Donor Iron Deficiency Study

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A randomized trial to determine if RBCs from donors with iron deficient erythropoiesis have decreased post-transfusion RBC recovery and whether iron repletion improves recovery

ClinicalTrials.gov #NCT02889133

Concept Synopsis and Study Schema
Each year, ~5 million regular donors meet almost half the transfusion needs of the United States by voluntarily donating their blood. Despite fulfilling all requirements for blood donation, almost two-thirds of the women and half of the men who are regular blood donors are iron deficient. In order to manage the inventory, red blood cell (RBC) units destined for transfusion can be stored for up to 42 days prior to transfusion based on FDA guidelines. This guideline is based on a post-transfusion recovery study in which one must prove to the FDA that, on average, using healthy volunteers, more than 75% of transfused red cells remain in circulation for 24 hours at outdate. **This research will determine if RBC units from iron-deficient volunteer blood donors fail to meet U.S. FDA standards for 24-hour post-transfusion recovery.** RBCs from iron-deficient donors may be specifically damaged by refrigerated storage. In studies with a mouse model of iron deficiency (with hemoglobin levels similar to those that are acceptable for human blood donation), we found a mean 10.6% further decrease in the 24-hour post-transfusion recovery of refrigerator-stored red cells from iron-deficient animals, as compared to the post-transfusion recovery from iron-replete littermates. A comparable decrement in post-transfusion recovery of refrigerator-stored red cells from iron-deficient human volunteer blood donors would result in many of these units failing to meet established quality standards for clinical use. This study will also test whether giving iron deficient donors their iron back will improve the quality of their red cells during refrigerator storage.

Research Question(s)/Hypothesis(es):

Primary
1. The 24-hour post-transfusion RBC recovery from units obtained from iron-deficient donors will not meet FDA standards for clinical use.

2. The 24-hour post-transfusion RBC recovery from units obtained after intravenous iron repletion will improve significantly and will meet FDA standards for clinical use.

Secondary
1. Iron therapy will improve neurocognition and emotional well-being in donors with iron deficient erythropoiesis.

2. Metabolite levels in the transfusate will be associated with 24-hour post-transfusion RBC recovery and iron status.

Study Schema:
This is a randomized, controlled, double-blind clinical trial. 60 healthy regular donors who meet donation standards, while exhibiting iron-deficient erythropoiesis by laboratory test criteria, will donate a single standard RBC unit that will be leukoreduced and stored under standard conditions for 42 days. The FDA gold standard measure for the quality of RBCs destined for transfusion is the 51Cr-radiolabeled 24-hour RBC recovery study. Thus, a small aliquot of the transfused RBCs will be radiolabeled and injected into the volunteers on day 41 or 42 of storage. RBC recovery will be calculated from samples obtained at 5min, 7.5min, 10min, 12.5min, 15min, 30-minutes, 1-hour and 24 hours after autologous infusion as per the standardized protocol.[4] For secondary outcomes, study surveys and neurocognitive assessments to assess symptoms of anemia and iron deficiency will also be performed prior to donation and prior to infusion.

In a prospective, randomized, double-blind manner, these donors will then receive either intravenous saline or low-molecular weight iron dextran (or ferric carboxymaltose if iron dextran is unavailable due to supply) within 1-day to 4-weeks after the first post-transfusion recovery study. After five months, they will donate a second RBC unit, similarly stored for 42 days, and autologous 51-chromium 24-hour post-transfusion RBC recoveries will again be determined. The primary outcome will be the group mean difference in 24-hour post-transfusion recovery difference from after treatment to baseline, between the groups receiving intravenous saline and iron.

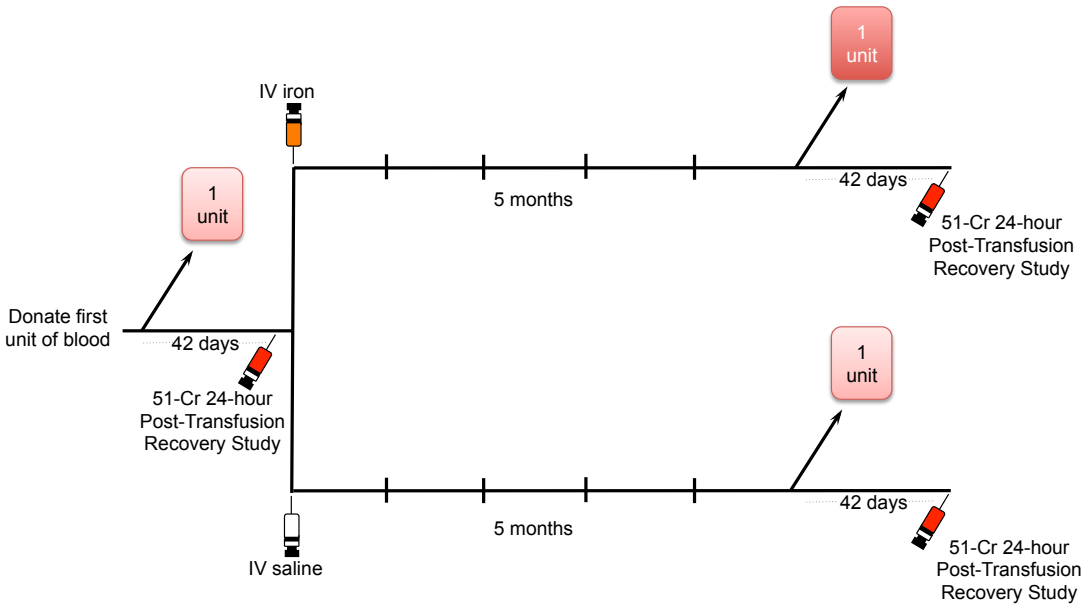

Figure 1. Study Schema. 60 frequent blood donors with iron-deficient erythropoiesis will donate one unit of RBCs and a ^{51}Cr-labeled post-transfusion RBC recovery study will be performed. Each volunteer will then be randomized to placebo (intravenous saline) or iron repletion (intravenous low molecular weight iron-dextran or ferric carboxymaltose) within 4-weeks of completing the post-transfusion RBC recovery study. 5 months ± 4 weeks later (target 5 months) they will donate another RBC unit. 42 days after the second blood donation, another ^{51}Cr-labeled post-transfusion RBC recovery study will be performed and the result compared to the first ^{51}Cr-labeled post-transfusion RBC recovery study.

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1. Background and Significance:

General Introduction. Iron deficiency is common among regular blood donors, but the recovery and quality of RBC units from iron-deficient donors has not been rigorously examined. Evidence from both animal and human studies indicate that when the iron supply for erythropoiesis is inadequate, the RBCs produced have multiple metabolic defects that impair their ability to tolerate refrigerated storage. Our studies in a mouse model demonstrated decreased post-transfusion recovery of refrigerator-stored RBCs obtained from iron-deficient donors. The planned studies will identify human donors at greatest risk of providing RBCs with poor post-transfusion recovery by using a combination of a decreased serum ferritin concentration and increased RBC zinc protoporphyrin, as described below. To evaluate unequivocally the role of iron deficiency in poor post-transfusion RBC recovery, intravenous iron will be used for iron repletion.

Serum ferritin and RBC zinc protoporphyrin detect iron-deficient erythropoiesis in volunteer blood donors. Iron deficiency, a decrease in the amount of body iron, is detected clinically by measuring indicators of iron storage (e.g., serum ferritin), and of iron supply (e.g., RBC zinc protoporphyrin). In the absence of complicating factors, as iron stores decrease, serum ferritin levels decline; a serum ferritin level less than 12 µg/L is virtually diagnostic of the absence of marrow iron stores. RBC zinc protoporphyrin monitors the supply of iron available for RBC production. In the developing RBC, the insertion of iron into protoporphyrin IX is the final step in producing heme for incorporation into hemoglobin. If iron is unavailable, divalent zinc is incorporated instead, producing zinc protoporphyrin, which binds to hemoglobin and persists for the life of the RBC as a biochemical indicator of an inadequate supply of iron for RBC production.[1,3]

Four successive stages of iron deficiency can be distinguished (Fig. 2). **Reduced iron stores (not shown)**: As iron stores decrease, serum ferritin levels decline proportionally. **Iron depletion**: Iron depletion develops when iron stores are absent, but iron delivery to the erythroid marrow for producing hemoglobin and other functional iron compounds is maintained by the combination of iron recycling from senescent RBCs and gastrointestinal iron absorption. With absent iron stores, the serum ferritin falls to <15 µg/L. Because the iron supply for RBC production is maintained, RBC zinc

protoporphyrin levels remain in the reference range. **Iron-deficient erythropoiesis**: With further reductions in body iron, the lack of iron limits production of hemoglobin and other iron-requiring compounds, resulting in iron-deficient erythropoiesis. Nonetheless, the effect on the circulating hemoglobin concentration is insufficient to be detected by the standards used to screen blood donors, which currently only includes a hemoglobin test. As newly formed RBCs replace senescent RBCs, RBC zinc protoporphyrin progressively increases, providing an index reflecting the severity and duration of the inadequate supply of iron for erythropoiesis. Further decreases in the nominal serum ferritin levels have no physiological meaning. Thus, **the combination of a serum ferritin <15 µg/L and an increased RBC zinc protoporphyrin (i.e., >80 µmol/mol heme), is highly specific for iron-deficient erythropoiesis**.[1,3]
Iron-deficiency anemia: Further diminution in body iron produces frank iron-deficiency anemia, which would result in donor deferral.

Iron deficiency is common in volunteer blood donors. In the United States in 2011, of the donors who provided the ~15.7 million units of RBCs that were collected, 69% were repeat donors.[5] In addition, in Canada, ~90% of RBC units collected for transfusion are provided by repeat donors.[6] Although iron deficiency is surprisingly prevalent in first-time donors,[7,8] its prevalence is even higher in the particularly altruistic frequent donors, especially among women of childbearing age.[9,10] In the REDS-II Donor Iron Status Evaluation (RISE) study,[11] up to 49% and 66% of male and female frequent donors, respectively, manifested either iron depletion (i.e., absent iron stores) or iron-deficient erythropoiesis. Similar frequencies of iron deficiency were also reported in Canadian,[6] Austrian,[12] Danish,[13] and Dutch[14] populations.

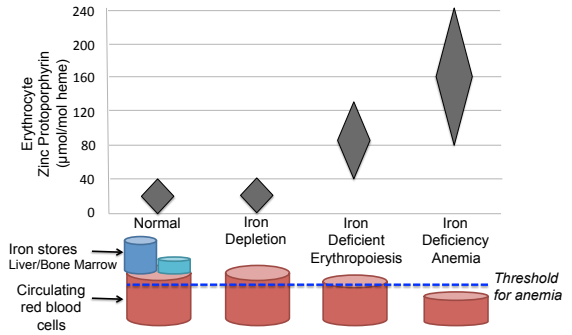

Figure 2. RBC zinc protoporphyrin progressively increases with increasing severity of iron deficiency. Iron deficiency progresses from reduced iron stores to iron depletion, in which there is normal erythropoiesis with normal zinc protoporphyrin levels, but decreased ferritin due to absent iron stores. The next stage is iron-deficient erythropoiesis, in which increased RBC levels of zinc protoporphyrin and very low serum ferritin levels reflect both absent iron stores and the lack of sufficient iron for normal hemoglobin production. As iron deficiency progresses further, frank iron deficiency anemia develops with insufficient iron for maintaining adequate hemoglobin levels. **Our target population is human donors with iron-deficient erythropoiesis**. Adapted from Hastka et al.[1] and Brittenham[3].

RBCs from donors with iron-deficient

erythropoiesis have impaired tolerance for refrigerated storage and decreased post-transfusion RBC recovery. RBCs from individuals with iron deficiency anemia have decreased levels of endogenous antioxidants,[15,16] have evidence of oxidative damage,[17,18] and are more sensitive to oxidative stress[15,18] and low pH;[16] the latter decreases progressively during RBC storage.[19] Furthermore, refrigerated storage induces oxidative stress in donor RBCs and inhibits their oxidative stress defense mechanisms.[17,20-24] Oxidative damage *per se* also impairs RBC deformability[25] and impaired deformability was seen in humans,[18] rats,[18] and rabbits with iron deficiency anemia[26] and in stored RBCs from healthy human donors.[27] Rigid RBCs are less able to pass through previously negotiable microcirculatory beds and are more prone to undergo extravascular hemolysis in the spleen.[28] Indeed, circulatory RBC lifespan is decreased in humans with iron deficiency anemia[16,29-31] and in relevant animal models.[26,32] In humans, decreased circulatory lifespan is most likely due to extravascular hemolysis in the spleen[29-31] and is corrected by iron repletion.[16,30]

Remarkably, in several older studies,[16,29,33] RBCs obtained from donors with iron deficiency anemia were transfused into healthy recipients, without prior refrigerated storage. In each study, the transfused iron-deficient RBCs had a decreased circulatory lifespan/recovery, most likely due to splenic clearance. In addition, when RBCs obtained from healthy donors were transfused into recipients with iron deficiency anemia, the transfused RBCs had a normal lifespan, suggesting that the iron deficiency-induced defect was intrinsic to the RBC and not due to enhanced clearance mechanisms.[33,34]

Iron deficiency decreased the 24-hour post-transfusion RBC recovery in a mouse model. Replicating the human studies described just above from the 1940-70's is no longer ethically permissible. Although the RBCs transfused in these studies were from donors with iron deficiency anemia, not from donors with iron-deficient erythropoiesis, deliberate allogeneic human transfusion studies using such donors and healthy volunteer recipients are no longer feasible. Instead, we used a mouse model to obtain proof-of-principle preliminary data. Three mouse donor cohorts were prepared: iron replete, iron-

deficient erythropoiesis, and iron deficiency anemia (Fig. 3). Similar to our prior studies,[35] refrigerator-stored, transfused RBCs from iron-replete donors had a normal 24-hour post-transfusion recovery (mean 77.1%). In addition, as expected from other publications,[16,29,33] the 24-hour post-transfusion recovery was poor using RBCs from donors with severe iron-deficiency anemia (mean 46.7%; $p<0.001$). In contrast, results using donors with iron-deficient erythropoiesis were subnormal (mean 66.5%; $p<0.05$) and would be less than the FDA-mandated minimum (in this mouse model). Taken together, **these data support our hypothesis that, after refrigerated storage, RBCs from donors with iron-deficient erythropoiesis without anemia are suboptimal**.

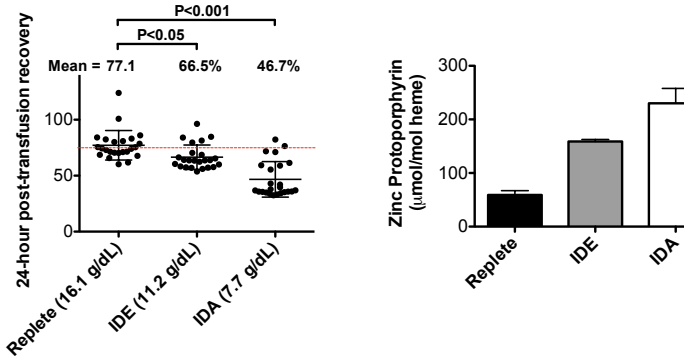

Figure 3. RBCs from iron-deficient donor mice have reduced 24-hour recovery and increased zinc protoporphyrin levels. Weanling male C57BL/6 mice were placed on a defined iron control diet of 45ppm iron (TD.110593, Harlan Laboratories) or an iron-deficient diet of 0-4ppm iron (TD.110592). A third group was fed the iron-deficient diet and was also phlebotomized weekly (50-100 µL per bleed) to generate a total of three groups of mice with varying iron status: (1) iron replete, (2) iron-deficient erythropoiesis (**IDE**) with mild iron deficiency, and (3) severe iron deficiency anemia (**IDA**). At ~10 weeks of age, blood was collected from each cohort, leukoreduced, packed, and refrigerator stored in CPDA-1. After 12 days of storage, 24-hour post-transfusion recovery was determined in recipient transgenic mice expressing enhanced Green Fluorescent Protein under the direction of the human ubiqutin C promoter using flow cytometric detection of transfused RBCs (left panel). The experiment was repeated three times in its entirety (N=24 per group total). Mean hemoglobin levels prior to blood donation are in parenthesis. The dotted red line represents 75% RBC recovery. Statistical significance determined by one-way ANOVA with Dunnett's

2. Objectives

2.1 Primary Hypothesis

1) The 24-hour post-transfusion RBC recovery of units obtained from donors exhibiting iron-deficient erythropoiesis will not meet FDA standards for clinical use.

2) The 24-hour post-transfusion RBC recovery of units obtained after intravenous iron repletion will improve significantly and will meet FDA standards for clinical use.

2.2 Secondary Hypothesis

1) The extent of iron-deficient erythropoiesis, as measured by RBC zinc protoporphyrin levels, will predict the 24-hour post-transfusion RBC recovery.

2) There will be a good correlation (R^2>0.5) between RBC zinc protoporphyrin levels and other markers of iron status: ferritin, hepcidin, soluble transferrin receptor, reticulocyte hemoglobin.

3) Iron repletion will improve fatigue score[36] and self-reported health and wellbeing score.[37]

4) Iron repletion will decrease RBC zinc protoporphyrin and soluble transferrin receptor levels.

5) Iron repletion will increase hepcidin, ferritin, and hemoglobin levels.

6) Iron repletion will improve the following neurocognitive scores:

a) Cognitive Function Composite Score
b) Executive Function
c) Attention
d) Episodic Memory
e) Language
f) Processing Speed
g) Working Memory

7) Iron repletion will improve the following emotional well-being scores:

a) quality of life
b) fatigue
c) depression
d) anxiety

8) Metabolite levels in the RBC unit will correlate with iron status and 24-hour post-transfusion RBC recovery.

3. Study Population

3.1 Enrollment Inclusion Criteria:

1) 18-75 years old;

2) healthy (by self report);

3) body weight >110 lbs;
4) female hematocrit >38%, male hematocrit >39%;
5) frequent blood donor (men ≥2 and female ≥1 RBC unit donations in past year);
6) ferritin <15 ng/mL;
7) zinc protoporphyrin >60 μmol/mol heme.

3.2 Enrollment Exclusion Criteria:

1) ineligible for donation based on the New York Blood Center donor autologous questionnaire;
2) C-reactive protein >10 mg/L;
3) sickle cell trait (by self report);
4) systolic blood pressure >180 or <90 mm Hg, diastolic blood pressure >100 or <50 mm Hg;
5) heart rate <50 or >100;
6) temperature >99.5°F prior to donation (attempts will be made to reschedule donation if possible);
7) temperature >100.4°F or subjective feeling of illness prior to ^{51}Cr 24-hour RBC recovery study (to avoid the subject having a concurrent illness that may affect post-transfusion recovery); (attempts will be made to reschedule donation and ^{51}Cr 24-hour RBC recovery study);
8) positive results on standard blood donor infectious disease testing;
9) pregnancy;
10) taking, or planning to take, iron supplements and not willing to stop for duration of study;
11) history of severe asthma requiring hospitalization, allergic eczema (atopic dermatitis), or other atopic allergy causing anaphylaxis.

4. Trial Enrollment

4.1 Screening/Recruitment

The New York Blood Center study staff will send a recruitment letter/email (see Appendix 1) to potential subjects who are 18-75 years old and frequent blood donors donating in the Manhattan catchment area. We will define frequent blood donors as (1): men who have donated the equivalent of at least 2, and women who have donated the equivalent of at least 1, RBC units in the past year.

Volunteers responding to the recruitment email will be screened for participation in the study by phone or email (see Appendix 7 for all screening forms) and then invited for a

screening meeting (P&S 14-434) to confirm eligibility and provide informed consent.

Screening day (P&S 14-434): All volunteers will be given the health questionnaire they would receive upon donation at the New York Blood Center (see Appendix 7). All volunteers who decide to proceed with screening and sign consent will have four tubes of blood drawn to determine baseline laboratory values and to perform a blood type and antibody screen. If the baseline hematocrit is >38% (female) or >39% (male), ferritin < 15ng/mL, zinc protoporphyrin level is > 60 umol/mol heme, and C-reactive protein is <10mg/L (see inclusion/exclusion criteria above) then the volunteer will be scheduled for an autologous whole blood donation at the New York Blood Center, and scheduled for an autologous 51-Chromium red cell recovery study 6 weeks after donation. At that time, neurocognitive assessments and emotional wellbeing will be performed as well (see Appendix 2-5 for surveys and section 5.4). Re-screening: Those subjects who meet the ferritin <15ng/mL and the zinc protoporphyrin > 60umol/mol heme criteria, but do not qualify due to a low hematocrit may be re-screened between 2 weeks to 3 months later.

4.2 Randomization

Randomization will be performed using a computerized system with equal allocation (1:1) to iron repletion or placebo. Randomization will be stratified by gender; randomly permuted block sizes of 4, 6, or 8 will be used. The moment of randomization will be recorded and will occur only after successful completion of the first post-transfusion RBC recovery study (i.e., between Day #43-71; target is the day after the post-transfusion recovery study; performed on Harkness Pavillion 10). Volunteers will be admitted to the Columbia General Clinical Research Center (GCRC) (supported by the Columbia Clinical and Translational Science Award (CTSA)). One peripheral intravenous line will be inserted and blood (2 tubes, 10mL) will be drawn for determining the 24-hour post-transfusion recovery. The subject will be randomized at this point. The randomization scheme will be created by a statistician unaffiliated with the study team and provided to the research pharmacy and DSMB prior to study initiation. The CUMC research pharmacy will be responsible for randomizing each subject to the appropriate treatment arm and will prepare either the iron treatment or saline placebo and provide it to the study team in a blinded

fashion. If a subject arrives on the day of randomization and has a fever (T>100.4F) or feels ill, then the 24-hour post-transfusion recovery sample will be drawn, but randomization may be held until the subject feels better and reschedules.

4.3 Blinding

The randomized group will only be known to the Columbia University Medical Center Research Pharmacy and the statistician who generated the randomization scheme. The study will be double-blind. As described in section 5.3, both a test dose of the iron treatment/placebo and the treatment/placebo will be blinded. The test dose will be administered prior to giving the full dose to ensure safety of treatment. A research pharmacist will provide the placebo (IV saline) or treatment (IV low molecular weight iron-dextran or ferric carboxymaltose) and test dose of iron/placebo in a tinted infusion bag and tubing specifically designed to maintain blinding in clinical research studies (Medipak). A research nurse unaffiliated with the study team, from the Outpatient or Inpatient Center at the Columbia General Clinical Research Center (Harkness Pavillion 10th Floor), will be responsible for the test infusion and total dose iron infusion. With this design, volunteers and study investigators will be blinded to whether volunteers receive the active intervention or placebo. Subjects in both groups will receive similar discharge instructions as if they had received low molecular weight iron-dextran (see Appendix 6 for discharge instructions). Only the research pharmacist and the statistician on the DSMB will not be blinded. Scheduling and logistic communications with volunteers will be made by the study coordinator, who will also be blinded to the treatment group. The research pharmacy will provide a standard 1-gram dose of low molecular weight iron-dextran, ferric carboxymaltose, or saline in special bags designed to conceal group assignment.

Situations may arise in which breaking the blind earlier would be in the best interest of the volunteer. In any situation in which a physician or the subject asks to be un-blinded to study treatment, a sealed copy of the randomization scheme for each subject will be kept in the investigator's office and will be broken upon request. Furthermore, the research pharmacy can be reached on an emergency basis to provide this information. Finally, any un-blinding that occurs will be reported to the DSMB and ultimately reported in the resulting publication.

5. Interventions

5.1 Blood Donation:

The blood donation will be performed at the New York Blood Center at 310 East 67th Street, New York, NY per standard protocol for autologous RBC donations. A urine pregnancy test will be performed on all female participants < age 55 on the day of donation. A positive pregnancy test will result in exclusion from the study (see Appendix 7 for donation day eligibility form). At the New York Blood Center, subjects will be screened per the standard protocol for autologous RBC donations. This will include health questionnaire, finger stick hemoglobin determination, temperature, heart rate and blood pressure measurement. Following donation, the blood will be leukoreduced, packed, and stored in AS-3 solution. The unit will be transferred to the CUMC-NYPH blood bank for storage following standard hospital procedures.

5.2 ^{51}Chromium 24-hour post-transfusion RBC recovery study

Volunteers will be admitted to the Columbia General Clinical Research Center (GCRC) (supported by the Columbia Clinical and Translational Science Award (CTSA) on Harkness Pavillion 10). A urine pregnancy test will be performed on all female participants < age 55 years on the day of infusion. A positive pregnancy test will result in exclusion from the study (see Appendix 7 for infusion eligibility form). Two intravenous lines will be placed in contralateral arms. Four tubes of blood will be drawn to determine laboratory values and to perform a repeat blood type and antibody screen. If the subject chooses to come in for an optional blood draw within 30 days of this study visit, only three tubes will be drawn as the type and screen will not be necessary.

A 30 mL aliquot of the autologous blood unit donated 6 weeks prior will be removed using sterile technique by a licensed radiopharmacist into a syringe. This syringe will be labeled with the volunteer's identifying information per hospital policy and radiolabeled in the Kreitchman PET Center. The radiolabeling will be performed using 20 microcurie of sodium chromate (^{51}Cr) based on the methods of Moroff et al[4] and the recommendations from the International Committee for Standardization in Hematology.[38] The ^{51}Cr labeled red cells will be washed with saline and then infused intravenously

(over 1 minute through one IV line) into the volunteer. For calculating counts per minute per ml of RBC at time zero (T0), samples (two tubes, 10 mL) from the contralateral arm will also be obtained after 5, 7.5, 10, 12.5, 15 minutes, 30 minutes, and 1-hour after infusion. Thus, in total, the volunteers will be infused approximately 30mL of packed radiolabeled RBCs and will have 16 tubes or 80 mL of blood drawn overall (8 time points x 2 tubes x 5mL per tube = 80 mL). All blood draws will be performed from the peripheral intravenous line to avoid multiple needle sticks. Finally, because 30mL of blood will be infused and only 80mL of blood removed, this will leave the volunteer with a net loss of only 50mL of blood on the day of infusion. Following the 1-hour timed blood draw, the subject will be discharged.

5.3 Low Molecular Weight Iron Dextran (LMWID) or Ferric Carboxymaltose (INJECTAFER) Infusion

Volunteers will be admitted to the Columbia General Clinical Research Center (GCRC) (supported by the Columbia Clinical and Translational Science Award (CTSA) on Harkness Pavillion 10). One peripheral intravenous line will be inserted and blood (2 tubes, 10mL) will be drawn for determining the 24-hour post-transfusion recovery. The allowable time frame for this blood draw will be from 20-28 hours of infusion the prior day. The subject will be randomized from this point to 4-weeks later by the research pharmacy. A research pharmacist will provide the placebo (IV saline) or treatment (IV low molecular weight iron-dextran or ferric carboxymaltose) and test dose (25 mg LMWID, 12.5 mL of a 2 mg/mL solution of iron dextran or 0.5 mL of 50 mg/mL solution of ferric carboxymaltose, diluted in normal saline) in tinted infusion bags and tubing specifically designed to maintain blinding in clinical research studies (Medipak). If low molecular weight iron dextran is unavailable due to shortages, the research pharmacist will provide ferric carboxymaltose instead. A research nurse unaffiliated with the study team, from the Outpatient or Inpatient Center at the Columbia General Clinical Research Center (Harkness Pavillion 10th Floor), will be responsible for the test infusion and total dose iron infusion. The research pharmacy will provide a standard 1-gram dose of low molecular weight iron-dextran, ferric carboxymaltose, or saline in special bags designed to conceal group assignment. In addition, the clinical pharmacy will provide Solumedrol 125

mg IV both before and after infusion and Tylenol 650mg PO and Benadryl 25mg PO before the infusion. The Solumedrol has been shown to reduce the risk of myalgias and arthralgias after LMWID infusions.[39] Premedication with Tylenol and Benadryl have also been shown to be effective in reducing adverse events,[40] and this is the current standard of care at Columbia University Irving Medical Center for adults receiving LMWID. After the intravenous test dose (25 mg of LMWID or ferric carboxymaltose, 12.5 mL of the complete dose, infused over 20 minutes), patients will be observed for any side effects for 40 minutes (one hour from start of infusion); if no adverse effects are seen, then the entire dose diluted in 500 mL normal saline (i.e., 2 mg/mL of LMWID) will be infused over a period of 2 to 6 hours as tolerated (target 2 hours). If ferric carboxymaltose is used, the same test dose and the full 1 gram dose will be administered in 250 mL of normal saline over the same time frame. The same premedication will be provided to minimize differences in treatment effects. Adverse events will be identified by observation, direct inquiry, and physical examination of each volunteer. Vital signs will be measured before, during (after 15 minutes and then hourly), and after each infusion. Resuscitation equipment and personnel trained in the detection and treatment of anaphylactic-type reactions will be readily available during drug administration.

Recent studies support the convenience, safety, and efficacy of a single infusion of 1g of LMWID as therapy for iron deficiency in adults.[41] The calculation for iron repletion using LMWID (from INFeD package insert) in adults is as follows:
Dose (mL) = 0.0442 (Desired Hb - Observed Hb) x LBW + (0.26 x LBW)

Based on:
Desired Hb = the target Hb in g/dl.
Observed Hb = the patient's current hemoglobin in g/dl.

LBW = Lean body weight in kg. A patient's lean body weight (or actual body weight if less than lean body weight) should be utilized when determining dosage.

For males: LBW = 50 kg + 2.3 kg for each inch of patient's height over 5 feet

For females: LBW = 45.5 kg + 2.3 kg for each inch of patient's height over 5 feet
To calculate a patient's weight in kg when lbs are known: patient's weight in pounds = weight in kilograms/2.2

Using this formula, the minimum dose that would be required to raise the hemoglobin by 2 g/dL would be ~800mg (minimum donation requirement is 110lbs = 50 kg) in a female and almost 900mg in a male. Thus, given that all subjects will be healthy volunteers with ferritin < 15 ng/mL with evidence of iron-deficient erythropoiesis, and will be dosed following a whole blood donation for study purposes, 1 gram of LMWID was chosen for simplicity, safety, consistency, and design considerations. Based on similar considerations, 1 gram of ferric carboxymaltose would provide equivalent total dose iron repletion with a similar safety profile.[42]

5.4 Cognitive and Emotional Status Assessment

As outlined in the *Schedule of Measurements* section below, cognitive and emotional functioning will be assessed with a thorough battery of neuropsychological tests and questionnaires assessing overall wellbeing. Tests will be administered in person by a psychometrician who has received training on test administration.

Neuropsychological tests will be administered to subjects on an iPad or Tablet. Subjects will be supervised at all times during this portion of the evaluation and will be encouraged to complete the tests within one session (i.e. without interruption). Total time for test administration is approximately 30 minutes and the time of start and completion of the test battery will be recorded. When the neuropsychological battery has been completed, the subject will be provided with a clipboard containing printed copies of four questionnaires (see appendices 4-7). The subject will be asked to complete the surveys on his/her own without interruption. The time of start and completion of the surveys will be recorded.

Neuropsychological tests included were selected based on the following criteria: All (1) meet high psychometric standards, (i.e. are reliable, valid, and well standardized); (2) have appropriate normative data; (3) maintain the lowest potential

for patient burden, e.g. duration; (4) can be administered serially without significant practice effects.

5.4.1 Cognition

Subjects will be administered subtests from the NIH Toolbox Cognition Battery (NIHTB-CB), a computerized battery of neuropsychological tests. The NIH Toolbox provides a standard set of royalty-free, comprehensive assessment tools that can be used by researchers and clinicians in a variety of settings, with a particular emphasis on measuring outcomes in longitudinal epidemiologic studies and prevention or intervention trials. The battery has been normed and validated across the lifespan in subjects age 3-85 and its use ensures that assessment methods and results can be used for comparisons across existing and future studies. By providing a "common currency" for the study of neurological research, the NIH Toolbox enables economies of scale and enhances efficiency. The NIH Toolbox is capable of monitoring neurological and behavioral function over time, and measuring key constructs across developmental stages.

Scores from the NIH Toolbox Cognition Battery will produce a Cognitive Function Composite Score. Individual measure scores reflecting Executive Function, Attention, Episodic Memory, Language, Vocabulary, Processing Speed and Working Memory will also be obtained.

NIH Toolbox Cognition Battery (NIHTB-CB)

Vocabulary

NIH Toolbox Picture Vocabulary Test

Memory

NIH Toolbox Auditory Verbal Learning Test (Rey)
NIH Toolbox Picture Sequence Memory Test

Attention/Executive Functioning

NIH Toolbox Flanker Inhibitory Control and Attention Test

NIH Toolbox Dimensional Change Card Sort Test (DCCS)

Processing Speed
NIH Toolbox Pattern Comparison Processing Speed Test

Working Memory
NIH Toolbox List Sorting Working Memory Test

5.4.2 Emotion

Subjects will complete four paper and pencil self-administered questionnaires to evaluate quality of life, fatigue, depression, and anxiety.

Short Form 36 Health Survey (SF-36)
36-item, patient-reported survey assessing overall health status and quality of life (Appendix 4).[43]

Multidimensional Assessment of Fatigue (MAS)
16 item scale used to measure fatigue according to four dimensions: degree/severity, distress that it causes, timing of fatigue, and its impact on ADLs (Appendix 5).[44]

Beck Depression Inventory-II (BDI-II)
21 item self-report multiple choice inventory used to assess for depression (Appendix 6).[45]

Beck Anxiety Inventory (BAI)
21-question multiple-choice self-report inventory that is used for measuring how the subject has been feeling in the last week, focusing primarily on somatic symptoms (Appendix 7).[46]

5.4.3 Hearing

Words-In-Noise (WIN) Test
Measures a person's ability to recognize single words amid varying levels of background noise, measuring difficulty a person might have hearing in a noisy environment. A recorded voice tells the participant to listen and repeat words. Background noise gets louder, reducing the signal-to-noise ratio.
Hearing Handicap Inventory Screening
10 questions to assess hearing impairment.

5.4.4 Restless Leg Syndrome (RLS)

Restless Leg Syndrome Rating Scale
10 questions to assess restless leg symptoms.

6. Schedule of Measurements

6.1 Summary Timeline

The schedule of measurements is summarized in **Table 1**. Following successful screening and signed informed consent, subjects will donate a unit of autologous red blood cells (Donation1). This will be followed 40-42 days later by the baseline 51-Chromium post-transfusion recovery study. Following the 24-hour post-chromium infusion blood draw, the subjects will be randomized to iron infusion or placebo (Iron infusion1). Five months ± 4 weeks later, subjects will donate the second unit of autologous blood (Donation2). The second 51-Chromium post-transfusion recovery study will be performed 40-42 days after this donation. Subjects will have to return 24-hours after chromium infusion to determine the 24-hour post-transfusion recovery. Surveys and neurocognitive assessments will be conducted prior to Donation1, PTR1, Donation2, and PTR2.

Because an active type and screen must be obtained before the blood bank issues the stored RBC unit and the radioactive 51-Cr-labeling can occur, we will provide subjects with the option of coming in up to 30 days prior to the scheduled PTR study to obtain a type and screen sample (1 tube of blood by standard venipuncture). This will provide for a shorter day for the PTR study because there will no longer be the need to wait for the type and screen testing and the study can begin once the subject arrives. If a subject chooses not to provide this optional blood draw, a blood sample will be taken when the IV lines are placed for the PTR study and the PTR study will proceed after the blood bank issues the blood and it is radiolabeled per hospital guidelines.

Table 1: Schedule of Measurements

Day of study	Urine pregnancy test	CBC with reticulocyte count	Iron parameters*	C-reactive protein	Type and Screen	51-Cr PTR**	Iron/Placebo infusion	Surveys/ Neuro-cognitive	Metabolomics****
Screening		X	X	X	X				
Donation1	X	X	X	X				X	X
Blood draw (optional)***					X				
PTR1 (40-42 days after Donation1)	X	X	X	X	X	X		X	
Iron infusion1 (1 day after PTR1)	X						X		
Donation2 (5months ± 4 weeks after iron infusion1)	X	X	X	X				X	X
Blood Draw (optional)***					X				
PTR2 (40-42 days after Donation2)	X	X	X	X	X	X		X	
Final blood draw (1 day after PTR2)						X – collection of 24 hour (+/- 4hr) time point for final measurement			

*Iron parameters = Zinc Protoporphyrin (ZPP), iron, total iron binding capacity (TIBC), transferrin saturation, ferritin, soluble transferrin receptor, hepcidin.

**PTR = post-transfusion recovery study with associated blood draws for hemoglobin and Chromium-51 determination taken pre-infusion and 5, 7.5, 10, 12.5, 15, 30, 60 minutes and 24-hours post-infusion (+/- 4 hours).

***The option to come in up to 30 days prior to the PTR study will be provided to obtain a type and screen sample. This will provide an active type and screen specimen so that the PTR study can begin without delay. Alternatively, the type and screen blood sample will be drawn when the IV is paced for the PTR study and then the study will begin once the type and screen is tested and the blood is issued and labeled.

****Metabolomics will be performed by mass spectrometry on blood obtained from the unit at the time of radiolabeling by metabolomics facilities at BloodWorks NW and University of Colorado.

6.2 Assessment Procedures

6.2.1 Zinc Protoporphyrin, Serum Iron, Total Iron Binding Capacity, Transferrin Saturation, Ferritin, C-Reactive Protein, Soluble Transferrin Receptor

Samples for zinc protoporphyrin and soluble transferrin receptor will be sent to ARUP Laboratories for testing. The other parameters will be measured in the Columbia University Medical Center Automated Core Laboratory using clinically-validated instruments.

6.2.2 Hepcidin

This will be measured using an ELISA kit (Bachem) following the manufacturer's instructions.

6.2.3 Complete Blood Counts

Blood counts will be performed in the Columbia University Medical Center Automated Core Laboratory using clinically-validated instruments.

6.2.4 Urine Pregnancy Test

Point of care urine pregnancy testing will be performed using QuickVue One-Step hCG Urine tests (Quidel Corp) following the manufacturer's instructions.

6.2.5 Type and Screen Testing

All type and screen testing will be performed by licensed medical technologists in the Columbia University Medical Center – New York Presbyterian Hospital blood bank following standard procedures.

6.2.6 Metabolomics Testing

Comprehensive metabolomics testing will be performed for research use only at the metabolomics core facilities at the University of Denver and Bloodworks NW using coded specimens. CUIMC investigators will not share identifying information with the collaborating investigators.

7. Specimen Collection Procedures

All blood samples will be obtained by peripheral venipuncture or from an indwelling peripheral intravenous line following institutional protocols.

8. Compensation

Subjects will be provided monetary compensation in cash for participation per visit. $20 will be provided for the screening. $10 will be provided for re-screening for those who meet criteria to be invited to be screened again. $80 will be provided for each donation and PTR study ($320 total). $80 will be provided for the iron/placebo infusion and for the final blood draw at the end of the study ($160 total). Finally, $40 will be provided for each of the optional blood draws. Thus, the maximum total compensation for subjects is $590 and the minimum compensation is $500 for completing the study. Subjects who dropout of the study prior to completion will keep any compensation received up to the dropout.

9. Adverse Event Criteria and Reporting

9.1 Definitions

Adverse Event (AE) – Any untoward or unfavorable medical occurrence in a human subject, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in the research, whether or not considered related to the subject's participation in the research.

Grades were developed using the following guidelines:
Grade 1: Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.

Grade 2: Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL.

Grade 3: Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self care ADL.

Grade 4: Life-threatening consequences; urgent intervention indicated.

Grade 5: Death related to AE.

Serious Adverse Event (SAE) – Any adverse event temporally associated with the subject's participation in

research that meets any of the following criteria: results in death; is life-threatening (places the subject at immediate risk of death from the event as it occurs); requires inpatient hospitalization or prolongation of existing hospitalization; results in a persistent or significant disability/incapacity; results in a congenital anomaly/birth defect; or any other adverse event that, based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the other outcomes listed in this definition.

Note that seriousness and severity are separate concepts. The term "severe" refers to the intensity of a specific event; a severe event may be of minor medical significance (e.g., a severe leg cramp). The term "serious" is based on outcome or action criteria that are usually associated with events that pose a threat to the patient's life or functioning. An event that is mild in severity is serious if it leads to one of the outcomes defined above.

Grade 4 and 5 events will always be considered Serious Adverse Events. Many Grade 3 events and some Grade 1 and 2 events may meet the definition of a Serious Adverse Event.

Unexpected Adverse Event – Any adverse event occurring in one or more subjects in a research protocol, the nature, severity, or frequency of which is not consistent with either: the known or foreseeable risk of adverse events associated with the procedures involved in the research that are described in (a) the protocol–related documents, such as the IRB-approved research protocol or the current IRB-approved informed consent document, and (b) other relevant sources of information, such as product labeling and package inserts; or the expected natural progression of any underlying disease, disorder, or condition of the subject(s) experiencing the adverse event and the subject's predisposing risk factor profile for the adverse event.

Unanticipated problem involving risks to subjects or others (UP): Any incident, experience, or outcome that meets all of the following criteria: unexpected (in terms of nature,

severity, or frequency) given (a) the research procedures that are described in the protocol-related

Attribution – the determination of whether an adverse event is related to a medical treatment procedure.
Attribution categories:

1. Not Related - Event clearly related to other factors (e.g., clinical state, other therapies; concomitant drugs)
2. Possibly Related - Sequence of event is compatible with study drug, device, or procedure, but could have been produced by other factors
3. Probably Related - Sequence of event is compatible with study drug, device, or procedure and cannot be explained by other factors without much doubt
4. Definitely Related - Sequence of event is compatible with study drug, device, or procedure and beyond doubt cannot be explained by other factors

9.2 Types of Adverse Events Reported

Strict definitions and monitoring protocols of adverse effects (AEs) and serious AEs (SAEs) will be developed with the Data Safety Monitoring Board (DSMB). An SAE defined as being one or more of the following: (i) life threatening, (ii) results in hospitalization, or (iii) causes irreversible, persistent, or significant disability/incapacity; these will be reported to regulatory agencies within 7 days of their occurrence. Any AE or SAE temporally related with study procedures will be reported by the site investigators or coordinators. An alert will be sent to the DSMB, and the Institutional Review Board and Sponsor (NIH-NHLBI). Safety outcomes will be assessed at each study visit and with a follow-up phone call the day after each visit using a checklist of known adverse events and an open-ended question for volunteers to describe other adverse events. Participants will be encouraged to call the coordinator throughout the study if they experience 1) a new symptom, 2) change in severity of an existing symptom, 3) see a doctor/ER, 4) start a new medication. The most common serious acute adverse reaction of blood donation and insertions of intravenous lines is a vasovagal reaction, which may lead to loss of consciousness. Witnessed or un-witnessed vasovagal reactions will be considered as AEs, unless resulting in hospitalization (e.g., due to loss of consciousness causing

head trauma), in which case the event will be considered an SAE. Permanent or persistent peripheral arm nerve damage (in a phlebotomized arm) causing disability will be considered an SAE. Bruising and pain at the site of needle insertion that results in volunteer complaint at the follow-up phone call a day after phlebotomy will be considered an AE. Anaphylactoid reactions to iron infusions are usually evident within a few minutes, and close observation is necessary to ensure recognition. If at any time during the intravenous administration, any signs of a hypersensitivity reaction or intolerance are detected, administration will be stopped immediately. The reaction will be considered an SAE if it meets one of the criteria listed above. Furthermore, subjects will be called one day and one week after infusion to inquire about delayed side effects, such as myalgias, arthralgias, and gastrointestinal problems. Finally, iron deficiency is associated with fatigue, restless leg syndrome, decreased physical endurance and work capacity, and impaired concentration, attention, and other cognitive functions. Thus, subjects randomized to the placebo group may have AEs due to continued iron deficiency. These will be assessed using the validated Multidimensional Assessment of Fatigue, Beck Depression Inventory-II, Beck Anxiety Inventory, and the overall well-being SF-36 Health Survey incorporating both physical and mental summary measures. Each subject will take these short surveys as outlined in Table 1.

Medical follow-up of any Serious Adverse Event will be provided by the medical staff of Columbia University Medical Center in the New York Presbyterian Hospital or, if needed, in the out-patient clinic at appropriate intervals until resolution of the condition related to the Serious Adverse Event. An Adverse Event that does not meet the criteria of a Serious Adverse Event will be reported promptly to Drs. Spitalnik and Hod, appropriate management will be provided by the medical staff at Columbia University Medical Center, and the occurrence of any adverse events reviewed periodically by Dr. Spitalnik, Dr. Hod, and their Co-Investigators. A Data Safety Monitoring Board will be established and will review all reported events at least annually and all serious adverse events within one month of occurrence. A report summarizing the Data Safety Monitoring Board meeting and

recommendations will be submitted to the Institutional Review Board.

9.3 Reporting timelines

Table 2 below details the SAE and UP safety reporting requirements and timelines. Reporting timelines for all non-serious AEs will follow the Data and Safety Monitoring Plan for the study.

Table 2: SAE and UP event reporting timelines

What Event is Reported	When is Event Reported	By Whom is Event Reported	To Whom is Event Reported
Fatal or life-threatening unexpected, suspected serious adverse reactions	Within **7 calendar days** of initial receipt of information	Investigator	Local/internal IRBs and Data Safety Monitoring Board (DSMB)
		Sponsor	NHLBI
Non-fatal, non-life-threatening unexpected, suspected serious adverse reactions	Within **15 calendar days** of initial receipt of information	Investigator	Local/internal IRBs and Data Safety Monitoring Board (DSMB)
		Sponsor	NHLBI
Unanticipated adverse effects	Within **10** working days of investigator first learning of effect	Investigator	Local/internal IRBs and Data Safety Monitoring Board (DSMB)
		Sponsor	Local/internal IRBs and Data Safety Monitoring Board (DSMB)
Unanticipated Problem that is not an SAE	Within **14 days** of the investigator becoming aware of the problem	Investigator	Local/internal IRBs/Institutional Officials, NHLBI and/or DCC
All Unanticipated Problems[1]	Within **30 days** of the IRB's receipt of the report of the UP from the investigator.	IRB	OHRP
		Investigator	External IRBs

[1.] Per OHRP guidance: only when a particular AE or series of AEs is determined to meet the criteria for an UP should a report of the AE(s) be submitted to the IRB at each institution under the HHS regulations at 45 CFR part 46. Typically, such reports to the IRBs are submitted by investigators.

10. Interim Reporting

This section describes scheduled reports that will be sent to the DSMB. Reporting requirements for events that will be monitored continuously (i.e. all fatal events and all serious adverse events possibly, probably, or definitely related to red cell transfusion) are described above.

10.1 Semi-Annual DSMB Reports

The DSMB will meet twice a year, either in-person or via teleconference. Reports will include:

- Baseline characteristics overall and by group
- Primary study endpoint overall and by group, (p-values to compare to early stopping boundary will only be provided after 20, 40, and 60 volunteers have been completed)
- Number, type, and severity of serious adverse events, overall and by treatment arm, with p-value for comparison of number of serious adverse events per group. This p-value will be compared to the p-value boundary from an alpha-spending approach approximating O'Brien-Fleming boundaries. No formal stopping rule is set for this comparison.
- Number, type, and severity of adverse events attributed as possibly, probably, definitely related to donation, iron or red cell infusion, overall and by treatment arm, with p-value for comparison of number of such adverse events per group. This p-value will be compared to the p-value boundary from an alpha-spending approach approximating O'Brien-Fleming boundaries. No formal stopping rule is set for this comparison.
- Proportion of subjects in each treatment arm with at least one serious adverse event possibly, probably, or definitely related to a donation, red cell or iron infusion, with p-value. This p-value will be compared to the p-value boundary from an alpha-spending approach approximating O'Brien-Fleming boundaries. No formal stopping rule is set for this comparison.
- Unexpected adverse events, and unanticipated problems overall and by treatment arm
- Site status

- Accrual
- Site/study compliance issues
- Data entry completeness and data QA

11. Statistical Considerations

11.1 Analysis Plan

The primary null hypothesis will be tested in an intent-to-treat analysis using a t-test, or nonparametric equivalent, of the between-group difference in means of the within-subject change in the post-transfusion RBC recovery from the initial study (under iron-deficient erythropoiesis conditions) and the subsequent study performed after randomization to iron repletion or placebo. A similar approach will be used to test the secondary objectives (i.e., the between-group difference in means of the within-subject change in fatigue score,[36] self-reported health and wellbeing score,[37] zinc protoporphyrin, hepcidin, soluble transferrin receptor, serum ferritin and hemoglobin concentration).

Furthermore, because we expect variable responses to iron repletion in the experimental group, and some crossover in the placebo group, we will also perform a secondary analysis to explore the effect of iron status on post-transfusion RBC recovery. We will use multiple regression to assess whether RBC zinc protoporphyrin level increases the R^2 of a model of post-transfusion recovery predicted by treatment group membership. We will examine pre-specified demographic variables (gender [male/female], race [white/not white], age [<50/≥50 years]) to see if we can identify one or more that may be effect modifiers. These factors will be considered for inclusion in an adjusted model. The specific criteria for inclusion are: (i) difference by treatment group significant at $\alpha = 0.10$ two-sided, and (ii) related to outcome at level $\alpha = 0.10$ two-sided. If any of the pre-specified covariates meet the criteria for inclusion, they will be incorporated in an adjusted model, and that model will become the primary analysis. Otherwise, the simple model will be primary. With 60 subjects, we will have 80% power to detect a partial correlation coefficient increase of 0.33 for the unique contribution of RBC zinc protoporphyrin level.[47]

11.2 Sample Size Estimate

Based on preliminary data from our prior ^{51}Cr RBC recovery studies (IRB protocol #AAAI-0835), the standard deviation of the measure in our single site is 5.0%. Furthermore, the expected mean difference in post-transfusion RBC recovery

between iron replete mice and mice with iron-deficient erythropoiesis is 10.6%. If the difference were this large in humans, we would require <6 subjects (alpha = 0.05, two-sided, power = 0.80). However, we expect the difference to be less conspicuous in humans than in inbred mice. Thus, we power the study to detect a clinically relevant difference in post-transfusion recovery of 4%. Under this assumption, the calculated sample size required for each arm is 26 (alpha = 0.05, two-sided, power = 0.80). Furthermore, to allow for up to a 15% dropout rate (i.e., 4/26 subjects), we plan to randomize 30 subjects per arm for a total sample size of 60 subjects. Of note, randomization will occur after successful completion of the first post-transfusion recovery study; thus, we predict up to 70 individuals will need to be recruited.

11.3 Interim Analyses

Interim analyses will be performed twice (after every 20 subjects have completed study participation) in addition to the final analysis. The DSMB will conduct the analyses using a two-sided asymmetric Lan-DeMets alpha-spending approach with an O'Brien-Fleming two-sided symmetric stopping boundary and overall alpha = 0.05. The DSMB criteria for early stopping will include: (i) the Z-score at interim analysis lies outside of the group sequential boundaries as calculated (Table 3); (ii) major safety violations; and (3) convincing evidence of futility in the context of AEs. Interim boundaries together with terminal criteria (z-scores and associated p-values) calculated using the WinLD version 2 program are provided in Table 3.

Table 3. Lan-DeMets Group Sequential Boundaries Calculations

Volunteers completed	Lower boundary	Upper boundary	Nominal upper alpha	Cumulative alpha
20	-3.7103	3.7103	0.00010	0.00021
40	-2.5114	2.5114	0.00601	0.01210
60	-1.9930	1.9930	0.02313	0.05000

12. Data Collection and Validation

Data will be collected and entered into an encrypted web-based data management system (DMS). Reports of outstanding edits, generated upon completion of data entry, will enable continuous cleaning of the data.

Confidentiality – each subject is assigned a unique number to assure confidentiality. Any publication or presentation will refer to subjects by this number and not by name. The medical records department, affiliated with the institution where the subject receives medical care, maintains all original inpatient and outpatient chart documents. Subject research files will be kept in a locked office in a cabinet.

Data Management – The principal investigators will monitor timely entry of data into the study database. Access to all source documentation maintained by the Investigator, including correspondence and source data, will be available for monitoring and audit purposes.

Data archives – at all times, appropriate backup copies of the database and related software files will be maintained and the information will be appropriately protected from illegitimate access.

13. Protection of human subjects

All aspects of this proposed research will be conducted according to the defined protocol, relevant FDA regulations, ICH-GCP Guidelines, and HIPAA for protection of human subjects under local IRB oversight.

14. Investigator Responsibility

14.1 Institutional Review Board (IRB) Approval

No patient will be enrolled in the study until the Columbia University Medical Center and New York Blood Center IRB has approved the protocol and the Informed Consent Form. Copies of all submissions to and correspondence (approvals and disapprovals) from the IRB must be maintained on file at the study site.

14.2 Informed Consent

If a subject is potentially eligible for the study and responds to the letter sent by the New York Blood Center, the subject will be contacted to be screened and obtain written informed consent. The background of the study and the potential benefits and risks will be explained. The subject or legally authorized representative must sign the consent form that has been approved by the IRB prior to enrollment. Failure to obtain signed informed consent renders the subject ineligible for the study. Copies of the signed informed consent shall be kept in the study files.

14.3 Subject Data Protection

Subjects will be identified in the electronic case report form (eCRF) by a subject identification number. All information and data sent to NHLBI, concerning subjects or their participation in this study, will be considered confidential. All data used in analysis and reports will be used without identifiable reference to the subject. At all times throughout the study, confidentiality shall be observed by all parties involved. All data shall be secured against unauthorized access. All subjects enrolled in this study will be informed and must agree to the use and disclosure of their study information by the institution and investigators to NHLBI, their agents and representatives, or other review boards.

15. Records and reports

15.1 Case Report Forms

Case Report Forms (CRFs) will be used to collect all subject data during the course of the study. The Principal Investigator or predetermined designated individual shall be responsible for completion of the CRFs. All protocol deviations shall be documented and a justification for any missed assessments shall be provided on the protocol deviation log. The Investigator will allow regulatory bodies to review the study files, subject CRFs, medical records and other study-related documents.

15.2 Source documents

Good Clinical Practice Guidelines require that investigators maintain information in the subject's medical records, laboratory reports, clinic charts, etc. that corroborate data recorded on the CRFs. In order to comply with these requirements, the following information should be maintained:

- Laboratory data before enrollment sufficient to verify protocol entry criteria
- Dated and signed notes for specific results of procedures and exams

15.3 Record Retention

NHLBI and the investigators must establish and maintain records and reports. The Investigator must maintain the signed Informed Consent Forms, CRFs, study documentation (listed above) and source documents for at least 3 years and 3 months after study completion or termination. In addition, the

Investigator must not discard or destroy any study-specific materials unless otherwise instructed by NHLBI.

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