Title:	***RH* genotype matched red cells for patients with sickle cell disease and anti-D**
Short Title	RHD genotype matching for anti-D
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ABBREVIATIONS AND DEFINITIONS OF TERMS

°C	Degrees centigrade
AE	Adverse event
CBC	Complete blood count
DHTR	Delayed hemolytic transfusion reaction
Hgb	Hemoglobin
PI	Principal investigator
RBC	Red blood cells
SCD	Sickle cell disease

ABSTRACT

Context:

Red blood cell transfusion remains a critical therapy for patients with sickle cell disease (SCD). A major problem is the high rate of alloimmunization (antibody formation against transfused red cells) that occurs in patients with SCD. Recent studies performed by our group and others demonstrate *RH* genetic variants in patients and donors is a major risk factor leading to Rh alloimmunization. Anti-D formation in D+ patients occurs frequently, and once identified, providing D- cells for all subsequent transfusions can be challenging. These anti-D antibodies in D+ patients suggest exposure to different or variant D protein on donor cells. We will test whether transfusion of patients with anti-D with *RHD* genotyped matched red cells is feasible, safe and can decrease D- donor unit demand.

Objectives:

Primary: we will prospectively study whether provision of *RHD* genotype and C, E, K matched red cells to chronically transfused patients with a history of anti-D is feasible and safe.

Study Design:

This is a pilot study to evaluate the feasibility and safety of providing *RH* genotype matched D+ RBCs to chronically transfused patients with SCD who type D+ but have formed anti-D and are currently transfused with D- RBC units.

Setting/Participants:

The study setting will be the apheresis unit, the outpatient day hospital or the inpatient units at the Children's Hospital of Philadelphia.

There will be two sites: The Children's Hospital of Philadelphia, New York Blood Center.

We will recruit 20 subjects overall from The Children's Hospital of Philadelphia. The first 5 subjects enrolled will be adults.

Inclusion Criteria include:

- Diagnosis of SCD, all genotypes
- Require a period of chronic red cell transfusion therapy
- Require a minimum of 3 units per transfusion visit
- History of anti-D (anti-D not detectable at screening visit and past 6 months)
- *RH* genotype predicts D+ expression

Study Interventions and Measures:

We will provide one red cell unit of D+ *RH* genotype matched RBCs at the first transfusion study visit. The remainder of units will be provided per clinical standard of care, i.e. D-, CEK-matched, and negative for all other antigens the patient is alloimmunized against. If laboratory monitoring shows no reappearance of anti-D and no signs of increased red cell hemolysis, the patient will receive one unit of D+ *RH* genotype matched RBCs at the 2nd transfusion study visit, and if tolerated, D+ red cell exposures will increase by one unit per study visit until all units required are D+.

For subjects with a history of stroke/recurrent transient ischemic attack or other indication who require tight control of Hb S and *RH* genotyped blood is not available, standard of care serologic matched blood would be administered rather than delaying transfusion and risking higher Hb S level.

We will collect a pre-transfusion sample to prospectively store plasma on all study subjects.

Study participants will return 5-12 days post-transfusion for a CBC, reticulocyte count, hgb quantification, bilirubin level, and antibody screen to evaluate for anti-D reappearance or red cell hemolysis.

Main study outcome measures are feasibility of identifying sufficient *RH* genotype matched units (identifying sufficient *RH* genotype match red cells without delays in transfusion), and safety (no anti-D reappearance or evidence of hemolysis of transfused red cells).

PROTOCOL SYNOPSIS

Study Title	*RH* genotype matched red cells for patients with sickle cell disease and anti-D
Funder	National Institutes of Health
Clinical Phase	Pilot
Study Rationale	Patients with SCD exhibit significantly increased rates of alloimmunization compared to other chronically transfused populations, and antibodies formed against Rh are the most common, despite provision of standard serologic Rh matched red cells. Recent studies reveal *RH* gene variation is common in patients with SCD and in African-American blood donors, and contributes to Rh alloimmunization. Many D+ patients have formed anti-D, and now require D- units. These anti-D suggest exposure to different or variant D antigen on donor cells. We will test whether providing *RH* genotype matched D+ RBCs to chronically transfused patients with SCD is feasible, safe, and can lessen demand for D- RBC units.
Study Objective(s)	**Primary** • To determine whether provision of *RHD* genotype and C, E, K matched red cells to chronically transfused D+ patients with a history of anti-D is feasible and safe
Test Article(s)	*RH* genotyped D+ donor red cell units will be matched to the patient's *RH* genotype.
Study Design	This is a pilot study to evaluate the feasibility and safety of providing *RH* genotype matched D+ RBCs to patients with SCD who type D+ but have formed anti-D and currently require D- RBC units. We will identify D+ patients with a historical anti-D that is not currently detectable, and on a chronic red cell exchange program. *RH* genotyped donor units will be obtained from the New York Blood Center. We will transfuse one D+ unit matched by *RHD* genotype at the first two study visits and increase D+ units incrementally with subsequent transfusion visits. We will determine whether sufficient *RH* matched donor units can be identified for the patient's *RH* genotype (identifying sufficient *RH* genotype match red cells without delays in transfusion), and its safety (no anti-D reappearance or evidence of increased red cell hemolysis). For subjects with a history of stroke/recurrent transient ischemic attack or other indication who require tight control of Hb S and *RH* genotyped blood is not available, standard of care serologic matched blood would be administered rather than delaying transfusion and risking higher Hb S level. For all subjects, standard of care serologic matched blood would be administered rather than delaying transfusion beyond 7 days.

	We will enroll at least 5 adults to collect safety and preliminary efficacy data, before enrolling children to the study.
Subject Population **key criteria for Inclusion and Exclusion:**	**Inclusion Criteria** 1. Subjects age ≥ 8 years old 2. Diagnosis of SCD, all genotypes 3. Require chronic red cell transfusion therapy 4. History of anti-D 5. *RH* genotype predicts D+ expression **Exclusion Criteria** 1. Rare *RH* genotype that would preclude sufficient RBC units 2. Antigen negative requirements due to alloimmunization that would preclude sufficient RBC units
Number of Subjects	Total Number of Subjects = 20 Total Number at CHOP = 20 Total Number of Sites = 2
Study Duration	Each subject's participation will last up to 2 years depending on how many red cells units are transfused per visit. The entire study is expected to last 5 years.
Study Phases **Screening** **Study Treatment** **Follow-Up**	(1) Screening: screening for eligibility and obtaining consent (2) Intervention: study intervention of D+ *RH* genotype matched red cell units for transfusion for patients with SCD and history of anti-D (3) Follow-up: monitor feasibility of identifying sufficient *RH* genotype matched red cell units and formation of Rh antibodies by ongoing chart review
Safety Evaluations	Study participants will return 5-12 days post-transfusion for a CBC, reticulocyte count, hgb quantification, bilirubin level, and antibody screen to evaluate for anti-D reappearance or red cell hemolysis.
Statistical and Analytic Plan	This a pilot feasibility and safety study that will evaluate qualitative measures and determine whether adequate *RH* genotyped units can be identified for chronically transfused patients with SCD in a timely fashion, and whether patients experience anti-D reappearance and/or red cell hemolysis of transfused cells.
DATA AND SAFETY MONITORING PLAN	The PI will be responsible for data quality management and ongoing assessment of safety.

TABLE 1: SCHEDULE OF STUDY PROCEDURES

Study Phase	**Screening and baseline assessment**	**Intervention visit**
Visit Number	**1**	**2+**
Informed Consent/Assent	X	
Review Inclusion/Exclusion Criteria	X	
Demographics/Medical History	X	
Interim Medical History		X
Physical Examination	X	
Vital Signs	X	X
Review of Height and Weight	X	X
Medication review	X	X
Clinical Laboratory Review	X	X
Blood draw for study sample		X
Transfusion of *RH* genotype matched red cell units		X
Blood draw for follow-up labs 5-12 days after transfusion		X
Adverse Event Assessment		X

1 BACKGROUND INFORMATION AND RATIONALE

1.1 Introduction

Red blood cell transfusion remains a life-saving therapy for patients with sickle cell disease (SCD). A major problem is the high rate of alloimmunization (antibody formation against transfused red cells) that occurs in transfused patients with SCD. Alloimmunization leads to delays in care, increases costs, and makes transfusion therapy unsafe and impossible for some patients. The most common antibodies formed by patients with SCD are directed against the Rh blood group system. Genetic diversity in Rh antigens in patients and blood donors of African descent contributes to the high incidence and complexity of antibodies found in patients with SCD.[1] Recent studies performed by our group and others demonstrate *RH* genetic variants in patients and donors is a major risk factor leading to Rh alloimmunization. Routine blood bank assays cannot identify these variants, so although Rh-matched red cells by standard serologic assays are transfused to patients with SCD, they would only be truly Rh-matched with DNA-based matching. Many D+ patients have formed anti-D, and now require D- units, resulting in new challenges. These anti-D suggest exposure to different or variant D protein on donor cells. The major goal of this study is to perform pilot clinical studies to test whether providing *RH* genotype matched D+ RBCs to chronically transfused patients with SCD and anti-D is feasible, safe, and can lessen demand for D- RBC units. *RH* genotype matched red cells are not currently available for transfusion outside this study except for rare cases. We will determine the feasibility of identifying adequate genotype matched donor units in real clinical practice, identify barriers, and prospectively monitor patients for anti-D reappearance or signs of hemolysis.

1.2 Name and Description of Investigational Product or Intervention

Chronically transfused patients with SCD and anti-D will receive D+ *RH* genotyped matched red cell units for transfusion in addition to standard C, E, and K antigen matching and being hemoglobin S negative, which is our institutional standard of care for patients with SCD. *RH* genotyping of donor units will be performed by the NYBC Immunogenetics laboratory, led by Dr. Connie Westhoff, co-investigator.

1.3 Relevant Literature and Data

Alloimmunization is a major complication of blood transfusion in patients with SCD

One major explanation for high alloimmunization rates in patients with SCD is the disparate distribution of RBC antigens between patients who are primarily of African ancestry, and blood donors of non-African ethnic backgrounds.[2] The frequency of C, E, K, Jk^b, Fy^a, Le^a and S antigens is significantly lower in individuals of African descent compared to blood donors who are primarily of European descent. Outside of ABO, the Rh blood group system is the most immunogenic. Since sensitization to Rh antigens (D, C, c, E, e) and to K (a Kell system antigen) comprise a majority of the RBC antibodies encountered in patients with SCD, consensus guidelines including the 2014 NHLBI expert panel report, recommend provision of C, E, K-matched RBCs to this patient population.[3] Transfusion with RBC units from African American donors with the same ethnic background, who are more likely to have similar blood group antigen profiles, has also been suggested to mitigate exposure to foreign antigens that cause high rates of alloimmunization. This approach has been combined with C, E, K matching for over two decades at The Children's Hospital of Philadelphia (CHOP).

RBC alloimmunization despite transfusion from Rh matched minority donors

We performed a 15-year retrospective analysis to assess antigen matching for D, C, E, K and transfusion from African American donors on alloimmunization rates, antibody specificity, and clinical significance in pediatric and young adult patients with SCD (featured article in BLOOD).[1] Our study included 182 patients who received a total of 44,482 RBC units. Surprisingly, we found 58% of 123 chronic and 15% of 59 episodically transfused patients were alloimmunized (median units transfused per patient was 230 and 3, respectively). Two-thirds of the antibodies formed were directed against the same Rh blood group antigens that were targeted for prevention (D, E, e, C, c, Fig. 1A). Notably, the majority of Rh antibodies occurred in patients whose red cells were phenotypically positive for the corresponding Rh antigen and would not be expected to form antibodies to a "self" antigen. Anti-D was identified in 29 patients whose cells all typed D+ (Fig. 1B). Overall, one-third of these antibodies were clinically significant with lower hemoglobin (hgb) or % hgb S levels than their baseline pre-transfusion values, suggestive that the patient hemolyzed the transfused RBCs.

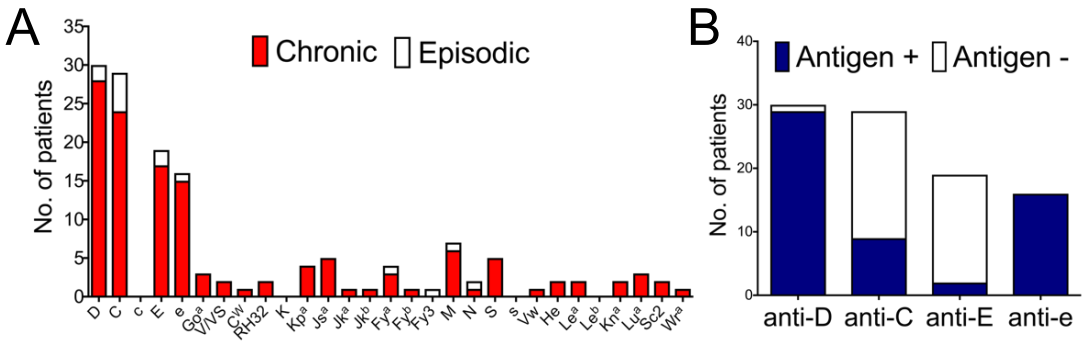

Figure 1. Alloimmunization in 182 patients with SCD transfused with D,C,E,K-matched RBCs from minority donors. A. *146 antibodies were identified and 70% had Rh specificity.* ***B.*** *56 Rh antibodies occurred in patients whose RBCs typed positive for that antigen (sometimes mistaken for "autoantibodies"), and 35 were identified in antigen-negative patients despite transfusion with Rh-matched RBCs (i.e. negative for that antigen).*

Genetic analysis of the two genes that encode the Rh antigens, *RHD* and *RHCE,* revealed that >85% of our patients carry variant alleles that result in loss or alteration of Rh antigenic epitopes.[1] We subsequently showed that African American donors share a similar degree of *RH* genetic variation.[4] Variant Rh antigens are not detected by traditional blood bank tests which identify only the common Rh antigens (D,C,c,E,e). Consequently, patients with SCD are at risk of Rh antibody formation when exposed to conventional Rh antigens on donor red cells,[1] or other foreign Rh variants on African American donor cells.[4] This problem is unique to patients with SCD who are primarily of African Black ethnicity, as *RH* variation occurs in <3% of individuals from other ethnic populations, but up to 85% of Blacks. For anti-D, we identified numerous D+ patients with at least one conventional *RHD* allele, yet still formed anti-D antibodies, suggesting an immune response to variant D exposure from African American donor units.

RH genes and proteins

The Rh system is the most complex of all blood group systems and includes greater than 50 different antigens encompassing polymorphic epitopes, but standard serologic "Rh typing" of red cells tests for the five common antigens in all populations (D,C,c,E,e). The Rh proteins are encoded by two genes: *RHD* encodes the D antigen and *RHCE* encodes the CE antigens in various combinations (ce, cE, Ce, CE) (Fig. 2). Individuals with D antigen expressed are "Rh positive" and the absence of D, usually due to *RHD* gene deletion, are "Rh negative." The highest incidence of "Rh negative" (~15%) occurs in those of European ancestry. The *RH* genes are inherited as a haplotype, and allele frequencies differ in various populations.

Rh variants encode "partial" Rh antigens undetectable with current typing reagents

RHD and *RHCE* genetic variants are frequent in individuals of African ethnicity and result in altered epitopes often termed "partial" Rh antigens because they lack common epitopes. Patients with variant *RH* who lack commonly encoded epitopes are at risk of antibody production if exposed to these Rh epitopes via transfusion or pregnancy. Thus, D+ individuals with "partial D" antigen may form anti-D (to the epitopes of D they lack) when exposed to conventional D antigen.[1] For example, *RHD*DAU4* encodes a protein in which lysine replaces glutamic acid at amino acid position 233 resulting in loss or alteration of one or more common RhD epitopes (Fig. 2). Variant *RHCE* alleles encoding "partial C, c, or e antigens" occurs frequently in African Blacks, and their RBCs often lack high prevalence Rh antigenic epitopes, such as hrB and hrS, and express novel antigenic epitopes (V, VS). The relatively common altered allele, *RHCE*ce(733G),* encodes a new antigen VS and loss of the high prevalence antigen hrB (Fig. 2). We demonstrated that variant *RHD* or *RHCE* contributes to Rh alloimmunization and delayed transfusion reactions in patients with SCD.[1,5] Inheritance of variant *RH* alleles explained ~1/3 of antibodies, but the remainder were found in patients with the corresponding conventional alleles or in patients who were C- or E-negative and had received donor units typed as negative. These observations suggested that Rh antibodies are not only a result of inheriting variant *RH* alleles but may also be the result of variant or altered epitopes on African American donor red cells.[4] However, recruitment of African American donors is important to identify an adequate supply of CEK negative units. Genotype matching of donors to patients may be feasible in the future, but the cost and infrastructure of genotyped red cells for patients with SCD is currently prohibitive. With improved sequencing approaches, we anticipate cost will not remain a longstanding challenge. Feasibility of *RH* genotyping for patients with SCD is an important first step.

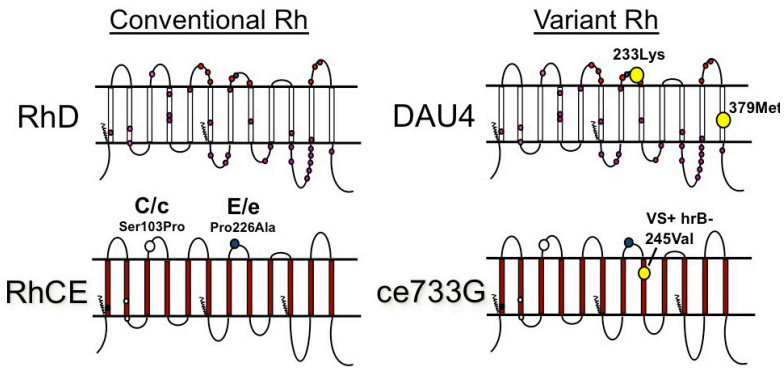

Figure 2. Rh proteins. *Predicted 12 transmembrane RhD and RhCE proteins (left). The amino acid differences between conventional RhD and RhCE are shown as red circles. Positions 103 and 226 in RhCE are critical for C or c and E or e expression. Yellow circles depict amino acid changes on variant Rh proteins DAU4 and ce(733G) (right).*

In a recent publication from Brazil, 11 patients with SCD requiring chronic transfusion who typed D+ and had a history of anti-D, were switched from D- to D+ units after *RH* genotyping revealed at least one conventional *RHD* allele.[6] The D+ donor units were not *RH* genotype matched, so they only switched patients who had at least one conventional RHD allele. The authors reported that no patient exhibited adverse effects or hemolytic transfusion reactions to transfusion with D+ red cells, and over 200 D- units remained in the inventory for emergencies or transfusion of D- individuals on an annual basis.

1.4 Compliance Statement

This study will be conducted in full accordance of all applicable Children's Hospital of Philadelphia Research Policies and Procedures and all applicable Federal and state laws and regulations including 45 CFR 46, and the Good Clinical Practice: Consolidated Guideline approved by the International Conference on Harmonisation (ICH). All episodes of noncompliance will be documented.

The investigators will perform the study in accordance with this protocol, will obtain consent and assent, and will report unanticipated problems involving risks to subjects or others in accordance with The Children's Hospital of Philadelphia IRB Policies and Procedures and all federal requirements. Collection, recording, and reporting of data will be accurate and will ensure the privacy, health, and welfare of research subjects during and after the study.

2 STUDY OBJECTIVES

The purpose of the study is to determine the feasibility, safety and challenges of transfusing *RHD* genotype matched donor red cells for D+ patients with SCD and history of anti-D.

2.1 Primary Objective

The primary objective of this study is to determine the feasibility and safety of *RH* genotype matched red cells (intervention) for chronically transfused D+ patients with SCD and history of anti-D. We will determine whether sufficient *RHD* genotyped units can be matched to the patient's own *RH* genotype, and whether re-exposure to D+ units that are *RHD* genotype matched is safe, as monitored by anti-D reappearance or signs of hemolysis.

3 INVESTIGATIONAL PLAN

3.1 General Schema of Study Design

There will be a screening and baseline assessment, and a treatment phase. See Table 1 for an overview of the study visits and procedures.

We will enroll at least 5 adults to collect safety and preliminary efficacy data, before enrolling children to the study. The data gathered will be submitted to the IRB via an amendment to be reviewed by the convened board. We will obtain IRB approval before opening enrollment to children.

3.1.1 Screening Phase and Baseline Assessment

Potential subjects will be screened using the protocol inclusion and exclusion criteria. We will identify D+ patients with a historical anti-D that is not currently detectable, and on a chronic transfusion program (by apheresis preferably). Eligible subjects will be approached in the hematology clinic, the Apheresis Unit, the Day Hospital (where patients receive straight transfusions), or the inpatient units at The Children's Hospital of Philadelphia.

Subject/parental/guardian permission (informed consent) and, if applicable, child assent, will be obtained prior to any study related procedures being performed, including discontinuation of current therapy.

A baseline assessment will be performed at the time of a clinic, day hospital transfusion, or apheresis visit. At the baseline assessment, one of the clinical study team members will obtain a medical history, physical exam, and review pertinent laboratory studies.

3.1.2 Intervention visit

Intervention visits will occur at the time of each of the patient's scheduled transfusion visits. Typically, patients requiring chronic transfusion therapy present for transfusion every 3-5 weeks to maintain their target hemoglobin S level.

At these intervention visits, a member of the research team will obtain an interim medical history, as well as review clinically obtained vital signs and laboratory evaluations. At the time

of pre-transfusion blood draw, IV placement or central line access, 5-10 ml of blood will be drawn for study evaluations (for additional antibody evaluation as needed). Patients will receive their transfusions as ordered for their clinical indication, but will receive red cell units from the blood bank that are *RH* genotype matched in addition to standard C, E, and K antigen matching. Other red cell processing such as irradiation, leukoreduction and/or washing will occur as clinically indicated. We will transfuse one D+ unit matched by *RHD* genotype at the first study visit, increase D+ units incrementally with subsequent transfusion visits, and monitor for anti-D re-demonstration and signs of hemolysis or other adverse effects. Adverse events will be assessed at each of these intervention visits. The next transfusion and intervention visit will be scheduled prior to the subject's discharge from their transfusion visit.

A laboratory visit will be scheduled 5 to 12 days after each transfusion to screen for antibodies and laboratory signs of increased red cell hemolysis from baseline.

If there is an inability to identify sufficient *RH* genotype matched units, standard of care serologic matched red cells will be administered rather than delaying transfusion for more than 7 days.

3.1.3 Follow-up Phase

The follow-up phase involves medical chart review and will continue for up to 6 months after the subject's last transfusion and intervention visit.

3.2 Study Duration, Enrollment and Number of Sites

3.2.1 Duration of Study Participation

The study duration per subject will be up to 2 years, with up to 1 day of screening and baseline assessment, up to 1.5 years of Intervention visits, and 6 months follow-up.

3.2.2 Total Number of Study Sites/Total Number of Subjects Projected

The study will be conducted at 2 investigative sites in the United States. The Children's Hospital of Philadelphia, and The New York Blood Center (no subject enrollment, providing *RH* genotyped red cell units and engaged in research activities).

Recruitment will stop when approximately 20 subjects are enrolled. It is expected that approximately 20 subjects will be enrolled to produce 18 evaluable subjects.

3.3 Study Population

We will enroll at least 5 adults to collect safety and preliminary efficacy data, before enrolling children to the study

3.3.1 Inclusion Criteria

1) Males or females age $\geq$ 8 years.

2) Diagnosis of SCD, all genotypes

3) Requires chronic red cell transfusion therapy

4) Requires a minimum of 3 units per transfusion visit

5) History of anti-D

6) *RH* genotype predicts D+ expression

7) Anti-D not detectable at screening visit and past 6 months

3.3.2 Exclusion Criteria

1) Rare *RH* genotype that would preclude sufficient RBC units

2) Antigen negative requirements due to alloimmunization that would preclude sufficient RBC units

3) Parents/guardians or subjects who, in the opinion of the Investigator, may be non-compliant with study schedules or procedures.

Subjects that do not meet all of the enrollment criteria may not be enrolled. Any violations of these criteria must be reported in accordance with IRB Policies and Procedures.

For subjects with a history of stroke/recurrent transient ischemic attack or other indication who require tight control of Hb S and *RH* genotyped blood is not available, standard of care serologic matched blood would be administered rather than delaying transfusion and risking higher Hb S level.

4 STUDY PROCEDURES

4.1 Screening Visit and Baseline Assessment

The screening visit and baseline assessment will occur on the same day with the following procedures:

- Informed consent
- Review inclusion/exclusion criteria
- Demographics/medical history
- Medication review
- Physical exam
- Vital signs
- Laboratory test review
- Medical Record Review

4.2 Study Treatment Phase

Intervention visits will be scheduled with each of the subject's transfusion visits as per the subject's clinical indication for chronic transfusion therapy. We will provide one unit of D+ *RH* genotype matched RBCs at the first transfusion study visit (Figure 3). The remainder of units will be D-, CEK-matched, and negative for all other antigens the patient is alloimmunized

against. In this manner, should anti-D be re-stimulated, only one unit of the 3 to 11 units required for red cell exchange will be at risk for hemolysis, and the patient would not become

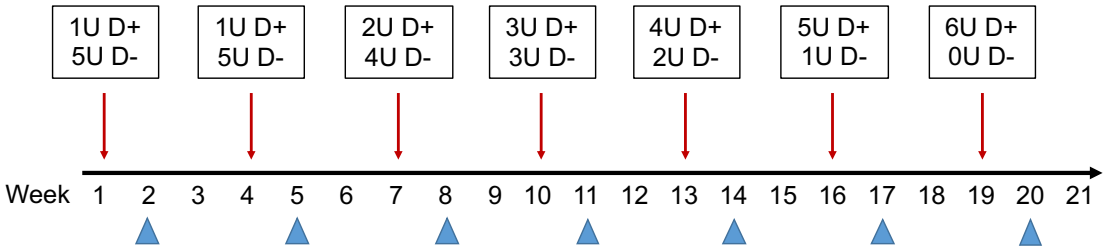

Figure 3. Example transfusion protocol for D+ patient with history of anti-D that is not currently detectable, and requires 6 units per RBC exchange every 3 weeks. One D+ unit will be matched by *RHD* genotype and transfused at the first two study visits, and an increasing number of D+ units will be issued at consecutive RBC exchange visits. U, unit. Triangle denotes a study visit for laboratory studies including an antibody screen, CBC, hemoglobin quantification and bilirubin level

acutely anemic. If laboratory monitoring shows no anti-D re-appearance or signs of increased hemolysis, the patient will receive one unit of D+ *RH* genotype matched RBCs at the 2nd transfusion study visit, and again if no anti-D re-appearance or signs of increased hemolysis, we will increase by one D+ unit per study visit until all units are D+. If a patient re-demonstrates anti-D, we will end that subject's participation and he/she will resume transfusions with D negative RBC units.

All patients with SCD on chronic transfusion therapy have a pre-transfusion complete blood count (CBC), Hgb quantification to determine %hgb A and S levels, and an antibody screen. We will collect a pre-transfusion sample to prospectively store plasma on all study subjects. Patients undergoing chronic red cell exchange will have an immediate post-procedure CBC and hgb quantification as standard of care. Study participants will return 5-12 days post-transfusion for a CBC/reticulocyte count, hgb quantification, bilirubin level, and antibody screen to evaluate for anti-D recrudescence or red cell hemolysis (Table 2).

Table 2. Laboratory monitoring

	Pre-transfusion	Immediate Post-transfusion	5-12 days Post-transfusion
Complete blood count	✓	✓	✓
Hemoglobin quantification	✓	✓	✓
Reticulocyte count	✓		✓
Antibody screen/identification	✓		✓
Bilirubin (total, unconjugated)			✓
Creatinine			✓
Plasma sample frozen	✓		✓
Donor red cells frozen	✓		

Any time a patient shows a re-appearance of anti-D on an antibody screen, the medical monitor will be notified, and all safety data will be reviewed. As stated above, if a subject re-demonstrates anti-D, we will end that subject's participation and he/she will resume transfusions with D negative RBC units.

If any subject shows signs of transfused red cell hemolyis, with or without an anti-D identified on the antibody screen, the medical monitor will be notified, and all safety data will be reviewed.

Study stopping criteria include:

1) Two of the first 5 enrolled subjects, or after the first 5 enrolled subjects, greater than 30% of enrolled patients, develop re-appearance of anti-D
2) Any subject with anti-D re-appearance and clinical or laboratory evidence of transfused red cell hemolysis (dark urine, increased scleral icterus, exacerbated anemia, higher Hb S level than expected based on timing from last transfusion

If either of these criteria occur, the study will be stopped, the safety medical monitor will review all clinical and laboratory evidence to determine safety risks, and will meet with the investigative team to determine a safe course of action. The IRB would be notified and the study procedures would be suspended until approval of the IRB.

4.2.1 Visit 1

At each intervention visit the following procedures and measurements include:

- Interim medical history
- Medication review
- Review of vital signs
- Review of clinical laboratory evaluation
- Peripheral blood sample collection
- Transfusion of 1 unit D+ *RH* genotype matched red cell units. Remainder of units transfused will be serologic CEK matched red cells that are D negative.
- Adverse event assessment

4.2.2 Visit 2

- Laboratory tests (see table 2)
- Assess possible adverse events

4.2.3 Visit 3 plus

- Same as Visit 1, keep repeating until all units transfused are D+ or anti-D reappearance

4.3 Follow-up Phase

The follow-up phase involves medical chart review and will continue for up to 6 months after the subject's last transfusion and intervention visit. The medical chart review will abstract data regarding the transfusion visit, as well as blood bank evaluations including antibody screens and antibody identification.

4.3.1 End of Study

There are no special procedures for the end of study participation.

4.4 Unscheduled Visits

We do not anticipate unscheduled visits but these will be recorded. If a patient presents to Emergency Department, or is admitted to the Inpatient Hospital service, and requires transfusion, we will order D- red cells matched for C, E, and K by standard serologic methods which is the standard of care.

4.5 Subject Completion/Withdrawal

Subjects may withdraw from the study at any time without prejudice to their care. They may also be discontinued from the study at the discretion of the Investigator for lack of adherence to study treatment or visit schedules, or AEs. The Investigator may also withdraw subjects who violate the study plan, or to protect the subject for reasons of safety or for administrative reasons. It will be documented whether or not each subject completes the clinical study. If the Investigator becomes aware of any serious, related adverse events after the subject completes or withdraws from the study, they will be recorded in the source documents and on the CRF.

4.5.1 Early Termination Study Visit

Subjects who withdraw from the study will have all procedures enumerated for the last transfusion/intervention as the early termination visit.

5 STUDY EVALUATIONS AND MEASUREMENTS

5.1 Screening and Monitoring Evaluations and Measurements

5.1.1 Medical Record Review

The following variables will be abstracted from the medical chart (paper or electronic).

- Date of birth
- Height and weight
- Vital signs (temperature, blood pressure, heart rate, respiratory rate)
- Medications
- Medical history including SCD clinical course, complications, and indication for chronic red cell transfusion therapy
- Transfusion and blood bank history including date of first transfusion, number of lifetime red cell exposures, alloimmunization history, antibody evaluations, history of transfusion reactions, *RH* genotype, extended red cell antigen profiles
- Additional clinical laboratory studies including complete blood counts, reticulocyte counts, antibody evaluations, direct antiglobulin test, chemistry panels, lactate dehydrogenase levels

5.1.2 Physical Examination

A physical examination will be performed for the baseline assessment.

5.1.3 Vital Signs

Vital signs (temperature, blood pressure, heart rate, respiratory rate) will be measured as part of clinical care. The study team will review and record the vital signs from the chart.

5.1.4 Laboratory Evaluations

The following laboratory evaluations are obtained as part of routine clinic and transfusion visits, which the study team will review and record from the patient's chart.

5.1.4.1 Table: Clinical Laboratory Tests

Category	Tests
Hematology	Hemoglobin, hematocrit, platelet count, WBC +/- differential, reticulocyte count, hemoglobin quantification
Liver function tests	AST, ALT, total bilirubin (total and unconjugated)
Renal function tests	BUN, creatinine
Chemistry tests	Lactate dehydrogenase
Blood bank tests	Type and screen, compatibility testing, antibody identification, direct antiglobulin test, *RH* genotype, extended red cell antigen profile

5.1.4.2 Table: Study Laboratory Tests 5-12 Days After Transfusion

Category	Tests
Hematology	Hemoglobin, hematocrit, platelet count, WBC +/- differential, reticulocyte count, hemoglobin quantification
Liver function tests	Bilirubin (total and unconjugated)
Renal function tests	BUN, creatinine
Blood bank tests	Antibody screen, antibody identification, +/- direct antiglobulin test

5.1.5 Diagnostic Tests

Pre-transfusion and post-transfusion laboratory studies will be monitored for anti-D re-appearance or signs of increased hemolysis (i.e. lower hemoglobin/hematocrit, lower hgb A and higher hgb S level, elevated bilirubin).

5.2 Safety Evaluation

Subject safety will be monitored by adverse events, vital signs, anti-D reappearance and other laboratory data (complete blood count, hemoglobin quantifications, red cell antibody evaluations). It will also include evaluation for any delays in transfusion of red cells. If a patient re-demonstrates anti-D, we will end that subject's participation and he/she will resume transfusions with D negative RBC units.

We will have a medical monitor independent of the study team who is familiar with SCD and transfusion, and blood bank procedures. The medical monitor will review safety data, including antibody screens for anti-D reappearance, and other laboratory studies, i.e blood counts and hemoglobin quantifications, for signs of transfused red cell hemolysis. Details about the untis transfused, including the *RH* genotype, and the red cell antigen profile, will be reviewed.

6 STATISTICAL CONSIDERATIONS

This is a pilot study to determine the feasibility and safety of providing *RH* genotype matched D+ red cells to patients with SCD and anti-D who require chronic transfusion therapy.

6.1 Primary Endpoint

The primary endpoint is the feasibility and safety of providing *RH* genotype matched D+ red cells for patients with SCD and history of anti-D who require chronic transfusion. *RH* genotype matched red cells are currently not available clinically except for rare circumstances. Descriptive, qualitative data, i.e. anti-D reappearance and signs and symptoms of increased red cell hemolysis, or delays in transfusion, will be collected to inform future multi-center studies of *RH* genotype matched red cells designed to test the effectiveness of providing *RH* genotype matched D+ red cells for patients with SCD and history of anti-D.

Given that there is donor center scalability with *RH* genotyping if more than one institution was requesting *RH* genotype matched red cells, we would consider this matching strategy feasible if provision of matched units were possible in > 85% of the transfusion study visits without a delay in transfusion.

6.2 Statistical Methods

6.2.1 Baseline Data

Baseline and demographic characteristics will be summarized by standard descriptive summaries (e.g. means and standard deviations for continuous variables such as age and percentages for categorical variables such as gender).

6.2.2 Efficacy Analysis

The primary efficacy endpoints will be anti-D re-appearance, signs of increased hemolysis, and timely issue of units for transfusion. These will be descriptive data.

6.2.3 Safety Analysis

All subjects entered into the study at Visit 1 will be included in the safety analysis. The frequencies of AEs by type, severity and relationship to study intervention will be summarized. SAEs (if any) will be described in detail. The analysis will be a qualitative description of AEs.

6.3 Sample Size and Power

The sample size for this feasibility and safety study is based on our current chronically transfused patient population who receive apheresis *and* have a historical anti-D, which is approximately 20-25 active patients. We expect an additional 2-3 eligible patients per year. Therefore, we anticipate that with ~20 subjects will enroll over the 5 years of the study.

6.4 Interim Analysis

If a patient re-demonstrates anti-D, we will end that subject's participation and he/she will resume transfusions with D negative RBC units. If a patient's genotype precludes readily identified RH genotype matched RBC units and a delay in transfusion is documented in 3 consecutive visits, we will consider termination of study participation for that individual.

7 STUDY INTERVENTION

7.1 Description

The study intervention is provision of *RH* genotype matched D+ red cells for transfusion in chronically transfused patients with SCD and history of anti-D. All other clinical care will remain as usual, including the decision to transfuse, timing of transfusions, and the quantity of red cells to transfuse. Red cells will be ordered from NY Blood Center using a secure online ordering system, per usual clinical practice guidelines established at CHOP for patients with SCD. Specifically, serologic C, E, and K antigen matched donor units are ordered. All units are leukoreduced, CMV-safe, and irradiated per CHOP policy. The CHOP blood bank aims to issue < 21 day old for all patients with hemoglobinopathies, particularly for those chronically transfused.

The study intervention is D+ donor units that are *RH* genotyped by the blood supplier, and will be matched according to the patient's *RH* genotype. *RH* genotyping assays are not FDA approved, but are performed in an American Association of Blood Banks (AABB) accredited and CLIA certified lab, and validated for sensitivity, specificity, reproducibility and repeatability by NY State regulations as laboratory developed tests (LDTs). There are no current FDA approved *RH* genotyping assays that distinguish Rh variants from conventional Rh antigens. In fact, many assays including the *RH* genotyping that are currently used clinically to aid in antibody identification and red cell matching for patients with SCD are not FDA approved.

7.1.1 Packaging

Red cell units will have the typical packaging from the blood supplier.

7.1.2 Labeling

Red cell unit labels will have the typical labels from the blood supplier but the donor unit ID will be linked to *RH* genotype data.

7.1.3 Dosing

The volume of red cells ordered for transfusion will be based solely on the clinician order. Typically, patients receive between 2 and 11 units of RBCs for a red cell exchange, depending on the pre-transfusion hgb and hgb S level.

7.1.4 Treatment Compliance and Adherence

Compliance with transfusion visits will be monitored. Termination of study participation will be considered for patients who miss multiple scheduled transfusion visits.

7.1.5 Transfusion Product Accountability

Adequate records of study red cell unit receipt and disposition will be maintained by the CHOP Blood Bank including blood orders, dispensing records, and disposition forms, which is standard for all blood products issued. These will be examined during the course of the study. The study coordinator will extract this data for study records. The purpose of these records is to ensure regulatory authorities and the Sponsor that the investigational product is accounted for. Should a patient miss their appointment, the blood may be issued to non-study individuals at the discretion of the investigators.

8 SAFETY MANAGEMENT

8.1 Clinical Adverse Events

Clinical adverse events (AEs) will be monitored throughout the study.

We will have a medical monitor independent of the study team who is familiar with SCD and transfusion, and blood bank procedures. The medical monitor will review safety data, including antibody screens for anti-D reappearance, and other laboratory studies, i.e blood counts and hemoglobin quantifications, for signs of transfused red cell hemolysis. Details about the untis transfused, including the *RH* genotype, and the red cell antigen profile, will be reviewed.

Any time a patient shows a re-appearance of anti-D on an antibody screen, the medical monitor will be notified, and all safety data will be reviewed. As stated above, if a subject re-demonstrates anti-D, we will end that subject's participation and he/she will resume transfusions with D negative RBC units.

If any subject shows signs of transfused red cell hemolyis, with or without an anti-D identified on the antibody screen, the medical monitor will be notified, and all safety data will be reviewed.

Study stopping criteria include:

1) Three of the first 5 enrolled subjects, or greater than 50% of enrolled patients, develop re-appearance of anti-D
2) Any subject with anti-D re-appearance and clinical or laboratory evidence of transfused red cell hemolysis (dark urine, increased scleral icterus, exacerbated anemia, higher Hb S level than expected based on timing from last transfusion

If either of these criteria occur, the study will be stopped, the safety medical monitor wll review all clinical and laboratory evidence to determine safety risks, and will meet with the investigative team to determine a safe course of action. The IRB would be notified and the study procedures would be suspended until approval of the IRB.

8.2 Adverse Event Reporting

Unanticipated problems related to the research involving risks to subjects or others that occur during the course of this study (including SAEs) will be reported to the IRB in accordance with CHOP IRB SOP 408: Unanticipated Problems Involving Risks to Subjects. AEs that are not serious but that are notable and could involve risks to subjects will be summarized in narrative or other format and submitted to the IRB at the time of continuing review.

8.3 Definition of an Adverse Event

An adverse event is any untoward medical occurrence in a subject who has received an intervention (drug, biologic, or other intervention). The occurrence does not necessarily have to have a causal relationship with the treatment. An AE can therefore be any unfavorable or unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

All AEs (including serious AEs) will be noted in the study records and on the case report form with a full description including the nature, date and time of onset, determination of non-serious versus serious, intensity (mild, moderate, severe), duration, causality, and outcome of the event.

8.4 Definition of a Serious Adverse Event (SAE)

An SAE is any adverse drug experience occurring at any dose that results in any of the following outcomes:

- death,
- a life-threatening event (at risk of death at the time of the event),
- requires inpatient hospitalization or prolongation of existing hospitalization,
- a persistent or significant disability/incapacity, or
- a congenital anomaly/birth defect in the offspring of a subject.

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug event when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

A distinction should be drawn between serious and severe AEs. A severe AE is a major event of its type. A severe AE does not necessarily need to be considered serious. For example, nausea which persists for several hours may be considered severe nausea, but would not be an SAE. On the other hand, a stroke that results in only a limited degree of disability may be considered a mild stroke, but would be an SAE.

8.4.1 Relationship of SAE to study drug or other intervention

The relationship of each SAE to the study intervention should be characterized using one of the following terms in accordance with CHOP IRB Guidelines: definitely, probably, possibly, unlikely or unrelated.

8.5 IRB/IEC Notification of SAEs and Other Unanticipated Problems

The Investigator will promptly notify the IRB of all on-site unanticipated, serious Adverse Events that are related to the research activity. Other unanticipated problems related to the research involving risk to subjects or others will also be reported promptly. Written reports will be filed using the eIRB system and in accordance with the timeline below. External SAEs that are both unexpected and related to the study intervention will be reported promptly after the investigator receives the report.

Type of Unanticipated Problem	Initial Notification (Phone, Email, Fax)	Written Report
Internal (on-site) SAEs Death or Life Threatening	24 hours	Within 2 calendar days
Internal (on-site) SAEs	7 days	Within 7 business days

All other SAEs		
Unanticipated Problems Related to Research	7 days	Within 7 business days
All other AEs	N/A	Brief Summary of important AEs may be reported at time of continuing review

8.5.1 Follow-up report

If an SAE has not resolved at the time of the initial report and new information arises that changes the investigator's assessment of the event, a follow-up report including all relevant new or reassessed information (e.g., concomitant medication, medical history) will be submitted to the IRB. The investigator is responsible for ensuring that all SAE are followed until either resolved or stable.

8.6 Investigator Reporting of a Serious Adverse Event to Sponsor

Reporting will be consistent with regulatory requirements.

8.7 Medical Emergencies

Describe any plans or procedures for taking care of medical emergencies that might develop during the course of the study. Should a patient demonstrate anti-D reappearance that is associated with increased hemolysis, he/she will be treated with immunosuppression congruent to the degree of anemia and hemolysis (i.e. oral steroids, IV steroids, and/or IVIg depending on severity). If moderate to severe hemolytic transfusion reaction, he/she may need to be admitted to the hospital and may require transfusion of D- RBC units.

9 STUDY ADMINISTRATION

9.1 Data Collection and Management

Primary records (source documents) and data abstraction forms for entering the data into our study database (Filemaker) will be used. The plan is consistent with CHOP Policy A-3-6: Acceptable Use of Technology Resources that defines the requirements for encryption and security of computer systems, and addresses the following:

1. Confidentiality. Confidentiality of the data will be ensured with the use of password-protected files. Specifically, a Filemaker database is hosted on the CHOP research network and password protected such that only the study team members have access. Data may be exported into Excel files, which can be both coded with the UPIN and a separate master list, and password-protected. Blood ordering is performed via a secure online blood ordering system hosted by the blood supplier (NY Blood Center).

2. Security. Data is backed up intermittently as a copy of the password-protected file on the PI's office computer and the original is hosted on one of the Hospital's secure servers for research community.

3. Anonymization, de-identification or destruction. Analyses from this study will be conducted over many years and we would like to retain PHI to link the samples with the subjects. The identifiers and data will be retained per CHOP Data Retention Policy A-3-9 for Human subjects research that is greater than minimal risk.

9.2 Confidentiality

All data and records generated during this study will be kept confidential in accordance with Institutional policies and HIPAA on subject privacy. The Investigator and other site personnel will not use such data and records for any purpose other than conducting the study. Subject confidentiality will be maintained by the use of password protected files that are accessible only to study personnel and regulatory agencies overseeing the research.

No identifiable data will be used for future study without first obtaining IRB approval. The investigator will obtain a data use agreement between the provider (the PI) of the data and any recipient researchers (including others at CHOP) before sharing a limited dataset (PHI limited to dates and zip codes).

9.3 Regulatory and Ethical Considerations

9.3.1 Data and Safety Monitoring Plan

The study has greater than minimal risk. The study intervention of *RH* genotype matched D+ red cells to patients with SCD and anti-D provides a higher level of matching than current standard of care, but there is a risk of anti-D reappearance, increased red cell hemolysis and delay to transfusion for up to 7 days. If a patient re-demonstrates anti-D, we will end that subject's participation and he/she will resume transfusions with D negative RBC units. In the case of acute need for transfusion, subjects may receive non-*RH* genotype matched red cells that would be matched by standard of care methods at the discretion of the attending physician, i.e. D-, CEK matched red cells. Oversight for the emerging safety information will include the PI. Red cell units will continue to be issued per standard institutional blood bank policies.

We will have a medical monitor independent of the study team who is familiar with SCD and transfusion, and blood bank procedures. The medical monitor will review safety data, including antibody screens for anti-D reappearance, and other laboratory studies, including blood counts and hemoglobin quantifications, for signs of transfused red cell hemolysis. Details about the units transfused, including the *RH* genotype, and the red cell antigen profile, will be reviewed.

Any time a patient shows a re-appearance of anti-D on an antibody screen, the medical monitor will be notified, and all safety data will be reviewed. As stated above, if a subject re-demonstrates anti-D, we will end that subject's participation and he/she will resume transfusions with D negative RBC units.

If any subject shows signs of transfused red cell hemolyis, with or without an anti-D identified on the antibody screen, the medical monitor will be notified, and all safety data will be reviewed.

Study stopping criteria include:

1) Two of the first 5 enrolled subjects, or after the first 5 enrolled subjects, greater than 30% of enrolled patients, develop re-appearance of anti-D
2) Any subject with anti-D re-appearance and clinical or laboratory evidence of transfused red cell hemolysis (dark urine, increased scleral icterus, exacerbated anemia, higher Hb S level than expected based on timing from last transfusion

If either of these criteria occur, the study will be stopped, the safety medical monitor will review all clinical and laboratory evidence to determine safety risks, and will meet with the investigative team to determine a safe course of action. The IRB would be notified and the study procedures would be suspended until approval of the IRB.

The PI will monitor study progress, ensure subject safety, and the accuracy and security of the data at CHOP, and will report any adverse events in accordance with the FDA regulations as they pertain to blood products and IRB policies.

9.3.2 Risk Assessment

Risks are greater than minimal. Providing D+ RBC units to a patient with history of anti-D may cause re-appearance of the anti-D with or without transfused red cell hemolysis. *RH* genotype matched red cells provide a higher level of matching, and thus the risk of anti-D reappearing is predicted to be very low. The study was also designed to minimize the risk of harm from anti-D reappearance by transfusing D+ *RH* genotype matched units for only 1 out of a typical 3 to 8 RBC units for the first two visits, and then titrating up by one unit with each subsequent transfusion. There is the possibility that transfusion would be delayed up to 7 days to identify adequate *RH* genotype red cell units, this may cause inconvenience (waiting or rescheduling transfusion visit).

There are risks involved with red cell transfusion, i.e. allergic reaction, infection, alloimmunization, but these risks are associated with routine clinical care and not a result of the research activities.

The risks involved with a venipuncture are pain, bleeding or bruising at the venipuncture site.

9.3.3 Potential Benefits of Trial Participation

Direct benefits to the study subject as a result of participation may be demonstrating that the patient can be transfused safely with D+ *RH* genotype matched red cells and no longer need to rely on D- red cell units, resulting in improved ability to identify compatible units. The indirect benefits are that all patients with SCD may receive safer transfusions in the future if we find *RH* genotype matching is feasible.

9.3.4 Risk-Benefit Assessment

RH genotype matched red cells are predicted to reduce Rh alloimmunization and result in safer transfusion products, thus, providing more benefit than risk. The risks of providing D+ units to D+ patients with history of an anti-D are greater than minimal due to risk of anti-D re-appearance and potential for increased hemolysis, but the benefit of *RH* genotype matched red cells that provide a higher level of matching than standard clinical practice outweigh those risks.

9.4 Recruitment Strategy

Patients will be recruited from the chronic transfusion program for SCD at the Children's Hospital of Philadelphia. This will primarily include patients who receive their red cell transfusions erythrocytapheresis in the Apheresis Unit, but could include patients transfused by simple transfusion in the Day Hospital. Since the principal investigator is a pediatric hematologist and transfusion medicine specialist, she will refer her own patients as well as other care providers. All patients with SCD receiving chronic transfusion therapy will be screened for eligibility. If the prospective subjects are not patients of the investigator, one of the investigators or the research coordinator will approach the subjects and offer participation in the study in person during a transfusion visit. If recruitment is poor from the apheresis transfusion cohort at CHOP, patients receiving 2-3 units for simple red cell transfusion can be recruited from the Day Hospital.

9.5 Informed Consent/Assent and HIPAA Authorization

All prospective subjects eligible for participation in this study will be enrolled after obtaining informed consent. The study coordinator or one of the investigators will obtain consent in the Hematology Clinic, Day Hospital, or Apheresis Unit. The consent process will take place in the clinic exam room, or other private room where privacy is assured. The investigational nature of the study, the study objectives, procedures involved and the potential risks and benefits will be explained to the subject and/or guardian using lay language. Consent forms will be written in a well-organized format using direct language. We will explicitly state that the choice of study participation will not impact their access to clinical care. Assent will be obtained from all children seven years and older before study enrollment when deemed appropriate by the investigator and the parent(s). Written informed combined consent/HIPAA authorization and assent (if applicable) will be obtained. Individuals with limited English proficiency will only be enrolled in person after obtaining written consent using the short form consent process.

9.6 Payment to Subjects/Families

Subjects will be compensated a nominal amount for the inconvenience of participating in the study, which requires a return visit for laboratory studies. Subjects will be compensated $50 per laboratory only visit, since this is a separate visit outside of routine clinical care. For patients who encounter economic hardship to travel to and from CHOP for the laboratory only

visit despite the $50 compensation, we can offer reimbursement for travel for the follow up lab visits that are obtained 5-12 days after the transfusion since these are outside the visits needed for routine clinical care. Money will be added after each laboratory visit to a debit card.

10 PUBLICATION

The study investigators using standard publication guidelines will prepare manuscript(s) and abstract(s) from the data collected during this trial.

11 REFERENCES

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