CLINICAL INVESTIGATION PLAN

Study Title:	Evaluate the Efficacy and Safety of RBCs Derived from Mirasol-treated Whole Blood Compared with Conventional RBCs in Patients Requiring Chronic Transfusion Support (PRAISE)
Study Number:	CTS-5056
Study Device:	Mirasol® System for Whole Blood
Manufacturer:	Terumo BCT, Inc. 10811 West Collins Avenue Lakewood, CO 80215; USA Phone: +1 (877) 339-4228
Sponsor:	Terumo BCT Biotechnologies, LLC 1215 Quail Street Lakewood, CO 80215; USA Phone: +1 (877) 339-4228 Funded by the US Department of Defense Joint Warfare Medical Research Program, administered by United States Army Medical Research & Materiel Command (JWMRP, Contract #W81XWH-13-C-0160)
Sponsor Contact:	Heather Pidcoke, MD, PhD Phone: +1 (303) 231-4805 Email: Heather.Pidcoke@terumobct.com
Lead Investigator:	Steven Sloan, MD, PhD Blood Bank Medical Director, Boston Children's Hospital 300 Longwood Avenue Boston, MA 02115 Email: Steven.Sloan@childrens.harvard.edu

This study will be conducted according to this protocol, Good Clinical Practice as described in the International Conference of Harmonisation Guidance for Industry E6, and as applicable, US Food and Drug Administration 21 CFR 812, International Organization for Standardization 14155:2011(E), and other regulatory requirements of the regions where the study is conducted. All essential documents will be archived.

Version/Date:	Version 5.0 / 15 FEB 2018

Confidentiality Statement

CLINICAL INVESTIGATION PLAN APPROVAL

Name: Steven Sloan, MD, PhD
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Name: Shannon Godbold, RN, BSN
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Signature: _____ **Date:** _____

Name: Bethany L. Brown, MSCS
Title: Senior Clinical Research Lead

Signature: _____ **Date:** _____

Name: William Coar, PhD
Title: Consultant Statistician

Signature: _____ **Date:** _____

AMENDMENT 4.1 SUMMARY OF CHANGES

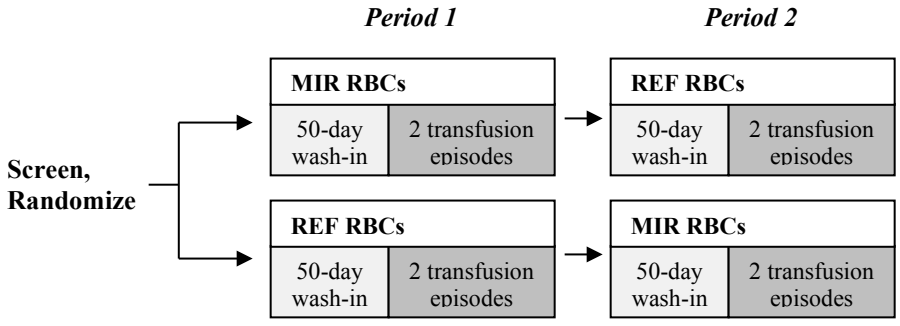
Study Title:	Evaluate the Efficacy and Safety of RBCs Derived from Mirasol-treated Whole Blood Compared with Conventional RBCs in Patients Requiring Chronic Transfusion Support (PRAISE)	
CIP #:	CTS-5056	
Amendment Version / Date:	Version 5.0/15 FEB 2018	
Replaces CIP Version / Date:	Version 4.1/28 AUG 2017	
Rationale:	This revision history captures updates from Version 4.1/16 AUG 2017 to the current Version 5.0/15 FEB 2018. Version 5.0 is the first version of the protocol under which patients are anticipated to be enrolled. No Significant changes were made. Administrative changes, section numbering, typographical error corrections, and minor wording changes for clarity as a result of this amendment have been made and will not be reflected in the table below.	
Section(s)	**Used to Read:**	**Now Reads:**
Section 14.3 With each RBC Transfusion Episode	The date and time of transfusion, number of units, type of product transfused, and reason for administration will be documented in the eCRF.	The date and time of transfusion, number of units, **and** type of product transfused will be documented in the eCRF.

Section 19.12.1 Research Monitor	A designated DMC member will fulfill the role of the Research Monitor as required for studies funded by the United States Department of Defense. (added) Responsibilities will include the following: Should the Research Monitor determine that SAEs or UADEs present an unreasonable risk to study subjects, he/she may make recommendations to the Sponsor regarding suspension of enrollment or termination of the study based on safety concerns. However, all final decisions regarding trial modifications rest with the Sponsor. Discussion of Study: May discuss the protocol with the Investigators, interview subjects, and consult with others outside the study about the research. Subject Protection: Shall have the authority to stop the protocol, remove subjects from the protocol, and take any necessary steps to protect the safety and well-being of subjects until the IRB/EC can assess the Independent Medical Monitor's report. Reporting: Shall have the responsibility to promptly report their observations and findings to the IRB/EC or other designated official.	A designated DMC member **whose expertise is consonant with the nature of risk(s)** will fulfill the role of the **independent** Research Monitor as required for studies funded by the United States Department of Defense. The independent research monitor may discuss the research protocol with the investigators, interview human subjects, consult with others outside of the study about the research; shall have the authority to stop a research protocol in progress, remove individual human subjects from a research protocol, and take whatever steps are necessary to protect the safety and well-being of human subjects until the IRB can assess the monitor's report; shall have the responsibility to promptly report their observations and findings to the IRB or other designated official and the human research protections office (HRPO). Responsibilities will **also** include the following: (deleted)

SYNOPSIS

<table>
<tr><td>Sponsor:</td><td>Terumo BCT Biotechnologies, LLC and Terumo BCT, Inc. hereafter referenced as Terumo BCT</td></tr>
<tr><td>Study Title:</td><td>Evaluate the Efficacy and Safety of RBCs Derived from Mirasol-treated Whole Blood Compared with Conventional RBCs in Patients Requiring Chronic Transfusion Support (PRAISE)</td></tr>
<tr><td>Study Number:</td><td>CTS-5056</td></tr>
<tr><td>Target Population:</td><td>Transfusion-dependent thalassemia patients</td></tr>
<tr><td>Device Description:</td><td>The Mirasol System for WB consists of 3 main components:
Components of the Mirasol System for WB
<table>
<tr><th>Component</th><th>Description</th></tr>
<tr><td>Mirasol Illuminator</td><td>A microprocessor-controlled, electro-mechanical device capable of delivering a controlled amount of ultraviolet (UV) light. The Mirasol Illuminator is programmed to deliver, record, and store intensity and duration of UV light.</td></tr>
<tr><td>Mirasol Whole Blood Disposable Kit</td><td>Sterile, non-pyrogenic, single use, closed fluid path system comprising:
• 1 illumination bag, 1 whole blood storage bag, tubing and frangible connectors packaged within a clear poly/Tyvek package, sterilized by ethylene oxide gas; and
• 1 riboflavin bag containing 35 mL, 500 µM riboflavin in 0.9% sodium chloride packaged within a clear PVC bag, steam sterilized, and packaged within an opaque foil pouch to protect from light.</td></tr>
<tr><td>Mirasol Manager Software</td><td>Software installed on a separate computer which integrates with the Mirasol Illuminator(s) to manage and report data.</td></tr>
</table>
Additional equipment includes a barcode reader, a sterile connection device and an electronic scale.</td></tr>
<tr><td>Intended Use:</td><td>The Mirasol System for Whole Blood (WB) is an extracorporeal blood treatment process which uses ultraviolet (UV) light energy and riboflavin (vitamin B_2) to inhibit nucleic acid replication in WB for the production of Mirasol-treated RBC.
• Mirasol System treatment is used to inactivate Babesia species in order to prevent transfusion-transmitted Babesiosis
• Mirasol System treatment is used to inactivate T-lymphocytes in order to reduce the risk of transfusion-associated graft-versus-host disease (TA-GvHD). Mirasol treatment is at least as effective as gamma irradiation at inactivating lymphocytes</td></tr>
<tr><td>Study Centers Planned:</td><td>Up to 10 transfusion centers and corresponding blood centers with the majority of the sites in the United States and a few sites outside of the United States (eg, Europe, Canada, or the Middle East). Each transfusion center and each blood center will have a designated Investigator.
• Transfusion centers will screen, enroll, treat, and perform assessments on study subjects.
• Blood centers will be responsible for the manufacturing, processing, storage, and shipment of study related blood products to transfusion centers. One blood</td></tr>
</table>

	center may supply study related blood products to more than 1 transfusion center.
Objectives:	**Primary Objective** • To determine if percent survival of RBCs derived from Mirasol-treated WB is non-inferior to conventional RBCs when transfused into patients requiring chronic RBC transfusion support. **Secondary Objectives** • To compare other efficacy and safety endpoints between treatment groups.
Efficacy Endpoints:	**Primary Efficacy Endpoint** 1. Normalized hemoglobin (Hb AUC) calculated from normalized Hb between successive transfusions as a measure of percent surviving RBCs **Secondary Efficacy Endpoints** 1. Hb increment. 2. Actual Hb level post-transfusion (15min). 3. Proportional decline in post-transfusion Hb level. 4. RBC mass infused (volume x Hb/unit).
Safety Endpoints:	1. Incidence of treatment-emergent antibody with confirmed specificity to RBCs derived from Mirasol-treated WB. 2. Human leukocyte antigen (HLA) alloimmunization rates. 3. Treatment emergent adverse events (TEAEs). 4. Transfusion-related adverse events (AEs). 5. Serious adverse events (SAEs). 6. Unanticipated adverse device effects (UADEs).
Inclusion Criteria:	1. Transfusion dependent thalassemia patient with mean 2-4 week transfusion intervals for the prior 6 months. 2. Age ≥ 12 years. 3. Negative pregnancy test for women of childbearing potential and agreement to practice a medically acceptable contraception regimen throughout the participation in the clinical trial. Not required if female subjects are not of child-bearing potential (ie, prior to menses onset, surgically sterilized, 1-year postmenopausal). 4. Signed informed consent from the patient, or if the patient is < 18 years of age, signed assent from patient and consent from parent/guardian, according to local Institutional Review Board/Ethics Committee (IRB/EC) requirements.
Exclusion Criteria:	1. Historical RBC transfusion requirement of more than 200 mL/kg/year. 2. Presence of RBC antibodies that make procurement of compatible RBC units not feasible per the treating physician's clinical judgment for reasonable execution of the study. 3. Prior treatment with pathogen-reduced RBCs with subsequent development of known antibodies to the associated RBCs. 4. Planned treatment requirement of frozen RBC products. 5. Treatment requirements for any medication that is known to cause hemolysis. 6. Receiving cardiac medications for heart failure. 7. Patients anticipated to receive massive transfusion, per the treating physician's clinical judgment. 8. Known human immunodeficiency virus (HIV) infection (defined as HIV RNA positive) with changes to antiviral regimen within the 12 months prior to screening.

	9. Acute or chronic medical disorder that, in the opinion of the Investigator, would impair the ability of the patient to receive study treatment. 10. Participation in another clinical study, either concurrently or within the previous 28 days, in which the study drug or device may influence study endpoints or patient safety, according to Investigator discretion. 11. Participation in another study within the past 3 months if investigational RBCs or treatment or drugs were received that are likely to have long term effect on RBC function. 12. Pregnant or breastfeeding. 13. Planned concurrent treatment with other pathogen reduction blood products during participation in this study. 14. Patients who received prior treatment with pathogen-reduced RBCs within the past 120 days. 15. Inability of the patient to comply with study procedures and/or follow-up.
Number of Study Subjects Planned:	Assuming a 20% dropout rate, it is anticipated that approximately 97 patients will be randomized to achieve 77 subjects in the Full Analysis Set (FAS). The FAS will include all randomized patients who have at least 1 normalized Hb AUC measurement.
Study Design:	This is a prospective, multi-center, randomized, crossover trial to evaluate the clinical effectiveness of RBCs derived from Mirasol-treated WB versus conventional RBCs in transfusion dependent thalassemia patients. Throughout the clinical study, 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 RBCs derived from Mirasol-treated WB (MIR RBCs) followed by conventional RBCs (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, prior to 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.
RBC Product:	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. MIR RBCs will be prepared from WB treated with the Mirasol System for WB according to the Blood Processing and Storage Guidelines and the Mirasol System for WB Illuminator Version 6.2 Operator's Manual. MIR RBCs must not be irradiated. Irradiating blood products that have been Mirasol-treated is redundant and may further compromise RBC viability. In vitro studies demonstrated Mirasol treatment was as effective as irradiation in preventing leukocyte proliferation and was more effective than gamma irradiation at preventing antigen presentation, cytokine production, and T-cell activation. Modifications such as RBC washing (MIR RBCs or REF RBCs) or irradiation (REF RBCs only) may be done according to Hospital Site and/or Local Blood Center standard procedures and following established guidelines as to impact to storage durations and transfusion practices. Any modifications will be captured in the electronic case report form (eCRF). Throughout the clinical trial, RBC transfusion volume and frequency will be determined by each subject's treating physician.
Study Duration:	Subject participation will be approximately 7-9 months, including a screening period (up to 28 days) and 2 treatment periods (each including 50-day wash-in period and 2 transfusion episodes). An end of study treatment follow-up visit will occur 2-4 weeks after the last per protocol transfusion, prior to the next standard of care transfusion. A final study visit will occur at least 60 days after the last per protocol transfusion. Study duration will be approximately 29 months.

Safety Assessments:	Safety will be evaluated by assessment of physical examinations, vital signs, clinical laboratory values, TEAEs, UADEs, SAEs, and discontinuations due to treatment-related AEs. Safety will be assessed according to the National Cancer Institute (NCI) Common Toxicity Criteria for Adverse Events (CTCAE) scale, Version 4.03. This trial will be monitored using an independent Data Monitoring Committee (DMC) operating under a ratified charter. The DMC will review SAE/UADE reports on an ongoing basis. Additionally, the DMC will review safety reports summarizing the frequency and severity of TEAEs, UADEs, and other relevant safety information as defined in the DMC charter. Enrollment will not be placed on hold during the DMC review process. The DMC will determine if the observed AEs are acceptable for this patient population, and will make recommendations to the Sponsor regarding study continuation.
Statistical Methodology:	Eligible subjects will be randomized to treatment sequence via an electronic system using a permuted-block schedule stratified by investigational site. The primary endpoint will be the normalized Hb AUC calculated from normalized Hb between successive transfusions as a measure of the percent surviving RBCs and will be evaluated for the first 2 transfusions after a 50-day wash-in for each treatment period. These 2 measurements of normalized Hb AUC within each period will be used for analysis. The primary hypothesis is that the normalized Hb AUC as a measure of RBC survival derived from Mirasol-treated WB is not inferior to normalized Hb AUC after transfusion of conventional untreated RBCs. The study has approximately 80% power to show the decrease in normalized Hb AUC of RBCs derived from Mirasol-treated WB is not more than 20% of the decrease in normalized Hb AUC of conventional untreated RBCs, assuming a 1-tailed type 1 error rate of 2.5%.

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LIST OF ABBREVIATIONS AND DEFINITION OF TERM

AABB	Formerly known as American Association of Blood Banks
AE	adverse event
ALT	alanine aminotransferase
AS-3	Additive Solution Formulation 3
AST	aspartate aminotransferase
ATP	adenosine triphosphate
AUC	Area under the curve
BP	blood pressure
BUI	Blood Unit Identification
BUN	blood urea nitrogen
CBC	Complete Blood Count
CE	Conformité Européenne
CFR	Code of Federal Regulations
CHIKV	Chikungunya Virus
CIP	Clinical Investigation Plan
CMV	Cytomegalovirus
CPD	citrate phosphate dextrose
CRA	Clinical Research Associate
CRF	case report form
CTA	Clinical Trial Agreement
CTCAE	Common Terminology Criteria for Adverse Events
DCF	data clarification form
DENV	Dengue Virus
DMC	Data Monitoring Committee
DNA	deoxyribonucleic acid
DoD	Department of Defense
eCRF	electronic case report form
EDC	Electronic Data Capture
eg	for example
FAS	Full Analysis Set
FDA	US Food and Drug Administration
GCP	Good Clinical Practice
GvHD	Graft-versus-host disease
Hb	hemoglobin
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
HR	heart rate
HRPO	Human Research Protections Office
HTLV	Human T cell lymphotropic virus
IB	Investigators Brochure
ICF	Informed Consent Form
ICH	International Conference of Harmonization

ID	Identification
ie	that is
IRB/EC	Institutional Review Board/Ethics Committee
ISO	International Organization for Standardization
IWRS	interactive web response system
kg	kilogram
LDH	lactate dehydrogenase
LR	leukoreduced
MedDRA	Medical Dictionary for Regulatory Affairs
mg	milligram
mL	milliliter
µL	microliter
mm	millimeter
µM	micromolar
MOP	Manual of Procedures
ORP	Office of Research Protections
PPS	Per Protocol Set
PT	Preferred Term
RBC	red blood cell
RNA	ribonucleic acid
RR	respiratory rate
SAE	serious adverse event
SAP	Statistical Analysis Plan
SI	International System of Units
SOC	System Organ Class
SpO_2	arterial oxygen saturation via pulse oximetry
SS	Safety Set
TA-GvHD	Transfusion-associated graft-versus-host disease
TEAE	treatment emergent adverse event
TRIM	transfusion-related immunomodulation
TTB	transfusion-transmitted babesiosis
TTI	transfusion-transmitted infection
UADE	unanticipated adverse device effect
USAMRMC	United States Army Medical Research and Materiel Command
UV	ultraviolet
WB	whole blood
WBC	white blood cell
WNV	West Nile virus
ZIKV	Zika Virus

1 INTRODUCTION

To date, there is no artificial product that can substitute for human blood. Because of the short shelf life of some components and the relative shortage of willing blood donors, blood for transfusion is constantly in demand and a safe, practical, economical, and efficient usage of our blood supply is imperative.

Whole blood (WB) contains red blood cells (RBCs), white blood cells (WBCs), and platelets suspended in plasma; approximately 45% (by volume) are the components and approximately 55% (by volume) is plasma. The RBCs contain hemoglobin (Hb), a complex, iron binding protein that transports oxygen and other gases (eg, carbon dioxide and nitric oxide) through the body. Hematocrit (Hct) is the percent of blood volume composed of RBCs. There are approximately 1 billion RBCs in 2-3 drops of blood, and for every 600 RBCs, there are approximately 40 platelets and 1 WBC. Synthesized in bone marrow, RBCs and Hb are continuously produced and broken down systemically. Under normal circumstances, RBCs persist in the circulatory system for approximately 110-120 days.[1,2]

Following volunteer donation, WB is separated into constituent components by centrifugation. Isolated RBCs are mixed with storage solutions which provide nutrients to preserve RBC viability. This increases storage survival and maintains functionality during refrigerated storage, thereby limiting so called “storage lesions” of isolated RBCs.[2]

Red blood cells are the most common blood component used in transfusion medicine. Approximately 85 million units of RBCs are transfused annually worldwide with approximately 15 million units transfused annually in the United States.[1] Therapeutic RBC transfusions increase the recipients’ Hct and Hb levels without significantly increasing blood volume. Red blood cell (RBC) transfusions are commonly employed for symptomatic anemia, prophylaxis in life-threatening anemia, restoration of oxygen-carrying capacity in hemorrhage, exchange transfusion in sickle cell disease, treatment of severe parasitic infection, severe methemoglobinemia, and severe hyperbilirubinemia of the newborn.[1]

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 Hb production.[3] Beta thalassemia is classified as thalassemia minor/trait (heterozygous), which is more common and asymptomatic; thalassemia intermedia (homozygous, heterozygous, or compound heterozygous) is a non-transfusion dependent thalassemia with features of iron loading and ineffective erythropoesis; and thalassemia major (homozygous) is the most severe form of the disease, requiring RBC transfusions from an early age. Consequently, thalassemia is the most RBC-transfused syndrome worldwide with as many as 2,000 thalassemia patients estimated to live in the United States. Additionally, there are more than 60,000 births each year of serious forms of thalassemia, globally.[4] The current standard of care for transfusion-dependent thalassemia patients dictates chronic RBC transfusions with the

goal of controlling anemia and suppressing ineffective erythropoiesis.[3] 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.

Transfused blood products provide great therapeutic benefits and can save lives, but carry the inherent risk of transferring not only the needed blood components but also elements that are potentially dangerous, such as pathogens that can transmit disease and WBCs that can elicit harmful immune responses.

The pathogen risk is reduced by pre-screening of blood donors and testing of the blood. These safety measures decrease the risk of harmful pathogens being present in the blood but have limitations. One limitation of blood testing is the window period between the time of infection and the time when pathogen reaches the threshold of detection. Also, there are many pathogens for which tests are not readily available, and developing new tests is a time consuming and costly process. On average 5.3 viruses per year, of which 60%-70% are human pathogens, have emerged from 1940 to 2004.[5]

Although the vast majority of WBCs in blood products can be removed by leukoreduction, residual levels of WBCs remain and can cause transfusion-associated graft-versus-host disease (TA-GvHD).[6,7] Inactivation of WBCs in blood products can be achieved with the use of gamma irradiation. Currently, this method is accepted as a standard of care in many parts of the world for use with blood products to be transfused to immune-compromised patients or patient populations that are particularly susceptible to TA-GvHD. The use of gamma irradiation has disadvantages such as the difficulty of maintaining radiation sources and the public health and safety risks associated with the use of cesium chloride.[8,9] Another disadvantage is that patients at risk of TA-GvHD may not be properly identified as such.

1.1 Pathogen Risk

Transfusion-transmitted infections occur when pathogens, such as viruses, bacteria, and parasites, are introduced to a person via a blood transfusion. The risk of TTIs has decreased over the last decades due to extensive donor screening and improved pathogen testing, but a residual risk remains; for some patients, the risk can be very serious or even fatal. Serology and/or nucleic acid testing (NAT) tests detect pathogens efficiently,[10] but these tests require that the concentration of infectious particles or antibodies directed against the pathogens is above a certain threshold. If the donor is at the early phase of an acute infection or at a silent chronic phase, the threshold of detection may not be reached. A greater concern is the pathogens for which no testing is conducted. Emerging infectious diseases (EIDs) that have recently started to pose a threat because of expanding vector range (eg, dengue virus[DENV]),[11-13] viral mutations

(eg, chikungunya virus [CHIKV]), [14] or zoonotic events (eg, swine flu)[15] can have very serious consequences, especially since it is costly and time-consuming to develop new tests for blood screening. In the United States and Canada, pathogens such as variant Creutzfeldt-Jakob prions, DENV, and the red-cell parasites that cause babesiosis, are classified as highest priority due to a known transfusion-transmission threat and severity to recipients.[5] Babesia is of special interest because it is an erythrocytic parasite.

Babesia microti and *Babesia divergens,* as well as other Babesia species, have been identified as the causative agent of transfusion-transmitted babesiosis (TTB), for which the incidence is steadily increasing over the last decade.[16-20] For 2013, among the 27 states in which babesiosis was a reportable condition, the Centers for Disease Control was notified of 1,762 cases of babesiosis; babesiosis had become a nationally notifiable condition in January 2011.[21,22] Of note, the AABB has classified TTB as a "red agent" or one with the greatest risk to United States blood safety.[16] General Babesia screening of donors is currently not cost/benefit effective.[23]

The recent outbreak of Zika virus (ZIKV) provides an example of the impact of EIDs on blood safety. To address this risk, donor deferral and sourcing of blood from non-outbreak areas has been implemented.[24] Historically, specific and sensitive diagnostic tests are not immediately available during EID outbreaks. In the French Polynesia 2013-2014 outbreak, 2.8% of samples from asymptomatic blood donors contained ZIKV.[25] In 2016 in Brazil, 2 cases of transfusion-transmitted ZIKV were suspected.[26] Dengue virus and CHIKV are also endemic in regions at risk for ZIKV, and DENV is present in more than 100 countries.[27,28] Donated blood products are not routinely screened for DENV or CHIKV. In a study by Sabino et al., transfusion-transmission of DENV occurred in 37.5% of blood recipients who received DENV RNA+ blood.[29] A more universal approach to blood safety is needed to mitigate risks posed by rapidly emerging infectious threats.

In a survey conducted using 8 thalassemia centers, 3 centers (37.5%) reported at least 1 case of transfusion-transmitted baesiosis.[30] As many thalassemia major patients are asplenic and therefore immunocompromised, they are more vulnerable to babesiosis, adding to the general increased risk of TTIs that chronically transfused patients experience as a consequence of repetitive exposure to blood products from multiple donors.

It is important to note that the risk of TTIs varies dramatically in different parts of the world. In developing countries, blood transfusion safety is challenged by a much higher incidence of infected donors and by far less efficient screening procedures. Because of factors such as these, blood transfusions carry a substantially higher risk in these countries. Using human immunodeficiency virus (HIV) as an example, the prevalence of TTI in high-income countries is 0.002%; in low-income countries, it is 0.85%.[31]

Blood safety has been dominated by measures to reduce the risk of HIV in the past 20 years, particularly in sub-Saharan Africa, but many other pathogens are also of serious concern. At

present, blood transfused in sub-Saharan Africa is typically not tested for *Plasmodium* species, the parasite that causes malaria, although the likelihood of donor parasitemia is high.[32] In the United States, a rapid diagnostic test for Plasmodium is available, but microscopy confirmation is essential and testing is not routinely used to screen donor blood.[33] Prevention of transfusion-transmitted malaria relies on donor questionnaires and donor deferrals of tourists (for 1 year) and former residents (for 3 years) of malarious regions.[34]

The Mirasol System offers a means to make transfusions significantly safer by providing pathogen reduction that targets both unscreened and undetected pathogens, including pathogens that have not yet been identified as a threat (data provided in Sections 4 and 5 below). It is especially suitable for the reduction of pathogens such as *Babesia* and *Plasmodium* that reside in RBCs, for WB transfusions in emergency trauma situations including military combat, and for use in the developing countries where the majority of blood donations are transfused as WB rather than components.

1.2 White Blood Cell Risk

White blood cell related complications after blood and blood component transfusion are well recognized in transfusion medicine and can be life-threatening. Residual WBCs in blood components have been implicated in TA-GvHD, alloimmunization, Febrile Non-hemolytic Transfusion Reaction, and transfusion-related immunomodulation (TRIM). Leukoreduction removes the vast majority of WBCs from blood component products, but residual levels of WBCs remain and have caused TA-GvHD.[6,35]

Inactivation of WBCs in blood products can be achieved with the use of irradiation. In many parts of the world, this method is the standard treatment of blood products to be transfused in immunocompromised patients or those susceptible to TA-GvHD. Even so, gamma irradiated WBCs are still capable of inducing a proliferative response in allogenic responder cells, and producing similar levels of pro-inflammatory cytokines as untreated control cells. Mirasol-treated blood components eliminate WBCs capability of mediating such responses. The use of gamma irradiation also has disadvantages such as the difficulty of maintaining radiation sources and the public health and safety risks associated with radioactive cesium chloride.[8,9]

The performance of the Mirasol System for inactivation of leukocytes in WB has been evaluated in vitro and in vivo and has been directly compared with gamma irradiation.[36] The Mirasol System for WB inactivates WBC proliferation and viability to the same extent as gamma irradiation and reduces the risk of TA-GvHD as efficiently as gamma irradiation. Mirasol treatment surpasses gamma irradiation in terms of T-cell inactivation and has the added advantage of inhibiting cytokine production in WBC, which gamma irradiation fails to do.[36]

1.3 Novel Device

Terumo BCT has developed the Mirasol System, which is an extracorporeal blood treatment process that uses ultraviolet (UV) light energy and riboflavin (vitamin B_2) to inhibit nucleic acid replication in WB for the production of Mirasol-treated RBCs, thus improving the safety of transfused blood by significantly reducing the possibility of TTIs and of immune reactions resulting from transfusion (data provided in Sections 4 and 5 below). Importantly, the approach that the Mirasol System offers addresses not only currently known threats to blood safety, but also has the potential to safeguard the blood supply against emergent pathogens that have not yet been identified as a risk.

Riboflavin associates with genomic nucleic acids and mediates an oxygen-independent electron transfer process, leading to the modification of nucleic acids. Compared with the use of UV light alone, which mainly causes repairable nucleic acid dimerization, damage induced by the combination of UV light and riboflavin is irreversible due to guanine base modifications. Thereby, deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) replication is inhibited.

Over the last decade, the Mirasol System has been implemented to treat platelets and plasma in 17 countries in Europe, the Middle East, Africa, and Asia. The Mirasol System for Platelets and Plasma received Conformité Européenne (CE) mark as a Class IIb medical device and has been in commercial use since 2007. The Mirasol System for WB is a medical device with a new indication utilizing existing technology, based on the same platform as the Mirasol System for Platelets and Plasma. The Mirasol System for WB received CE mark in 2015. The Mirasol Systems are not commercially available in the United States.

There have been no recalls of the Mirasol System for Platelets or Plasma.

There are no known contraindications for use of the Mirasol System for WB, platelets, or plasma; however, data for the investigator is provided below in Section 19.3.4 and in the associated Investigator's Brochure.

2 DEVICE DESCRIPTION

2.1 Device Description

The Mirasol System for WB consists of 3 main components:

Components of Mirasol System for WB

Component	Description
Mirasol Illuminator	A microprocessor-controlled, electro-mechanical device capable of delivering a controlled amount of ultraviolet (UV) light. The Mirasol Illuminator is programmed to deliver, record, and store intensity and duration of UV light.

Mirasol Whole Blood Disposable Kit	Sterile, non-pyrogenic, single use, closed fluid path system comprising: • 1 illumination bag, 1 whole blood storage bag, tubing and frangible connectors packaged within a clear poly/Tyvek package, sterilized by ethylene oxide gas; and • 1 riboflavin bag containing 35 mL, 500 µM riboflavin in 0.9% sodium chloride packaged within a clear PVC bag, steam sterilized, and packaged within an opaque foil pouch to protect from light.
Mirasol Manager Software	Software installed on a separate computer which integrates with the Mirasol Illuminator(s) to manage and report data.

Additional equipment includes a barcode reader, a sterile connection device and an electronic scale.

2.2 Principles of Operation

The WB is collected into a 450mL bag with anticoagulant citrate phosphate dextrose (CPD) in accordance with blood center standards. WB units meeting incoming specifications are transferred to the illumination bag. The riboflavin solution is added and mixed with the WB. The riboflavin/WB mixture is placed in the Illuminator. The Illuminator delivers a dose of 80 Joules of energy per milliliter of RBC (80 J/mL$_{RBC}$) to the riboflavin/WB mixture while agitating in a horizontal motion.

The spectral energy of the Mirasol System for WB is centered at 313 nm; 99% of the spectral energy reaching the product falls within the ultraviolet B (UVB, 280–315 nm) and ultraviolet A (UVA, 315–400 nm) region, which is the optimal range for riboflavin-mediated photosensitization of nucleic acids. Riboflavin associates with genomic nucleic acids and mediates an oxygen-independent electron transfer process, leading to the modification of nucleic acids. Compared with the use of UV light alone, which mainly causes repairable nucleic acid dimerization, damage induced by the combination of UV light and riboflavin is irreversible due to guanine base modifications. Thereby, DNA and RNA replication is inhibited.

2.3 Principles of Use

Once illuminated, the Mirasol-treated WB is transferred to the attached WB Storage Bag and centrifuged at hard spin to separate RBCs according to site standard practices. Using a commercially available blood processing set, the product is then leukoreduced (LR) and transferred to the final RBC product storage bag which then may be transfused or stored in additive solution-3 (AS-3) within 9 hours of collection. The Mirasol-treated RBCs are stored at 1-6°C and have an expiration date of ≤ 21 days from collection.

Steps to remove riboflavin are not required following treatment with the Mirasol System for WB since the photosensitizer, riboflavin, and its possible photoproducts are present in human blood.

No new chemical compounds are introduced into the body when RBCs derived from Mirasol-treated WB are transfused.

The function of the Mirasol System for WB is entirely extracorporeal. The therapeutic benefit of Mirasol treatment derives from the blood product itself, not from the Mirasol treatment. Riboflavin does not act as a medicinal product in this application. There are no intended pharmacologic functions associated with the device or its components.

For detailed information about the device, refer to the current Mirasol System for WB Investigators Brochure (IB), the Blood Processing and Storage Guidelines associated with this protocol, and the Mirasol System for WB Illuminator Version 6.2 Operator's Manual.

3 INTENDED USE STATEMENT

The Mirasol® System for Whole Blood (WB) is an extracorporeal blood treatment process which uses ultraviolet (UV) light energy and riboflavin (vitamin B_2) to inhibit nucleic acid replication in WB for production of Mirasol-treated RBC.

- Mirasol System treatment is used to inactivate Babesia species in order to prevent transfusion-transmitted Babesiosis

- Mirasol System treatment is used to inactivate T-lymphocytes in order to reduce the risk of transfusion-associated graft-versus-host disease (TA-GvHD). Mirasol treatment is at least as effective as gamma irradiation at inactivating lymphocytes

4 NONCLINICAL STUDIES

The safety and efficacy of the Mirasol System for WB has been characterized extensively. The nonclinical studies include evaluations of toxicity, pathogen reduction, WBC inactivation, and blood product quality. Taken together, these studies support the safety of the Mirasol System for WB for further evaluation in clinical studies.

Mirasol-treated WB and RBCs derived thereof match the functional properties of untreated products, maintain a good quality throughout storage for up to 14 (WB) and 21 (RBCs) days, respectively, and offer the added benefit of pathogen reduction and WBC inactivation.

For detailed information about the nonclinical studies, refer to the current Mirasol System for WB IB.

A summary of in vitro pathogen reduction data is summarized in Table 4-1, below.

Table 4-1: Pathogen Reduction Capability of the Mirasol System for Whole Blood

Virus	**Model for**	**Log reduction factor**
Human Immunodeficiency Virus (HIV)	Enveloped retroviruses	4.5 ± 0.5
Zika Virus (ZIKV)	ssRNA flavivirus	3.0
Bacteria	**Gram stain**	**Low titer reduction: #pos/ #tested**
Yersinia enterocolitica	Negative	1/8
Parasite	**Disease**	**Log reduction factor**
Babesia microti	Babesiosis	≥ 5.0 ± 0.2
Babesia divergens	Babesiosis	7.3 ± 0.7
Trypanosoma cruzi	Chagas' disease	≥ 3.5
Leishmania donovani	Leishmaniasis	2.3 ± 0.12
Plasmodium falciparum	Malaria	≥ 6.4 ± 0.8

5 CLINICAL TRIAL EXPERIENCE

There have been 3 completed clinical trials of the Mirasol System for WB, overviews of the results are provided below.

The first 2 clinical studies evaluated radiolabeled recovery and survival of autologous RBCs derived from Mirasol-treated fresh WB and reinfused in healthy adult subjects.

In the third clinical study, the efficacy of the Mirasol System for WB was evaluated for reducing the transfusion transmission of malaria by transfusion of Mirasol-treated WB. Additional measured endpoints included bacteria reduction, hematological parameters, biochemistry parameters, and coagulation parameters.

For detailed information about clinical trial experience, refer to the current Mirasol System for WB IB.

5.1 The IMPROVE Study

The clinical study titled Inactivation of WB with Mirasol: Performance in Red Blood Cells, Platelets and Plasma Investigation (IMPROVE) was a prospective, single-center, open-label, feasibility study performed at 1 site in the United States and included 12 healthy volunteer subjects. Units of WB were treated with the Mirasol System for WB using 3 different UV light energies (22, 33, and 44 J/mL$_{RBC}$), separated into blood components, and RBCs were stored at 1 – 6ºC for 42 days. The in vitro parameters and in vivo recovery and survival of the RBCs were tested.

Results suggested that 2 key RBC quality parameters, hemolysis and ATP concentration, may be predictive of 24-hour RBC recovery and T_{50} survival. The correlation led to the choice of the current 21 day storage period for RBCs derived from Mirasol-treated WB and 80 J/mL$_{RBC}$ as the energy dose that balances blood quality with pathogen kill. Five subjects reported minor adverse

events (AEs), and none were device related (allergic rhinitis, upper respiratory infection, dyspepsia [n = 2], and bronchitis). No serious adverse events (SAEs) or deaths were reported.

5.2 The IMPROVE II Study

The clinical study, Inactivation of Whole Blood with Mirasol: Performance in Red Blood Cells in Healthy Volunteers (IMPROVE II) utilized a prospective, 2-center, single-blind, randomized, 2-period crossover design to evaluate recovery and survival of autologous RBCs derived from Mirasol-treated WB, stored as LR- RBCs and reinfused into healthy subjects. The primary objective was to determine whether LR-RBCs treated at 80 J/mL$_{RBC}$ and stored at 1 – 6°C for 21 days met FDA criteria of 24-hour RBC recovery. Secondary endpoints included RBC survival, half-life and area under the curve (AUC), neoantigenicity, AEs, in vitro characteristics of test and control RBCs, and correlations between in vitro and in vivo parameters.

Twenty nine (29) healthy adult volunteers were enrolled. Five (5) subjects in the Enrolled Population discontinued prior to or on Day 21 (3 subjects on Day 0 and 2 subjects by Day 21); thus, 24 of the 29 enrolled subjects were re-infused and included in the Evaluable Population.

The results from this study demonstrated that RBCs derived from Mirasol-treated WB maintained an acceptable cell quality and survival as compared to untreated RBCs in accordance with US Food and Drug Administration (FDA) criteria, which includes the following 3 criteria:

- The 1-sided 95% lower confidence limit for the population proportion of success is greater than 70%, where success of a unit is defined as the RBC in vivo 24-hour percentage recovery ≥ 75%;
- The sample mean of percent recovery ≥ 75%; and
- The sample standard deviation (SD) of in vivo 24-hour RBC recovery ≤ 9%.

Subjects reinfused with Mirasol-treated LR-RBCs demonstrated no evidence of neoantigenicity or other major safety signals. There were no device related treatment emergent adverse events (TEAEs), TEAEs resulting in procedure and/or study discontinuation, SAEs, or unanticipated adverse device effects (UADEs). Procedure-related TEAEs were mild in severity and limited to bruising at the puncture/infusion site. One device deficiency was reported but did not affect study treatment or subject data. The device deficiency was related to communication between the illuminator to the Mirasol Manager software. The IIlluminator did not transfer the log files as intended; however, the files remained on the Illuminator and were therefore accessible, with no loss of study data.

The results from this study indicated that RBCs derived from Mirasol-treated WB maintained an acceptable cell quality and recovery as compared to untreated RBCs in accordance with FDA criteria. The geometric least squares (LS) mean of the AUC of RBC survival following Mirasol treatment was approximately 83% of that for untreated control. The mean linear RBC survival

was shorter following Mirasol treatment (60.49 days) compared with the mean RBC survival of untreated control (81.57 days). Subjects reinfused with Mirasol-treated LR-RBCs demonstrated no evidence of neoantigenicity or other major safety concerns. In light of previous nonclinical data supporting the efficacy of Mirasol treatment in reducing pathogens and residual WBCs, this study further supported the use of Mirasol treatment of WB as a means to increase the safety of RBC transfusions.

5.3 The AIMS Study

The clinical study titled African Investigation of Mirasol System for Whole Blood Clinical and Biological Efficacy of Mirasol-treated Fresh Whole Blood for the Prevention of Transfusion transmitted Malaria (AIMS) was a prospective, randomized, double-blind, controlled, single center clinical study designed to evaluate the efficacy of the Mirasol treatment in reducing the incidence of malaria transmission by blood transfusion. The planned primary endpoint was incidence of transfusion transmitted malaria (TTM). Secondary endpoints included measurement of bacterial contamination, hematological parameters, biochemistry parameters, and coagulation parameters.

The primary efficacy endpoint of this study was incidence of TTM. Non-parasitemic subjects receiving parasitemic WB were assessed for TTM by detection of identical *Plasmodium* in subjects post-transfusion and in the transfused WB confirmed by *Plasmodium* allelic discrimination. The presence of *Plasmodium* in WB products and in study subject blood samples were routinely assessed via microscopy and by qPCR, including species identification. While microscopy and PCR were both performed on blood samples, allelic discrimination was used to determine TTM incidence. There were 65 subjects transfused with parasitemic blood who were non-parasitemic pre-transfusion, 49 of whom remained non-parasitemic post-transfusion. Sixteen (16) subjects became parasitemic post-transfusion after receiving parasitemic WB and were evaluated for TTM. There were 28 subjects in the Mirasol-treated group and 37 subjects in the untreated group, making a total of 65 subjects in the Evaluable Population. One transmission was identified in the Mirasol-treated group and 8 transmissions were identified in the untreated group ($P = 0.039$).

There were 145 TEAEs reported in a total of 92 (41.3%) subjects in the safety population. Forty eight (48) subjects (43.8%) in the Mirasol group reported a total of 75 TEAEs and 44 (39.3%) subjects in the untreated group reported 70 TEAEs. The most common TEAEs in all subjects were allergic transfusion reaction (6.3%), malaria (6.3%) and febrile non-hemolytic transfusion reaction (4.0%). The TEAEs of malaria were reported based upon clinical symptoms suggestive for malaria and/or treatment initiated for malaria after at least 1 blood transfusion. There were no reported UADE and no TEAEs that led to study discontinuation. There were 22 serious TEAEs reported during the study. Four (4) serious TEAEs were related to the device and/or procedure. After unblinding, the serious TEAE of malaria in the Mirasol group was unlikely to be related to

the device, due to a negative *Plasmodium* detection test in both units of transfused blood. There was 1 serious TEAE of malaria in the untreated group that was related to the procedure as 1 unit of untreated transfused blood tested positive for *Plasmodium* detection. Thirteen (13) deaths were reported during the conduct of this study with 8 deaths occurring in the Mirasol group and 5 in the untreated group. None of the deaths were related to the study device or procedure.

Treatment of WB using the Mirasol System for WB reduced the incidence of TTM. The primary endpoint of the study was achieved, as treatment of WB using the Mirasol System for WB reduced the incidence of TTM. Additional study information is available in the Mirasol System for WB Investigator's Brochure.

6 RATIONALE FOR THE CURRENT STUDY

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 Hb production.[3] Thalassemia major (homozygous) is the most severe form of the disease, requiring RBC transfusions from an early age. Consequently, thalassemia is the most RBC-transfused syndrome worldwide with as many as 2,000 thalassemia patients estimated to live in the United States. Additionally, there are more than 60,000 births each year of serious forms of thalassemia, globally.[4] The current standard of care for transfusion-dependent thalassemia patients dictates chronic RBC transfusions with the goal of controlling anemia and suppressing ineffective erythropoiesis.[3] There are 85 million units of RBCs transfused annually worldwide with approximately 15 million in the United States. Treatment with chronic RBC transfusions requires that these thalassemia patients are exposed to multiple blood products from multiple donors, thus increasing the risk of TTIs and transfusion-related immune reactions. In Vichinsky 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 WBC inactivation may provide a safer primary treatment for this population.

An available device which reduces the pathogen load and inactivates WBCs in WB will improve treatment for thalassemia patients as well as other patient populations receiving RBC transfusions and ultimately lead to safer available blood products.

This study will provide valuable information about Mirasol-treated blood products in regards to blood safety. A secondary objective of this study is to compare safety endpoints including incidence of alloimmunization, neoantigenicity, TEAEs, transfusion-related AEs, SAEs, and UADEs between groups.

The normalized Hb AUC will be calculated from normalized Hb between successive transfusions as a measure of percent surviving RBCs. The decision to transfuse, generally and specifically in thalassemia, is not based solely on Hb levels but also individual patient characteristics and

symptoms.[37] Defining appropriate measures of RBC transfusion efficacy has been an extremely challenging aspect of transfusion medicine, and there are no accepted standards for evaluation. Previous evaluations of RBC therapy have utilized in vivo RBC recovery and survival as surrogate markers for efficacy. This study will provide valuable information about Hb AUC measurement, which has been demonstrated to be an objective, reliable, and clinically meaningful summary statistic.[38]

7 OBJECTIVES

7.1 Primary Objective

The primary objective of this clinical study is to determine if percent survival of RBCs derived from Mirasol-treated WB is non-inferior to conventional RBCs when transfused into patients requiring chronic RBC transfusion support.

7.2 Secondary Objective

The secondary objectives include comparing other efficacy and safety endpoints between treatment groups.

8 EFFICACY ENDPOINTS

8.1 Primary Endpoint

1. Normalized Hb AUC calculated from normalized Hb between successive transfusions as a measure of percent surviving RBCs.

8.2 Secondary Endpoint

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

9 SAFETY ENDPOINTS

1. Incidence of treatment-emergent antibody with confirmed specificity to RBCs derived from Mirasol-treated WB.
2. Human leukocyte antigen (HLA) alloimmunization rates.
3. Treatment emergent adverse events.
4. Transfusion-related adverse events.
5. Serious adverse events.

6. Unanticipated adverse device effects.

10 INVESTIGATIONAL PLAN

10.1 Study Design

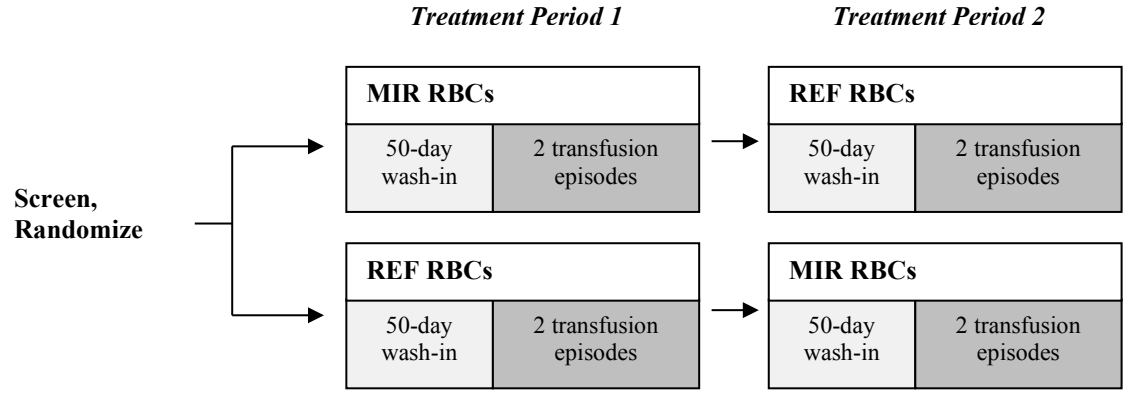
This is a prospective, multi-center, randomized, crossover trial to evaluate the clinical effectiveness of MIR RBCs versus REF RBCs in transfusion dependent thalassemia patients. Throughout the clinical trial, RBC transfusion volume and frequency will be determined by each subject's treating physician.

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 conventional RBCs, or to receive conventional 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.

Blood transfusion is the mainstay of care for individuals with thalassemia major. The purpose of transfusion is twofold: to improve the anemia and to suppress the ineffective erythropoiesis. A transfusion episode for these thalassemia patients are the routine transfusions administered on a regulator schedule for the life of the patient. An end of study treatment follow-up visit will occur 2-4 weeks after the last per protocol transfusion, prior to the next standard of care transfusion. A final study visit will occur at least 60 days after the last per protocol transfusion.

Figure 10-1: Study Schematic



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 in order to provide study product for transfusion.

10.2 Red Blood Cell Products

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 CPD solution, treated with the Mirasol System for WB, LR, and stored in 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.

MIR RBCs will be prepared from WB treated with the Mirasol System for WB according to the Blood Processing and Storage Guidelines and the Mirasol System for WB Illuminator Operator's Manual.

The MIR RBCs must not be irradiated. Irradiating blood products that have been Mirasol-treated is redundant and may further compromise RBC viability. In vitro studies demonstrated Mirasol treatment was as effective as irradiation in preventing leukocyte proliferation and was more effective than gamma irradiation at preventing antigen presentation, cytokine production, and T-cell activation.

Modifications such as RBC washing (MIR RBCs or REF RBCs) or irradiation (REF RBCs only) may be done according to Hospital Site and/or Local Blood Center standard procedures and following established guidelines as to impact to storage durations and transfusion practices. Any modifications will be captured in the electronic case report form (eCRF). Anticipated incidence of washing is 35% for conventional RBCs and 33% for irradiated RBCs.[30]

Mirasol increases extracellular potassium in WB derived RBC products. Use with caution in patients at risk of hyperkalemia (eg, patients with renal failure) and/or requiring administration via central venous access device.

10.3 Off-Protocol Transfusions

Off-protocol transfusions are defined as any RBC transfusions other than those to which the subject was randomized (MIR RBCs or REF RBCs), regardless of the indication or reason for the transfusion. Following the off-protocol transfusion, the subject will continue to receive transfusions according to the allocated treatment arm and will continue study participation.

For each off-protocol transfusion, if possible, all of the same data as for protocol specified transfusions will be captured in the eCRF, including the indication and reason for the off-protocol transfusion and any modifications to the RBC product.

10.4 Study Duration

Subject participation will be approximately 7-9 months, including a screening period (up to 28 days) and 2 treatment periods, each including a 50-day wash-in period followed by 2 transfusion episodes. An end of study treatment follow-up visit will occur 2-4 weeks after the last per protocol transfusion, prior to the next standard of care transfusion. A final study visit will occur at least 60 days after the last per protocol transfusion.

Study duration is approximately 29 months.

11 PATIENT POPULATION

11.1 Number of Subjects and Subject Selection

Assuming a 20% dropout rate, it is anticipated that approximately 97 subjects will be randomized to achieve 77 subjects in the Full Analysis Set (FAS). The FAS will include all randomized subjects who have at least 1 normalized Hb AUC measurement.

11.2 Inclusion Criteria

Subjects will be eligible for inclusion only if all of the following criteria apply:

1. Transfusion dependent thalassemia patient with mean 2-4 week transfusion intervals for the prior 6 months.
2. Age ≥ 12 years.
3. Negative pregnancy test for women of childbearing potential and agreement to practice a medically acceptable contraception regimen throughout the participation in the clinical trial. Not required if female subjects are not of child-bearing potential (ie, prior to menses onset, surgically sterilized, 1-year postmenopausal).
4. Signed informed consent from the patient, or if the patient is < 18 years of age, signed assent from patient and consent from parent/guardian, according to local Institutional Review Board/Ethics Committee (IRB/EC) requirements.

11.3 Exclusion Criteria

Subjects will not be eligible for inclusion if any of the following criteria apply:

1. Historical RBC transfusion requirement of more than 200 mL/kg/year.
2. Presence of RBC antibodies that make procurement of compatible RBC units not feasible per the treating physician's clinical judgment for reasonable execution of the study.
3. Prior treatment with pathogen-reduced RBCs with subsequent development of known antibodies to the associated RBCs.
4. Planned treatment requirement of frozen RBC products.
5. Treatment requirements for any medication that is known to cause hemolysis.
6. Receiving cardiac medications for heart failure.
7. Patients anticipated to receive massive transfusion, per the treating physician's clinical judgment.
8. Known HIV infection (defined as HIV RNA positive) with changes to antiviral regimen within the 12 months prior to screening.
9. Acute or chronic medical disorder that, in the opinion of the Investigator, would impair the ability of the patient to receive study treatment.
10. Participation in another clinical study, either concurrently or within the previous 28 days, in which the study drug or device may influence study endpoints or patient safety, according to Investigator discretion.
11. Participation in another clinical study within the past 3 months if investigational RBCs or treatment or drugs were received that are likely to have long term effect on RBCs function.
12. Pregnant or breastfeeding.
13. Planned concurrent treatment with other pathogen reduction treated blood products during participation in this study.
14. Patients who received prior treatment with pathogen-reduced RBCs within the past 120 days.
15. Inability to comply with study procedures and/or follow-up.

12 ENROLLMENT

12.1 Recruitment Process

This is a multicenter study; each investigational site (Hospital Site) will recruit subjects according to the site's standard procedures. Recruitment material, if applicable, will be reviewed and approved by the site's IRB/EC prior to implementation.

12.2 Informed Consent/Assent Process

Prior to subject participation in this study, the Investigator must obtain written IRB/EC approval for the Clinical Investigation Plan (CIP), the ICF/parental consent form and assent form, where applicable. Children under the age of 18 will sign an assent form and their parent or legal guardian will sign the ICF or a parental consent form, as specified by the IRB/EC. The approved ICF/parental consent form/assent form will clearly reflect the IRB/EC approval date.

A copy of the site-specific subject ICF/parental consent form/assent form must be provided to the Sponsor for review and approval prior to submission to the IRB/EC for approval. Once approved, the subject ICF/parental consent form/assent form must be provided to the Sponsor prior to implementation in this study. All study subjects/legal guardians must provide written informed consent/parental consent/assent using the sponsor and IRB/EC approved ICF/parental consent form/assent form, where applicable.

Once the subject's initial eligibility has been determined, the Investigator or designee, who has been trained on the CIP, will explain in lay terms the nature and scope of the study, potential risks and benefits of participation, answer questions for the subject and, if applicable, the subject's legal guardian and obtain informed consent/parental consent/assent if the subject is interested in participating in the study. Potential subjects/legal guardians will be given as much time and privacy as necessary to review the ICF, to ask any questions and to provide an opportunity to discuss potential participation with others outside the study team. If the subject/legal guardian agrees to participate, the ICF/parental consent form/assent form must be read by the subject/legal guardian, and signed and dated by the subject/legal guardian and the person completing the consent process. The ICF/parental consent form/assent form, where applicable will be obtained prior to initiating any study specific procedure that is not considered standard of care. The original ICF/parental consent form/assent form will be kept in the subject's record, and a copy will be provided to the subject. The original ICF/parental consent form/assent form will remain in the study files. A note about the subject's participation will be written in the subject's medical record. Study subjects will be assured that they may withdraw from the study at any time and for any reason.

Failure to obtain a signed ICF/parental consent form/assent form prior to enrollment constitutes noncompliance with the Declaration of Helsinki, International Conference of Harmonization (ICH) Good Clinical Practice (GCP), US Code of Federal Regulations (21 CFR 812), and International Organization for Standardization (ISO) 14155.

12.3 Enrollment

Subjects will be considered enrolled upon giving informed consent. Screen failures will be subjects who do not continue to meet enrollment criteria or withdrawal consent or discontinue study participation for any reason prior to randomization.

Once a subject/parent/legal guardian has signed the ICF/parental consent form/assent form, where applicable, a designated site staff member will enter the subject information into the Electronic Data Capture (EDC) system which will automatically assign a site number and subject specific identification (ID) number.

The investigational site will maintain a screening/enrollment log.

12.4 Randomization/Stratification

Subjects will be randomized as close to the initiation of the first transfusion as possible (study day 0), not to exceed 10 days prior to the planned initiation of transfusions. The 10-day window is designed to allow sufficient time for the procurement and shipment of appropriate RBC product from Central or Local Blood Centers. Subjects will be randomized using a permuted-block schedule stratified by investigational site in a 1:1 allocation to 1 of 2 sequence groups. (Section 10.1).

Randomization will be performed centrally using web-based system such as an interactive web response system (IWRS). When a patient has met eligibility criteria and provided informed consent/parental consent/assent to participate, a designated site staff member will log into the system and enter the required subject information to obtain the randomization ID number and treatment group assignment. The system access will be controlled by site and designated personnel for data entry.

13 BLOOD CENTER PROCEDURES

Blood products to be used in the study, MIR RBCs and REF RBCs, are defined in Section 10.2.

The Sponsor of the study is responsible for conducting the medical device implementation, training Blood Center personnel, and monitoring at the Blood Centers during the course of the study.

Test products (MIR RBCs) are collected, tested, stored, and transfused like the REF RBC products but they undergo the following additional steps: WB will be collected in a 450 mL bag with CPD; WB will be Mirasol-treated within 6 hours of collection, transferred to the storage bag, centrifuged, LR, and the derived RBCs transferred to a final storage bag with AS-3 within 9 hours of collection; and they will have an expiration date of ≤ 21 days.

Notably for MIR RBCs, after adding riboflavin solution and until after RBC separation, the WB product should be protected from exposure to direct sunlight or prolonged exposure (1 hour or more) to ambient light. For detailed information about appropriate ambient light protection measures throughout the Mirasol system treatment process, see the “Ambient Light Protection” tables located in each processing section of the Blood Processing and Storage Guidelines. The process of producing RBCs will remove most of the remaining riboflavin solution from the

product with the removal of plasma, so the RBC product is much less sensitive to light exposure, but should be kept in a darkened cooler during long term storage.

The MIR RBC product must not be irradiated.

Refer to the Blood Processing and Storage Guideline associated with this document for detailed instructions pertaining to MIR RBC production. Mirasol-treatment and storage will be per the Blood Processing and Storage Guideline associated with this protocol and the Mirasol System for WB Illuminator Version 6.2 Operator's Manual provided to the Blood Centers.

Reference RBCs will be collected, tested, and stored per site standard operating procedures.

All RBC products must meet standard release criteria for transfusion.

Mirasol-treated products are not to be administered in the event massive transfusion is warranted.

13.1 Mirasol RBCs

Donated WB for the production of MIR RBCs will be collected at designated Blood Centers according to the standard processes for donor consent and will be collected into a 450 mL bag with CPD anticoagulant. Mirasol RBC product will be manufactured according to the Blood Processing and Storage Guidelines and the Mirasol System for WB Illuminator Operator's Manual. Refer to the Blood Process and Storage Guidelines for cautions and warnings, kit overview and product specifications, labeling, procedural steps, and storage requirements.

In addition to medical device implementation, training, and monitoring during the study, the Sponsor will review Mirasol Manager content and the RBC product information during the course of the study to ensure appropriate use of the medical device equipment.

13.2 Reference RBCs

The Local Blood Bank at each Hospital Site will be responsible for acquiring REF RBC product from their routine use inventory.

13.3 Data Collection and Procedures for RBC Units

Data will be recorded in source documents and entered into eCRFs for each MIR RBC, REF RBC, and off-protocol unit unless otherwise noted. Table 13-1 summarizes these procedures.

1. RBC product details:
    a. Blood Unit Identification (BUI) number
    b. Donation type (WB or apheresis, REF RBCs only)
    c. Donation collection date

d. ABO and Rh type of unit

e. Unit expiry date

f. Washed RBCs (yes/no)

g. Irradiated RBCs (yes/no; date) (REF RBC only)

2. Mirasol treatment details (MIR RBCs only)

a. Mirasol Illuminator serial number

b. Mirasol Whole Blood disposable set lot number

c. Donation collection start time

d. Mirasol Illumination start date/time

e. Date/time MIR RBCs placed in refrigerated storage

3. Product disposition (MIR RBCs only)

Table 13-1: Blood Center Procedures and Data Collection

	MIR RBCs	REF RBCs
Blood Unit Identification (BUI) number	X	X
Donation type (WB or apheresis) [a]		X
Donation collection date	X	X[b]
ABO and Rh type of unit	X	X
Unit Expiry date	X	X
Washed RBCs (yes/no)	X	X
Irradiated RBCs (yes/no; date)		X
Mirasol Illuminator serial number	X	
Mirasol Whole Blood disposable set lot number	X	
Donation collection start time	X	
Mirasol Illumination start date/time	X	
Date/time MIR RBCs placed in refrigerated storage	X	
Product disposition	X	

[a] MIR RBCs derived from WB only. REF RBC may be derived from WB or via apheresis.

[b] REF RBC: Record collection date if available.

14 STUDY PROCEDURES

14.1 Screening

Subject information may not be captured or study procedures conducted until informed consent/parental consent/assent has been obtained. The informed consent/parental consent/assent process may take place prior to the 28-day screening window.

The following screening procedures will be conducted within 28 days prior to the anticipated initiation of the first RBC transfusion (study day 0), unless otherwise specified below. Retrospective review of data such as demographics and prior medical history may be used to determine potential subjects for study participation and to obtain screening data if it is available from standard of care procedures conducted prior to the screening visit.

The following procedures will be completed during screening:

1. Review and sign the ICF/parental consent form/assent form (may take place prior to the 28-day screening window)
2. Review eligibility for study participation
3. Record demographic information (date of birth, sex, race, and ethnicity)
4. Record subject's height and weight
5. Perform a full physical examination, including examination of general appearance, skin, eyes/ears/nose/throat, head/neck, pulmonary, chest, cardiovascular, abdominal and liver/spleen, lymphatic, musculoskeletal, and neurological
6. Record vital signs. Vital signs include heart rate (HR), blood pressure (BP), respiratory rate (RR), arterial oxygen saturation via pulse oximetry (SpO_2), and temperature
7. Collect medical and surgical history
    a. Including thalassemia diagnosis information, genotype (if available), phenotype, history of antibody formation (list of antibodies) and date of diagnosis
    b. Including transfusion history: date of initiation of transfusion therapy for thalassemia, history of transfusion reactions in the previous 6 months (type, date), mean transfusion interval for the prior 6 months
    c. Including splenectomy status, immunizations, infection exposures in the 3 months prior to the screening visit, current organ dysfunction
8. Collect blood for Local Laboratory Tests (Section 16.2.1)
    a. Serum or urine pregnancy test; not required if female subjects are not of child-bearing potential (ie, prior to menses onset, surgically sterilized, 1-year postmenopausal)
9. Identify and document the target post-transfusion (15 min) Hb level

14.2 Randomization (Day -10 to 0)

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

14.3 With Each RBC Transfusion Episode

The following procedures will be completed before, during, and after each study transfusion unless otherwise noted. These procedures will be completed for all transfusions occurring during the 50 -day wash-in period plus the subsequent 2 transfusion episodes (for endpoint assessment) during each of the 2 crossover treatment periods. The date and time of transfusion, number of units, and type of product transfused will be documented in the eCRF. For off-protocol RBCs transfusion, the following characteristics will also be collected: transfused volume, type of RBC unit (per ISBT code), and hematocrit (if known).

Visits on 1 Day Post Transfusion and 7 Days Post Transfusion may be conducted in the clinic or may utilize a remote nursing visit.

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[39]

        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[39,40]

14.3.1 Prior to Each Transfusion Episode

Blood Product Procedures

1. Collect specimen from each unit prior to the transfusion (Section 16.1)
    a. Local: Complete Blood Count (CBC)
    b. Central: supernatant potassium, supernatant sodium, and supernatant hemoglobin

Subject Procedures

1. Measure weight
2. Conduct physical examination (only prior to the first transfusion of Treatment Period 2)

a. Perform an abbreviated physical examination, including examination of general appearance, eyes/ears/nose/throat, pulmonary/chest, cardiovascular, abdominal, and neurological

3. Record vital signs within 1 hour prior to transfusion. Vital signs include HR, BP, RR, SpO_2, and temperature

4. Local Laboratory Tests (may be collected/tested at any time within 5 days before the transfusion unless otherwise noted)

   a. Prior to Treatment Period 2 only: Serum or urine pregnancy test; not required if female subjects are not of child-bearing potential (ie, prior to menses onset, surgically sterilized, 1-year postmenopausal)

   b. Routine blood typing and crossmatch per site standard practice

   c. RBC antibody screen

      - If positive, conduct RBC antibody identification and record only new RBC antibodies, if applicable

   d. Crossmatch with RBCs derived from Mirasol-treated WB if the previous study transfusion was a MIR RBC product

   e. CBC, plasma free hemoglobin; and haptoglobin within 1 hour prior to transfusion (Section 16.2.1)

   f. Chemistry within 1 hour prior to transfusion (Section 16.2.1)

5. Collect blood from subject for Central Laboratory Tests (Section 16.2.2)

   a. Collect and hold blood sample for RBC antibody testing, when the antibody screen and crossmatch performed at the local lab either is positive for an antibody specific to MIR-treated RBCs or inconclusive. See Section 16.2.2.1 for details regarding criteria for sending samples to the central laboratory

   b. Collect blood sample for HLA testing, only during Treatment Period 1 (Section 16.2.2.2)

6. Record all concomitant medications taken starting from the day of the first study transfusion and throughout the study, including all blood products transfused (eg, off-protocol RBCs, platelets) (Section 17)

7. Record AEs, SAEs, and UADEs (Section 19). All AEs will be recorded from the time of the first study transfusion through the final follow-up or early termination visit

14.3.2 During and Within 15 Minutes Post-End Transfusion

1. Begin RBC transfusion per site standard procedures

   Note: As per the AABB Technical Manual, the initial flow rate for RBC transfusions should be 1-2 mL/min (60-120 mL/hr) for the first 15 minutes, with the total infusion duration ≤ 4 hours[41]

2. Record start/end date/time(s) of transfusion(s)
3. Record volume of RBCs transfused
4. Record vital signs (HR, BP, RR, SpO_2, temperature) during transfusion at 15 (± 5) minutes after starting transfusion and hourly, at end of transfusion, and 15 (± 5) minutes after end of transfusion. A ± 15 minute window is allowed unless otherwise specified
5. Collect blood for Local Laboratory Tests at 15 (+ 5) minutes post-end transfusion
    a. CBC, plasma free hemoglobin and haptoglobin (Section 16.2.1)
    b. Serum potassium and sodium concentrations
    6. Record concomitant medications and all blood products transfused (eg, off-protocol RBCs, platelets) (Section 17)
7. Record AEs, SAEs, and UADEs (Section 19)

14.3.3 1 Day (24 ± 6 hours) Post-Transfusion

1. Record vital signs (HR, BP, RR, SpO_2, temperature)
2. Collect blood for Local Laboratory Tests
    a. CBC, plasma free hemoglobin and haptoglobin (Section 16.2.1)
    b. Chemistry (Section 16.2.1)
3. Record concomitant medications and all blood products transfused (eg, off-protocol RBCs, platelets) (Section 17)
4. Record AEs, SAEs, and UADEs (Section 19)

14.3.4 7 Days (± 1 day) Post-Transfusion

1. Collect blood for Local Laboratory Tests
    a. CBC and haptoglobin (Section 16.2.1)
    b. Chemistry (Section 16.2.1)
    c. RBC antibody screen
        - If positive, conduct RBC antibody identification and record only new RBC antibodies, if applicable
    d. Crossmatch with Mirasol-treated RBCs if the previous study transfusion was a MIR RBC product
2. Collect blood for Central Laboratory Tests (Section 16.2.2)
    a. Collect and hold blood sample for central RBC antibody testing, when the antibody screen and crossmatch performed at the local lab either is positive for an antibody

specific to MIR-treated RBCs or inconclusive. See Section 16.2.2.1 for details regarding criteria for sending samples to the central laboratory

3. Record concomitant medications and all blood products transfused (eg, off-protocol RBC, platelets) (Section 17)
4. Record AEs, SAEs, and UADEs (Section 19)

14.4 End of Study Treatment Follow-up/Early Termination

All enrolled subjects who received at least 1 post-randomization RBC transfusion will complete a follow-up visit approximately 2-4 weeks after the last per protocol transfusion episode, prior to the next transfusion performed as standard of care.

In the event that a subject withdraws consent for study participation, an early termination visit should be scheduled as close as possible to the time when consent was withdrawn.

The following procedures will be completed for the follow-up visit and/or the early termination visit:

1. Conduct physical examination
    a. Perform an abbreviated physical examination, including examination of general appearance, eyes/ears/nose/throat, pulmonary/chest, cardiovascular, abdominal, and neurological
2. Collect blood for Local Laboratory Tests (Section 16.2.1)
    a. CBC, plasma free hemoglobin (only if subject is terminating within 24 hours after previous study transfusion) and haptoglobin
    b. Chemistry
    c. RBC antibody screen
        - If positive, conduct RBC antibody identification and record only new RBC antibodies, if applicable
    d. Crossmatch with Mirasol-treated RBCs if the previous study transfusion was a MIR RBC product
3. Collect blood for Central Laboratory Tests (Section 16.2.2)
    a. Collect and hold blood sample for central RBC antibody testing, if applicable. See Section 16.2.2.1 for details regarding criteria for sending samples to the central laboratory
    b. Collect blood sample for HLA testing, only if subject terminated from the study during Treatment Period 1 (Section 16.2.2.2)
4. Record concomitant medications and all blood products transfused (eg, off-protocol RBCs, platelets) (Section 17)

5. Record AEs, SAEs, and UADEs (Section 19)

14.5 Final Study Follow-up

All enrolled subjects will complete a final study follow-up visit, which can be concurrent with routine standard of care, at least 60 days after the last per protocol study transfusion episode.

1. Collect blood for Local Laboratory Tests (Section 16.2.1)
    a. RBC antibody screen
    b. If positive, conduct RBC antibody identification and record only new RBC antibodies, if applicable
2. Collect blood for Central Laboratory Tests (Section 16.2.2)
    a. Collect and hold blood sample for central RBC antibody testing, if applicable. See Section 16.2.2.1 for details regarding criteria for sending samples to the central laboratory
3. Record AEs, SAEs, and UADEs (Section 19)

Table 14-1: Schedule of Procedures for Subjects

	Screening	**With Each RBC Transfusion**				**End of Study Treatment Follow-up/ Early Termination**	**Final Study Follow-up**
	(Days -28 to 0)	Prior to Transfusion	During and Within 15 min Post-Transfusion	1 Day (24 ± 6 hrs) Post-Transfusion[p]	7 Days (± 1 day) Post-Transfusion[p]	2-4 weeks after last transfusion	≥ 60 days after last transfusion
ICF/assent,[a] and Eligibility	X						
Demographics	X						
Medical/surgical history[b]	X						
Height and weight	X	X[c]					
Randomization	X[d]						
Identify target post-transfusion (15 min) Hb	X						
Record RBC details[e]			X				
Begin transfusion			X[f]				
Calculate the volume of RBCs required to achieve the target Hb		X					
Physical examination[g]	X	X				X	
Vital signs	X	X[h]	X[h]	X			
Routine blood typing and crossmatch, per site standard practice		X					
CBC, plasma free hemoglobin and haptoglobin[i]		X	X	X	X	X	
Chemistry		X	Serum K and Na only[j]	X	X	X	
Serum/urine pregnancy test[k]	X	X[k]					
RBC antibody screen and identification[l]		X			X	X	X
Crossmatch with Mirasol-treated RBCs[m]		X			X	X	

	Screening	**With Each RBC Transfusion**				**End of Study Treatment Follow-up/ Early Termination**	**Final Study Follow-up**
	(Days -28 to 0)	Prior to Transfusion	During and Within 15 min Post-Transfusion	1 Day (24 ± 6 hrs) Post-Transfusion[p]	7 Days (± 1 day) Post-Transfusion[p]	2-4 weeks after last transfusion	≥ 60 days after last transfusion
Central RBC antibody screen[n]		X			X	X	X
Central HLA antibody screen[o]		X				X	
Central supernatant K, Na and hemoglobin from RBC product		X					
Record concomitant medications		X	X	X	X	X	
Record all blood products, RBC details		X	X	X	X	X	
Record AEs, SAEs, and UADEs		X	X	X	X	X	X

Abbreviations: AE = adverse event, BP = blood pressure, CBC = complete blood count, HR = heart rate, ICF = informed consent form, K = potassium, Na = sodium, RBC = red blood cell, RR = respiratory rate; SAE = serious adverse event, SpO_2 = arterial oxygen saturation via pulse oximetry, UADE = unanticipated adverse device effect.

[a] The ICF/parental consent/assent process may take place prior to the 28-day screening window.

[b] Medical/surgical history should include thalassemia diagnosis information (ie, genotype [if available], phenotype, history of antibody formation [list of antibodies] and date of diagnosis); transfusion history (date of transfusion initiation, history of transfusion reactions in the previous 6 months [type, date], mean transfusion interval for the prior 6 months); and splenectomy status, immunizations, infection exposures in the 3 months prior to the screening visit, current organ dysfunction.

[c] Height will be measured once at the screening visit. Weight will be measured prior to each transfusion.

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

[e] Document RBC product details for each unit transfused per Section 13.3.

[f] As per the AABB Technical Manual, the initial flow rate for RBC transfusions should be 1-2 mL/min (60-120 mL/hr) for the first 15 minutes, with the total infusion duration ≤ 4 hours.[41]

[g] A full physical exam will be conducted at the screening visit and will include examination of general appearance, skin, eyes/ears/nose/throat, head/neck, pulmonary, chest, cardiovascular, abdominal and liver/spleen, lymphatic, musculoskeletal, and neurological. Abbreviated physical exams will be conducted prior to the first transfusion of Treatment Period 2 and at the Final Follow-up Visit, and will include examination of general appearance, eyes/ears/nose/throat, pulmonary/chest, cardiovascular, abdominal, and neurological.

[h] Vital sign measurements will include HR, BP, RR, SpO_2, and temperature. On the days of transfusion, vital sign measurements will be obtained within 1 hour prior to transfusion, during transfusion at 15 (± 5) minutes and hourly, at end of transfusion, and 15 (± 5) minutes after end of transfusion. All vital sign measurements should be collected within ± 15 minutes of the nominal time point unless otherwise specified.

[i] A blood sample for the CBC, plasma free hemoglobin, and haptoglobin tests will be collected from the subject within 1 hour prior to transfusion, within 15 (± 5) minutes post-transfusion, and on Day 1 after each transfusion. In addition, on Day 7 (± 1 day) after each transfusion, a blood sample for CBC and haptoglobin tests will be collected.

[j] Serum potassium and sodium from the subject within 15 (+ 5) minutes post-end transfusion.

[k] Serum or urine pregnancy test to be conducted only at the screening visit and prior to the first transfusion of Treatment Period 2. Not required if female subjects are not of child-bearing potential (ie, prior to menses onset, surgically sterilized, 1-year postmenopausal).

[l] Record history of all RBC antibodies detected prior to first study transfusion; record only new antibodies thereafter, if applicable.

[m] Test with Mirasol-treated RBCs if the previous study transfusion was a MIR RBC product. A segment from the prior MIR RBC unit should be used to conduct this test.

[n] Collect and hold blood sample for central RBC antibody testing when the antibody screen and crossmatch performed at the local lab either is positive for an antibody specific to MIR-treated RBCs or inconclusive. Samples will be sent to the central laboratory when the crossmatch to MIR RBCs and antibody identification at the Local Laboratory confirms the presence (or suspected presence) of an antibody specific to Mirasol-treated RBCs, or when the results are inconclusive (eg, positive crossmatch but unidentifiable antibody).

[o] Samples for HLA antibody testing will be collected from all subjects prior to each transfusion in Period 1 only and sent to a central laboratory. The samples will be collected at the Early Termination visit if subject terminated early from the study during Period 1.

[p] Visits on 1 Day Post Transfusion and 7 Days Post Transfusion may be conducted in the clinic or may utilize a remote nursing visit.

15 STUDY AND/OR TREATMENT DISCONTINUATION

All subjects are free to withdraw from study participation at any time and for any reason, specified or unspecified, and without prejudice. Similarly, an Investigator may discontinue any subject from study participation for any reason. The reason for the study discontinuation will be recorded in the eCRF.

15.1 Early Discontinuation/Study Termination

Reasons for a subject's withdraw from study participation or termination of the study by the Sponsor may include, but are not limited to the following:

1. Study completion as per protocol
2. Development of an AE that interferes with the subject's continued participation
3. Subject refuses further treatment and/or follow-up and withdraws consent
4. Investigator decision
5. Sponsor decision
6. Subject is lost to follow-up
7. Subject death
8. Development of an antibody with confirmed specificity to Mirasol-treated RBCs

16 LABORATORY TESTS

16.1 RBC Product Laboratory Testing

The following tests will be performed on the RBC products (MIR RBCs and REF RBCs) prior to transfusion for in vitro and in vivo correlation.

16.1.1 Local Laboratory

CBC: WBC count, RBC count, Hb concentration, and hematocrit.

16.1.2 Central Laboratory

Testing will be conducted for supernatant potassium, supernatant sodium, and supernatant hemoglobin (for calculation of percent hemolysis). Samples will be processed, stored, and shipped according to the instructions specified in the Manual of Procedures (MOP).

The percent hemolysis will be derived from the supernatant hemoglobin and CBC results, using the following formula:

% Hemolysis = [(plasma free Hb) *(1 – Hct)/total Hb] * 100

16.2 Clinical (Subjects) Laboratory Testing

16.2.1 Local Laboratory Tests

The following laboratory tests on subject blood samples will be performed locally. Copies of the current laboratory certifications and normal ranges will be provided to the Sponsor or designee prior to initiation of the study and upon every renewal throughout study conduct.

Pregnancy testing: Serum or urine pregnancy at Screening and prior to the first transfusion of Treatment Period 2. Not required if female subjects are not of child-bearing potential (ie, prior to menses onset, surgically sterilized, 1-year postmenopausal).

CBC: WBC count, RBC count, Hb concentration, hematocrit, and platelet count.

Haptoglobin: At the same time points when blood samples are collected for CBC analysis, samples will also be analyzed for haptoglobin concentration.

Plasma free hemoglobin: Prior to each transfusion, at 15 (± 5) min post-transfusion, and on Day 1 after each transfusion.

Chemistry: bilirubin, lactate dehydrogenase (LDH), alkaline phosphatase, aspartate aminotransferase (AST), alanine aminotransferase (ALT), potassium, sodium, blood urea nitrogen (BUN), creatinine, and ferritin.

Routine blood typing and crossmatch: Prior to each transfusion according to site standard procedures.

RBC antibody screen and identification: Local laboratories will conduct antibody screen prior to transfusions, on Day 7 (± 1 day) after each transfusion, at the End of Study Treatment Follow-up Visit, and at the Final Study Follow-up Visit according to site standard procedures. If the screen is positive, local laboratories will conduct RBC antibody identification.

If the identification is positive for MIR RBCs (or suspected positive) or is inconclusive, a sample will be sent to the central laboratory for further testing.

Crossmatch to MIR RBCs: This will occur at Day 7 (± 1 day), prior to the next transfusion, and at the End of Study Treatment Follow-up Visit whenever the previous study transfusion was a MIR RBC product per the randomized treatment allocation. A segment from the prior MIR RBC unit should be used to conduct this test.

16.2.2 Central Laboratory Tests

Refer to the MOP for additional information and detailed instructions.

16.2.2.1 RBC Antibody Testing

Samples will be collected from subjects prior to each study transfusion, at 7 days (± 1 day) after each transfusion, at the End of Study Treatment Follow-up Visit, and at the Final Study Follow-up Visit, and held pending antibody screen and identification results from the Local Laboratory. Samples that meet the requirements for shipment to the designated Central Laboratory as noted below will be processed, stored, and shipped according to the instructions specified in the MOP. Samples that do not meet these requirements will be destroyed after study completion according to the Hospital Site's standard procedures.

Requirements for sample shipment: This will be conducted when the crossmatch to MIR RBCs and antibody identification at the Local Laboratory confirms the presence (or suspected presence) of an antibody specific to MIR RBCs, or when the results are inconclusive (eg, positive crossmatch but unidentifiable antibody).

16.2.2.2 HLA Antibody Testing

A central laboratory will conduct HLA antibody testing. Samples will be collected from all subjects prior to each transfusion in Period 1 only, since 1 full treatment period will be adequate to assess chronic transfusions and will incorporate both study treatments (MIR RBCs and REF RBCs). Samples will be processed, stored, and shipped according to the instructions specified in the MOP.

17 CONCOMITANT MEDICATIONS/BLOOD PRODUCTS

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 eCRF.

All study related RBC transfusions will be recorded as per Section 14.3.

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.

No other pathogen reduction treated blood products including platelets and/or plasma may be used for transfusion in subjects until the end of study treatment follow-up visits.

18 INVESTIGATIONAL DEVICE

18.1 Device Deficiencies

All device deficiencies involving any device component must be reported to the Sponsor within 24 hours upon knowledge of the event. The Device Deficiency Report Form (located in the study MOP) will be used for reporting. Every attempt should be made by the investigational site to save or collect the defective device, and if appropriate, the packaging, for return to the Sponsor. If the deficiency occurs with the Illuminator, a service technician from the Sponsor will evaluate and determine whether service or replacement is necessary. A qualified Sponsor representative will investigate and determine root cause and corrective actions as applicable, and directives will be provided to the site if warranted.

Examples of device deficiencies warranting reporting to the Sponsor include the following:

- Any situation where the device or accessory to the device physically breaks for any reason.
- Any situation where a component of the device or the disposables kit fails to perform as it is specified in the study CIP or Blood Processing and Storage Guidelines.
- Potential manufacturing or shipping failures where device contamination, potential for device contamination, or a break in sterility or sterile barrier upon opening the device packaging is identified.

18.2 Receipt, Storage, Accountability

The investigational devices and accessories will be received, stored, and accounted for at the Blood Centers.

18.2.1 Receipt of Study Device

The contents should be examined upon receipt to ensure that packaging and labeling is intact and the devices have not been damaged. Potential damages must be reported to the Sponsor.

18.2.2 Storage

The devices should be stored in a cool, dry place. Devices should be with their original shipping materials. Proper care should be taken to ensure that the devices will not be damaged.

18.2.3 Accountability

The Investigator or designated site staff must maintain accountability records throughout the course of the study. The Clinical Research Associate (CRA) will review inventory and accountability of disposables and study supplies during monitoring visits.

The Investigator will not supply medical device materials to other Investigators who are not participating in the study. Device use, other than as directed by this CIP, is not allowed without prior authorization from the Sponsor.

Used materials are to be handled as per the study site's standard procedures. At the conclusion of the study, unused materials will be returned to the Sponsor/designee.

19 ADVERSE EVENTS/EFFECTS

19.1 Potential Transfusion Risks

Blood component transfusion is a common and usually safe procedure. However, even with advanced donor screening and other safety mitigation strategies employed, adverse transfusion reactions may still occur. It has been estimated that 5%-6% of all transfusion recipients experience adverse reactions from transfusion of blood or blood products.[42] Transfusion-related adverse reactions may include nausea, chills, fever, urticaria, hives, itching, bronchospasm, hemoglobinuria, dyspnea, hypoxia, hypotension, tachycardia, transfusion-associated circulatory overload, bacterial and/or viral contamination, allergic transfusion reaction, anaphylaxis, acute lung injury, hemolytic transfusion reaction, nonimmunologic hemolysis, alloimmunization, iron overload, and sepsis. Transfusion-related adverse reactions may be classified by temporal relationship to the transfusion (ie, immediate, delayed) or by relationship to immunological factors.[42,43]

Transfusion-related adverse reactions are summarized in Table 19-1. Notably, these adverse reactions are general blood transfusion risks and are not specific to Mirasol System treatment.

19.1.1 Venipuncture-Related Risks

With each venipuncture performed to collect a blood sample there may be some extravasation of blood into the tissues during the venipuncture causing a hematoma. Other common, mild side effects are: stinging or pain during insertion of the needle, upset stomach or vomiting, dizziness, thirst, sweating, rapid pulse, or fainting.

If the skin is not adequately cleaned before the venipuncture there may be a risk of infection at the venipuncture site. Other serious side effects may include: hives, bronchospasm, allergic reactions, muscle spasms or cramps, convulsions, or vessel damage (including dissection or puncture).

19.1.2 Risks Pertaining Specifically to RBC Transfusions

Red blood cell transfusions present similar risks as for blood or blood component transfusions, namely the risk of infectious or immunological phenomena. However, several adverse reactions

are specifically associated with RBC transfusions (Table 19 1).[37] Notably, these adverse reactions are general RBC transfusion risks and are not specific to Mirasol System treatment.

The potential risks of RBC transfusions described below apply generally to RBC transfusions and may not specifically apply to thalassemia patients. Knowledge of the potential risks described below should be combined with the Investigators' best clinical judgment for assessing safety throughout this clinical study.

Table 19-1: Transfusion-Related Adverse Reaction

Adverse Reactions	Description
General Adverse Reactions for Transfusion	
Acute hemolytic transfusion reaction	• Destruction of RBCs, characterized by increased temperature, pulse rate, and BP instability. • Incompatibility may be represented by hemoglobinuria, hypotension, DIC. • Hemoglobinemia, hemoglobinuria, elevated serum bilirubin, positive DAT
Delayed hemolytic transfusion reaction	• Occurs 2-14 days post-transfusion in previously red-cell-alloimmunized patients. • Fever, positive DAT, decreased hemoglobin/hematocrit, elevated LDH or bilirubin
Febrile nonhemolytic reaction	• Manifested as a temperature elevation ≥ 1ºC or 2ºF, occurring during or immediately after transfusion.
Allergic reaction	• Mild or self-limiting uticaria or wheezing (1%-3% of plasma containing components). • May include respiratory or cardiovascular complications and resemble an anaphylactic reaction.
Transfusion-associated circulatory overload	• Results from a transfusion rate greater than the recipient's cardiac output. • Transfused product adds circulating volume without increasing oxygen-carrying capacity. • Characterized by acute respiratory distress, elevated BNP, elevated CVP, leaf heart failure, positive fluid balance, or pulmonary edema.
Transfusion-related acute lung injury	• Acute onset of hypoxemia and noncardiogenic pulmonary edema within 6 hours of transfusion in the absence of other causes of acute lung injury or circulatory overload.
Post-transfusion purpura	• Destruction of PLTs (thrombocytopenia) in a patient with history of sensitization, occurring 7-10 days after transfusion.
Transfusion-associated graft-versus-host disease	• Occurs when viable T lymphocytes in the transfused component react against recipient tissue antigens. • Immunocompromised patients are at greatest risk.
Transfusion-transmitted infection	• Characterized by laboratory testing evidence of pathogen (ie, viruses, bacteria, parasites, variant Creutzfeldt-Jakob disease agent) in the transfusion recipient. • Occurs despite donor screening and blood testing.
Hypotensive transfusion reactions	• Hypotension occurs during or within 1 hour of transfusion. • Characterized in adults by SBP ≤ 80 mmHg and SBP decrease ≥ 30 mmHg. • Characterized in children by SBP decrease > 25% from baseline.
Transfusion-associated dyspnea	• Acute respiratory distress within 24 hours of transfusion, not attributable to allergic reaction, TACO, or TRALI.

Adverse Reactions	Description
Adverse Reactions Specific to Red Blood Cell Transfusion	
Hemolytic transfusion reaction	• Immunologic destruction of transfused RBCs, resulting from preformed antibodies to incompatible transfusion product (1:76,000) or ABO incompatibility (1:40,000).
Alloimmunization	• Characterized by clinically significant recipient antibodies to transfused RBC antigens. • May be detected in pretransfusion antibody screening.
Transfusion-associated circulatory overload	• Chronic anemia patients with increased plasma volumes are at risk of circulatory overload with RBC transfusions.
Iron overload	• Characterized by significant iron accumulation following multiple RBC transfusion. • Greater risk for patients with decreased RBC production or increased RBC destruction; less risk for patients with hemorrhage.
Nonimmunologic hemolysis	• RBC lysis resulting from hypotonic fluid exposure, drug effects, bacterial toxins, thermal injury (ie, freezing, overheating), metabolic damage, or mechanical injury/osmotic stress

Source: AABB. Primer of Blood Administration. 2010; AABB, ARC, ABC, ASBP ; and CDC NHSN Hemovigilance Module (Aug 2014)

Abbreviations: BNP = brain natriuretic peptide, BP = blood pressure, CVP = central venous pressure, DAT = direct antiglobulin testing, DIC = disseminated intravascular coagulopathy, LDH = lactate dehydrogenase, PLT = platelet, RBC = red blood cell, SBP = systolic blood pressure, TACO = transfusion-associated circulatory overload, TRALI = transfusion-related acute lung injury.

19.2 Potential Transfusion Complications in Thalassemia Patients

Thalassemia is the most RBC-transfused syndrome worldwide with as many as 2,000 thalassemia patients estimated to live in the United States. Additionally, there are more than 60,000 births each year of serious forms of thalassemia, globally.[4] The current standard of care for transfusion-dependent thalassemia patients dictates chronic RBC transfusions with the goal of controlling anemia and suppressing ineffective erythropoiesis.[3] Treatment with chronic RBC transfusions requires that these thalassemia patients are exposed to multiple blood products from multiple donors, increasing the risk of TTIs by unscreened or undetected pathogens in the blood supply and transfusion-related immune reactions. In Vichinsky et al., 80% of the thalassemia patients in this study were chronically transfused at the time or enrollment and 24% of transfused thalassemia patients had laboratory evidence of previous exposure to one or more transfusion-associated pathogens.[4] In this chronically transfused group, patients received an average of 15.6 transfusions during the year prior to enrollment.

Table 19-2: Reported Adverse Reactions in Thalassemia Patients

Adverse Reaction	Description
General Complications	
Hemosiderosis	• Median ferritin was 1,376 µg/L, 356 µg/L, and 81 µg/L for chronic, intermittent, and nontransfused patients, respectively ($p < 0.0001$) • 78% of patients had received chelation therapy
Organ dysfunction	• 16.5% had more than 1 organ system involved • Included cardiac disease (13%), gonadal failure (17%), growth hormone deficiency (8%), hypothyroidism (8%), hypoparathyroidism (1%), diabetes (10%), cirrhosis (2%), and thrombotic events (7%)
Thrombotic events	• Included 6 incidence of pulmonary embolism, 5 superior vena cava syndromes, 4 deep vein thromboses, 3 cardiac thromboses, 2 portal vein thromboses, and 2 renal thrombosis • 26 chronic transfusion patients had thrombosis – 25 of the 26 had a splenectomy – 21 of the 26 had concomitant central venous access • 31 patients were receiving chronic anticoagulant therapy
Surgical procedures	• Splenectomy occurred in chronically (52%), intermittently (29%), and nontransfused (7%) patients ($p < 0.0001$) • Central venous access (27%) and cholecystectomy (14%) were reported
Infections	
Transfusion transmitted infection	• 24% of transfused patients had laboratory evidence of previous infectious disease exposure • Included HAV, HBV, HCV, HIV, malaria, *S. aureus*, babesia
Transfusion Complications	
Transfusion reactions	• Self reported by 48% of transfused patients • More likely in chronically transfused (50%) than intermittently transfused patients (22%; $p = 0.002$) • More frequent in males (55%) than females (42%) • Not related to race or splenectomy status • 27% had multiple, different transfusion reactions
Types of reactions	• 52% reported allergic reaction – 2 anaphylactic reactions – 1 transfusion-associated hypotensive event – 1 transfusion-associated dyspnea • 16% reported febrile reactions • 2.2% reported confirmed hemolytic immunologic reactions • Tachycardia, acute vertigo, and transient vomiting were reported • There were no incidents of TRALI
Alloantibodies	

Adverse Reaction	Description
Alloimmunized	• Chronically (23%) and intermittently transfused (13%) patients were both alloimmunized • Multiple antibodies were present in 47% of alloimmunized patients • 31% of splenectomy patients were alloimmunized whereas 11% of nonsplenectomized patients were ($p < 0.0001$) • In chronically transfused patients, years of transfusion was a significant independent predictor of alloimmunization

Source: Vichinsky. Transfusion complications in thalassemia patients, a report from CDC. Transfusion, 2014
Abbreviations: HAV = hepatitis A virus, HBV = hepatitis B virus, HCV = hepatitis C virus, HIV = human immunodeficiency virus, TRALI = transfusion-related acute lung injury.

19.3 Potential Risk of the Mirasol System for Whole Blood

The risk of using the Mirasol System incorrectly is minimized by an inherently safe design. The disposable kit comes prefilled with the correct dose of riboflavin and sterility is maintained throughout the process. The Illuminator has built-in safety features, including visual and auditory alarms that ensure correct illumination. The Mirasol Manager Software prevents the risk of double illumination. Labeling prevents the risk of illumination by the Mirasol System and further irradiation.

Mirasol System treatment of blood or blood components has been evaluated for hemocompatibility, cytotoxicity, genotoxicity, immunotoxicity, and embryo-fetal development both in vitro and in vivo in several animal species. These nonclinical studies have shown no observable or Mirasol-related changes to the toxicity profile. Terumo BCT has extensively evaluated the toxicological profile of riboflavin, its photoproduct (lumichrome), and blood products treated by the Mirasol System in several in vivo and in vitro studies.[44] Three (3) potential risks have been identified that are related to blood quality: putative toxic effects of riboflavin and its photoproduct, potential for transient hyperkalemia due to increased extracellular potassium in RBCs derived from Mirasol-treated WB, and the reduced survival which may impact the need for more frequent RBC transfusions.

Mirasol-treated products are not to be administered in the event massive transfusion is warranted.

19.3.1 Risk of Toxic Effects of Riboflavin and its Photoproducts

Riboflavin and its photoproduct are present in the human diet and, as a result, are also present in human blood. Terumo BCT has extensively evaluated the toxicological profile of riboflavin, its photoproduct, and blood products treated by the Mirasol System in several in vivo and in vitro studies.[44] There is an abundance of published toxicology data for riboflavin, administered orally, periorally, sub-cutaneously, intraperitoneally, or intravenously in several species. Acute, sub-chronic and chronic toxicity tests as well as tests for development, reproduction, mutagenicity, and pharmacokinetics have not displayed any health concerns despite exposure at high

concentrations and/or long duration. This suggests that the slight and transient increase of riboflavin and lumichrome in human blood following transfusion with RBCs derived from Mirasol-treated WB is unlikely to cause any health concerns due to the presence of riboflavin specifically. As riboflavin and its photoproduct are naturally occurring in human blood as well, albeit at a lower concentration, no new compounds are introduced and there is no need to remove riboflavin from blood components after Mirasol treatment. Additionally, the process of producing RBCs will remove most of the remaining riboflavin with the removal of plasma from the product.

19.3.2 Risk of Hyperkalemia

Hyperkalemia, high serum potassium, is a recognized risk of RBC transfusions. Many factors may contribute to hyperkalemia, including patient factors (acidosis, hypothermia, hypocalcemia, hyperglycemia, beta-adrenergic blockade, shock/low cardiac output state, low body weight [pediatric]), mode of blood administration (large volume transfusions, rapid administration, central intravenous access administration, pressure infusing devices), and high potassium load in the blood product (due to, eg, excessive hemolysis, long storage time, irradiation).[45]

Mirasol treatment increases extracellular potassium in derived RBC products; the potassium concentration after 21 days of storage (the proposed limit) is similar to what has been observed in gamma-irradiated products after 28 days of storage[46,47] and in conventional RBCs stored for 42 days (the out date).[48] Mirasol treated WB and derived RBCs should be used with caution in patients at risk of hyperkalemia (eg, patients with renal failure) and/or requiring administration via central venous access device.

Hyperkalemia is generally transient because of rapid dilution, redistribution into cells and excretion.[41] However, patients with renal failure, premature infants, and newborns receiving large transfusions may be at increased risk for hyperkalemia.[41] Notably, hypokalemia is a more frequent complication of massive RBC transfusion than is hyperkalemia. Transfusion-associated hypokalemia occurs as a result of rapid potassium reuptake in potassium-depleted RBCs; release of aldosterone, antidiuretic hormone and catecholamines; co-infusion of potassium-poor crystalloids; and lowering of potassium due to the metabolism of citrate present in anticoagulants or additive solutions.[49]

19.3.3 Risk of Increased Transfusion Frequency

The recovery and survival in vivo studies have demonstrated that compared to conventional untreated RBCs, RBCs derived from Mirasol-treated WB have reduced recovery, although still within acceptable therapeutic ranges. Specifically, the mean recovery of Mirasol-treated RBCs was 82.5% compared with 91.7% for untreated RBCs. The RBC area under the curve (RBC AUC), a measure of RBC survival, was 16.7% lower in transfused Mirasol-treated RBCs. Because of these differences, patients receiving RBCs derived from Mirasol-treated WB may require more frequent RBC transfusions and/or more units of RBCs per transfusion as compared to conventional RBCs. Transfusion frequency will be evaluated in this study to determine if there

is an increase for subjects receiving RBCs derived from Mirasol-treated WB when compared to conventional RBCs.

19.3.4 Data for the Investigator

The following tables provide in vitro data for Investigators who will utilize RBCs derived from Mirasol-treated WB.

The data below allow Investigators to compare in vitro parameters and in vivo survival of RBCs prepared from Mirasol-treated WB and gamma-irradiated RBCs prepared from WB as reported in 3 separate studies.

Table 19-3: In Vitro Parameters and In Vivo Survival of Stored RBCs Derived from Mirasol-Treated Whole Blood

	Day 0		Day 21	
	Untreated mean (SD)	**Mirasol-treated** mean (SD)	**Untreated** mean (SD)	**Mirasol-treated** mean (SD)
Hemolysis (%)	0.091 (0.035)	0.103 (0.034)	0.150 (0.061)	0.215 (0.100)
Supernatant potassium (mEq/L)	1.78 (0.465)	2.17 (0.497)	36.73 (3.749)	65.50 (2.497)
Glucose (mg/dL)	559.5 (45.9)	560.3 (17.6)	398.6 (33.8)	408.5 (30.9)
ATP (µmol/g Hb)	4.54 (0.428)	4.66 (0.737)	5.40 (1.011)	4.42 (1.131)
2,3-DPG (µmol/mL)	12.49 (3.75)	12.29 (2.14)	0.33 (0.41)	0.25 (0.26)
pH at 37°C	6.83 (0.041)	6.81 (0.031)	6.57 (0.048)	6.55 (0.048)
Survival of 21 Day old RBC After Reinfusion				
	Untreated		**Mirasol-treated**	
Linear Survival (days)	81.57 (15.5)		60.49 (5.6)	
Half-life (days) Linear equation	35.83 (7.9)		22.58 (4.3)	

Source: Clinical Investigation Report for the IMPROVE II clinical study; Terumo BCT data.

Abbreviations: AS-3 = Additive Solution Formula 3, ATP = adenosine triphosphate, CPD = citrate phosphate dextrose, DPG = diphosphoglycerate, NA = not available, RBC = packed red blood cells, WB = whole blood.

Notes: In these studies, WB was collected into CPD and treated with the Mirasol System (80 J/mL_{RBC}). Packed RBCs were prepared from Mirasol-treated WB and were stored in AS-3 solution at 1 - 6°C for 21 days.

Table 19-4: In Vitro Parameters and In Vivo Survival of Gamma-Irradiated Stored RBCs Derived from Whole Blood

	Day 28			
	N	**Untreated** mean (SD)	**Gamma-irradiated** mean (SD)	**P value**
Hemolysis (%)	16	0.3 (0.1)	0.6 (0.4)	< 0.01
Supernatant potassium (mEq/L)	16	44.9 (5.3)	72.3 (6.4)	< 0.01
Glucose (mg/dL)	16	NA	NA	> 0.05
ATP (µmol/g Hb)	16	3.3 (0.9)	2.9 (0.7)	NS
pH at 37°C	16	NA	NA	> 0.05
RBC Survival After Reinfusion				
Half-life (days) Dornhorst equation	16	28.0 (3.5)	26.0 (2.5)	NS

Source: Moroff G, Holme S, AuBuchon JP, Heaton WA, Sweeney JD, Friedman LI. Viability and in vitro properties of AS-1 red cells after gamma irradiation. *Transfusion*. 1999;39:128-134.[50]

Abbreviations: AS-1 = Additive Solution Formula 1, ATP = adenosine triphosphate, CPD = citrate phosphate dextrose, NA = not available, NS = not significant, RBCs = packed red blood cells, WB = whole blood.

Notes: This table provides data from blood storage Protocol 1 described in the manuscript. In Protocol 1, WB was collected into CPD, and RBCs were prepared and stored in AS-1 solution at 1 - 6°C. In Protocol 1, the units of RBCs in AS-1 were gamma-irradiated (2,500 cGy) on Day 1 and stored to Day 28. No Day 1 data were presented in the manuscript, and no Day 28 data were provided for glucose and pH (other than statistical comparison data).

Table 19-5: WB/RBC Parameters of RBCs Derived from Mirasol-Treated WB

	Control Collection Mean ± SD (range)	**Mirasol Collection** Mean ± SD (range)	**Control Day 0** Mean ± SD (range)	**Mirasol Post-Trt Day 0** Mean ± SD (range)	**Control 21 Days** Mean ± SD (range)	**Mirasol 21 Days** Mean ± SD (range)
Hct (%)	35.53 ± 3.33 (28.70 – 40.90)	35.60 ± 3.17 (29.60 - 41.70)	57.12 ± 2.86 (48.90 - 61.70)	56.94 ± 2.60 (50.90- 61.40)	57.74 ± 2.66 (51.40- 62.90)	56.66 ± 2.90* (50.70 - 63.00)
pH_{22C}	7.25 ± 0.03 (7.18 – 7.30)	7.25 ± 0.02 (7.19 – 7.31)	7.00 ± 0.04 (6.91 – 7.06)	7.00 ± 0.03 (6.91 – 7.09)	6.71 ± 0.04 (6.61 – 6.83)	6.70 ± 0.04* (6.55 – 7.80)
Hemolysis (%)	0.17 ± 0.06 (0.12 – 0.57)	0.17 ± 0.04 (0.12 – 0.38)	0.07 ± 0.01 (0.05 – 0.09)	0.08 ± 0.05 (0.06 – 0.44)	0.21 ± 0.20 (0.06 – 1.25)	0.35 ± 0.18* (0.07 – 0.96)
Plasma Hb (g/dL)	0.03 ± 0.01 (0.03 – 0.08)	0.03 ± 0.01 (0.03 – 0.08)	0.03 ± 0.00 (0.03 – 0.03)	0.03 ± 0.02 (0.03 – 0.20)	0.10 ± 0.09 (0.03 – 0.58)	0.15 ± 0.08* (0.03 – 0.41)
Hb Conc (g/dL)	12.06 ± 1.23 (9.60 – 14.50)	12.06 ± 1.26 (9.30 – 14.30)	19.07 ± 1.15 (16.60 – 21.6 0)	19.07 ± 1.17 (16.30 – 21.20)	19.35 ± 1.08 (17.20 – 21.70)	19.16 ± 1.19 (16.50 – 21.10)
Total Hb (g)	64.08 ± 6.32 (51.08 – 76.06)	63.92 ± 6.60* (49.24 – 75.65)	56.32 ± 5.86 (45.87 – 66.6 9)	54.00 ± 5.69 (42.43 – 65.83)	52.37 ± 5.16 (42.27 – 61.45)	50.69 ± 5.71 (39.12 – 62.70)
MetHb (%)	0.77 ± 0.46 (0.10 – 1.80)	0.64 ± 0.43* (0.10 – 1.70)	0.29 ± 0.16 (0.00 – 1.10)	2.22 ± 1.68* (0.40 – 6.90)	0.28 ± 0.07 (0.00 – 0.30)	0.30 ± 0.02* (0.20 – 0.30)
WBC ($x10^6$/mL)	4.90 ± 1.36 (2.90 – 8.30)	4.86 ± 1.21 (3.00 – 9.10)	0.03 ± 0.05 (0.00 – 0.10)	0.01 ± 0.03* (0.00 – 0.10)	0.06 ± 0.05 (0.00 – 0.10)	0.02 ± 0.05* (0.00 – 0.20)
RBC Recovery (%)	100	100	89 ± 4 (76 – 105)	85 ± 2* (81 – 94)	82 ± 4 (70 – 96)	79 ± 2* (75 – 87)
MCV (fL)	87.04 ± 6.79 (71.30 – 101.3 0)	88.18 ± 6.67* (69.70 – 101.6 0)	87.64 ± 6.75 (71.70 – 101. 10)	88.72 ± 6.66 (70.20 – 102.30)	88.40 ± 6.70 (73.30 – 103.6 0)	88.38 ± 6.94 (69.60 – 104.5 0)
Platelets, $x10^3/\mu L$	196.66 ± 44.34 (111.00 – 331. 00)	191.36 ± 41.07 (119.00 – 315. 00)	3.11 ± 7.93 (0.00 – 60.00)	0.90 ± 1.47* (0.00 – 10.00)	3.62 ± 7.01 (0.00 – 44.00)	1.85 ± 2.80 (0.00 – 16.00)
Potassium (K+) (mmol/L)	3.24 ± 0.21 (2.70 – 3.64)	3.15 ± 0.19* (2.77 – 3.60)	2.08 ± 0.37 (1.27 – 3.03)	2.23 ± 0.34* (1.53 – 3.06)	37.35 ± 4.61 (28.65 – 49.04)	68.42 ± 4.12* (56.20 – 77.31)
Sodium (Na+) (mmol/L)	155.22 ± 2.05 (150.00 – 160. 30)	154.90 ± 1.92 (151.20 – 160. 20)	142.87 ± 1.68 (139.50 – 14 8.60)	142.39 ± 1.41 (140.00 – 146.9 0)	117.57 ± 3.22 (108.90 – 123. 70)	88.24 ± 2.94* (82.30 – 96.50)
2,3-DPG (µmol/gHb)	12.22 ± 4.12 (3.90 – 31.03)	13.85 ± 4.38* (6.56 – 24.00)	12.03 ± 3.09 (0.00 – 18.39)	12.95 ± 3.23* (7.18 – 21.94)	NA	NA
2,3-DPG (µmol/gHb) Regenerated[a]	NA	NA	NA	NA	13.79 ± 4.57 (0.00 – 20.90)	14.99 ± 4.56 (3.13 – 24.04)
ATP in RBC (µmol/gHb)	4.31 ± 0.83 (1.51 – 5.95)	4.34 ± 0.52 (2.99 – 5.61)	4.50 ± 0.84 (1.59 – 6.22)	4.62 ± 0.59 (3.32 – 6.05)	5.50 ± 0.80 (3.46 – 7.29)	4.90 ± 0.87* (2.10 – 6.89)
pCO_2 (mmHg)	85.62 ± 7.98 (68.00 – 104.3 0)	84.22 ± 7.45 (69.20 – 105.4 0)	78.32 ± 6.55 (61.60 – 93.4 0)	68.65 ± 5.65* (58.10 – 84.60)	122.99 ± 12.53 (100.50 – 148. 90)	122.75 ± 10.72 (100.70 – 144. 50)

pO_2 (mmHg)	26.90 ± 5.43 (18.90 – 45.80)	29.18 ± 5.37* (21.70 – 43.30)	33.50 ± 6.03 (23.50 – 56.80)	32.78 ± 5.76 (22.80 – 47.70)	41.06 ± 5.91 (31.00 – 59.60)	36.46 ± 4.64* (27.90 – 47.70)
HCO_3^- (mmol/L)	14.96 ± 1.09 (12.80 – 18.20)	15.07 ± 0.96 (13.10 – 17.00)	7.79 ± 0.92 (5.70 – 9.40)	7.40 ± 0.87* (5.40 – 9.10)	6.11 ± 0.87 (3.90 – 7.60)	5.79 ± 0.92* (2.90 – 7.30)
Glucose (mmol/L)	18.95 ± 1.77 (15.20 – 24.10)	19.43 ± 1.34 (16.90 – 25.50)	27.80 ± 2.34 (23.60 – 31.60)	27.58 ± 1.59 (23.70 – 30.20)	19.91 ± 1.74 (16.40 – 25.30)	19.14 ± 1.87* (12.70 – 23.40)
Lactate (mmol/L)	2.80 ± 0.79 (1.38 – 5.27)	2.46 ± 0.66* (1.32 – 5.01)	2.11 ± 0.46 (1.03 – 3.51)	2.00 ± 0.44 (1.27 – 3.51)	15.28 ± 2.11 (11.14 – 20.18)	15.53 ± 2.52 (10.53 – 20.75)

Source: In Vitro Evaluation of RBCs from WB treated with the Mirasol System (DRP-0084); Terumo BCT data.

Hct = hematocrit; Hb = hemoglobin; MetHb = methemoglobin; WBC = white blood cell; RBC = red blood cell; MCV = mean corpuscular volume; fL = femtoliters; 2,3-DPG = 2,3-bisphosphoglyceric acid; ATP = adenosine triphosphate; pCO_2 = partial pressure of carbon dioxide; pO_2 = partial pressure of oxygen; mmHg = millimeter of mercury; HCO_3 = bicarbonate; Trt = treatment.

Values reported are mean ± 1 SD for n = 61 paired units.

* Significantly different from paired control, $P < 0.05$, Student's t-test.

[a] Regenerated value, with Rejuvesol.

Risks associated with the Mirasol System treatment and storage of RBCs derived from WB include putative toxic effects of riboflavin and its photoproduct, potential for transient hyperkalemia due to increased extracellular potassium in RBCs derived from Mirasol-treated WB, and the reduced survival which may impact the need for more frequent RBC transfusions. Details of these risks are described above in Sections 19.3.1, 19.3.2, and 19.3.3. For additional data for Investigators, refer to the Investigator's Brochure.

19.4 Risk-Benefit Assessment

The clinical study will be conducted in accordance with ICH, FDA 21 CRF 812, and ISO 14155 guidelines for clinical study conduct to minimize risk of clinical trial participation to study subjects.

The study population is at risk because the Mirasol System is an investigational medical device used to treat WB. To minimize risk due to medical device function, blood center staff will be trained in proper use of the Mirasol System, and the blood center staff will be given written instructions for operating the Mirasol System. The Sponsor will provide maintenance and repair for each Mirasol System throughout study conduct.

Mirasol treatment inhibits nucleic acid replication in WB and RBCs derived thereof, thus decreasing the likelihood of TTIs and of AEs such as TA-GvHD associated with residual WBCs. Cell quality characteristics of RBCs derived from Mirasol-treated WB have been investigated by in vivo and in vitro studies and in clinical RBC survival studies. Values for potassium were higher in treated units on Day 21, similar to that observed with gamma-irradiated RBCs or in untreated RBCs at the end of storage. High potassium in blood products is one potential risk factor in developing transfusion-associated hyperkalemia in susceptible patients. The recovery

and survival in vivo studies have demonstrated that compared to conventional untreated RBCs, RBCs derived from Mirasol-treated WB have reduced recovery, although still within acceptable therapeutic ranges. The RBC area under the curve (RBC AUC), a measure of RBC survival, was lower in transfused Mirasol-treated RBCs. These data suggest the possibility that the study population may be at risk of requiring slightly more frequent RBC transfusions compared to those receiving conventional RBC transfusions. To mitigate these risks, clinical laboratory tests for subject safety will be conducted between transfusions; RBC transfusion volume and frequency will be determined by each subject's treating physician. Red blood cells derived from Mirasol-treated WB meet criteria for transfusion, are of good quality throughout the 21-day storage period, and have benefits due to pathogen reduction and WBC inactivation demonstrated both in vitro and in vivo. Currently, there are no approved technologies available in the United States to reduce the risks posed by transfusion of pathogens and residual WBCs in RBC preparations from WB.

Device-related risks are mitigated by design elements which prevent user errors and cell quality is maintained in Mirasol-treated blood products. The thalassemia population is specifically at higher risk for TTIs and immune-related reaction due to chronic RBC transfusions, thus they will derive great benefit from safer blood products.

The Mirasol System offers a means to make transfusions significantly safer by providing pathogen reduction that targets both unscreened and undetected pathogens. Overall, the substantial clinical benefit of using the Mirasol System for WB, as compared to the risk of no pathogen reduction and no WBC inactivation, outweighs the low risks associated with the use of the system.

19.5 Adverse Event Recording/Reporting

19.5.1 Adverse Event/Effect Definitions

An AE is defined as any untoward medical occurrence in a patient or clinical investigation subject, temporally associated with the use of a medical device, whether or not considered related to the medical device and/or procedure. Therefore, an AE can be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of the medical device and/or procedure.

Abnormal laboratory results must be recorded as AEs if any of the following conditions are met:

- The abnormal laboratory value leads to a therapeutic intervention.
- The abnormal laboratory value is considered to be clinically significant by the investigator.
- The abnormal laboratory value is considered to be related to the medical device and/or procedure by the investigator.

Examples of an AE **do not include**:

- Expected outcomes of the procedure.
- Transient yellow discoloration of urine (known side effect of riboflavin) following infusion of RBCs derived from Mirasol-treated WB.
- A medical or surgical procedure (eg, endoscopy), although the condition that leads to the procedure may be an AE.
- Anticipated day-to-day fluctuations of pre-existing conditions present or detected at the start of the study that do not worsen.

In the current study, changes to the frequency of transfusion or dose of RBCs are considered outcomes rather than AEs. However, the Investigator's best clinical judgment should be used to identify changes of frequency and dose that exceed changes described in literature or typical clinical practice.

19.6 Reporting of Adverse Events

Timely and complete reporting of AEs is essential. Monitoring and documentation of AEs allows for identification of treatment-related trends and for adherence to regulatory requirements.

19.6.1 Recording of Adverse Events

All AEs will be recorded from the time of the first study transfusion through the final follow-up visit or early termination.

19.6.2 Grading of Adverse Events

All AEs will be graded according to the Common Terminology Criteria for Adverse Events (CTCAE) Scale (version 4.03; http://ctep.cancer.gov/reporting/ctc.html). The CTCAE includes a grading (severity) scale for each AE term. Grades were developed using the following guidelines:

Grade 0 – No adverse event or within normal limits

Grade 1 – Mild

Grade 2 – Moderate

Grade 3 – Severe

Grade 4 – Life threatening or disabling

Grade 5 – Fatal

19.6.3 Transfusion-Related Adverse Events

Any untoward events or constellation of events that may represent a transfusion reaction can be identified and named using the Adverse Reaction Case Classification Criteria Table presented in the Centers for Disease Control and Prevention's National Healthcare Safety Network Biovigilance Component Hemovigilance Module Surveillance Protocol (version 2.1.3, dated August 2014; http://www.cdc.gov/nhsn/PDFs/Biovigilance/BV-HV-protocol-current.pdf) (Appendix 1).

Knowledge of potential transfusion risks, guidance on pre-specified transfusion reaction definitions, and the Investigators' best clinical judgment should be combined for assessing safety and naming AEs throughout this clinical study.

All confirmed, named transfusion reactions should be recorded on the AE pages of the CRF.

19.7 Follow-up of Adverse Events

All AEs must be followed at a minimum through the final follow-up visit and until return to baseline, resolution or until the Investigator deems the event to be chronic, the patient stable, or the patient is lost to follow-up. All AEs must be followed in accordance with the ICH-GCP guidelines, and other applicable regulatory requirements (eg, 21 CFR 812 and/or ISO 14155-2011).

19.8 Relationship

The Investigator at each site will determine and document the relationship between the AE and study treatment and/or procedure. The following criteria, in addition to good clinical judgment, should be used as a guide for determining relationship:

Not Related: The event is clearly related to factors other than the study treatment and/or procedure(s), such as the patient's clinical state.

Possibly Related: The event follows a reasonable temporal sequence from the time of study treatment administration/procedure, and/or follows a known response pattern to study treatment/procedure(s) but could have been produced by other factors, such as the patient's clinical state or other therapeutic interventions.

Probably Related: The event follows a reasonable temporal sequence from the time of study treatment/procedure(s) and cannot be reasonably explained by other factors, such as the patient's clinical state or therapeutic interventions.

Definitely Related: The event follows a reasonable temporal sequence from the time of study treatment/procedure(s), and follows a known response pattern, and cannot be reasonably

explained by other factors. In addition, the event occurs immediately following study treatment/procedure(s), improves on stopping the study treatment/procedure, and/or reappears on resumption of study treatment/procedure(s).

19.9 Serious Adverse Event/Unanticipated Adverse Device Effect

In the interest of patient care and to allow the Sponsor to fulfill regulatory requirements, any SAE and/or UADE, regardless of causal relationship to study treatment or procedure(s), must be reported to the Sponsor within 24 hours of knowledge of the event.

19.9.1 Definitions

SAEs are defined (21 CFR 812 and ISO 14155:2011) as those AEs meeting any of the following criteria:

1. Results in death
2. Led to serious deterioration in the health of the patient, that either resulted in
    - a life-threatening illness or injury, or
    - a permanent impairment of a body structure or a body function, or
    - in-patient or prolonged hospitalization, or
    - medical or surgical intervention to prevent life-threatening illness or injury or permanent impairment to a body structure or a body function.
3. Led to fetal distress, fetal death or a congenital abnormality or birth defect

NOTE: Planned hospitalization for a pre-existing condition or a planned procedure, without serious deterioration in health, is not considered a SAE.

UADEs are defined (21 CFR 812.3) as any serious adverse effect on health or safety or any life-threatening problem or death caused by, or associated with a device, if that effect, problem or death was not previously identified in nature, severity, or degree of incidence in the CIP or IB, or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of patients.

NOTE: Anticipated adverse device effects are effects, which by nature, incidence, severity or outcome have been identified in the CIP and/or the IB.

19.9.2 SAE/UADE Reporting

Any SAE/UADE that occurs from the first study transfusion through the final follow-up visit, regardless of relationship to the study treatment or procedures, must be reported within 24 hours of knowledge of the event, and the Sponsor/designee may request additional information from

the Investigator to ensure the timely completion of accurate safety reports. Reporting of the event will occur by submitting a SAE/UADE Form to:

Terumo BCT

Email: ClinicalAffairs@TerumoBCT.com

Fax: (303) 876-9146

Additionally, the SAE/UADE must be entered on the AE pages of the CRF. Follow-up reports relating to the subject's subsequent medical course after the SAE/UADE must be submitted to the Sponsor/designee and the reviewing IRB/EC, according to the IRB/EC reporting requirements, until either the event has subsided/resolved or until the subject's condition stabilizes and the likely overall clinical outcome has been ascertained (eg, in the case of permanent impairment).

The Sponsor will be responsible for the following:

- Providing all SAE/UADE reports to the independent Data Monitoring Committee (DMC) members upon receipt
- Promptly reporting unanticipated problems involving risk to volunteers or others, SAEs and UADEs related to participation in the study and all volunteer deaths related to participation in the study to the United States Army Medical Research and Materiel Command (USAMRMC)

  Office of Research Protections

  Human Research Protections Office

  (ORP HRPO)

  Phone: +1 301-619-2165

  Fax: +1 301-619-7803

  Email: hsrrb@det.amedd.army.mil
- Follow-up reporting (ie, a complete written report) to the USAMRMC

  United States Army Medical Research and Materiel Command

  ATTN: MCMR-ZB-PH

  504 Scott Street

  Fort Detrick, Maryland 21702-5012
- Reporting SAE/UADEs to the regulatory authorities in accordance with applicable reporting regulatory guidelines
- Monitoring the safety of the study on an ongoing basis

The investigational site will be responsible for reporting all SAE/UADEs to the following:

- IRB/EC, as required by local policy

19.9.3 Exclusions to SAE/UADE Reporting Requirements

The following are not considered SAEs/UADEs:

- Planned hospitalization.
- Anticipated day-to-day fluctuations of pre-existing conditions present or detected at the start of the study that do not worsen.

19.10 Reproductive Risks

There are no known risks during pregnancy or breastfeeding associated with Mirasol-treated blood products. However, women who are pregnant or breastfeeding will be excluded from study participation. Appropriate methods of birth control are required during study participation and include hormonal methods (such as birth control pills or vaginal rings), intrauterine devices (IUDs), double barrier methods (ie, condoms plus spermicide, or diaphragm plus spermicide), or total abstinence. Female subjects who are not of child-bearing potential will not be required to utilize a contraception regimen. Non-childbearing potential describes females who are prior to menses onset, are postmenopausal (continuous amenorrhea for ≥12 months) or surgically sterile (bilateral oophorectomy, bilateral tubal ligation, or hysterectomy.)

19.10.1 Pregnancy Notification

Pregnancies involving a study subject and occurring during participation in the study must be immediately reported to the Sponsor/designee. Pregnancies must also be reported to the IRB/EC if required per institution policy. The study sites will notify the sponsor if there are any pregnancy or fetal complications related to the study device or investigational product.

19.11 Clinical Investigation Plan Deviations

Deviations from the CIP will be documented and verified during monitoring. Protocol deviations must be reported to the IRB/EC according to local policies and procedures.

19.12 Medical Monitoring

It is the responsibility of the Investigators to oversee the safety of the study at his/her site. In conjunction with the DMC, the Sponsor/designee will review patient data, SAEs, UADEs, and device deficiency reports to oversee the safety throughout the study.

19.12.1 Research Monitor

Throughout the clinical trial, patient care for the thalassemia population will be a collaboration between each subject's treating physician/hematologist, the transfusion site (overseeing transfused blood products), and the blood center (manufacturing, process, and stores the blood

products).A designated DMC member whose expertise is consonant with the nature of risk(s) will fulfill the role of the independent Research Monitor as required for studies funded by the United States Department of Defense.

The Research Monitor should be a qualified physician, other than the Principal Investigator, or a qualified health care provider other than a physician, who is not associated with the study. The independent Research Monitor may discuss the research protocol with the investigators, interview human subjects, consult with others outside of the study about the research; shall have the authority to stop a research protocol in progress, remove individual human subjects from a research protocol, and take whatever steps are necessary to protect the safety and well-being of human subjects until the IRB can assess the monitor's report; shall have the responsibility to promptly report their observations and findings to the IRB or other designated official and the human research protections office (HRPO).

Responsibilities will also include the following:

SAEs/UADEs: The Research Monitor will review all unanticipated problems involving risks to subjects or others, SAEs and UADEs in a timely fashion and will provide an independent causality assessment of reported SAEs or UADEs. The Research Monitor is required to review all UADEs involving risk to patients or others, SAEs, and all patient deaths associated with the CIP and provide an unbiased written report of the event. At a minimum, the Research Monitor must comment on the outcomes of the event or problem and in case of an SAE or death, comment on the relationship to participation in the study. The Research Monitor must also indicate whether he/she concurs with the details of the report provided by the Investigator. Reports for events determined by either the Investigator or Research Monitor to be possibly or definitely related to participation and reports of events resulting in death must be promptly forwarded to the ORP HRPO.

Follow-up Reports: Follow-up reports relating to the subject's subsequent medical course after a UADE or SAE must be submitted to the sponsor and the reviewing IRB/EC until the event has subsided or resolved, in the case of permanent impairment, until the subject's condition stabilizes and the likely overall clinical outcome has been ascertained.

20 INDEPENDENT DATA MONITORING COMMITTEE

This study will be monitored using an independent DMC operating under a ratified charter. Additional administrative and functional details will be provided in the DMC charter.

The DMC will review SAE/UADE reports on an ongoing basis. Additionally, the DMC will review safety reports summarizing the frequency and severity of TEAEs, SAEs, and UADEs and other relevant safety information throughout conduct of the study as defined in the DMC charter. Enrollment will not be placed on hold during the DMC review process. The DMC will determine

if the observed AEs are acceptable for this patient population and will make recommendations to the Sponsor on study continuation.

21 STATISTICAL PLAN

The following section summarizes the statistical methods that will be used in the analysis of the clinical data from this active-controlled trial. A statistical analysis plan (SAP) will be written and approved prior to the database lock.

21.1 Analysis Sets

21.1.1 Full Analysis Set

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.

21.1.2 Safety Set

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 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.

21.1.3 Per Protocol Set

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.

21.2 Reporting Conventions and Definitions

Duration of events will be calculated in days as (stop date – start date + 1). Conversions to weeks, months, or years will be calculated as days/7, days/30.4375, or days/365.25, respectively.

For analysis purposes, baseline will be defined as the day of the first transfusion within each treatment period. In general, missing values will be handled as follows. For continuous variables at baseline, missing values will be excluded from calculation of summary statistics and the number and percent of patients with missing values will be displayed. For categorical values at baseline, the number and percent of patients with missing values will be displayed.

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 ≤ 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.

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

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.

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.

21.3 Patient Disposition

Enrollment and extent of participation in the study will be summarized by sequence (MIR RBCs/REF RBCs or REF RBCs/MIR RBCs) 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 (prior to complete follow-up for the primary end point), and reason, will be presented.

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

21.4 Deviations from the Clinical Investigation Plan

Deviations from the CIP will be documented and verified during monitoring.

21.4.1 Major Deviations from the Clinical Investigation Plan

Patients who are randomized into the study but who do not meet all major inclusion or exclusion criteria will be considered as having major CIP deviations. The major inclusion/exclusion criteria will be defined in the SAP.

21.5 Patient Demographics and Baseline Characteristics

All demographic and baseline characteristics will be presented in by-patient listings by sequence using the FAS and the SS. Summary tables will be presented by sequence for each analysis set.

Demographic and baseline patient characteristics that are only measured or assessed prior to randomization will be summarized using descriptive statistics. No formal comparisons between treatments will be performed.

Medical history and baseline signs and symptoms will be presented in data listings and summarized by sequence for the FAS and the SS.

21.6 Treatments (Study Treatment and Concomitant Medication)

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.

Concomitant medications will only be listed.

21.7 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 to check the robustness of the results to the missing data (eg, early dropouts). Details will be provided in the SAP.

21.7.1 Endpoint Measures

21.7.1.1 Primary Endpoint

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

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

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 and carry-over will be modeled along with treatment, where Mirasol-treated WB is coded 1 and conventional treatment is 0. 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.

Sensitivity analysis will be performed to assess whether results of the primary efficacy analysis (based on treatment effect plus subject effect) are robust to the assumption of no carry over or period effects. The primary analysis of the primary efficacy endpoint will also be performed using the per protocol analysis set.

A secondary analysis will be conducted to evaluate whether irradiation of REF RBCs has an impact on the primary treatment comparison. This analysis will include a binary classification to identify REF RBC transfusions that utilized irradiated RBCs. Tests will then be carried out comparing the MIR RBCs to irradiated REF RBCs as well as MIR RBCs to non-irradiated REF RBCs, and a final test will compared these to treatment effects to assess whether they are statistically significantly different. Similar analyses will be conducted to evaluate the impact of older REF RBCs on the primary endpoint. This analysis will distinguish the effect of treatment with MIR RBCs versus REF RBCs when units were stored for ≤21 days and MIR RBCs versus REF RBCs stored when units were stored for > 21 days. Note that the study is powered to assess the primary endpoint of MIR RBCs compared to REF RBCs administered per current standard of care.

The frequency of off-protocol transfusions will be estimated during the run-in period and treatment periods for MIR 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 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 intention-to-treat and per-protocol non-inferiority analyses.

The linear mixed-effect model accommodates incomplete data arising if some patients do not yield responses to two transfusions in each treatment period. Moreover as this model is likelihood based findings will be insensitive to missing at random mechanisms. In a tertiary analysis any missing AUCs will be imputed using the SAS procedure for Multiple Imputation (Proc MI). The variables used in the MI process will be (pooled) treatment, age, gender, normalized AUC from transfusion episodes during the wash-in period. The number of imputations will be n=10 and the treatment effect for each imputation dataset will be analyzed using Proc MIANALYZE. The result will be summarized as the MI estimate of the treatment effect, standard error, and a 95% confidence interval.

21.7.1.2 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)

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 simply 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 follow-up 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.

21.8 Sample Size Rationale

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 RBCs derived from Mirasol-treated WB. 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 Hb AUC. Assuming a within subject variance of 0.007706 and a 16.7% reduction in Hb AUC, approximately 77 subjects are needed to obtain at least 80% power to show the decrease in Hb AUC from RBCs derived from Mirasol-treated WB is not more than 20% of the Hb AUC less from conventional untreated RBCs assuming a 1 tailed type 1 error rate of 2.5%. Algebraically if ln (μ_M) is the mean of the natural log-transformed Hb AUC for RBCs derived from Mirasol-treated WB and ln (μ_C) is the mean of the natural log-transformed Hb AUC for conventional untreated 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.

21.9 Safety Analyses

21.9.1 Adverse Events

The Medical Dictionary for Regulatory Activities (MedDRA) version 18.0 or later will be used to code all adverse events to a system organ class (SOC) and a preferred term (PT) within the SOC. The National Cancer Institute Common Terminology Criteria for Adverse Events (version 4.03; CTCAE) will be used to grade severity of all AEs.

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 TEAEs
- Treatment-related TEAEs
- Adverse transfusion reactions
- TEAEs by maximum CTCAE grade
- SAEs and UADEs
- 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.

21.9.2 Laboratory Tests

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: change from baseline to the time point prior to each transfusion and change from the pre transfusion time point between each consecutive transfusion.

22 STUDY MANAGEMENT

22.1 Investigator Responsibilities

22.1.1 Roles and responsibilities of Key Study Personnel

- Lead Investigator: overall responsibility over the trial
- Research Coordinator: procedures with the patients, maintain study records, enter data into database, contact with the Sponsor

This is a multicenter study and the delegation of authority log at each investigational site will further define the roles and responsibilities of study personnel.

22.1.2 Statement of Investigator

Each Investigator will provide to the Sponsor a current curriculum vitae and a signed Statement of Investigator prior to initiation of the study.

22.1.3 Institutional Review Board/Ethics Committee

The Institution's IRB/EC or other similarly functioning committee will review and approve the CIP, CIP amendments, initial/revised ICF documents, and recruitment materials, if applicable. Upon IRB/EC approval, documentation of that approval and the approved ICF documents will be sent to the Sponsor before any subject is enrolled into this study.

22.1.4 Informed Consent

The Investigator is responsible for preparing the written informed consent/parental consent/assent documents for this study. The Sponsor or designee will provide to the Investigator ICF/parental consent form/assent form templates. The Investigator may rearrange or reword the contents of the templates or may add other elements or language, provided the meaning and content are not changed or deleted. The site-specific ICF/parental consent form/assent form will be reviewed/approved by the Sponsor prior to submission to the IRB/EC.

Written informed consent/parental consent/assent will be obtained from all subjects/legal guardians participating in this study. The case history for each subject must document that the informed consent/parental consent/assent process was conducted prior to participation in the study. The original ICF/parental consent form/assent form will be kept in the subject's record, and a copy will be provided to the patient.

All subjects are free to withdraw from participation in this study at any time, for any reasons, specified or unspecified, and without prejudice. The reason for the patient discontinuing or terminating from the study must be recorded on the eCRF.

22.1.5 Record Retention

Investigational sites will maintain all records pertaining to this study for the longer of the following time periods:

- A minimum of 2 years following premarket approval (or applicable application); OR
- 2 years after the study was discontinued

Thirty (30) days before destroying any study-related records, investigational sites will provide written notice to the Sponsor.

Investigators must notify the Sponsor in the event they are leaving the institution.

22.2 Recording and Collecting of Data

The Investigator will maintain complete, accurate, legible, and easily retrievable data. The Investigator will provide access for Sponsor authorized personnel to all study data at any time. Such data shall also be secured in order to prevent loss of data.

All required data for this study will be recorded from the source documentation onto standardized eCRFs.

For the duration of the study, the Investigator will maintain complete and accurate documentation, including but not limited to, medical records, study progress notes, laboratory reports, signed patient informed consent forms, device accountability logs, correspondence with the reviewing IRB/EC, sponsor and study monitor, adverse event reports, and information regarding patient discontinuation or completion of the study.

The Investigator/institution will permit direct access to source data and documents in order for study-related monitoring, audits, IRB/EC reviews, event adjudication and regulatory inspections to be performed. The Investigator will obtain, as part of the informed consent process, permission for sponsor, authorized sponsor employees or representatives, representatives of the United States Army Medical Research and Materiel Command, study monitors or regulatory authorities to review, in confidence, records that identify patients in this trial.

Accurate and complete study records will be maintained and made available to representatives of the United States Army Medical Research and Materiel Command. These representatives are authorized to review research records as part of their responsibility to protect human research volunteers. Research records will be stored in a confidential manner so as to protect the confidentiality of patient information.

22.2.1 Source Documents

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of that trial. Source data are contained in source documents. Examples of these original documents and data records include hospital and clinic records, evaluation checklists, scan or laboratory results, etc.

22.2.2 Case Report Forms

The study CRF via EDC is the primary data collection instrument for the study. All data must be recorded in English. Any missing data must be explained.

Completed CRFs will be reviewed and signed by the Investigator. The CRA will verify the EDC data with the patient's source data, evaluate the data for accuracy, consistency, and completeness, and will ensure that all forms with missing data and/or errors are ultimately addressed. Accurate and complete CRFs for a patient must be completed in a timely manner.

22.3 Clinical Investigation Plan Compliance

The Investigator is responsible for ensuring the study is conducted in accordance with the procedures and evaluations described in this CIP.

22.3.1 Deviations from the Clinical Investigation Plan

A CIP deviation is defined as any event where the investigator or site personnel deviate from the study CIP or study procedures for any reason.

It is the Investigator's responsibility to ensure that there are no CIP deviations throughout the lifetime of the study and that the study is conducted in full compliance with the CIP and all established procedures and conditions of the reviewing IRB/EC. CIP deviations that are due to unforeseen circumstances beyond the Investigator's reference, (eg, if the patient did not arrive for their scheduled follow-up and had to be re-scheduled outside of the CIP-defined time-window) are required to be reported on the CIP deviation form in order to ensure that all deviations from the standard subject population are adequately documented and reported.

The Investigator will inform the sponsor of all deviations and will inform the reviewing IRB/EC of CIP deviations per IRB/EC requirements. The occurrence of CIP deviations will be monitored

by the Sponsor for evaluation of Investigator compliance to the CIP, GCP compliance, and regulatory requirements. The Sponsor will report all deviations that are considered a modification to the CIP, pose a risk to the patient or that affect the scientific integrity of the study to HRPO. In the event that an Investigator does not comply with the clinical study/Investigator agreement, and/or the CIP, the Investigator will be notified of their noncompliance, and the Sponsor will review the circumstances. Continued noncompliance may result in discontinued shipment of study devices and termination of the Investigator's and/or investigational site's participation in the study.

The Investigator will not deviate from the CIP for any reason without prior written approval from the sponsor, except in cases of medical emergencies. A medical emergency is defined as a medical condition where no other medical option exists for satisfactory survival of the patient. In the case of a medical emergency, the Investigator should notify the sponsor as soon as possible, in writing, of the emergency use of the device.

22.4 Sponsor Responsibilities

22.4.1 Amendments to the Clinical Investigation Plan

Any amendment to the CIP, as deemed appropriate by the Sponsor, will be implemented as the study progresses. Amendments will be submitted to the IRB/EC for written approval before implementation.

22.4.2 General Responsibilities of Sponsors

As per ISO 14155 Section 8 and 21 CFR 812, the Sponsor is responsible for selecting qualified Investigators and providing them with the information they need to conduct the investigation properly, ensuring quality study conduct and proper monitoring of the investigation, ensuring required approvals are obtained and that significant new information about an investigation is promptly reported to reviewing IRB/EC and government authorities as well as annual reports as required. Sponsor or designee will maintain essential study documentation.

22.4.3 USAMRMC Review, Approval, and Continuing Review

The study will be conducted in accordance with the CIP submitted to and approved by the ORP HRPO and will not be initiated until written notification of approval of the research project is issued by the ORP HRPO.

A copy of the continuing review report for the research study will be submitted to the local IRB/EC. A copy of the approved continuing review report and the local IRB/EC approval notification must be submitted to the ORP HRPO as soon as these documents become available. In addition to reporting progress of the approved research to the local IRB/EC, as described

above, the sponsor will provide progress reports to the ORP HRPO as often as requested, but not less frequently than once per year.

22.5 Joint Investigator/Sponsor Responsibilities

22.5.1 Training

The Sponsor will train applicable study team members to the device, CIP, and study procedures and will provide updated information as it becomes available during the course of the study, if applicable. The Investigator is responsible for ensuring that additional site personnel that were not trained by the Sponsor receive applicable documents and training.

22.5.2 Compliance

The study will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki, United States CFR, ISO14155/2011 and any regional or national regulations, as appropriate. The study will not begin until the required approvals/favorable opinion from the local IRB/EC and the regulatory authority have been obtained.

22.5.3 On Site Audits

In accordance with the Sponsor's operating procedures, the Sponsor or designee may request access to all study records, including source documents, for inspection and duplication. In the event that an Investigator is contacted by a regulatory agency in relation to this study, the Investigator will notify the Sponsor immediately.

The Investigator or designee must be available to respond to reasonable requests and audit queries made by authorized regulatory representatives during the audit process. The Investigator must provide the Sponsor with copies of all correspondence that may affect the review of the current study or their qualification as an Investigator in clinical studies conducted by the Sponsor. The Sponsor will provide any needed assistance to regulatory audits or correspondence.

Accurate and complete study records will be maintained and made available to representatives of the USAMRMC. These representatives are authorized to review research records as part of their responsibility to protect human research volunteers. Research records will be stored in a confidential manner so as to protect the confidentiality of patient information.

The knowledge of any pending compliance inspection/visit by the FDA, Office of Human Research Protections, or any other government agency concerning this clinical investigation or research, the issuance of Inspection Reports, FDA Form 483, warning letters, or actions taken by any regulatory agencies including legal or medical actions and any instances of serious or continuing non-compliance with the regulations or requirements will be reported immediately to USAMRMC ORP HRPO.

22.5.4 Termination of the Study

For reasonable cause, either the Investigator or the Sponsor may terminate the Investigator's participation in this study, provided a written notice is submitted within the time period provided for in the Clinical Trial Agreement (CTA). In addition, the Sponsor may terminate the study at any time upon immediate notice for any reason, including but not limited to, the Sponsor's belief that termination is necessary for the safety of patients.

The ORP HRPO will be notified upon completion of the study. A copy of the final study report will be submitted to the local IRB/EC. A copy of the approved/accepted final study report and the local IRB/EC approval/acceptance notification must be submitted to the ORP HRPO as soon as these documents become available.

22.5.5 Financing

The Sponsor will compensate the Investigator Sites according to the terms of a CTA between the Sponsor, the Investigator, and the Investigator Site that will list the anticipated cost and expenses relating to the conduct of this study. However, the Sponsor will not compensate the Investigator for evaluation of patients in a manner other than specified in the CIP.

The Investigator is not financially attached to private enterprises, foundations, etc., that may have interests in this clinical study.

22.5.6 Publication Policy

The Sponsor recognizes the importance of communication of medical study data. The Sponsor encourages the publication of such data in reputable scientific journals and the presentation of such data at scientific seminars and conferences. Any proposed publication or presentation of the data generated from the study must be provided to the Sponsor for timely review in accordance with the terms of the CTA between the Investigator, the Institution, and the Sponsor. The Sponsor shall not, in its scientific publications or promotional material, quote from publications by Investigators without full acknowledgment of the source. Because this will be a multisite trial, all Investigators agree not to publish individual site data without prior approval from the Sponsor, as negotiated in the CTA. All trial data will be published as 1 or more manuscripts based on the accumulated data from all trial sites.

23 REFERENCES

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24 INVESTIGATOR SIGNATURE

Study Title:	Evaluate the Efficacy and Safety of RBCs Derived from Mirasol-treated Whole Blood Compared with Conventional RBCs in Patients Requiring Chronic Transfusion Support (PRAISE Trial)
Study Number:	CTS-5056
Version/Date:	Version 5.0: 15 FEB 2018

I have read the clinical investigation plan, including all appendices, and I agree that it contains all necessary details for my staff and me to conduct this study as described. I will conduct this study in compliance with the clinical investigation plan, Good Clinical Practices, and all applicable regulations. I will make a reasonable effort to complete the study within the time designated.

I will provide all study personnel under my supervision with copies of the clinical investigation plan and grant access to all information provided by Terumo BCT. I will discuss this material with all study personnel under my supervision to ensure that they are fully informed about the study.

Investigator Name (printed)	Signature
Date	

Appendix 1: Centers for Disease Control and Prevention's National Healthcare Safety Network Biovigilance Component Hemovigilance Module Surveillance Protocol (version 2.2, dated January 2016)