchers who would like to adapt them to their own studies.

*- The trial preparations have been manufactured to enable concealment and therefore blinding of the children. Are there blinded and unblinded assessors?*

*How is concealment bias ensured if there are weekly hb testing - are these results kept blinded also do all children attend or only some – will there be reporting bias as only the sicker children return for repeat testing?*

The trial is double-blinded. All participants and field and lab staff are blinded. Each child has a completely unique 'drug' code and therefore we don't even know which children are in the same treatment arm. Haemoglobin in 3 months does not change that much and the field staff who measure Hb every week cannot determine which children will be on iron or placebo.

All children return to the clinics weekly unless they are travelling or mothers busy (~15% maximum of missed visits) and this does not depend on their well status. Children do additional visits to the clinics if they have an adverse event.

*- Will children and healthcare workers be asked if they have an idea of what intervention was given to ensure lack of bias? It may be clear that a child's health is improving and this may in turn lead to higher compliance compared to a group where the intervention is causing side effects.*

The health of every child in the study is improving simply due to all the extra care they receive whilst taking part in the study, with daily visits by the field staff and weekly visits to a nurse. All children that are sick with fever, diarrhoea etc are immediately treated. We must

remember, this is a setting where there is very limited health care and children hardly ever would see a nurse or clinician unless they were severely sick. This is why it was so crucial in this study to have the placebo arm to determine the true effects of giving iron above just providing standard health care and no iron.

#### **- Outcomes**

*Resolving iron deficiency and anaemia correction are two separate endpoints. What is the definition of resolving iron deficiency and also for correcting anaemia. A patient can have no anaemia but be iron deficient and vice versa.*

The model we will use for iron deficiency in the presence of inflammation is referenced and we will use the recommended method of the BRINDA group.

*- It seems the endpoint is Hb correction but hb rise > 1g/dl is not the same as an absolute correction of anaemia. Should this endpoint be change in Hb?*

This is justified in the Discussion.

- *If 705 to be consented and enrolled please use this number throughout for enrolment to the trial.*

We have changed this.

#### **- Discussion**

*As many of the patients have been enrolled, can the authors please highlight any interim analysis and also (I assume) the DSMC have approved continuation.*

Thanks, we have added a statement on this.

**Competing Interests:** No competing interests were disclosed.