TRANSFUSION MEDICINE

Original article

The impact of red cell storage age on transfused patients with sickle cell disease: protocol of a pilot randomized clinical trial to evaluate changes in inflammation and clinical transfusion efficacy

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Background - Despite fulfilling all requirements for donor blood units as defined by the FDA, a number of patients with sickle cell disease (SCD) are transfused with red blood cell (RBC) units that are near the end of their storage life, exposing them to the potentially adverse components of the red cell storage lesion. Due to their chronically inflamed state, patients with SCD may be particularly susceptible to these components. We present here a pilot study protocol for testing the impact of fresh *vs* older red cell units in chronically transfused adults with SCD.

Materials and methods - This is a randomized, prospective, clinical trial. We aimed to recruit forty chronically transfused adults or adolescents with SCD who receive regular RBC transfusions for their clinical care and randomize these patients to receive either units greater than or equal to 30 days, or units less than or equal to 10 days for 3 consecutive outpatient transfusion events.

Results - The primary endpoint is the metabolic differences identified between units transfused that are greater than or equal to 30 days, and those units less than or equal to 10 days. The secondary endpoint evaluates the change in blood monocyte activation at 2 hours after transfusion between the two groups. Lastly, we evaluate unit RBC efficacy via changes in hemoglobin/day, hemoglobin A%/day, hospitalization rate, pain scores, and infections as documented via blood and urine cultures.

Discussion - This study promises to provide evidence as to whether metabolically older red cell units affect the quality and efficacy of chronic transfusion therapy for adults with SCD and has the potential to guide the need for future study on this important clinical issue.

Keywords: *red blood cells, sickle cell disease, storage lesion, randomized trial, methods.*

BACKGROUND

Transfusion of red blood cells (RBC) remains the most important therapy available for patients with sickle cell disease (SCD), with many adults on long-term, monthly regimens of chronic transfusion to prevent stroke or critical illness[1-3]. However, not all RBC units are optimal for a patient with SCD[4-7]. Many profound physiological changes occur during RBC storage including alterations to the lipid composition of the red cell membrane, such as increased surface exposure of phosphatidylserine (PS) and phosphatidylethanolamine

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(PE)[8-11], as well as hemolysis with release of cell-free hemoglobin, heme and iron[12]. While randomized clinical trials reveal that these changes may be of little clinical consequence to other patient populations[13-16], to vulnerable populations, such as chronically inflamed patient with SCD, these changes may reduce the quality and efficacy of the unit[12,17,18].

Increasing evidence now demonstrates that storage age alone may not be the best marker of individual unit quality[19], and other variables need to be considered. Recent studies have suggested that there is inherent storage variability in the blood donor population and in the manufacturing process that can affect blood component quality[20-22]. Moreover, the clearance of transfused RBC units is variable, both among different individuals[23-25], but also among sequential transfusions within the same individual[26-27], suggesting that recipient, manufacturing, and donor unit characteristics can all influence RBC unit effectiveness.

The variable metabolism of RBCs whether from storage duration or inherited donor characteristics results in a cascade of changes that may be detrimental to patients with SCD[28]. High levels of surface PS and PE from damaged donor RBCs may contribute to the increased clearance of these cells, promoting circulating inflammatory monocyte upregulation (typically CD14+, CD16–, and CD62L+)[29,30], and potentially leading to reduced RBC survival post-transfusion[31,32]. Increases in red cell microparticles can promote coagulation[33], promote adhesion of sickle red cells to vascular endothelium[34], promote complement activation[35], and stimulate an increase of inflammatory cytokines[36]. These donor red cells may further overwhelm the phagocytic capacity of macrophages and promote release of cell-free hemoglobin, heme, and iron[37]. Cell-free hemoglobin and microparticles interact with nitric oxide, and can promote inflammation, reactive oxygen species, and endothelial injury[38]. Heme has been shown in mouse models of SCD to trigger vaso-occlusion through activation of toll-like receptors on vascular endothelial cells[39], and excess circulating iron is injurious to tissues and can promote microbial growth[40].

Thus, there is a strong rationale to suggest that the transfusion of metabolically older RBC units may stimulate a cascade of events that ultimately cause either inefficient transfusions, increased risk for vaso-occlusions, or even infections in a patient with SCD[22,28].

Based on these data, we performed a pilot randomized clinical trial to define and clarify the impact that the exposure to increased levels of aberrant red cell surface lipids (PS and PE) or metabolically older transfused donor red cells has on patient circulating monocytes, circulating iron, cytokines, RBC survival, and the risk of infection in adults with SCD.

MATERIALS AND METHODS

Study design

This was a three site (Medical College of Wisconsin [MCW]), University of North Carolina at Chapel Hill [UNC], and Emory University), prospective, pilot randomized clinical trial that started in 2017 and ended in 2024. The goal of this study was to correlate the storage age of transfused RBCs with metabolic age (PS- and PE-exposure), and then evaluate how this exposure modulated SCD recipient immune systems, as quantified by levels of CD62L+ monocytes and subsequent clinical outcomes.

We aimed to recruit 40 adults (16-60 years) with SCD who are treated with chronic outpatient RBC transfusions (i.e. one or two RBC units transfused every 3-8 weeks per a medically defined protocol). Consenting subjects were randomized to receive ≥30 day or ≤10 day old units for 3 consecutive outpatient transfusion events (**Figure 1**). The study statistician provided the blood bank at each site with the randomization arm of each subject. Site blood banks were responsible for providing appropriately aged units to these subjects. Study participants and all other investigators were blinded to treatment assignment, but inadvertent unblinding was theoretically possible.

A baseline venous blood sample for flow cytometry was obtained just prior to the first randomized transfusion. Subjects were asked to hold their iron chelation for 72 hours before each study transfusion so that we could more clearly define the change in circulating serum iron after transfusion. Pre-transfusion unit link samples were obtained from each RBC unit for flow cytometry and metabolomic analysis. Patient venous blood samples were also obtained pre-transfusion, 2 hours post-transfusion, and 24 hours post-transfusion for clinical testing, flow cytometry and metabolomic analysis (**Figure 1**, **Table I**). Patient venous blood samples were obtained 2 weeks post-transfusion for clinical testing only. Participants were provided standardized diaries for each day on study

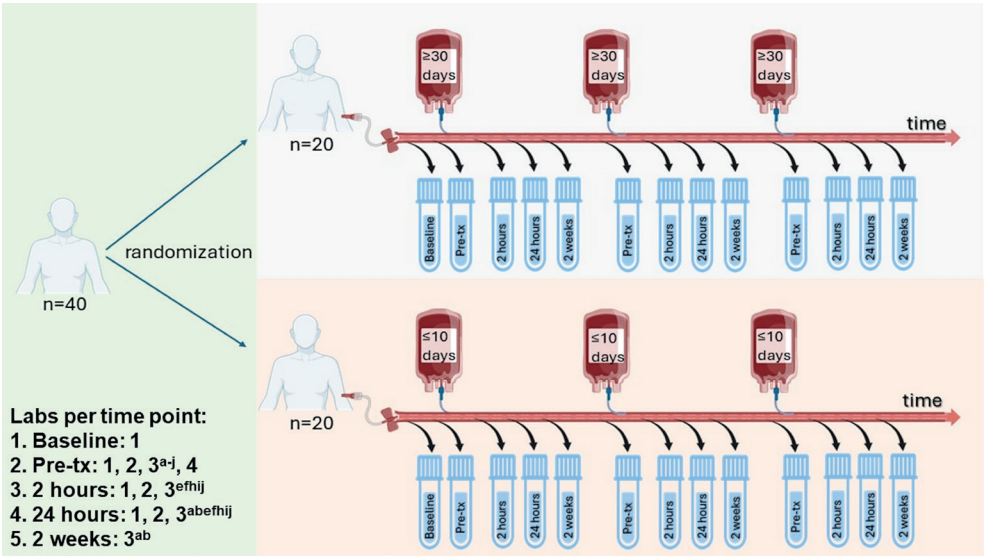

Figure 1 - Study schema after randomization
Forty patients with sickle cell disease (SCD) who received chronic transfusion were randomized to receive units less than or equal to 10 days old (No.=20) or greater than or equal to 30 days old (No.=20). Blood samples were taken over 3 consecutive outpatient transfusion events while randomized at baseline (prior to the first transfusion), and then pre-transfusion, 2 hours post transfusion, 24 hours post transfusion, and at 2 weeks post transfusion. Samples from each unit transfused were also obtained. Each sample (both patient and unit) were tested for red cell surface phosphatidylserine and phosphatidylethanolamine. Patient samples were tested for clinical, metabolomic, and flow-cytometric markers of inflammation. Tx: red cell transfusion; 1) patient flow cytometry samples; 2) metabolomic samples; 3) clinical laboratory samples (a-complete blood count, b-hemoglobin electrophoresis, c-blood culture, d-urine culture, e-haptoglobin, f-plasma hemoglobin, g-basic metabolic panel, h-iron panel, i- lactate dehydrogenase, j-high sensitivity c-reactive protein); 4) unit flow cytometry red cell samples.

Table I - *Schedule of study measurements*

Study measure	Daily	Pre-every transfusion	2 hours post every transfusion	24 hours post every transfusion	2 weeks post every transfusion	End of study
Physical exam, adverse event assessment, medical assessment		X				X
Complete blood count with differential*		X		X	X	
Hemoglobin electrophoresis		X		X	X	
Blood culture		X				
Urinalysis with reflex culture		X				
Haptoglobin		X	X	X		
Plasma hemoglobin		X	X	X		
Basic metabolic panel+		X				
Ferritin & iron panel#		X	X	X		
Lactate dehydrogenase		X	X	X		
High sensitivity- c-reactive protein		X	X	X		
Red blood cell Unit blood donor sample		X				
Patient flow cytometry sample		X	X	X		
Metabolomic sample		X	X	X		
Study diary	X					

*White blood cell count, hemoglobin, hematocrit, platelet count; +Creatinine, bicarbonate, chloride, blood urea nitrogen, potassium (K), sodium (Na), glucose, calcium; #Iron, serum iron, transferrin, total iron binding capacity, % saturation.

to document subjective symptoms of infection, pain, and emergency department (ED) or hospital utilization. Diaries were collected and participants were reassessed by physical examination prior to each transfusion encounter, for up to 3 transfusion events, and 4 weeks after the last transfusion. To ensure compliance, coordinators at each site were instructed to contact subjects regularly to remind them to complete their diaries. All data were captured with standardized case report forms and entered into an electronic database.

Subject screening

Patients 16-60 years of age undergoing chronic outpatient red cell transfusion therapy were identified through either the adult outpatient sickle cell clinic at UNC, the Infusion Clinic lists at UNC and at Emory/Grady Health System, or the infusion clinic at the Medical College of Wisconsin (MCW). Of note, of the three sites, Emory/Grady Health System only enrolled subjects who were ≥18 years of age. Those subjects that met eligibility (**Table II**) were approached for consent. Individual center scheduling practices influenced how the contact was arranged, which included in-person at the infusion clinic or in the sickle cell patient clinic. Subjects who consented to the study were assigned a study ID number which was communicated to the blood bank for randomization assignment. Additionally, basic features of subject medical and surgical histories (i.e. age, gender, reason for receiving chronic transfusion therapy, past medical histories, medication list, and previous hospitalizations) were recorded from the electronic health record and entered into the study electronic database.

If a subject was enrolled and received an RBC transfusion prior to randomization, that subject was still randomized, and started the study at their next outpatient red cell transfusion appointment so long as the appropriately aged unit was available. Only when appropriately aged RBC units stored in AS-1, AS-3, or AS-5, were available would the study start for that subject (**Figure 1**). After that initial point, the remaining 2 consecutive outpatient transfusions involved crossmatch compatible, CEK antigen matched, and sickle negative RBC units that may or may not meet study unit age criteria, but best efforts were made by each transfusion service to keep each subject to their specific study arm for the remaining transfusion events.

Table II - *Study inclusion and exclusion criteria*

Inclusion criteria
1. Ages 16 to 60 years old 2. Subject must have sickle cell anemia confirmed by hemoglobin analysis 3. Must be receiving red cell transfusion therapy 4. Must be outpatient at the time of study transfusions
Exclusion criteria
1. History of severe reactions to transfusion therapy 2. Receiving manual or automated exchange transfusions 3. Receive red cell transfusions that are crossmatch incompatible 4. Current participation in another therapeutic trial for sickle cell disease 5. Current pregnancy 6. History of HIV infection 7. Having an uncontrolled inter-current illness, or psychiatric illness/social situations that would limit compliance with study requirements as determined by the principal investigator

Laboratory measures and flow cytometry analyses

Two mL from each donor unit were obtained from 3 sterile unit links/segments on the day of the 3 randomized outpatient transfusion events. PE and PS exposure were measured by flow cytometry within 48 hours of collection using an LSR2 (BD Biosciences, Franklin Lakes, NJ, USA) and the LSRFortessa (BD Biosciences). All flow cytometry analyses were recorded using FACSDiVa software (BD Biosciences, v9.0) and analyzed using FlowJo (BD Biosciences, v10.9). The remaining samples were centrifuged and the supernatant and RBC fraction from each RBC unit were snap frozen for metabolomic studies. Subject whole blood samples were obtained by port or peripheral access using trained phlebotomists as available prior to transfusion, 2 hours after transfusion, 24 hours after transfusion, and 14 days after transfusion (**Table I**). One Cytochex tube (Streck Laboratories, La Vista, NE, USA) was used pre-transfusion, 2 hours and 24 hours post transfusion to preserve the white cell surface markers for flow cytometry. Patient white cells were isolated from RBC using BD FACS lysing solution, 10X concentrate (BD Biosciences), and the monocytes were tested by flow cytometry as above for identity and markers of activation (CD16, CD14, HLA-DR, CD11b, CD35, CD64, CD66b, CD69, CD192, CD86, CD63, and CD62L) 7-14 days after collection due to the Cyto-Chex tube preservative. The remaining samples were centrifuged and the plasma and RBC fractions from each sample and each time point (pre-transfusion, 2 hours and 24 hours post transfusion) were frozen and stored at –80°C. One plasma sample at each time point was used in a Bio-Plex multiple cytokine assay analysis kit (Bio-Rad Laboratories, Hercules, CA, USA). The cytokines and chemokines evaluated included

CCL2, CXCL1, CXCL9, CXCL10, CXCL11, IFN-γ, IL-1a, IL-1b, IL-1ra, IL-4, IL-6, IL-8, IL-10, IL-12 (p70), CCL5, MPO, TGFβ1, TGFβ3, and TGFβ2.

The snap frozen plasma and RBC samples were de-identified and sent to Dr. Angelo D'Alessandro at the University of Colorado to explore the metabolomic profiles of the units transfused and the subject's plasma and RBCs before transfusion, 2 hours post-transfusion, and 24 hours post transfusion. Metabolomics extraction and analyses in 96 well-plate format were performed as has been described in detail previously by this laboratory[41,42].

Clinical measurements obtained from each subject included complete blood count (4 mL EDTA tube), reticulocyte count (4 mL EDTA tube), hemoglobin electrophoresis (3 mL EDTA tube), haptoglobin (1 mL serum tube), standard chemistry labs (ferritin (0.5 mL serum tube), serum iron and iron saturation (1 mL serum tube), lactate dehydrogenase (1 mL serum tube), urinalysis (12 mL) and blood culture (8-10 mL /bottle) (**Table I**).

Daily diary

Each subject was provided a daily diary. Each day, subjects rated their pain on an ordinal numeric pain rating scale (0-10), indicate whether the pain was consistent with a "crisis", indicate whether they utilized a healthcare facility, and record the type and amount of opioids used for pain control. Subjective infection symptoms were also documented along with the type, duration, and dose of any antibiotics taken if applicable. Subject emergency department and hospitalization events, and etiologies, were confirmed and recorded at regular intervals by the study coordinator during the study period using patient report, and the available electronic medical record. Of note, diary completeness was assessed at each face-to-face visit.

Study randomization scheme

Subjects were randomized to receive RBCs stored either ≤10 days or ≥30 days at the time of transfusion. Storage arm assignment applied to up to 3 consecutive outpatient transfusions, beginning at the time of randomization and continuing through the end of the 3rd randomized outpatient transfusion. Randomization occurred at any time between the date of consent until 2-3 days prior to the first study transfusion. Only the transfusion service had access to the randomization assignment as designated by the study biostatistician. The randomization methodology used was blocks of 5 (S. Piantadosi block randomization software, 2010) to achieve a 1:1 ratio between those that received ≤10 day or ≥30 day units. Due to the small sample size (No.=20 per group), randomization could not be stratified by gender, age, ethnicity, or hemoglobinopathy type.

Study blinding

Only blood bank staff with the appropriate security level had access to the treatment arm assignment devised by the study biostatistician. Access to case report forms containing information about the age of the RBC products sent for each subject was also restricted at the site to the appropriate blood bank staff. The clinical staff overseeing the subject's participation in the trial, including the study principal investigator and study coordinators, did not have access to the treatment arm assignment or information about the age of the RBC products transfused.

No alteration was made to the labels on the RBC units. The expiration date, collection date, and any processing dates (e.g. irradiation dates) were not obscured. Medical infusion clinic personnel that physically provided the RBC transfusions verified product and patient identity according to hospital-specific procedures. These personnel were instructed to not divulge the patients' randomization assignments. Other infusion clinic staff (other than those infusing the RBCs), were instructed not to seek to identify the age of the products the patients are receiving. The subjects themselves were not informed of their randomization assignment and were also instructed not to seek to identify the age of the products they were receiving. However, as the key components of this study were laboratory-based, inadvertent unblinding of the randomization assignment was not felt to compromise the validity of the study.

Statistical analysis plan for each study aim, and sample size calculations

The analysis is an intent to treat (ITT) with all those randomized being included in the study. For all aims, the distribution of, and relationship between, variables were explored with summaries, plots, and tree analyses. All statistical estimates of population parameters were tabulated along with corresponding confidence intervals (CIs). All hypothesis tests yielding large p-value (e.g., $p > \alpha$) were reported as being inconclusive.

The lead biostatistician or designee performed these analyses. Where necessary for parametric assumptions, appropriate transformations were employed with

justification for their use; otherwise, non-parametric tests were used. P-values were reported without dichotomization wherever applicable. Data obtained from transfusion events where units of the appropriate age could not be obtained (study deviation) were excluded from a subanalysis of the dataset, and the same distribution of, and relationship between, variables were explored with similar summaries, plots, and tree analysis as in the ITT analysis.

All subjects, regardless of age and gender, were analyzed together as one cohort. Statistical software used in this study was Cytel (Cambridge, MA, USA), StatXact and LogXact, SAS version 9.4 (SAS Institute, Cary, NC, USA), and Salford systems (San Diego, CA, USA) Classification and Regression Trees (CART). Regarding missing data, a logistic regression approach was used to investigate the possible causes for missing data and to investigate if data were missing at random (MAR). Assuming data were MAR, multiple imputations were used for items, and for repeated measures, random effects models were used; the structure for the variance covariance matrix was explored under the constraints of sample size. A sensitivity analysis was also performed to evaluate the robustness/fragility of the study's main results to reasonable perturbations of the statistical methods and assumptions used. Results of the sensitivity analyses were only used to guide trust in the main results.

RESULTS

Primary outcome

The primary outcome measure was the proportion of metabolically old RBCs. Previous studies and our own work have suggested that PS and PE increase over storage time, but as noted previously[8,17], different donors contribute different sensitivities to storage, and thus we hypothesized that some proportion of units stored ≤10 days might be metabolically old, and some units ≥30 days might be considered metabolically young. For this study, the cut-off for a metabolically old unit was the point at which an estimated sigmoidal curve enters its log phase, which for PE and PS was the mean value at day 21, or 8%, and 3% respectively based on our preliminary studies[11]. This cutoff was biologically meaningful and was supported temporally by the metabolic changes that occur with red cell storage[43]. Consequently, metabolically old units were defined as having either a surface PE or PS concentration ≥ the *a priori*-defined cutoff. We compared the transfusions provided to the two groups using a Fisher exact test at an alpha of 0.05. With a total sample size of 40 patients (20 in each study arm), we determined that we would have at least 80% power (α=0.05) to detect a difference of at least 47% between the two randomized groups for metabolically old RBC units.

We further calculated the mean, median, minimum, and maximum levels of each metabolic compound analyzed by Dr. Alessandro in his laboratory at the University of Colorado. The metabolic markers of interest included carnitine[44], kynurenine[45], 2,3-BPG[46], hypoxanthine[47], ATP[47], and sphingosine 1-phosphate[48], which have all been shown to be important to red cell quality and metabolic age. While exploratory in nature, we compared these key metabolomic markers with the PS and PE measurements obtained by flow cytometry, and the clinical laboratory findings pre and post transfusion, including hemoglobin, hemoglobin A%, ferritin, serum iron, and plasma free hemoglobin.

Secondary outcome

The key secondary outcome measure was the change in CD62L+ circulating monocytes at 2 hours post-transfusion compared to the subject pre-transfusion. We compared the two groups using a two-sample two-sided t-test at an alpha of 0.05. With the planned 40 subjects, we had at least 80% power to detect a change in the ratio of 1.35 post-transfusion.

We additionally used a general(ized) linear mixed model to include metabolically old units transfused (primary outcome) and unit storage solution, regardless of unit age, as a covariate in our analysis. Other co-variates in this model included free hemoglobin and serum iron. We will similarly compare other obtained activation measures as outcomes: activation markers for monocytes and measured plasma cytokine concentrations. We will also plan for auxiliary analyses of the outcome variables in which the explanatory variable is the age (days) of the RBC units to characterize the degree to which age (days) can accurately predict the clinical and physiologic outcome measures defined. Lastly, as an exploratory aim, we evaluated the *in vivo* change in recipient red cell PE/PS positivity at 2 and 24 hours post transfusion.

Tertiary outcome

We evaluated the rate of post-transfusion infections in randomized subjects. For this study, infections were

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defined as a positive blood or urine culture detected after the start of study transfusions. The primary estimate was the treatment effect defined as the difference between the regimen-specific proportions of patients in the target populations that would experience post-transfusion infections. This population parameter was estimated as a function of the proportions observed in the sample of patients studied. The point estimate was reported along with the corresponding 95% confidence interval estimate. The presence of an indwelling catheter was also used as a co-variate in this analysis. An infection rate difference of 20% would be of clinical interest. While not expected, if recurrent infections per patient are identified, Poisson modeling could be utilized for this analysis. We did not expect to have adequate power in this study, but at an alpha of 0.05 with 20 subjects in each group, determined that we could detect a difference of 47% between the proportions.

We further explored the relationship of blood storage age and transfusion of metabolically old RBC units on the change in hemoglobin and hemoglobin A% over time, pain scores (0-10 as an ordinal scale), opioid use and dose, ED and hospitalization rate, infection symptoms, new alloantibody formation, and antibiotic use during the 3-month study period using a Fisher exact test or 2-sample t-test as applicable.

Study approval and registration

The study was conducted according to the Declaration of Helsinki and in accordance with good clinical practice guidelines. The Medical College of Wisconsin, University of North Carolina at Chapel Hill, and Emory University Review Boards approved the protocol independently and there was no central IRB. All research participants provided written informed consent prior to study participation. The study was registered prior to initial enrolment in ClinicalTrials.gov with the identifier #NCT 03704922 (https://clinicaltrials.gov/study/NCT03704922?term=NCT03704922&rank=1).

Data and safety monitoring plan

For this trial, the principal investigator appointed three members to a safety monitoring committee (SMC). These three members were investigators from institutions with expertise in transfusion medicine, sickle cell disease, and biostatistics, respectively. They were charged with monitoring the accruing data to confirm that the patients in the trial were being cared for safely. The SMC met at least once a year and was responsible for:

1. reviewing and analyzing the progress of the study;
2. approving amendments to the trial protocol, if warranted;
3. monitoring the safety of the study treatments and diagnostic procedures;
4. ensuring data quality;
5. reviewing recruitment and adverse event rates.

The data safety monitoring plan ensured that all recruitment sites followed Federal regulations and Good Clinical Practice Guidelines.

Stopping guidelines were determined by the SMC. The study was expected to take three years to complete, but due to COVID, continued until 2024. Stopping guidelines were proposed for two outcomes, group differences in serious infections and pain crises requiring ED or hospital admission. Serious infections for this study were defined as documented infections of any type serious enough to warrant an inpatient hospitalization or prolongation of hospitalization. For these two outcomes, yearly looks at the data took place.

Subject study termination occurred under the following 5 conditions: 1) the subject completed the 3 study transfusions; 2) the subject decided to withdraw from the study; 3) the subject moved away, died, or was lost to follow-up; 4) the patient no longer needed outpatient RBC transfusions as determined by the patient's provider; or 5) subjects could be removed from the study, or treatment stopped by the Investigator.

Current study status

Patient follow-up and donor sample acquisition was completed in March 2024 with final analyses for clinical, metabolomic, flow cytometric, and cytokine results reporting projected to be completed in 2025. The summary achieved patient enrollment and sample acquisition are provided (**Table III**).

Table III - *Summary of patient enrollment and sample acquisition*

Study group	≤10 day unit arm	≥30 day unit arm	Total
Randomized subjects	13	13	26
RBC unit samples acquired	65	67	132
Subject metabolic/flow cytometric samples acquired	89	66	155
Subject clinical samples acquired	110	77	187

DISCUSSION

There remains equipoise among transfusion medicine physicians and hematologists regarding the importance of older or metabolically old units for adults with SCD[51-53]. This prospective, randomized study will add to our knowledge by defining how well correlated storage age is to markers of metabolic age, as defined by surface PS, PE, and other metabolomic markers. Second, this study will define the changes in circulating monocytes and serum markers of inflammation post transfusion in this unique population, and clearly determine whether storage age or markers of metabolomic age correlate with these immunologic changes. Third, this study will evaluate key clinical markers of transfusion efficacy, including the transfusion increment post transfusion, the rate of vaso-occlusive crises, the rate of culture positive infections, and determine whether storage age or markers of metabolomic age correlate with these important clinical outcomes. Lastly, this pilot clinical trial will both clarify the safety of using older red cell units in this population and define whether this study design is feasible, especially if a larger multi-site clinical trial is warranted.

This study does have limitations. Given the pilot nature of this study, the relatively small number of subjects recruited were not adequately powered to determine important clinical differences that would be of interest, especially since we did not reach our recruitment goals. However, we hope that the findings from the hundreds of units and patient samples from this trial will demonstrate a need for a larger trial powered adequately for these important clinical differences. Second, the study design, while randomized, was not optimized to study the key questions of interest. Study subjects and coordinators, for instance, could be inadvertently unblinded due to the fact that the units were not altered. That said, we felt that the main study aims (PS, PE, and CD62L measures) would not be altered by any unintentional unblinding. In the future, the study design itself could be better optimized if using each subject as their own control. While a cross-over study design might have been the ideal study method, that methodology was practically complicated due to the limited time for study recruitment (due in part due to the COVID pandemic) and the concern that subjects would not be able to stay on study for a year or more. Future more definitive clinical trials could address these practical concerns.

CONCLUSIONS

Despite the now increasing use of curative therapies, finding ways to maximize the safety and efficacy of chronic red cell transfusion therapy for adult patients with SCD is critically needed. If found to be clinically important, restricting RBC units to the most effective units, whether it be defined by storage age or metabolic age, has the potential to improve patient outcomes and significantly alter the field of transfusion medicine.

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ETHICAL CONSIDERATION

The study was conducted according to the Declaration of Helsinki and in accordance with good clinical practice guidelines. The Medical College of Wisconsin, University of North Carolina at Chapel Hill, and Emory University Review Boards approved the protocol independently and there was no central IRB. All research participants provide written informed consent prior to study participation. The study was registered prior to initial enrolment in ClinicalTrials.gov with the identifier #NCT 03704922. Written informed consent was obtained from each participant/patient for study participation and data publication.

AUTHORS' CONTRIBUTIONS

MSK drafted the first version of the manuscript. All other Authors reviewed and edited the manuscript.

CONFLICTS OF INTEREST

MSK is a paid consultant for Westat Inc (Rockville, MD, USA). RMF serves on a medical advisory board for Pfizer (New York, NY, USA) and Cerus (Concord, CA, USA), and has received research funding from Cerus; he also serves as a consultant for REDSIV-P which is funded by the NIH/NHLBI (Bethesda, MD, USA). JJF has received honoraria from Bayer (Leverkusen, Germany), and receives research funding from FORMA Therapeutics (Watertown, MA, USA), Shire/Takeda (Lexington, MA, USA), and Rigel (San

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