The Impact of Oxidative Stress on Erythrocyte Biology

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CLINICAL STUDY PROTOCOL

Red Blood Cell Survival Study:

The Impact of Oxidative Stress on Erythocyte Biology

Clinical Trials.gov Number: NCT04028700

Principal Investigator: Matthew S. Karafin, MD, MS

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Title: The Impact of Oxidative Stress on Erythocyte Biology

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1	30 JUL 2019
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4	12 Nov 2021
5	11 Feb 2022
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PROTOCOL SUMMARY

Title	The Impact of Oxidative Stress on Erythrocyte Biology
Principal Investigator	Matthew S. Karafin MD, MS
Target Population	Adults with sickle cell disease who receive RBC transfusions
Primary Objective	**Aim 1. Evaluate the relative effects of blood from a donor with G6PD-deficiency on 24-hour post-transfusion recovery (PTR) as measured by Chromium 51 (^{51}CR).** * Using a crossover experimental design, we will compare the two treatment regimens (G6PD-deficient vs. G6PD-normal donor units) in terms of the mean response on the ^{51}CR scale. The same approach will be used for Aims 2a – 2d.
Secondary Objectives	**Aim 2a. Evaluate the relative effects of blood from a donor with G6PD-deficiency on 4-week post-transfusion outcomes as measured by mean change in hemoglobin A.** **Aim 2b. Evaluate the relative effects of blood from a donor with G6PD-deficiency on 4-week post-transfusion outcomes as measured by mean changes in twenty-three other clinically-relevant measures.** **Aim 2c. Evaluate the relative effects of blood from a donor with G6PD-deficiency on the longitudinal series of metabolic profiles obtained from blood samples.** **Aim 2d. Evaluate the relative effects of blood from a donor with G6PD-deficiency on longitudinal pain scores, infection symptoms, and antibiotic use.**
Outcome Measures	For Aims 1and 2 the outcome measures of interest will be obtained from blood samples on 15 occasions within each of Period1 and Period2: At baseline prior to the infusion treatment. Post-infusion at 5, 7.5, 10, 12.5, 15, 30 minutes, and at 1, 24, 48, 72 hours, and at 1, 2, 3, 4 weeks. For Aim 1, the outcome measure is 24-hour ^{51}CR-labeled PTR of RBCs. For Aim 2a, the outcomes are Hemoglobin A. For Aim 2b, the outcome variables are 26 clinically-relevant measures. For Aim 2c, the outcomes of interest are the metabolomic profile from blood samples obtained longitudinally. For Aim 2d, pain scores, occurrence of infection, and antibiotic use will be obtained via a daily 4-week diary for evaluation of pain on an ordinal scale (0,1,2,…,10), occurrence of infection symptoms, and use of antibiotics (type, duration, dose).

Study Design	For this Phase II, single-blind, longitudinal controlled trial, the experimental design is a two-treatment, two-period, two-sequence cross-over design. The two treatment regimens are (**A**) infusion of G6PD-deficient donor units, and (**B**) infusion of G6PD-normal donor units. Due to the scarcity of G6PD-deficient units in the donor pool, we will exchange units that are available at the time of scheduled study exchange (likely normal units) and not utilize a randomization scheme. Each 4-week study exchange period will be separated from the second study exchange by a 4-month washout interval. During the washout period, we will look for suitable units (likely G6PD-deficient) for the second study exchange.
Sample	N = 16 eligible patients will be enrolled
Participation Time	Each enrollee's participation will last at least 6 months
Enrollment Time	Recruitment and enrollment of the participants will require 4 years

SCHEMA

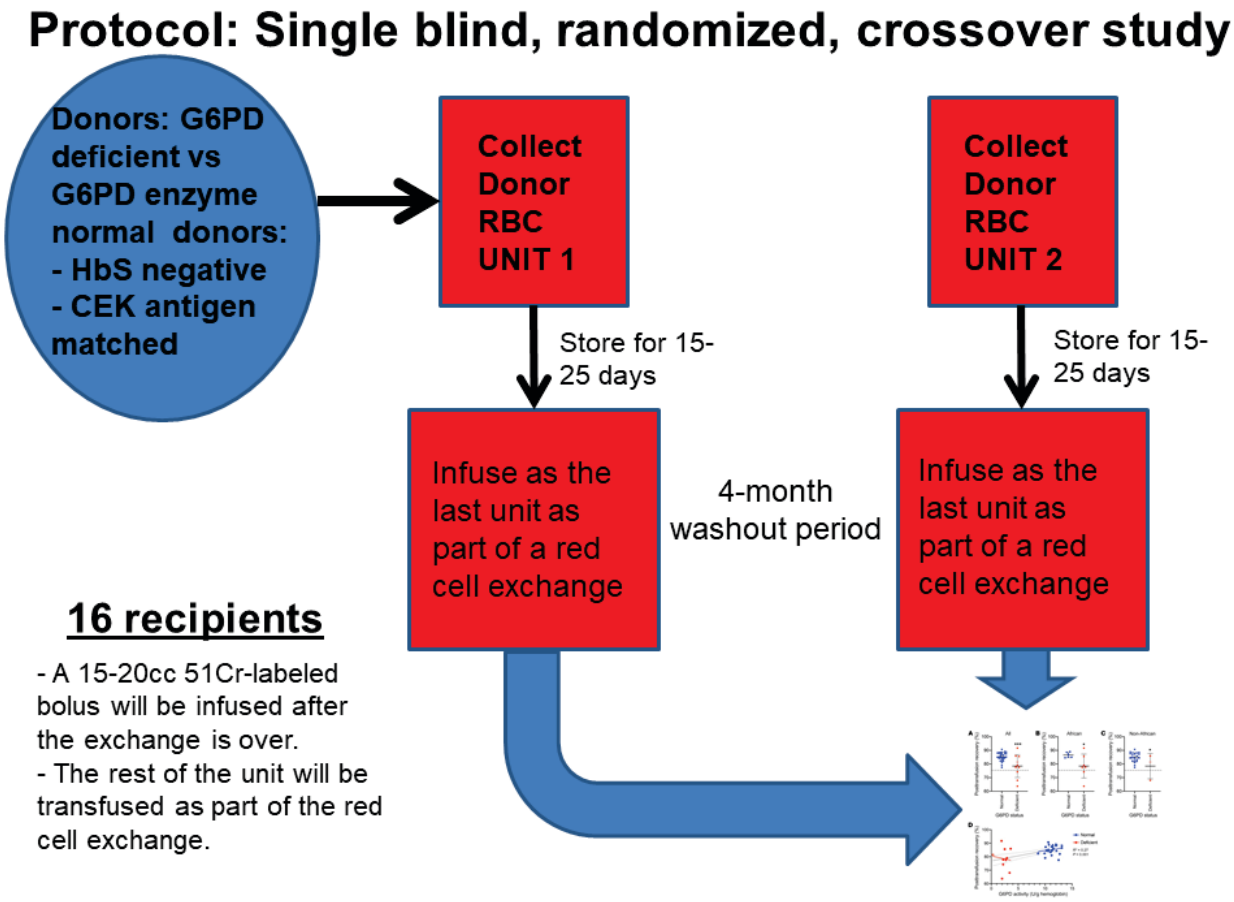

Figure 1: Study Schema: We will compare two treatment regimens via is a two-treatment, two-period, two-sequence cross-over design: CO(2,2,2). The two treatment regimens are (**A**) infusion of G6PD-deficient donor units, and (**B**) infusion of G6PD-normal donor units.
The two sequences (**AB** and **BA**) will be assigned to the N=16 enrollees by RBC unit availability at the time of the first study exchange transfusion .
The two 4-week periods will be separated by a 4-month washout interval. The outcome measures of interest are 24-hour ^{51}CR-labeled PTR of RBCs, and Hemoglobin A evaluated before and 4 weeks after infusion.

Period/ Procedure						Follow-up visits					
Study Day/Visit Day	**24-72 hrs pre-infusion**	**Pre-transfusion**	**5,7.5,10,12.5,15, 30 min post infusion**	**1 hr post transfusion/ infusion (+/-15 min)**	**24hr post infusion (+/- 3 hours)**	**48 hr post infusion (+/- 3 hours)**	**72hr post infusion (+/- 3 hours)**	**1week post infusion (+/- 1 day)**	**2week post infusion (+/- 2 days)**	**3 week post infusion (+/- 2 days)**	**4 week post infusion (+/- 2 days)**
Informed consent	X										
Study Diary				X	X	X	X	X	X	X	X
AE assessment		X		X	X	X	X	X	X	X	X
Type and screen sample	X										
Clinical procedures											
Physical exam		X									X
Vital signs		X									
Medical history	X										
Midline Placement		X									
Laboratory procedures											
CBC w/ Diff [3]		X		X	X	x	X	X	X	X	X
Reticulocyte count/%		X		X	X	X	X	X	X	X	X
Hb profile		X		X	X	X	X	X	X	X	X
Haptoglobin (hapto)		X		X	X			X	X	X	X
Iron Panel		X		X				X	X	X	X
Cell free hemoglobin		X		X	X			X	X	X	X

Period/ Procedure						Follow-up visits					
Study Day/Visit Day	**24-72 hrs pre-infusion**	**Pre-transfusion**	**5,7.5,10,12.5,15, 30 min post infusion**	**1 hr post transfusion/ infusion (+/-15 min)**	**24hr post infusion (+/- 3 hours)**	**48 hr post infusion (+/- 3 hours)**	**72hr post infusion (+/- 3 hours)**	**1week post infusion (+/- 1 day)**	**2week post infusion (+/- 2 days)**	**3 week post infusion (+/- 2 days)**	**4 week post infusion (+/- 2 days)**
Total, direct, indirect bilirubin		X		X	X			X	X	X	X
Labile plasma Iron (NTBI)		X		X	X			X	X	X	X
Ferritin & Transferrin saturation		X		X	X			X	X	X	X
hsC-reactive protein (HSCRP)		X		X	X			X	X	X	X
Myeloperoxidase (MPO)		X		X	X			X	X	X	X
Lactate dehydrogenase (LDH)		X		X	X			X	X	X	X
Patient Metabolomic Samples		X	X	X	X	X	X	X	X	X	X
RBC Unit blood donor sample		X									
Subject Chromium samples		X	X	X	X	X	X	X	X	X	X
Pregnancy test (urine)		X									

LIST OF ABBREVIATIONS

AE	adverse event
CBCD	complete blood cell (count) with differential
CRC	clinical research coordinator
CRF	case report form
CR51	Chromium 51
CTCAE	Common Terminology Criteria for Adverse Events
DSMC	Data and Safety Monitoring Committee
FDA	Food and Drug Administration
GCP	Good Clinical Practice
G6PD	Glucose 6 phosphate dehydrogenase
HbA	Hemoglobin, type A
HbS	Hemoglobin, type S
HCT	hematocrit
HGB	hemoglobin
IRB	Institutional Review Board
IV	intravenous
LDH	lactate dehydrogenase
LFT	liver function test
NTBI	Non-transferrin bound iron (synonym is LPI)
PTR	post transfusion recovery
RBC	red blood cell
SAE	serious adverse event
SCD	sickle cell disease
UNC	University of North Carolina
WBC	white blood cell (count)

1 BACKGROUND

Sickle Cell Disease and Red Blood Cell Transfusions

For logistical purposes, RBCs are stored for up to 42 days in the United States, resulting in altered RBC biology (i.e., the "storage lesion").[8] Indeed, if the storage lesion does nothing else, it does decrease the effective dose of transfused RBCs by up to 25% in healthy autologous recipients, as mandated by FDA criteria.[6,9] However, this is only an average; thus, some units store even worse than this.[9] More importantly, this is also an underestimate, because stored RBCs do more poorly when transfused into sick patients,[10] whereas FDA approval studies are performed in healthy volunteers. Although controversy exists as to whether stored RBC transfusions are dangerous in certain settings, there is consensus that iron overload from red blood cell transfusions is a very serious adverse outcome in chronically transfused, non-bleeding patients (e.g., in SCD and thalassemia).[11,12] Such patients can have significant morbidity, and even mortality, from iron overload, despite chelation therapy. As such, providing them with RBC units with better recovery and survival (post transfusion recovery, PTR), thereby transfusing fewer units and less iron, would be of substantial benefit.

Of equal significance to improving understanding of the storage lesion, is realizing that it results from oxidant stress.[8,13] As such, storage biology is also relevant for elucidating RBC oxidant stress mechanisms which are important for various diseases. G6PD deficiency, the most common human enzymopathy, affects >400 million humans worldwide and is a serious barrier to malaria treatment and eradication.[14] The only drugs capable of completely eliminating *Plasmodium vivax* cannot be given to patients with G6PD deficiency, due to hemolysis *in vivo* from oxidant damage.[14] It is well established that G6PD deficiency causes red cell hemolysis and anemia during oxidant stress. While this deficiency is critical to red cell biology and survival, blood donor centers currently do not screen for this deficiency, and about 1 in 10 RBC units provided to patients with SCD currently have this deficiency.[46] The impact of these units on current transfusion protocols for patients with SCD is unknown. **By better defining known oxidant stress pathways (e.g., G6PD deficiency), and also elucidating novel pathways, this project has broad significance to RBC oxidant biology, in general, in addition to its immediate relevance to RBC transfusion therapy.**

Study Rationale

Mounting evidence suggests that stored G6PD-deficient RBCs have reduced transfusion quality. Thus, the PTR of stored autologous G6PD-deficient RBCs is modestly, but significantly, worse than that of G6PD-normal RBCs in healthy volunteers;[78,79] however, the effects in actual patients have not been evaluated systematically. Patients with SCD are regularly transfused with units from G6PD-deficient donors, with an exposure incidence of around 10%. Nonetheless, case reports suggest that intra- and extra-vascular hemolysis can occur in patients transfused with G6PD deficient donor RBCs.[45] Furthermore, in our observational study of children with SCD,[80] transfusions of G6PD deficient RBCs reduced the persistence of HbA-containing RBCs, reflected by increased post-transfusion HbS and reticulocyte levels. In adults with SCD, we would anticipate that these changes would still occur, but may be proportionally reduced due to differences in patient blood volumes. Because patients with SCD have increased iron, inflammation, and oxidant stress at baseline,[81] we hypothesize that G6PD-deficient RBCs will circulate more poorly in all patients with SCD than in healthy controls. Therefore, our pilot study described herein is critical to begin to define the clinical and biological impact associated with the frequent transfusions of G6PD-deficient RBCs in patients with SCD, who often require chronic transfusion therapy.

2 SPECIFIC AIMS

2.1 Primary Objective (Aim 1)

Aim 1. Evaluate the effects of blood from a donor with G6PD-deficiency on 24-hour post-transfusion recovery (PTR) as measured by Chromium 51 (^{51}CR).

We hypothesize that, in contrast to the decreased PTR we found in healthy adult recipients, adults with SCD have a greater decrease in PTR when receiving older, stored RBCs from G6PD-deficient donors, as compared to G6PD-normal donors. We anticipate that the mean difference (i.e., the treatment effect) is larger than we observed in our previous study of healthy controls (6.8%).

The Aim 1 investigation will be considered a success if the confidence interval estimate for the treatment effect (i.e., the mean difference in PTR between G6PD-deficient donors and G6PD-normal donors) in SCD patients is not too wide. With an n = 16 participants with complete data, with a hypothesized difference in PTR of 10%, the chance of drawing a sample of patients that would yield a p-value smaller than $\alpha = 0.05$ is 99%.

2.2 Secondary Objectives (Aims 2a-2d)

Aim 2a. Evaluate the relative effects of blood from a donor with G6PD-deficiency on 4-week post-transfusion outcomes as measured by mean change in hemoglobin A (HbA).

Aim 2b. Evaluate the relative effects of blood from a donor with G6PD-deficiency on 4-week post-transfusion outcomes as measured by mean changes in twenty-three clinically-relevant measures.

Aim 2c. Evaluate the relative effects of blood from a donor with G6PD-deficiency on the longitudinal series of metabolic profiles obtained from blood samples.

Aim 2d. Evaluate the relative effects of blood from a donor with G6PD-deficiency on longitudinal pain scores, infection symptoms, and antibiotic use.

The investigations of Aims 2a-2d will be considered success if the confidence intervals for the treatment effects are not too wide.

2.3 Rationale for the Outcome Measures Selected

For Aim 1, the primary outcome (24-hour PTR) is the only in-vivo FDA approved method for guiding quality of red cell products. This method has been used for multiple decades, and a large volume of literature can be applied to help interpret the proposed study findings.

For Aim 2a, hemoglobin A is the leading clinically-relevant measure of interest.

For Aims 2b and 2d, the selected secondary measures are fundamentally important clinical measures.

For Aim 2c, we expect the metabolomic data to provide insights for generation of new hypotheses.

3 STUDY DESIGN

3.1 General Description

This is a phase II, single blind, crossover study in 16 adults with SCD who are actively on a chronic transfusion protocol. Patients with SCD, >18-years old, will be recruited from the UNC Adult Sickle Cell Clinic. Subjects will receive 15-25 day old RBCs from sickle trait-negative, ABO-compatible (but not identical, if non-O), CEK-matched, cross-match compatible, G6PD-deficient or G6PD-normal donors. Subjects will be blinded to infusion assignment. Subjects will be transfused 1 experimental unit of RBC per the UNC blood transfusion protocol as part of a routine red cell exchange. During the last 30-45 minutes (+/- 30 minutes) of the exchange, a 50mL sterile sample will be removed from the experimental RBC unit, one aliquot used for 51Cr-labeling and one for "omics" studies. Once the exchange is complete, the subject will then be rapidly infused with the 51Cr labeled aliquot per Nuclear Medicine's protocol (*Please refer to RDRC documents*).

Blood samples will be obtained pre-infusion, and at approximately 5, 7.5, 10, 12.5, 15, 30 minutes; 1, 24, 48, 72 hours; and 1, 2, 3, 4 weeks post-infusion. We will measure PTR and long-term lifespan of the donor RBCs, accounting for the expected elution of 51Cr using standard formulas. Hemolysis markers, CBCs, and HbA/S% levels will be measured at defined time points post-transfusion to explore correlations with the PTR. After a >120-day washout period (i.e., the RBC lifespan), the same procedures will again be performed on each subject, this time receiving RBCs from a donor of the alternative G6PD status (i.e., normal vs. deficient). During this time, the patient will remain on their clinical treatment protocols as ordered by their provider, and no changes to their clinical care will be made as a result of their participation in the study.

3.2 Study Completion

The study will reach completion once all 16 subjects have completed the study. This study is estimated to take 4 years to complete once the study opens to accrual.

4 SUBJECT PARTICIPATION, DISCONTINUATION, AND WITHDRAWAL

We will follow all UNC IRB requirements and policies regarding subject participation:

4.1 Subject Status

Subject statuses throughout the trial are defined as follows:

- Prescreening: preconsent (subject considering trial or study staff considering patient for the trial per institutional recruitment methods).
- Screening: period after consent, but prior to eligibility confirmation.
- Consented: consented, prior to eligibility confirmation.
- Eligible: the local investigator confirms all eligibly criteria apply.

- On study/enrolled: date eligibility is confirmed.
- Off study: no additional subject data gathered.
- Withdrawn: subject fully withdraws consent (i.e., refuses ALL follow-up) or is taken off study by the local principal investigator.

4.2 Prescreening and Screening Log

The UNC study principal investigator regularly reviews screen failure reasons to understand barriers to accrual and consider amending eligibility criteria. Screen failures are defined as participants who were considered for the trial to participate in the clinical trial with or without consent, but are not subsequently assigned to the study intervention or enrolled in the study. Prescreening and screening tracking will follow standard practice for research at UNC.

4.3 Consent

Investigators or their appropriate designees will identify potentially eligible subjects from their clinics, subject self-referrals, referrals from other clinicians, and/or other IRB-approved recruitment methods. No study conduct, including subject prescreening, can occur until after IRB approval.

A written, signed informed consent form (ICF) and a Health Insurance Portability and Accountability Act (HIPAA) authorization must be obtained before any study-specific assessments are initiated. A signed ICF copy will be given to the subject and a copy will be filed in the medical record (per local IRB policies and SOPs). The original will be kept on file with the study records. UNC exchange and blood consent will also be obtained prior to any RBC transfusion activities, if not already up to date.

4.4 Screening Procedures

For women of childbearing potential, a negative pregnancy test must be obtained prior to RBC transfusion. Visit procedures that were performed as standard of care prior to consent (without the specific intent to make the subject eligible for the trial), may count toward screening tests and eligibility if they are within the screening window.

4.5 Eligibility Confirmation

All patient eligibility criteria will be confirmed by a trained study investigator prior to ordering any blood products for infusion.

4.6 Study Eligibility Criteria

*No waivers of protocol eligibility will be granted.

Inclusion Criteria

1. Age 18-60 years
2. A diagnosis of sickle cell disease
3. Steady state (no pain or baseline pain and ≥1 month from any hospital admission)
4. Receiving chronic transfusions (i.e regular transfusion every 4-8 weeks).

Exclusion Criteria

1. History of transfusion reactions not adequately managed by antihistamines
2. No available crossmatch compatible red cell units
3. Known G6PD deficiency
4. Hepato- or splenomegaly
5. Participation in another therapeutic trial
6. Pregnant or nursing
7. HIV positive
8. At investigator discretion for uncontrolled inter-current illness or social situation limiting compliance with study requirements.
9. Inability to speak and/or read English

4.7 Enrollment

Subject enrollment logistics are defined as follows:

Patients ≥ 18 years of age with sickle cell disease will be identified through the adult outpatient sickle cell clinic at UNC through the sickle cell providers trained on the study protocol.

Potentially eligible patients, as described in the previous paragraph, will be approached for study consent prior to their transfusion or infusion. Individual center scheduling practices will influence how this contact is arranged. Subjects who consent to the study will be assigned a study ID number and have their eligibility status determined. If the subject is eligible for the study based on the inclusion/exclusion criteria (section 4.7, 4.8), they will be enrolled in the study and will be considered for randomization (see sections 4.2 and 5.1).

4.8 Unit Selection, Identification, and Study Activities

At enrollment, basic features of patient medical and surgical histories (i.e. age, gender, past red cell transfusions, red cell phenotype) will be collected. The subject's eligibility status and ABO/RBC phenotype will be entered into the electronic data capture system (EDC). If eligible, the subject's first data of chromium labeling will be determined, and appropriate RBC units at the UNC transfusion service will be identified.

At a minimum, potential RBC units will be selected from the appropriate RBC unit inventory to ensure that the unit is: 1) ABO compatible with the subject, 2) D, Cc, Ee, and Kk matched to the subject (**rr, Ror, or RoRo phenotype)**, 3) stored in AS1, 4) sickle negative, 5) G6PD positive or negative based on testing. African black donor units will be preferred, but not required, for selection due to the known increased prevalence for G6PD deficiency. All other standard quality metrics for RBC units will be adhered to per standard blood bank service practice.

For G6PD testing: Two sterile segment links from the units assigned for the subject's red cell exchange identified by UNC will be codified and tested for G6PD enzyme activity in the clinical laboratory (McClendon Laboratories). Units identified as G6PD deficient/not deficient will be selected for use. Given the rarity of G6PD deficient units, these units will be selected first, if available. If not, a G6PD normal unit will be selected. Cross-matching for the unit with the subject will be done at UNC once the unit is 15-25 days old from collection, if possible.

Only study staff with the appropriate security level will be able to access the arm assignment (G6PD deficient first, or G6PD normal first). Access to case report forms containing information about the RBC products sent for each subject will also be restricted at the site to the appropriate study staff. Clinical staff overseeing the subject's participation in the trial, collecting data, and reporting the data into EDC will have access to the treatment arm assignment and information about the G6PD-status of the RBC products transfused.

No alteration will be made to the labels on the RBC units. The expiration date, collection date, and any processing dates (e.g. irradiation dates) will not be obscured. Trained personnel at the adult Translational Research Unit will verify product and patient identity according to hospital-specific procedures. These personnel will be instructed to not divulge the patients' unit type. Other ancillary staff (other than those actually infusing RBCs), will be instructed not to seek to identify the G6PD status of the products transfused. The subjects themselves will not be informed of the identity of the labeled unit and will also be instructed not to seek to identify the G6PD status of the products they are receiving. However, as the key components of this study are objective, **inadvertent unblinding of the unit-status will not compromise the validity of the study data**.

Study Activities

24-72 hours *prior to the 51Cr-labeled RBC infusion:*

Draw 10mL of whole blood for type and screen

Day 1

- Physical exam by principal investigator or designee.
- Double-lumen Midline catheter placement by Interventional Radiology or VAT
- Provide daily diary
- Urine sample for urine pregnancy screen (females only)
- Vital signs will be checked for eligibility (subjects who are pregnant, have SBP >180 or <90mm Hg, DBP >100 or <50mm Hg, Heart rate <50 or >100, Temperature >100.4 F or feeling ill, will not continue study activities, and will be evaluated to be rescheduled).
- Informed consent for a blood transfusion, if not up to date
- Draw 10 mL of whole blood for CBCD, reticulocyte count, and hemoglobin electrophoresis.
- Draw 4 mL of whole blood for cell free hemoglobin.
- Draw 15 mL of whole blood for Hapto, Iron panel, BILI T/D, HSCRP, LDH, Ferritin, Iron Saturation, MPO, and NTBI.
- Clinic RN will transfuse the experimental RBC unit to the patient as the last unit of the red cell exchange or transfusion following hospital standard procedures
- Toward the end of the exchange/transfusion (about 30-45 minutes prior to completion), draw 30 mL from the unit into a syringe for chromium labeling. The syringe will be kept in a cooler once collected, and will be transported to Nuclear Medicine in the cooler for Chromium labeling once the transfusion is completed.
- Draw 6 mL of whole blood for in vivo chromium measurement (baseline- pre infusion).
- Infuse Chromium labeled blood into study subject.

Day 1 – *post infusion*

- Draw 6 mL of whole blood for chromium measurements will be taken at time **5, 7.5, 10, 12.5, 15, 30 minutes, and 1 hour** post labeled red cell infusion (samples for Omics will be taken from this tube).

- At 1 hour (+/- 15 minutes), draw 4 mL of whole blood for cell free hemoglobin.
- At **1 hour (+/- 15 minutes) post-RBC transfusion**, draw 10 mLfor CBCD, reticulocyte count, and hemoglobin electrophoresis.
- At 1 hour (+/-), draw 15 mL Hapto, Iron panel, BILI T/D, HSCRP, LDH, Ferritin, Iron Saturation, MPO, NTBI.
- Process and store samples for NTBI and metabolomics studies.

24hrs - Post Infusion

- Draw 6 mL of whole blood for chromium measurements taken at **24 hrs (+/3 hours)** post infusion (samples for Omics will be taken from this tube).
- Draw 4 mL of whole blood for cell free hemoglobin
- Draw 10 mL of whole blood for CBCD, reticulocyte count, and hemoglobin electrophoresis.
- Draw 15 mL of whole blood for Hapto, Iron panel, BILI TD, HSCRP, LDH, Ferritin, Iron Saturation, and MPO at **24 hrs (+/- 3 hours)** post infusion.
- Process and store samples for NTBI and metabolomics studies.
- Review of daily diary

48hrs - post infusion

- Draw 6 mL of whole blood for chromium measurements taken at **48 hrs (+/- 3 hours)** post infusion (samples for Omics will be taken from this tube).
- Draw 10 mL of whole blood for CBCD, reticulocyte count, and hemoglobin electrophoresis.
- Draw 4 mL of whole blood for cell free hemoglobin
- Process and store samples for NTBI and metabolomics studies.
- Review of daily diary

72hrs - post infusion

- Draw 6 mL of whole blood l for chromium measurements taken at **72 hrs (+/- 3 hours)** post infusion (samples for Omics will be taken from this tube).
- Draw 10 mL of whole blood mL for CBCD, reticulocyte count, and hemoglobin electrophoresis.
- Process and store samples for NTBI and metabolomics studies.
- Review of daily diary

1 Week – post infusion

- Draw 6 mL of whole blood for chromium measurements taken at **1 week (+/- 1 day)** post infusion (samples for Omics will be taken from this tube).
- Draw 10 mL of whole blood for CBCD, reticulocyte count, and hemoglobin electrophoresis.
- Draw 4 mL of whole blood for cell free hemoglobin
- Draw 15 mL of whole blood for Hapto, Iron panel, BILI TD, HSCRP, LDH, Ferritin, Iron Saturation, and MPO at **1 week (+/- 1 day)** post infusion.
- Process and store samples for NTBI and metabolomics studies.
- Review of daily diary

2 Week – post infusion

- Draw 6 mL of whole blood for chromium measurements taken at **2 weeks (+/- 2 days)** post infusion (samples for Omics will be taken from this tube).

- Draw 10 of whole blood for mL for CBCD, reticulocyte count, and hemoglobin electrophoresis.
- Draw 4 mL of whole blood for cell free hemoglobin
- Draw 15 mL of whole blood for Hapto, Iron panel, BILI TD, HSCRP, LDH, Ferritin, Iron Saturation, and MPO at **2 weeks (+/- 2 days), 3 weeks (+/- 2 days)** post infusion.
- Process and store samples for NTBI and metabolomics studies.
- Review of daily diary

3 Week – post infusion

- Draw 6 mL of whole blood for chromium measurements taken at **3 weeks (+/- 2 days)** post infusion (samples for Omics will be taken from this tube).
- Draw 10 mL of whole blood for CBCD, reticulocyte count, and hemoglobin electrophoresis.
- Draw 4 mL of whole blood for cell free hemoglobin
- Draw 15 mL of whole blood for Hapto, Iron panel , BILI TD, HSCRP, LDH, Ferritin, Iron Saturation, and MPO at **3 weeks (+/- 2 days)** post infusion.
- Process and store samples for NTBI and metabolomics studies.
- Review of daily diary

4 Week - post infusion

- Physical Exam
- Draw 6 mL of whole blood for chromium measurements taken at **4 weeks (+/- 2 days)** post infusion (samples for Omics will be taken from this tube).
- Draw 10 mL of whole blood for CBCD, reticulocyte count, and hemoglobin electrophoresis.
- Draw 4 mL of whole blood for cell free hemoglobin
- Draw 15 mL of whole blood for Hapto, Iron panel, BILI TD, HSCRP, LDH, Ferritin, Iron Saturation, and MPO at **4 weeks (+/- 2 days)** post infusion.
- Process and store samples for NTBI and metabolomics studies.
- Review of daily diary

Follow-Up Visit

After day 28, the subject will be given a (washout period) break of about 4 months (120 days). The patient will continue their transfusion treatements as prescribed by their physician. After this break, the subject will be contacted to repeat the study blood draws and visits.

Specimen Processing

NTBI: 0.2mL from SST tube will be obtained from each required time point, snap frozen, and stored at -80C until mailing. Samples will be sent yearly to the research laboratory of Dr. Eldad Hod (Columbia University)

Metabolomics: Unit samples: 0.5 ml (max, Min 0.1 ml) transfusate (no separation); 0.1 ml of packed RBC (pellet) and 0.1 ml of supernatants after gentle centrifugation at 2,500g at 4C for 10 Min. All samples will be snap frozen and stored at -80C until mailing. **Patient samples**: 20 ul of plasma and 50 ul of RBCs after gentle centrifugation at 2,500g at 4C for 10 Min will snap frozen and stored at -80 until mailing. Samples will be sent yearly to the research laboratory of Dr. Angelo D'Alessandro (University of Colorado)

4.9 Discontinuation of Study Treatment, Withdrawal, and Compliance

Discontinuation from the study does not mean discontinuation from any clinically guided medical care. Subjects will be monitored for AEs/SAEs during the 4 week infusion testing period so long as an infusion took place.

In the absence of delays due to adverse events, study activities may continue until:

- Disease progression or instability as determined by the principal investigator.
- General or specific changes in the subject's condition renders the subject unacceptable for further treatment in the investigator's judgment.
- Intercurrent illness that prevents further participation.
- Subject decides to withdraw from the study.
- The subject has significant noncompliance with the protocol (defined as greater than 3 missed appointments).
- Unacceptable adverse event(s)
- Study stopping rules are met.

Subjects who sign the informed consent form, and are enrolled and receive the study intervention, but subsequently withdraw, or are withdrawn or discontinued from the study, will not be replaced.

Consent Withdrawal

A subject may decide to withdraw from the study at any time. UNC will follow its IRB of record's SOPs regarding consent withdrawal.

If a subject intends on withdrawing consent, staff should confirm which of the following options the subject chooses and document the discussion:

- Full consent withdrawal with no study follow-up.

- Selective consent withdrawal from interventional portion of the study, but agree to continued follow-up of associated clinical outcome information (i.e. study diary and/or blood draws).

Investigator-initiated Withdrawal

The investigator will withdraw a subject whenever continued participation is no longer in the subject's best interests. Reasons for withdrawing a subject include, but are not limited to, disease progression or instability, the occurrence of an severe adverse event or a concurrent illness, a subject's request to end participation, a subject's noncompliance or simply significant uncertainty on the part of the investigator that continued participation is prudent. The reason for study withdrawal and the date the subject was removed from the study must be documented.

4.10 Lost to Follow-up

The following actions must be taken if a participant fails to return to the clinic for a required study visit and/or is unable to be reached for follow-up:

- The investigator or designee must make every effort to regain contact and/or reschedule a missed visit with the participant.
- A participant is deemed lost to follow-up if his/her status cannot be obtained after *all* of the following occurs at two consecutive scheduled protocol calendar timepoints:
    - Three telephone calls (at least one day apart) from the study team are unanswered
      AND
    - A letter to the participant's last known mailing address goes unanswered
      AND
    - These contact attempts must be documented in the participant's study file.
- Update the EDC when a participant is officially considered lost to follow-up.
- If a subject is considered lost to follow-up, but subsequently contacts the participating site study team, the subject should be considered in follow-up again.

4.11 Accrual Suspension and Closure

The UNC PI facilitates the suspension and closing of accrual in the following manner:

- EDC tracks accrual throughout the study.
- If the study must be suspended, EDC and Clinical trial management software (ie Velos) is updated to a 'suspended' status.
- When the accrual number is reached, PI notifies staff of study closure.

4.12 End of Study Definition

A participant is considered to have completed the study if he or she has completed all phases of the study including the last visit or the last scheduled procedure shown in the calendar of events or has been discontinued.

4.13 Study Discontinuation and Closure

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause (as determined by the study principal investigator, DSMC, sponsor, and/or IRB). Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to study participants, investigator, funding agency, and regulatory authorities. If the study is prematurely terminated or suspended, the principal investigator (PI) will promptly inform the MCW Institutional Review Board (IRB) and sponsor and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes.

5 STUDY PLAN

5.1 RBC Unit Administration

Treatment will be administered on an outpatient basis. The study RBC unit will be administered per hospital protocol by the UNC RN staff per normal administration protocols. Subject vital signs will be taken at regular intervals per hospital protocol. The study staff will aseptically remove 50 ml (~4 tablespoons) of the same crossmatch-compatible RBCs into a syringe, and members of the Nuclear Medicine Department will label that sample with a radioisotope (^{51}Chromium) per standard protocols. A portion of that tagged aliquot will be transferred into a

syringe to be infused into the subject. A second portion from that sample, pre chromium labeling, will be saved for future “omics” studies. The UNC *Nuclear Medicine department* study staff will be specifically responsible for the preparation and infusion of the chromium-labeled aliquot to the patient. The RN staff will be responsible for the transfusion of the non-labeled compatible red cell unit and lab blood draws.

5.2 Monitoring Subject Compliance

Pain and infection diary: The diary is adapted from previously published SCD studies.[87] Pain and infection symptoms will also be documented daily for 4 weeks along with the type, duration, and dose of antibiotics, if applicable. Daily, participants will rate pain on a numeric pain rating ordinal scale (0-10), indicate whether the pain was consistent with a “crisis,” indicate whether they utilized a healthcare facility, and record the amount of opioids used. Infection symptoms will also be documented daily along with the type, duration, and dose of antibiotics, if applicable.

5.3 Follow-up Period

Patients will be followed for up to 28 days following their last study-related blood transfusion. Patients will not be followed after completion of the study protocol.

Patients removed from the study treatment for unacceptable SAEs will be followed until resolution or stabilization of the adverse event. SAEs will be followed until completion.

6 DOSING DELAYS/DOSE MODIFICATIONS

6.1 Monitoring and Toxicity Management

Each patient receiving G6PD-deficient and G6PD-normal RBCs will be evaluated for safety. The safety parameters include all laboratory tests and hematological abnormalities, physical findings and spontaneous reports of adverse events reported to the investigator by patients, and are outside laboratory values and clinical events considered usual for SCD.

The chromium agent used for red cell labeling has no known or previously reported risks of adverse events. Consequently, general tracking of adverse events will be conducted as noted in the following sections.

Each patient will be assessed periodically for any toxicity development. Toxicity will be assessed according to the CTCAE v 4.0.

We will specifically monitor for evidence of red cell hemolysis as a result of the red cell transfusion. Evidence of hemolysis will be obtain from clinical symptoms (fever, chills) and laboratory values obtained per study protocol (LDH, haptoglobin, and hemoglobin values).

Acute toxicity will be managed according to the symptoms and determined etiology. Most adverse events from transfusion can be managed symptomatically by standard over-the-counter medications, such as Tylenol for fevers, or Benadryl for allergic symptoms. Further management will depend upon the judgment of the clinician and may include ED admission or inpatient hospitalization.

Patients will also be monitored for exacerbations of the subjects underlying sickle cell disease: Vaso-occlusive pain crises, acute chest crises, stroke, serious infection, and priapism. This will be monitored by review of the patients electronic medical record, subject verbal report, and/or review of the daily diary.

7 ADVERSE EVENTS: DEFINITIONS AND REPORTING REQUIREMENTS

7.1 Definitions

1) Vaso-occlusive pain crisis: an episode of acute pain with no cause other than a vaso-occlusive event lasting at least 24 hours that requires the administration of oral or parenteral opioids in a medical facility.

2) Acute Chest Syndrome: an acute illness requiring medical facility attention characterized by fever and/or respiratory symptoms, accompanied by a new pulmonary infiltrate on a chest X-ray.

3) Priapism: an acute illness requiring medical facility attention characterized by *an* erect penis that does not return to its flaccid state, despite the absence of both physical and psychological stimulation, within four hours.

4) Acute infection: an acute illness requiring medical facility intervention (i.e prescription of antibiotics or hospital admission) characterized by any subjective symptom.

5) *Chronic transfusion*: receiving red cell units on a 4-8 week schedule as part of routine, non-acute care).

6) Acute transfusion: receiving red cell units on an unscheduled basis to resolve an acute issue.

7.1.1 Adverse Event (AE) and Serious Adverse Events (SAE)

The investigator and his or her team will follow UNC policies related to adverse event reporting. This information may be found on the Human Research Protection Program website.

Serious AE (SAE) means any untoward medical occurrence that at any dose:

- **Death.** Results in death.
- **Life threatening**. Is life threatening (refers to an AE in which the patient was at risk of death at the time of the event. It does not refer to an event which hypothetically might have caused death if it were more severe).
- **Hospitalization**. Requires inpatient hospitalization or prolongation of an existing hospitalization (see clarification in the paragraph below on planned hospitalizations).
- **Disability/incapacity**. Results in persistent or significant disability or incapacity. (Disability is defined as a substantial disruption of a person's ability to conduct normal life functions).
- **Medically important event**. This refers to an AE that may not result in death, be immediately life threatening, or require hospitalization, but may be considered serious when, based on appropriate medical judgment, may jeopardize the patient, require medical or surgical intervention to prevent one of the outcomes listed above, or involves suspected transmission via a medicinal product of an infectious agent. Examples of such

medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse; any organism, virus, or infectious particle (e.g., prion protein transmitting transmissible spongiform encephalopathy), pathogenic or nonpathogenic, is considered an infectious agent.

Clarification should be made between a serious AE (SAE) and an AE that is considered severe in intensity (Grade 3 or 4), because the terms serious and severe are NOT synonymous. The general term severe is often used to describe the intensity (severity) of a specific event; the event itself, however, may be of relatively minor medical significance (such as a Grade 3 headache). This is NOT the same as serious, which is based on patient/event outcome or action criteria described above, and is usually associated with events that pose a threat to a patient's life or ability to function. A severe AE (Grade 3 or 4) does not necessarily need to be considered serious. For example, a white blood cell count of 1000/mm^3 to less than 2000 is considered Grade 3 (severe) but may not be considered serious. Seriousness (not intensity) serves as a guide for defining regulatory reporting obligations.

7.1.2 Unanticipated Problem Involving Risk to Subject or Other

The investigator and his or her team will follow UNC policies related to unanticipated problems involving risks to subjects or others. This information may be found on the Human Research Protection Program website.

7.1.3 AE Attribution and Grading

Adverse Event Grading

Grade	Description
0	No AE (or within normal limits).
1	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
2	Moderate; minimal, local or noninvasive intervention (e.g., packing cautery) indicated; limiting age-appropriate instrumental activities of daily living (ADL).
3	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL.
4	Life-threatening consequences; urgent intervention indicated.
5	Death related to AE.

Adverse Event Attribution

Attribution is an assessment of the relationship between the AE and the medical intervention.

Relationship	Attribution	Description
Unrelated to investigational agent/intervention	Unrelated	The AE *is clearly* ***NOT*** *related* to the intervention
	Unlikely	The AE *is* ***doubtfully related*** to the intervention
Related to investigational agent/intervention	Possible	The AE ***may be related*** to the intervention
	Probable	The AE ***is likely related*** to the intervention
	Definite	The AE ***is clearly related*** to the intervention

Relationship Assessment: In-Depth Definitions

For all collected AEs, the clinician who examines and evaluates the subject will determine the adverse event's causality based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories below:

Definitely Related: There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event, including an abnormal laboratory test result, occurs in a plausible time relationship to drug administration and cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the drug (dechallenge) should be clinically plausible. The event must be pharmacologically or phenomenologically definitive, with use of a satisfactory rechallenge procedure if necessary.

Probably Related: There is evidence to suggest a causal relationship, and the influence of other factors is unlikely. The clinical event, including an abnormal laboratory test result, occurs within a reasonable time sequence to administration of the drug, is unlikely to be attributed to concurrent disease or other drugs or chemicals, and follows a clinically reasonable response on withdrawal (dechallenge). Rechallenge information is not required to fulfill this definition.

Possibly Related: There is some evidence to suggest a causal relationship (e.g., the event occurred within a reasonable time after administration of the trial medication). However, the influence of other factors may have contributed to the event (e.g., the subject's clinical condition, other concomitant events). Although an adverse drug event may rate only as "possibly related" soon after discovery, it can be flagged as requiring more information and later be upgraded to "probably related" or "definitely related," as appropriate.

Unlikely: A clinical event, including an abnormal laboratory test result, whose temporal relationship to drug administration makes a causal relationship improbable (e.g., the event did not occur within a reasonable time after administration of the trial medication) and in which other drugs or chemicals or underlying disease provides plausible explanations (e.g., the subject's clinical condition, other concomitant treatments).

Unrelated: The AE is completely independent of study drug administration, and/or evidence exists that the event is definitely related to another etiology. There must be an alternative, definitive etiology documented by the clinician.

7.2 Known AEs List

Below are know AE's related to blood cell transfusion. Many of these risks are common to sickle cell disease and are known to occur. Reporting of these instances will be based on frequency and severity under the determination of trained study staff.

- Myocardial infarction
- Pulmonary embolism
- Stroke
- Renal failure
- Sepsis
- Ventricular tachycardia
- Ventricular fibrillation
- Red cell hemolysis (immune and non-immune)
- Transfusion-associated congestive heart failure/transfusion-associated circulatory overload (TACO)
- Transfusion-related acute lung injury (TRALI)
- Anaphylaxis
- Graft vs. Host Disease (GVHD)
- **Grade 2** or higher events of the following types:
    - Allergic reaction
    - Sinus bradycardia
    - Sinus tachycardia
    - Hypertension
    - Hypotension
    - Dyspnea
    - Hypoxia
    - Wheezing
    - Cough
    - Fever
    - Chills
    - Hemolysis
    - Hyperkalemia
    - Hypocalcemia
    - Hyperbilirubinemia
    - Hemoglobinuria.

7.2.1 Other Risks

7.2.1.1 Loss of confidentiality.

- While participating in this project all study records kept electronically will be on encrypted drives and all paper copies will be kept in locked cabinets in a room requiring badge access for entry but there is always the potential some of the subjects information could be stolen.

Risks of Transfusion/Apheresis catheter

- Injury to local structures
- Phlebitis at insertion site
- Air embolism
- Hetamtoma
- Arrhythmia

- Catheter malposition
- Infection
- thrombosis

7.3 Time Period and Grade of AE Capture

The study staff will start recording AE's from the time of the pre-transfusion type-and-cross blood sample until the end of the subject's last visit on week 4. This process will occur twice due to the cross-over design of the study.

7.4 Monitoring and Recording an Adverse Event

Definition. Any clinically relevant deterioration in laboratory assessments in the opinion of the study investigator or other clinical finding determined relevant by the study investigator is considered an AE.

Reporting source. AEs may be spontaneously reported by the patient and/or in response to an open question from study personnel or revealed by observation, physical examination or other diagnostic procedures.

Prior to the trial. Planned hospital admissions or surgical procedures for an illness or disease that existed before the patient was enrolled in the trial are not to be considered AEs unless the condition deteriorated in an unexpected manner during the trial (e.g., surgery was performed earlier or later than planned).

Pretreatment events following signed informed consent. For serious pretreatment events, the investigator must determine both the intensity of the event and the relationship of the event to study procedures.

Treatment events. For serious AEs, the investigator must determine both the intensity of the event and the relationship of the event to study drug administration.

Not serious AEs. For non-serious AEs, the investigator must determine both the intensity of the event and the relationship of the event to study drug administration.

Follow-up of Adverse Events

All adverse events will be followed with appropriate medical management 30 days following the last dose of the study transfusion or until they are resolved, if they are related to the study treatment.

7.4.1 Procedure for Reporting Drug Exposure during Pregnancy and Birth Events

If a woman becomes pregnant, or suspects that she is pregnant, while participating in this study, she must inform the investigator immediately and permanently discontinue study participation. The investigator must notify the DSMC by email. The pregnancy must be followed for the final pregnancy outcome.

7.4.2 Subject Complaints

If a complaint is received by anyone on the study staff, it will be discussed with the study staff and will be addressed on a case-by-case basis. The PI will be notified of any complaints. Complaints will be reported to the IRB if indicated.

If the subject has questions about his or her rights as a study subject, wants to report any problems or complaints, obtain information about the study or offer input, the subject can call the UNC Hospital research subject advocate or the study PI at 984-974-1583. This information is provided to the subject in their consent.

7.4.3 Routine Reporting Procedures for AEs

Study staff must report the Adverse Event form within 7 days of each study transfusion. The Adverse Event Checklist will indicate whether or not one or more of the events listed in Section 8.2 occurred during the transfusion. The PI will be also be notified of the identified event within 24 hours of learning of the event by phone or email.

Since this is an investigator-initiated study, the principal investigator, also referred to as the sponsor-investigator, is responsible for reporting serious adverse events (SAEs) to any regulatory agency and to the sponsor-investigator's IRB. Regardless of expectedness or causality, all SAEs (including serious pretreatment events) must also be reported to the DSMB as soon as possible, but no later than five calendar days after the sponsor-investigator's observation or awareness of the event.

Signs or symptoms reported as adverse events will be graded and recorded by the investigator, according to the CTCAE. When possible, signs and symptoms indicating a common underlying pathology should be noted as one comprehensive event.

The investigator will assess all adverse events and determine reporting requirements to the UNC Safety Monitoring Committee (SMC) and MCW's Institutional Review Board. The investigator will report SAEs to any required regulatory agency and to the sponsor-investigator's IRB.

All adverse events, whether or not unexpected, and whether or not considered to be associated with the use of the study drug, will be entered into the studies AE log.

Reporting to the Safety Monitoring Committee

Regardless of expectedness or causality, all SAEs (including serious pretreatment events) must also be reported to the SMC as soon as possible, but no later than five calendar days of the sponsor-investigator's observation or awareness of the event.

Report Method: The investigator will use email to report SAEs to the SMC. The SAE report must include event term(s), serious criteria and the sponsor-investigator's or sub-investigator's determination of both the intensity of the event(s) and the relationship of the event(s) to study drug administration. Intensity for each SAE, including any lab abnormalities, will be determined by using the NCI CTCAE as a guideline whenever possible.

Reporting to MCW Committee Institutional Review Board

The principal investigator must report events to the MCW IRB within five business days of his/her awareness of the event.

Event Type	Report Recipients					
	PI/Study Chair/ Coordinating Center	**Institutional Review Board**	**DSMC**	**FDA**	**CTO Regulatory Office**	**Other**
Serious Adverse Event	ASAP	5 days (or annual CPR)[1]	5 days	7 or 15 days[2]	ASAP	
Unanticipated Problems Involving Risks to Subjects of Others	ASAP	5 days (or annual CPR)[1]	5 days	7 or 15 days[2]	ASAP	
Evidence of Causal Relationship between Drug and AE	ASAP	5 days (or annual CPR)[1]	5 days	7 or 15 days[2]	ASAP	

Contacts

Role	Name	Entity/Department	Institution	Telephone	Email
Investigator	Matthew Karafin		UNC	984-974-1583	Matthew.karafin@unc.edu
Research Coordinator	David Wichlan		UNC	*919-966-6876*	*david_wichlan@med.unc.ed*

Footnotes

[1] Consult UNC IRB Policies (contact your regulatory representative)

[2] FDA guidelines: Suspected adverse reaction, Unexpected and Serious = 7 Days; If not = 15 days

8 INTERVENTION INFORMATION

8.1 Agent #1*: Chromium-labeled red cells*

8.1.1 Product Description: Chromium-labeled red cells is an FDA approved cross-match compatible red cell transfusion product.

Contraindications: Pregnancy

Side Effects: None known.

Solution Preparation

1. Prepare the Cr-51 dose.
2. With a 20 gauge needle, withdraw 50 millliter of the unit and immediately transfer syringe contents to the ACD vial using a 25 gauge needle as an airway. Mix gently.
3. From the ACD vial, withdraw 4-6 milliliter of whole blood and place in a lavender top tube labelled background.
4. Label the red blood cells by adding 200 microcurie of Cr-51 to the ACD vial and mix gently. Incubate at room temperature for 30 minutes, mixing gently every 10 minutes.
5. After the 30-minute incubation of Cr-51 with blood, add 50 milligram of ascorbic acid to the ACD vial, mix gently and let stand for 5 minutes.
6. Withdraw all of the Cr-51 labelled RBC into a 60 milliliter syringe and inject the patient. Make note of the location of injection.

8.1.2 Investigational Agent Administration

The 51Cr labeled red cells will be infused intravenously (over 1 minute through one IV line) into the subject.

8.1.3 Storage Requirements

The 51Cr labeled red cells will be stored at 1-6° C per standard blood bank storage conditions for red cells.

8.1.5 Route of Administration

The 51Cr labeled red cells will be infused intravenously (over 1 minute through one IV line) into the subject via a midline catheter that is placed by interventional radiology prior to the infusion.

8.1.6 Nursing Implications

The infusion will be provided by a trained assigned staff member of the Nuclear Medicine Department.

8.1.7 Handling

The 51Cr labeled red cells will be stored at 1-6° C per standard blood bank storage conditions for red cells. The prepared red cell product is intended to be infused as soon as the labeling step is complete.

8.1.8 Agent Ordering

The UNC Nuclear Medicine Department will order and create the compound as necessary for this single site study.

9 STATISTICAL CONSIDERATIONS

9.1 Study Measures

9.1.1 Outcome Variables

For Aims 1 and 2, the outcome measures of interest will be obtained from blood samples on 15 occasions within each of Period1 and Period2: At baseline prior to the infusion treatment.
Post-infusion at 5, 7.5, 10, 12.5, 15, 30 minutes, and at 1, 24, 48, 72 hours, and at 1, 2, 3, 4 weeks.

For Aim 1, for purposes of the main analysis the measurements of ^{51}CR-labeled PTR of RBCs prior to infusion and at 24 hours post-infusion are of interest. The units of measurement will be % recovery.

For Aim 2a and b, the outcome measures are Hemoglobin A and the following other clinically-relevant measures. We will evaluate their longitudinal tragectories over 4 weeks:

- complete blood count with differential (CBCD: RBCs, WBCs, PLTsHCT, PCV, Hgb, MPV, throbocytes)
- reticulocyte count per RBCs (%),
- Hgb A, Hgb A2, Hgb F (% of total by electrophoresis)
- cell-free hemoglobin (mg/dL),
- haptoglobin (mg/dL),
- Iron (mcg/dL), TIBC (mcg/dL), transferrin saturation (%), ferritin (mcg/L),
- bilirubin values (total, direct, indirect) (mg/dL),
- high-sensitivity C-reactive protein (HSCRP) (mg/L),
- lactate dehydrogenase (LDH) (U/L),
- myeloperoxidase (MPO) (pmol/L),
- labile plasma iron (LPI) (µmol/L)
- non-transferrin bound iron (NTBI) (µmol/L)

Note: normal and valid values of these measurements will be determined by the clinical laboratories performing these assays.

For Aim 2c, the outcomes of interest are the metabolomic profile estimates obtained for each blood sample. The unit of measure will be (µmol/L)

For Aim 2d, pain scores, occurrence of infection, and antibiotic use will be obtained via a daily 4-week diary for evaluation of pain on an ordinal scale (0,1,2,… , 10), occurrence of infection symptoms, and use of antibiotics (type, duration, dose).

9.1.2 Baseline Characteristics of the Participants

At enrollment, the following will be recorded: age, gender, history of past red cell transfusions, red cell phenotype, medical history information, and surgical history information.

9.2 Study Design

For this Phase II, single-blind, controlled trial, the experimental design is a two-treatment, two-period, two-sequence cross-over design: CO(2,2,2). The two treatment regimens are

Regimen **A** transfusion of one G6PD-deficient donor units stored 15-25 days

Regimen **B** transfusion of one G6PD-normal donor units stored 15-25 days

The two sequences of treatment are **AB** and **BA**. The two 4-week periods will be separated by a 4-month washout interval. We believe the 4-month washout interval will be adequate to prevent carryover effects from period1 into period2 because RBCs normally only survive 120 days (4 months) at their maximum normal survival.

9.3 Randomization, Concealment and Blinding

The two sequences (**AB** and **BA**) will be assigned to the N=16 enrollees as determined by RBC unit availability at the time of the first exchange transfusion. The two 4-week periods will be separated by a 4-month washout interval.

In crossover studies, reliably concealed unit assignment is the basis for the important assumption that 'sequence effects are zero'. Per the CONSORT Statement, allocation concealment will be used to prevent selection bias by concealing the allocations from the study personnel who are responsible for enrolling and assigning participants --until the moment of assignment.

After consent, one RBC unit will be requested for the study transfusion. The study staff member will then contact UNC transfusion service to confirm that the transfusion service has a units that meet the crossmatch requirements (ABO, sickle negative, antigen matched) for this subject. The patient will then be enrolled in the study and assigned to a treatment sequence (**AB** or **BA**) depending upon RBC unit availability. The subject will be contacted regarding the date of their study transfusion once a suitable unit (G6PD deficient or G6PD normal) becomes available, and reaches the appropriate storage age, per protocol.

9.4 Replacement Policy

Replacement of enrolled participants who discontinue the study is not recommended because it be a source of selection bias. It is preferable to choose a target enrollment that is sufficiently large to be able to cope with dropout.

9.5 Accrual Estimates

The Adult Sickle Cell Disease clinic at UNC sees about 450 patients per year, of which only 50 would be eligible due to being on chronic transfusion. Given known estimates of alloimmunization, 75% of these patients would be eligible to approach for consent (N=37). Due to the complexity of the study, target enrollment will aim for 4-8 research subjects per year, with a 3-4 year timeframe for study completion. Additional sites may be considered at the discretion of the principal investigator to meet enrollment goals.

9.6 Statistical Analysis Plans

Overview. The aim-specific analysis plans include (1) detailed steps for the major estimators and inferential analyses, (2) sensitivity analyses performed to assess the robustness of the main results to reasonable perturbations/modifications of the *a priori* assumptions, choices, and methods used, (3) a role for outcome-dependent exploratory analyses for hypothesis generation, and (4) necessary descriptive graphical and tabular methods used to characterize the sample of cows, visualize the data, and examine relationships among variables. Assuming the reasons for missing data values were appropriately documented in/with the database, then best practices for dealing with incomplete data will depend on the documented causes of missing values.

The main analyses will focus on the magnitude and direction of point- and interval-estimates of the population parameters of interest; e.g., the treatment effect and the regimen-specific means and variances. To indicate precision, all statistical estimates of population parameters will be tabulated along with corresponding confidence intervals (CI) and standard errors (SE). The CI will be interpreted as a set of plausible values of the population parameter that are most compatible with the observed data. The point- and interval-estimates will be treated as the most important results of the study because it is most appropriate to

ask quantitative research questions and obtain quantitative answers. P-values for hypothesis tests will be of less interest because they only address binary questions such as “Is the treatment effect exactly zero in the target population of cows?” Following the recent recommendations of the American Statistical Association as explained by Wasserstein, et al. (2019), and Wasserstein, et al. (2016), p-values will be reported to four decimal places and will not be dichotomized. This approach acknowledges that no p-value can reveal the plausibility, presence, truth, or importance of an association or effect. Smaller p-values indicate a larger degree of evidence against the (null) hypothesis tested and/or assumptions made, whereas larger p-values indicate that the test is inconclusive due to scarcity of information.

Uncertainty about the optimal choice of methods and assumptions is best handled by relegating competing approaches to an important role in the domain of sensitivity analyses. The various sensitivity analyses will be used to evaluate the robustness/sensitivity of the study’s main results to reasonable perturbations/modifications of the statistical methods and assumptions used. Results of the sensitivity analyses will be used to guide our level of trust in the main results.

[Reference: 2019 ICH E9-R1 addendum, “Estimands and Sensitivity Analysis in Clinical Trials”. www.gmp-compliance.org/guidemgr/files/E9-R1_Step4_Guideline_2019_1203.pdf]

Exploratory analyses will be distinguished from confirmatory analyses. The purpose of exploratory analyses will be to generate new hypotheses or to refine hypotheses; p-values will not be used in exploratory analyses.

Statistical computations will be performed using software from Cytel (StatXact and LogXact), SAS Institute (SAS version 9.2), and Salford systems (CART).

Analysis Plan for Aim 1. The longitudinal analysis of the effects of treatment on 24-hour ^{51}CR-labeled PTR of RBCs will rely on a generalized linear mixed-effects model which expresses mean response as a function of *treatment_regimen*, *treatment_period*, and *occasion*. The baseline PTR value will be treated as being one of the outcomes. The fitted model will be used to obtain parameter estimates that characterize the 24-hour trajectory of the mean response for each of the two regimens. Graphical figures will be used to display the resulting point- and interval-estimates.

Hypothesis testing. An F-test procedure will be used to test the null hypothesis “the treatment effect is exactly zero in the target population”.

Missing data. For this main analysis, all enrollees with at least one post-exchange transfusion evaluation will be included. We anticipate that the main analyses will rely on an assumption that missing data values, if any, are attributable to ignorable causes (i.e., the mechanisms satisfy the “missing at random” (MAR) criteria.)

Sensitivity analyses. After the main analysis has been conducted, sensitivity analyses will be used to evaluate the robustness/fragility of the main results to reasonable perturbations of the statistical methods and assumptions used. Results of the sensitivity analyses will only be used to guide trust in the main results. The sensitivity analyses will include, for example: diagnostics for goodness of fit and influential observations, and analysis of residuals for examination of distributional assumptions; evaluation of the impact of using alternative statistical procedures (for coping with missing values); investigation of the impact of including / excluding questionable data values. As part of the sensitivity analyses, investigation of missing values will include use of logistic regression methods to investigate apparent causes for missing data, and multiple imputations methods. The impact of using alternative assumptions regarding the variance covariance structure will also be included in the sensitivity analyses. The impact of using alternative distributional assumptions and transformations of scales will also be considered.

Auxiliary analyses. Longitudinal analyses of the PTR data spanning all 15 occasions per period will also be performed to characterize the trajectory of the mean response for each regimen over 4 months.

Analysis Plan for Aim 2a. The longitudinal analysis of the effects of treatment on Hemoglobin A will rely on the same analysis strategy as described for Aim 1 for Estimation, Hypothesis testing, handling Missing data, and Sensitivity analyses.

Analysis Plan for Aim 2b. For each of the 26 outcome variables, a longitudinal analysis of the effects of treatment on mean response will rely on an analysis strategy similar to that described in Aim 1 for Estimation, handling Missing data, and Sensitivity analyses. Regardless of scale, the *strategy* for obtaining the main results is the same for each regimen and for the difference between the regimens, point estimates of central

tendency and variance will be obtained along with 95% confidence intervals to indicate precision.

Analysis Plan for Aim 2c. For the purpose of hypothesis generation, exploratory statistical methods (reference: Tukey, 1977) and exploratory predictive modeling methods will be used to investigate associations between *treatment_regimen* and longitudinal metabolomic profiles.

Analysis Plan for Aim 2d. For each of the outcome variables [pain on an ordinal scale {0,1,2,… , 10}, occurrence of infection symptoms, and use of antibiotics (type, duration, dose)], a longitudinal analysis of the effects of treatment on mean response will rely on an analysis strategy similar to that described in Aim 1 for Estimation, handling Missing data, and Sensitivity analyses.

9.7 Interim Analyses

No interim analyses will be performed.

9.8 Analysis of Safety Data

Descriptive and tabular analyses of the safety data will be performed based on information from all patients having received at least one transfusion. The study will use the NCI CTCAE v4.0.

9.9 Sample Size Rationale

Given that the experimental design is a 2-treatment 2-period 2-sequence crossover, the number of patients enrolled should be an even number; thus, we will enroll N=16 participants and assign them depending upon RBC unit availability for the first exchange transfusion. The primary considerations in choosing N = 16 enrollees as the target sample size included:

- the anticipated frequency of missing data,
- anticipated levels of precision of estimators,
- anticipated levels of power of hypothesis tests,
- costs and time requirements.

Our assessment of anticipated levels of precision and power focused on Aim 1. Those considerations were informed by a previous study[79] of 24-hour posttransfusion RBC recovery (PTR %), in which the point-estimates and interval-estimates of the population standard deviation (σ) of PTR were as follows:

Previous study[79]		Estimates of the mean		Estimates of σ	
	n	estimate	95%CI	estimate	95%CI
Normal donors	27	85.3%	[84.1, 86.5]	3.2%	[2.5, 4.4]%
G6PD-deficient	10	78.5%	[73.2, 83.7]	8.4%	[5.8, 15.3]%

Precision of estimators of the means in Aim 1. The above table suggests conjectures about the magnitudes of standard errors (SE) of the means that can be anticipated for the proposed crossover study:

Proposed crossover study (n=16)	SE of the mean = SD/($16^{1/2}$)	
	Expected	Plausible Range
A: units from normal donors	0.8%	[0.6, 1.1]%
B: units from G6PD-deficient donors	2.1%	[1.5, 3.8]%
A vs **B**: mean difference (if ρ=0.5)[1]	1.8%	[1.3, 3.4]%
A vs **B**: mean difference (if ρ=0.0)[2]	2.2%	[1.6, 4.0]%

1 The SEs in the 3rd row correspond to SD = 7.3% and [5.0, 13.6]%.

2 The SEs in the 4th row correspond to SD = 9.0% and [6.3,15.9]%.

This table of SEs suggests that precision will be adequate for estimation of regimen-specific mean PTR% in the proposed crossover study of n=16 patients. For example, the width of the 95%CI for the regimen-**A** mean is expected to be ± 1.6% (i.e., 1.96 standard errors). The width of the 95%CI for the regimen-**B** mean is

expected to be ± 4.2%, approximately. The third row in this table conservatively assumes that the correlation between a pair of PTR values is "moderate" (ρ=0.5). The fourth row's assumption (ρ=0) is ultra-conservative.

Power of a test procedure in Aim 1. We consider a test of the null hypothesis that "The PTR(%) mean difference between regimens **A** and **B** is exactly zero in the target population of patients." If we assume that
(1) the mean absolute PTR difference is Δ=10% in the target population of current and future SCD patients,
(2) the SD of the differences in the target population is σ_{diff} =7.3% (or plausibly 5.0% $\leq \sigma_{diff} \leq$ 13.6%)
(3) the correlation between paired PTR measures (for **A** and **B**) is ρ = 0.50 in the target population, and
(4) the sample size is n = 16 participants with complete data,
then the chance of drawing a sample of patients that would yield a p-value smaller than α = 0.05 is 99%; this power level drops to 78% if σ_{diff} = 13.6%. If Δ=5% and σ_{diff} = 7.3% in the target population, then the power level would be 72%; this power level drops to 28% if σ_{diff} = 13.6%.

While the planned analysis strategy for Aim 1 will rely on a longitudinal linear mixed-effects model that accounts for period effects, the above estimates of anticipated precision and power are based on the assumptions of a simplistic model for paired 24hr PTR (%) outcomes. The resulting estimates are thus approximate but provide reasonable guidance about precision and power for the proposed study.

In summary, our sample size analysis suggests that a target enrollment of N=16 participants is adequate to provide satisfactory levels of precision for the estimators of mean PTR(%) and is expected to easily provide a high level of power for a test of difference when the treatment effect is near 10% or larger --even if a few participants do not have complete data. The estimates suggest that a smaller sample size would not provide adequate precision for key estimators (i.e., the confidence intervals would be too wide).

10 PLANS TO ENSURE DATA QUALITY

Database System for Data Capture
Data will be collected and entered into a web-based data management system (RedCAP). The lead coordinator will routinely verify that all data entry fields are entered. Verifications are question-by-question checks that give immediate feedback to help catch data entry errors, form completion errors, and out-of-range values. Reports of outstanding edits, generated upon completion of data entry, will enable continuous cleaning of 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 room or locked cabinet.

Data Management Plans
The Principal Investigator will serve as the trial coordinator for this study. The PI or designee will monitor timely entry of data into the study database. Access to all source documentation maintained by the lead coordinator, including correspondence and source data, will be available for monitoring and audit purposes.

11 DATA AND SAFETY MONITORING PLAN (DSMP)

Disposition of Participants

1) The subject completes the 2 chromium infusions and associated lab draws
2) The subject decides to withdraw from the study
3) The subject moves away, dies, or is lost to follow-up
4) Subjects may be removed from the study, or treatment stopped by the Investigator for any of the following reasons:
    - Occurrence of CTCAE Criteria of grade 3 or higher that are deemed attributable to the chromium infusion per the opinion of the PI
    - PI decides to withdraw the subject due to noncompliance
    - Withdrawal by the PI or subject because of transfusion side effects or complications

Data and Safety Management Overview

The UNC Safety Monitoring Committee (SMC) and the UNC Institutional Review Board (IRB) will approve protocol-specific DSM plans. A local, investigator-initiated trial will be required to be continuously monitored by the principal investigator of the study with annual safety and progress reports submitted to the SMC.

11.1 Study Team

The study team minimally consists of the principal investigator, the clinical research coordinator, regulatory specialist and the study biostatistician. While subjects are on treatment, the principal investigator will meet regularly with the research coordinator and the study biostatistician to review study status. This review will include but not be limited to reportable SAEs and update of the ongoing study summary that describes study progress in terms of the study schema. The appropriateness of further subject enrollment and the specific intervention for a next subject enrollment is addressed.

11.2 Quality Assurance

The UNC Clinical Trials Office provides ongoing quality assurance audits.

11.3 SMC

UNC places the highest priority on ensuring the safety of patients participating in clinical trials. Every clinical trial conducted at UNC includes a plan for safety and data monitoring.

The SMC is an independent board appointed by the PI composed of three members. The principal role of the SMC is to regularly monitor the data from the clinical trial, review and assess the performance of its operations, and make recommendations, as appropriate, to the PI and IRB.

The PI will be notified within 24 hours of learning of an event when a serious adverse event possibly, probably or definitely related to red cell transfusion or an event resulting in death (regardless of attribution) is reported. The PI will review the event as soon as the materials are available. The PI may request additional information regarding the event and may request the subject's treatment arm assignment. Following the review, the PI will sign a log summarizing the event.

The SMC chairperson will receive information on all serious adverse events possibly, probably, or definitely related to red cell transfusion and all events resulting in death (regardless of attribution) within 7 days of the PI becoming aware of the event. The SMC chair will have expertise in benign hematology and transfusion medicine, will review the serious adverse event materials, and determine if the information is complete.

The SMC members will meet annually via teleconference. All adverse events will be reviewed during the call, and the group will determine if additional SMC review is required, and make recommendations about the study.

This study will be reviewed by a Safety Monitoring Committee (SMC). A summary of the SMC activities are as follows:

- Review the clinical trial for data integrity and safety.
- Review all unexpected grade 3, and all grade 4, and 5 adverse events, as well as any others requiring expedited reporting as defined in this protocol. (Grades 4 and 5 events must be reported to the SMC within five calendar days of study staff's knowledge.)
- Submit a summary of any recommendations related to study conduct.
- Terminate the study if deemed unsafe for patients.

A copy of the UNC Data and Safety Monitoring Plan and membership roster will be maintained in the study research file and updated as membership changes. The committee will review reports from the study principal investigator annually (or more frequently if needed) and provide recommendations on trial continuation, suspension or termination as necessary.

For this pilot study, the site-PI will appoint three members to a Safety Monitoring Committee (SMC). These will be investigators from institutions with expertise in transfusion medicine, SCD, and biostatistics. They will be charged with monitoring the accruing data to confirm that the patients in the trial are being cared for safely.

The SMC will meet at least once per year, and will be responsible for:
1) reviewing and analyzing the progress of the study
2) approving amendments to the trial protocol
3) monitoring the safety of the study treatments and diagnostic procedures
4) ensuring data quality
5) reviewing interim analyses and recommending early stopping or continuation of the trial
6) reviewing recruitment and event rates.

The data safety monitoring plan will ensure that the site is in compliance with Federal regulations, Good Clinical Practice and Good Manufacturing Practice Guidelines, as applicable.

The SMC will meet at least once per year, either in-person or via teleconference. Prior to the meeting, the site-PI will provide the SMC with:
• Accrual totals
• Adverse event log reports for each study subject
• Serious adverse event reports for each study subject
• Study compliance issues
• The SMC will also be provided each subject's study arm assignment by the primary site biostatistician.

The SMC will determine:
• All-cause mortality
• Number, type, and severity of serious adverse events
• Number of subjects in each treatment arm with at least one clinically documented pain crisis leading to an ED or hospital admission

• Unexpected adverse events and unanticipated problems
• Serious infection or pain admissions

For the sessions, results will be overall and by RBC unit group.

Any available SMC letters will be submitted to the IRB of record as required.

12 REGULATORY COMPLIANCE, ETHICS AND STUDY MANAGEMENT

12.1 Ethical Standard

This study will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki as stated in 21 CFR §312.120(c)(4); consistent with GCP and all applicable regulatory requirements.

12.2 Regulatory Compliance

This study will be conducted in compliance with:

- The protocol
- Federal regulations, as applicable, including: 21 CFR 50 (Protection of Human Subjects/Informed Consent); 21 CFR 56 (Institutional Review Boards) and §312 (Investigational New Drug Application; and 45 CFR 46 Subparts A (Common Rule), B (Pregnant Women, Human Fetuses and Neonates), C (Prisoners), and D (Children), GCP/ICH guidelines, and all applicable regulatory requirements. The IRB must comply with the regulations in 21 CFR §56 and applicable regulatory requirements.

12.3 Prestudy Documentation

Prior to implementing this protocol at UNC, the protocol, informed consent form, HIPAA authorization and any other information pertaining to participants must be approved by the UNC IRB.

13.4 Institutional Review Board

The protocol, the proposed informed consent form and all forms of participant information related to the study (e.g., advertisements used to recruit participants) will be reviewed and approved by the MCW Institutional Review Board. Prior to obtaining MCW approval. The initial protocol and all protocol amendments must be approved by the IRB prior to implementation.

Informed Consent Process

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. Extensive discussion of risks and possible benefits of this therapy will be provided to the subjects and their families. Consent forms describing in detail the study interventions/products, study procedures and risks are given to the subject and written documentation of informed consent is required prior to starting intervention/administering study product.

Consent forms will be IRB-approved and the subject (and legally authorized representative, if necessary) will be asked to read and review the document. Upon reviewing the document, the investigator will explain the research study to the subject and answer any questions that may arise. In accordance with 46 CR 46.111, the subject will sign and date the informed consent document prior to any procedures being done specifically for the study.

A witness should only sign when required, per UNC IRB policy. If a witness signs the document when not required, the study staff should document in the legal medical record (or note to file) the relationship to the patient and why a witness signed. (i.e., “Although not required, the subject’s spouse was present during the consenting process and signed as the witness.” Or “Although not required, hospital staff was present for consenting process and signed as a witness.”)

The subjects will have the opportunity to discuss the study with their surrogates or think about it prior to agreeing to participate. The subjects may withdraw consent at any time throughout the course of the trial.

A copy of the informed consent document will be given to the subjects for their records. The rights and welfare of the subjects will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study. If there are changes to the consent form, all revisions will be reviewed with study subject at the next appropriate opportunity. Patients who require reconsenting will be defined in the IRB approved amendment submission. The process for obtaining informed consent will again be performed. Study subjects will not be reconsented for continuing reviews. The UNC research staff will follow the UNC IRB’s policy for subjects who demonstrate limited English proficiency or limited literacy.

After the subject’s visit in which the consent is signed, it is documented in the clinic chart that the consent has been signed and that all questions have been answered to the subject’s satisfaction after adequate time for review of the consent. It is also documented that a copy of the consent is given to the subject. The original consent is kept with the subject’s study file, and a copy of the consent is sent to the OCRICC office, which will then submit to HIM a copy of the signed consent to be scanned into EPIC, the legal medical record.

13.5 Subject Confidentiality and Access to Source Documents/Data

Subject confidentiality is strictly held in trust by the sponsor/sponsor-investigator, participating investigators, and any staff. This confidentiality includes the clinical information relating to participating subjects, as well as any genetic or biological testing.

The study protocol, documentation, data and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the principal investigator.

The conditions for maintaining confidentiality of the subjects’ records are required for the life of the data.

One risk of taking part in a research study is that more people will handle the personal health information collected for this study. The study team will make every effort to protect the information and keep it confidential, but it is possible that an unauthorized person might see it. Depending on the kind of information being collected, it might be used in a way that could embarrass the subject or affect his/her ability to get insurance.

While data are being collected and after all data have been collected but are still in the process of being analyzed, the subject’s data/PHI are stored in the locked UNC Clinical Research Office. Databases in which the study subject information is stored and accessed are password protected, allowing for limited access by authorized personnel only. Data/PHI kept in the case report forms contain the study identifiers, subject initials, date of birth and date of service.

Personal identifiers, such as name and medical record number, will be removed from accompanying lab reports and test results. Any data/PHI that are not stored for the purposes of the study are shredded in the Clinical Trials Office.

After all study queries and analyses are completed, the data/PHI will not be destroyed but will be archived in a secure long-term storage site in order to keep an accurate record of screened and enrolled subjects for the sponsor and potential audit purposes only specific for this study.

The Investigator will maintain the signed Informed Consent Forms, CRFs, study documentation and source documents for at least 10 years after study completion or termination per UNC Institutional policy. In addition, the Investigator will not discard or destroy any study-specific materials unless otherwise instructed.

The principal investigator will allow access to all source data and documents for the purposes of monitoring, audits, IRB review and regulatory inspections.

13.6 Protection of Human Subjects

13.6.1 Protection from Unnecessary Harm

The PI and study personnel are responsible for protecting all subjects involved in human experimentation. This is accomplished through the IRB mechanism and the informed consent process. The IRB reviews all proposed studies involving human experimentation and ensures that the subject's rights and welfare are protected and that the potential benefits and/or the importance of the knowledge to be gained outweigh the risks to the individual. The IRB also reviews the informed consent document associated with each study in order to ensure that the consent document accurately and clearly communicates the nature of the research to be done and its associated risks and benefits.

13.6.2 Protection of Privacy

As noted, patients will be informed of the extent to which their confidential health information generated from this study may be used for research purposes. Following this discussion, they will be asked to sign informed consent documents. The original signed document will become part of the patient's medical records, and each patient will receive a copy of the signed document.

13.7 Changes in the Protocol

Once the protocol has been approved by the UNC IRB, any changes to the protocol must be documented in the form of an amendment. The amendment must be signed by the investigator and approved by IRB prior to implementation.

If it becomes necessary to alter the protocol to eliminate an immediate hazard to patients, an amendment may be implemented prior to IRB approval. In this circumstance, however, the investigator must then notify the IRB in writing within five working days after implementation.

The IRB may provide, if applicable regulatory authority(ies) permit, expedited review and approval/favorable opinion for minor change(s) in ongoing studies that have the approval /favorable opinion of the IRB. The investigator will submit all protocol modifications to the sponsor and the regulatory authority(ies) in accordance with the governing regulations.

Changes to the protocol may require approval from the sponsor.

Any departures from the protocol must be fully documented in the source documents.

13.8 Investigator Compliance

The investigator will conduct the study in compliance with the protocol given approval/favorable opinion by the IRB and the appropriate regulatory authority(ies).

<u>Onsite Audits</u>

Auditing is essential to ensure that research conducted at the UNC is of the highest quality and meets UNC and regulatory agency standards.

Regulatory authorities, the IRB may request access to all source documents, data capture records and other study documentation for on-site audit or inspection. Direct access to these documents must be guaranteed by the investigator, who must provide support at all times for these activities.

13 DATA HANDLING AND RECORD KEEPING

Case Report Forms

Case Report Forms (CRFs) will be used to collect all subject data during the course of the study. Data from these forms will be entered into the study EDC. 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. Completed CRFs will be verified at regular intervals throughout the study by the principal investigator.

Record Retention

The Investigator will maintain the signed Informed Consent Forms, CRFs, study documentation (listed above) and source documents for at least 10 years per after study completion or termination per UNC Institutional policy. In addition, the Investigator will not discard or destroy any study-specific materials unless otherwise instructed.

13.1 Overview

Every effort is made to uphold the integrity of the project, the research, the institution and the researchers involved. Data collection guidelines and methodologies are carefully developed before the research begins. Investigators focus on the following to ensure data integrity: well-trained data collectors/recorders to ensure consistency and quality, well-designed data collection protocols and ongoing monitoring. In this way, study rigor and validity are maintained. Data is protected from physical damage as well as from tampering, loss or theft. This project's data management is a multidisciplinary activity that includes investigators, research coordinators and nurses, data mangers, support personnel, biostatisticians and database programmers. Quality control will be applied to each stage of data handling to ensure that all data are reliable and have been processed correctly.

14.2 Data Management Responsibilities

14.2.1 Principal Investigator
The principal investigator oversees the management of patient records/case report forms and ensures that a) complete and accurate data will be obtained and provided to the sponsor; b) patient records are maintained to include history, prescribed medication and investigational product(s), measurements, exams, evaluations and adverse events; c) corrections are applied to clinical research data according to principles of good research practice (i.e., single-line delete, date and initial). He or she will ensure that there is correlation between the case report forms and the source documents**.**

14.2.2 Research Coordinator
A research coordinator creates, collects and organizes clinical trial documentation. He or she ensures that source documentation and data abstraction and entry are being done at protocol specified time points.

14.2.3 Research Nurse/Medical Staff
The research nurse and medical staff document protocol-required care or assessment of the subject's outcomes, adverse events and compliance to study procedures.

14.2.4 Biostatistician

The biostatistician may assist in CRF development (content and design), dataset specifications (annotation of CRFs and record layout) and validation. The study biostatisican will all perform all statistical computations associated with Aims 1 and 2a-d and be involved in manuscript preparation from this data.

14.4 Source Documents

Good Manufacturing 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:

- Medical history/physical condition of the subject before enrollment sufficient to verify protocol entry criteria
- Dated and signed notes for specific results of procedures and exams
- Good Manufacturing Practices require the nuclear pharmacist (or designee) to record date, time, and responsible person completing all critical steps in the manufacturing processing, including but not limited to the receipt, preparation, processing/manufacturing, labeling, distribution, and infusion of the radiolabeled red blood cell product. This should include records of accountability and chain of identity / chain of custody for the radiolabeled product.

All source documents will be written following ALCOA standards:

ALCOA Attribute	Definition
Attributable	Clear who has documented the data.
Legible	Readable and signatures identifiable.
Contemporaneous	Documented in the correct time frame along with the flow of events. If a clinical observation cannot be entered when made, chronology should be recorded. Acceptable amount of delay should be defined and justified.
Original	Original, if not original should be exact copy; the first record made by the appropriate person. The investigator should have the original source document.
Accurate	Accurate, consistent and real representation of facts.
Enduring	Long-lasting and durable.
Available and accessible	Easily available for review by treating physicians and during audits/inspections. The documents should be retrievable in reasonable time.
Complete	Complete until that point in time.
Consistent	Demonstrate the required attributes consistently.
Credible	Based on real and reliable facts.
Corroborated	Data should be backed up by evidence.

14.5 Case Report Forms

The principal investigator and/or his/her designee will prepare and maintain adequate and accurate participant case histories with observations and data pertinent to the study. Study-specific case report forms (CRFs) will document safety and treatment outcomes for safety monitoring and data analysis. All study data will be entered into the EDC via standardized CRFs, in accordance with the study calendar, using single data entry with a secure access account. The clinical research coordinator will complete the CRFs as soon as possible upon completion of the study visit; the investigator will review and approve the completed CRFs.

The information collected on CRFs shall be identical to that appearing in original source documents. Source documents will be found in the patient's medical records maintained by UNC personnel. All source documentation should be kept in separate research folders for each patient.

In accordance with federal regulations, the investigator is responsible for the accuracy and authenticity of all clinical and laboratory data entered in CRFs. The principal investigator will approve all completed CRFs to attest that the information contained on the CRFs is true and accurate.

All source documentation and data will be available for review/monitoring by the SMC and regulatory agencies.

14.6 Study Record Retention

Source documents will be maintained for 10 years after data analysis has been completed and the study in closed. After which all materials will be destroyed.

The principal investigator is required to maintain adequate records of the disposition of the drug, including dates, quantity and use by subjects, as well as written records of the disposition of the drug when the study ends.

The principal investigator is required to prepare and maintain adequate and accurate case histories that record all observations and other data pertinent to the investigation on each individual administered the investigational drug or employed as a control in the investigation. Case histories include the case report forms and supporting data including, for example, signed and dated consent forms and medical records including, for example, progress notes of the physician, the individual's hospital chart(s), and the nurses' notes. The case history for each individual shall document that informed consent was obtained prior to participation in the study.

Study documentation includes all CRFs, data correction forms or queries, source documents, and regulatory documents (e.g., protocol and amendments, IRB correspondence and approval, signed patient consent forms).

Source documents include all recordings of observations or notations of clinical activities and all reports and records necessary for the evaluation and reconstruction of the clinical research study.

14.7 Publishing Data

The UNC PI will possess the data and be responsible for publishing. The University of Columbia will have access to the data and may also utilize the data for publishing with the approval of the UNC’s PI.

REFERENCES

1. Pfuntner A, Wier LM, Stocks C. Most Frequent Procedures Performed in U.S. Hospitals, 2010: Statistical Brief #149. Healthcare Cost and Utilization Project (HCUP) Statistical Briefs. Rockville (MD); 2013.
2. Spitalnik SL, Triulzi D, Devine DV, Dzik WH, Eder AF, Gernsheimer T, Josephson CD, Kor DJ, Luban NL,Roubinian NH, Mondoro T, Welniak LA, Zou S, Glynn S, State of the Science in Transfusion Medicine Working G. 2015 proceedings of the National Heart, Lung, and Blood Institute's State of the Science in Transfusion Medicine symposium. Transfusion 2015;55:2282-2290.
3. Zimring JC, Smith N, Stowell SR, Johnsen JM, Bell LN, Francis RO, Hod EA, Hendrickson JE, Roback JD, Spitalnik SL. Strain-specific red blood cell storage, metabolism, and eicosanoid generation in a mouse model. Transfusion 2014;54:137-148.
4. de Wolski K, Fu X, Dumont LJ, Roback JD, Waterman H, Odem-Davis K, Howie HL, Zimring JC. Metabolic pathways that correlate with post-transfusion circulation of stored murine red blood cells. Haematologica 2016;101:578-586.
5. D'Alessandro A, Rapido F, Thomas TA, Fu X, Francis RO, Spitalnik SL, Zimring JC, Hod EA. Metabolic Pathways That Correlate with In Vivo Hemolysis Following Storage of Human RBCs. AABB National Meeting 2017;San Diego:B16-A03I.
6. Rapido F, Brittenham GM, Bandyopadhyay S, La Carpia F, L'Acqua C, McMahon DJ, Rebbaa A, Wojczyk BS, Netterwald J, Wang H, Schwartz J, Eisenberger A, Soffing M, Yeh R, Divgi C, Ginzburg YZ, Shaz BH, Sheth S, Francis RO, Spitalnik SL, Hod EA. Prolonged red cell storage before transfusion increases extravascular hemolysis. J Clin Invest 2017;127:375-382.
7. Hod EA, Brittenham GM, Billote GB, Francis RO, Ginzburg YZ, Hendrickson JE, Jhang J, Schwartz J, Sharma S, Sheth S, Sireci AN, Stephens HL, Stotler BA, Wojczyk BS, Zimring JC, Spitalnik SL. Transfusion of human volunteers with older, stored red blood cells produces extravascular hemolysis and circulating non-transferrin-bound iron. Blood 2011;118:6675-6682.
8. Zimring JC. Established and theoretical factors to consider in assessing the red cell storage lesion. Blood 2015;125:2185-2190.
9. Dumont LJ, AuBuchon JP. Evaluation of proposed FDA criteria for the evaluation of radiolabeled red cell recovery trials. Transfusion 2008;48:1053-1060.
10. Luten M, Roerdinkholder-Stoelwinder B, Schaap NP, de Grip WJ, Bos HJ, Bosman GJ. Survival of red blood cells after transfusion: a comparison between red cells concentrates of different storage periods. Transfusion 2008;48:1478-1485.
11. Porter JB, Garbowski M. The pathophysiology of transfusional iron overload. Hematol Oncol Clin North Am 2014;28:683-701, vi.
12. Walter PB, Fung EB, Killilea DW, Jiang Q, Hudes M, Madden J, Porter J, Evans P, Vichinsky E, Harmatz P. Oxidative stress and inflammation in iron-overloaded patients with beta-thalassaemia or sickle cell disease. Br J Haematol 2006;135:254-263.
13. Nemkov T, Sun K, Reisz JA, Song A, Yoshida T, Dunham A, Wither MJ, Francis RO, Roach RC, Dzieciatkowska M, Rogers SC, Doctor A, Kriebardis A, Antonelou M, Papassideri I, Young CT, Thomas TA, Hansen KC, Spitalnik SL, Xia Y, Zimring JC, Hod EA, D'Alessandro A. Hypoxia modulates the purine salvage pathway and decreases red blood cell and supernatant levels of hypoxanthine during refrigerated storage. Haematologica 2018;103:361-372.
14. Recht J, Ashley EA, White NJ. Use of primaquine and glucose-6-phosphate dehydrogenase deficiency testing: Divergent policies and practices in malaria endemic countries. PLoS Negl Trop Dis 2018;12:e0006230.
15. Kuypers FA. Red cell membrane lipids in hemoglobinopathies. Curr Mol Med 2008;8:633-638.
16. Yawata Y, Kanzaki A, Inoue T, Ata K, Wada H, Okamoto N, Higo I, Yawata A, Sugihara T, Yamada O. Red cell membrane disorders in the Japanese population: clinical, biochemical, electron microscopic, and genetic studies. Int J Hematol 1994;60:23-38.
17. Morse EE. Mechanisms of hemolysis in liver disease. Ann Clin Lab Sci 1990;20:169-174.
18. Deplaine G, Safeukui I, Jeddi F, Lacoste F, Brousse V, Perrot S, Biligui S, Guillotte M, Guitton C, Dokmak S, Aussilhou B, Sauvanet A, Cazals Hatem D, Paye F, Thellier M, Mazier D, Milon G, Mohandas N, Mercereau-Puijalon O, David PH, Buffet PA. The sensing of poorly deformable red blood cells by the human spleen can be mimicked in vitro. Blood 2011;117:e88-95.

19. Macdougall LG. Red cell metabolism in iron deficiency anemia. III. The relationship between glutathione peroxidase, catalase, serum vitamin E, and susceptibility of iron-deficient red cells to oxidative hemolysis. Journal of Perinatology 1980;80:775-782.
20. Macdougall LG, Judisch JM, Mistry S. Red cell metabolism in iron deficiency anemia. II. The relationship between red cell survival and alterations in red cell metabolism. Journal of Perinatology 1970;76:660-675.
21. Ogunro PS, Ogungbamigbe TO, Muhibi MA. The influence of storage period on the antioxidants level of red blood cells and the plasma before transfusion. African Journal of Medicine and Medical Sciences 2010;39:99-104.
22. Yip R, Mohandas N, Clark MR, Sushil J, Shohet SB, Dallman PR. Red cell membrane stiffness in iron deficiency. Blood 1983;62:99-106.
23. Bennett-Guerrero E, Veldman TH, Doctor A, Telen MJ, Ortel TL, Reid TS, Mulherin MA, Zhu H, Buck RD, Califf RM, McMahon TJ. Evolution of adverse changes in stored RBCs. PNAS 2007;104:17063-17068.
24. Dumaswala UJ. Glutathione loading prevents free radical injury in red blood cells after storage. Free Radical Research 2000;33:517-529.
25. Dumaswala UJ, Zhuo L, Mahajan S, Nair PNM, Shertzer HG, Dibello P, Jacobsen DW. Glutathione protects chemokine-scavenging and antioxidative defense functions in human RBCs. American Journal of Physiology - Cell Physiology 2001;280:867-873.
26. Dumont LJ, Yoshida T, Aubuchon JP. Anaerobic storage of red blood cells in a novel additive solution improves in vivo recovery. Transfusion 2009;49:458-464.
27. Jozwik M, Jozwik M, Jozwik M, Szczypka M, Gajewska J, Laskowska-Klita T. Antioxidant defence of red blood cells and plasma in stored human blood. Clinica Chimica Acta 1997;267:129-142.
28. Rinalducci S, Marrocco C, Zolla L. Thiol-based regulation of glyceraldehyde-3-phosphate dehydrogenase in blood bank–stored red blood cells: a strategy to counteract oxidative stress. Transfusion 2014;54:in press.
29. Mohanty JG, Nagababu E, Rifkind JM. Red blood cell oxidative stress impairs oxygen delivery and induces red blood cell aging. Frontiers in Physiology 2014;5:1-6.
30. Rodvien R, Gillum A, Weintraub LR. Decreased glutathione peroxidase activity secondary to severe iron deficiency: A possible mechanism responsible for the shortened life span of the iron-deficient red cell. Blood 1974;43:281-289.
31. Knight JA, Searles DA, Clayton FC. The effect of desferrioxamine on stored erythrocytes: lipid peroxidation, deformability, and morphology. Annals of Clinical and Laboratory Science 2010;26:283-290.
32. Diez-Ewald M, Layrisse M. Mechanisms of hemolysis in iron deficiency anemia. Further studies. Blood 1968;32:884-894.
33. Farid Z, Nichols JH, Schulert AR, Bassily S. Chromium-51 red cell half-life in severe iron deficiency anemia. American Journal of Tropical Medicine and Hygiene 1965;14:605-609.
34. Layrisse M, Linares J, Roche M. Excess hemolysis in subjects with severe iron deficiency anemia associated and nonassociated with hookworm infection. Blood 1965;25:73-91.
35. Kempe DS, Lang PA, Duranton C, Akel A, Lang KS, Huber SM, Wieder T, Lang F. Enhanced programmed cell death of iron-deficient erythrocytes. The FASEB Journal 2005; 20:368-370.
36. Bandyopadhyay S, Brittenham GM, Francis RO, Zimring JC, Hod EA, Spitalnik SL. Iron-deficient erythropoiesis in blood donors and red blood cell recovery after transfusion: initial studies with a mouse model. Blood Transfus 2017;15:158-164.
37. Gilson CR, Kraus TS, Hod EA, Hendrickson JE, Spitalnik SL, Hillyer CD, Shaz BH, Zimring JC. A novel mouse model of red blood cell storage and posttransfusion in vivo survival. Transfusion 2009;49:1546-1553.
38. Loria A, Sanchez-Medal L, Lisker R, de Rodriguez E, Labardini J. Red Cell Life Span in Iron Deficiency Anaemia. British Journal of Haematology 1967;13:294-302.
39. D'Alessandro A, Nemkov T, Kelher M, West FB, Schwindt RK, Banerjee A, Moore EE, Silliman CC, Hansen KC. Routine storage of red blood cell (RBC) units in additive solution-3: a comprehensive investigation of the RBC metabolome. Transfusion 2015;55:1155-1168.
40. Pretsch W, Charles DJ, Merkle S. X-linked glucose-6-phosphate dehydrogenase deficiency in Mus musculus. Biochem Genet 1988;26:89-103.
41. Nemkov T, Hansen KC, D'Alessandro A. A three-minute method for high-throughput quantitative metabolomics and quantitative tracing experiments of central carbon and nitrogen pathways. Rapid Commun Mass Spectrom 2017;31:663-673.

42. Gehrke S, Reisz JA, Nemkov T, Hansen KC, D'Alessandro A. Characterization of rapid extraction protocols for high-throughput metabolomics. Rapid Commun Mass Spectrom 2017;31:1445-1452.
43. Karafin MS, Fu X, D'Alessandro A, Thomas T, Hod EA, Zimring JC, Field JJ, Francis RO. The clinical impact of glucose-6-phosphate dehydrogenase deficiency in patients with sickle cell disease. Curr Opin Hematol 2018, *In press*.
44. Zimring JC, Spitalnik SL. On the appropriate use and interpretation of animal models in transfusion medicine research. Transfusion 2013;53:2334-2339.
45. Francis RO, Jhang JS, Pham HP, Hod EA, Zimring JC, Spitalnik SL. Glucose-6-phosphate dehydrogenase deficiency in transfusion medicine: the unknown risks. Vox Sang 2013;105:271-282.
46. Francis RO, Jhang J, Hendrickson JE, Zimring JC, Hod EA, Spitalnik SL. Frequency of glucose-6-phosphate dehydrogenase-deficient red blood cell units in a metropolitan transfusion service. Transfusion 2013;53:606-611.
47. Wojczyk BS, Kim N, Bandyopadhyay S, Francis RO, Zimring JC, Hod EA, Spitalnik SL. Macrophages clear refrigerator storage-damaged RBCs and subsequently secrete cytokines in vivo, but not in vitro, in a murine model. Transfusion 2014; 54:3186-97.
48. Hendrickson JE, Hod EA, Perry JR, Ghosh S, Chappa P, Adisa O, Kean LS, Ofori-Acquah SF, Archer DR, Spitalnik SL, Zimring JC. Alloimmunization to transfused HOD red blood cells is not increased in mice with sickle cell disease. Transfusion 2012; 52:231-40.
49. Hendrickson JE, Hod EA, Hudson KE, Spitalnik SL, Zimring JC. Transfusion of fresh murine red blood cells reverses adverse effects of older stored red blood cells. Transfusion 2011; 51:2695-702.
50. Hendrickson JE, Hod EA, Cadwell CM, Eisenbarth SC, Spiegel DA, Tormey CA, Spitalnik SL, Zimring JC. Rapid clearance of transfused murine red blood cells is associated with recipient cytokine storm and enhanced alloimmunogenicity. Transfusion 2011; 51:2445-54.
51. Hod EA, Zhang N, Sokol SA, Wojczyk BS, Francis RO, Ansaldi D, Francis KP, Della-Latta P, Whittier S, Sheth S, Hendrickson JE, Zimring JC, Brittenham GM, Spitalnik SL. Transfusion of red blood cells after prolonged storage produces harmful effects that are mediated by iron and inflammation. Blood 2010;115:4284-4292.
52. Hod EA, Hendrickson JE, Sharma S, Francis RO, Wojczyk BS, Zimring JC, Spitalnik SL. Transfusion of Mouse RBCs Stored in AS-1 Induces Inflammation and Exacerbates Bacterial Sepsis. Transfusion 2010;50 (Abstract).
53. Hod EA, Arinsburg SA, Francis RO, Hendrickson JE, Zimring JC, Spitalnik SL. Use of mouse models to study the mechanisms and consequences of RBC clearance. Vox Sanguinis 2010;99:99-111.
54. Hendrickson JE, Hod EA, Spitalnik SL, Hillyer CD, Zimring JC. Storage of murine red blood cells enhances alloantibody responses to an erythroid-specific model antigen. Transfusion 2010;50:642-648.
55. Hod EA, Sokol SA, Zimring JC, Spitalnik SL. Hypothesis: hemolytic transfusion reactions represent an alternative type of anaphylaxis. Int J Clin Exp Pathol 2009;2:71-82.
56. Kraus T, Gilson C, Hod E, Hendrickson J, Spitalnik SL, Hillyer C, Shaz B, Zimring J. An analysis of in vivo survival of murine RBCs following storage in CPDA-1. Transfusion 2008;48 (Suppl):37A (Abstract).
57. Hod EA, Zimring JC, Spitalnik SL. Lessons learned from mouse models of hemolytic transfusion reactions. Curr Opin Hematol 2008;15:601-605.
58. Hod EA, Cadwell CM, Liepkalns JS, Zimring JC, Sokol SA, Schirmer DA, Jhang J, Spitalnik SL. Cytokine storm in a mouse model of IgG-mediated hemolytic transfusion reactions. Blood 2008;112:891-894.
59. Zhang P, Gao X, Ishida H, Amnuaysirikul J, Weina PJ, Grogl M, O'Neil MT, Li Q, Caridha D, Ohrt C, Hickman M, Magill AJ, Ray P. An in vivo drug screening model using glucose-6-phosphate dehydrogenase deficient mice to predict the hemolytic toxicity of 8-aminoquinolines. Am J Trop Med Hyg 2013;88:1138-1145.
60. Wickham KS, Baresel PC, Marcsisin SR, Sousa J, Vuong CT, Reichard GA, Campo B, Tekwani BL, Walker LA, Rochford R. Single-Dose Primaquine in a Preclinical Model of Glucose-6-Phosphate Dehydrogenase Deficiency: Implications for Use in Malaria Transmission-Blocking Programs. Antimicrob Agents Chemother 2016;60:5906-5913.
61. Dholakia U, Bandyopadhyay S, Hod EA, Prestia KA. Determination of RBC Survival in C57BL/6 and C57BL/6-Tg(UBC-GFP) Mice. Comp Med 2015;65:196-201.
62. Picot J, Ndour PA, Lefevre SD, El Nemer W, Tawfik H, Galimand J, Da Costa L, Ribeil JA, de Montalembert M, Brousse V, Le Pioufle B, Buffet P, Le Van Kim C, Francais O. A biomimetic microfluidic chip

to study the circulation and mechanical retention of red blood cells in the spleen. Am J Hematol 2015;90:339-345.
63. Nemkov T, Sun K, Reisz JA, Song A, Yoshida T, Dunham A, Wither MJ, Francis RO, Roach RC, Dzieciatkowska M, Rogers SC, Doctor A, Kriebardis A, Antonelou M, Papassideri I, Young C, Thomas T, Hansen KC, Spitalnik SL, Xia Y, Zimring JC, Hod EA, D'Alessandro A. Hypoxia modulates the purine salvage pathway and decreases red blood cell and supernatant levels of hypoxanthine during refrigerated storage. Haematologica 2018;103:361-372.
64. D'Alessandro A, Nemkov T, Yoshida T, Bordbar A, Palsson BO, Hansen KC. Citrate metabolism in red blood cells stored in additive solution-3. Transfusion 2017;57:325-336.
65. Ohgami RS, Campagna DR, Greer EL, Antiochos B, McDonald A, Chen J, Sharp JJ, Fujiwara Y, Barker JE, Fleming MD. Identification of a ferrireductase required for efficient transferrin-dependent iron uptake in erythroid cells. Nat Genet 2005;37:1264-1269.
66. Farwer SR, der Boer BC, Haddeman E, Kivits GA, Wiersma A, Danse BH. The vitamin E nutritional status of rats fed on diets high in fish oil, linseed oil or sunflower seed oil. Br J Nutr 1994;72:127-145.
67. Sanders TA, Hinds A. The influence of a fish oil high in docosahexaenoic acid on plasma lipoprotein and vitamin E concentrations and haemostatic function in healthy male volunteers. Br J Nutr 1992;68:163-173.
68. Sanford K, Fisher BJ, Fowler E, Fowler AA, Natarajan R. Attenuation of Red Blood Cell Storage Lesions with Vitamin C. Antioxidants (Basel) 2017; 6:55.
69. Pallotta V, Gevi F, D'Alessandro A, Zolla L. Storing red blood cells with vitamin C and N-acetylcysteine prevents oxidative stress-related lesions: a metabolomics overview. Blood Transfus 2014;12:376-387.
70. Tsao CS, Leung PY, Young M. Effect of dietary ascorbic acid intake on tissue vitamin C in mice. J Nutr 1987;117:291-297.
71. Wu H, Bogdanov M, Zhang Y, Sun K, Zhao S, Song A, Luo R, Parchim NF, Liu H, Huang A, Adebiyi MG, Jin J, Alexander DC, Milburn MV, Idowu M, Juneja HS, Kellems RE, Dowhan W, Xia Y. Hypoxiamediated impaired erythrocyte Lands' Cycle is pathogenic for sickle cell disease. Sci Rep 2016;6:29637.
72. deWolski KS, Waterman HR, Thomson PC, Gorham JD, Ranson MS, Howie HL, Munday A, Johnsen JM, Zimring JC. Steap3 Is a Novel Candidate Gene for Regulating the RBC Blood Storage Lesion By Mitigating Peroxidation of Membrane Lipids in a Mouse Model. Blood 2014;124:760 (Abstract).
73. Patel SR, Cadwell CM, Medford A, Zimring JC. Transfusion of minor histocompatibility antigenmismatched platelets induces rejection of bone marrow transplants in mice. J Clin Invest 2009;119:2787-2794.
74. Fitts DA. Minimizing animal numbers: the variable-criteria sequential stopping rule. Comp Med 2011;61:206-218.
75. Mouritsen OG, Zuckermann MJ. What's so special about cholesterol? Lipids 2004;39:1101-1113.
76. Liu D, Yi S, Zhang X, Fang P, Zheng C, Lin L, Cai R, Ye Y, Zhou Y, Liang Y, Cheng F, Zhang X, Zhou W, Mohandas N, An X, Xu X. Human STEAP3 mutations with no phenotypic red cell changes. Blood 2016;127:1067-1071.
77. Peters LL, Cheever EM, Ellis HR, Magnani PA, Svenson KL, Von Smith R, Bogue MA. Large-scale, highthroughput screening for coagulation and hematologic phenotypes in mice. Physiol Genomics 2002;11:185-193.
78. Francis RO, D'Alessandro A, Eisenberger A, et al. Donor glucose 6 phosphate dehydrogenase deficiency decreases blood quality for transfusion. J Clin Invest. 2020;130(5):2270-2285.
79. Orlina AR, Josephson AM, McDonald BJ. The poststorage viability of glucose-6-phosphate dehydrogenase-deficient erythrocytes. J Lab Clin Med 1970;75:930-936.
80. Sagiv E, Fasano RM, Luban NLC, Josephson CD, Stowell SR, Roback JD, Francis RO, Yee MEM. Glucose-6-phosphate-dehydrogenase deficient red blood cell units are associated with decreased posttransfusion red blood cell survival in children with sickle cell disease. Am J Hematol 2018;93:630-634.
81. Karafin MS, Koch KL, Rankin AB, Nischik D, Rahhal G, Simpson P, Field JJ. Erythropoietic drive is the strongest predictor of hepcidin level in adults with sickle cell disease. Blood Cells Mol Dis 2015;55:304-307.
82. Szymanski IO, Valeri CR, McCallum LE, Emerson CP, Rosenfield RE. Automated differential agglutination technic to measure red cell survival. I. Methodology. Transfusion 1968;8:65-73.
83. Holt JT, Spitalnik SL, McMican AE, Wilson G, Blumberg N. A technetium-99m red cell survival technique for in vivo compatibility testing. Transfusion 1983;23:148-151.
84. Moroff G, Sohmer PR, Button LN. Proposed standardization of methods for determining the 24-hour survival of stored red cells. Transfusion 1984;24:109-114.

85. Recommended method for radioisotope red-cell survival studies. International Committee for Standardization in Haematology. Br J Haematol 1980;45:659-666.
86. Mock DM, Widness JA, Veng-Pedersen P, Strauss RG, Cancelas JA, Cohen RM, Lindsell CJ, Franco RS. Measurement of posttransfusion red cell survival with the biotin label. Transfus Med Rev 2014;28:114-125.
87. Smith WR, Penberthy LT, Bovbjerg VE, McClish DK, Roberts JD, Dahman B, Aisiku IP, Levenson JL, Roseff SD. Daily assessment of pain in adults with sickle cell disease. Ann Intern Med 2008;148:94-101.
88. Field JJ, Majerus E, Ataga KI, Vichinsky EP, Schaub R, Mashal R, Nathan DG. NNKTT120, an anti-iNKT cell monoclonal antibody, produces rapid and sustained iNKT cell depletion in adults with sickle cell disease. PLoS One 2017;12:e0171067.
89. Field JJ, Majerus E, Gordeuk VR, Gowhari M, Hoppe C, Heeney MM, Achebe M, George A, Chu H, Sheehan B, Puligandla M, Neuberg D, Lin G, Linden J, Nathan DG. Randomized phase 2 trial of regadenoson for treatment of acute vaso-occlusive crises in sickle cell disease. Blood Adv 2017;1:1645-1649.
90. Beutler E, West C. Measurement of the viability of stored red cells by the single-isotope technique using 51Cr. Analysis of validity. Transfusion 1984;24:100-104.
91. Mollison PL. Methods of determining the posttransfusion survival of stored red cells. Transfusion 1984;24:93-96.