Terumo BCT Biotechnologies,LLC
Protocol #: CTS-5056

Evaluate the Efficacy and Safety of RBCs Derived from Mirasol treated Whole Blood Compared with Conventional RBCs in Patients Requiring Chronic Transfusion Support (PRAISE)

Statistical Analysis Plan

Version 1.0
Document last saved: 5-Feb-2018 3:42 PM

Prepared for:
Terumo BCT Biotechnologies, LLC
1215 Quail Street
Lakewood, CO 80215; USA
Phone: +1 (877) 339-4228

Prepared by:
Jeffrey Botbyl
Novella Clinical
1700 Perimeter Park Dr.
Morrisville, NC 27560

Contents

I. Introduction

A. Background

Thalassemia is a congenital blood disorder, categorized as either alpha-thalassemia or beta thalassemia, in which production of alpha or beta globin is decreased or absent, respectively resulting in lack of hemoglobin (Hb) production. Thalassemia major (homozygous) is the most severe form of the disease, requiring red blood cell (RBC) transfusions from an early age.

The current standard of care for transfusion-dependent thalassemia patients dictates chronic RBC transfusions with the goal of controlling anemia and suppressing ineffective erythropoiesis. Treatment with chronic RBC transfusions requires that these thalassemia patients are exposed to multiple blood products from multiple donors, increasing the risk of transfusion transmitted infections (TTI) by unscreened or undetected pathogens in the blood supply and transfusion-related immune reactions. In Vinchinsky et al., 24% of transfused thalassemia patients had laboratory evidence of previous exposure to one or more infectious diseases. Mirasol-treated blood products resulting in pathogen reduction and white blood cell (WBC) inactivation may provide a safer primary treatment for this population.

This study will provide valuable information about Mirasol-treated blood products regarding blood safety.

B. Protocol and Amendment History

This Statistical Analysis Plan (SAP) is based on version 4.1 /28AUG2017 of Protocol CTS-5056, which will incorporate Amendment x.

This SAP will govern the analysis of data from this study. Any modifications to the SAP will be done by a blinded statistician without access to the study data prior to database lock. Any deviations from the SAP, including any after database lock, will be documented as such in the clinical study report.

II. Protocol Objectives

A. Primary

- To determine if percent survival of RBCs derived from Mirasol-treated whole blood (WB) is non-inferior to conventional RBCs when transfused into patients requiring chronic RBC transfusion support.

B. Secondary

- To compare other efficacy and safety endpoints between treatment groups

III. Study Endpoints

A. Efficacy Endpoint

1. Primary

- Normalized hemoglobin area under the curve (Hb AUC) calculated from normalized Hb between successive transfusions as a measure of percent surviving RBCs

2. Secondary

- Hb increment
- Actual Hb level post-transfusion (15min)
- Proportional decline in post-transfusion Hb level
- RBC mass infused (volume x Hb/unit)

B. Safety Endpoints

- Incidence of treatment-emergent antibody with confirmed specificity to RBCs derived from Mirasol-treated WB
- Human leukocyte antigen (HLA) alloimmunization rates
- Treatment emergent adverse events (TEAEs)
- Transfusion-related adverse events (AEs)
- Serious adverse events (SAEs)
- Unanticipated adverse device effects (UADEs)

IV. Study Design

A. Design Overview

This is a prospective, multi-center, randomized, crossover trial to evaluate the clinical effectiveness of RBCs derived from Mirasol-treated WB (MIR RBCs) versus conventional RBCs (REF RBCs) in transfusion dependent thalassemia patients. Throughout the clinical trial, RBC transfusion volume and frequency will be determined by each subject's treating physician.

Eligible subjects who have signed an informed consent form (ICF)/parental consent form and assent form, where applicable, will be enrolled and randomized 1:1 to a treatment sequence via an electronic system using a permuted-block schedule stratified by investigational site. Subjects will be randomized to receive either MIR RBCs followed by REF RBCs, or to receive REF RBCs followed by MIR RBCs.

The crossover trial design will consist of 2 treatment periods. Each period will include a 50-day wash-in phase (Day 0 of the wash-in = Day 0 of the treatment period) followed by 2 transfusion episodes for assessment of the primary endpoint. The 50-day wash-in serves to ensure that an adequate volume of RBCs from the assigned treatment allocation (MIR RBCs versus REF RBCs) have been transfused into the subject prior to collecting samples to support the primary endpoint.

An end of study treatment follow-up visit will occur 2-4 weeks after the last per protocol transfusion, which should coincide with the next standard of care transfusion. A final study visit will occur at least 60 days after the last per protocol transfusion.

During each treatment period, blood samples will be collected for safety and efficacy analysis.

Blinding is not feasible because riboflavin used in the Mirasol-treated WB may cause yellow colored urine in transfusion recipients of Mirasol-treated blood products. Also, there is a difference in MIR RBC and REF RBC storage bags and labels. However, subjects, investigational site staff, and the Sponsor will not be informed of the randomization assignment. Study team members conducting safety assessments on subjects should remain blinded, whenever possible, to ensure causality determination remains unbiased. Central and Local Blood Centers will be informed of the randomization assignment to provide study product for transfusion.

B. Study Population

The study population will consist of transfusion-dependent thalassemia patients. 97 patients will be randomized at up to 10 transfusion centers and corresponding blood centers with sites in the United States and outside of the United States (e.g., Europe, Canada, or the Middle East).

C. Sample Size Estimation

Data from the IMPROVE II study were available to obtain information on normalized Hb AUC associated with RBCs derived from Mirasol-treated WB as well as from untreated WB. Pairwise differences in the natural log-transform of normalized Hb AUC from treatment and control were used to obtain estimates required for determining the sample size needed for this study.

The mean difference in log-transformed Hb AUC values suggests a reduction of 16.7% resulting from the use of MIR RBCs. The sample variance of within subject differences is 0.015412. The estimate of the within subject variance required for the 2x2 crossover design is one-half of the variance of within subject differences (0.007706).

The primary hypothesis is that RBCs derived from Mirasol-treated WB are not inferior to conventional, untreated RBCs with respect to normalized Hb AUC. Assuming a within subject variance of 0.007706 and a 16.7% reduction in normalized Hb AUC, approximately 77 subjects are needed to obtain at least 80% power to show the decrease in normalized Hb AUC from RBCs derived from Mirasol-treated WB is not more than 20% of the normalized Hb AUC from conventional untreated RBCs assuming a 1 tailed type 1 error rate of 2.5%. Algebraically if $\ln(\mu_M)$ is the mean of the natural log-transformed Hb AUC for MIR RBCs and $\ln(\mu_C)$ is the mean of the natural log-transformed Hb AUC for REF RBCs, the sample size is calculated to detect an alternative hypothesis versus a null hypothesis stated as:

$$H_0 : \ln(\mu_M) - \ln(u_C) \leq -22.3\% \; versus \; H_a : \ln(\mu_M) - \ln(u_C) > -22.3\%$$

In the original scale (indicated by *), the above hypotheses translate to:

$$H_0 : {\mu_M^*}/{\mu_C^*} \leq 0.80 \; versus \; H_a : {\mu_M^*}/{\mu_C^*} > 0.80$$

Assuming a potential 20% discontinuation rate, approximately 97 patients may be enrolled.

The sample size was calculated using SAS 9.3 proc power for paired two samples t-test with option for finding difference with the null difference at the non-inferiority margin of -0.2231 (corresponding to 20% non-inferiority margin at the original scale), true mean of -0.1839 (corresponding to 16.7% observed

decrease in IMPROVE II), and correlation of 0.35. Should the correlation be higher, the proposed sample size will have higher power for the non-inferiority test.

D. Treatment Randomization

Subjects will be randomized 1:1 to a treatment sequence via an electronic system using a permuted-block schedule stratified by investigational site. Subjects will be randomized to receive either MIR RBCs followed by REF RBCs, or to receive REF RBCs followed by MIR RBCs.

E. Assessment Schedule

Patients will undergo a Screening Period, Treatment Assessments and End of Study Assessments. Please see Table 14-1 in Clinical Investigation Plan for a detailed Study schedule, including all measurements and evaluations for the entire study period (Screening to Follow-up) presented in tabular form.

During the Screening period (days -28 to 0), each patient will be screened to determine the eligibility.

If screening procedures confirm eligibility, the subject will be randomized within 10 days prior to initiation of the first study transfusion (Study Day 0).

During Treatment Phase, eligible patients will be followed by 2 transfusion episodes for assessment schedules specified in Table 14-1.

With Each RBC Transfusion Episode:

At Baseline the Investigator will identify and document the target post-transfusion (15 min) Hb level; with each transfusion episode, the Investigator will perform the following:

1. Calculate the volume of RBCs required to achieve the target Hb (identified at baseline)
   a. [(desired hemoglobin) - (current hemoglobin)] x (body weight [kg]) x (3/Hct of RBC units) = mL to transfuse

      Note: 3 is constant in the formula above
2. If the post-transfusion (15 min) Hb is under 15% of the target Hb, then an additional transfusion should be considered. If the post-transfusion Hb is over 15% of the target Hb, this will be documented.

The schedule will continue with the 50-day wash-in that serves to ensure that an adequate volume of RBCs from the assigned treatment allocation (MIR RBCs

versus REF RBCs) have been transfused into the subject prior to collecting samples to support the primary endpoint.

All patients who come off study should have an end of study treatment follow-up visit that will occur 2-4 weeks after the last per protocol transfusion. A final study visit will occur at least 60 days after the last per protocol transfusion.

V. Interventions

A. Clinical Trial Material

The RBCs to be utilized in this study will be as follows and will meet standard release criteria for transfusion.

- MIR RBCs: RBCs will be derived from WB collected in citrate phosphate dextrose (CPD) solution, treated with the Mirasol System for WB, LR, and stored in Additive Solution Formula 3 (AS-3) for ≤ 21 days at 1 - 6°C.
- REF RBCs: LR apheresis RBCs or WB-derived RBCs will be per site standard inventory.

No other pathogen reduction treated blood products including platelets and/or plasma may be used for transfusion in subjects during study participation.

VI. General Analytical Considerations

A. Study Time Points

Baseline

Baseline for analysis purposes is defined as the last assessment prior to treatment start date/time (or randomization date, for patients who never receive any treatment) for each treatment period. In cases where the date is the same and the times are unknown, assessments taken on the same day are considered baseline.

Study Day
Study day for analysis purposes is defined as (date of event – treatment start date) (+ 1 if the event occurs after treatment start date) within each period.

Treatment Start Date
Treatment start date is defined within each period as the date of the first transfusion received for each period.

B. Missing Data

In general, missing data will not be imputed. Missing values will generally be kept as missing in the data analyses. However, imputations will be made in the following cases described below:

Imputations will be made for partial dates and the relationship of an Adverse Event (AE) to the study agent. These imputations are explained in more detail below.

For partial dates, other than the start date of an AE, the following conventions will be used for dates if needed:

- For dates missing both the month and the day, the month will be imputed to 7 and the day will be imputed to 15.
- For dates missing only the day where the month and year correspond to the first day of dosing the day will be imputed to the first day of dosing. All other missing days will be imputed to 15.
- For dates missing the year, no imputation will be used.

For partial onset dates for AE, the following conventions will be used:

- For dates missing both the month and the day, if the year corresponds to the year of first dose, the date will be imputed as the same as the date of first dose. Otherwise, the month will be imputed to 7 and the day will be imputed to 15.
- For dates missing only the day where the month and year correspond to the first day of dosing the day will be imputed to the first day of dosing. All other missing days will be imputed to 15.
- For dates missing the year, no imputation will be used.

Missing severities of AEs will not be imputed and will be considered missing in any tabulations of AE severity. If an AE is missing a response to the question regarding relationship to study treatment, or relationship to study procedure, or relationship to study procedure, the event will be considered to be related.

The normalized Hb AUC is expected to be calculated using four timepoints: the 15-minute post transfusion, 24-hour, Day 7, and end of transfusion interval (ie, pre-transfusion measure for the subsequent interval). If either the 24-hour or Day 7 measure is missing, the AUC will still be calculated using the rules described below in Section VIII.A. If both are missing, or if either the 15-minute post transfusion or the end of transfusion interval are missing, then no AUC will be calculated for that interval.

As a sensitivity analysis to the primary analysis, multiple imputation will be used to estimate missing normalized Hb AUC. Further details are provided in Section VIII.B.

C. Multiple Study Centers

No adjustment for stratification by the study centers is planned. However, centers will be grouped by region (US vs. non-US) and a sensitivity analysis will be conducted to determine if region impacts the primary endpoint.

D. Covariate Adjustment in Primary Analysis

Pre-transfusion Hb level will be included as a covariate in the primary analysis.

E. Sample Size Reassessment

No sample size reassessment is planned.

F. Interim Analyses or Timing of Analyses

No interim analyses planned.

G. Test Sizes

All confidence intervals will be two-sided with 95% coverage.

H. Multiple Comparisons

No applicable for this study.

I. Analysis Sets

Three analysis sets will be defined for use with various analyses.

Full Analysis Set (FAS)

The Full Analysis Set (FAS) will include all randomized patients who have at least 1 normalized Hb AUC measurement. Patients will be analyzed according to the treatment (MIR RBCs, REF RBCs) to which they were to receive in each period as assigned at randomization.

Safety Set (SS)

The Safety Set (SS) will include all randomized patients who undergo at least 1 episode of treatment after randomization. Patients will be analyzed according to the actual treatment received (MIR RBCs, REF RBCs) within each period. If a patient receives MIR RBCs at any time during a given period, patients will be summarized in the SS as receiving MIR RBCs for

that period. If a patient randomized to receive RBCs derived from Mirasol-treated WB only receives untreated RBCs within a given period, the patient will be summarized in the SS as receiving RBCs from untreated WB for that period. The safety analysis set will be used in reporting the safety issues in the study.

Per Protocol Set (PPS)

The Per Protocol Set (PPS) will consist of all randomized patients who complete all episodes of treatment without any off-protocol transfusions, and who do not have any major CIP deviations. Patients in the PPS will be analyzed according to the treatment received. The study team will identify major CIP deviations prior to data analysis. The PPS will be used in the exploratory analysis of the primary efficacy endpoint.

J. Subgroups of Analysis Populations

Data from subgroups of subjects in the defined analysis populations will be analyzed as specified below. These analyses will comprise descriptive summaries; the goal will be to identify signals of additional effects that analyses of data from the defined populations do not take into account. Such analyses will be considered exploratory. They may not be sufficient for drawing conclusions; hence, these analyses will not involve hypothesis testing.

K. Data Display Characteristics

Data displays produced for this study will include three types: summary tables, data listings, and figures.

Data listings will simply list the data recorded on the case report form (CRF) or derived for each subject. They will be ordered by treatment, site, subject number, and time of assessment. Additional levels of ordering may be employed as appropriate. Data listings will not display subject initials.

Summary tables of post-baseline measures will be presented by treatment:

- MIR RBCs: RBCs will be derived from WB collected in CPD solution, treated with the Mirasol System for WB, LR, and stored in AS-3 for $\leq$ 21 days at 1 - 6°C
- REF RBCs: LR apheresis RBCs or WB-derived RBCs will be per site standard inventory

Patients will be randomized by treatment sequence:

- MIR RBCs followed by REF RBCs (MR) or
- REF RBCs followed by MIR RBCs (RM)

Summary tables of baseline measures will be presented by treatment sequence.

Continuous data will be summarized with the number of non-missing values, mean, standard deviation, minimum, median, and maximum. Categorical data will be summarized with the number of non-missing values and the numbers of values equal to each of the possible values. Percentages of patients with each of the possible values will be calculated from the number of patients in the relevant cohort of the corresponding analysis population, unless stated otherwise. Some continuous variables may also be grouped into categorical levels and evaluated in frequency tables.

All statistical analyses will be performed using SAS® software, Version 9.3, or higher.

VII. Subject Accountability

A. Subject Disposition

Enrollment and extent of participation in the study will be summarized by sequence (MR or RM) for all randomized patients. The number and percent of patients randomized, the number and percent of patients in each analysis set, and the number of patients who discontinued study early, and reason, will be presented.

Patient disposition data will be provided in a listing. A separate listing describing each patient's inclusion or exclusion status for each of the analysis sets will also be provided.

B. Protocol Deviations

Protocol deviations will be captured by the site and reviewed by medical monitor. A listing of protocol deviations will be included.

C. Subject Characteristics

Demographic and Baseline Characteristics

Demographic and baseline information will be summarized for the FAS (only if significantly different than SS) and SS by treatment sequence:

- Age will be calculated at using the formula:
  Age = (Date of informed consent - Date of birth + 1) / 365.25 and truncated to complete years
- Sex (Male, Female)
- Ethnicity (Hispanic or Latino, Not Hispanic or Latino, Not Reported)
- Race (White, Black or African American, Native Hawaiian or Other Pacific Islander, Asian, American Indian or Alaskan Native, Other, Not Reported)

- Height (cm)
- Weight (kg)
- Prior RBC phenotyping or genotyping performed (Yes, No)
- Phenotyping/Genotyping results

Conversion factors and calculations for height, and weight:

- Height (in cm) = height (in inches) * 2.54
- Weight (in kg) = weight (in lbs) * 0.4536

Medical and Surgical History

Medical and Surgical History will be summarized by treatment sequence for the FAS and SS as the number and percentages of subjects with histories significant for each of the medical history/surgical history categories on the Medical History form.

All medical history will be provided as a listing for all subjects in SS.

Thalassemia History and Transfusion History

Primary diagnosis for Thalassemia and transfusion history (number of transfusion episodes in the last year; mean transfusion interval for the past 6 months; transfusion reactions in previous 6 months, and type of reaction) be summarized by treatment sequence for the FAS.

A subject listing will be presented for the SS.

Study Treatment and Concomitant Medications

All concomitant medications taken from the day of the first study transfusion and throughout the end of study treatment follow-up visit will be captured in the CRF. All study related RBC transfusions will be also recorded. Additionally, all blood products including off-protocol RBC transfusions, platelets, or plasma transfused until the end of study treatment follow-up visit will be recorded. The number of episodes of treatment received will be summarized by treatment (MIR RBCs, REF RBCs). The reason for treatment discontinuation will be associated with the last treatment arm prior to the time of discontinuation. Reasons for treatment discontinuation will be presented by treatment.

All concomitant medications will be coded to the therapeutic class and preferred term according to Mar 2016, Format C of World Health Organization (WHO) Drug Dictionary. Three listings will be provided:

1) All concomitant medications.
2) A listing of study related RBC transfusions
3) All other blood transfusions

VIII. Efficacy Analyses

All efficacy analyses will be performed using the FAS unless otherwise noted. For the primary analysis, no imputation will be used for missing data. Sensitivity analyses will be performed on the primary endpoint using multiple imputation to impute missing values of the primary endpoint.

A. Efficacy Outcomes

Primary Endpoint

The primary endpoint is normalized Hb AUC as a measure of the percent surviving RBCs. The normalized Hb AUC is calculated using the trapezoidal method on normalized Hb. The normalization is accomplished by dividing all post-transfusion Hb values by the 15-minute post-transfusion Hb level. The ratio is expressed as a percentage. A natural log-transform of the observed normalized Hb AUC will be utilized.

The normalized Hb AUC is expected to be calculated using four timepoints: the 15-minute post transfusion, 24-hour, Day 7, and end of transfusion interval (ie, pre-transfusion measure for the subsequent interval). If either the 24-hour or Day 7 measure is missing, the AUC will still be calculated using the following rule. If both are missing, or if either the 15-minute post transfusion or the end of transfusion interval are missing, then no AUC will be calculated for that interval.

A single value imputation approach will be used to estimate the missing percent decrease at either the 24 hour or Day 7 (but not both). The median of the observed normalized Hb levels (ie, percent decreases) for the treatment received at that particular time point and transfusion interval number will be used so that the AUC is based on 4 values of normalized Hb. This approach takes advantage of all the observed data rather than assuming an entire AUC is missing in the presence of a single missing Hb value.

The approximations are conservative in the sense that they bias towards inferiority in the case of missing Hb values for the normalized Hb AUC calculation.

The Hb will be collected within 1 hour before transfusion, 15 (+ 5) minutes, 24 (± 6) hours, and 7 (± 1) days after transfusion and at the end of study treatment follow-up. The transformed normalized Hb AUC values from the first 2 transfusion episodes after the 50-day wash-in for each period will be used to obtain a single measure associated with each treatment. If only a single

normalized Hb AUC value is available after the wash-in for an individual period, that single value will be used for analysis.

If a patient randomized to receive RBCs derived from Mirasol-treated WB in a particular period receives 2 successive off-protocol transfusion episodes (i.e., untreated RBCs) after completing the 50-day wash-in period, the primary endpoint for that period will be excluded from the analysis. If only 1 of the 2 successive transfusion episodes after the 50-day wash-in period is per protocol, only the primary endpoint associated with the per protocol transfusion episode will be analyzed. If at least 1 off-protocol transfusion occurs during a transfusion episode, the transfusion episode will be considered off-protocol. No off-protocol transfusions will be used for the primary analysis to minimize bias towards non-inferiority. The study will be executed to eliminate or minimize the risk of off-protocol transfusions.

Secondary Endpoints

There are 4 secondary endpoints of interest in this study. No adjustments for multiple comparisons/testing will be performed for the other secondary endpoints. Analyses will be based on the observed data using the FAS.

The secondary endpoints are:

- Hb increment
  - Calculation: [(post-transfusion Hb – pre-transfusion Hb)/Hb transfused]/RBC volume in subject at pre-transfusion
  - Hb transfused in the above formula is the sum of Hb content of each unit transfused calculated as (Hb/dL x volume)/100
  - Note: RBC volume in subjects at pre-transfusion is based on weight, height, sex, and pre-transfusion Hct. Example formulas are provided below where weight is in pounds and height is in inches.
  - RBC Volume (Male) = [(0.006012 x height^3)/(14.6 x weight) + 604] x (Hct/100)
  - RBC Volume (Female) = [(0.005835 x height^3)/(15 x weight) + 183] x (Hct/100)
- Actual Hb level post-transfusion (15 min)
- Proportional decline in post-transfusion Hb level
  - Calculation: [(Hb (t0) – Hb(t1))/Hb(t0)]/time between transfusion (days)
- RBC mass infused (volume x Hb/unit)

B. Primary Efficacy Endpoint Analysis

The primary analysis will use a mixed effect repeated measures model on the log-transformed data to assess differences between treatments using the FAS. A

random subject-level effect will be used to accommodate dependence in responses within subjects over time. Fixed main effect terms for period, treatment, treatment*period interaction and baseline normalized Hb AUC will be included in the model Mirasol-treated WB will be coded as 1 and conventional treatment will be coded as 0 in the model.

A one-sided 97.5% confidence interval for treatment differences will be used to test the primary hypothesis that the normalized Hb AUC as a measure of RBC survival derived from Mirasol-treated WB is non-inferior to normalized Hb AUC after transfusion of conventional untreated RBCs. Assuming a non-inferiority margin of 20% (at the original scale), the use of RBCs derived from Mirasol-treated WB will be declared non-inferior to conventional, untreated RBCs if the lower limit of the one-sided confidence interval for the treatment effect (RBCs derived from Mirasol-treated WB mean minus conventional) is greater than ln(0.80) = -0.2231.

The following provides sample code for implementing the MMRM analysis:

```
ods output lsmeans=lsmeans diffs=diffs;
proc mixed data=test;
class subjid trt period trannum;
model resp=period trt trt*period trannum basehb/ddfm=kr;
random subjid(sequence)/sub=subjid type=cs;
lsmeans trt/diff alpha=.05;
```

where

- RESP is the log(normalized Hb AUC)
- TRT is the variable for treatment (0 for REF RBCs; 1 for MIR RBCs)
- BASEHB is the pre-treatment Hb
- PERIOD is the variable for period
- TRANNUM is the variable to identify the order of the two transfusions following the 50-day washin-in period for each treatment period (1 for first transfusion, 2 for second transfusion)
- SUBJID is for subject

The carryover effect will be tested with the TRT*PERIOD interaction term. If the interaction is significant, then a carryover effect may be present and alternative analysis methods may be considered.

Several secondary/exploratory analyses will be conducted on the primary endpoint. Each are described below:

Per Protocol Analysis
As as secondary analysis, the primary endpoint will be analyzed as described above using the PPS.

Imputation of Missing Values Analysis
As a sensitivity analysis, missing normalized Hb AUC values will be imputed. In this analysis, any missing normalized Hb AUCs will be imputed using the SAS procedure for Multiple Imputation (Proc MI).

1) The pattern of missingness will be analyzed and determined to be either monotone or arbitrary.
2) If the missingness pattern is arbitrary, then the MCMC method will be used to impute just enough missing values to make the imputed data sets have monotone missing patterns.
3) PROC MI will then be used to impute the remainder of the missing data (in a monotone pattern) using linear regression.
4) MMRM model will be used to produce point estimates and associated standard errors for the treatment difference.
5) PROC MIANALYZE will be used to combine the collection of point estimates and SEs and create a 95% CI to make inferences.

The variables used in the MI process will be (pooled) treatment, age, gender, normalized Hb AUC from transfusion episodes during the wash-in period, pre-transfusion Hb level, and normalized Hb AUC from previous transfusion in the same treatment/control period. The number of imputations used in the MI process will be 100.

The primary endpoint in each imputation dataset will then be analyzed similarly to the primary analysis of the primary endpoint described above. The point estimates and associated SEs of the treatment difference for each imputation dataset will be analyzed using PROC MIANALYZE. The result will be summarized as the MI estimate of the treatment difference and associated 95% confidence interval.

RBC ≤ 21 Day Old Analysis
This exploratory analysis will distinguish the effect of treatment with MIR RBCs versus REF RBCs when units were stored for ≤ 21 days. All MIR RBCs will be stored for ≤ 21 days; however, REF RBCs may be stored for up to 42 days. In this analysis, the primary endpoint of normalized Hb AUC will be analyzed similarly to the primary analysis, but all normalized Hb AUCs that resulted from REF RBCs stored longer than 21 days (at least 1 unit per transfusion is stored longer than 21 days) will be excluded. Note that the study is powered to assess the primary endpoint of MIR RBCs compared to REF RBCs administered per current standard of care which allows REF RBCs to be stored longer than 21 days. This analysis is exploratory and is not powered to determine non-inferiority.

Non-Irradiated RBC Only Analysis
This exploratory analysis will be conducted to evaluate whether irradiation of REF RBCs has an impact on the primary treatment comparison. In this analysis, the primary endpoint of normalized Hb AUC will be analyzed similarly to the

primary analysis, but all normalized Hb AUCs that resulted from irradiated REF RBCs will be excluded.

Region Analysis
This exploratory analysis will be conducted to evaluate the impact of region on the primary treatment comparison. Each center will be classified as either US-based or Non-US based. In this analysis, the primary endpoint of normalized Hb AUC will be analyzed similarly to the primary analysis; however, region and the region*treatment interaction will be included in the model as fixed effect terms. If the region*treatment interaction term is significant at 0.05 significant level in the model, then the point estimates and associated 95% confidence intervals for the treatment differences may be calculated for each region.

RBC Storage Solution Analysis
This exploratory analysis will be conducted to evaluate the impact of RBC storage solution on the primary treatment comparison. In this analysis, the primary endpoint of normalized Hb AUC will be analyzed similarly to the primary analysis; however, storage solution type will be included in the model as a fixed effect. If the storage solution type term is significant in the model, then the point estimates and associated 95% confidence intervals for the treatment differences may be calculated by storage solution type.

C. Secondary Efficacy Endpoints Analyses

Hb increment, proportional decline in post-transfusion Hb and RBC mass infused will each be analyzed separately with a mixed effects repeated measures model fitting fixed effect terms for treatment, period and treatment*period interaction. Subject will be included in the model as a random effect. The point estimates and associated 95% confidence intervals will be presented for the treatment differences.

Two analyses will be carried out for the 15 minute Hb value.

- The first is directed at estimating the difference between the MIR RBC and REF RBC products and for this the mixed-effect model used for the primary outcome will be adopted to model the 15 minute Hb value as the response. A 95% confidence interval for the estimated mean difference in the 15 minute Hb value will be computed.
- A second analysis will report the proportion of 15 minute Hb values within the pre-specified range of plus or minus 15% of the target value. This target value is specified at the start of study and should remain fixed during the course of the study. This success rate will be estimated using generalized estimating equations with this binary response, a logistic link, a main effect for product type (MIR vs. REF RBCs) and an exchangeable correlation matrix to accommodate the dependence within patients. The resulting estimates will be provided as supplementary information to help in the

interpretation of the primary analyses. There will be no non-inferiority bound or formal tests associated with these event rates.

All secondary endpoints will be descriptively summarized by treatment. Additionally, Hb increment, proportional decline in post-transfusion Hb and RBC mass infused will be presented graphically.

As a secondary analysis, actual Hb AUC will also be analyzed similarly to the primary endpoint described in Section VIII.B. Specifically, the Hb AUC will not be normalized to the 15-minute post-transfusion Hb level in this analysis.

The frequency of off-protocol transfusions will be estimated during the wash-in period and treatment periods for MIR RBCs and REF RBC products. This frequency will be estimated using generalized estimating equations with this binary response (on- versus off-protocol), a logistic link, a main effect for assigned treatment (MIR RBCs vs. REF RBCs), and an exchangeable correlation matrix to accommodate the dependence within patients. The results of this descriptive analysis will give context to the FAS and PPS non-inferiority analyses. The raw patterns and frequencies of off-protocol transfusions will be reported.

IX. Safety Analyses

Safety analyses will use data from the SS.

A. Adverse Events

All subjects will be assessed regularly for potential occurrence of adverse events (AE) from the treatment period until the subject is off study. The Medical Dictionary for Regulatory Activities (MedDRA; Version 16.1) will be used to code all adverse events to a System Organ Class (SOC) and Preferred Term (PT). The incidence of treatment-emergent AEs (TEAE) will be summarized and tabulated by treatment group. A TEAE is defined as any AE that occurs on or after the date of first transfusion.

The National Cancer Institute Common Terminology Criteria for Adverse Events (version 4.03; CTCAE) will be used to grade severity of all AEs. The severity of each AE will be assessed as mild, moderate, severe, life-threatening or fatal. Several occurrences of the same AE in one subject will be counted once and the one with worst severity will be counted.

The relationship of each AE to the study treatment or to study procedure will be grouped as related or unrelated. Any AE with a CRF description of definitely related, probably related, or possibly related will be considered related. Any AE

with a CRF description of not related will be considered unrelated. Several occurrences of the same AE in one subject will be counted once and the one with the closest relationship to study treatment or to study procedure will be counted. If an AE is missing the relationship to study medication, the event will be assumed to be related for analysis and summarization.

The number and percentage of patients reporting each PT will be summarized by the treatment received in the period of onset. Incidence of AEs by maximum reported CTCAE grade will also be tabulated within each arm. The SAEs and AEs leading to study discontinuation will be displayed. The following summaries will be presented:

- Overall summary of TEAEs
- TEAEs by SOC and PT. The number and percentage of subjects with at least one TEAE, as classified by system organ class (SOC) and preferred term (PT), will be summarized by treatment received in the period of onset. For these summaries, subjects with multiple events will be counted only once per SOC and preferred term.
- Study Treatment-related TEAEs by SOC and PT
- Study Procedure-related TEAEs by SOC and PT
- TEAEs by maximum CTCAE grade, SOC and PT
- Serious TEAEs by SOC and PT
- UADEs by SOC and PT
- Adverse transfusion reactions
- TEAEs causing discontinuation from the study
- All-cause mortality
- Incidence of treatment-emergent antibody with confirmed specificity to RBCs derived from Mirasol-treated WB

Because all AEs will be recorded from the time of the first study-related transfusion through the end of study treatment follow-up visit or early termination, any AEs will by definition be TEAEs. However, causation by treatment may not be determined for AEs observed at the final follow-up visit due to off protocol transfusions administered since last study transfusion

B. Clinical Laboratory Results

All laboratory values will be converted to International System of Units (SI units).

Laboratory test covered by the CTCAE (version 4.03) will be assigned grades accordingly. A grade of 0 will be assumed for non-missing values not graded as 1 or higher. In the unlikely cases where a laboratory normal range overlaps in the higher (ie, non-zero) CTCAE grade, the laboratory value will still be taken as within normal limits and assigned a CTCAE grade of 0. Grade 5 will not be used.

For laboratory tests where grades are not defined by CTCAE, results will be graded by the low/normal/high classifications based on normal ranges.

The following summaries by treatment group will be presented separately for laboratory parameters: shift in CTCAE grade from pre-transfusion of wash-in period to each assessment timepoint for laboratory tests that are graded; for non-graded tests, shift in normal range classification from pre-transfusion of wash-in period to each assessment timepoint. For both shift tables, a summary of worst shift from pre-transfusion of wash-in period will be presented.

C. Vital Signs

Vital signs will be performed at baseline, during and within 15 min post-transfusion, 1 Day post-transfusion, and 7 Days post-transfusion. Vital signs include temperature, heart rate (HR), respiratory rate (RR), blood pressure (BP), and arterial oxygen saturation via pulse oximetry (SpO2), will be listed by treatment sequence, treatment group and time point. Vital signs will be summarized as actual and change from baseline for each treatment group at each scheduled assessment.

D. Physical Examination

Physical examination will include assessment of general appearance, eyes/ears/nose/throat, pulmonary/chest, cardiovascular, abdominal, and neurological. Physical examination will be captured at baseline and at the end of study treatment follow-up. All examination findings will be listed.

E. Pregnancy Test

For all females of child-bearing potential, a listing of pregnancy test including test date, test result, and reason if test not taken will be presented.

F. Treatment-Emergent Antibodies

The number and percentage of subjects with incident treatment-emergent antibodies with confirmed specificity to RBCs derived from Mirasol-treated WB will be presented by time intervals antibodies are detected.

G. HLA Alloimmunization Rates

HLA alloimmunization rates will be descriptively summarized by treatment.