Iron Supplementation and Nutritional Counseling Interventions to Improve Availability and Safety of Blood in Ghana

Short Title: Bloodsafe Ghana- Iron and Nutritional Counseling Strategy Pilot Study (BLIS)

Statistical Analysis Plan

Version 1.0

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Protocol version	Updated SAP version number	Section number(s) changed	Description and reason for change	Date changed

Contents

1 Introduction

1.1 Objective of the statistical analysis plan

The objective of this statistical analysis plan (SAP) is to describe how quantitative interim and final data from the pilot study entitled “Iron Supplementation and Nutritional Counseling Interventions to Improve Availability and Safety of Blood in Ghana” will be analysed. The short name is “Bloodsafe Ghana- Iron and Nutritional Counseling Strategy Pilot Study” with acronym BLIS. This version of the SAP was based on version 1.0 of the protocol and may be modified prior to modifications of version 1.0 of the protocol. Alternatively, newer versions of the study protocol may still use this version of the SAP.

This SAP:

- Provides a brief background of the study (section 1.2).
- Provides details on statistical aspects of the study design (sections 2.1-2.4).
- Describes goals of the interim review by the independent data and safety monitoring board (DSMB), the planned format of the review meetings, and timing of final analyses (sections 2.6-2.7).
- Describes the trial population used for analysis (section 3).
- Describes the planned data analyses that will be included in reports to the DSMB (sections 4-7).
- Describes data summaries to be provided regularly to the DSMB and study leadership to aid in monitoring trial conduct and data quality; these data summaries will be restricted to enrolment, baseline data, data completeness, and study conduct, and will be pooled across treatment groups (See section 8).

The SAP for BLIS may be updated after enrolment begins but will be finalized prior to database lock. These updates will be distinguished by new version numbers (with updated dates). A table after the title page of this document will summarize any changes.

1.2 Description of the study

This section briefly summarizes the rationale and approach of BLIS. The study protocol provides greater detail. Details on statistical topics (e.g., sample size and analysis) beyond what the study protocol provides will be described in later sections of this SAP.

Rationale

The major challenge for blood transfusion services in Sub-Saharan Africa is the juxtaposition of an increasing clinical demand for blood and a historically insufficient supply of safe blood in the region. The system of blood donation in Ghana has been identified as the major barrier to providing adequate and regular blood to meet the transfusion needs of the country. This study is therefore planned as a feasibility and acceptability pilot trial of iron supplementation to increase blood donation among blood donors deferred for low levels of haemoglobin within the catchment areas of the Southern Zonal Blood Centre (SZBC).

Design

This pilot study has 3 components: 1) a cross-sectional assessment designed to estimate the prevalence of anaemia leading to donor deferral, 2) a cross-sectional assessment designed to estimate the prevalence of iron deficiency (ID) and the prevalence of iron deficiency anaemia (IDA) among first-time donors, and 3) a longitudinal 2-arm parallel groups trial among first-time voluntary donors that compares haemoglobin levels at 4 months among those who receive iron supplementation at enrolment to those who do not receive iron supplementation at enrolment.

The primary outcome for the trial will be haemoglobin levels at 4 months. Participants will be followed for a total of 6 months with study visits at 2, 4 and 6 months after the baseline assessment. Blood draws for a full blood count (FBC), peripheral film comment, malaria rapid test and ferritin assessment and the administration of a structured questionnaire will occur at screening, 2 months (interim 1), 4 months (interim 2) and final visits (6 months). The malaria rapid test occurs at the enrolment visit which occurs within 3 weeks of the screening visit. There is not a blood draw at the enrolment visit unless the participant presents with signs of infection.

The parallel group's component is designed as a non-inferiority trial for haemoglobin levels at 4 months. At baseline, the 2 groups will be determined based on the need for iron supplementation: all individuals with ID, IDA or low haemoglobin (gender specific values), who satisfy our eligibility criteria regardless of deferral status, will receive iron supplements while all others will not. While a high prevalence of ID and IDA is expected, enrolment will be staggered so that an equal number of participants from each group are enrolled from each site with at least 1 participant who is assigned to iron supplementation. If there are more potential participants in one group at some site, randomization will be used to select the individuals to enroll in the trial to maintain balance over time. The extent of adherence is a central question for this study, and it is also unclear if the haemoglobin deficiency can be improved above what is seen in the control group, hence it is not hypothesized that those receiving iron supplementation will have higher levels of haemoglobin at 4 months. The hypothesis about the iron supplementation intervention is that it will improve haemoglobin levels to near that seen among those who did not receive iron supplementation. If haemoglobin levels are higher in those receiving iron supplementation the intervention would also be deemed a success. Hence a non-inferiority trial is most appropriate for this hypothesis.

Selection of the margin is a critical component of a non-inferiority trial. Here, we specify a margin of 1 g/dL for haemoglobin levels at 4 months. Under a collection of reasonable assumptions, this margin will ensure that the majority of those with low haemoglobin at screening will have haemoglobin levels at 4 months above the threshold for anaemia and should therefore qualify as donors. To see this, assume that half of donors are anaemic at screening (using the thresholds for anaemia of 13 g/dL for men and 11.5 g/dL for women). A simple calculation finds that if we assume haemoglobin levels are normally distributed (which is a reasonable assumption based on observed distributions for haemoglobin in unselected individuals in West Africa) and the standard deviation of screening haemoglobin levels is 1.89 g/dL (this estimate will be described below) then the mean haemoglobin level among men who are deferred is 11.5 g/dL and the mean haemoglobin level among men who are not deferred is 14.5 g/dL (these values for

women are 10.0 g/dL and 13.0 g/dL). If we assume that the haemoglobin level among those who are not deferred is the same 4 months later then a margin of 1 g/dL implies a mean haemoglobin level among those with anaemia at screening of at least 13.5 g/dL for men and 12.0 g/dL for women. Thus, the mean haemoglobin level after 4 months of iron supplementation among those who should have been deferred will exceed the threshold for anaemia and consequently, if the distribution of haemoglobin levels is symmetric, the majority of those who were anaemic at screening would qualify as donors (in fact at least 60.4% would pass the threshold for both sexes if this distribution is normal). Note that the threshold for the copper sulphate test used for women is 12 g/dL, not 11.5 g/dL, so under these assumptions 50% of women would pass the copper sulphate test. Nonetheless, since well over 50% of donors are men the majority of deferred donors would pass the copper sulphate test. It is likely that fewer than half of all donors will be anaemic at screening. In this case the difference in the means between those with anaemia and those without anaemia will be larger, the gain in haemoglobin levels in the anaemic group will be larger and the proportion of donors who would be deferred at screening but would not be deferred after 4 months of iron supplementation will be even larger. It is possible that the difference between the 2 groups is smaller than the margin at screening. This seems unlikely since the 2 groups are defined by a threshold for haemoglobin levels, but if the standard deviation of haemoglobin levels is 0.62 then the difference between the groups among men would be less than the margin under the assumptions of the previous paragraph (this standard deviation is less than one third of the standard deviation used for design). To address this, when enrolment reaches 50% of the total planned sample size, the statisticians supporting the trial at the data coordinating centre (DCC) will compute the standard deviation of haemoglobin levels at screening and revisit the margin calculation and the sample size. If the standard deviation is low enough so that the difference between the groups is less than the margin, then the study will be overpowered for the current margin and a smaller margin will be selected so that the trial is still testing for differences at 4 months that exceed the screening difference. Any changes to the study design based on these analyses will be discussed with the DSMB.

In addition, we will use a qualitative approach to identify barriers and facilitators of blood donation and the use of dietary and iron supplementation strategies to address ID and/or anaemia. This will involve conducting focus group discussions (FGDs) during the last month of the intervention. The interviews will focus on mulitple issues including food selection and consumption, cost of micronutrient-rich foods as well as other potential issues that might contribute to the success or otherwise of the dietary and iron supplementation interventions.

Objectives

Primary Objectives

The primary objective of the Iron Supplementation Trial is to determine the potential for an iron supplementation strategy to increase blood donations in Ghana. To determine this potential, the study will investigate the feasibility and acceptability of this intervention along with key return blood donation measures among first-time blood donors found to have low haemoglobin or found to have IDA or be ID within the catchment areas of the SZBC. These aspects of the potential for this intervention will be

operationalized as follows:

- **Feasibility** of recruitment, enrolment, retention, and adherence will be evaluated with a target retention rate > 90% of all participants recruited completing the trial. If we are unable to achieve this target, alternative strategies will be considered for the UH3 phase.
- **Acceptability** will involve establishing the safety profile of 3 times weekly low dose ferrous sulphate (65mg elemental iron) supplements among those assigned to iron supplementation at enrolment. This safety profile will be established by comparison to a similar group of participants who do not receive iron supplementation. Participant and key informant views and perspectives of barriers and recommendations for using iron supplementation and nutritional counselling to increase the blood donor pool will also be investigated with participant questionnaires. In addition, FGDs and key informant interviews will provide critical insights into the acceptability of this intervention.
- **Key blood donation measures** include haemoglobin levels at 4 months, the change in haemoglobin concentration, ferritin levels at 4 months, the proportion of participants with corrected ID, the proportion of participants who return to the blood centre to donate blood and the proportion of returners who are able to successfully donate (not deferred). In addition, the incidence of ID and IDA among blood donors will be determined among those who consent to the Iron Supplementation Trial at screening.

To achieve this objective, a pilot parallel groups trial will be conducted for one year. This allows for enrolment over a maximum of 6 months and 6 months of follow-up for the last individual enrolled. The follow-up period incorporates 4 months of the iron supplementation intervention.

The **primary endpoint for the parallel groups trial** will be haemoglobin levels at 4 months, and this will be compared between those assigned iron supplementation at enrolment and those not assigned iron supplementation at enrolment.

Secondary Objectives

- To determine changes in haemoglobin in blood donors over a 6-month period.
- To determine the impact of iron supplementation on the change in haemoglobin.
- To estimate the prevalence of ID and IDA in prospective first-time blood donors.
- To determine the factors associated with ID and IDA among prospective blood donors.
- To determine the prevalence of malaria or adverse events among the prospective first-time blood donors.
- To identify the barriers and facilitators of repeat donation and the use of dietary and iron supplementation interventions to address ID and/or anaemia among blood doors.
- To determine feasibility of the intervention, through documenting implementation fidelity, intervention adaptation, and intervention strengths and weaknesses.
- To understand participant and key informant acceptance of the intervention.
- To develop and implement key strategies aimed at increasing the blood donor pool.

- To compare haemoglobin levels as determined by copper sulphate versus automated haematology analyser.

Duration

The duration of this UG3 phase (Pilot trial) is 1 year.

Sample size

Qualitative study: In all, ten focus groups of 8-10 participants purposively selected with one group from each of the following blood donation categories: (i) religious organizations, (ii) educational institutions, (iii) corporate institutions, (iv) organized community groups, and (v) walk-in blood donors. The aim of the focus groups is to identify barriers and recommendations for using iron supplementation and nutritional counselling to increase the blood donor pool. These discussions may involve gender-sensitive topics and gender may impact participation in the discussion, so two groups of each blood donation category by gender will be formed (e.g., 8-10 females, 8-10 males). The female and male focus groups will meet in separate spaces so only one gender will be interviewed at a time. Thus, there is one male and one female group for each donation category.

Quantitative method: Cross-sectional study to determine the prevalence of anaemia among prospective blood donors: There are 2 distinct components of the cross-sectional study: estimation of the proportion of potential donors deferred due to low haemoglobin and the proportion of first-time potential donors who have ID and IDA. The determination of deferral due to low haemoglobin is routinely collected by the National Blood Service Ghana (NBSG) and does not require consent of potential donors in this study. The proportion deferred will be determined using the same donation sites as the other components of the study with an estimated sample size of 600 and resulting margin of error less than 0.04. Consequently, the 95% confidence intervals for deferment prevalence will have a total width of less than 0.08 giving a more precise estimate than the year-to-year variation in the total percentage deferred. Participant consent is needed to draw blood for determination of ID and IDA. We anticipate that 292 participants will consent and be enrolled with resulting margin of error less than 0.057 and total confidence interval width of less than 0.11 for likely values of the prevalence of ID and IDA among first-time potential donors (conditional on deferral status). Thus, the ID and IDA prevalence estimates will have comparable precision to the estimated proportion of those deferred using all potential donors at recruitment sites.

Parallel groups' trial to assess the acceptability of iron supplementation: Using a non-inferiority design with a non-inferiority margin of 1 g/dL for haemoglobin levels at 4 months, a 1-sided alpha level of 0.025, at least 85% power and under the assumption that the true difference in the means is 0.3 requires 262 participants (131 per arm). This number has been increased to allow up to 10% loss to follow-up or iron supplementation for those initially not requiring supplements (such individuals will be excluded from the primary analysis: see Section 7.2.1 for further details), thus the sample size is 292. For efficiency, participants will be enrolled into both parts of the quantitative study.

Study site and Population

The NBSG SZBC located in the capital Accra annually collects voluntary blood donations from 200-300 blood donor groups that are categorized as (i) religious organizations, (ii) educational institutions, (iii) corporate institutions, (iv) organized community groups, (v) walk-in blood donors and (vi) mass/media events. Data for the prevalence of deferral assessment will be collected from all prospective voluntary blood donors within the catchment areas of the SZBC. The prevalence study of ID and IDA as well as the parallel groups trial will be restricted to first-time donors. The parallel groups trial will be restricted to voluntary donors. Generally, prospective voluntary blood donors are healthy, unlikely to be severely anaemic and are more likely to be male.

The study population will be all adults between the ages of 17 and 60 years who visit blood donation sites at the time of recruitment. A stringent screening and selection process is performed to evaluate the suitability of prospective donors. This is essential to guarantee the safety and adequacy of the blood supply; protecting the wellbeing of the recipients of transfusion as well as the fitness of the donors while ensuring that qualified donors are not deferred needlessly.

Stratification and Sampling

The SZBC contributes to the blood service needs of the Western, Central, Greater Accra, Eastern and the Volta Regions of Ghana. In 2018 and 2019, the SZBC attended to an average of 550 prospective voluntary blood donors a week, including 80 walk-in donors. Out of this number, the study will recruit all participants (who fall within the inclusion criteria) reporting per day for up to 6 months until the sample size is attained. Eligible participants will form the sampling frame for the study.

Donors will be informed about the trial and the prevalence study of ID and IDA. Due to a need to recruit approximately equal numbers of participants in the 2 groups in the trial, donors who fail the copper sulphate test will be disproportionately approached for participation. This creates a stratification of donors by status with regard to the outcome of the copper sulphate test. Within these strata the proportion of donors who are ID and IDA will be determined. The estimates of these proportions within strata will be unbiased but if one simply averages over the strata, we expect that the estimates of the proportion who are ID or IDA will have positive bias. However, since we will have estimates of the proportion of donors who fail the copper sulphate test, we can post-stratify the proportions of donors who are ID or IDA by the proportion of donors who fail the copper sulphate test.

Out of the sampling frame, participants will be purposively sampled for FGDs. In all there will be ten FGDs i.e., two groups (male-only and female-only) selected to represent each of the five NBSG voluntary blood donation categories (excluding mass/media event donors), and with each group composed of both first-time and repeat donors. In-depth interviews will also be conducted with blood donation staff and medical officers responsible for blood donation.

Monitoring

A DSMB will provide independent monitoring of this pilot study in accordance with the BLOODSAFE DSMB charter. The DSMB will have an opportunity to review the protocol

and informed consent and provide feedback prior to study initiation. An open and closed report will be prepared by a DCC statisticians. Interim data summaries will be reviewed at regular intervals by the DSMB (at least twice a year). The DSMB will review adverse event data, other safety data, quality and completeness of study data, and enrolment data at each meeting to ensure proper trial conduct. Study personnel should provide any new literature pertinent to the trial, along with their recommendation as to whether it affects the trial conduct or design. The DSMB will review the consent periodically and/or as needed and consider whether the consent form requires revision in light of any new findings or amendments. In addition to regular meetings, it may be necessary to convene the DSMB urgently on an *ad hoc* basis to discuss new data or other information that raises questions about equipoise, safety, or other issues identified by DSMB members.

2 Study Methods

2.1 Design

This pilot study has 3 components: 1) a cross-sectional assessment designed to estimate the prevalence of anaemia leading to donor deferral, 2) a cross-sectional assessment designed to estimate the prevalence of ID and IDA among first-time donors, and 3) a longitudinal 2-arm parallel groups trial among first-time voluntary donors that compares haemoglobin levels at 4 months among those who receive iron supplementation to those who do not receive iron supplementation.

2.2 Randomization

There is no randomization in the cross-sectional component. In the parallel groups' component, the 2 groups will be determined based on the need for iron supplementation, so the treatment is not assigned by randomization.

2.3 Blinding

The participants and the investigators are not blinded.

2.4 Sample size

Cross-sectional study: Since all potential donors will contribute to the estimate of the proportion of potential donors who are deferred due to low haemoglobin, the estimate of this proportion will be sufficiently accurate to allow for comparisons against yearly rates. As stated above, the annual proportion of deferrals from 2015-2018 ranged from 33-53%. If we assume that 5 blood donors attempt to donate each business day, then there should be about 600 donation attempts over the 6-month enrolment period. Table 1 shows values for the prevalence of low haemoglobin, the corresponding margin of error and the corresponding 95% confidence interval. For example, if the prevalence of low haemoglobin leading to deferral is 50% then a 95% confidence interval would be (46%, 53%) which allows for determination of the prevalence in a range that is smaller than the range of yearly estimates. Note: these estimates were obtained using the usual normal approximation to the sampling distribution of a proportion without a continuity correction.

Table 1: Precision of prevalence estimates for low haemoglobin leading to deferral.

Prevalence	Error Margin	95% Confidence Interval
0.2	0.032	0.17, 0.23
0.3	0.037	0.26, 0.34
0.4	0.039	0.36, 0.44
0.5	0.040	0.46, 0.54
0.6	0.039	0.56, 0.64

Since not all participants will consent to participate in the study and only a subset of donors are first-time donors, the precision of estimates of the prevalence of ID and IDA will be lower. Based on the unpublished estimates of the prevalence of ID and IDA provided above (i.e., 63.9% of those with anaemia had IDA and 86.1% of those with anaemia had ID, so 30.7% of the total had IDA and 41.3% had ID), estimates of the prevalence of these conditions cover almost the same range as our estimates of the prevalence of low haemoglobin. Since enrolment for this component of the study entails enrolment in the longitudinal study, the sample size was determined by the requirements for the longitudinal study which is 292 participants. Since some participants in the cross-sectional study of ID and IDA may be found to have haemoglobin (Hb)<10g/dl and will therefore be excluded from the longitudinal study, the sample size for the cross-sectional study may exceed 292. This will require that, on average, almost 2.5 participants per day consent to the study to get to about 300 over the 6-month enrolment period. Table 2 shows values for the prevalence of ID/IDA within deferral strata, the corresponding margin of error and the corresponding 95% confidence interval. For example, if the prevalence of IDA is 30% within one of the strata then a 95% confidence interval would be (25%, 35%) which has a total width comparable to the confidence intervals obtained using all potential donors. The intervals for the post-stratified estimates will be slightly larger due to the sampling variability of the estimated post-stratification weights.

Table 2: Precision of prevalence estimates for ID and IDA.

Prevalence	Error Margin	95% Confidence Interval
0.2	0.046	0.15, 0.25
0.3	0.053	0.25, 0.35
0.4	0.056	0.34, 0.46
0.5	0.057	0.44, 0.56

Parallel groups study: The primary outcome for this portion of the study is haemoglobin levels at 4 months. Formally the null hypothesis for this outcome is inferiority of treatment of low haemoglobin with iron supplementation among those with

low haemoglobin at screening to no treatment among those without low haemoglobin at screening. The non-inferiority margin is fixed at a value of 1.0 g/dL. Based on recent work in West Africa, the DCC has access to haemoglobin measurements from 1548 individuals between the ages of 17 and 60 who weigh at least 50 kg. Among these individuals the standard deviation of haemoglobin was 1.89 g/dL (the mean was 13.4 g/dL). These individuals did not pass the strict screening criteria used by the NBSG, so this estimate might be slightly inflated and so our power calculations may be somewhat conservative. We specify an alpha level of 0.025 and require power of at least 85%. With these values we can determine the number of participants per arm if we assume a value for the true difference between the groups (the absolute value of this difference must be less than our margin and will generally be negative when the margin is positive to be consistent with the lack of superiority of iron supplementation). Table 3 presents the number of participants per arm under these assumptions for a selection of values for the true difference. We assume that the true difference is -0.3 g/dL and obtain a sample size of 131 participants per arm. If the true difference is larger than -0.3 g/dL (e.g., if there is no difference) then the power will exceed 85%. To allow for loss to follow-up and some participants requiring iron supplementation due to incident anaemia, ID or IDA the sample size has been increased to 292.

Table 3: Number of participants per arm for 1-sided alpha of 0.025 and power of at least 85%.

True Hb Mean Difference (treated – control)	Sample size (per group)
0	65
-0.1	80
-0.2	101
-0.3	131
-0.4	179

2.5 Framework and statistical principles

The qualitative study that precedes the trial will be used to guide implementation and evaluation of the larger study at the UH3 Phase, including identifying barriers and recommendations for using iron supplementation and nutritional counselling to increase the blood donor pool. Moreover, qualitative findings resulting from focus groups to be conducted at the end of the trial and key informant interviews will be used to guide the planning, implementation, and evaluation of the UH3 phase.

The following general principles will apply to this pilot. All confidence intervals (CIs) are 2-sided and at the 95% level and hypothesis tests are 2-sided at the 5% significance level unless otherwise specified. Percentages, means, medians and quartiles will be rounded to one significant digit. P-values will be given to 2 significant figures. Version

9.3 (or later) of SAS and/or version 3.5 (or later) of R will be used to conduct the analysis and generate tables and figures.

Descriptive statistics for enrolment rates over time and the proportion of participants completing the 6-month study visit will be assessed by treatment group. Those statistics will be used to determine the feasibility of the larger study (UH3) and may lead to changes in how consent, enrolment, and follow-up are conducted.

Cut-date for interim reviews: Analysis data sets will be frozen (locked) several days (or weeks) prior to the review date, to allow the statisticians time to prepare a consistent report. The cut-date may be earlier than the date of the data freeze, to allow for additional time for the reporting of events.

2.6 Interim analyses and DSMB reviews

Goals of the interim reviews:

Protect the safety of study participants.
Review the conduct of the trial.

Reviews will be timed according to the recommendations of the DSMB and study leadership.

Review meetings will typically consist of an executive session (optional; closed), open session, closed session, and a second open session to give feedback to study leadership (optional).

Open report to the DSMB

The open reports will contain:

A synopsis of the trial design and current status of the study.
Responses of the study team to DSMB requests.
A summary prepared by the study leadership.
Data summaries for
- Key Informant Interviews
- Anaemia Deferment Study
  - Baseline characteristics overall and stratified by consent into the Iron Supplementation Trial
- Prevalence of ID and IDA Study
  - Screening, baseline characteristics
- Iron Supplementation Trial
  - Screening, enrolment, eligibility violations, protocol deviations, and baseline characteristics overall.
- FGD

Summary reports for data completeness and study conduct (pooled across treatment groups for the Iron Supplementation Trial)
New literature relevant to the trial and recommendation as to whether it affects the trial conduct or design, will also be provided to the DSMB by the study leadership. This is usually included with the open report but may be shared confidentially if needed.

All data summaries in the open report involving safety or follow-up data will be pooled across the treatment arms for the Iron Supplementation Trial. The open reports will be prepared by a DCC statistician.

While the study is ongoing, summaries and comparisons by treatment group are restricted to the confidential closed report to the DSMB. Additionally, all summaries of follow-up data other than the data completeness and study conduct reports will be restricted to the confidential closed report. On a case-by-case basis, other pooled follow-up data may be provided if explicitly approved by the DSMB.

Closed report to the DSMB

The closed reports for a full review will contain:

Specific data summaries requested by the DSMB or study leadership.
Data summaries for the trial in the open report presented by treatment group.
Safety and follow-up for the trial (including adherence to iron supplementation) overall and by treatment group.
Listings of grade 3, 4 adverse events (AEs) events and serious adverse events (SAEs).
Analysis of the efficacy of the intervention.

The closed reports will be prepared by a DCC statistician with access to the full data set.

2.7 Timing of final analysis

The primary study report will be written after enrolment and follow-up for the primary outcome of the Iron Supplementation Trial is completed or the study is stopped by the sponsor after recommendation by the DSMB (e.g., safety, futility or efficacy reasons).

3 Study Population

3.1 Eligibility

Inclusion Criteria

There are 3 components of the quantitative study (observational study of deferral, observational study of the prevalence of ID and IDA, and the parallel groups trial of iron supplementation) with slightly different inclusion and exclusion criteria, hence we indicate which criteria apply to which component of the study below.

Items 1-3 are required for the observational study of deferral, items 1-4 are required for the observational study of the prevalence of ID and IDA and items 1-6 are required for the trial. Only participants who meet ALL the following inclusion criteria will be eligible for the trial:

1) Males or females aged between 17 and 60 years who weigh at least 50kg.
2) Pass pre-donation screening using the NBSG standardised donor screening questionnaire for medical conditions and lifestyle risks for transfusion transmissible infections (TTIs) (Appendix 5).

3) Vital signs must meet the NBSG requirement for blood donation: systolic and diastolic blood pressures between 90-140 mmHg and 60-90 mmHg, respectively; pulse rate between 50-100 bpm; non-contact forehead temperature not exceeding 37.5°C; meeting acceptable requirements for skin lesions, needle marks and physical appearance.
4) Must be willing and able to give study consent or assent.
5) Intend to remain in the study location/site during the entire length of the study.
6) Voluntary non-remunerated blood donor

Exclusion Criteria

There are no additional exclusion criteria for the observational study of deferral, items 1-2 are required for the observational study of the prevalence of ID and IDA, and items 1-5 pertain to the clinical trial. All persons who meet ANY of the following exclusion criteria will be excluded from participating in the clinical trial.

1) Persons who have used iron supplementation within the past one month.
2) Participant reports having previously donated blood.
3) Evidence for a TTI at screening among those who successfully donated.
4) Evidence of malaria (malaria rapid test at screening) and helminthic infections from specimens obtained at screening.
5) Participants who have Hb <10g/dl at screening.

Potential donors who are found to have Hb < 10g/dl at screening will be referred for further evaluation and care by a haematologist. Any trial participant found to have Hb < 10g/dl at any follow-up visit will be referred for evaluation by a haematologist.

Since participants will only be tracked if they meet eligibility criteria for the study, the consolidated standards for reporting trials (CONSORT) diagram will start with those who consented to the Iron Supplementation Trial. Due to the complexity of the interrelated components of the study, multiple diagrams may be necessary for an accurate characterization of enrolment and follow-up.

For the open report, the following enrolment and eligibility summaries will be provided for those who consented to the Iron Supplementation Trial:

Screening and enrolment over calendar time: plot by day and week, cumulative and increments.
Consented but unable to recontact: number (%).
Eligibility: number (%) and reasons for eligibility violations.
For the Iron Supplementation Trial: Number (%) eligible at screening pending laboratory results and not eligible for enrolment visit with % for each reason (e.g., expiration of laboratory result (timing of expiration and by which laboratory result), Hb < 10 g/dL, Malaria, Helminthes, TTI).

3.2 Withdrawal/follow-up

A case report form will document participant withdrawal and capture whether the participant initiated the withdrawal, or the withdrawal was administrative. The form will also capture if participants want their data removed from the study database, and the

reason for withdrawal. For participants who initiate withdrawal prior to ascertainment of the primary efficacy endpoint, primary outcome data may be imputed. The withdrawals and how these impact the analysis datasets will be indicated in the CONSORT diagram.

3.3 Analysis population

The analysis population for the anaemia deferment study will include all eligible participants, while the analysis population for the ID/IDA study and the trial will include all eligible and consented participants.

3.4 Baseline characteristics

Baseline characteristics for those who enrol in the trial will come from information collected on the screening and enrolment forms. The only characteristics captured for those in the anaemia deferment study are age, sex, if the person has previously donated, and the type of donation (i.e., replacement/family or voluntary). For those in the study of ID/IDA information on abnormal peripheral blood film, malaria, haemoglobin, ferritin, and TTI status will also be obtained. Additional information will be available for those in the trial. For the open report, baseline characteristics will be summarized pooled across the two treatment groups and presented separately for those in the anaemia deferment study, the ID/IDA study, and the trial. Note that these study populations are related as follows: everyone in the trial is in the ID/IDA study, and everyone in the ID/IDA study is in the anaemia deferment study. Note that someone who consents to the trial but is found to have levels of haemoglobin that are too low at screening will participate in the ID/IDA study but will not participate in the trial.

For the closed report, baseline characteristics will be summarized by treatment group for the Iron Supplementation Trial.

The following baseline characteristics will be summarized with counts and percentages for binary and categorical variables, medians and quartiles for continuous variables and ordinal variables when available for each of the 3 components of the study.

Demographics:

- Age: summary as continuous variable.
- Sex.
- First-time donation status (Yes or No).
- Number of children (an integer equal or above 0).
- Marital status (6 options).
- Home situation (6 options).
- Main method of travel (private or public).
- Highest level of education completed (7 options).
- Employment category (8 options).
- Number of expensive, durable consumer items (an integer in the range 0-7).
- Ethnic background (6 options).
- Religion (5 options).

Screening laboratory results:

- Haemoglobin concentration.
- Plasma ferritin concentration.

- Malaria test results from a malaria rapid diagnostic test.
- Abnormal peripheral blood film results reported by type of abnormality (i.e., helminthic infection, malaria, neutrophilia, leucocytosis, and lymphocytosis).
- ID present
- IDA present.

Medical History:

- Diagnoses (19 options, select all that apply).

Nutritional History:

- Number of meals yesterday (4 options).
- Vegetarian status (No, Strict, or Partial).

History of Blood Loss:

- Ever given medicine for the treatment of malaria (Yes or No).
- Given medicine for the treatment of malaria within the last 3 months (Yes or No).
- Bleeding piles in the last 3 months (Yes or No).
- Stomach ulcer in the last 3 months (Yes or No).
- Bled from an injury in the last 3 months (Yes or No).
- Surgery in the last 3 months (Yes or No).
- Currently on NSAID painkillers (Yes or No).
- Have sibling(s) with known repeated anaemia (Yes or No).

4 Administration of Study Treatment

Potential first-time donors will be asked if they are interested in participating in a study of the prevalence of ID and IDA and the impact of iron supplementation on haemoglobin levels and repeat donation. Those who express interest, consent to the study, and are eligible will be asked to return within 3 weeks. During this 3-week period a FBC, a peripheral blood film analysis, and a plasma ferritin measurement will be conducted, and participants will be classified as iron supplement participants (e.g., anaemic, ID, IDA) or otherwise (which we refer to as controls in this protocol).

Those found to be anaemic, ID or IDA will receive oral iron supplementation and nutritional counselling. The oral iron supplements will be ferrous sulphate 200mg (65mg Elemental iron). The iron supplements are already available at the Korle-Bu Teaching Hospital Pharmacy. The study principal investigators and study pharmacist will ensure the medicine is stored properly and dispensed only when needed for study participants. The following will be monitored and completed on a periodic basis to ensure the medicine is within study guidelines, pharmaceutical compliance, and manufacturer guidelines:

Drug Accountability Record.
Iron supplement Safety and Handling Sheet.

Controls will not be given iron supplementation at enrolment but will receive the standard practice of nutritional counselling for blood donors. If someone not given iron supplements at enrolment is found to be anaemic, ID or IDA at a follow-up visit that individual will receive iron supplements.

All study participants will have nutritional counselling, dietary advice, and for those

receiving iron, the need for iron therapy to be used consistently 3 days a week.

5 Safety Assessment

Definitions

Adverse Event (AE)

An AE is any untoward or unfavourable medical occurrence in a study participant, including any abnormal sign (e.g., abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with their participation in research. Only AEs potentially related to iron supplementation will be recorded as AEs.

At each follow-up visit all participants will be asked if they have experienced each item from a list of common adverse reactions to iron supplementation. Furthermore, the occurrence of additional AEs will be sought by non-directive questioning of the study participant at each visit during the study. AEs also may be detected when they are volunteered by the study participants during or between visits or through physical examination findings, laboratory test findings, or other assessments.

Adverse events will be recorded in the Adverse Events electronic case report form (eCRF) under the signs, symptoms, or diagnosis associated with them, accompanied by the following information (as far as possible):

AEs will be assessed.
Its relationship to the study treatment.
Its duration (start and end dates) or if the event is ongoing, an outcome of not recovered/not resolved must be reported.
The grade of the AE.
Whether it constitutes a SAE.
Action taken regarding with study treatment.
All AEs will be treated appropriately. Treatment may include one or more of the following:

- Dose not changed.
- Dose Reduced/increased.
- Drug interrupted/withdrawn.
- Its outcome (i.e., recovery status or whether it was fatal).

If the event worsens the event will be reported a second time in the eCRF noting the start date when the event worsens. For grade 3 and 4 AEs only, if improvement to a lower grade is determined a new entry for this event will be reported in the eCRF noting the start date when the event improved from having been grade 3 or 4.

Adverse events (including lab abnormalities that constitute AEs) will be described using a diagnosis whenever possible, rather than individual underlying signs and symptoms.

Once an AE is detected, it will be followed until its resolution or the end of follow-up and an assessment will be made at each visit (or more frequently, if necessary) of any changes in severity, the suspected relationship to the interventions required to treat it, and the outcome. If an AE has not resolved by the end of follow-up for a participant, the outcome of the AE will be recorded as unknown.

Common adverse reactions to iron supplementation are minor and include:

Unpleasant taste.
Constipation.
Dark stools.
Nausea.
Vomiting.
Abdominal pain.
Diarrhea.

Abnormal laboratory values or test results constitute AEs only if they fulfil at least one of the following criteria:

They induce clinical signs or symptoms.
They are considered clinically significant.
They require therapy.

Clinically significant abnormal laboratory values or test results will be identified through a review of values outside of normal ranges/clinically notable ranges, clinically significant changes from screening or the previous visit.

Serious Adverse Event (SAE)

Serious adverse events (SAEs) are defined as the following:

Death.
Life-threatening (i.e., an immediate threat to life).
Hospitalization or prolongation of hospitalization.
Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
Congenital abnormalities/birth defects.

Other important medical events that may jeopardize the participant and/or may require intervention to prevent one of the outcomes listed above.

Information about all SAEs will be collected and recorded on the electronic Serious Adverse Event Report Form; all applicable sections of the form will be completed in order to provide a clinically thorough report. The investigator will assess and record the relationship of each SAE to the study intervention, complete the SAE Report Form in English, and submit the completed form within 24 hours to the College of Health Sciences IRB.

All follow-up information for the SAE including information on complications, progression of the initial SAE and recurrent episodes will be reported as follow-up to the original episode within 24 hours of the investigator receiving the follow-up information. An SAE occurring at a different time interval or otherwise considered completely unrelated to a previously reported one will be reported separately as a new event.

Follow-up information will be submitted in the same way as the original SAE Report and will describe whether the event has resolved or continues, if and how it was treated, and whether the study participant continued or withdrew from study participation.

We do not anticipate any SAEs directly related to iron supplementation in this study. However, accidental ingestion of a large amount of oral iron preparation may result in acute iron poisoning which can manifest as severe diarrhoea, acidosis, shock, and death. Study participants will be educated and strict instructions on iron supplementation given and the need to store them away from children. Study pills will be distributed in child-safe containers. The pills will be taken three times weekly, specifically Mondays, Wednesdays and Fridays.

Unanticipated Problems

An Unanticipated Problem (UP) is any incident, experience or outcome that is:

Unexpected in terms of nature, severity, or frequency in relation to:

- The research risks that are described in the IRB-approved research protocol and informed consent document; Investigator's Brochure (IB) or other study documents.
- The characteristics of the population being studied.
- Possibly, probably, or definitely related to participation in the research.

Places study participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized per the IB.

Furthermore, an UP could be an expected event that occurs at a greater frequency than would be expected based on current knowledge of the disease and treatment under study. The DSMB providing oversight to the study may make such an assessment based on an aggregate analysis of events.

Severity

The investigator will evaluate all AEs related to iron supplementation with respect to both seriousness (results in outcomes as above) and **severity** (intensity or grade).

The following generic scale will be used:

Grade 1	Symptoms causing no or minimal interference with usual social and functional activities
Grade 2	Symptoms causing greater than minimal interference with usual social and functional activities
Grade 3	Symptoms causing inability to perform usual social and functional activities
Grade 4	Symptoms causing inability to perform basic self-care functions, or medical or operative intervention indicated to prevent permanent impairment, persistent disability, or death
Grade 5	Events resulting in death

Causality

Causality refers to the likelihood that the event is related to the study intervention. It will be assessed for SAEs and UPs.

The causality assessment is based on available information at the time of the assessment of the event. The investigator may revise these assessments as additional information becomes available.

Expectedness

The expectedness assessment is based on available information at the time of the assessment of the event. The investigator may revise these assessments as additional information becomes available.

Schedule for Data Collection and Reporting of Specific Events

Safety assessments will consist of monitoring and recording all AEs related to iron supplementation. It will also include regular monitoring of laboratory assessments including FBC and plasma ferritin.

Halting Enrolment for Safety Reasons

The study may be discontinued at any time by the IRB, DSMB or National Heart, Lung, and Blood Institute (NHLBI) as part of their duties to ensure that research participants are protected.

6 Qualitative Data Analysis

Qualitative data collection and analysis will proceed simultaneously. In all, ten focus groups of 8-10 participants purposively selected with one group from each blood donation group categories (i) religious organizations, (ii) educational institutions, (iii) corporate institutions, (iv) organized community groups, and (v) walk-in blood donors. The aim of the focus groups is to identify barriers and recommendations for using iron supplementation and nutritional counselling to increase the blood donor pool.

FGDs will be audio recorded with consent from the participants. Information obtained will be transcribed verbatim and translated when appropriate. The qualitative analysis will include: (1) preliminary exploration of the data by reading through the transcripts and writing memos; (2) coding the data by segmenting and labelling the text; (3) using codes to develop themes by aggregating similar codes together; (4) connecting and interrelating themes; (5) constructing a narrative. Data will be coded and analysed for themes using the Qualitative Software and Research Nvivo 1.0 (March 18, 2020).

The qualitative study will be used to guide implementation and evaluation of the larger study at the UH3 phase, including identifying barriers and recommendations for using iron supplementation and nutritional counselling to increase the blood donor pool. Moreover, qualitative findings resulting from focus groups to be conducted at the end of the trial at the UG3 phase and key informant interviews will be used to guide the planning, implementation, and evaluation of the UH3 phase.

7 Quantitative Data Analysis

7.1 Cross-sectional study

Analytical approaches to the analysis of data from the prevalence study will focus on univariate summaries but will also investigate risk factors for deferral. An exact

confidence interval for the probability of deferral will be computed using the method first described by Clopper and Pearson. Logistic regression will be used to assess risk factors for deferral. These risk factors will include age, sex, previous donation status and type of donor.

Since recruitment at donation events focuses on informing individuals who fail the copper sulphate test about the study, we expect that the proportions of those who are ID and IDA will be higher than the proportion among those attending a donation event. For this reason, we will estimate the level of ID and IDA overall by weighting the estimates within deferral strata by the observed probability of deferral based on the cross-sectional study of deferral (but restricted to first time donors, or voluntary first time donors depending on who enrols into the ID/IDA study). To obtain 95% confidence intervals for the overall proportion of those who are ID and IDA we will use a Bayesian approach in which we sample the probability that first-time donors are deferred for low haemoglobin from its posterior distribution (which is a beta distribution) and we sample the probability of being ID and IDA conditional on deferral status from their posterior distributions (which will also be beta distributions). We can then combine these draws from the posterior distributions to obtain weighted estimates of the probability of being ID and IDA that will be consistent for these probabilities in the overall population. Risk factors for being ID and IDA conditional on the outcome of the copper sulphate test will be examined using logistic regression.

7.2 Parallel group trial

Analysis cohort: The intervention group is the group assigned oral iron supplementation at enrolment and the control group consists of those not assigned oral iron supplementation at enrolment.

7.2.1 Primary outcome

The **primary outcome** of the trial is haemoglobin level (g/dl) after 4 months.

Primary analysis

The analysis for the primary outcome of haemoglobin levels at 4 months will be conducted using a linear model with haemoglobin levels at 4 months as the response variable. It is unlikely that there is a need to transform the response variable as haemoglobin levels are usually symmetrically distributed with tails that decay at rates consistent with the normal distribution. However, if informal univariate assessments of normality (e.g., qq-plots) indicate strong departures from these expectations, the Box-Cox transformation will be used to select a transformation. The set of explanatory variables will be group membership (i.e., received iron supplementation at enrolment or not), screening haemoglobin, sex, and age. A 1-sided t-test for the regression coefficient associated with group membership will be conducted with a significance level of 0.025 to test the inferiority hypothesis. This analysis will exclude individuals who did not receive iron supplementation at enrolment but received it during follow-up due to changes in ferritin or haemoglobin values. The frequency of this will be closely tracked and if more than 10% of participants in the group initially not receiving iron supplementation receive iron supplementation during follow-up, alternative analysis approaches will be considered and presented to the DSMB.

7.2.2 Secondary outcomes

Secondary outcomes related to efficacy

Several secondary outcomes relate to the efficacy of iron supplementation. The treatment of those not requiring iron supplementation at enrolment but requiring iron supplementation during follow-up will be the same as for the primary outcome. These secondary outcomes include:

Change in haemoglobin over 4 months (g/dl).
Ferritin levels at 4 months (µg/L).
Diagnosis of ID or IDA at 4 months (Yes or No).
Attempt to return for donation within 6 months (Yes or No).
Successful donation within 6 months (Yes or No).

Secondary analyses of secondary outcomes related to efficacy

The first two secondary outcomes that related to efficacy (i.e., change in haemoglobin over 4 months and ferritin levels at 4 months) will be treated as continuous variables and will serve as response variables in multiple regression models. It is anticipated that there will not be a need to transform the change in haemoglobin (however the Box-Cox transformation will be pursued if there is evidence for skewness), but it is anticipated that ferritin will need to be transformed. Usually logarithmic transformations are required to reduce the skewness of variables of this sort (i.e., measured by an ELISA), and this will be pursued, but other transformations may be more appropriate. The multiple regression models will include group membership, screening haemoglobin, screening ferritin (log transformed if substantial skewness is present), sex, and age. The remaining 3 outcomes that related to efficacy (i.e., diagnosis of ID or IDA at 4 months, attempt to return for donation within 6 months, and successful donation within 6 months) will be examined using logistic regression models with group membership, screening haemoglobin, screening ferritin (perhaps transformed), sex, and age as covariates. A significance level of 5% will be used to determine statistical significance of effects and 95% confidence intervals will be used to summarize effect sizes.

Secondary outcomes related to safety and feasibility

Other secondary outcomes related to the safety of iron supplementation, as listed below:

1. Acceptability of iron supplementation among participants as indicated by:
   incidence of SAEs.
   incidence of grade 3 or 4 AEs.
   response to questions about known adverse reactions to iron supplementation.
2. Incidence of gastrointestinal adverse events.
3. Incidence of suspected malaria or bacterial infections.

Secondary analyses of secondary outcomes related to safety and feasibility

To maximize the power to detect an association between group membership and safety outcomes, a composite of grade 3 and 4 AEs, SAEs, gastro-intestinal problems, malaria, and bacterial infections will be constructed and compared between the groups.

The frequency of this composite will be compared between groups using Fisher's exact test. Furthermore, logistic regression models will be fit with group membership, sex, and age as covariates and the odds ratio associated with group membership will be estimated along with a 95% confidence interval. These models will be parameterized so that an OR greater than 1 indicates that the intervention group has a higher incidence of negative outcomes. Components of the composite will also be investigated using a similar approach.

The questions about known side effects to iron supplementation ask: Has the participant experienced any of the following since the last visit (mark all that apply)?

Unpleasant taste.
Constipation.
Dark tools.
Nausea.
Vomiting.
Abdominal pain.
Diarrhea.
Other; specify.

The incidence of each of these outcomes over follow-up will be computed and logistic regression models will examine the impact of group membership, sex, and age on these outcomes. The odds ratio and a 95% confidence interval for the group membership variable will be used to summarize the association. Models which look at the incidence of any of these outcomes will also be examined and an odds ratio and 95% confidence interval will be used to summarize the relationship.

The incidence of ID or IDA will be examined among participants in the control group (who are not ID and do not have IDA at baseline by definition). These estimates will be in the form of events per unit time. The generalized estimating equation (GEE) version of Poisson regression will be used with the robust standard error. A model with just an intercept and an offset (for those without complete follow-up for the final analysis and for interim analyses) will be used to estimate the rate and models with the covariates age and sex will be used to test for sex and age specific differences. Age will be treated as a categorical variable in these models with ranges 17-20, 21-40 and greater than 40. Note that data from different subjects will be treated as independent in this model: GEE are being used here to account for potential overdispersion. The same approach will be used to examine the incidence of ID and IDA separately.

Adherence will be assessed by pill counting and administering adherence questionnaires. Adherence will be calculated as the percent of follow-up days/expected pill consumption days, participant did consume the pill. A similar analysis will be conducted based on dosage if more than 10% of participants have a change in their dosage during follow-up. We will also use a validated simplified self-report medication adherence measure with the following six items:

Number of remaining pills (integer).
Ever forgot to take the study medicine (Yes or No).
Are you careless at times about taking the study medicine (Yes or No).

Sometimes if you feel worse, do you stop taking your study medicine (Yes or No).
How often have you not taken the study medicine in the last week (4 options: 0, 1 day, 2 days, 3 days).
Days missed taking pills in the last two weeks (integer).

These items will be summarized with counts and proportions.

Additional Analyses

Additional analyses will include longitudinal models for diagnoses of ID, diagnoses of IDA, haemoglobin levels and ferritin levels. The first two outcomes will be treated as binary variable and the latter two will be continuous variables. These models will use GEE to account for the repeat measurements from subjects. A significance level of 0.05 without multiplicity adjustment will be utilized for model interpretation.

Comparison of the results from the copper sulphate test and haemoglobin levels from the FBC will be examined with logistic regression models. These models will model the probability of the copper sulphate test being positive as a function of haemoglobin levels. Models will be fit separately for men and women. Exploratory analyses will investigate if age or factors relating to diet (as listed below) impact the nature of this relationship. The interaction terms between these factors and haemoglobin levels will be added to the model as explanatory variables and the null hypothesis that the regression coefficient associated with the interaction is zero will be tested. Factors relating to diet (responses are obtained from baseline and follow-up questionnaires) include:

Baseline vegetarian status (No, Strict, or Partial).
Number of meals yesterday (4 options: 0, 1, 2, 3 or more).
Frequency of consuming specific foods and beverages category (5 options: daily, weekly, monthly, rarely, never). The foods and beverages categories include fruits and vegetables, meat, poultry, fish, beans and peas, tea/coffee. Each category will be analyzed separately.

7.2.3 Sensitivity analyses

In the primary analysis, individuals are excluded if they did not receive iron supplementation at enrolment but received it during follow-up due to changes in ferritin or haemoglobin values. Sensitivity analyses will be conducted for the primary outcome by including those requiring iron supplementation during follow-up in the control group.

Since the treatment is not assigned at random there is a strong potential that confounders will introduce bias into the estimates of the treatment effects. As an attempt to remedy this, sensitivity analyses using inverse probability weighting will be conducted for the primary outcome and the secondary outcomes that relate to efficacy. If we use X_i to represent group membership for participant i and Z_i is a vector of predictors for this participant, the first step of the analysis is to estimate the stabilized inverse probability of "treatment" weights W_i for subject i, $W_i = P(X_i = x_i)/P(X_i = x_i|Z_i)$. The predictors Z_i include age, sex, screening haemoglobin concentration, screening plasma ferritin concentration, socio-economic status, medical history, and diet. An estimate of $P(X_i = x_i|Z_{ij})$ can be obtained by logistic regression

and $P(X_i = x_i)$ can be estimated by the sample proportion. One then uses these weights in a regression model to adjust for participant characteristics that differ between the groups.

7.2.4 Subgroup analyses

Subgroup analyses for the primary endpoint and secondary outcomes will be performed to determine whether the intervention differs qualitatively across various baseline-defined subgroups. The following baseline characteristics define those subgroups:

Age treated as categorical with levels 17-20, 21-40 and greater than 40.
Sex.

Subgroup analyses for the primary endpoint will use linear models as used for the primary analysis. Subgroup analyses for the secondary outcomes related to efficacy will use linear models for the first two outcomes (i.e., change in haemoglobin over 4 months and ferritin levels at 4 months), and use logistic regression models for the other three outcomes (i.e., diagnosis of ID or IDA at 4 months, attempt to return for donation within 6 months, and successful donation within 6 months). The explanatory variables will be the subgroup factor and its interaction with the group membership indicator. The null hypothesis that the regression coefficient associated with the interaction is zero will be tested at a significance level of 0.05.

Subgroup analyses will be interpreted with caution since no adjustments for multiple hypothesis testing are planned.

7.2.5 Preventing and accounting for missing data

Missing data will be prevented by several procedures. These start with prioritizing retention through follow-up in the recruitment and consenting procedures, and this will be emphasized during site training and on protocol team calls. During conduct of the trial, visit completion rates and case report form data completeness will be monitored closely. Missing data from the analysis of the primary and secondary endpoints will be handled by multiple imputation via chained equations (MICE), as implemented in SAS PROC MI procedure. This method can handle both binary outcomes and ordinal categorical outcomes. The multiple imputation model will include the demographic characteristics listed in section 3.4. Twenty rounds of imputation will be used.

8 Interim Monitoring Guidelines for the DSMB

The DSMB will periodically review and evaluate the accumulated study data for participant safety, study conduct and progress, and, when appropriate, efficacy. Recommendations to continue, terminate, or modify the study will be made by the DSMB, however there are no formal stopping criteria for this small pilot study. Interim data summaries will be prepared by the DCC statisticians and reviewed at regular intervals by the DSMB (at least twice a year). Regulatory requirements and site-level interim monitoring will be tracked by the DCC. Safety data reports will be generated and reviewed by the DSMB at more frequent intervals if the DSMB requests such reports. Additional data summaries may be requested by the DSMB. The DSMB will make

recommendations concerning continuation, modification, or termination of the study after each meeting.

9 Data Completeness and Study Conduct

Data completeness and study conduct reports will be provided to DSMB by treatment group (for the closed report) and pooled across treatment groups (for the open report).

The following data summaries will be provided to assess data completeness and study conduct:

Number and percent of participants with deviations from protocol, and type of protocol deviation.
Number and percent of participants who consented and were administratively withdrawn. This will be further summarized by withdrawal due to ineligibility (due to laboratory values or expiration of laboratory values) or not.
Number and percent of participants who withdrew consent or were lost to follow-up (i.e., the participant did not return for the 6-month visit and the window for that visit has closed).
If more than 10% of participants are lost to follow-up, the cumulative proportion of participants who are lost to follow-up over time will be estimated by the Kaplan-Meier method. These estimates will be provided by treatment group (closed report only).
Listing of participant identifiers who initiated withdrawal of consent, the dates of enrolment of those participants, study treatment assignment, date of withdrawal, and reason for withdrawal.
The number of each eCRF that has been completed and the expected values for these numbers.
Length of follow-up summarized by median, IQR, and range.
The number and percentage of participants who have completed follow-up.

10 Distribution of Reports

Open report: BLIS leadership team; selected staff from the National Institutes of Health (NIH) and the NHLBI of the United States; and all recipients of the closed report.
Closed report: DSMB members.
The results at the end of the study: NBSG, Ghana Health Service, NIH and NHLBI of the United States, publications in peer-reviewed journals, conference presentations and mass media.

Appendix A. Schedule of Assessments

Assessment	Screening (Day -14 to 0)	Enrolment (Day 0)	Biweekly (Throughout)	Interim Visit 1 (Week 8-10)	Interim Visit 2 (Week 16-18)	Final Visit (Week 24)
NBSG Pre-Donation Assessment	X					X
Informed Consent	X					
Inclusion and Exclusion Criteria	X					
Demographics	X					
Full Blood Count and peripheral blood comment	X			X	X	X
Plasma Ferritin	X			X	X	X
Malaria Parasites Testing (RDT)	X	X		X	X	X
Nutritional Counselling		X		X	X	
Qualitative Assessment						X
Follow-Up Calls			X			
Return Blood Donation Status				X	X	X
Assessment for Adverse Events		X	X	X	X	X
Adherence Assessment (for those receiving supplemental iron)			X	X	X	X
RDT – Rapid Diagnostic Test						

Appendix B. List of Acronyms

AE	Adverse event
BLIS	Bloodsafe Ghana- Iron and Nutritional Counselling Strategy Pilot Study
DCC	Data Coordinating Centre
DSMB	Data and Safety Monitoring Board
eCRF	Electronic Case Report Forms
FBC	Full Blood Count
FGD	Focus group discussion
GEE	Generalized estimating equations
Hb	Haemoglobin
ID	Iron Deficiency
IDA	Iron Deficiency Anaemia
IRB	Institutional Review Board
NBSG	National Blood Service Ghana
NHLBI	National Heart, Lung, and Blood Institute
SAE	Serious adverse event
SAP	Statistical analysis plan
SZBC	Southern Zonal Blood Centre
TTI	Transfusion Transmissible Infection