REd CEll Storage Duration Study (RECESS)

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Study Sponsor: National Heart, Lung, and Blood Institute

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Conducted by the Transfusion Medicine/Hemostasis Clinical Trials Network

A randomized trial to determine if there is a clinically important difference between the effect of shorter storage-age RBCs vs. longer storage-age RBCs on clinical outcome and mortality risk

Concept Synopsis and Study Schema
The desired impact of red blood cell (RBC) transfusion is to increase oxygen delivery as a means to protect tissues from ischemia and hasten post-operative recovery. However, controversy exists over transfusions with blood banked for short vs. long storage intervals. No large, randomized prospective human study has attempted to correlate age of transfused RBCs with measures of end-organ dysfunction or death, or with changes in standard hemodynamic variables or immediate oxygen delivery enhancement.

Primary Hypothesis:
There is a clinically important difference between the effect of shorter storage-age RBCs vs. longer storage-age RBCs on clinical outcome and mortality risk.

Study Schema:
This study is a randomized, controlled, partially-blinded clinical trial. Cardiac surgery patients ≥ 12 years old and ≥ 40 kg who are likely to be transfused with allogeneic RBCs during or after complex cardiac surgery will be identified by a screening transfusion risk assessment tool. Study consent will be sought in these eligible subjects pre-operatively. Subjects will be randomized prior to surgery, but no earlier than the calendar day prior to surgery, to receive RBCs stored either ≤ 10 days at the time of transfusion, or ≥ 21 days at the time of transfusion. Pre-operative, intra-operative and post-operative transfusions should be given according to the randomized treatment arm, whenever inventory allows. Randomization will only occur when BOTH of the following criteria are met:
- The transfusion service has enough suitable units stored ≤10 days to meet the crossmatch request.
- The general inventory of the transfusion service has enough suitable units stored ≥ 21 days to meet the crossmatch request. In other words, the crossmatch request would be filled with RBC units stored ≥ 21 days if issued according to standard inventory management (oldest product released first).
    - **The transfusion service is <u>not</u> allowed to maintain a special study inventory of units stored ≥ 21 days, or to specifically order units stored ≥ 21 days.**

Only the blood bank personnel will be told of the assigned study arm.

The primary endpoint of RECESS will be the change in clinical outcome assessed using the Multiple Organ Dysfunction Score (MODS), which is a composite endpoint of multi-system organ dysfunction including death. The MODS calculated immediately prior to surgery will be compared to the MODS calculated as the sum of the worst individual component scores for each of the systems up until post-operative Day 7, hospital discharge, or death, whichever occurs first. The change in the MODS value (ΔMODS), among subjects who receive one or more RBC transfusions, will be compared between the subjects who were randomized to receive RBCs which were ≤ 10 days old vs. those randomized to receive RBCs which were ≥ 21 days old at the time of transfusion.
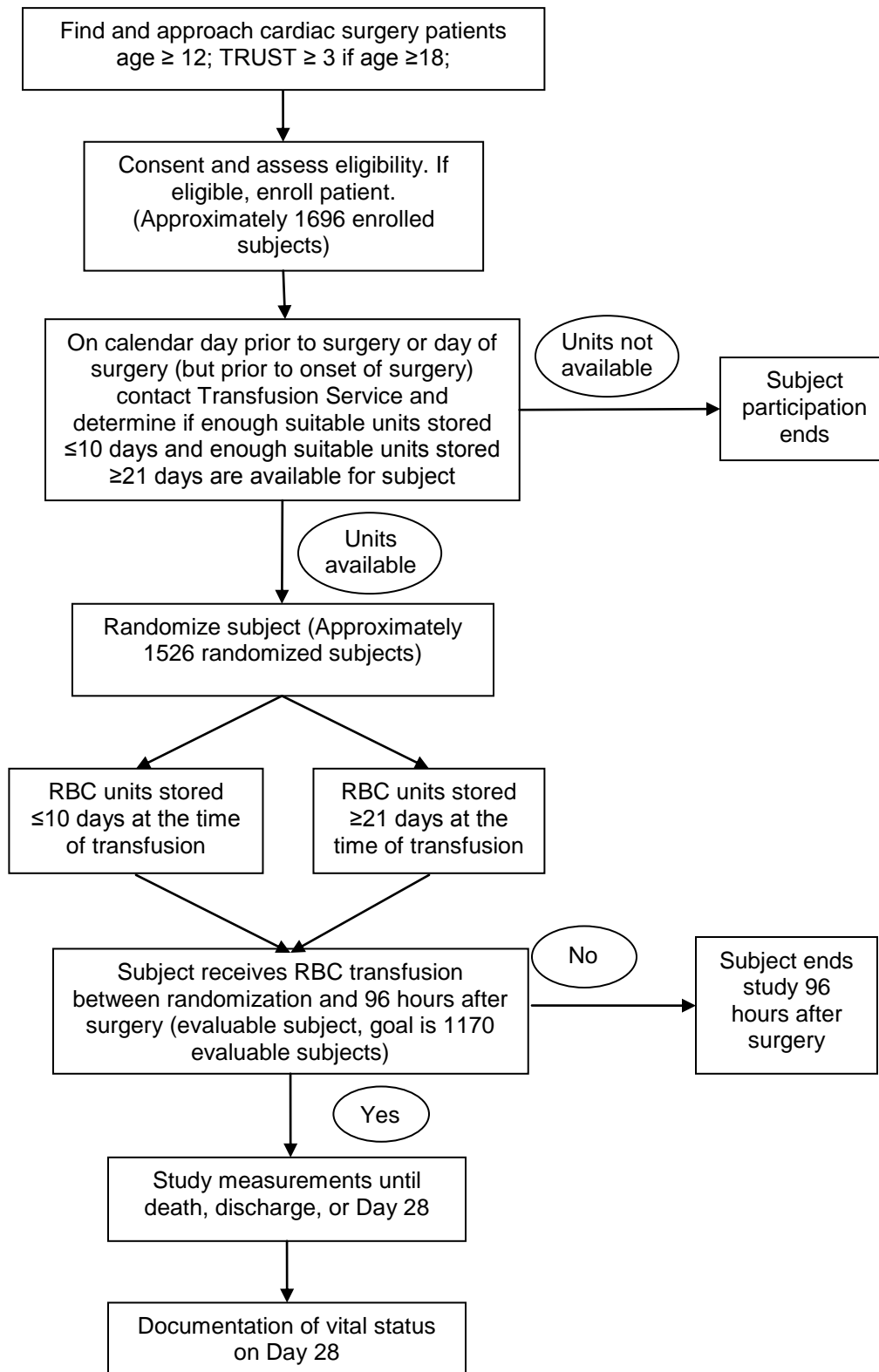
Secondary endpoints are:
1. All-cause mortality through 28 days
2. ΔMODS through post-operative day 28, hospital discharge, or death, whichever comes first
3. Composite of major in-hospital post-operative complications through post-operative Day 7, hospital discharge, or death, whichever occurs first
4. Composite of major cardiac events through post-operative Day 7, hospital discharge, or death, whichever occurs first
5. Composite of major pulmonary events through post-operative Day 7, hospital discharge, or death, whichever occurs first

6. Ventilation duration through post-operative day 28, hospital discharge, or death, whichever comes first
7. Days alive and ventilator free through post-operative day 28.
8. Any mechanical ventilation from 48 hours post-operative to Day 28, hospital discharge, or death, whichever occurs first
9. Changes in the following laboratory parameters, from pre-op to worst recorded post-op value through post-operative Day 7, hospital discharge, or death, whichever occurs first:
    - Serum creatinine
    - Troponin-I
    - Lactate
    - Liver function tests (bilirubin, and for children also ALT)
10. Days to first bowel movement through post-operative day 28, hospital discharge, or death, whichever comes first
11. Days to first solid food through post-operative day 28, hospital discharge, or death, whichever comes first

The precision for the 95% confidence interval for the difference in ΔMODS between treatment groups is designed to be ±0.85 points or narrower.

Figure 1: Study Schema

Find and approach cardiac surgery patients age ≥ 12; TRUST ≥ 3 if age ≥18;

↓

Consent and assess eligibility. If eligible, enroll patient. (Approximately 1696 enrolled subjects)

↓

On calendar day prior to surgery or day of surgery (but prior to onset of surgery) contact Transfusion Service and determine if enough suitable units stored ≤10 days and enough suitable units stored ≥21 days are available for subject

→ (Units not available) → Subject participation ends

↓ (Units available)

Randomize subject (Approximately 1526 randomized subjects)

↙   ↘

RBC units stored ≤10 days at the time of transfusion   RBC units stored ≥21 days at the time of transfusion

↘   ↙

Subject receives RBC transfusion between randomization and 96 hours after surgery (evaluable subject, goal is 1170 evaluable subjects)

→ (No) → Subject ends study 96 hours after surgery

↓ (Yes)

Study measurements until death, discharge, or Day 28

↓

Documentation of vital status on Day 28

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1. Background and Significance

The desired impact of red blood cell (RBC) transfusion is to increase oxygen delivery as a means to protect organs/tissues from ischemia and hasten post-operative recovery. The FDA has approved transfusion of RBC products stored for up to 42 days.

Standard inventory management practice for RBC products is to release the oldest products first. The reason for this practice is to avoid, to the extent possible, having units exceed 42 days of storage, after which time they cannot be transfused. This policy helps to maintain adequate inventory and avoid wasting donated blood.

In a survey conducted in four TMH centers, cardiac surgery patients were categorized according to whether they received only RBCs stored ≤ 10 days, only RBCs stored 11-20 days, only RBCs stored 21-42 days, or RBCs from more than one category. Approximately 32% of the included cardiac surgery patients received only RBCs stored 21-42 days and approximately 11% received only RBCs stored ≤ 10 days. However, there were wide variations between hospitals and between patients of different blood types. Among RBC units given to patients who received units from more than one category, over 40% of the units were stored 21-42 days.

Longer RBC storage is associated with structural, biochemical, and cytokine level alterations such as: loss of RBC deformability, decreased levels of ATP and 2,3-diphosphoglycerate, and decreases in both Anion Exchange Protein 1 and hemoglobin-bound nitric oxide which appear to diminish the vasodilatory properties of red cells (Bennett-Guerrero, Kristiansson, Reynolds, Shanwell, Tinmouth, Zimrin). However, there is controversy about whether these changes have clinical significance for transfusion recipients.

A number of observational studies and some small randomized studies have been carried out to investigate the effects of RBC storage age in various patient populations.

Observational Studies
Several observational studies in cardiac surgery patients have demonstrated an association between longer RBC storage time and worse outcomes. One retrospective study (Vamvakas 1999) found an association between longer RBC storage and increased risk of post-operative pneumonia. In a prospective, non-randomized study of 585 patients undergoing a variety of cardiac operations, receipt of RBCs stored > 28 days was statistically associated with the development of pneumonia, although no association was found between median RBC age and ICU length of stay, ventilator days, new myocardial infarction and other outcomes (Leal-Noval 2003). A recent large retrospective study (Koch) showed an increased risk of post-operative complications, including reduced short-and long-term survival, in patients undergoing cardiac surgery who received RBCs stored more than 14 days (compared to patients who received RBCs stored for 14 days or less). The groups were imbalanced at baseline in terms of prior disease characteristics, in the proportion receiving leukoreduced RBC, and in ABO blood group.

Conversely, several other observational studies in cardiac surgery patients have not found an adverse effect of longer stored units. A study in 268 CABG patients found no relationship between RBC storage duration and post-operative morbidity after adjustment for confounding factors (Vamvakas 2000). A large retrospective single center study (Van de Watering) showed no difference in 30 day mortality between 945 CABG patients who received only RBCs < 18 days old vs. 950 patients who had received only RBCs > 18 days old. Another more recently published retrospective study of 670 patients undergoing

cardiopulmonary bypass for non-emergent, non-redo cardiac operations found no RBC storage age effect on mortality (Yap).

Two observational studies in ICU patients with sepsis found that longer storage of RBCs was associated with worse outcomes (Marik, Purdy). Another observational study in ICU patients with sepsis found no difference between longer and shorter storage RBCs with respect to change in microcirculation (Sakr).

Several observational studies in colorectal cancer surgery have been carried out. One study found longer storage duration RBCs were associated with increased risk of infection (Mynster 2000). Another study found no difference in infection risk based on storage duration of RBCs (Edna). A third study found that RBCs stored for less than 21 days were associated with increased risk of cancer recurrence (Mynster 2001).

A number of observational studies in trauma patients have found that longer storage of RBCs is associated with worse outcomes (Leal-Noval 2008, Offner, Spinella, Weinberg, Zallen).

A population-based study of all red blood cell recipients in Sweden and Denmark (Edgren) analyzed 7-day and 2-year mortality risk in patients receiving only RBC units stored 0-9 days, 10-19 days (reference group), 20-29 days, 30-42 days, or RBC units from more than one of these categories. The models adjusted for number of transfusions and several other relevant covariates. There were no significant differences in 7-day mortality between the reference group and any other group. Compared to the reference group, patients receiving only RBCs stored 30-42 days had a slightly elevated mortality risk over 2 years (hazard ratio 1.05 with 95% confidence interval 1.02 to 1.08), but the authors stated that this may be due to residual confounding. Patients receiving RBCs of mixed ages also had a slightly elevated mortality risk over 2 years, compared to the reference group (hazard ratio 1.03, 95% confidence interval 1.02 to 1.05). However, patients who received blood of mixed ages received many more RBC units than patients in the other groups, so there is a possibility of residual confounding even though the analysis adjusted for number of transfusions. In the subset of patients undergoing CABG surgery there were no statistically significant differences between the reference group and any of the other groups with respect to 7-day or 2-year mortality.

All non-randomized studies are subject to confounding by measured and unmeasured covariates and some of the studies did not adjust for important covariates. Some of the studies investigated a large number of possible outcomes with differing conclusions by outcome and with no consideration given to the number of analyses done. A number of articles have pointed out these concerns (Dzik, Zimrin, LeLubre, Vamvakas 2010, Tinmouth).

Randomized Studies
A few small studies have randomized patients to different storage duration of RBCs. None of these studies found an adverse effect associated with longer storage duration.

A pilot study comparing < 8 day old blood vs. >15 day old blood in critically ill and cardiac surgery patients enrolled 66 patients, 57 of whom were transfused including 42 cardiac surgery patients. This study reported higher mortality in recipients of the < 8 day old units but these associations were not statistically significant and the treatment groups seem to differ on some important baseline characteristics (Hebert 2005).

A small randomized study in 22 anemic ICU patients did not find deleterious effects on gastric tissue oxygenation with transfusion of 2 units of RBCs ≥ 20 days old vs. ≤ 5 days old (Walsh).

A study of 17 trauma patients randomized to <11 day vs. >20 day RBCs did not find a significant difference in mortality (Schulman).

A randomized study of 9 normal volunteers showed that fresh and older blood did not have different effects on how well the transfusions reversed anemia-induced deficits in brain oxygenation (Weiskopf).

Meta-analyses and Reviews
Two recent comprehensive reviews, each including over 20 cited studies, support that equipoise exists and identify the need for prospective, randomized trials to answer the question of whether RBC stored for longer vs. shorter periods of time negatively impact patient outcomes (Zimrin, LeLubre). A third review (Tinmouth) suggested possible detrimental effects associated with longer storage RBCs, but also called for randomized trials. A fourth review (Vamvakas 2010) includes a meta-analysis of two randomized studies (Hebert 2005, Schulman) suggesting that longer stored RBCs may be associated with a significant *reduction* in mortality and a meta-analysis of observational cardiac surgery studies that showed no significant association between RBC storage duration and pneumonia.

Summary
Thus, equipoise exists regarding the effect of RBC storage on patient outcomes, as summarized in the comprehensive reviews cited above. Furthermore, under standard inventory practice there is wide variation in the storage duration of RBCs received by cardiac surgery patients. Many cardiac surgery patients receive only RBCs stored 10 days or less, and many receive only RBCs stored 21 days or more (the two treatment arms in RECESS). In addition, many cardiac surgery patients receive RBCs stored 11 to 20 days or receive RBCs of varying storage durations.

Other On-going Studies
The Red Cell Storage Duration and Outcomes in Cardiac Surgery clinical trial is a phase III randomized clinical trial which is currently being conducted at the Cleveland Clinic (NCT00458783). This trial opened in 2007 and plans to randomize 2800 cardiovascular surgery patients to receive either RBCs stored for <14 days or RBCs stored >20 days. The primary aim of the study is to determine whether length of storage of red blood cells is related to postoperative morbidity outcomes in patients undergoing cardiac surgery.

Perhaps only ICU patients have been studied more carefully with respect to RBC transfusion. A multi-center clinical trial, the Age of Blood Evaluation (ABLE) study, comparing the effects of RBC storage time on clinical outcomes in ICU patients has been funded in Canada (MCT-90648). In addition to the different patient population, the ABLE study also differs in that the primary outcome will be mortality and the RBCs in the two arms will be either ≤ 10 day of storage or "standard of care". Hence the two studies will be complementary, and between them encompass two of the patient populations accounting for a large share of blood utilization.

Another study addressing the impact of RBC storage on clinical outcomes is being conducted in Canada in premature infants. The Age of Red Blood Cells in Premature Infants (ARIPI) study (NCT00326924) is a multi-center, blinded, randomized clinical trial in which infants weighing ≤ 1250 g are being randomized to receive transfusions of RBCs

stored for < 7 days or to the current standard practice of using multiple aliquots from a single RBC unit for a specific infant until its expiration date. The primary outcome measure is a composite endpoint of mortality, necrotizing enterocolitis, retinopathy of prematurity, bronchopulmonary dysplasia and intraventricular hemorrhage by 90 days (Fergusson).

RECESS Population

Patients undergoing cardiac surgery were chosen as the study population for several reasons: 1) these patients commonly require multiple RBC transfusions and might be expected to manifest complications ascribed to exposure to allogeneic blood; 2) cardiac surgery patients comprise a very large group and account for a significant proportion of blood component utilization; 3) there are conflicting results from several retrospective studies and a few small randomized studies testing the effects of RBC storage time on clinical outcomes in this population ; and 4) cardiac surgery patients generally undergo invasive cardiorespiratory monitoring, which provides information on oxygen consumption/delivery and other physiologic parameters impacted by red cell transfusion.

RECESS will study patients undergoing cardiac surgery regardless of whether the procedure is performed on-pump or off-pump. A recent Veterans Administration study included 2203 cardiac surgery patients randomized between on- and off-pump urgent or elective coronary artery bypass graft (CABG) procedures. The study found no significant difference between groups with respect to the proportion requiring red cell transfusion (52% vs. 56%); 30 day mortality; or a composite endpoint including adverse events such as re-operation, cardiac arrest, stroke, coma, renal failure requiring dialysis, or new mechanical support. It must be recognized that these were all male patients who were generally undergoing procedures with lower risk for transfusion than the procedures eligible for RECESS, that there were more operations being performed by trainees in the off-pump group, off-pump operations took longer and mortality and graft patency at one year were poorer in the off-pump group (Shroyer). Nevertheless, in an evolving surgical practice environment and to make RECESS results applicable to the greatest population possible, both on-pump and off-pump patients will be included.

Measurements of Organ Dysfunction

Multiple scoring systems have been developed to standardize risk assessment of clinical parameters and/or response and outcomes to medical interventions. The Multiple Organ Dysfunction Score (MODS) has been used to standardize mortality risk based on cumulative organ dysfunction, including assessing the effects of liberal vs. restrictive red cell transfusion practice in the TRICC trial and the subgroup analysis of patients with cardiac disease (Hebert 2001, Marshall 1995, Marshall 1997). Other scoring systems such as APACHE II define and tabulate organ failures as risk factors contributing to morbidity and mortality (Knaus). Yet other scoring systems assign mortality risk by including the intensity of clinical interventions, such as the SOFA score, which includes mechanical ventilation and use of vasopressors (Vincent) and the new CASUS score, which includes in the scoring system use of intra-aortic balloon pump, vascular assist devices, and renal replacement therapy (Hekmat). However, clinical studies employing these latter scoring systems should standardize patient management. Otherwise, patient scores can be modified by clinical staff decisions on the type and timing of interventions.

Several scoring systems have been applied to the cardiac surgery population. Outcomes comparing leukoreduced vs. non-leukoreduced RBC transfusions given to valve surgery patients defined multiple organ dysfunction as ≥ two systems failing and found that a high incidence of two system failures was strongly associated with the number of transfusions given (Bilgin). Several series of cardiac surgical patients demonstrated that the SOFA score (Ceriani, Mazzoni, Patila) or M/SOFA score (Shime) and its change over time correlated

well with morbidity and mortality. However, those studies did not include standardized management.

Several pediatric-specific scoring systems have been developed and validated. These include PRISM (Pollak), P-MODS (Graciano) and a pediatric adaptation of Marshall's MODS, named the PELOD score, which includes mechanical ventilation interventions (Leteurtre). Change in PELOD score was a secondary endpoint in the recent TRIPICU study evaluating liberal vs. standard transfusion practices in pediatric ICU patients (Lacroix).

MODS was chosen as the primary outcome for RECESS because it has been validated, it has been used in previous transfusion studies in cardiac surgery, and it does not depend on the type and timing of clinical interventions.

The lack of a scoring system validated in both children and adults limits the patient population for this study to older children whose size and physiology approximates that of adults.

2. OBJECTIVES

2.1   Primary Hypothesis

There will be a clinically important difference in subject outcomes measured as the change in composite of multiple organ dysfunction (ΔMODS) (Marshall 1994, Marshall 1997) from the pre-operative baseline through post-operative Day 7, hospital discharge, or death, whichever occurs first, in cardiac surgery subjects transfused with shorter storage-age RBC units vs. longer storage-age RBC units.

2.2   Secondary Hypotheses

There will be clinically relevant differences between treatment groups in one or more of the following outcomes.
1. All-cause mortality through 28 days
2. ΔMODS through post-operative day 28, hospital discharge, or death, whichever comes first
3. Composite of major in-hospital post-operative complications through post-operative Day 7, hospital discharge, or death, whichever occurs first:
   a. Death
   b. Stroke
   c. Myocardial infarction (MI)
   d. Renal Failure
   e. Culture proven sepsis/septic shock
4. Composite of major cardiac events through post-operative Day 7, hospital discharge, or death, whichever occurs first:
   a. Death
   b. Myocardial infarction (MI)
   c. Low cardiac output
      i. Intra-aortic balloon pump placement post-op
      ii. Use of inotropic and/or vasoconstrictive agents 48 hours post-surgery or later (Use of such agents within 48 hours following surgery is not considered part of this composite outcome)
   d. Ventricular tachycardia
   e. Ventricular fibrillation

5. Composite of major pulmonary events through post-operative Day 7, hospital discharge, or death, whichever occurs first:
   a. Any mechanical ventilation from 48 hours post-operative to Day 28, hospital discharge, or death, whichever comes first.
   b. Pulmonary embolism
6. Ventilation duration through post-operative day 28, hospital discharge, or death, whichever comes first
7. Days alive and ventilator free through post-operative day 28
8. Any mechanical ventilation from 48 hours post-operative to Day 28, hospital discharge, or death, whichever occurs first
9. Changes in the following laboratory parameters, from pre-op to worst recorded post-op value through post-operative Day 7, hospital discharge, or death, whichever occurs first:
   - Serum creatinine
   - Troponin-I
   - Lactate
   - Liver function tests (bilirubin, and for children also ALT)
10. Days to first bowel movement through post-operative day 28, hospital discharge, or death, whichever comes first
11. Days to first solid food through post-operative day 28, hospital discharge, or death, whichever comes first

3. STUDY POPULATION

3.1 Enrollment Inclusion Criteria:

1) Patients ≥ 12 years old
2) Patients ≥ 40 kg
3) Scheduled complex cardiac surgery with planned use of median sternotomy. The procedure may be performed either on-pump or off-pump. Procedures that qualify as complex cardiac surgery include the following ("Repeat procedure" means that the subject had a previous cardiac surgery with median sternotomy.):
   - Single Vessel Coronary Artery Bypass Graft, repeat procedure
   - Multiple Coronary Artery Bypass Grafts, first or repeat procedure
   - Single Valve Repair or Replacement, repeat procedure
   - Multiple Valve Repair or Replacement, first or repeat procedure
   - Surgery involving both Coronary Artery Bypass Graft(s) and Valve Repair(s), first or repeat procedure
   - One or more of the following procedures, with or without Coronary Bypass Graft(s):
     - left ventricular aneurysm repair,
     - ventricular and/or atrial septal defect repairs,
     - batista (surgical ventricular remodeling),
     - surgical ventricular restoration,
     - congenital defect repair, and
     - aortic root procedures
4) Patients ≥ 18 years must have a TRUST (Alghamdi) probability score ≥ 3, which corresponds to a high likelihood of receiving RBC transfusions during surgery or within 96 hours post-operatively (see Appendix 1). Calculate the TRUST score using the most recent test results done within the previous 60 days. If data for some components of the TRUST score are not available, but enough data are available to know that the TRUST score is at least 3, the patient meets this eligibility criterion. For patients < 18 the TRUST score need not be calculated.

3.2 Enrollment Exclusion Criteria:
1) Refusal of blood products
2) Planned surgery is minimally invasive
3) Known transfusion reaction history
4) <u>Requirement</u> for washed products, volume reduced products, or products with additive solution removed
5) Expected residual cyanosis with O_2 saturation < 90
6) Left ventricular assist device (LVAD) or Extracorporeal membrane oxygenation (ECMO) support pre-operatively or planned need post-operatively
7) Cardiogenic shock requiring pre-operative placement of an Intra-aortic balloon pump (IABP) (IABP done for unstable angina or prophylactically for low ejection fraction is not excluded)
8) Planned Deep Hypothermic Circulatory Arrest (DHCA)
9) Renal dysfunction requiring pre-operative renal replacement therapies such as hemodialysis (HD) or continuous venovenous hemofiltration (CVVH)
10) Planned use of alternative to heparin, e.g. bivalirudin
11) Planned use of autologous or directed donations
12) Prior RBC transfusion during hospitalization for the study-qualifying surgery
13) Prior randomization into the RECESS study

4. TRIAL ENROLLMENT

4.1 Screening/Recruitment
Patients ≥ 18 years of age undergoing complex cardiac surgery will be identified through pre-operative scheduling procedures in advance of their operations. Patients already hospitalized may be included. Basic features of patient medical and surgical histories (i.e. age, gender, type of surgery) will be scored using the TRUST screening risk assessment tool as discussed in Section 3.1 inclusion criterion 4 (Alghamdi). Patients <18 years need not have TRUST scores determined since this scoring system is not validated for these patients, but the available components of the TRUST score will be collected. Patients 12-17 years old weighing at least 40 kilograms and scheduled to receive eligible surgeries as defined in section 3.1 have a high likelihood of receiving a RBC transfusion and are potentially eligible.

Potentially eligible patients, as described in the previous paragraph, will be approached for study consent prior to their operation. Individual center scheduling practices will influence how this contact is arranged. Subjects who consent to the study will be assigned a study ID number and have their eligibility status determined. If the subject is eligible for the study based on the inclusion/exclusion criteria, they will be enrolled in the study and will be considered for randomization, pending the availability of suitable products (see sections 4.2 and 5.1).

If a subject is enrolled and receives a red cell transfusion prior to randomization, that subject will no longer be considered for randomization, and their participation in the study will end.

4.2   Stratification and Randomization

Subjects will be randomized to receive RBCs stored either ≤ 10 days at time of transfusion or ≥ 21 days at the time of transfusion. Storage arm assignment applies to all pre-operative, intra-operative and post-operative RBC transfusions, beginning at the time of randomization and continuing through hospital discharge, death, or 28 days following the end of surgery, whichever occurs first.

Randomization will be stratified by age (≥18 years or <18 years) and according to whether or not the subject had been pre-admitted to an ICU prior to surgery. The randomization scheme will use permuted blocks within strata and institutional balancing (Zelen).

After the number of units requested for crossmatch is known, and prior to surgery but no earlier than 1 calendar day before surgery, a study staff member will contact the institution's transfusion service to determine whether both of the following criteria are met.
- The transfusion service has enough suitable units stored ≤ 10 days to meet the crossmatch request for this subject.
- The general inventory of the transfusion service has enough suitable units stored ≥ 21 days to meet the crossmatch request for this subject. In other words, the crossmatch request would be filled with RBC units stored ≥ 21 days if issued according to standard inventory management (oldest product released first).

If these criteria are both met, the study staff member will immediately randomize the subject.

If there is not sufficient inventory to meet both criteria, the subject will not be randomized and their participation in the study will end.

4.3   Masking of Treatment Allocation

At enrollment, the subject's eligibility status and ABO/Rh type will be entered into the data management system (DMS). If eligible, and there is sufficient inventory in both treatment arms, the subject will be randomized, prior to surgery but no earlier than 1 calendar day before the surgery. Only blood bank staff with the appropriate security level in the DMS will be able to access the treatment arm assignment. Access to case report forms containing information about the age of the RBC products sent for each subject will also be restricted at the site to the appropriate blood bank staff. Clinical staff caring for the subject, collecting data and reporting the data in the DMS will not have access in the DMS to the treatment arm assignment or information about the age of the RBC products transfused.

No alteration will be made to the labels on the RBC units. The expiration date, collection date, and any processing dates (e.g. irradiation dates) will not be obscured. Medical personnel physically providing the RBC transfusions will verify product and patient identity according to hospital-specific procedures. These personnel will be instructed to not divulge the patients' randomization assignments. Operating room staff (other than those actually infusing RBCs), surgical staff, ICU staff, and others caring for RECESS subjects will be instructed not to seek to identify the age of the products the patients are receiving. The subjects themselves will not be informed of their randomization assignment and will also be instructed not to seek to identify the

age of the products they are receiving. However, as the components of the MODS are objective physiology measures, as are the majority of secondary outcome measures, inadvertent unblinding of the randomization assignment will not compromise the validity of the study. A blinded study of 20 red cell units was performed to determine whether the age of the red cell unit could be predicted based on the appearance of the unit. The study demonstrated that the age could not be reliably predicted from the appearance of the unit.

Throughout the study, withdrawal rates and reasons for withdrawal will be monitored by treatment arm and site.

5. INTERVENTIONS

5.1 Preparation

In Section 5 of this protocol, the term "study RBC units" includes all RBC units provided to a randomized RECESS subject, beginning at the time of randomization and continuing through hospital discharge, death, or 28 calendar days after the end of surgery, whichever occurs first.

Transfusions will be ordered based on patient need, following all institutional policies and practices. The decision to order (or not order) an RBC transfusion is not dictated in any way by the RECESS protocol.

5.1.1 Preparation criteria applying to both treatment arms
The following criteria apply to all study RBC units in both treatment arms. Any study RBC unit which does not meet all these criteria will be considered a protocol violation.
- Institutional processing standards will be maintained for all units.
- All study RBC units should be pre-storage leukoreduced.
- All study RBC units should be stored in either AS1, AS3, or AS5.
- No study RBC unit should be washed before release from the transfusion service.
- No study RBC unit should be washed intra-operatively.
- No study RBC unit should be volume-reduced.
- No study RBC unit should include a frozen product.
- No study RBC unit should be deglycerolized.

The use of irradiated products is not prohibited. However, if it is standard practice at an institution or in a transfusion service to irradiate products before they are issued, the study strongly recommends that the irradiation be done no more than 12 hours prior to releasing the unit from the blood bank. Likewise, when selecting a product for the study, whenever possible sites should avoid selecting units that were irradiated more than 12 hours prior to the time they would be released from the blood bank.

5.1.2 Preparation of RBC units for subjects randomized to receive units stored ≤ 10 days

All study RBC units released from the blood bank for subjects randomized to receive units stored ≤10 days should have been stored ≤10 days at the time of transfusion.

If there are not enough suitable units stored ≤10 days at the time a study RBC transfusion is needed, the blood bank should release suitable units with the minimum available storage time. The transfusion should not be cancelled or postponed because there are not enough suitable units stored ≤ 10 days.

Units stored longer than 10 days will be considered protocol deviations for subjects in this treatment arm.

The transfusion service is allowed to maintain an inventory of red cells stored ≤10 days.

Subjects randomized to this treatment arm are likely to receive units with shorter storage time than the units they would receive if not in the study, but this is not guaranteed to be true for any particular subject.

5.1.3 Preparation of RBC units for subjects randomized to receive units stored ≥ 21 days

All study RBC units released from the blood bank for subjects randomized to receive units stored ≥ 21 days should have been stored ≥ 21 days at the time of transfusion.

If there are not enough suitable units stored ≥ 21 days at the time a study RBC transfusion is needed, the blood bank should release suitable units with the maximum available storage time. The transfusion should not be cancelled or postponed because there are not enough suitable units stored ≥ 21 days.

Units stored less than 21 days will be considered protocol deviations for subjects in this treatment arm.

The transfusion service is <u>not</u> allowed to maintain a special study inventory of units stored ≥ 21 days, or to specifically order units stored ≥ 21 days.

Therefore, because standard inventory management is oldest unit released first, subjects assigned to receive units stored ≥ 21 days will never receive units with longer storage duration than the units they would receive if they were not in the study and were being transfused using standard inventory management.

5.2 Administration
RBC units will be administered according to local institutional policy and safety standards as ordered by the medical team for patient care needs.

5.3 Concomitant Treatments
Standard medical care according to the local institutional standard will be provided to the subject.

6. SCHEDULE OF MEASUREMENTS

6.1 Pre-operative to Post-operative Day 7, Hospital Discharge, or Death

Hemodynamic and laboratory measures will be assessed pre-operatively and daily from post-operative Day 1 through post-operative Day 7, hospital discharge or death, whichever occurs first. If a subject is discharged prior to Day 7 but returns to the study site for standard care on Day 7, attempts will be made to perform phlebotomy on that day for study laboratory tests. Subjects who do not receive an RBC transfusion after randomization, either pre-operatively, intra-operatively, or within the first 96 hours after their operation, will not be followed any longer.

- Hemodynamic parameters:
    - Heart rate
    - Blood pressure
    - Mean arterial pressure
    - Central venous pressure (CVP)
    - Systemic oxygen saturation via pulse oximetry probe
    - PO_2 arterial
    - FiO_2
    - Arterial oxygen saturation (SaO_2)
- Laboratory Measurements:
    - BUN
    - Creatinine
    - ALT(required for subjects < 18 years and collected for subjects ≥18 years only if done as part of standard of care)
    - Fibrinogen (required for subjects < 18 years and collected for subjects ≥18 years only if done as part of standard of care)
    - Bilirubin
    - Troponin-I
    - Lactic acid
    - Hemoglobin
    - Platelet count
- Glasgow Coma Score
- Hemostatic Agents (plasma derived or recombinant clotting factors, ε-aminocaproic acid, tranexamic acid or desmopressin). The pre-op period for reporting hemostatic agents is 7 days prior to surgery.
- Data Related to Surgical Procedure:
    - Type of procedure performed
    - Start and end time of procedure
    - Medications
    - Intraoperative cell recovery and reinfusion
    - Hemodilution
    - Nadir temperature
- 12-lead EKG
    - Required pre-operatively for all subjects
    - Required within 18 hours after the first time an elevated Troponin-I (defined as a Troponin-I in excess of 5 times the upper limit of normal per local standards) is collected post-operatively. If a 12-lead EKG was performed within the six hours prior to drawing the elevated Troponin-I, this EKG may be substituted.
    - Required within 18 hours after each subsequent elevated Troponin-I (defined as a Troponin-I in excess of 5 times the upper limit of normal per local standards) is collected post-operatively, if this elevated value is at

least 20% higher than the previous Troponin-I value. If a 12-lead EKG was performed within the six hours prior to drawing the elevated Troponin-I, this EKG may be substituted.

Pre-operative measurements are defined as those performed closest to the time of surgery but no earlier than 30 days prior to the start of surgery. However, if the subject receives any RBC transfusion(s) after randomization but before the start of surgery, the pre-operative MODS measurements reported must be based on measurements obtained prior to the RBC transfusion(s) but no earlier than 30 days prior to the start of surgery. If some MODS parameters were not measured before the pre-operative transfusion, they will be assumed to be normal. The elements of the MODS include: platelet count, Glasgow Coma Score, bilirubin, creatinine, PaO_2/FiO_2, and hemodynamic parameters (Marshall 1997).

If central vascular and/or arterial access has/have been removed, the invasive hemodynamic parameters will not be assessed (i.e. no new central venous access will be placed if the PA catheter and/or CVP line have been removed) and unless clinically indicated, an arterial blood gas will not be drawn.

6.2 Post-operative Day 8 through Day 28, Hospital Discharge, or Death
Hemodynamic and laboratory measures in Table 1 will continue to be tabulated if obtained during standard local practice after Day 7, until hospital discharge, death, or Day 28, whichever comes first.

MODS comparisons will be made for day of hospital discharge or death (if before Day 28), or Day 28 (end of study). It is expected that for most subjects, once they leave the ICU it will be evident the maximal MODS perturbations have been experienced during earlier hospital days. However, some subjects will be readmitted to the ICU and some will deteriorate and die on the ward, and daily assessment will allow recovery and analysis of data from these subjects. Laboratory studies will be obtained at end of study, which is at a maximum Day 28, or upon hospital discharge if that occurs earlier.

As above, no additional monitoring labs will be obtained unless deemed clinically indicated by the medical team. If central vascular and/or arterial access has/have been removed, the invasive hemodynamic parameters will not be assessed (i.e. no new central venous access will be placed if the PA catheter and/or CVP line have been removed) and unless clinically indicated, an arterial blood gas will not be drawn.

6.3 Day 28, All Evaluable Subjects
All randomized subjects who receive an RBC transfusion between randomization and 96 hours after surgery must have their vital status documented at post-operative Day 28, or earlier if the subject dies prior to Day 28. If a subject has been discharged prior to Day 28, the vital status may be obtained through medical records, the subject's physician, or a telephone interview with either the subject or a family member.

6.4 RBC Transfusions
The following data will be collected for each RBC unit ordered for the subject while on study:
- Unit ID number
- Source of unit (apheresis or whole blood collection)
- Blood group of unit (ABO and Rh)
- Leukoreduction status*
- Irradiation status
- Volume reduction status*

- Washing status*
- Date and time of product irradiation
- Storage medium (AS1, AS3, or AS5)
- Collection or expiration date
- Start and end time of transfusion

*As discussed in Section 5.1.1, all units should be leukoreduced and no units should be volume reduced or washed. However, these parameters will be recorded to identify any potential protocol deviations.

Table 1: Schedule of Measurements

Study Measures	Pre-op	Procedure	Post-op	Daily from Post-operative Day 1-7 or discharge/ death	As done as part of standard care Day 8–27 or until discharge/ death	At Hospital Discharge or Death or max Day 28 (study end)
TRUST score components	X					

MODS Components
• **PO₂**
• **FiO₂**
• **Creatinine††**
• **Total Bilirubin††**
• **PAR**
  ▪ **Heart rate**
  ▪ **Central venous pressure†**
  ▪ **Mean arterial pressure†**
• **Platelet Count††**
• **Glasgow Coma score**
	X		X	X	X†	X

Systemic Oxygen Saturation (via probe)	X		X	X		X
Blood pressure	X		X	X		X
Arterial O₂ sat	X		X	X		X
Lactic acid	X		X	X		X
Hemoglobin††	X		X	X		X
Fibrinogen*††	X		X	X		X
ALT*††	X		X	X		X
BUN	X		X	X		X
Troponin-I	X		X	X		X
12-lead EKG‡	X‡		X‡	X‡		
Hemostatic Agents	X	X	X	X	X	X
RBC transfusion data	X	X	X	X	X	X
Data on transfusions of plasma, platelet, and cryoprecipitate	X	X	X	X	X	X
Procedure data		X				
Documentation of vital status						X Day 28

*Fibrinogen and ALT will be required at these time points for subjects < 18 years and collected for subjects ≥18 years only if done as part of standard of care

†If available

††For subjects who are discharged from the hospital before Day 7, but return to the hospital on Day 7 for a post-operative visit as part of standard of care, blood for these laboratory tests will be collected. ALT and Fibrinogen values will only be collected on Day 7 for returning subjects ≥ 18 years if done as part of standard of care.

‡ A pre-op 12-lead EKG will be required from all subjects. Additionally, the protocol requires that an EKG be collected within the six hours prior to or 18 hours after the first time an an elevated Troponin-I suggestive of MI (defined as a Troponin-I in excess of 5 times the upper limit of normal per local standards) is collected post-operatively, and within the six hours prior to 18 hours after each subsequent elevated Troponin-I is collected, if that elevated value is at least 20% higher than the previous Troponin-I value.

6.5 Ending of Study Participation

- Enrolled subjects who receive an RBC transfusion before undergoing randomization will end study participation when they receive the transfusion.
- Enrolled subjects who are not randomized before their surgery begins will end study participation when the surgery begins.
- If a subject is randomized, and surgery does not take place within the next 30 days, study participation will end 30 days after randomization.
- If a subject is randomized and undergoes surgery within the next 30 days, but does not receive any RBC transfusion between randomization and 96 hours after the end of surgery, study participation ends 96 hours after the end of surgery or at death or hospital discharge, whichever occurs first.
- All remaining subjects (that is, all randomized subjects who do undergo surgery within the next 30 days, and do receive at least one RBC transfusion) will remain in the study until one of the following occurs (whichever occurs first)
  - 28 days have elapsed after the end of surgery
  - The subject dies

If the subject (or the subject's guardian) withdraws consent for further follow-up in the study, the subject ends study participation at that time. Note that the subject should still be followed if consent is withdrawn for study transfusions, but consent is not withdrawn for study follow-up.

6.6 Assessment Procedures and Definitions

6.6.1 TRUST Score
The TRUST score ascertains the likelihood of needing allogeneic RBC transfusions during or within 96 hours after surgery. A TRUST score of ≥3 corresponds to a high likelihood of requiring red cell transfusion. All available components of the TRUST score will be collected on the case report form. The TRUST score (or the minimum and maximum possible TRUST scores, if there are any missing components) will be calculated according to the published algorithm (Appendix 1).

6.6.2 Multiple Organ Dysfunction Score (MODS)
The MODS will be calculated from physiologic and laboratory data according to the method used in Hebert's red cell pilot study, an adaptation of Marshall's MODS (Appendix 2) (Hebert 2005).

Pre-operatively (within 30 days prior to the start of surgery):
- If multiple values for platelet count, bilirubin, serum creatinine, Glasgow coma score or the cardiovascular parameters are obtained in the 30 days prior to the start of surgery, in general the value of each of these measurements obtained closest to the start of surgery will be reported. However, if a subject has an RBC transfusion after randomization but before the start of surgery, the measurements done closest, but prior to, the start of the first RBC transfusion after randomization will be reported. If the cardiovascular parameter is a calculation, the individual measurements used in the calculation must be collected at the same time

On each study day, post-operative Day 1 through Day 7, hospital discharge, or death (whichever occurs first):

- At least one platelet count, bilirubin and serum creatinine level must be obtained; if multiple values are obtained on one day, the value of each of these laboratory measurements from blood drawn closest to 7:00 am local time will be reported.
- A Glasgow coma score must be calculated; if multiple scores are calculated on a study day, the score obtained closest to 7:00 am local time will be reported
- At least one of each of the respiratory and cardiovascular parameter assessments must be performed
  - If the parameter is a calculation, the individual measurements used in the calculation must be collected at the same time
  - If there is more than one of a parameter done on a study day, report the value obtained closest to 7:00 am local time.

However, if the surgery which began on Day 0 did not conclude until Day 1, the Day 1 measurements that are reported must be those taken closest to 7:00 am but after the end of the surgery.

If the subject has not been discharged from the hospital by post-operative Day 7, the protocol does not require that the components of the MODS score be assessed after post-operative Day 7. However, for each calendar day from post-operative day 8 through post-operative day 28 or death, whichever occurs first, the components of MODS will be reported if available in the medical record. If multiple measurements of a particular element are available for the same calendar day, the measurement obtained closest to 7:00 am will be reported.

6.6.3 Myocardial Infarction (MI)

For purposes of pre-defined study endpoints, an MI will be defined as having occurred if the Troponin-I value is in excess of 5 times the upper limit of normal per the local laboratory standards, and there are EKG changes which meet the central reading facility's criteria for myocardial infarction. These criteria will be pre-specified by the central reading facility before any adjudication of possible RECESS MI events begins.

All subjects are required to have a pre-operative EKG performed. After surgery and through Day 7, discharge, or death, if a subject experiences an elevated Troponin-I (defined as a value in excess of 5 times the upper limit of normal per local standards) (Thygesen), an EKG is required the first time there is an elevated Troponin-I post-operatively, and after each subsequent elevated Troponin-I if the elevated value is at least 20% higher than the previous Troponin-I value. If an EKG was performed within the six hours prior to collecting the elevated Troponin-I sample, this EKG is acceptable. Otherwise, an EKG will need to be performed within 18 hours of collecting the elevated Troponin-I sample. The two EKGs will be read by a central reading facility, and the central reading facility will determine if the subject has had a myocardial infarction (MI) meeting the study definition. If multiple EKGs are performed within this window, the EKG closest to when the elevated Troponin-I sample was drawn will be submitted to the central reading facility.

Any clinical event which is considered to be a myocardial infarction by the clinical staff caring for the subject will be reported as a Serious Adverse Event on a Myocardial Infarction case report form, whether or not the above definition is met.

Data on locally-defined MI's, as well as data on study-defined MI's, will thus be available.

6.6.4 Renal Failure
Renal failure is defined per the RIFLE (Bellomo) criteria as
- an increase in creatinine to 3 times the baseline level, or
- absolute creatinine $\geq$ 4.0 mg/dl with an acute rise of > 0.5mg/dl, or
- new need for dialysis modality

6.6.5 Culture proven sepsis/septic shock
Sepsis/septic shock is defined as a positive culture from blood and/or CSF with at least 3 of the following SIRS signs (Levy):
- Temperature < 36°C or > 38°C
- Heart rate > 90bpm
- Respiratory rate > 20 breaths/min or $PaCO_2$ < 32 mmHg
- White blood cell count > 12,000 or < 4,000 cell/mm3 or > 10% bands

7. SPECIMEN COLLECTION PROCEDURES
Blood samples will be obtained per institutional protocol and assayed in local laboratory facilities, or at the bedside for the cardiac output measures.

8. ADVERSE EVENT CRITERIA AND REPORTING
Serious adverse events and unexpected adverse events relating to RECESS transfusion arm allocation may overlap events expected during recovery from complex cardiovascular surgery. These events will include, but are not limited to: myocardial infarction, clinical thromboembolic events, reintubation, acute renal dysfunction, serious infections, readmission to the ICU, and death.

Reporting of serious and unexpected adverse events will be consistent with standard TMH CTN procedures described in the TMH Manual of Procedures (MOP), Chapter 6: Guidelines for Reporting Adverse Events. Reporting requirements are calibrated to the seriousness of the event and the perceived relationship to the study intervention (RBC transfusion). For this study the reporting requirements will be based on the type and severity of adverse event.

The TMH CTN will be using the descriptive terminology developed by the National Cancer Institute for use in reporting adverse events: Common Toxicology Criteria for Adverse Events (CTCAE) version 4.0, dated May 29, 2009. The website for the CTCAE is http://evs.nci.nih.gov/ftp1/CTCAE/About.html.. The CTCAE includes a grading (severity) scale for each adverse event term. Grades were developed using the following guidelines:

Grade 1: Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
Grade 2: Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL.
Grade 3: Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self care ADL.
Grade 4: Life-threatening consequences; urgent intervention indicated.
Grade 5: Death related to AE.

In general, investigators should report adverse events as diseases or syndromes whenever possible, instead of reporting individual component symptoms, signs, laboratory abnormalities or sequelae.

8.1 Definitions

Adverse Event (AE) – Any untoward or unfavorable medical occurrence in a human subject, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in the research, whether or not considered related to the subject's participation in the research

Serious Adverse Event (SAE) – Any adverse event temporally associated with the subject's participation in research that meets any of the following criteria:

- results in death;
- is life-threatening (places the subject at immediate risk of death from the event as it occurs);
- requires inpatient hospitalization or prolongation of existing hospitalization;
- results in a persistent or significant disability/incapacity;
- results in a congenital anomaly/birth defect; or
- any other adverse event that, based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the other outcomes listed in this definition

Note that *seriousness* and *severity* are separate concepts. The term "severe" refers to the intensity of a specific event; a severe event may be of minor medical significance (e.g., a severe leg cramp). The term "serious" is based on outcome or action criteria that are usually associated with events that pose a threat to the patient's life or functioning. An event that is mild in severity is serious if it leads to one of the outcomes defined above.

Grade 4 and 5 events will always be considered Serious Adverse Events. Many Grade 3 events and some Grade 1 and 2 events may meet the definition of a Serious Adverse Event.

Unexpected Adverse Event – Any adverse event occurring in one or more subjects in a research protocol, the nature, severity, or frequency of which is not consistent with either:

- the known or foreseeable risk of adverse events associated with the procedures involved in the research that are described in (a) the protocol–related documents, such as the IRB-approved research protocol or the current IRB-approved informed consent document, and (b) other relevant sources of information, such as product labeling and package inserts; or
- the expected natural progression of any underlying disease, disorder, or condition of the subject(s) experiencing the adverse event and the subject's predisposing risk factor profile for the adverse event.

Unanticipated problem involving risks to subjects or others (UP): Any incident, experience, or outcome that meets all of the following criteria:

- unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied;

- related or possibly related to a subject's participation in the research; and
- suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) related to the research than was previously known or recognized.

Attribution – the determination of whether an adverse event is related to a medical treatment procedure.

Attribution categories:
1. Not Related - Event clearly related to other factors (e.g., clinical state, other therapies; concomitant drugs)
2. Possibly Related - Sequence of event is compatible with study drug, device, or procedure, but could have been produced by other factors
3. Probably Related - Sequence of event is compatible with study drug, device, or procedure and cannot be explained by other factors without much doubt
4. Definitely Related - Sequence of event is compatible with study drug, device, or procedure and beyond doubt cannot be explained by other factors

8.2. Types of Adverse Events to be Reported in RECESS

Cardiac surgery patients incur risks from their pre-existing conditions and surgical/anesthetic events. Major risks following cardiac surgery include:
- Damage to any major organ as a result of surgical or anesthetic event
- Life-threatening infections because of indwelling catheters, prosthetic material or pre-existing debilitation. These can be of a bacterial, viral, parasitic, or fungal nature.
- Progression of the underlying pre-existing disease.

All of these events may be severe enough to result in death.

Subjects receiving red cell transfusions incur the following major risks:
- Anaphylaxis
- Acute lung injury
- Volume overload (Transfusion-Associated Circulatory Overload)
- Transmission of infectious disease

All of these events may be severe enough to result in death.

Information about the following events will be collected for RECESS:

1. Serious adverse events, regardless of attribution to red cell transfusion

2. Specific expected adverse events of Grade 2 or higher, regardless of attribution to red cell transfusion

- Myocardial infarction
- Pulmonary embolism
- Stroke
- Renal event
- Infection/sepsis
- Ventricular tachycardia
- Ventricular fibrillation
- Transfusion-associated congestive heart failure/transfusion-associated circulatory overload (TACO)

- Transfusion-related acute lung injury (TRALI)
- Anaphylaxis
- Graft vs. Host Disease (GVHD)
- Allergic reaction
- Sinus bradycardia
- Sinus tachycardia
- Hypertension
- Hypotension
- Dyspnea
- Hypoxia
- Wheezing
- Cough
- Fever
- Chills
- Hemolysis
- Hyperkalemia
- Hyperphosphatemia
- Hypocalcemia
- Hyperbilirubinemia
- Hemoglobinuria.

3. Unexpected adverse events (all grades), attributed as possibly, probably or definitely related to red cell transfusion

4. Unanticipated problems

8.3. Timeline for Reporting of Adverse Events to the DCC

Site personnel must data enter an Adverse Event Checklist eCRF daily for each subject enrolled in RECESS. The Adverse Event Checklist will indicate whether or not one or more of the events listed in Section 8.2 occurred during the previous calendar day on study. The data entry of the checklist will serve as DCC notification of the event. If a site is unable to data enter the checklist on a particular day, they may also notify the DCC of the event within 24 hours of learning of the event by phone or email but must also enter the checklist as soon as possible.

If a subject experiences an event listed in Section 8.2, site personnel must also data enter the appropriate adverse event form within 3 days of learning of the event. Relevant medical records must be sent to the DCC within 5 business days of learning of the event.

8.4. Review of Events for Subjects Enrolled in RECESS

The DCC will contract with two Medical Monitors, with expertise in cardiovascular surgery and transfusion medicine respectively, to review RECESS adverse events.

The Data Safety Monitoring Board (DSMB) is an independent board appointed by the NHLBI. The principal role of the DSMB is to regularly monitor the data from the clinical trial, review and assess the performance of its operations, and make recommendations, as appropriate, to the NHLBI.

The Medical Monitors and the NHLBI will be notified immediately when a serious adverse event possibly, probably or definitely related to red cell transfusion or when an event resulting in death (regardless of attribution) is reported to the DCC. The Medical Monitors will review the event as soon as the materials are available. The Medical Monitors may request additional information regarding the event and may request the subject's treatment arm assignment. Following their review, the Medical Monitors will complete a form summarizing their findings.

A subset of the adverse events that undergo expedited review by the Medical Monitors will also undergo expedited DSMB review after the Medical Monitor review has been completed. This will include

- All serious adverse events that are both unexpected and possibly, probably, or definitely related to red cell transfusion
- All serious adverse events that are possibly, probably or definitely related to RBC transfusion and either result in death or are ongoing at the time a subject dies.

The DSMB Executive Secretary will send information on these adverse events to two designated DSMB members with expertise in cardiovascular surgery and transfusion medicine respectively. These members will review the adverse event materials, determine if the information is complete, determine if additional DSMB review is required, and make recommendations to the NHLBI concerning continuation of the study. The adverse event materials provided to the DSMB members will include all available summaries provided by the Medical Monitor(s) and relevant medical history data, surgical procedure data, clinical notes, medications, laboratory and other test reports provided by the site. .

The Medical Monitors and representatives from the DCC will meet monthly via teleconference. All events reported or updated since the last conference call will be reviewed during the call.

Each month the DCC will send NHLBI and the two designated DSMB members (via the DSMB Executive Secretary) summary information about all adverse events reported or updated since the last monthly report.

8.5. Reporting Events to Local Institutional Review Boards
The investigator must notify the local Institutional Review Board (IRB) of all adverse events at his /her institution, including death, in accordance with institutional policy.

9. INTERIM REPORTING TO SITES, NHLBI AND DSMB

This section describes scheduled reports that will be sent to the study sites and the DSMB. Reporting requirements for events that will be monitored continuously (i.e. all fatal events and all serious adverse events possibly, probably, or definitely related to red cell transfusion) are described above.

9.1. Monthly Reports to Sites

Reports of accrual information, outstanding queries, and protocol violations will be distributed to the sites monthly.

9.2. Monthly Reports to NHLBI and Two Designated DSMB Members

- Summary of monthly medical monitor review of all events reported or updated since last monthly review
- Number of subjects ending study in previous month, and percent of these who died. If this percentage is at least 20%, then the results of an interim look at mortality will be included in the report (see section 10).

9.3. Quarterly DSMB Reports

- Primary study endpoint overall and by treatment arm, with p-value to compare to pre-specified early stopping boundary
- All-cause mortality, with p-value to compare to pre-specified early stopping boundary. Both time from randomization to death and time from cardiac surgery procedure to death will be presented.
- Number of serious adverse events per subject, overall and by treatment arm, with p-value. This p-value will be compared to the p-value boundary from an alpha-spending approach approximating O'Brien-Fleming boundaries. No formal stopping rule is set for this comparison.
- Number of adverse events per subject attributed as possibly, probably, or definitely related to red cell transfusion, overall and by treatment arm, with p-value. This p-value will be compared to the p-value boundary from an alpha-spending approach approximating O'Brien-Fleming boundaries. No formal stopping rule is set for this comparison.
- Proportion of subjects in each treatment arm with at least one serious adverse event possibly, probably, or definitely related to a red cell transfusion, with p-value. This p-value will be compared to the p-value boundary from an alpha-spending approach approximating O'Brien-Fleming boundaries. No formal stopping rule is set for this comparison.

9.4. Semi-Annual DSMB Reports

The DSMB will meet twice a year, either in-person or via teleconference. Reports will include:

- Baseline characteristics overall and by treatment arm
- Primary study endpoint overall and by treatment arm, with p-value to compare to early stopping boundary
- All-cause mortality, with p-value to compare to early stopping boundary. Both time from randomization to death and time from cardiac surgery procedure to death will be presented.
- Number, type, and severity of serious adverse events, overall and by treatment arm, with p-value for comparison of number of serious adverse events per subject. This p-value will be compared to the p-value boundary from an alpha-spending approach approximating O'Brien-Fleming boundaries. No formal stopping rule is set for this comparison.

- Number, type, and severity of adverse events attributed as possibly, probably, definitely related to red cell transfusion, overall and by treatment arm, with p-value for comparison of number of such adverse events per subject. This p-value will be compared to the p-value boundary from an alpha-spending approach approximating O'Brien-Fleming boundaries. No formal stopping rule is set for this comparison.
- Proportion of subjects in each treatment arm with at least one serious adverse event possibly, probably, or definitely related to a red cell transfusion, with p-value. This p-value will be compared to the p-value boundary from an alpha-spending approach approximating O'Brien-Fleming boundaries. No formal stopping rule is set for this comparison.
- Unexpected adverse events, and unanticipated problems overall and by treatment arm
- Site status
- Accrual overall and by site
- Site/study compliance issues

10. SAFETY MONITORING AND STOPPING GUIDELINES

Interim monitoring plans and stopping guidelines will be determined by the TMH DSMB. This section describes proposed plans.

The study is expected to take approximately 4 years to complete, depending on the number of participating sites.

Stopping guidelines are proposed for two outcomes, ΔMODS and all-cause mortality. For these two outcomes, quarterly looks at the data will take place. Therefore, approximately16 looks at the data (including the final look) are anticipated.

In designing the study, a difference in ΔMODS between the treatment groups of less than 1.2 points was judged to be too small to necessitate a major change in transfusion practice. Therefore, there should be a low probability of stopping the trial early if the absolute value of the true treatment difference is less than 1.2 points. However, if the true treatment difference is very large, in either direction, it would be desirable to have a high probability of halting the trial early. At each interim analysis, the p-value for the significance of the difference in ΔMODS between the group randomized to receive shorter-storage RBCs and the group randomized to receive longer-storage RBCs will be calculated. If this p-value is less than 0.000002, then the criteria for halting early will be met. The p-value to declare statistical significance at the final look will be calculated using an alpha spending approach so that the overall type I error will be no more than 0.05. This plan has very low probability of halting early if the true difference is < 1.2 in either direction. This will still be the case even if the standard deviation is smaller than expected (6 rather than 7.3). See Table 2 for the performance characteristics of this plan, based on 10,000 simulations of each scenario. In order to obtain as much data as possible about the possible effects of RBC storage age on clinical outcomes, no stopping boundary for futility is planned. (In other words, even if the treatment groups look so similar at an interim look that it is unlikely that the null hypothesis will be rejected at the end of the study, the study will not halt early for that reason.)

Table 2. Performance of interim monitoring plan for ΔMODS

Number of Interim Analyses	Standard Deviation in ΔMODS	Difference in ΔMODS between groups	ρ = 0				ρ = 0.2			
			Stop Early	Expected Number of Analyzable Subjects Per Arm	Conclude There Is A Difference (Reject H_0)	Do not reject H_0	Stop Early	Expected Number of Analyzable Subjects Per Arm	Conclude This Is A Difference (Reject H_0)	Do not reject H_0
15	7.3	0	0.00%	585	4.76%	95.24%	0.01%	585	4.82%	95.18%
		1	0.84%	584	64.33%	35.67%	0.93%	584	62.33%	37.67%
		1.2	2.41%	582	78.52%	21.48%	2.54%	581	78.44%	21.56%
		1.6	14.93%	563	95.93%	4.07%	13.85%	564	95.99%	4.01%
		2	44.64%	508	99.57%	0.43%	41.79%	514	99.66%	0.34%
		3	97.04%	309	100.00%	0.00%	98.62%	291	100.00%	0.00%
	6	0	0.00%	585	4.81%	95.19%	0.00%	585	5.07%	94.93%
		1	2.95%	581	79.56%	20.44%	3.18%	580	82.43%	17.57%
		1.2	8.34%	572	92.06%	7.94%	9.59%	571	93.72%	6.28%
		1.6	39.70%	516	99.51%	0.49%	44.91%	507	99.73%	0.27%
		2	80.33%	409	99.98%	0.02%	83.39%	400	100.00%	0.00%
		3	99.96%	217	100.00%	0.00%	100.00%	204	100.00%	0.00%
	8	0	0.00%	585	4.84%	95.16%	0.00%	585	5.17%	94.83%
		1	0.39%	585	51.34%	48.66%	0.42%	585	53.54%	46.46%
		1.2	1.60%	583	70.75%	29.25%	1.65%	583	72.70%	27.30%
		1.6	7.91%	574	91.89%	8.11%	9.02%	571	92.63%	7.37%
		2	26.50%	543	98.77%	1.23%	28.57%	539	98.72%	1.28%
		3	91.68%	356	100.00%	0.00%	91.49%	358	100.00%	0.00%

ρ is the correlation between the baseline MODS and ΔMODS

Interim looks for all-cause mortality will be carried out quarterly. In addition, an additional interim look for all-cause mortality will be taken if the number of evaluable subjects who die on study during a particular calendar month is at least 20% of the subjects who ended the study during that calendar month. For example, suppose the quarterly interim looks are scheduled to take place at the beginning of January, April, July, and October of each year. If 50 evaluable subjects end the study in August 2012 (through death, completing the 28-day follow-up, or withdrawing from the study) and 10 or more of these 50 died, an extra interim look for all-cause mortality will be taken at the beginning of September 2012. The 20% "trigger" for extra looks was chosen so that extra interim looks are unlikely if the true 28-day mortality is no more than 7% in each treatment arm because that mortality rate would not be unusual in this patient population. At each interim look for mortality, treatment groups will be compared on time to death, using a Cox proportional hazards model, and data on all evaluable subjects enrolled to date. The p-value stopping guidelines at each interim look, and the p-value to declare statistical significance at the final look, will be calculated using an alpha spending approach approximating O'Brien-Fleming boundaries. These boundaries require a very significant difference to stop very early in the trial and become less stringent as the number of subjects increases. Table 3 shows performance characteristics for this plan, based on 10,000 simulations for the four scenarios where there is truly no difference between treatment arms, and 5,000 simulations for each of the other scenarios. The simulations assume a constant hazard function in each group. For ease of presentation, it is assumed that the group randomized to receive shorter-storage RBCs has a lower mortality rate than the group randomized to receive longer-storage RBCs; since the test will be two-sided, the results would be the same if the longer-storage group has the lower mortality rate.

Table 3. Performance of interim monitoring plan for all-cause mortality.

Number of Interim Analyses	28-Day Mortality Rate in Group Randomized to Receive Shorter-Storage RBCs	28-Day Mortality Rate in Group Randomized to Receive Longer-Storage RBCs	Stop Early	Expected Number of Evaluable Subjects Per Arm	Conclude Different (Reject H_0)	Do not reject H_0
15	7%	7%	3.94%	580	4.95%	95.05%
		8%	6.82%	576	8.72%	91.28%
		9%	18.86%	557	23.30%	76.70%
		10%	37.60%	526	43.80%	56.20%
		11%	57.82%	483	64.82%	35.18%
		12%	77.10%	434	82.34%	17.66%
	4%	4%	3.35%	581	4.52%	95.48%
		5%	9.02%	574	11.16%	88.84%
		6%	26.82%	547	32.76%	67.24%
		7%	50.26%	502	57.88%	42.12%
		8%	74.20%	446	80.04%	19.96%
		9%	89.12%	393	92.80%	7.20%

11.   STATISTICAL CONSIDERATIONS

11.1. Selection of Smallest Clinically Important Treatment Difference

The Multiple Organ Dysfunction Score (MODS) predicts an increase in mortality risk of approximately 15% for every 4-point increase. In a subset of the TRICC data (Hebert 2001) which compared restrictive to standard transfusion strategies in patients with cardiovascular disease, the treatment arm difference in the clinical outcome (ΔMODS) was 1 point with a standard deviation of 7.3. The discussion of the TRICC Trial indicated that a 1 point difference in ΔMODS was not clinically significant. In the ABLE pilot study, Hebert states in his discussion that "absolute differences in major outcomes such as mortality, organ failure and infections less than 3-4% between RBC (ages) may not be worth pursuing" (Hebert 2005).

The smallest treatment group difference in ΔMODS that would justify changing practice is a matter of debate, given the impact a change might have on blood product availability. Therefore, rather than power the study for a particular treatment difference, the sample size was guided by the precision of a 95% confidence interval for the treatment difference.

11.2. Sample Size and Power

The sample size for RECESS was calculated using the following assumptions.
- Precision of a two-sided 95% confidence interval for the treatment group difference of ± 0.85 points or narrower
- Equal sample size in each treatment group
- Baseline MODS are integer values between 0 and 24 with mean 7.8 and standard deviation 3.9. ΔMODS are also integer values with a range that depends on the baseline MODS value and a standard deviation of 7.3 (Hebert 2001).
- No correlation between baseline MODS and ΔMODS. (If these are correlated, the confidence interval will be narrower.)
- The sample size is inflated by 3% to account for interim monitoring, and the adequacy of the inflated sample size was confirmed through simulation studies (refer to section 10)

With these assumptions, the study will need 585 patients per arm who are evaluable for the primary endpoint. Evaluable patients are those who are randomized, undergo cardiac surgery, receive at least one RBC transfusion between randomization and 96 hours after the end of surgery, and who have data available to calculate baseline MODS and MODS through Day 7, hospital discharge, or death, whichever occurs first. Therefore, enrollment is planned to continue until the target of 1170 evaluable subjects have been obtained.

Based on retrospective data from 4 TMH sites on transfusions in cardiac surgery patients with TRUST scores of 3 or greater, it was estimated that 87% of RECESS subjects would receive at least one RBC transfusion. However, practice patterns have changed since the retrospective data were collected, and many hospitals now do not give RBC transfusions to some cardiac surgery patients that might in the past have received transfusions. Based on RECESS data entered in the data management system by July 12, 2011, it is now estimated that approximately 76.7% of randomized subjects will receive at least one RBC transfusion. Therefore, it is estimated that it will be necessary to randomize approximately 763 subjects per arm, or 1526 subjects in all, to obtain the

target number of 1170 evaluable subjects. This number has not been further inflated to take into account patients who are randomized and receive a transfusion but for whom ΔMODS cannot be calculated for the time period extending through Day 7, hospital discharge, or death, whichever occurs first. However, there is expected to be very little if any missing data for this outcome, because the patients will be hospitalized during the entirety of the relevant time period. In the TRICC trial, approximately 1% of subjects withdrew, none were lost to follow-up at 30 days, and less than 1% were lost to follow-up at 60 days (Hebert 2001).

It is estimated that approximately 10% of eligible and consenting subjects will not be able to be randomized, due to lack of inventory or to RBC transfusion before randomization can take place. Therefore, it is estimated that approximately 1696 subjects need to be enrolled to achieve the target number of 1170 evaluable subjects.

11.3. Analysis Plan

Unless otherwise specified, all analyses will be by modified intention-to-treat, analyzing all subjects as belonging to their assigned treatment group, regardless of what storage-duration RBCs they actually received. The modification is that, unless otherwise specified, only subjects who were randomized, underwent cardiac surgery and received at least one RBC transfusion between randomization and 96 hours following the end of the surgery, will be analyzed. Subjects who are not randomized, or do not undergo cardiac surgery within 30 days of being randomized, or who do not receive at least one RBC transfusion, will be excluded from all analyses.

11.3.1 Primary Outcome Analysis

The primary outcome is 7-day ΔMODS. The pre-operative MODS will be calculated for each subject. The follow-up MODS used to calculate 7-day ΔMODS from pre-operative baseline will be based on the worst value of each component of MODS observed through post-operative Day 7, day of hospital discharge, or death (whichever occurs first), even if the worst values occur on different dates (Marshall 1995, Marshall 1997). Analysis of covariance will be used to construct a 95% confidence interval for the difference in 7-day ΔMODS between the two treatment arms, with baseline MODS treated as a covariate in this model. If the ΔMODS has a skewed distribution, attempts will be made to find a suitable transformation that can be performed prior to analysis. In addition, a p-value for the difference between the two treatment arms will be calculated and reported, to assess how likely a difference at least as large as that observed between the two groups could have occurred by chance.

Secondary analyses of the primary outcome will include subgroup analyses by subject's ABO group, by gender, by race/ethnicity, by on-pump vs. off pump surgery, by pediatric vs. adult status, and by whether they were in the ICU prior to randomization. Interaction tests will be performed to determine whether the treatment effect (if any) differs between subgroups.

Exploratory regression analyses will be used to investigate which baseline subject characteristics are associated with large increases in the MODS. In addition, secondary analyses will explore the effect of treatment group on the primary outcome after adjusting for such predictors.

Additional secondary analyses of the primary outcome will be carried out. One will restrict analysis to subjects who only received RBC units in accordance with their

assigned treatment arms. This analysis, however, may be biased. For example, subjects who require a large number of transfusions may be more likely to receive units that are not in accordance with their assigned treatment, and may be more likely to be dropped from this "per-protocol" analysis. Another analysis will address the concern that any effect of storage time may only be important in multiply-transfused subjects, by adjusting for the number of RBC units received and investigating whether there is a significant interaction between treatment group and number of units transfused.

11.3.2 Secondary Outcome Analysis

The secondary outcome of the ΔMODS from pre-operative baseline to worst variables observed through post-operative Day 28 or day of hospital discharge/death (whichever occurs first) will be analyzed in a manner similar to the primary outcome.

A Cox proportional hazards model will be used to compare the treatment groups with respect to time to all-cause mortality, time to first discontinuation of ventilation, days to first bowel movement, and days to first solid food. Baseline MODS will be included in these models. For all-cause mortality, an attempt will be made to contact the subject or his/her family at Day 28 to ascertain vital status. If the subject or his/her family cannot be reached, the subject will be censored at the time of hospital discharge. For the other endpoints, subjects will be censored at the end of the study, which includes hospital discharge, reaching day 28 post-op, or withdrawing from the study. Because some subjects may experience multiple periods of ventilator use, the total duration that they are on a ventilator will also be compared between the two groups using analysis of variance. It is expected that ventilator duration will be non-normally distributed, and an appropriate transformation will be performed prior to this analysis. If no suitable transformation can be found, a non-parametric test will be used.

The secondary outcome "days alive and ventilator-free" will be defined as the number of days the subject was alive (up to a maximum of 28 days after surgery), minus the number of calendar days the subject was on a ventilator at least part of the day. An unadjusted linear regression will be used to compare treatment groups with respect to this outcome. Secondary exploratory analyses will also be done, adjusting for baseline characteristics associated with this outcome.

In addition, an exploratory analysis is planned to determine which baseline patient characteristics are associated with increased mortality, and whether the treatment groups differ in time to death after adjustment for these variables.

Differences between groups for composite of major in-hospital post-operative complications, composite of major cardiac events, composite of major pulmonary events, and ventilation for more than 48 hours (all binary variables) will be analyzed using Fisher's exact tests. In addition, logistic regression will be utilized to determine which baseline patient characteristics are associated with increased risk for each of these outcomes, and whether the treatment groups differ after adjustment for these variables.

For changes in laboratory parameters analysis of variance will be used to compare the treatment groups. The baseline value of the laboratory parameter will be included as a covariate. Appropriate transformations will be performed prior to any analysis. If no suitable transformation can be found, non-parametric tests will be used.

12. DATA COLLECTION AND VALIDATION

Data will be collected and entered into a web-based data management system (DMS) at each site participating in the TMH RECESS Study, and transferred electronically to the Data Coordinating Center. The DMS is programmed to validate all data entry fields as the data are entered. Validations are question-by-question checks that give immediate feedback to help catch data entry errors, form completion errors, and out-of-range values. Reports of outstanding edits, generated upon completion of data entry, will enable continuous cleaning of data at each site.

The DCC will regularly monitor all data for consistency and correctness. If the DCC observes inconsistent data or patterns of protocol violations or missing data, site staff will be contacted immediately to address the finding.

Confidentiality – each subject is assigned a unique number to assure confidentiality. Any publication or presentation will refer to subjects by this number and not by name. The medical records department, affiliated with the institution where the subject receives medical care, maintains all original inpatient and outpatient chart documents. Subject research files will be kept in a locked room or locked cabinet.

Data Management – New England Research Institutes, Inc. will serve as the trial coordinator for this study. THE DCC will monitor timely entry of data into the study database. Access to all source documentation maintained by the Investigator, including correspondence and source data, will be available for monitoring and audit purposes.

Data archives – at all times, appropriate backup copies of the database and related software files will be maintained and the information will be appropriately protected from illegitimate access.

13. PROTECTION OF HUMAN SUBJECTS

This study will be conducted according to Good Clinical Practices (GCP), the rules and regulations of the Institutional Review Board at each participating institution, and in accordance with state and federal agencies.

14. INVESTIGATOR RESPONSIBILITY

14.1 Institutional Review Board (IRB) / Ethics Committee (EC) Approval

No patient will be enrolled in the study until the IRB/EC has approved the protocol and the Informed Consent Form. Documentation of approval must be sent to the DCC. At study termination, a site specific study summary must be submitted by the Investigator to their IRB and to the DCC. Copies of all submissions to and correspondence (approvals and disapprovals) from the IRB/EC must be maintained on file at the study site.

14.2 Informed Consent

If a patient is potentially eligible for the study, based on type of surgery, age, and (for patients ≥ 18 years of age) TRUST score, the patient will be approached to obtain written informed consent. The background of the study and the potential benefits and risks will be explained. The patient or patient's legally authorized representative must sign the consent form that has been approved by the IRB or EC prior to enrollment. Failure to

obtain signed informed consent renders the patient ineligible for the study. Copies of the signed informed consent shall be kept in the patient's medical records and study files.

14.3 Subject Data Protection

Subjects will be identified in the electronic case report form (eCRF) by a subject identification number. All information and data sent to NHLBI or the DCC, concerning subjects or their participation in this study, will be considered confidential. All data used in analysis and reports will be used without identifiable reference to the subject. At all times throughout the study, confidentiality shall be observed by all parties involved. All data shall be secured against unauthorized access. All subjects enrolled in this study will be informed and must agree to the use and disclosure of their study information by the institution and investigators to NHLBI, their agents and representatives, or other review boards.

15. MONITORING and QUALITY CONTROL

15.1 Site Training

Only trained personnel can perform study related procedures. All investigators and relevant staff such as study coordinators and blood bank staff members will be trained on the protocol. These individuals will be trained by DCC representatives.

Each site will be responsible for ensuring that the hospital staff directly responsible for patient care from the pre-operative period to hospital discharge and outpatient follow-up (e.g. ICU nurses, staff nurses and physicians) are adequately trained in the management of these cardiac surgery patients and blood bank procedures.

15.2 Monitoring of the Study

DCC or designee will monitor the investigation to ensure that it is conducted in accordance with the protocol and the following guidelines and standards: ISO 3826, the Code of Federal Regulations and country specific regulations. During monitoring visits, the following documents must be made available for review: all CRFs, all source documents such as medical records and clinic charts and any other study related documents. The DCC intends to monitor each enrolling investigational site at an interval consistent with the enrollment rate. Additional monitoring of the study will continue on a regular basis through written correspondence and/or telephone calls. If a severe protocol deviation is noted, NHLBI or the DCC will recommend corrective action. If there is no response by the Investigator, NHLBI or the DCC will discontinue the investigation at that site and notify the IRB/EC of the site in which the Investigator is conducting the study, if applicable.

15.3 Investigational Site Termination

NHLBI reserves the right to terminate an investigational site from the study for any of the following reasons:

- Repeated failure to complete case report forms
- Failure to obtain Informed Consent
- Failure to report Serious Adverse Events
- Repeated protocol violations

16. RECORDS AND REPORTS

16.1 Case Report Forms

Case Report Forms (CRFs) will be used to collect all subject data during the course of the study. The Principal Investigator or predetermined designated individual shall be responsible for completion of the CRFs. All protocol deviations shall be documented and a justification for any missed assessments shall be provided on the protocol deviation log. Completed CRFs will be verified by the DCC appointed clinical monitor at the site at regular intervals throughout the study. The Investigator will allow the monitor and regulatory bodies to review the study files, subject CRFs, medical records and other study-related documents.

16.2 Source Documents

Good Clinical Practice Guidelines require that investigators maintain information in the subject's medical records, laboratory reports, clinic charts, etc. that corroborate data recorded on the CRFs. In order to comply with these requirements, the following information should be maintained:

- Medical history/physical condition of the subject before enrollment sufficient to verify protocol entry criteria

- Dated and signed notes for specific results of procedures and exams

16.3 Record Retention

NHLBI, the DCC and all participating Investigators must establish and maintain records and reports. The Investigator must maintain the signed Informed Consent Forms, CRFs, study documentation (listed above) and source documents for at least 3 years and 3 months after study completion or termination. In addition, the Investigator must not discard or destroy any study-specific materials unless otherwise instructed by NHLBI or the DCC

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Appendix 1 TRUST Score Calculation (for those patients ≥ 18 years of age)

Transfusion Risk Understanding Scoring Tool (TRUST) Overview (Alghamdi):

Alghamdi, et al. developed the Transfusion Risk Understanding Scoring Tool (TRUST) for identifying cardiac surgery patients who may need blood transfusion.

Parameter	Finding	Points
age of the patient in years	≤ 65 years	0
	> 65 years	1
Gender	male	0
	female	1
hemoglobin	≥ 13.5 g/dL	0
	< 13.5 g/dL	1
body weight in kilograms	≥77 kilograms	0
	< 77 kilograms	1
elective surgery	yes	0
	no (nonelective)	1
serum creatinine	≤ 1.36 mg/dL (120 μmol/L)	0
	> 1.36 mg/dL (120 μmol/L)	1
history of previous cardiac surgery	no	0
	yes	1
surgical task	isolated	0
	Non-isolated (CABG + valve replacement, etc)	1

Total TRUST Score =

= SUM (points for all 8 parameters)

Interpretation:

minimum score: 0

maximum score: 8

The higher the score the greater the probability of requiring a blood transfusion.

Total Score	Risk Group	Probability of Transfusion
0	baseline risk	< 20%
1	low risk	20 - 39%
2	intermediate risk	40 - 59%
3	high risk	60 - 79%*
4 to 8	very high risk	80 - 100% *

X = (0.8377 * (total score)) - 1.9503

probability of blood transfusion = 1 / (1 + EXP((-1) * X))

Appendix 2 Multiple Organ Dysfunction Score (MODS) (Marshall 1997)

	Score				
Organ System	**0**	**1**	**2**	**3**	**4**
Respiratory [a] (P0$_2$/Fi0$_2$ ratio)	>300	226-300	151-225	76-150	≤75
Renal [b] (serum creatinine)	≤100	101-200	201-350	351-500	>500
Hepatic [c] (serum bilirubin)	≤20	21-60	61-120	121-240	>240
Cardiovascular [d] (PAR)	≤10.0	10.1-15.0	15.1-20	20.1-30	>30
Hematologic [e] (platelet count)	>120	81-120	51-80	21-50	≤20
Neurologic [f] (Glasgow Coma Score)	15	13-14	10-12	7-9	≤6

[a] The P0$_2$/Fi0$_2$ ratio is calculated without reference to the use of mode of mechanical ventilation, and without reference to the use or level of positive end-expiratory pressure

[b] the serum creatinine concentration is measured in μmol/L, without reference to the use of dialysis

[c] the serum bilirubin concentration is measured in μmol/L

[d] the pressure-adjusted heart rate (PAR) is calculated as the product of the heart rate (HR) multiplied by the ratio of the right atrial (central venous) pressure (RAP) to the mean arterial pressure (MAP): PAR= HR x RAP/mean BP

[e] the platelet count is measured in platelets/mL 10^{-3}

[f] the Glasgow Coma Score is preferably calculated by the patient's nurse and is scored conservatively (for the patient receiving sedation or muscle relaxants, normal function is assumed, unless there is evidence of intrinsically altered mentation)

ICU mortality by MOD score. White bars indicate development data set. Black bars indicate validation data set.
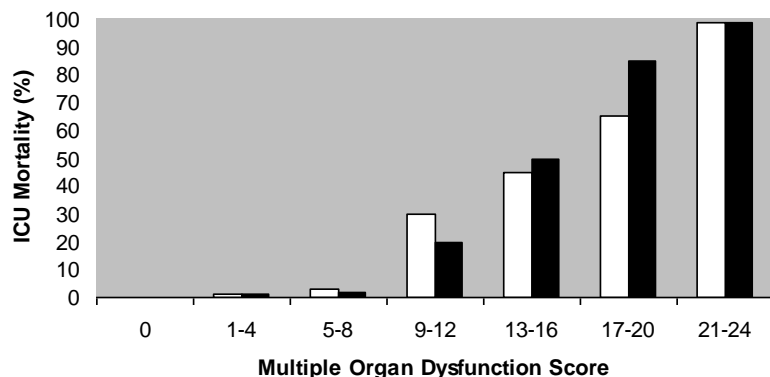
Hospital mortality by MOD score
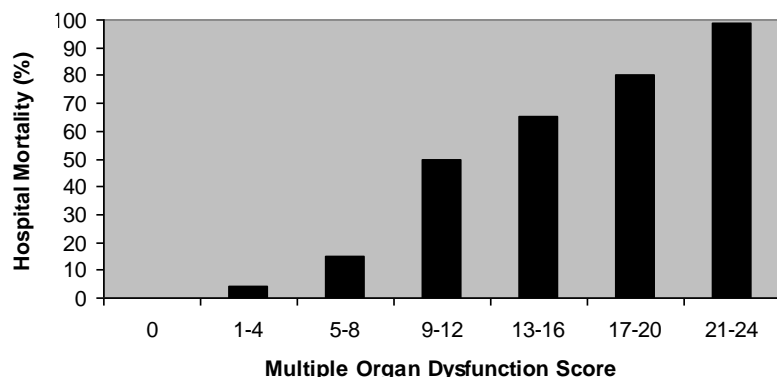
PROTOCOL SIGNATURE PAGE

I have read the foregoing protocol and agree that it contains all necessary details for carrying out this study. I will conduct the study in accordance with the design and specific provisions outlined herein; deviations from the protocol are acceptable only with a mutually agreed upon protocol amendment.

I will provide copies of the protocol and all pertinent information to all individuals responsible to me who assist in the conduct of this study. I will discuss this material with them to ensure they are fully informed regarding the treatment and the conduct of the study.

I will use the informed consent form approved by NHLBI and will fulfill all responsibilities for submitting pertinent information to the Institutional Review Board or Ethics Committee responsible for this study.

I also agree to report all information or data in accordance with the protocol and, in particular, I agree to report any serious adverse experiences as defined in Section 8 of this protocol.

I further agree that NHLBI and their representatives have access to any source documents from which case report form information may have been generated.

I also agree to handle all clinical supplies provided by NHLBI and collect and handle all clinical specimens in accordance with the protocol.

The below signed confirm herewith to have read and understood this trial protocol and/or amendment and appendices; furthermore, to accomplish this study in accordance to the protocol and Good Clinical Practice guidelines, as well as local regulations; and to accept respective revisions conducted by authorized personnel of NHLBI and by competent authorities.

PRINTED OR TYPED NAME(S)   SIGNATURE   DATE

_____   _____   _____
Principal Investigator(s)

_____   _____   _____
Principal Investigator(s)