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PROTOCOL SIGNATURES

This study is intended to be conducted in compliance with the protocol, Good Clinical Practice and the applicable regulatory requirements.

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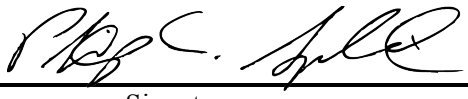

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TABLE of CONTENT

1. GENERAL INFORMATION 1

2. BACKGROUND INFORMATION 1

2.1. CHANGES IN RBCS DURING THE STORAGE PROCESS 1
2.2. PHYSIOLOGICAL EFFECTS ASSOCIATED WITH RBC STORAGE 1
2.3. PEDIATRIC STUDIES EXAMINING RBC STORAGE AGE AND OUTCOMES 2

3. TRIAL HYPOTHESIS AND PURPOSE 2

4. TRIAL DESIGN 2

4.1. PRIMARY AND SECONDARY OUTCOME MEASURES 3
4.1.1. Primary outcome 3
4.1.2. Secondary outcomes 3
4.2. TYPE OF TRIAL 3
4.3. TARGET POPULATION 3
4.4. MEASURES TO AVOID BIAS 3
4.5. RANDOMIZATION 4
4.5.1. Concealment of Randomization 4
4.5.2. Stratification 4
4.5.3. Blinding 4
4.5.4. Trial Treatment and Co-Interventions 5
4.6. STUDY PARTICIPANT DURATION 5
4.7. SELECTION AND WITHDRAWAL OF PATIENTS 5
4.7.1. Screening and consent procedures 5
4.7.2. Eligibility criteria 7
4.7.3. Inclusion criteria 7
4.7.4. Exclusion criteria 7
4.8. DURATION OF TREATMENT PERIOD AND FOLLOW UP 8
4.9. STOPPING RULES OR DISCONTINUATION CRITERIA 8
4.10. PATIENT WITHDRAWAL 8
4.11. SAMPLE SIZE 9
4.12. RECRUITMENT RATE 9
4.13. COMPLIANCE 9
4.13.1. Measures to ensure compliance 9
4.13.2. Compliance with the intervention 10
4.14. RATE OF LOSS TO FOLLOW-UP 10
4.15. TYPE AND FREQUENCY OF ANALYSES 11
4.15.1. Philosophy of analysis 11
4.15.2. Baseline characteristics 11
4.15.3. Intervention and co-interventions 11
4.15.4. Analysis of primary outcome 11
4.15.5. Analyses of secondary outcome measures and subgroup analyses 12
4.15.6. Other statistical analyses and specific considerations 13
4.15.7. Data collected 14
4.15.8. Data Safety Monitoring Board and Interim Analyses 14
4.16. ADVERSE EVENTS/ SERIOUS ADVERSE EVENTS 16

5. SECURITY 19

5.1. COMPLIANCE TO PRIVACY-SECURITY REGULATIONS 19
5.2. ESTABLISHMENT OF CODING 19
5.3. STUDY MONITORING AND STUDY ORGANIZATION 19

5.4. STUDY MONITORING 20
5.5. STUDY ORGANIZATION AND COMMITTEE RESPONSIBILITIES 20
5.6. STUDY ORGANIZATION AND COMMITTEE RESPONSIBILITIES ARE DESCRIBED IN THE MOP. 20

6. *APPENDIX 1: DATA COLLECTION TIMELINE* **21**

7. APPENDIX 2: PARTICIPATING INSTITUTIONS AND PRINCIPAL INVESTIGATORS **22**

PART A: US SITES 22
7.1. 22
PART B: CANADIAN SITES 29
7.2. 29
7.3 PART C: SITES IN FRANCE 30

8. APPENDIX 3: DEFINITIONS OF AE'S/SAES **32**

9. APPENDIX 4: INTENT TO TREAT ANALYSIS **37**

10. PROTOCOL REFERENCES: **40**

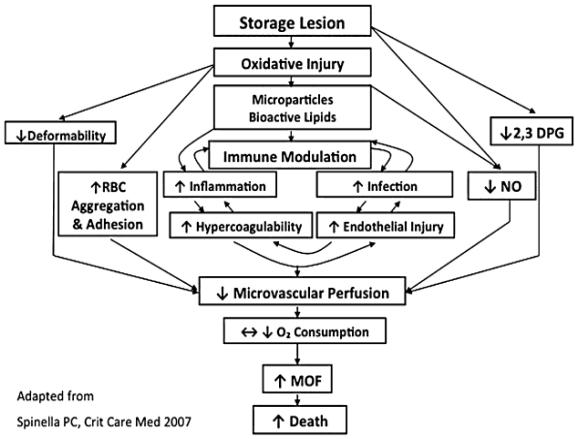
1. General Information

The ABC PICU Trial is a randomized clinical trial (RCT) that will compare the clinical consequences of red blood cell (RBC) storage duration in 1538 critically ill children. Laboratory and observational evidence points to serious concerns about the lack of safety and effectiveness of older RBCs, especially in more vulnerable populations. Physicians and institutions have been systematically transfusing fresh RBCs to some pediatric patients primarily because of beliefs that short storage cells improve outcomes. Conversely, the standard practice of blood banks is to deliver the oldest RBC unit in order to decrease blood wastage. To provide much needed high quality evidence to answer the question "do RBCs of reduced storage duration improve outcomes", ABC PICU will conduct a RCT comparing development of New or Progressive Multiple Organ Dysfunction Syndrome (NPMODS) in critically ill children transfused with either RBCs stored ≤ 7 days or standard issue RBCs (expected mean RBC storage duration of 17-21 days).

2. Background Information

2.1. Changes in RBCs during the storage process

Blood procurement agencies determine the upper limit of RBC shelf life based upon a mean hemolysis of less than 1% (0.8% in Europe) and having > 75% of transfused circulating RBCs still alive in healthy volunteers 24 hours after transfusion[1, 2]. These regulations do not consider the numerous biochemical, structural, inflammatory, and physiologic changes that occur in stored RBCs in proportion to storage duration, sometimes referred to collectively as the ''RBC storage lesion", which are indisputable, have been extensively described and may be deleterious [1-10]. Biologic pathways and potential causes of the RBC storage lesions are displayed in the adjacent figure 1[11].



2.2. Physiological effects associated with RBC storage

Plausible mechanisms linking prolonged RBC storage to adverse effects in critically ill patients are shown in the figure above and include decreased microvascular perfusion, abnormal rheologic properties, increased recipient inflammation[12,13] , increased production of oxidative injury mediators, hypercoagulability[11, 14], decreased tissue oxygenation, and decreased tissue perfusion and oxygen consumption[12, 15-25]. Many of these could have substantial impact in the critically ill. One very important physiologic impact relates to changes in microcirculatory flow, tissue oxygen delivery and utilization. RBCs play a role in oxygen-dependent vasoregulation[26, 27]. RBC storage affects intra-erythrocyte hemoglobin in banked RBCs which can impair RBC vasodilatory capacity in the microvasculature, which in turn can compromise regional oxygen delivery[27-30]. Impaired microcirculation can also result from increased generation of thrombin and procoagulant phospholipids which are increased in leukoreduced RBC units with prolonged storage time[18-20]. The combination of significantly impaired microcirculatory dysfunction and decreased RBC deformability may dramatically affect tissue oxygen delivery in patients with sepsis and septic shock. Another important physiologic impact of transfusion in the critically ill is its potential immunomodulatory effect[39,50-54]. Indeed, prestorage leukoreduced RBC s with

increased storage times have been associated with immune effects that can be pro- and anti-inflammatory[31-36].

Immunosuppressive effects of RBC transfusions have been documented by an increase in live births in women with a history of spontaneous abortions and increased post-operative infection risks[12, 37, 38] as well as in reports describing transfusion-related immune modulation[31, 33, 35, 39]. Conversely, pro-inflammatory effects have also been attributed to bioactive lipids which accumulate with RBC storage and increase risk of acute lung injury[12, 22, 40, 41].

2.3. Pediatric studies examining RBC storage age and outcomes

RBC transfusions are a frequent event in critically ill children. In Bateman et al, almost 50% of critically ill children in PICU for more than 2 days received at least 1 RBC transfusion[42]. There are few clinical studies that adequately explore the clinical consequences of prolonged RBC storage – and there have been no RCTs performed in children Most of the published data on the effect of RBC storage time in children has emanated from our group in two manuscripts that report an independent association between transfusion of RBC unit(s) with > 14 days of storage and increased risk of MODS[43, 44]. Karam et al[43] published a secondary analysis of a prospective descriptive transfusion study conducted in 29 North American PICUs that included 296 transfused children with a PICU length of stay > 48 hours. An independent association was measured between transfusion of ≥ 1 units of RBCs stored > 14 days and the primary outcome NPMODS, with an odds ratio of 1.87 (95% confidence interval, 1.06-3.31, p=0.03); an adjusted increase in PICU length of stay (+3.7 days, p<0.001) was also observed. Another study from our group, an analytic cohort analysis involving 455 transfused children enrolled in the (Transfusion Requirements in Pediatric Intensive Care Units (TRIPICU) RCT[45], showed that transfusion of RBCs stored > 14 days was independently associated with increased NPMODS after adjusting for multiple variables at randomization. When all transfused patients were analyzed, the adjusted odds ratio for NPMODS was 2.3 (95% confidence interval, 1.24-4.28, p<0.05). No cause and effect relation could be established between RBC storage time and development of NPMODS in either study due to uncontrolled confounding by indication. An association between increased RBC age and mortality was not measured because mortality was too low (< 5%) in both cohorts[43, 44].

3. Trial Hypothesis and Purpose

We hypothesize that transfusion of RBC units stored for ≤ 7 days to critically ill children will reduce the number of patients developing NPMODS by at least 6% (33% relative risk reduction), from 18% in children receiving standard issue RBCs to 12% in the short storage group.

We have adopted a pragmatic approach to all design elements and have engaged a wide variety of pediatric hospitals in Canada and the US, with the potential to add sites from additional countries. There are minimum restrictions in patient eligibility, no controls on clinical practice and have chosen clinically important outcomes. The purpose of the ABC PICU Trial is to determine if the transfusion of RBCs ≤ 7 days will improve outcomes compared to the transfusion of standard issue RBCs. Results of the ABC PICU Trial will be generalizable since it is comparing an intervention with standard care.

4. Trial Design

4.1. Primary and secondary outcome measures

4.1.1. Primary outcome

The primary outcome measure of this RCT is NPMODS defined as the proportion of patients who die during the 28 calendar days after randomization or who develop NPMODS. For patients with no organ dysfunction at randomization, New MODS is the development of ≥ 2 concurrent organ dysfunctions during the 28 calendar days after randomization. For patients with 1 organ dysfunction at randomization, New MODS is the development of at least 1 other concurrent organ dysfunction after randomization. Patients with MODS (i.e. concurrent dysfunction of ≥ 2 organ systems) at randomization can develop Progressive MODS defined as development of at least 1 additional concurrent organ dysfunction at during the 28 calendar days after randomization. All deaths will be considered Progressive MODS. NPMODS will be monitored up to 28 calendar days or PICU discharge because it is almost never observed beyond this time in children[46].

4.1.2. Secondary outcomes

Clinically important secondary outcomes will include 28-day, and 90-day all-cause mortality. Nosocomial infections will be recorded, including nosocomial pneumonia and blood stream infection. Other secondary outcomes include PELOD2 score, severe sepsis, septic shock, acute respiratory distress syndrome (ARDS), mechanical ventilation, ICU free days and transfusion associated delirium using the Cornell Assessment for Pediatric Delirium (CAPD) with the exception of Canadian participating centers. We are not requesting to add this secondary outcome at Canadian sites due to the reduced reimbursement per patient in Canada. Site coordinators are already providing more effort than they are being reimbursed, therefore it is not feasible to add an additional outcome measure at these sites.

4.2. Type of Trial

The ABC PICU Trial is a multicenter international, pragmatic, double-blind, superiority, two-arm RCT, in 1538 critically ill children, comparing the risk of NPMODS between patients transfused RBCs of decreased storage age (length of storage ≤ 7 days) and those transfused standard issue RBCs (stored 2-42 days with a documented average length of storage of about 17-21 days).

4.3. Target Population

Based upon previously published data, for critically ill children who are expected to be in a ICU for > 24-48 hours and transfused, we expect the incidence rate of NPMODS to be 18% in the control and 12% in the experminental group.

4.4. Measures to Avoid Bias

Patients will be randomized to receive either standard issue RBCs or RBCs stored for ≤ 7 days. This intervention will be maintained in effect until 3 possible events, whichever occurs first: 1) 28 calendar days elapse post-randomization; 2) hospital discharge; or 3) death.

All RBC units will be prepared in accordance to existing local and national standards and collection and expiration date will be recorded by blood bank personnel not involved in the ABC PICU Trial as site investigators. While the decision to transfuse RBCs will not be protocolized, a summary of the current literature with guidance on transfusion thresholds is provided in the MOP. All management decisions will be at the discretion of the clinical team. Whenever

possible, during the first 28 calendar days after randomization, only RBCs with shorter storage times will be given to patients allocated to the "short storage" arm; we recognize that this might be impossible in specific situations. Because the number of patients per site in the "short storage" arm of the trial will be small (1-3 patients per month), this trial will not affect the age of RBC units available to patients allocated to the usual care arm; this will be monitored during the trial by the Data and Safety Monitoring Board (DSMB) from data collected on total inventory RBC age at selected sites.

4.5. Randomization

The randomization process will consist of a computer-generated random listing of treatment allocation using a pre-established algorithm. The blood bank will contact the web randomization system for any eligible patient meeting inclusion and exclusion criteria. Time zero will be the time a patient is randomized to one arm of the study within the web randomization system (a date and time will be provided for each patient). Allocation will be in a 1:1 ratio. Randomization will be stratified by center and age. The central randomization system will be a web-based automated system that will require confirmation of patient age and will have a backup in the form of an on-call statistician or designate at the Data Management Center (DMC) or Data Coordinating Center (DCC). Only the study statistician and DMC/DCC designate will have knowledge of randomization codes. Once a patient is randomized, an automated notification will be sent back by email to the blood bank.

4.5.1. Concealment of Randomization

Research personnel, ICU staff and other caregivers will not have access to the randomization schedule, nor to the allocation of participating patients. In order to conceal future allocation, 3 sizes of blocks permutation (2, 4 and 6 patients/block) will be randomly used.

4.5.2. Stratification

Patients will be stratified at randomization according to center and age (≤ 28 days (as assessed by 28 days after the day of birth), 29 to 365 days, and ≥ 1 year).

Stratification by site and age will be employed since the possibility for unbalanced treatment allocation is possible given the diversity in case mix within each of the participating ICUs. The stratification tree will consist of 6 branches per center; two treatment strategies ("short storage" or "standard delivery" RBC units) and three age groups.

Blocking will also be employed. Each stratification tree will contain multiple fixed blocks of 2, 4 or 6 patients per block. An equal number of patients will be assigned to one of two treatments within each block, with the order of blocks (2, 4 or 6 patients per block) and the order of treatment assignment within blocks selected at random according to the computer-generated randomization scheme. This approach ensures good randomization concealment.

4.5.3. Blinding

Blinding will be used in the allocation process (concealment of randomization). Physicians, nurses, other caregivers and research staff will not be given any information regarding individual entries from the computer generated random list. To blind clinicians and research personnel from the treatment group patients were allocated to, opaque stickers will be put on expiration dates on the labels affixed to bags of RBC units; this will be done in the blood bank before any RBC unit is delivered to a patient participating in the ABC PICU Trial. The procedure for blinding expiration dates is in the MOP. Other important patient identifiers and blood grouping will not be masked from the clinical team. Accidental un-blinding of the expiration date of the unit of RBCs

will be documented and reported via the protocol deviation process. The data of study patients with accidental un-blinding will be included in the intent to treat analysis. The incidence of accidental un-blinding will be reported within the trial manuscript.

The use of a removable non-defacing label is necessary in case the unit that is sent to the study patient is not used and returned to the blood bank for use in another non-study patient. For local paperwork which accompanies the RBC unit, sites may use any method they see fit to conceal the dates on these locally generated documents. Only ABC PICU study approved opaque stickers may be used on RBC units.

The diagnosis of NPMODS and the determination of the PELOD score will be done by research assistants who will be kept unaware of treatment allocation. All statistical analyses done at the end of the trial will be done without knowing treatment allocations. Dummy codes will be used to designate treatment allocation.

4.5.4. Trial Treatment and Co-Interventions

The 2 trial treatments are either RBCs ≤7 days of storage or standard issue RBCs. This period of eligibility is the first 7 days after ICU admission. Patients who require a RBC transfusion after 7 days from ICU admission will not be eligible to participate in the trial. All blood products including the RBCs used in this trial will be supplied by the hospital blood bank according to local and national regulations. Only pre-storage leuko-reduced RBC units will be used in this trial. Since RBCs are collected, processed and stored in multiple methods and solutions we will record each of these parameters for all RBCs transfused during the intervention period. Major co-interventions including use and volume per kg of all blood products (including frozen plasma, platelets, and cryoprecipitate). Other co-interventions that will not be protocolized due to the pragmatic nature of the trial but that will be monitored include fluid balance per day, and the proportion of patients receiving erythropoietin, vasoactive drugs, mild to moderate hypothermia treatment, systemic corticosteroids, insulin, starch colloids and/or gelatins and other events (plasmapheresis and Molecular Adsorbent Re-Circulating System or MARS). Data on these co-interventions will be collected daily up to 28 calendar days or ICU discharge or death so that the effect of any imbalances on the primary outcome can be examined after the trial is completed.

4.6. Study Participant Duration

Patients who are randomized remain in either study group for 28 calendar days. All randomized patients will be followed for a total of 90 days for both mortality and ICU readmission. All other outcomes will be measured within the first 28 calendar days after randomization or until ICU discharge or death.

For CAPD scoring, patients will be evaluated by clinical providers prior to every transfusion and twice a day for 72 hours following each transfusion for 28 calendar days post randomization or 72 hours following the last study transfusion, whichever is latter.

4.7. Selection and Withdrawal of Patients

4.7.1. Screening and consent procedures

Patients will be screened, and consented for randomization via three main scenarios in this trial (See figure 2 below; other scenarios may occur):

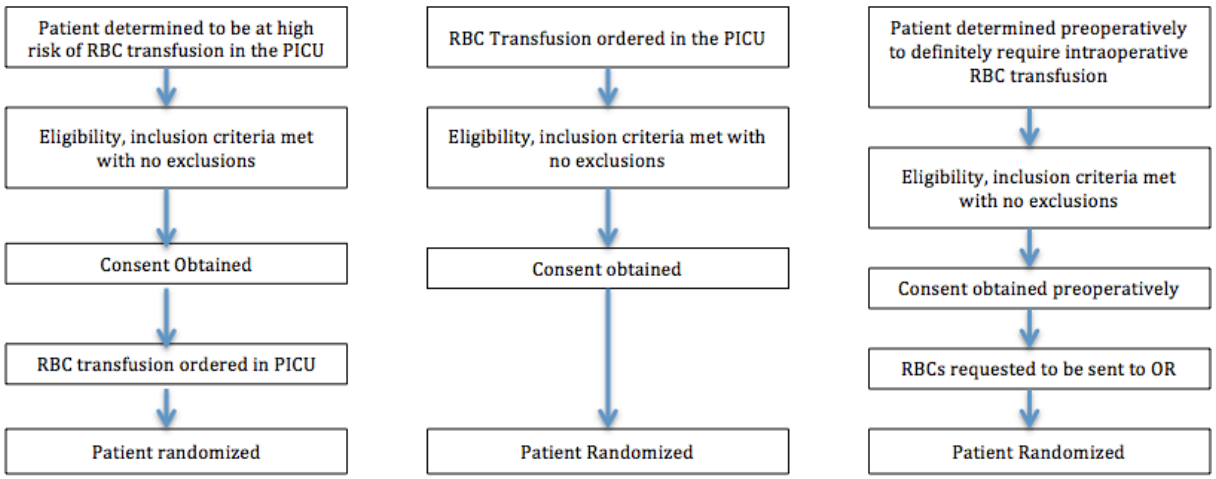
1. First scenario: Patients at high risk for RBC transfusion in the ICU.
    a. We will monitor clinical status and laboratory hemoglobin values (see MOP for

guidance on determining high risk of transfusion).
   b. Research staff will verify eligibility, inclusion and exclusion criteria.
   c. If patient meets all criteria, consent will be obtained.
   d. Then, if RBC transfusion is ordered in the ICU within first 7 days after admission, the patient will be randomized.

2. Second scenario: RBC transfusion is ordered in the ICU when the PICU clinical team is involved in the care of a patient:
   a. Research staff will verify, inclusion and exclusion criteria.
   b. If patient meets all criteria, consent will be obtained.
   c. Then, patient will be randomized.

3. Third scenario: Patient for whom ICU admission is requested post-operatively and who definitively requires a RBC transfusion intra-operatively:
   a. Research staff will verify eligibility, inclusion and exclusion criteria.
   b. If patient meets all criteria, consent will be obtained pre-operatively.
   c. When RBCs are requested in the OR in preparation for surgery, the patient will be randomized.

Figure 2: Screening, eligibility, consent, and randomization scenarios



In order to detect potential selection bias, a screening log will be maintained at each site to record the number of eligible patients who were not randomized and reason for their exclusion. Screening and assessment of study eligibility will be reassessed daily for a total of 7 days from ICU admission or from the moment the ICU team assumes care responsibilities for the patient. This period of eligibility is justified because the rate of New or Progressive MODS is low after 7 days in ICU (< 2%)[46]. A patient discharged from ICU and subsequently readmitted after > 24 hours will be considered a new ICU admission. Therefore, if a patient is readmitted > 24 hours after ICU discharge and has not been previously been enrolled and randomized in the ABC PICU Trial he will be eligible for the first 7 days of this new admission.

Written informed consent will be required prior to randomizing a patient in the ABC PICU Trial. Assent should be obtained from the child according to the REB/IRB requirements at each site.

4.7.2. Eligibility criteria

A patient will be entered in the screening log and considered eligible to participate in the trial if one of the following occurs:

1. A first RBC transfusion is requested within the first 7 days (168 hours) of PICU admission.
   OR
2. A patient assessed pre-operatively and for whom ICU admission is planned post-operatively, and who is determined to definitively require a first RBC transfusion during surgery.

Patients will be considered for randomization if they meet all inclusion criteria and have none of the exclusion criteria.

4.7.3. Inclusion criteria

Eligible critically ill pediatric patients who have an expected length of stay after transfusion in the ICU > 24 hours based on the best judgment of the attending ICU staff.

4.7.4. Exclusion criteria

Patients who meet one of the following criteria will be excluded:

1. Age at time of enrollment < 3 days from birth or has reached their 16th birthday
2. Post-conception age < 36 weeks at time of enrollment
3. Documented RBC transfusion within the 28 days prior to fulfilling the eligibility criteria
4. Previously randomized in this study
5. Weight < 3.0 kg at ICU admission
6. Known pregnancy
7. Conscious objection or unwillingness to receive blood products
8. Not expected to survive beyond 24 hours, brain death or suspected brain death
9. Limitation or withdrawal of care decisions have been made
10. Enrollment in another randomized clinical trial which has not been approved for co-enrollment
11. Patients for whom autologous and/or directed donation RBCs will be provided
12. Patients for whom the treating physician routinely and systematically requests RBC ≤ 14 days of storage
13. Patients for whom there systematically exist RBC aliquoting policies that mandate the initial use of units stored ≤ 14 days (ex: Pedi-Pack).
14. On ECMO or plan to be immediately placed on ECMO at time of enrollment
15. Patient predicted or presumed to require a massive transfusion according to treating physician judgment.
16. Refusal by physician
17. Inability to obtain consent
18. Blood bank personnel experiences difficulties in securing blood products (difficult cross matches, rare blood groups and diseases like IgA deficiency)
19. Insufficient number of ABO type compatible RBC units available in the blood bank at randomization with a storage time ≤ 7 days (minimum 1 unit regardless of patient age)
20. All RBC units available for the patient are not leukocyte-reduced prior to storage

Exclusion criteria # 1 to 17 will be ascertained by the research staff with the assistance of the attending ICU team. Exclusion criteria # 18 to 20 will be ascertained by blood bank personnel.

4.8. Duration of treatment period and follow up

Proposed duration of treatment (study intervention) and follow-up for the primary outcome NPMODS is 28 calendar days following randomization or up until ICU discharge or death. NPMODS will be monitored daily for the first seven days following randomization and then weekly until 28 calendar days or ICU discharge and through ICU stay. Follow up will occur at 90 days to determine mortality and ICU readmission rates.

4.9. Stopping Rules or Discontinuation Criteria

The stopping rules for the trial will be based upon the interim analysis or due to safety concerns as assessed by the DSMB. We are planning to do one interim analysis while conducting the ABC PICU Trial once 50% of patients have been accrued. The interim statistical analysis will compare NPMODS rates in the "short storage" and the "standard issue" transfusion strategy groups, using O'Brien and Fleming's stopping rules, with a two-tailed p value. The p-values for the 2 looks (1 interim + 1 final) will be 0.003051 and 0.046946; upper and lower boundaries of these z values are ±2.96259 and ±1.96857. The data and safety monitoring board (DSMB) of the ABC PICU Trial may consider terminating enrollment if the statistical analysis described above shows a statistically significant difference. Both positive and negative findings from the ABC PICU Trial will be considered of clinical interest.

4.10. Patient withdrawal

Study patients will be able to withdraw from the trial at any time. The data collected for all patients until the time of study withdrawal will be analyzed unless it is requested in writing that all data prior to study withdrawal is not to be analyzed. Despite the very low patient withdrawal rate expected, secondary analyses will be performed to understand the influence of withdrawals on the robustness of the intention-to-treat analysis of our primary outcome. The general approaches will include a best-case, worse-case scenario for all patients lost to follow-up or voluntarily withdrawing from the study prior to the 28-day follow up period. To accomplish this for patients with missing NPMODS data, we will first, in a best-case scenario, assume that none developed NPMODS and then, for the worst-case scenario, we will assume that every patient with missing NPMODS data did develop NPMODS. For both scenarios NPMODS will be compared between both treatment groups just as described for the primary analysis.

4.11. Sample Size

Table 1: Estimates for the absolute risk, reduction expected in ABC PICU

	RCT Gauvin et al [44]	Epidemiologic study – Karam et al [43]
RBC storage time cutoff	7 days	7 days
PICU expected LOS	> 24 hours	> 48 hours
Hemodynamic unstable patients	No	Yes
Percentage of NPMODS in all transfused patients	15%	39.2%
Odds ratio for development of NPMODS in older vs fresher (confidence interval)	1.39 (0.42-4.61)	1.54 (0.80-2.96)
Estimated risk in experimental group 1/OR =(p1/(1-p1))/(p0/(1-p0))	11%	22%

Patient eligibility criteria and short storage definition for RBCs in the ABC PICU Trial will be similar to those used in our preliminary studies (Table 1). [43,44] As a result we expect the incidence of NPMODS and estimated Relative Risk Reduction (RRR) to be accurate based on these prior results. When compared to our previous studies, the definition of short storage (7 day cut off) is the same in the ABC PICU Trial as that in these prior studies. [43,44] ABC PICU Trial inclusion criteria of ICU Length Of Stay (LOS) > 24 hours after transfusion is the same it was in the Gauvin study[44] (Karam study used PICU LOS > 48 hours), whereas the ABC PICU Trial inclusion of hemodynamically unstable patients will be the same as patients in the Karam study[43] (Gauvin study only included hemodynamically stable patients). The incidence of NPMODS ranged between 15-39%. Based on these results we have conservatively chosen to use an expected incidence of 18% for NPMODS in the control group and 12% in the treatment group for a RRR of 33%. According to these estimates, the sample size required for the trial is 1538 participants. The ABC PICU Trial Steering Committee, CCCTG and PALISI Networks support our above estimates and the choice of a 33% RRR because it is considered clinically important and sufficiently significant to change practice. Sample size calculations were estimated based on the above estimates and the formula for two independent proportions (chi-square) using a two-tailed α of 0.05 and a (1-β) of 0.90. While we expect that "short storage" RBCs are superior to standard stored RBCs, we cannot rule out that it may be inferior and thus have incorporated a conservative two-side alpha. Minor attrition at 1.7% is expected based on results of the TRIPICU study. To account for possible randomization failures, a conservative 1.7% non-compliance factor was incorporated in the sample size calculation.

4.12. Recruitment rate

Conservative assessment of estimated recruitment shows that we can expect to enroll an average of 1.5-2 patients per center per month. With up to 30 sites actively recruiting at this rate, the ABC PICU Trial patient recruitment will be completed within 36-48 months. We are seeking support from several sites in Europe to maximize recruitment. The trial should be completed within 5 years including study preparation and data analysis time.

4.13. Compliance

4.13.1. Measures to ensure compliance

The conduct of the trial depends on blood banks dispensing appropriately aged RBCs to patients in both study arms. As required, a record of patients actively participating in the ABC PICU Trial will be kept at each participating blood bank in order to deliver RBC units with the shortest storage time possible to patients in the short storage arm; sites with automated blood bank management systems, such as Traceline, will not require this. Several measures will be instituted to highlight trial participation and to maximize compliance. We have: 1) ensured full

participation of Transfusion Medicine/blood bank directors by having them as co-site investigators, and by including transfusion medicine expertise on all important trial committees; 2) developed institute-specific protocols for inventory management of RBC units for the trial and tracking of patients enrolled in the trial; 3) organized meetings prior to trial initiation at every site; 4) in some sites, we will monitor RBC supply to ensure adequate supplies are available for randomization; 5) conduct audits on the age of RBCs entered in the system to identify specific sites that may have issues to address; 6) implement mechanisms to allow for appropriate RBCs (short storage vs. standard issue) to be transferred from other local sites or from blood providers; 7) sequestered additional RBC units to ensure an adequate supply of short storage issue units for specific blood groups following randomization and allocation of patients to a study arm.

4.13.2. Compliance with the intervention

For the purpose of this study, patients in the "short storage" arm of the study will be considered adherent to protocol if 80% or more of transfused RBCs were stored ≤ 7 days and they receive no RBC unit stored > 14 days during the 28-day period follow-up period. Patients who have compliance rate less than 80% will be considered non-compliant and as such will be removed from the "per-protocol" primary analysis. The derived proportion will be used in a logistic regression model to assess the effect of compliance on outcome. It will rarely occur that a patient in the standard issue arm receives "short storage" RBCs; this may happen, for example, if the oldest available RBCs in inventory happen to be ≤ 7 days old or in a patient randomized to the standard issue arm who requires unplanned cardiac surgery after ICU admission and for whom a specific request is made for fresh RBCs (≤ 7 days old). These patients will also be considered as non-compliant. The reverse situation, i.e. any patient who goes from the "short storage" arm to standard issue arm, will be considered to fulfill criteria of non-adherence to protocol as described above.

A secondary analysis will be conducted on an intent-to-treat basis. Using this approach, patients allocated to the standard issue arm who are intentionally or unintentionally moved to the "short storage" arm of the study, will be analyzed as being in the standard issue group. We will also identify the rationale for the non-compliance in each instance.

The clinical team can administer any available RBCs regardless of storage time for patients who become unstable and have transfusion requirements that do not allow for adherence to the short storage arm of the study. The decision to withhold or to withdraw critical care will not be considered an exclusion criterion if taken after patient entry in the trial. These cases will be kept in the intent-to-treat analysis.

4.14. Rate of loss to follow-up

Loss to follow-up is defined as any patient who is lost after randomization, but before data analysis is performed, and for whom no information is available on the primary outcome (for example, a lost medical record). We will count the number of losses to follow-up.

It is expected that the number of losses to follow-up will be very low because most patients will remain in the ICU or in the hospital during the entire extent of their participation into the trial. Therefore, rate of loss to follow-up should be very low, and hospital outcomes should be easy to collect. We expect a 1.7% loss-to-follow-up. Mortality outcomes after hospital discharges will be determined by hospital, state or province registries or by telephone/email contact with the family.

However, some dropouts may happen. If there is an imbalance between the number of dropouts in both arms, we will undertake a sensitivity analysis, using an intent-to-treat approach and a

worst-case scenario where all the surplus in losses and dropouts in one arm will be allocated to the other arm.

4.15. Type and frequency of analyses

4.15.1. Philosophy of analysis

As this is an effectiveness trial, all statistical analyses will be based on an intention-to-treat (ITT) approach per Appendix 4. All participants randomized will be analyzed according to the intervention to which they were allocated, regardless of whether they received it or not. All p values will be reported as two-sided. Hypothesis testing for the primary analysis will be carried out with an overall level of significance set using a p value < 0.05, taking into account one interim analysis. All statistical analysis will be conducted under the supervision of Dean Fergusson (epidemiologist and biostatistician, Ottawa Hospital Research Institute) with input and collaboration from Thierry Ducruet (biostatistician from the Unité de Recherche Clinique Appliquée (URCA) of the Research Centre at Sainte-Justine Hospital) and Kenneth Schechtman (Washington University in St-Louis).

4.15.2. Baseline characteristics

Baseline characteristics of patients, intervention and co-interventions in both study arms will be assessed using frequency distributions and univariate descriptive statistics including measures of central tendency and dispersion. This analysis will also include blood storage duration. Means (± standard deviation) will be used to report central tendencies of data that can be reasonably approximated using a normal distribution, whereas medians (inter quartile ranges) will be used to report data with non-normal distributions. Percentages will be reported for categorical data. Any clinically relevant imbalances may be considered for adjusted analyses of primary and secondary outcomes. Baseline characteristics will be reported in a table.

4.15.3. Intervention and co-interventions

Post-randomization characteristics of our intervention (short storage vs standard issue RBC units) and major co-interventions such as other blood products (platelets, plasma), fluid balance, etc) in the ***two*** treatment arms will be presented using frequency distributions with measures of central tendency and dispersion and analyzed using relative risks & 95% confidence intervals for dichotomous data (e.g. proportion transfused with platelets) and Wilcoxon-Rank Sum tests for difference in continuous data (e.g. difference in median platelet use). These characteristics will be reported in a table.

Primary statistical hypothesis

The principal research question is whether RBC storage time affects outcome in critically ill children. The primary outcome measure of the ABC PICU Trial is New or Progressive MODS. We hypothesize that transfusion of RBCs stored for ≤ 7 days (compared to standard issue RBCs) will result in a reduction in New or Progressive MODS in critically ill children. As compared to standard issue RBCs (length of storage 2-42 days) we expect to find that RBCs stored for ≤ 7 days decrease the incidence of New or Progressive MODS by an ARR of 6% (from 18% to 12%) and a relative risk reduction of 33%.

4.15.4. Analysis of primary outcome

The analysis of the primary outcome measure will be conducted on an “intent-to-treat” basis with all patients randomized in the ABC PICU Trial.

The principal analysis, i.e. the influence of treatment groups (“short storage” versus standard

issue) on the primary outcome, will be compared using an absolute relative risk estimate with 95% confidence intervals.

Secondary analyses of the primary outcome include a logistic regression model to further elucidate the measure of effect while adjusting for known prognostic risk factors. For adjusted models, risk factors such as site, age, co-morbid illnesses, and severity of illness scores will be added to all logistic models based on clinical (not statistical) rationale. Continuous risk factors (e.g. PRISM III, number of transfusions per patient) will be entered into the models as a continuous measure rather than categorical to improve statistical efficiency. Regression diagnostics will be performed on all models. Odds ratios will be estimated from coefficients and confidence intervals will be constructed using Robbins-Greenlands procedures. We will also compare Kaplan-Meier curves using a log rank test followed by proportional hazards modeling for NPMODS rates: this analysis will compare the length of time between randomization and appearance of NPMODS.

4.15.5. Analyses of secondary outcome measures and subgroup analyses

Secondary outcome analyses

As with our primary outcome, secondary outcome measures will be analyzed by an "intent-to-treat" approach. The effect of treatment on dichotomous secondary outcomes will be compared by calculating absolute relative risks followed by logistic regression procedures to adjust for important prognostic risk factors. Continuous outcome measures such as the PELOD score, mechanical ventilation and ICU free days, will be analyzed using either parametric procedures (independent t test) or non-parametric procedures (Wilcoxon Rank Sum).
In keeping with our philosophy of study design and analysis used in other large pragmatic peer-reviewed clinical trials, we have chosen to not adjust for multiple testing, for the nine secondary outcomes. The primary rationale is twofold. First, we have placed considerable effort in selecting and defending our primary outcome of NPMODS and our secondary outcomes including their clinical importance, biological plausibility, and disease pathway. Our primary and secondary outcomes are explicit in the protocol, trial registration and will be made explicit in the publication of the trial protocol and primary publication of the results. As such, the number and nature of our outcomes are transparent.

Secondly, the choice of adjustment for multiple comparison is not straightforward (investigator set vs Bonferroni vs Hochberg vs Hommel etc) and, more importantly, clinicians who interpret the results will be confused by results that have different levels of significance presented in the publication. Typically high impact journals often dissuade authors from adjusting for multiple testing. Rather, it is less confusing to use traditional 5% level of significance (or 95% confidence intervals in our case) when presenting secondary and tertiary outcome analyses, subgroup analyses, and sensitivity analyses. To address the statistical consequences of not adjusting, we will be very transparent in the discussion of the trial results and state that any secondary results that have a p-value of < 0.05 should be interpreted with caution. We will also state in the manuscript the false positive rate based on the number of secondary outcomes measured. This has been our approach with a large number of peer-reviewed, funded, and published clinical trials, which have been successfully published in very high impact journals.

Categorical variables including mortality and infectious complications will be analyzed using an unadjusted Chi square followed by logistic regression procedures.

Subgroup analyses

Subgroup analyses are planned for the following subgroups of patients according to: 1) severity of illness at baseline, as evaluated by the PRISM III score; 2) stable vs unstable patients at the time of first transfusion (as defined in the TRIPICU study); 3) ABO type; 4) cardiac surgery patients; 5)volume of RBCs transfused in volume / kg (analyzed by quartiles). Subgroup analyses also include the stratification subgroup analyses. We will also perform a stratified analysis after collapsing cells with small strata. This analysis will include both age and center strata.

The analytic approach used for all subgroup analyses will be the intent-to-treat analysis described above for secondary outcome measures. Interactions between treatment group subgroup categories specified above will be calculated. Interactions will be assessed by adding the treatment, subgroup of interest (categorized), and its interaction term (treatment X subgroup) in a logistic regression model. We recognize the limitations of subgroup analyses (low power, Type I error, difficulties in interpretation). These analyses will be hypothesis-generating and hypothesis-supporting in nature.

4.15.6. Other statistical analyses and specific considerations

The issue of multiple transfusions and varying transfusion unit ages.

The proposed research will determine whether "short storage" RBC units (defined as all units stored at most 7 days) reduce the incidence of New or Progressive MODS when compared to standard issue RBCs. Multiple transfusions with RBCs of different ages will occur in both treatment arms; we expect randomization will balance this between study arms. In spite of this, because there will be overlap across the groups defined by randomization according to RBC unit storage time, because 7 days is an arbitrary cut-off point, and because some patients will receive multiple transfusions, several exploratory analyses will be performed to enhance our understanding of the impact of these realities.

The metric of interest will be the maximum storage time for all transfusions received by the patient. Independent of the group into which a patient was randomized, initial chi square tests will use a 7-day cut-off to determine whether the incidence of New or Progressive MODS differs according to whether the maximum storage time as defined above is less than or equal to vs greater than 7 days. This will be supplemented with logistic regression models that evaluate this relationship after adjusting for age, sex, severity of illness as measured by the PRISM III score, stable versus unstable baseline status as defined in the TRIPICU study [45], and patient ABO type. To facilitate an assessment of whether cut-points other than 7 days are preferable, sensitivity and specificity values will be computed for alternative cut-off points. More generally, the logistic models described will be repeated using alternative cut-points, the associated area under the ROC curve will be computed for each model, and each ROC curve will be compared statistically with the reference curve that uses 7 days as the cut-point. The latter comparisons will be performed using PROC LOGISTIC in SAS version 9.2 which contains options that permit the statistical comparison of the area under correlated ROC curves.

Excluded patients.

A limited analysis will be conducted on all patients meeting inclusion and exclusion criteria but who were not randomized, using data in the screening log, in order to see if these patients were different than the patients randomized in the ABC PICU Trial. This analysis will only be used to describe the excluded patients and highlight differences between patients who were randomized and patients who were eligible but not randomized. In doing so, we hope to identify possible selection bias and report on the generalizability of our results.

Variations of the intervention due to RBC processing methods

We will record and report blood bank processing methods for each RBC unit in the ABC PICU Trial to ensure that they are similar between study groups. As we include patients from several sites in different countries, we will record and report any processing differences. A short survey will be sent to the blood bank of all sites that are ready to start enrolling patients to inquire about RBC processing in their center that will need to be recorded per randomized patient. The data that will be recorded and compared for each patient includes the processing method of whole blood to RBCs, preservative solution, irradiation and washing status. It is expected that the randomization and stratification process will balance the differences between RBC processing methods. If this is not the case, we will conduct a sensitivity analysis to control for processing methods.

Co-interventions

Interventions other than transfusions which augment oxygen delivery (such as inotropic agents, fluids and vasodilators) will be adjusted for in the analysis if we observe some imbalance in their distribution into the two arms of the trial. Using logistic regression, we will assess the effect of these co-interventions on outcomes.

4.15.7. Data collected

We will collect baseline data both at ICU entry and at randomization. Data collected will include PRISM III score, The Cornell Assessment of Pediatric Delirium (CAPD), demographic and chronic health status information, and transfusion history. Following randomization we will continue to collect CAPD scores 2 times daily and for 72 hours following each transfusion for 28 days post randomization or 72 hours following the last transfusion whichever is latter. If after 72 hours following a transfusion a patient continues to score positive for Delirium(>9), we will continue to collect scores until the participant returns to baseline or 7 days following transfusion. Additionally, we collect data on intervention, co-interventions and outcomes including 28-day all-cause mortality, other mortality rates, organ failures and organ support information. Data will be gathered through direct patient visits in hospital, medical charts, blood bank databases, communication with clinicians such as attending's and nursing staff as well as family physician and family members, and vital statistics registries in each respective jurisdiction. Blood bank personnel will provide data on processing method, preservative solution, RBC storage duration and CMV status (if available) for each RBC unit.

Baseline characteristics of patients, intervention and co-interventions in both study arms will be assessed as described in section 4.15.2.

4.15.8. Data Safety Monitoring Board and Interim Analyses

Selection of DSMB members will be conducted according to NIH-NHLBI guidelines. Decision processes will follow both NHLBI and CIHR policy. The DSMB established for the ABC PICU Trial will monitor the implementation and safety of this study. NHLBI staff will convene the DSMB and provide an executive secretary. The protocol team will report all study related information to the FDA since this trial required an IND that has been approved. The US Study Coordinator will be responsible for communicating and submitting all required documents to the FDA.

The DSMB will confidentially review interim/cumulative data for evidence of study-related adverse events and for quality, completeness, and timeliness. The DSMB will assess compliance with study goals for patient recruitment and retention, adherence to protocol, and factors external to the study that may impact patient safety or the ethics of the study. The DSMB will also review

data regarding reported adverse events and all outcomes.

The following guidelines are proposed by the study investigators to assist the DSMB in their recommendations for suspension or termination:

- If a safety concern arises, it is expected that the DSMB will recommend suspension of the study and will advise the principal investigators, the NHLBI and the CIHR. Safety should not be an issue in the standard issue transfusion strategy group (control group), since the standard practice will be used in this group. However, safety can be an issue in the short storage group, which is not a standard transfusion strategy. NPMODS and mortality rates will be closely monitored.
- If efficacy is shown sooner than expected, i.e. if RBCs $\leq$ 7 days is better at the time of interim analysis, it is expected the DSMB will notify the NHLBI and the CIHR.

The PIs (Drs. Philip C. Spinella and Marisa Tucci) are responsible for providing the DSMB with new safety information relevant to the study. Information about the conduct of the study, any adverse events, and study outcomes will be reported to the DSMB by the study statisticians (Dean Fergusson and Kenneth Schechtman) using formats approved by the DSMB. The DSMB will also receive all protocol revisions, reports, and manuscripts relating to this study. After each data evaluation, the DSMB Chair will make recommendations to the Project Officer at the NIH/NHLBI and the US Lead Coordinating Center will notify the FDA Regulatory Project Manager regarding the continuation, modification, or termination of the trial.

Meetings: The DSMB will meet prior to the initiation of the study and at a minimum of at least every six months or as needed should safety or serious adverse events concerns arise. There will be a scheduled DSMB meeting after the completion of the Vanguard Phase. Ad hoc meetings may occur at the request of the DSMB members, the Washington University or Sainte-Justine Hospital IRB/REB, or the PIs. Meetings are face-to-face, or by telephone conferences, and may comprise up to 3 portions:

The Open Session to discuss general conduct and progress of the study including adverse events, patient accrual and follow-up, demographic characteristics of participants, and adherence to the study protocol. This session will be attended by DSMB members, the PIs, the study statistician and the selected members of study team by the study PI's.

The Closed Session to discuss grouped safety data will be attended by DSMB members and the study statistician. Data will be coded such that the intervention and control arms will not be identified.

The Closed Executive Session is to discuss results, make decisions and formulate recommendations. At this session, data may be unmasked if necessary at the discretion of the DSMB. A quorum of voting members must be present at the Closed Executive Session for decisions to be made based upon majority vote. A quorum is defined as at least 3 voting members. A representative of NIH/NHLBI or FDA may attend all meeting sessions of the DSMB.

DSMB Reports: The DSMB Chair will notify the NIH/NHLBI Program Officer and FDA Regulatory Project Manager immediately of any critical problems that arise or failure to comply satisfactorily with recruitment criteria. The DSMB Chair will prepare a written summary for all DSMB meetings, the findings and recommendations, and will submit the report to the NIH/NHLBI Program Officer, FDA Regulatory Project Manager and the PIs (Drs. Spinella and Tucci) within 2 weeks of the meeting. The PIs are responsible for providing this report to the Washington University and Sainte-Justine Hospital IRBs.

Interim analysis: We are planning to do one interim analysis while conducting the ABC PICU Trial once 50% of patients have been accrued. The interim statistical analysis will compare NPMODS rates in the "short storage" and the "standard issue" transfusion strategy groups, using O'Brien and Fleming's stopping rules, with a two-tailed p value. The p-values for the 2 looks (1 interim + 1 final) will be 0.003051 and 0.046946; upper and lower boundaries of these z values are ±2.96259 and ±1.96857. Both efficacy and futility will be analyzed at the interim analysis. The course of action regarding continuation of the study will be discussed between the DSMB, NHLBI, and protocol team.

If necessary, the issue of adjusting the sample size will need to be discussed between the DSMB and the Principal Investigators since there will be many factors that will influence the feasibility of accruing additional patients. At the interim analysis, to determine if the trial has sufficient power, the conditional power to detect the hypothesized 33.3% relative risk reduction will be computed based on the observed overall event rate (i.e. the two treatment groups combined) at the one designated interim monitoring time point.

4.16. Adverse Events/ Serious Adverse Events

Adverse Events: Patient safety is the highest priority in ABC PICU. Unfortunately, events that are unintended or unfavorable may occur. These events need to be tracked and reported to ensure safety and accurate interpretation of the ABC PICU outcomes.

The ABC PICU Trial will utilize the following definition of a Serious Adverse Event (SAE) for collection and reporting events as provided by the NIH NHLBI:

Serious Adverse Event (SAE) – an adverse event or suspected adverse reaction is considered serious if in the review of the investigator or the sponsor, it results in a Grade 4 or 5 adverse reaction defined by any of the following:

a) An adverse event, that is life threatening or prolongs the existing hospitalization. (Grade 4)

b) Death (Grade 5)

Adverse Events/ Serious Adverse Events that Require Reporting:

The AE/SAE screening starts at randomization and stops after the patient reaches 28 days post randomization or 72 hours after the last study transfusion, whichever is later and provided that the patient is still in the hospital. The only exception is Transfusion Associated Graft vs Host Disease for which screening will occur throughout the hospitalization (even if the hospitalization lasts more than 28 calendar days post randomization)

The 11 events listed below will be considered reportable if they are diagnosed after an RBC transfusion regardless of grade. This trial utilizes a modified tool to grade the following events and is provided in the eCRF instructions. Each is defined in appendix 3 of this document:

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1. Hemolytic transfusion reaction

2. Major allergic reaction
3. Transfusion-related acute lung injury (TRALI)
4. Septic shock
5. Circulatory overload (also named transfusion-associated cardiac overload or TACO)
6. Hyperkalemia
7. Acute Respiratory Distress Syndrome (ARDS)
8. Nosocomial pneumonia
9. Deep vein thrombosis
10. Transfusion Associated Graft vs. Host Disease
11. Hypocalcemia
12. Delirium

Additionally, All Grade 4 or 5 Serious Adverse Events as defined by the CTCAE V.4 will be reported in the eCRF according to reporting timelines listed in figure 3 below.

NOTE: A patient's **death** per se is not an event, but an outcome. The event which resulted in patient's death must be fully documented and reported without respect of being considered treatment-related or not.

If the rate of related or unexpected SAEs grade 3-5 rate is > 50% per patients enrolled (after 100 patients enrolled) an interim safety analysis will be required.

Critically ill children who are transfused RBCs in the intensive care unit have an expected 90-day mortality rate of 15.2% (95%CI 4-21%). We anticipate the 90-day mortality rate for children enrolled in ABC PICU to be between 4 to 21 %. Mortality rates will be reported to the DSMB every quarter. If we encounter mortality rates of greater than 25% for children enrolled in ABC PICU, an interim analysis will be performed and the results will be reported to the DSMB.

To evaluate the safety of the trial the DMS/DCC staff that is not blinded to treatment arms will perform an interim safety analysis for review by the DSMB.

SAE Reporting:

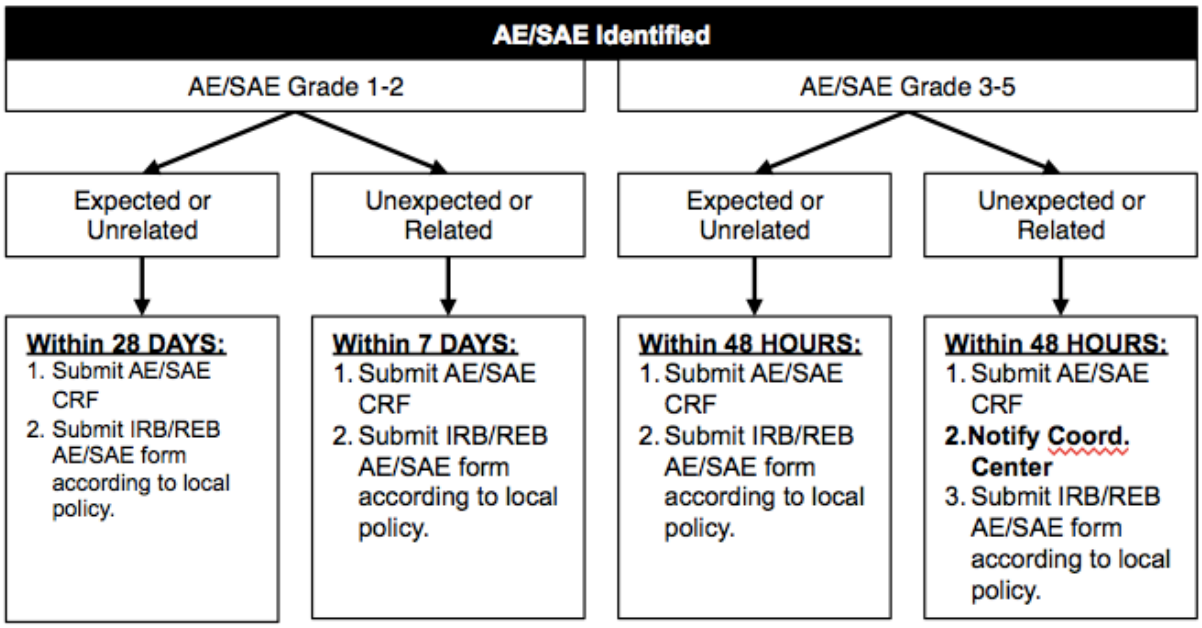
Grade 1-2 AE's/SAEs, as listed above, that are <u>expected or unrelated</u> will be reported to the DCC via the eCRF within 28 calendar days and local IRB/ERB according to site IRB/ERB policy.

Grade 1-2 AE's/SAEs, as listed above, that are <u>unexpected or related</u> will be reported to the DCC via the eCRF within 7 days and local IRB/ERB according to site IRB/ERB policy.

Grade 3-5 AE's/SAEs, as listed above and ALL Grade 4 SAE's, that are determined to be <u>unrelated or expected</u> will be reported within 48 hours of the event to the DCC via the eCRF and to the local IRB/ERB according to site policy.

Grade 3-5 AE's/SAEs, as listed above and ALL Grade 4 SAE's, that are determined to be <u>related or unexpected</u> will be reported to the DCC via the eCRF within 48 hours and to the Medical Monitor within 48 hours of the event, and the local IRB/ERB according to site IRB/ERB policy. Each of these will require documentation of resolution. The DCC will report each of these SAEs to the NHLBI who will forward them to the DSMB Chair.

Figure 3 AE/SAE Reporting Timelines:



5. Security

5.1. Compliance to Privacy-Security Regulations

Management activities will ensure that privacy and security regulations are defined and followed for all participating centers. For example, privacy legislation in Canada occurs at the provincial and federal levels. The Ontario provincial legislation (PHIPA) is deemed as substantially similar legislation and overrides the federal legislation (Personal Information Protection and Electronic Document Act PIPEDA) within the Province of Ontario and HIPAA in United States. Privacy issues also relate to the flow of data between a participating site, the DMS and the ICC/USCC. DMS will comply with guidelines concerning organizational, technological and physical environments. Access to data and preferred approaches will be established. Also a risk management approach will be taken to protect research data from loss, corruption, theft or any other unauthorized disclosure.

5.2. Establishment of Coding

Confidentiality is also part of standard operating procedures. Coding standards will be developed by ABC PICU Trial relevant staff, with the participation of the DMS group, in order to implement these standards throughout the eCRF.

Security (Authentication)

Processes, procedures and more importantly technical methods will be implemented to protect information contained in the Web-based application. A secure registration process and tool for application authentication will be implemented. Access to data will be provided in a secure and reliable manner. Safeguards for network and access will protect data in transit to authorized locations. Data will be monitored to prevent its unauthorized dissemination.

Data safety is maximized through several different mechanisms: physical access to computers and servers is electronically protected; server is behind a firewall; server is exclusively dedicated to database management (i.e. accessible only for researchers); access is controlled by keywords that are changed periodically; daily back-up of the full database and storage at 2 different locations will be made. Each center will have access to the data of its own patients, but not to the data of patients from other sites participating in the ABC PICU Trial. The data and clinical coordinating centers will have access to data from all centers.

Network Security

Network security will be performed with the involvement of various IT departments at OHRI, Ottawa Hospital, and remote site locations. Network security is used to minimize the risk of intrusion, virus infection and any danger arising from malicious software. On-going monitoring and maintenance of the network and its access will be provided for the duration of the development and the usage of the electronic applications.

SSL Certificate

With the implementation of a web application, a Secure Socket Layer (SSL) certificate will secure the online communications and transactions for all users accessing the application. The certificate will be purchased from a third party SSL certificate vendor, such as Entrust. (www.entrust.net)

5.3. Study monitoring and study organization

Approval for use of this protocol by the Institutional Review Board (IRB) must be obtained in

accordance with the institutional assurance policies of the U.S. Department of Health and Human Services. Institutional Review Board (IRB) approval of the ABC PICU protocol and consent forms will be required prior to patient participation on the trial. All reasonable measures will be taken to protect the confidentiality and identity of the patient and patient's records according to the applicable regulations. Patient identity will not be revealed in any publication.

5.4. Study Monitoring

Data management will include an audit trail, a security system, query functionality and quality control done according ICH-GCPs and US CFRs. Data management will be performed at the Ottawa Health Research Institute under the supervision of study statistician. Data will be entered on site in the web-based eCRF. For validation purposes only, double data entry will be used. During the validation phase, CRF and entries will be considered adequate if the frequency of discordance is lower than 2% in the eCRF. In addition, 5-10% of source documents from each site will be reviewed by a trained clinical monitor. The DCC will be responsible for data quality assurance done through eCRF (via regular data extraction) and queries.

Each site involved in ABC PICU will experience up to three routine site visits over the course of the study. Coordinators from the ICC will visit sites in Canada, and the USCC Coordinators will visit sites in the US. The first routine site visit should occur after the randomization of the first 2-3 patients (as logistics allow). The purpose of a routine site visit is to facilitate the site PI and Coordinator in carrying out the responsibilities necessary for the completion of the ABC PICU Trial. Site visits are viewed as a supportive measure with the opportunity to educate, not a punitive act.

A for cause site visit will result if a major protocol violation has occurred at the study site.

The site visit team will review study documents to determine whether the study has been conducted according to the protocol and that the inclusion and exclusion criteria were followed when enrolling patients. They will also be evaluating for signed documentation stating the family/patient was enrolled in the study, and that the consenting process took place per protocol and local policies. The site visit team will also visit the blood bank to assess procedures related to maintaining proper inventory and to ensure that staff training is properly documented.

5.5. Study Organization and Committee Responsibilities

5.6. Study organization and Committee responsibilities are described in the MOP.

6. *Appendix 1: Data collection timeline*

	Hospital Admission	ICU Admission	Just prior Randomization	Day 1 / randomization	Day 2 to Day 7	Day 14 or ICU discharge	Day 21 or ICU discharge	Day 28 (end of Calendar Day)	Day 90	ICU Discharge	Hospit Dischar
Inclusion/exclusion			X								
Consent			X								
Demographic			X								
Baseline			X								
PRISM III		X		X							
PELOD 2*		X	X							X	
NPMODS**		X	X	X	X	X	X	X		X	
CAPD***			X	X	X						
Clinical Data				X	X	X	X	X			
Fluid Balance				Daily from randomization to Day 28 or ICU Discharge (whichever comes first)							
Co-Interventions (Blood products and other therapies)				Daily from randomization to Day 28 or ICU Discharge (whichever comes first)							
6.1.1. PRBC Transfusion documentation and information				*6.1.2.* X	All transfusions should be documented through Day 28 (End of calendar day) or hospital discharge. If patient is discharged from ICU the patient remains on study treatment arm until end of calendar day 28 and all transfusion information is documented.						
Nosocomial infections, Sepsis and other clinical outcomes		Daily from ICU admission to Day 28 or ICU Discharge (whichever comes first)									
Mechanical ventilation		Daily from ICU admission to Day 28 or ICU Discharge (whichever comes first)									
Vasoactive and/or inotropic continuous intravenous drug		Daily from ICU admission to Day 28 or ICU Discharge (whichever comes first)									
Renal replacement therapy		Daily from ICU admission to Day 28 or ICU Discharge (whichever comes first)									
6.1.3. Adverse Events / Serious Adverse Events		Daily from randomization to Day 28 or 72 hours after the last study transfusion, whichever is later, provided that the patient is still in the hospital.									
Mortality status									X		X
Readmission information^									X		
Discharge status										X	X

*In addition to the times listed in this table, PELOD 2 score data will be collected on days 2, 5, 8, 12, 16 and 18 after ICU admission
**In addition to the times listed in this table, NPMODS data will be collected on the days when PELOD 2 data are collected
*** CAPD scores will be collected immediately prior to transfusion, and 2x daily at the beginning of each nursing shift for 72 hours following each transfusion

7. Appendix 2: Participating Institutions and Principal Investigators

7.1. Part A: US Sites

Critical Care	Transfusion Medicine
Kenneth Remy, MD Washington University School of Medicine Pediatric Critical Care Medicine 660 S. Euclid, CB 8116 St. Louis, MO 63110 Remy_K@kids.wustl.edu Office: 314-286-2894	**Ronald Jackups, MD** Washington University School of Medicine Laboratory Medicine 660 S. Euclid, CB 8118 St. Louis, MO 63110 rjackups@path.wustl.edu Office: 314-362-8413
Matthew Paden, MD Children's Healthcare of Atlanta Emory University Pediatric Critical Care Medicine 1405 Clifton Road NE Atlanta, GA 30322-1060 matthew.paden@choa.org Office: 404-785-1600	**Cassandra Josephson, MD** Children's Healthcare of Atlanta Emory University Transfusion Medicine 1405 Clifton Road NE Atlanta, GA 30322-1060 cjoseph@emory.edu Office: 404-785-4553
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8. Appendix 3: Definitions of AE's/SAEs

1. **Hemolytic transfusion reaction** – requires presence of hemoglobinuria or hemoglobinemia, new or unexplained by underlying condition, observed from the beginning of the transfusion up to 4 hours after end of the transfusion.
    1.1. Hemoglobinuria: macroscopic or microscopic hemoglobinuria, red, pink or very dark urine with positive test for hemoglobin in urine, observed from the beginning of the transfusion up to 4 hours after it was completed
    1.2. Hemoglobinemia: blood level of free hemoglobin above normal range or positive direct Coombs test (also know as the direct antiglobulin test or DAT)
    1.3. At least one of the following symptoms/signs.
        1.3.1. Fever (> 38°C) de novo.
        1.3.2. Dyspnea de novo.
        1.3.3. Hypotension and/or tachycardia de novo.
        1.3.4. Anxiety/agitation de novo.
        1.3.5. Pain de novo.
2. **Major allergic reaction** - A disorder characterized by an adverse local or general response (Type 1 hypersensitivity reaction[47]) from exposure to an allergen that includes at least 1 of the following:
    2.1. Cardiac Arrest
    2.2. Generalized allergic reaction or anaphylactic reaction de novo
    2.3 Angio-edema (facial and/or laryngeal) de novo (as reported by nurses).
    2.4 Upper airway obstruction de novo.
    2.5 Dyspnea de novo, wheezing de novo.
    2.6 Hypotension de novo, shock de novo.
    2.7 Precordial pain or chest tightness de novo.
    2.8 Cardiac arrhythmia de novo.
    2.9 Loss of consciousness de novo.
3. **Transfusion-related acute lung injury (TRALI)** – TRALI is caused de novo by a transfusion, and appearing during or less than 6 hours after the end of a transfusion.
    3.1 New onset or worsening of pulmonary function with hypoxemia that satisfies the international criteria for ALI (PaO_2/FiO_2 <300 mm Hg)

    AND

    3.2 Chest x-ray consistent with pulmonary edema
4 **Septic shock** – Septic Shock is defined as sepsis with cardiovascular dysfunction. Sepsis is defined as SIRS in the presence of or as a result of suspected or proven Infection.

SIRS requires the presence of at least two of the following four criteria, one of which must be abnormal temperature or leukocyte count (see section 1.2 of the Sepsis worksheet)

There are 4 criteria for SIRS:

4.1 Core temperature > 38.5°C or <35°C. Note that core temperature must be measured by rectal, bladder, oral or central catheter probe.

4.2 Leukocyte count elevated or depressed for age (not secondary to chemotherapy induced leucopenia) or > 10% immature neutrophils (bands) (Table 2)

Table 2
5th and 95th percentile for leukocyte count[48]

Age	Leukocyte Count
0 days – 1 week	>34
1 week to 1 month	>19.5 or <5
1 month to 1 year	>17.5 or <5
2 – 5 years	>15.5 or <6
6 – 12 years	>13.5 or <4.5
13 – 18 years	>11 or <4.5
Leukocyte Count (10^3 / mm^3 = K/Cumm = 10^9/L)	

4.3 Heart Rate:

4.4 Tachycardia, defined as: mean heart rate above the 95th percentile for age in the absence of external stimulus, chronic drugs, or painful stimuli (Table 3)

OR

4.5 Otherwise unexplained persistent elevation over a 0.5 to 4 hour time period. Unexplained refers to elevated heart rate not explained by obvious or known cause such as crying, agitated, administration of drugs causing elevated heart rate (atropine), etc.

OR

4.6 Bradycardia for children <1 yr old, defined as: Mean heart rate <10th percentile (Table 4)

OR

4.7 Otherwise unexplained persistent depression of the HR over a 0.5 time period. Unexplained refers to bradycardia not explained by obvious or known cause such as administration of beta-blocking agent, significant hypothermia, known intrinsic dysfunction of the heart's electrical conduction system (atrioventricular bloc, sinus bradycardia).

Table 3:
95th percentile values for heart rate in children[49]

Age	Beats/minute
0 days – 1 week	154
1 week to 1 month	159
1 -3 months	169
3 – 6 months	164
6 - 9 months	157
9 – 12 months	151
12 – 18 months	147
19 – 24 months	144
2 – 3 years	141
3 – 4 years	138
4 – 6 years	135
6 – 8 years	133
8 – 12 years	131
12 – 15 years	130
15 – 18 years	129

Table 4: 10th percentile for heart (HR) in children < 1 year[49]

Age	Beats/minute
0 – 3 months	123
3 – 6 months	120
6 – 9 months	114
9 – 12 months	109

4.8 **Respiratory rate or mechanical ventilation**

4.8.1 Mean respiratory rate above the 95th percentile for age (Table 5)

OR

4.8.2 On mechanical ventilation for an acute respiratory process not related to underlying neuromuscular disease or the receipt of general anesthesia.

Table 5:
95th percentile values for respiratory rate in children[49]

Age	Breaths/minute
0 – 3 months	60
3 – 6 months	57
6 – 9 months	55
9 – 12 months	52
12- 18 months	49
18 – 24 months	46
2 – 3 years	43
3 – 4 years	40
4 – 6 years	37
6 - 8 years	35
8 – 12 years	34
12 – 15 years	33
15 – 18 years	32

4.9 Mechanical ventilation: Invasive and Non-invasive ventilation.

Invasive ventilation: Mechanical ventilation delivered by positive pressure via endotracheal intubation or laryngeal mask use or a tracheostomy.

Non-invasive ventilation: Mechanical ventilation delivered by bi-level positive airway pressure (BiPAP) through a supra-laryngeal airway by a mask or nasopharyngeal tube. Continuous positive airway pressure (CPAP) throughout the breathing cycle is not considered Non invasive mechanical ventilation.

4.10 Infection is defined as:

4.9.1 A suspected or proven infection caused by any pathogen (by positive culture, tissue stain, or polymerase chain reaction test)

OR

4.9.2 A clinical syndrome associated with a high probability of infection. Evidence of infection includes positive findings on clinical exam, imaging, or laboratory tests (i.e. white blood cells in a normally sterile body fluid, perforated viscus, chest radiograph consistent with pneumonia, petechial or purpuric rash, or purpura fulminans).

6.0 Cardiovascular dysfunction requires the presence of one of the 3 following criteria:

6.1 Decrease in systolic BP (hypotension) <5th percentile for age (Table 6). Note that 2 low measurements within one hour are required.

OR

6.2 Need for vasoactive drug to maintain BP in normal range (dopamine >5 mcg/kg/min or any dose of dobutamine, epinephrine, norepinephrine, vasopressin/terlipressin, phenylephrine or milrinone)

OR

6.3 Two of the following

6.3.1 Unexplained metabolic acidosis, base deficit ≥5.0 mEq/L

6.3.2 Increased arterial lactate >2 times upper limit of normal

6.3.3 Oliguria: urine output < 1 mL/kg/hour for 4 hours

6.3.4 Prolonged capillary refill: > 5 secs

6.3.5 Core to peripheral temperature gap >3°C. Core temperature is measured by rectal, bladder, oral or central catheter probe. Peripheral temperature is measured by tympanic, toe, or axillary route.

Table 6:
<5th percentile for age for systolic BP in children[50]

Age	Boys	Girls
	Systolic BP	Systolic BP
0-7 days	57	57
8-30 days	64	62
1-6 months	72	72
6-12 months	71	70
1 yr	74	72
2 yr	77	77
3 yr	73	72
4 yr	71	69
5 yr	76	77
6 yr	80	78
7 yr	81	79
8 yr	82	81
9 yr	84	82
10 yr	85	84
11 yr	85	86
12 yr	88	89
13 yr	87	87
14 yr	89	88
15 yr	92	89
16 yr	94	91
17 yr	98	92

7.0 **Circulatory overload (also named transfusion-associated cardiac overload or TACO)** –TACO is a caused de novo by a transfusion as a result of fluid overload (positive fluid balance > 20/mL/kg in the last day) that appears within 6 hours after the end of a transfusion, with at least one of the following criteria:

7.1 Dyspnea or cyanosis de novo (as reported by nurses).

7.2 Pulmonary edema de novo.

7.3 Tachycardia de novo.

7.4 Hypertension de novo.

8.0 **Hyperkalemia** – Blood level of potassium > 5.5 mmol/L.

9.0 **Acute Respiratory Distress Syndrome (ARDS)**[51]

PaO_2/FiO_2 ratio ≤ 300 mmHg

AND

Confirmation of bilateral opacities (perihilar infiltrates alone are not considered) on chest imaging report

10.0 **Nosocomial pneumonia –**

A patient with a new or progressive radiographic infiltrate, along with a high clinical suspicion of pneumonia plus a definite cause established, must be intubated for at least 48 hours and diagnosis is made after patient has received a transfusion.

Microbiologically confirmed: The patient must have:

10.1 A new or progressive radiographic infiltrate, along with

10.2 A high clinical suspicion of pneumonia plus:

10.3 A definite cause established by

10.3.1 Recovery of a probable etiologic agent from an uncontaminated specimen (blood, pleural fluid, transtracheal aspirate, or transthoracic aspirate); **or**

10.3.2 Recovery from respiratory secretions of a likely pathogen that does not colonize the upper airways (e.g., Mycobacterium tuberculosis, Legionella species, influenza virus, or Pneumocystis jiroveci (carinii); **or**

10.3.3 Recovery of a likely/possible respiratory pathogen in high concentrations using quantitative cultures of a lower respiratory tract sample (endotracheal aspirate, BAL, or protected specimen brush); **or**

10.3.4 Positive serology.

11.0 **Deep vein thrombosis –**

Criteria for clinically suspected or proven deep vein thrombosis will include at least 1 of the following symptoms: superficial collateral circulation, jugular swelling, vena cava syndrome, edema, pain, inflammation of a limb, fever or signs of a thrombotic complication (e.g., pulmonary embolism), as described by Dubois et al. [52]

Criteria to identify deep vein thrombosis as diagnosed by ultrasonography will include direct visualization of the thrombus, non-compressibility of the vessel, incomplete filling of the vein and appearance of respiratory variations on venous flow as described by Prandoni et al. [53]

Symptomatic and asymptomatic deep vein thromboses will both be reported.

12.0 Transfusion Associated Graft vs. Host Disease -

Due to the complexity of this diagnosis, report as present if medical record shows has been diagnosed by clinical team within 28 calendar days after the last transfusion.

13.0 Hypocalcemia – Defined as an ionized calcium concentration < 0.8 mmol/L (<3.2 mg.dL)

14.0 Delirium- Defined as a CAPD score of ≥9 following the study transfusion.

15.0 **Grade 4 SAE-** An adverse event, that is life threatening or prolongs the existing hospitalization. (Grade 4)

16.0 Grade 5 SAE- Death

9. Appendix 4: Intent to Treat Analysis

Intention To Treat Approach for the ABC-PICU Trial

Purpose:
Patients can be randomized and not transfused in the ABC-PICU trial due to factors that occur prior to randomization and after randomization. Among the patients enrolled up to Oct 1st 2014 there have been 7/91 patients randomized but not transfused. For 5/7 of these, the patient was not transfused because the clinical team decided to not transfuse after randomization. This suggests that the clinical team prematurely and inappropriately determined the patient would need a transfusion prior to randomization. The other 2 patients were not transfused due to cancellation of the surgery in one case and a surgeon's decision to withdraw their patient from the study in the other case. This has prompted us to reevaluate our Intent To Treat approach to be used for our primary and secondary analyses in the ABC-PICU trial. It can be appropriate to legitimately exclude patients from the Intention To Treat (ITT) approach when the treatment is not received after randomization and allocation concealment has not been compromised. Every attempt is being made to minimize the number of "non-transfused" occurrences but such occurrences are inevitable due to the nature of transfusion trials in acute care settings.

Patients will be excluded from the ITT analysis if the factor that leads to non-transfusion occurred before randomization and our masked treatment allocation was not compromised. For example, if the surgeon feels a patient will "absolutely" receive a transfusion in the operating room before randomization yet eventually no transfusion takes place either during the surgical procedure or up to 28 days post-randomization, this patient will be excluded from the ITT analysis. For such a patient, the factor leading to non-transfusion was an incorrect assessment before randomization by the clinical team.

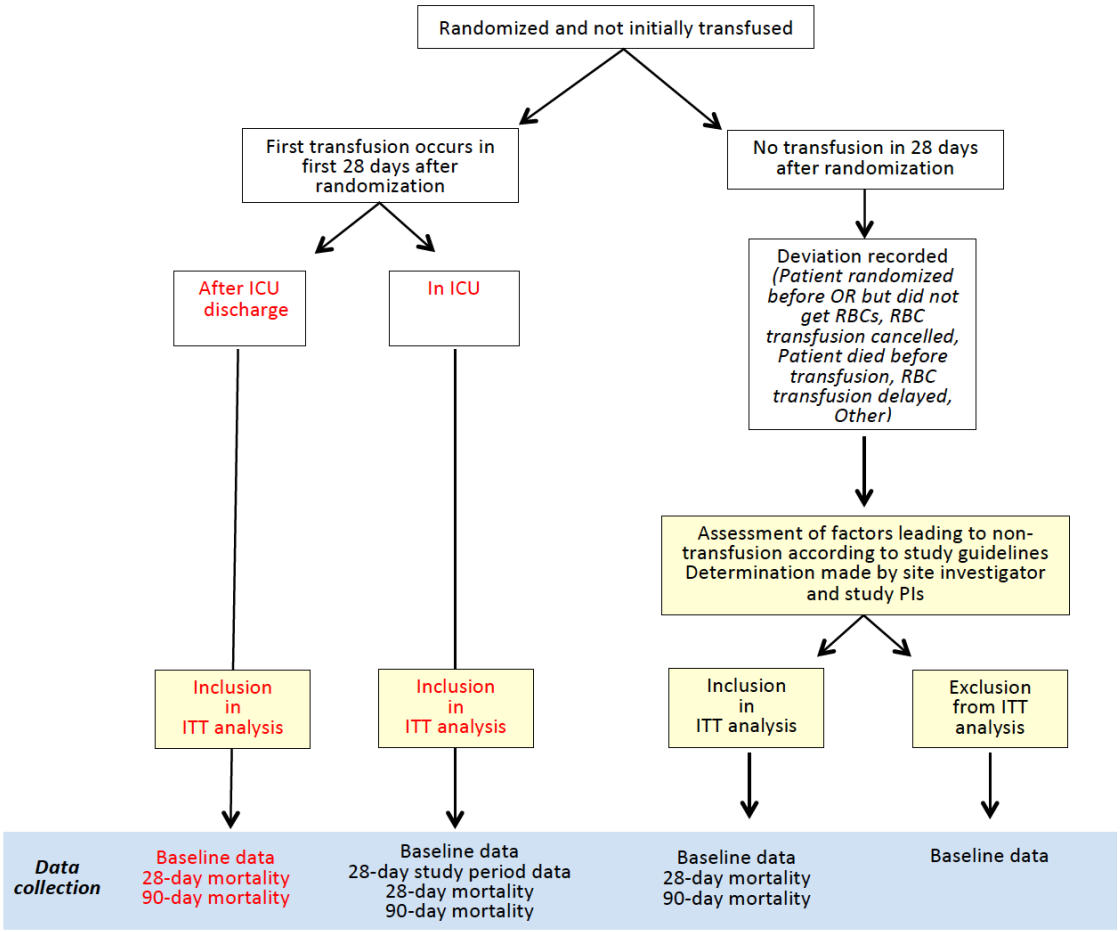
Process:
Determination of whether factors leading to non-transfusion according to study protocol were present before or after randomization will occur as follows:

- The basic approach involves inclusion in the ITT analysis of all subjects randomized regardless of the timing of transfusion, except if the child is no longer in the ICU when transfused. Baseline data will be obtained for all randomized subjects independent of whether they are included in the ITT analysis.
- When a patient is randomized and then not transfused during the 28 days post-randomization, the local site PI will determine if non-transfusion according to study guidelines was due to a factor that occurred before or after randomization.
- US and Canadian Study Coordinators and Principal Investigators will be accessible to assist in this determination.
- The advice provided to sites to make this determination is the following.
    - If a transfusion was ordered by the clinical team in the ICU or if the surgical team determined that the patient would definitively be transfused in the operating room, and subsequently no transfusion takes place after randomization, then this is a factor that occurred prior to randomization. The requirement for transfusion was

incorrectly assessed prior to randomization. Consequently, this patient will be excluded from the ITT analysis.

- If it is deemed that the issue leading to non-transfusion according to study guidelines occurred after randomization (for example, a surgeon decides during surgery that only fresh blood be administered), then the patient will remain in the ITT analysis.

- The diagram below shows the approach that will be used for patients randomized and not initially transfused:



- The US and Canadian coordinating center will inform the DCC whether the patient is to be included or excluded from the ITT analysis and will advise the clinical site of the data collection expected.

Data collection:

- All patients randomized and not initially transfused must continue to be monitored up to 28 days post-randomization.

- For a patient who is randomized and eventually transfused AND who will be included in the ITT analysis, the data collected will depend on whether the first transfusion occurs in ICU or after ICU discharge.

    - If the first transfusion occurs in ICU, baseline data, 28-day study period data, 28-day mortality and 90-mortality will be collected.
    - If the first transfusion occurs after ICU discharge, baseline data, 28-day mortality and 90-day mortality will be collected; 28-day study period data will not be collected for these patients because it is not possible to collect all the data required to ascertain development of NPMODS outside of an ICU.

- For a patient who is randomized and never transfused during the 28-day study period AND who will be included in the ITT analysis, clinical sites will collect baseline data, 28-day mortality and 90-day mortality. For patients not transfused following randomization, the eCRF allows for the clinical site coordinator to record the following deviations: *Patient randomized before OR but did not get RBCs, RBC transfusion cancelled, Patient died before being transfused, RBC transfusion delayed, Other(s) (specify).*

- For a patient who is randomized and never transfused during the 28-day study period and who is excluded from the ITT analysis, only baseline data will be collected.

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