CLI 00126

CLI-SAP 00126 v2.0

STATISTICAL ANALYSIS PLAN

August 28, 2026

NCT03037164



STATISTICAL ANALYSIS PLAN

CLI 00126

Protocol Title:	A Randomized, Double-Blind, Controlled, Parallel Group Study with the INTERCEPT Blood System for Red Blood Cells in Regions at Potential Risk for Zika Virus Transfusion-Transmitted Infections (**RedeS** Study)
Product:	Red Blood Cells treated with the INTERCEPT Pathogen Reduction System
Protocol Number:	CLI 00126 (Version 9.0)
Sponsor:	Cerus Corporation
SAP Author:	Kathy Liu, Ph.D.
Date:	August 28, 2026
Version:	2.0

CONFIDENTIAL

The information in this document is considered privileged and confidential by Cerus Corp. and may not be disclosed to others except to the extent necessary to obtain Institutional Review Board approval and informed consent, or as required by Federal and State Laws. Persons to whom this information is disclosed must be informed that this information is privileged and confidential and that it should not be further disclosed.

TABLE OF CONTENTS

ABBREVIATIONS **5**

1. INTRODUCTION **6**

2. CLINICAL BACKGROUND **6**

3. OBJECTIVE(S) AND ENDPOINTS **7**

3.1 Objectives 7

3.2 Endpoints 7

3.2.1 Primary Efficacy Endpoint 7

3.2.2 Secondary Efficacy Endpoints 7

3.2.3 Safety Endpoints 7

4. STUDY DESIGN **8**

4.1 General Study Design 8

4.2 Randomization and Blinding 9

4.3 Sample Size Estimation 10

4.4 Visits and Assessments 10

5. DEFINITIONS **16**

5.1 Time-Related Terms 16

5.1.1 Baseline 16

5.1.2 Study Day 16

5.1.3 RBC Exposure 16

5.1.4 Episode level Change 16

5.2 Other Definitions 16

5.2.1 Prior and Concomitant Medications 16

5.2.2 Non-Study RBC 17

6. STUDY POPULATION **18**

6.1 Modified Intent-to-Treat (mITT) Population 18

6.2 Non-Bleeding mITT Population 18

6.3 Non-Bleeding Per-Protocol Set (PPS) 18

6.4 Bleeding Extension Red Cell Exchange (RCE) Population 19

7. GENERAL CONSIDERATIONS **20**

7.1 Handling of Missing Data 20

7.1.1 Efficacy Measure 20

7.1.2 Safety Measures 20

7.1.3 Dates for Medical Events and Medications 21

7.2 Multi-Center Studies 21

7.3 Multiple Comparisons / Multiplicity 22

7.4 Interim Analysis 22
7.5 Data Safety and Monitoring Board 22
8. SUMMARY OF STUDY POPULATION DATA 23
8.1 Subject Disposition 23
8.2 Demographics and Baseline Characteristics 23
8.3 Medical, Surgical and Transfusion History 24
8.4 Protocol Deviations 24
8.5 Prior and Concomitant Medications 24
8.6 Exposure to Study RBC, Non-Study RBC and Other Blood products 25
9. EFFICACY ANALYSES 26
9.1 Analysis of Primary Efficacy Endpoint 26
9.2 Analysis of Secondary Efficacy Endpoints 28
9.2.1 Adjusted hemoglobin consumption 28
9.2.2 HbA clearance rate 29
9.3 Sensitivity Analyses 30
9.4 Exploratory Analyses 31
9.5 Subgroup Analyses 32
10. SAFETY ANALYSES 32
11. DEVIATIONS FROM THE PROTOCOL 33
12. REFERENCES 35

LIST OF TABLES

Table 1: Schedule Assessments for Subjects Requiring Simple or Repeated Simple RBC Transfusions....11

Table 2: Schedule Assessments for SCD subjects receiving 3 consecutive RCE procedures....13

Table 3: Handling of Missing Data for Medical Events and Medications 21

Table 4: Overview of Primary and Sensitivity Analysis Methods 30

Table 5: Overview of Exploratory Analyses 31

LIST OF FIGURES

Figure 1: Study Design Diagram....9

ABBREVIATIONS

AE	Adverse Event
AESI	Adverse Events of Special Interest
ANCOVA	Analysis of Covariance
ATC	Anatomical-Therapeutic-Chemical
CMH	Cochran-Mantel-Haenszel
CI	Confidence Interval
Control	Conventional RBCs
CSR	Clinical Study Report
eCRF	Electronic Case Report Form
Hb	Hemoglobin
INTERCEPT	INTERCEPT Blood System
MAR	Missing at Random
MCMC	Markov Chain Monte Carlo
MDS	Myelodysplastic syndrome
MedDRA	Medical Dictionary for Regulatory Activities
MI	Multiple Imputation
mITT	Modified Intention to Treat
MMRM	Mixed Models for Repeated Measures
MNAR	Missing Not at Random
NI	Non-Inferiority
PMM	Pattern Mixture Modeling
PPS	Per-Protocol Set
PRT	Pathogen Reduction Technology
PT	Preferred Term
RBC	Red Blood Cell
RCE	Red Cell Exchange
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAS	Statistical Analysis System
SCD	Sickle Cell Disease
SOC	System Organ Class
TBV	Total Blood Volume
TE	Treatment Emergent
TEAE	Treatment Emergent Adverse Event
Test	INTERCEPT RBCs
TR	Transfusion Reaction
TSP	Transfusion Support Period
TTI	transfusion transmitted infections

1. INTRODUCTION

This statistical analysis plan (SAP) specifies the statistical methods to be implemented for the analysis of data collected from the RedeS study within the scope of Cerus CLI00126: "a multi-center, randomized, double-blind, active-controlled, parallel-design study, with an extension option to evaluate subjects requiring repeated transfusion for chronic anemia or sickle cell disease (SCD) subjects undergoing regular repeated red cell exchange." It applies to the study protocol (version 9.0) dated 18 December 2023 and provides detailed instructions as to how each analysis will be performed.

Results obtained from the analyses specified in the final approved version of the SAP will become the basis of the clinical study report (CSR) for this study. Post-hoc exploratory analyses not necessarily identified in this SAP may be performed to further examine study data. Any post-hoc, or unplanned, exploratory analysis will be clearly identified as such in the final CSR.

2. CLINICAL BACKGROUND

Blood transfusion is a critical supportive therapy for healthcare. Subjects and physicians assume that, when required, blood transfusion will be available and safe. However, serious transfusion-transmitted infections (TTI) caused by viruses, bacteria and protozoa (Stramer, et al., 2009; MacLennan & Williamson, 2006); and fatal transfusion complications such as transfusion-associated graft-versus-host disease (TA-GVHD) still pose a threat to transfusion recipients (Kopolovic, et al., 2015). Currently, prevention of TTI includes pre-donation evaluation and selection of low-risk donors, followed by serologic and/or nucleic acid testing for selected known infectious pathogens.

Although the advent of testing has greatly reduced TTI from blood products, particularly viral disease, pathogens may still contaminate the blood supply because emerging infections are not predictable and may contaminate the blood supply for a period of time before discovery and availability of a test.

The INTERCEPT Blood System for platelets is the only pathogen reduction system currently approved by FDA for platelets, while Octaplas™ and the INTERCEPT Blood System are both approved for plasma. While there is no FDA approved pathogen reduction system yet for Red Blood Cells (RBCs), the INTERCEPT Blood System for RBCs has been shown to inactivate a range of pathogens, including arboviruses such as DENV, CHIKV and ZIKV (> 4 log10).

The present study has the following aims: 1) evaluate the safety and efficacy of the INTERCEPT Blood System for RBCs in subjects who require RBC transfusion support, 2) provide early access to the INTERCEPT Blood System for RBCs in regions where a substantial proportion of the population has been infected or is at risk of emerging transfusion transmitted infections, and 3) protect the blood supply and support self-sufficiency in these regions.

3. OBJECTIVE(S) AND ENDPOINTS

3.1 Objectives

The objective of this study is to evaluate the efficacy and safety of the INTERCEPT Blood System for RBCs pathogen reduction technology (PRT) in comparison to conventional RBCs in subjects who require acute and long- term RBC transfusion support in areas at risk of transfusion transmitted infections (TTI).

3.2 Endpoints

3.2.1 Primary Efficacy Endpoint

The primary efficacy endpoint is:

- Adjusted hemoglobin increment, defined as the difference between the pre-transfusion and post-transfusion episode hemoglobin values divided by the total hemoglobin content transfused, averaged over one or more transfusion episodes in non-bleeding subjects that were without active bleeding at baseline (active bleeding is defined as WHO Grade 3 or 4 bleeding).

3.2.2 Secondary Efficacy Endpoints

- Adjusted hemoglobin consumption, defined as total hemoglobin mass transfused in grams divided by body weight in kg at baseline and duration of the study transfusion period in days (g/kg/day), in non-bleeding subjects without active bleeding at baseline.
- HbA clearance rate in subjects with sickle cell disease (SCD) undergoing regular repeated red cell exchange (RCE).

3.2.3 Safety Endpoints

The primary safety endpoints are:

- Proportion of subjects with any treatment-emergent adverse events (TEAEs) possibly, probably, or definitely related to study RBC transfusion through 28 days after the last study transfusion.
- Proportion of subjects with treatment-emergent antibodies with confirmed specificity to INTERCEPT RBCs.

Additional safety assessments will include:

- Proportion of subjects with treatment-emergent AEs through 28 days after the last study transfusion.

- Proportion of subjects with transfusion reactions (TRs) related to study RBCs (test or control) (as defined by the CDC National Healthcare Safety Network [NHSN] Hemovigilance Module protocol), for 28 days after the last study transfusion.
- Proportion of subjects with treatment-emergent immunization to RBC allo-antigens; and
- All-cause mortality through 28 days after the last study transfusion
- Proportion of subjects with adverse events of special interest (AESI) through 28 days after the last study transfusion, in SCD subjects enrolled into the 6-Month extension with three RCE procedures.
- S-300 and Glutathione (GSH) plasma levels before and 15 minutes to 4 hours after RCE, in SCD subjects enrolled into the 6-Month extension with three RCE procedures.

Please note no formal hypotheses will be tested on the primary safety endpoints.

4. STUDY DESIGN

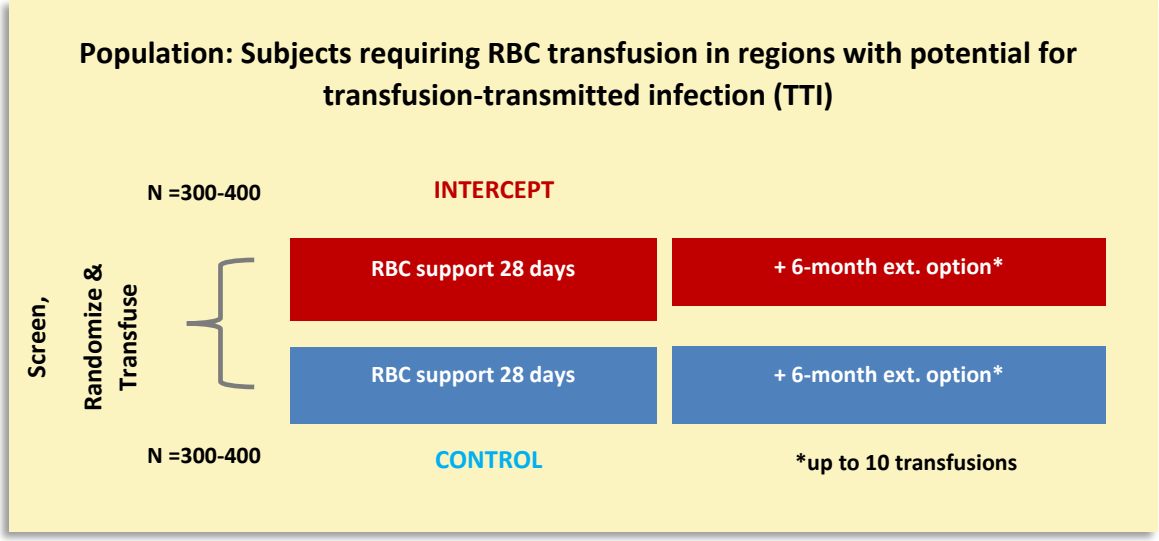
4.1 General Study Design

The study is a prospective, multicenter, randomized, double-blinded, active controlled, parallel-design, non-inferiority study, with an extension option to evaluate subjects requiring repeated transfusion for chronic anemia or sickle cell disease (SCD) subjects undergoing regular repeated RCE.

Eligible subjects will be consented and screened first to be considered for randomization. If eligible, the subject will be randomized to receive either INTERCEPT RBCs or conventional RBCs in a 1:1 ratio.

It is estimated that between 600 to 800 eligible subjects will be randomized and transfused with study RBC(s) (Figure 1).

Figure 1: Study Design Diagram



4.2 Randomization and Blinding

Subjects will be randomized to receive either Test or Control RBCs in 1:1 ratio. Randomization will be stratified by site, baseline bleeding status (indication for RBC transfusion due to active bleeding or for anemia without active bleeding), subjects' participation in the optional extension period (yes/no) and subjects' need for RCE. Active bleeding was defined as Grade 3 or 4 bleeding of the expanded WHO bleeding scale. Anticipated surgical bleeding and planned blood loss/replacement through RCE at baseline should be included as active bleeding by definition.

Randomized subjects who do not receive any study RBC transfusions through 28 days post-randomization will be discontinued from the study and replaced. Additional data will not be collected for these subjects, nor will they be part of the data analysis. These subjects may be reassessed for eligibility and re-enrollment after withdrawal.

Only the appropriate blood bank and transfusion service staff will be able to access the treatment arm assignment. Study RBC component labels will be indistinguishable for Test (INTERCEPT) and Control products. In order to protect the blind, on the day of planned transfusion, the final product (test and control) may be transferred into an FDA approved RBC storage container (Fenwal™ Transfer Pack™ Container – 600 mL, product code 4R2023) using a sterile docking technique. Blood transferred into an ancillary container should be stored at 1°C to 6°C and transfused within 7 calendar days of transfer.

Clinical staff and others caring for participating subjects, as well as the sponsor (and delegates) will be blinded to treatment assignments. Unblinded delegates will monitor the production of the RBC components at designated blood centers and distribution at hospital transfusion centers.

4.3 Sample Size Estimation

Assuming no treatment difference on the primary efficacy endpoint of adjusted hemoglobin increment, with a coefficient of variation of 50% and a non-inferiority margin of 15% of the control mean, 468 non-bleeding evaluable subjects (234 per treatment group) will provide approximately 90% power to declare non-inferiority at the two-sided 0.05 alpha level. Under the same assumptions, 402 non-bleeding evaluable subjects (201 per treatment group) will provide 85% power and 352 (176 per treatment group) will provide 80% power.

The study will also seek to include 140 non-bleeding subjects requiring repeated transfusions for chronic anemia enrolled into the 6-Month extension period. Subjects who receive 3 or more study transfusion episodes (including both bleeding and non-bleeding) and have an assessment of antibodies after the third study transfusion episode will be summarized as a subgroup representing subjects who are repeatedly exposed in terms of immunogenicity.

In addition, up to 30 SCD patients requiring regular repeated RCE will be enrolled into the extension period to allow a total of three consecutive RCE procedures in at least 20 subjects.

It is estimated that between 600 to 800 randomized and transfused subjects will be needed in order to meet the targets for sample size to power the primary efficacy endpoint and the 28-Day +6-Month extension period enrollment goal.

4.4 Visits and Assessments

Assessments at each visit and the time/visit window for each post-baseline assessment are specified in the Assessment Schedule (Table 1 and Table 2). For subjects whose study participation is terminated prior to Day 75 Visit, to the extent possible, physical exam, including general neurological examination, should be performed, concomitant medications and adverse events should be recorded. Standard laboratory tests should be collected, and other blood samples should be performed at the Exit Visit.

Table 1: Schedule Assessments for Subjects Requiring Simple or Repeated Simple RBC Transfusions

Study Assessments	Screening	Transfusion Support Period (TSP)*		Follow-Up Visit Day 28 (28±7 days) after last study transfusion	End of Study-Day 75 of Follow-Up (75±15 days) after last study transfusion
		Before each transfusion episode	15min - 24 hour after each transfusion episode		
Informed Consent (assent, if appropriate)	X[1]				
Medical, surgical, prior transfusion and medication history	X[1]				
Physical exam, including height	X[1]				
Weight	X[1]				
Demographics	X[1]				
ABO Blood type and Rh[3]	X[1]	X[3]			
Indirect Antiglobulin Test[3]	X[1]	X[3]		X	
DAT[8]	X[1]			X	X
Immune reactivity to INTERCEPT RBC (S-303 antibody testing)	X[1]	X[7]		X	X
Pregnancy test (if applicable)[3]	X				
Indication for RBC transfusion	X[1]				
Hematology panel[9]	X[1]	X		X	
Hemoglobin Measurement			X[2]		
Blood chemistry panel[10]	X[1]	X		X	
Coagulation panel[11]	X[1]			X	
Vital signs	X[1]	X	X	X	
RBC transfusion data (study or non-study)	X[1]	X		X[5]	
Concomitant medications	X[1]	X[4]		X	
Other blood Components transfused		X		X	
Procedure details (as applicable, e.g., surgery)		X			
Adverse events and transfusion reactions		X	X	X[13]	
SAEs		X	X	X[13]	
Surveillance Calls [12]		X		X	
Documentation of vital status		X		X	X

Assess the proportion of each RBC component transfused (e.g., 25%, 50%, 75% or 100%). For pediatric subjects, the volume of the intended transfusion should be recorded.			X		
Assessment of bleeding status[6]	X	X			

***** TSP includes Day 1 through Day 28 for simple transfusion subjects, and Day 1 through Day 28 plus 6-Month extension for repeated simple transfusion subjects.

[1] If results from the Screening assessments listed are available in the medical record within 30 days prior to randomization, those data may serve as the screening data and the assessments do not need to be repeated unless the subject has an RBC transfusion in the 30-day period. If an RBC transfusion occurs after determined eligibility but prior to randomization, the subject may be randomized, and those Screening assessments listed above should be repeated prior to the first study transfusion.

[2] Hb measurement at 15 minutes to 24 hours post transfusion episode (before the next RBC transfusion episode)

[3] Routine standard of care test order per local hospital SOPs

[4] Medications administered during anesthesia for surgical or other procedures are not collected.

[5] The date of non-study RBC transfusion, if any, during the 28-Day follow up period and 6-Month extension, if applicable, must be documented.

[6] Daily for inpatients when available through the end of the acute transfusion period.

[7] Performed in conjunction with every routine standard of care test for ABO Type, Rh and screen (IAT) test that is performed during the Acute Support Period

[8] If, during the acute support period or follow up assessments (Day 28), a positive DAT result is observed, participating sites will follow their routine standard practice for confirmation/characterization of DAT positive samples; this should only apply to any conversion from a negative to a positive DAT after enrollment, that is not part of an investigation of hemolysis or of a positive IAT with evidence of an S-303 antibody, in which case further investigations may be appropriate after discussion with the sponsor.

[9] Hematology Panel: WBC, RBC, Hb, Hct, MCV, MCH, MCHC, MPV, RDW, PLT, Neut %, Mono%, Lymph%, Baso%, Eos%

[10] Blood Chemistry Panel: BUN, Creat, EGFR, NA, K, GLU, Cl, CO_2, TP, ALB, ALKP, AST (SGOT), ALT (SGPT), LDH, CPK, TBIL, DBIL

[11] Coagulation Panel: PT, INR, APTT, Fibrinogen

[12] In the outpatient setting if the subject is not seen by study staff for any other reason, surveillance calls are to be completed at least monthly (at minimum every 35 days) to collect AEs, SAEs, TRs, and UADEs starting from the initiation of the first study transfusion through the entire transfusion support period and through 28 days after the final study transfusion. If the monthly surveillance call interval coincides with a study visit, the surveillance call is not required as the study visit assessments take precedent.

[13] AEs, SAEs, TRs, and UADEs are collected from initiation of the first study transfusion through exactly 28 days after the last study transfusion.

Table 2: Schedule Assessments for SCD subjects receiving 3 consecutive RCE procedures

Study Assessments	Screening	Transfusion Support Period (TSP) (Day 1 through Day 28 plus 6-Month extension or death – 3 RCE episodes)		Follow-Up Visit Day 14 (14±7 days) after each RCE episode	Follow-Up Visit Day 28* (28±7 days) after last study RCE or prior to next non-study RCE if less than 28 days since last study RCE	End of Study-Day 75 of Follow-Up (75±15 days) after last study transfusion
		Before each RCE episode	15min – 4 hours after each RCE episode			
Informed Consent (assent, if appropriate)	X[1]					
Medical, surgical, prior transfusion[2] and medication history, (incl. basis of SCD diagnosis and G6PD status)	X[1]					
Physical exam, including height	X[1]					
Weight	X[1]					
Demographics	X[1]					
ABO Blood type and Rh[3]. Extended Phenotype, if available	X[1]	X[3]				
Indirect Antiglobulin Test[3]	X[1]	X[3]			X	
DAT[8]	X[1]				X	X
Immune reactivity to INTERCEPT RBC (S-303 antibody testing)	X[1]	X[7]			X	X
Pregnancy test (if applicable)[3]	X					
Indication for RBC transfusion	X[1]					
Hematology panel and reticulocytes[9]	X[1]	X	X	X	X	
% HbS, HbA, HbF	X[1]	X[2]	X[2]	X	X	
Blood chemistry panel[10]	X[1]	X			X	
Coagulation panel[11]	X[1]	X	X		X	
S-300 and GSH (frozen plasma)		X	X		X	
Vital signs	X[1]	X	X		X	
RBC transfusion data	X[1]	X			X[5]	

Study Assessments	Screening	Transfusion Support Period (TSP) (Day 1 through Day 28 plus 6-Month extension or death – 3 RCE episodes)		Follow-Up Visit Day 14 (14±7 days) after each RCE episode	Follow-Up Visit Day 28* (28±7 days) after last study RCE or prior to next non-study RCE if less than 28 days since last study RCE	End of Study-Day 75 of Follow-Up (75±15 days) after last study transfusion
		Before each RCE episode	15min – 4 hours after each RCE episode			
(study or non-study)						
Concomitant medications	X[1]	X[4]			X	
Other blood components transfused		X			X	
RCE Procedure details			X			
Adverse events, AESI and transfusion reactions		X	X	X	X[13]	
SAEs		X	X	X	X[13]	
Surveillance Calls [12]		X		X	X	
Documentation of vital status		X			X	X
Assess the proportion of each RBC component transfused (e.g., 25%, 50%, 75% or 100%). For pediatric subjects, the volume of the intended transfusion should be recorded.			X			
Assessment of bleeding status[6]	X	X				

*Day 1 of the follow-up period is the first day after the last study transfusion.

1. If results from the Screening assessments listed are available in the medical record within 30 days prior to randomization, those data may serve as the screening data and the assessments do not need to be repeated unless the subject has an RBC transfusion in the 30-day period. If an RBC transfusion occurs after determined eligibility but prior to randomization, the subject may be randomized, and those Screening assessments listed above should be repeated prior to the first study transfusion.
2. Including pre- and post-transfusion Hb and % HbS, HbA, and HbF levels for prior 6 months if available and RBC volume transfused monthly for prior 6 months.
3. Routine standard of care test order per local hospital SOPs
4. Medications administered during anesthesia for surgical or other procedures are not collected.
5. The date of non-study RBC transfusion, if any, during the 28-Day follow-up period and 6-Month extension, if applicable, must be documented
6. Daily for inpatients when available through the end of the acute transfusion period
7. Performed in conjunction with every routine standard of care test for ABO Type, Rh and screen (IAT) test that is performed during the Acute Support Period and 6-Month Extension.
8. If, during the acute support period or follow up assessments (Day 28), a positive DAT result is observed, participating sites will follow their routine standard practice for confirmation/characterization of DAT positive samples; this should only apply to any

conversion from a negative to a positive DAT after enrollment, that is not part of an investigation of hemolysis or of a positive IAT with evidence of an S-303 antibody, in which case further investigations may be appropriate after discussion with the sponsor.

9. Hematology Panel: WBC, RBC, Hb, Hct, MCV, MCH, MCHC, MPV, RDW, PLT, Neut %, Mono%, Lymph%, Baso%, Eos%, reticulocytes
10. Blood Chemistry Panel: BUN, Creat, EGFR, NA, K, GLU, Cl, CO_2, TP, ALB, ALKP, AST (SGOT), ALT (SGPT), LDH, CPK, TBIL, DBIL
11. Coagulation Panel: PT, INR, APTT, Fibrinogen.
12. In the outpatient setting if the subject is not seen by study staff for any other reason, surveillance calls are to be completed at least monthly (at minimum every 35 days) to collect AEs, SAEs, TRs, and UADEs starting from the initiation of the first study transfusion through the entire transfusion support period and through 28 days after the final study transfusion. If the monthly surveillance call interval coincides with a study visit, the surveillance call is not required as the study visit assessments take precedence.
13. AEs, SAEs, TRs, AESIs and UADEs are collected from initiation of the first study transfusion through exactly 28 days after the last study transfusion.

5. DEFINITIONS

5.1 Time-Related Terms

5.1.1 Baseline

The *Baseline* measurement is the last non-missing measurement no later than the start of first study transfusion (defined as the first transfusion episode that includes at least one Test or Control RBC unit).

5.1.2 Study Day

The *study day* describes the relative day of an observation starting with the first study transfusion date designated as Study day 1. It will be calculated as:

- For days prior to the first study transfusion date, Study Day = Date – Date of first study Transfusion
- For days on/after the first study transfusion date, Study Day = Date – Date of first study Transfusion + 1

5.1.3 RBC Exposure

The *extent of exposure* to RBC transfusions will be assessed by units and grams of treatment exposure, derived as:

- Units of treatment exposure = RBC Units transfused during the transfusion support period.
- Total grams of treatment exposure (g) = Total Hemoglobin (g) transfused during the transfusion support period.

Both study and non-study RBCs will be counted.

5.1.4 Episode level Change

The change of a parameter for each episode will be derived as:

- Change = (Score at the Post-Episode) – (Baseline Score at Pre-Episode).

For the analysis of primary efficacy endpoint, the average of the adjusted hemoglobin change (hemoglobin increment) for each episode will be used.

5.2 Other Definitions

5.2.1 Prior and Concomitant Medications

Medications will be categorized into prior medications and concomitant medications which are coded using WHODrug September 2016 Dictionary. Specifically, prior medication is defined as

any medication taken and ended prior to the first study RBC start date. Concomitant medication is defined as any medication taken concurrently while receiving study RBC, i.e., the treatment period (period of time from first unit to last unit) of a concomitant medication taken by a subject must overlap with the treatment period.

5.2.2 Non-Study RBC

In this study, non-study RBC (conventional RBC) may be transfused according to the protocol to provide the patient with an appropriate and necessary treatment when study RBCs are unavailable or patient's need for RBC transfusions exceeds the quantity of study RBCs in inventory at the hospital blood bank.

The non-study RBC use will be summarized for Test and Control in terms of units, volume and proportions for the transfusion support period.

6. STUDY POPULATION

6.1 Modified Intent-to-Treat (mITT) Population

The mITT population includes all randomized subjects who received at least one study transfusion. Safety analyses will be performed using the mITT and summarized by actual treatment received. Depending on the baseline bleeding status and study period, safety analyses will be further broken out by the Non-Bleeding mITT Population, Bleeding Extension RCE and Bleeding Acute 28-Day populations.

6.2 Non-Bleeding mITT Population

Subset of the mITT, includes all mITT subjects who are classified as 'Non-Bleeding' at baseline. Efficacy analyses will be performed using the Non-Bleeding mITT and summarized using treatment as randomized. This is the analysis population for the primary efficacy endpoint and secondary efficacy endpoint of adjusted hemoglobin consumption. Depending on the study period, there are two subsets of this population: those who only participated in the initial Acute 28-Day transfusion support period (Non-Bleeding Acute 28 Day) and those who also participated in the 6-Month extension period (Non-Bleeding 6-Month Extension).

6.3 Non-Bleeding Per-Protocol Set (PPS)

The Non-Bleeding PPS is a subset of the Non-Bleeding mITT. It will be the analysis population for sensitivity analysis of the primary efficacy endpoint and will be summarized using treatment as randomized. Any subject affected by an important protocol deviation that might affect the primary efficacy analysis will be excluded from the PPS. The PPS will be identified before the unblinding treatment assignments.

Before the database lock and before unblinding the treatment assignments, Cerus' study team will review all protocol deviations, identify subjects with any protocol deviation that could impact the efficacy outcome, and determine whether or not to exclude the subject from the PPS.

The followings may lead to an exclusion of subjects from the PPS:

- Randomized to Test but received Control treatment.
- Randomized to Control but received Test treatment.
- Subjects who received non-study RBC unit(s) as part of their first study RBC(Test or Control) transfusion episode. For subjects who received non-study RBC unit after their first RBC transfusion episode, only data before the non-study RBC transfusion will be used for the analysis. Data collected after the start of non-study RBC transfusion will be omitted for the PPS analysis.

Additional protocol deviations may be considered important at the blinded data review meeting and will be documented appropriately.

6.4 Bleeding Extension Red Cell Exchange (RCE) Population

Subset of the mITT Population, consisting of SCD subjects enrolled into 6-Month extension to receive three consecutive RCE transfusions who were defined as ‘Bleeding’ at baseline. This is the analysis population for the secondary efficacy endpoint of hemoglobin clearance rate.

.

7. GENERAL CONSIDERATIONS

All measures will be summarized by treatment (planned or actual received) descriptively. Continuous variables will be summarized using descriptive statistics such as number of observations (n), mean, standard deviation, median, minimum, and maximum. Categorical variables will be tabulated using frequency (n) and percentage (%).

The statistical testing will be conducted at a significance level of 0.05 (two-sided) and the 95% confidence interval will be shown, unless specified otherwise. No formal statistical testing will be conducted for safety measures.

All data manipulations, descriptive summaries, and statistical hypothesis testing will be performed using Statistical Analysis System (SAS) Version 9.4 or later.

7.1 Handling of Missing Data

7.1.1 Efficacy Measure

For the primary analysis of average adjusted hemoglobin increment with Analysis of Covariance (ANCOVA) , missing primary efficacy endpoints will be imputed using multiple imputation . If a subject has no adjusted hemoglobin increment available for a particular episode, that episode will be ignored for the calculation of the average. If a subject has no adjusted hemoglobin increment available for all episodes, this subject will be treated as missing the primary efficacy endpoint. Missing adjusted hemoglobin increment values will be imputed via multiple imputations.

In the sensitivity analysis with Pattern-Mixture Model (PMM) approach, for subjects who received non-study RBC, any Hemoglobin values collected after the transfusion of non-study RBC will be censored (i.e., treated as missing). Missing hemoglobin increment data will be imputed using the Multiple Imputations (MI) approach.

Missing secondary efficacy endpoints will not be imputed.

7.1.2 Safety Measures

Descriptive summaries of safety measures will be based on observed data only. P-values are reported for exploratory purposes only and will not be adjusted for multiplicity. No imputation of missing scores will be implemented.

7.1.3 Datetimes for Transfusions

If only one of the timepoints (start datetime or end datetime) of transfusion component is available/reliable, the other timepoint will be imputed accordingly:

- If the start datetime is missing, it will be imputed as: End Datetime - 120 minutes.

- If the end datetime is missing, it will be imputed as: Start Datetime + 120 minutes.

7.1.4 Dates for Medical Events and Medications

Completely or partially missing onset and resolution dates of medical history (MH), AE, and concomitant medications (CM) will be imputed as follows:

Table 3: Handling of Missing Data for Medical Events and Medications

Date	Type of Missing Date	Handling of Missing Date
Event onset date (e.g., YYYY-MM-DD)	Completely missing	No imputation will be applied: For AE, the event will be considered treatment emergent. For CM, the event will be considered concomitant. For MH, the event will be considered to occur prior to Inform Consent date.
	Only YYYY is available	Use the first day of YYYY (1st January) unless YYYY is the same as year of the first transfusion and the event end date is on or after the first transfusion or ongoing then impute first transfusion date.
	YYYY and MM are available, but DD is missing	Use the first day of MM (1st) unless the MM is the same as month of the first transfusion and the event end date is on or after the first transfusion or ongoing then impute first transfusion date.
Event resolution date (e.g., YYYY-MM-DD)	Completely missing	No imputation will be applied. The event will be considered ongoing (i.e., not resolved) at the last visit date.
	Only YYYY is available	Use the last day of YYYY (31st December) unless YYYY is the same as year of study end then impute study end date.
	YYYY and MM are available, but DD is missing	Use the last day of MM unless MM is the same as month of study end, then impute as study end date

7.2 Multi-Center Studies

This is a multi-center study enrolling subjects from approximately fifteen sites. Analyses of primary and secondary efficacy endpoints will be adjusted for sites. Sites will also be a subgroup for efficacy and safety analyses.

7.3 Multiple Comparisons / Multiplicity

No multiplicity adjustment is performed for this study. The superiority of the primary efficacy endpoint will be tested only after the non-inferiority is met.

7.4 Interim Analysis

No formal interim analysis is planned for this study. For safety purposes, after approximately 300 subjects are transfused and at any other time that safety or enrollment rate concerns arise, safety data will be summarized in a group blinded manner for the DSMB's interim safety data review. Enrollment and study transfusions will continue during the interim safety evaluation, unless otherwise advised by the DSMB. Depending on the group blinded safety data reviewed, the DSMB may call for an additional unscheduled review, request to see unblinding summary, and/or suggest holding, stopping, or modifying the study for safety concerns.

As this planned interim analysis can only stop the trial for safety concerns, no adjustments will be made for the alpha level. If the study is discontinued as a result of this interim safety review, unblinded safety and efficacy data will be analyzed then and submitted to the DSMB and FDA.

7.5 Data Safety and Monitoring Board

The study data and safety will be monitored on a regular basis by the Data and Safety Monitoring Board (DSMB) to ensure patient safety and review protocol compliance. The DSMB will review group-blinded data generated by an independent statistician. All DSMB members are independent of the sponsor.

8. SUMMARY OF STUDY POPULATION DATA

8.1 Subject Disposition

The disposition of all randomized subjects will be summarized by treatment and overall. Summaries will include the number and percentage of subjects in the mITT, Non-Bleeding mITT, Non-Bleeding Acute 28-Day, Non-Bleeding 6-Month Extension, Non-Bleeding PPS, Bleeding mITT, Bleeding Acute 28-Day and Bleeding Extension RCE. The disposition summary will also include the number and percentage of completers and non-completers, as well as the number and percentage of discontinuations of the study and of the study RBC by the primary discontinuation reason.

8.2 Demographics and Baseline Characteristics

Subject demographics and baseline characteristics will be descriptively summarized for the mITT population by planned treatment and overall. Specifically, for subject demographics, the following variables will be summarized:

- Age at randomization (continuous and categorical: <18, 18 - 65 years and ≥ 65 years)
- Sex (categorical: Male or Female)
- Ethnicity (categorical: Hispanic/Latino or Not)
- Race (categorical: American Indian or Alaska Native, Asian, Black, Native Hawaiian or Pacific Islander, White, or Other)

For baseline characteristics, the following variables will be summarized:

- Baseline Hemoglobin
- Blood Type (A, B, O and AB)
- RH factor (Positive or Negative)
- Total blood volume (TBV), defined by Nadler's equation (Nadler, Hidalgo, & Bloch, 1962):

  Men: TBV (L) = 0.6041 + (0.3669 x H^3) + (0.03219 x W)

  Women: TBV (L) = 0.1833 + (0.3561 x H^3) + (0.03308 x W)

  where H is height in meters, and W is weight in kilograms.
- Baseline bleeding status (Bleeding vs. Non-Bleeding)
- Baseline disease status (Non-Bleeding 6-Month Extension subgroup only:- SCD, Thalassemia or Myelodysplastic syndrome (MDS))

8.3 Medical, Surgical and Transfusion History

For this study, medical history and adverse events will be coded using MedDRA 19.0, 2016. Each medical event will be classified into a System Organ Class (SOC) and mapped to a Preferred Term (PT).

The medical and surgical history will be summarized. Subjects reporting any medical and surgical history at baseline will be tabulated by SOC and PT for each planned treatment and overall.

8.4 Protocol Deviations

In this study, protocol deviations are categorized as follows:

- Informed Consent
- Inclusion/Exclusion Criteria and Concomitant Treatment
- Study Procedures/Treatment, including Study Tests/Assessments/ Laboratory
- Visit Scheduling
- Withdrawal Criteria
- Other

A protocol deviation is considered important if it may affect the study primary endpoints and the subject's rights, safety, or well-being, and/or the completeness, accuracy, and reliability of the study data. Cerus' study team will review all protocol deviations and determine the list of important protocol deviations prior to database lock. All randomized subjects with any important protocol deviation(s) will be tabulated by deviation category for each planned treatment and overall. In addition, protocol deviations will also be listed.

8.5 Prior and Concomitant Medications

For this study, prior and concomitant medications will be coded using WHODrug September 2016 Dictionary. Each medication will be classified mapped to a preferred drug name, summarized for the mITT population.

Subjects taking any prior medications will be tabulated by ATC level 3, level 4, and preferred drug name for each actual treatment received and overall. A subject will be counted at most once for each prior medication, even if the subject took the same prior medication on multiple occasions. Subjects taking any concomitant medications will be tabulated similarly. In addition, prior medications and concomitant medications will also be listed, separately.

8.6 Exposure to Study RBC, Non-Study RBC and Other Blood products

The extent of exposure to a study RBC is measured by the number of units and total Hb content transfused as described in Section 5.1.3. The following exposure data will be summarized using descriptive statistics:

- Transfusion episodes received
- Total components transfused
- Total days of transfusion: defined as the number of unique calendar days with any RBC transfusions. A day with two or more RBC transfusions is counted as one transfusion day.
- Total transfusion duration in hours: = transfusion episode end time – transfusion episode start time), summed over all episodes
- Average RBC age in days
- Total volume transfused
- Total hemoglobin transfused (g)

Use of non-study RBC, plasma, cryoprecipitate, platelet, or other blood product with their indication and transfused components will also be summarized by number of components received.

9. EFFICACY ANALYSES

The primary efficacy analyses will be performed on the Non-Bleeding mITT population, with subjects classified by planned treatment, irrespective of the actual treatment received.
It may be necessary to complete additional exploratory analyses after the planned analyses are completed. Full details of additional analyses will be presented in the CSR, and any such analyses will be clearly identified as post-hoc.

9.1 Analysis of Primary Efficacy Endpoint

The primary efficacy endpoint is adjusted hemoglobin increment, defined as the difference between the pre-transfusion and post transfusion episode hemoglobin values divided by the total hemoglobin content transfused, averaged over one or more transfusion episodes in non-bleeding mITT subjects. Both study and non-study RBCs initiated during the initial 28-Day transfusion support period and 6-Month extension transfusion period (if applicable) are included in the calculation.

The adjusted hemoglobin increment for a transfusion episode can be calculated by

$$\text{Adjusted Hgb Increment Per 100 grams Hgb transfused (g/dL)} = \frac{\text{Post Txn Episode Hgb (g/dL)} - \text{Pre Txn Episode Hgb (g/dL)}}{\text{Hgb Content Transfused (g)}}$$

The hemoglobin content transfused in grams from each transfusion episode is the sum of the hemoglobin content for all RBC components transfused, directly from the hemoglobin content reported in the blood center database for completely transfused units. For incompletely transfused units, it is derived as:

$$\text{Hemoglobin Content Transfused (g)} = \text{Hemoglobin Content (g)} \times \frac{\text{Volume of Transfused Study RBC (mL)}}{\text{Volume of a Unit of Study RBC (mL)}}$$

For each non-study RBC component received, the hemoglobin content will be imputed using the average hemoglobin content per study RBC component from the Control group within each clinical site.

The hypothesis for the primary efficacy endpoint is:

$$H_0: \; M_{Test} - M_{Control} < -0.15 \times \widehat{M}_{Control}$$

versus

$$H_A: \quad M_{Test} - M_{Control} \geq -0.15 \times \widehat{M}_{Control},$$

where M_{Test} and $M_{Control}$ are the means for Test (INTERCEPT) and Control groups, respectively, and $\widehat{M}_{Control}$ is the observed Control mean.

An analysis of covariance (ANCOVA) model will be used to analyze the primary efficacy endpoint including terms for treatment group, site, total blood volume and participation in extension period or not. Least square (LS) mean of average adjusted hemoglobin increment for each treatment group, standard error (SE) and differences between least squares treatment group means will be reported along with 95% confidence interval.

Non-inferiority will be declared if the lower bound of a 2-sided 95% confidence interval for the least square mean treatment difference (Test - Control) is greater than or equal to the negative of NI margin which is pre-specified as 15% of the Control mean. Further, if the lower bound is greater than 0, then superiority is achieved.

In the case that the normality assumption for ANCOVA is violated, a log transformation of the original data might be considered, or alternative statistical methods could be explored depending on the severity of the violation. In the case of log transformation, the above hypothesis can be written in the form of ratio of the means on the original scale, which can be translated into a hypothesis about the difference of two means on the logged scale as follows:

$$H_0: \frac{M_{Test}}{M_{Control}} < 0.85 \qquad versus \qquad H_A: \frac{M_{Test}}{M_{Control}} \geq 0.85$$

is equivalent to

$$H0: \ ln(M_{Test}) - ln(M_{Control)} < ln(0.85)$$

$$versus$$

$$HA: ln(M_{Test}) - ln(M_{Control)} \geq ln\ (0.85)$$

The same primary analysis ANCOVA model will be performed on the transformed data. The conclusion of the analysis will be drawn in terms of the original data. i.e., non-inferiority will be declared if the lower bound of a 2-sided 95% confidence interval for the least square mean treatment difference in log scale is greater than or equal to ln (0.85).

Multiple imputation

Missing data for the primary endpoint will be addressed using Multiple Imputation (MI) under the assumption that data are Missing at Random (MAR). The MI procedure will be implemented using SAS PROC MI with Fully Conditional Specification (FCS). A total of 100 imputed datasets will be generated using a regression-based approach. Imputation models will include treatment group, site, total blood volume, participation in extension period or not, age, race, baseline hemoglobin as well as additional variables predictive of missingness or the outcome, where appropriate. To ensure reproducibility of the imputation process, a fixed random seed 12345 will be used.

The imputed datasets will be analyzed using the ANCOVA model defined above. Estimates and standard errors from each imputed dataset will be combined using Rubin's rules (Rubin, 1996)

via PROC MIANALYZE to produce overall point estimates, standard errors and 95% confidence intervals.

Diagnostic checks will be conducted to assess the convergence of the imputation process and the plausibility of the imputed values, including comparisons between observed and imputed distributions. If convergence issues arise which cannot be achieved after reasonable adjustments, a complete-case analysis will be performed as a sensitivity analysis. Any deviations from the planned MI approach will be documented in the Clinical Study Report (CSR).

9.2 Analysis of Secondary Efficacy Endpoints

9.2.1 Adjusted hemoglobin consumption

The adjusted hemoglobin consumption is defined as total hemoglobin mass transfused in grams divided by body weight in kg at baseline and duration of the study transfusion period in days (g/kg/day), in Non-Bleeding mITT Population. This is calculated over all transfusion episodes (including both study and non-study) that are initiated transfusion period in the Non-Bleeding MITT Population. For each subject, the adjusted hemoglobin consumption value is calculated by:

$$\text{Adjusted Hgb Consumption (g/kg/day)} = \frac{\text{Total Hgb Mass (g) transfused/Baseline Body Weight(kg)}}{\text{Duration of Study Transfusion Support Period (day)}}$$

The total hemoglobin mass transfused is the sum of the hemoglobin content for all study and non-study RBC components transfused. For study RBC components, the hemoglobin transfused will come directly from the hemoglobin content reported in the blood center database for completely transfused units. For incompletely transfused units, the percentage of transfusion will be derived from the proportion of volume transfused (see calculation given in Section 9.1).

For each non-study RBC component received, the hemoglobin content will be imputed using the average hemoglobin content per study RBC component from the Control group within each clinical site.

Duration of the study transfusion support period will be 28 days for the acute 28-Day transfusion period and plus 6 months for the 6-Month extension period, if applicable, unless subjects prematurely discontinue from the study. In the case of early discontinuation during the study transfusion support period, the duration is calculated as:

$$(\text{Early discontinuation date}) - (\text{Start date of first study RBC transfusion}) + 1$$

The early discontinuation date is determined according to the following rules:

- For subjects who died, the date of death is used.
- For subjects lost to follow-up, use the date of the last valid record during the transfusion period, excluding the disposition form.
- For all other withdrawal reasons, use the date of the last valid record during the transfusion support period.

The resulting early discontinuation date is restricted to not exceed the maximum allowed duration of the transfusion period:

- Day 28 for subjects not enrolled in the 6-Month extension period, or
- Day 28 plus 6 months for those enrolled in the 6-Month extension period.

If the derived early discontinuation date exceeds the applicable maximum, it will be adjusted to the maximum allowed end date. The final duration is then calculated using this adjusted early discontinuation date.

The hypothesis testing is:

$$H_0: \ M_{Test} - M_{Control} > 0.15 \times \widehat{M}_{Control},$$

versus

$$H_A: M_{Test} - M_{Control} \leq 0.15 \times \widehat{M}_{Control}$$

where M_{Test} and $M_{Control}$ are the means for Test (INTERCEPT) and Control groups, respectively, and $\widehat{M}_{Control}$ is the observed Control mean.

An analysis of covariance (ANCOVA) model will be used to estimate the mean treatment differences while controlling for site, total blood volume and participation in extension period or not on Non-Bleeding MITT Population. Non-inferiority will be declared if the upper bound of a two-sided 95% confidence interval for the treatment difference (Test – Control) is less than or equal to 15% of the Control mean. This study is not powered for the endpoint of adjusted hemoglobin consumption.

9.2.2 HbA clearance rate

The second secondary efficacy endpoint is the HbA clearance rate for Bleeding Extension RCE Population.

HbA clearance rate is calculated using measurements from post-transfusion samples and the pre-transfusion sample from the next scheduled transfusion, adjusted by the duration in days. The HbA clearance rate will be averaged over all study RCE procedures for each subject in Bleeding Extension RCE population. Specifically, HbA clearance rate for each subject between two consecutive episodes will be calculated according to the following steps:

$$\text{HbA Clearance (g/dL/day)} = \frac{\text{Post Txn Episode Hgb} - \text{Pre Txn Episode Hgb (g/dL)}}{\text{Duration between transfusions (day)}}$$

Where Post Txn Episode Hgb is Hgb measurement collected 15 minutes to 4 hours after the transfusion episode and Pre Txn Episode Hgb is from the immediate next episode or from 28-day post-transfusion of the last episode. The duration is days between measurement times of Post Txn Episode Hgb and Pre Txn Episode Hgb in numerator. If there are additional Hb measurements collected between the two time points, i.e., at 14-days post first episode, the HbA

clearance rate between two consecutive episodes will be calculated as the average of the two clearance rates from below:

$$\text{HbA Clearance (g/dL/day)} = \frac{\text{Post Txn Episode Hgb } - \text{Hgb at Day 14 (g/dL)}}{\text{Duration between Post First Episode to Day 14 (day)}}$$

$$\text{HbA Clearance (g/dL/day)} = \frac{\text{Hgb at Day 14} - \text{ Pre Txn Episode Hgb (g/dL)}}{\text{Duration between Day 14 to Pre next Episode (day)}}$$

Descriptive statistics and two sample t-tests will be conducted to compare the HbA clearance rate (g/dL/day) between Test and Control arms for:

- the average HbA clearance rate across the three RCE episodes, and
- the HbA clearance rate for each individual RCE episode.

These analyses will be considered exploratory.

9.3 Sensitivity Analyses

To assess the robustness of the results from the primary analysis of the primary efficacy endpoint, two sensitivity analyses will be performed (Table 4). For subjects who received non-study RBC during the transfusion study period (TSP), any hemoglobin values collected after the transfusion of non-study RBC will be censored (i.e., treated as missing) in the sensitivity analysis with Pattern Mixture Modeling approach.

Table 4: Overview of Primary and Sensitivity Analysis Methods

Analysis Type	Statistical Method	Analysis Population	Handling of Missing Data
Primary Analysis	ANCOVA	Non-Bleeding mITT	Multiple Imputation (MI)
Sensitivity Analyses	Multiple regression	Non-Bleeding PPS	Observed cases
	Pattern Mixture Modeling (PMM)	Non-Bleeding mITT	Multiple Imputation (MI)

For the multiple regression approach, several covariates will be included in the model including age, race, gender, BMI, baseline hemoglobin and other relevant covariates.

While the multiple regression with multiple imputation (MI) in the primary analysis assumes missing at random (MAR) for missing primary efficacy endpoint, the second sensitivity analysis is to assess the sensitivity to departure from MAR assumption. Specifically, a Pattern-Mixture

Model (PMM) with delta-adjustment of imputations based on missing not at random (MNAR) assumption (Carpenter & Kenward, 2013; Van Buuren, 2012) will be implemented as follows:

a. Use MCMC to create monotone missingness first. Then obtain standard multiple imputations under MAR assumptions for missing hemoglobin values using monotone regression. The missing data are filled in and 100 complete data sets are created.

b. For each hemoglobin value that had been missing because of censoring after having received non-study RBC during TSP, regardless of treatment group, adjust these values by adding δ to the imputed values, where $\delta = 0.2$ g/dL.

c. Each of the 100 complete data sets will then be analyzed separately using the same ANCOVA method as used for the primary analysis.

d. The estimates obtained from the ANCOVA method of each complete data set are combined for inference purposes.

e. Repeat step b, c, d, with $\delta = 0.4, 0.6, ..., 2.0$ or until the conclusion is reversed from the primary analysis, whichever comes first. The larger the value of δ that is required to reverse the conclusion of the primary analysis, the more robust is the conclusion.

9.4 Exploratory Analyses

Exploratory analysis of the primary or secondary efficacy endpoints will also be conducted. A mixed model repeated measures (MMRM) of the primary efficacy endpoint will be explored. This model will include fixed effects for treatment group, episode, baseline hemoglobin, age, race, site, participation in extension period and total blood volume assuming a compound symmetry covariance matrix. For comparison, the primary efficacy endpoint will also be explored in the Bleeding Acute 28-Day subgroup using ANCOVA.

Table 5: Overview of Exploratory Analyses

Endpoints	Statistical Method	Analysis Population
Primary efficacy endpoint	MMRM	Non-Bleeding mITT
Primary efficacy endpoint	ANCOVA	Bleeding Acute 28-Day
Adjusted hemoglobin consumption	ANCOVA	Thalassemia subjects in Non-Bleeding 6-Month Extension subgroup

Adjusted hemoglobin consumption will also be explored in patients with Transfusion dependent Thalassemia as baseline disease status, a subgroup of Non-Bleeding 6-Month Extension. In this analysis, the first four study transfusion episodes will be considered a “wash in” period, and the analysis will be performed on the subsequent study transfusion episodes. An analysis on the

effect of 2-6 “wash-in” study transfusions will also be explored. The ANCOVA model will be used to estimate the mean treatment difference while controlling for the clinical site. Corresponding 95% confidence interval and p value will be reported for exploratory purposes.

Additional exploratory analyses may be conducted as suggested by data. Any P values presented from exploratory efficacy analyses will be interpreted in an exploratory sense only.

9.5 Subgroup Analyses

To assess the homogeneity of treatment effects among subgroups on primary efficacy endpoint, subgroup analyses with descriptive summaries will be performed by age groups (<18, 18-64, and ≥65 years old), sex (males or females), race (White or Non-white), clinical site, baseline hemoglobin value (<8 vs >= 8), total blood volume (< 5 vs >= 5), participation in extension period (yes or no), baseline disease status in Non-Bleeding 6-Month Extension Population (SCD, Thalassemia or MDS), , transfusion level (simple(<3) vs repeatedly (>=3)). As exploratory analyses, the treatment difference in the adjusted hemoglobin increment will also be summarized descriptively (with a 95% confidence interval provided).

In addition, the treatment difference in the adjusted hemoglobin consumption will also be summarized descriptively (with a 95% confidence interval provided) for the following Non-Bleeding 6-Month Extension subgroup:

- Subgroup by clinical site, age group (<18, 18-64, and >64 years old), sex, and race (White vs. other races), as appropriate.
- Baseline disease status and number of transfusion episodes (<5, 5-10, >10) within each disease status

Other subgroup analyses may be performed as suggested by the data.

10. SAFETY ANALYSES

The mITT population will be used for all safety summaries, and subjects will be classified by actual treatment received. Descriptive summary of each safety-related measure will be performed. In the event that a subject receives both Test and Control RBC during the study, the subject will be summarized with those receiving Test.

The primary safety endpoints (Related TEAEs and TE antibodies specificity to INTERCEPT RBCs) will be compared and tabulated by treatment using frequency (n) and percent (%). P values from a Cochran-Mantel-Haenszel (CMH) test stratified by actual baseline bleeding status (Bleeding vs. Non-Bleeding) and clinical site will be presented in an exploratory sense only. In the event of sparse counts, Fisher’s exact test may also be used to provide statistical interpretation.

Other safety endpoints including allo-antigens and mortality will be carried out in the same manner.

Subjects with any AE(s), SAE(s), TRs and AEs leading death will be tabulated by type of AE(s) for each actual treatment received and overall, by SOC, preferred term, severity, and relatedness. A subject who experienced multiple events within a SOC or preferred term will be counted only once for that SOC or preferred term. These subjects will also be listed separately.

For Bleeding RCE mITT population, the number and proportion of subjects with adverse events of special interest (AESI) through 28 days after the last study transfusion will be summarized, as well as the S-300 and Glutathione (GSH) plasma levels before and 15 minutes to 4 hours after RCE.

Subgroup analyses

Depending on the baseline bleeding status and study period, safety analyses will be further broken out by the Non-Bleeding mITT Population, Bleeding Extension RCE and Bleeding Acute 28-Day populations in order to compare events under homogenous clinical situations.

Subgroup analysis by irradiation status (yes vs. no) may be presented for selected safety data (including SAEs, AEs, and potassium level). Subgroup analyses by clinical site, age group (<18, 18-64, and >64 years old), sex (Male vs. Female), baseline bleeding status (Bleeding vs Non-Bleeding) and race (White vs. other races) will also be conducted, as appropriate.

Other subgroup analyses may be performed as suggested by the data.

11. DEVIATIONS FROM THE PROTOCOL

The following changes to the data analysis pre-specified in the protocol are made after the study started.

1. Primary analysis model

   Protocol states: An analysis of covariance (ANCOVA) model will be used to estimate the treatment effects while controlling the clinical sites.

   Justification: Clinically, total blood volume is considered strongly associated with the primary efficacy endpoint of adjusted hemoglobin increment. Therefore, it is added as a covariate in the primary analysis.

2. Added rational when primary analysis model assumption is violated.

   Justification: lab data can often be skewed. In the case the normality assumption for ANCOVA is violated, a log transformation of the original data might be considered, and the corresponding transformed hypothesis testing is specified.

3. Method to calculate the hemoglobin clearance rate.

   Protocol states: HbA clearance rate (g/dL/day) will be estimated from the area under the curve (AUC) calculated by the trapezoidal rule based on measurements from post-transfusion samples.

   Justification: HbA clearance rate is calculated using the difference between the post transfusion measurement and pre-measurement from next transfusion, divided by the duration. This assumes a straight line in decline in Hb concentration instead of AUC.

12. REFERENCES

Carpenter, J. R., & Kenward, M. G. (2013). *Multiple Imputation and Its Application.* New York: John Wiley & Sons.

Cro, S., Morris, T. P., Kenward, M. G., & Carpenter, J. R. (2020). Sensitivity analysis for clinical trials with missing continuous outcome data using controlled multiple imputation: A practical guide. *Statistics in medicine, 39(21),* 2815-2842.

Kopolovic, I., Ostro, J., Tsubota, H., Lin, Y., Cserti-Gazdewich, C. M., Messner, H. A., . . . Callum, J. (2015). A systematic review of transfusion-associated graft-versus-host disease. *Blood, 126(3),* 406-414.

MacLennan, S., & Williamson, L. (2006). Risks of fresh frozen plasma and platelets. *The Journal of trauma, 60(6 Suppl),* S46-S50.

Nadler, S. B., Hidalgo, J. H., & Bloch, T. (1962). Prediction of blood volume in normal human adults. *Surgery, 51(2),* 224-32.

Rubin, D. (1996). Multiple Imputation After 18+ Years. *Journal of the American Statistical Association, 91(434),* 473-89.

Stramer, S. L., Hollinger, F. B., Katz, L. M., Kleinman, S., Metzel, P. S., Gregory, K. R., & Dodd, R. Y. (2009). Emerging infectious disease agents and their potential threat to transfusion safety. *Transfusion, 49 Suppl 2,* 1S-29S.

Van Buuren, S. (2012). *Flexible Imputation of Missing Data.* Boca Raton, FL: Chapman & Hall/CRC.