CLINICAL RESEARCH PROTOCOL

TITRE: TRIAL OF INDICATION BASED TRANSFUSION OF RED BLOOD CELLS IN ECMO

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Study Summary

US Department of Defense Title	***TITRE***: **T**rial of **I**ndication-based **T**ransfusion of **R**ed Blood Cells in **E**CMO
Short Title	TITRE
Alternative Title	**Multicenter Trial of Indication- vs. Threshold-based Red Blood Cell Transfusion in ECMO**
Protocol Number	TITRE-002
Methodology	Multi-Center, Open Label, Blinded End-Point Adjudication, Randomized Clinical Trial
Study Duration	4 years
Study Center(s)	18 sites
Objectives	**OVERALL AIM** of this research is to conduct a multicenter randomized clinical trial to determine if indication-based red blood cell (RBC) transfusion based on reduced tissue oxygen delivery, compared with RBC transfusion based on an institutional-specific threshold reduces organ dysfunction and improves neurodevelopment, function, and health-related quality of life in critically ill infants and children receiving extracorporeal membrane oxygenation (ECMO). **SPECIFIC AIM 1:** To test whether children < 6 years of age on ECMO randomized to a strategy of indication-based *versus* center-specific threshold-based RBC transfusion will have greater improvement in organ function, as measured by change in the Pediatric Sequential Organ Failure Score (pSOFA). **SPECIFIC AIM 2:** To test whether children < 6 of age on ECMO randomized to indication-based compared to center-specific threshold-based RBC transfusion will have better outcomes of neurodevelopment, function, and health-related quality of life measured at 1-year post-randomization.
Number of Participants	228 participants

Diagnosis and Main Inclusion Criteria	Children supported with ECMO for any indication who meet all the following: 1. Age < 6 year at ECMO cannulation 2. Veno-arterial (VA) mode of ECMO 3. First ECMO run during the index hospital admission In the absence of any of the following at the time of screening: 1. Gestationally-corrected age <37 weeks 2. Veno-venous (VV) mode of ECMO 3. VV ECMO transitioned to VA ECMO 4. ECMO used for procedural support only, or ECMO duration expected to be < 24 h 5. Limitation of care in place or being discussed 6. Congenital bleeding disorders 7. Hemoglobinopathies 8. Primary Residence outside Unites States or Canada 9. Concurrent participation in a separate interventional trial that has the potential to impact neurodevelopmental status of the patient 10. Patients cannulated for ECMO at a non-trial center and transferred to a trial site 11. Randomization not possible within 36 h following ECMO cannulation (e.g., due to staffing or delays related to communication with participant family)
Study Intervention	This trial will not alter or introduce new medications or surgical or non-surgical procedures during ECMO care. Patient will be randomized to one of two arms: - Center-specific threshold-based RBC transfusion will use institutional standard Hemoglobin (Hb)/ Hematocrit (HCT) threshold for RBC transfusion during ECMO. -Indication-based RBC transfusion: When center-specific threshold for RBC transfusion is met, transfusion will be provided based on any one of three indications: 1. Bleeding; 2. Reduced tissue oxygen delivery, defined as a blood lactate level ≥ 5 mmol/L, or > 3 mmol/L and increasing on 2 measurements done 2 hours apart, after optimizing ECMO flow; or 3. Hb/HCT threshold: a. Hb < 10 g/dL or HCT <30%: if age≤28 days at randomization or if patient has single ventricle anatomy and is age <1 year at randomization b. Hb < 8 g/dL or HCT < 25%: for all other patients

Duration of administration	The trial transfusion protocols (indication-based and standard threshold-based) will be used from day #1 of ECMO (randomization) until ECMO decannulation (or 30 days post-randomization, whichever is earliest), patient management will be based on institutional standard care policies at the discretion of the institution and bedside clinician.
Reference therapy	Threshold-based transfusion current standard of care
Statistical Methodology	Assumptions include: • 85% power, two-sided $\alpha = 0.05$ • Standard deviation of change in pSOFA = 4.2 • 10% crossover from indication-based Strategy to center-specific threshold-based management • 5% crossover from center-specific threshold-based management to indication-based Strategy • 2% inflation for interim look. Based on these assumptions, the required sample size is 114 per group (total 228 participants). The primary analytic approach will be intention-to-treat (ITT). The primary clinical endpoint analysis will be analysis of covariance for the baseline-adjusted change score treatment group comparison. Multiple imputation will be used for the primary outcome for any participants who have insufficient clinical data to calculate a pSOFA score.
Safety evaluation	An independent Data and Safety Monitoring Board (DSMB) will review safety data throughout the trial. One interim look by treatment arm is planned at 60% information. All adverse events will be monitored closely throughout the trial.

1 General Investigational Plan

Extracorporeal Membrane Oxygenation (ECMO) can be life saving for children with cardiorespiratory failure unresponsive to conventional medical and surgical therapies [3-8]. However, only 50% of these critically ill children will survive to hospital discharge. ECMO is invasive, and serious adverse events, including bleeding, thrombosis, organ dysfunction, and cerebrovascular injury are common. These issues impact survival and long-term quality of life [9-15].

During ECMO, RBC transfusion is necessary to provide hemoglobin (Hb) to carry oxygen to tissues to manage bleeding. However, the Hb or hematocrit (HCT) level at which tissue oxygen delivery is optimal has not been determined [16, 17]. There is a critical knowledge gap regarding indications and management of RBC transfusion practice during ECMO, and this knowledge gap may contribute to poor outcomes in children supported with ECMO[9-11].

This **prospective, randomized clinical trial (RCT)**, *TITRE: Trial of Indication-Based Transfusion of Red blood cells in ECMO,* will evaluate whether a strategy of indication-based *vs.* center-specific threshold-based RBC transfusion improves organ recovery and survival in children aged < 6 y supported with ECMO. Additionally, the trial will evaluate whether indication-based RBC transfusion during ECMO improves neurodevelopmental and functional outcomes at 12 months post-randomization in ECMO survivors.

The **primary trial endpoint** is change in Pediatric Sequential Organ Failure Assessment Score (pSOFA) from pre-ECMO cannulation to decannulation (or 30 days post-randomization, whichever is earliest). The pSOFA is a validated instrument for assessment of end-organ dysfunction in critically ill children[18]. Change in pSOFA score during ECMO will be used to power the trial. *TITRE* is funded through a Fiscal Year 2021 Peer Reviewed Medical Research Program Clinical Trial Award sponsored by the United States Department of Defense, Office of Congressionally Directed Medical Research Programs. It is important to note that this RCT has the potential to influence the practice of RBC transfusion for children and adults supported with ECMO and optimize utilization of a scarce societal resource.

2 Study Objectives

The primary purpose of this trial is to improve survival and later neurodevelopmental outcomes in children < 6 years old who are supported with ECMO. The trial will assess whether end-organ dysfunction is improved by reducing unnecessary RBC transfusion during ECMO. Specifically, the trial will assess whether an *indication-based RBC transfusion*, where RBC transfusion is provided for reduced tissue oxygen delivery, bleeding, or HCT < 25%, compared with *center-specific threshold-based RBC transfusion*, where RBC transfusion is based on a standard-care institutional Hb or HCT threshold (usually HCT 30 – 35%) can reduce organ dysfunction in critically ill infants and children receiving ECMO, and improve their later neurodevelopment, function, and health-related quality of life. The study is designed to achieve the following objectives:

Primary Objectives

Clinical Objective (Aim 1): To test whether children < 6 years of age on ECMO support who are randomized to a strategy of indication-based *versus* center-specific threshold-based RBC transfusion will have greater improvement in organ function. The primary trial outcome will be a

change in Pediatric Sequential Organ Failure Score (pSOFA) from pre-ECMO cannulation to post-ECMO decannulation (or 30-days post-randomization, whichever is earliest).

Neurodevelopmental Objective (Aim 2): To test whether children < 6 of age who survive to hospital discharge following ECMO, randomized to indication-based compared to center-specific threshold-based RBC transfusion will have better outcomes of neurodevelopment, functional status, and health-related quality of life measured at 12 months post-randomization.

3 Study Design

3.1 General Design

This is a multi-center partially blinded randomized clinical trial. The primary aim of the study is to test the hypothesis that among children < 6 years of age supported with ECMO, those randomized to an indication-based RBC transfusion strategy will have greater improvement in organ function, assessed by change in pSOFA score from the pre-ECMO cannulation to decannulation (or 30 days post randomization, whichever is earliest), compared with a center-specific institutional threshold-based strategy. Study participants will be randomized <30 h after ECMO deployment and the transfusion strategy will begin 18-30 h after ECMO deployment. Adjudication of the primary endpoint, i.e., change in pSOFA score, will be blinded. The trial will also test whether 12-month neurodevelopmental outcomes of ECMO survivors are better in those assigned to an indication-based RBC transfusion protocol than those randomized to center-specific threshold-based management. The clinical trial schema is shown in **Figure 6-1**.

3.2 Primary Study Endpoints

3.2.1 Primary Clinical Endpoint

The primary clinical endpoint is the change in pSOFA score from pre ECMO cannulation to decannulation (or 30 days post-randomization, whichever is earliest). The pSOFA score is an objective assessment of organ dysfunction in critically ill children [33]. pSOFA assesses dysfunction in six organ systems and can be applied to children of all ages (**Table 3-1**). It has been validated as an excellent discriminator of mortality in critically ill children and those with severe sepsis (16). The premise for using a change score is based on the principle that patients requiring ECMO will have multi-organ dysfunction prior to cannulation, that ECMO support will help restore function of these organs, and that patients managed using an indication-based strategy will have higher resolution of organ dysfunction compared with those managed with a center-specific threshold-based RBC transfusion strategy. The pSOFA score ranges from 0 (no organ dysfunction) through 24 (severe dysfunction in all 6 organs assessed). Patients who die after randomization and prior to decannulation (or at 30 days post-randomization, whichever is earliest), will be assigned a score of 24. Patients supported with renal replacement therapy (continuous veno-venous hemofiltration (CVVH), CVVH dialysis, peritoneal dialysis, or intermittent hemodialysis) during ECMO, regardless of indication, will receive a score of 4 for the renal function component. When a patient is transitioned to a ventricular assist device (VAD) or is transplanted (heart, lung or both) from ECMO, for purposes of pSOFA measurement these events are equivalent to ECMO decannulation. For patients who go the operating room on ECMO and return without ECMO, day of surgery will be equivalent to the day of ECMO decannulation for primary endpoint assessment.

Table 3-1. Pediatric Sequential Organ Failure Assessment Score (pSOFA) Components

(Matics et al. JAMA Peds 2017; 171(10):e172352

	Score[a]				
Variables	0	1	2	3	4
Respiratory					
Pa_{O_2}:Fi_{O_2}[b] or Sp_{O_2}:Fi_{O_2}[c]	≥400	300-399	200-299	100-199 With respiratory support	<100 With respiratory support
	≥292	264-291	221-264	148-220 With respiratory support	<148 With respiratory support
Coagulation					
Platelet count, $\times 10^3/\mu L$	≥150	100-149	50-99	20-49	<20
Hepatic					
Bilirubin, mg/dL	<1.2	1.2-1.9	2.0-5.9	6.0-11.9	>12.0
Cardiovascular					
MAP by age group or vasoactive infusion, mm Hg or μg/kg/min[d]					
<1 mo	≥46	<46	Dopamine hydrochloride ≤5 or dobutamine hydrochloride (any)	Dopamine hydrochloride >5 or epinephrine ≤0.1 or norepinephrine bitartrate ≤0.1	Dopamine hydrochloride >15 or epinephrine >0.1 or norepinephrine bitartrate >0.1
1-11 mo	≥55	<55			
12-23 mo	≥60	<60			
24-59 mo	≥62	<62			
60-143 mo	≥65	<65			
144-216 mo	≥67	<67			
>216 mo[e]	≥70	<70			
Neurologic					
Glasgow Coma Score[f]	15	13-14	10-12	6-9	<6
Renal					
Creatinine by age group, mg/dL					
<1 mo	<0.8	0.8-0.9	1.0-1.1	1.2-1.5	≥1.6
1-11 mo	<0.3	0.3-0.4	0.5-0.7	0.8-1.1	≥1.2
12-23 mo	<0.4	0.4-0.5	0.6-1.0	1.1-1.4	≥1.5
24-59 mo	<0.6	0.6-0.8	0.9-1.5	1.6-2.2	≥2.3
60-143 mo	<0.7	0.7-1.0	1.1-1.7	1.8-2.5	≥2.6
144-216 mo	<1.0	1.0-1.6	1.7-2.8	2.9-4.1	≥4.2
>216 mo[e]	<1.2	1.2-1.9	2.0-3.4	3.5-4.9	≥5

3.2.2 Primary Neurodevelopmental Endpoint

The primary study neurodevelopmental endpoints will be measured at 12 months post-randomization and will be assessed using age-specific validated assessment instruments. The time required for assessment is shown in Table 3-2. These instruments include:

3.2.2.1 Bayley Scales of Infant and Toddler Development, Fourth Edition (Bayley-4): The Bayley-4 is an individually administered assessment of developmental functioning in infants and toddlers aged 16 days to 42 months. It is designed to monitor developmental progress in the domains of Cognitive, Language (Expressive and Receptive), and Motor (Gross and Fine), Social-Emotional, and Adaptive Behavior. Raw scores for each of the subtests are converted into scaled scores (mean=10, SD=3); standard scores (mean=100, SD=15) can be calculated for each of the domains. The Bayley-4 is highly correlated with the WPPSI-IV. The Bayley has been used in several prior studies examining ND outcomes in children surviving ECMO [13, 15, 19]. The Bayley-4 will be used in participants aged < 36 months at time of assessment.

3.2.2.2 Wechsler Preschool and Primary Scale of Intelligence (WPPSI – IV): The WPPSI – IV [42] is a standardized assessment of a child's cognitive abilities from 2 y, 6 mo – 7 y, 6 mo. It involves administering a variety of activities including verbal and nonverbal reasoning challenges, and as well as visual-spatial problem solving tasks. Index scores (Verbal Comprehension, Visual Spatial, Working Memory, and Full Scale IQ) will be reported (mean ± standard deviation, normal range 100 ± 15) along with subtest scaled scores (normal range 10 ± 3). It has been used in several prior studies with ECMO survivors [20]. The WPPSI-IV will be used in participants aged ≥ 36 months at the time of assessment.

Table 3-2. Neurodevelopmental Battery at 1 year Post-Randomization (Quality of Life at 9 months)

Test	Completion Time	Trial Participant Ages to be Administered to:
Bayley Scales of Infant and Toddler Development, Fourth Edition (Bayley-4)	60-90 minutes	< 36 months
Wechsler Preschool and Primary Scale of Intelligence (WPPSI – IV)	60 minutes	≥ 36 months
Adaptive Behavior Assessment System, Third Edition (ABAS-3)	15-20 minutes	All (1-7 years)
Child Behavior Checklist **1.5-5 year and 6-8 year versions**	10-20 minutes	1.5 – 7 years
PedsQL 4.0 General. Infant, and (if applicable) Cardiac Module 3.0	15 minutes	1 month – 7 years Infant scales: 1 month – 2 years Cardiac: ~40%
Total Time Required	**2 hr 40 min to 3 hr 25 min.**	

3.3 Secondary Study Endpoints

3.3.1: Secondary Clinical Endpoints

The secondary clinical endpoints will evaluate efficacy and safety of the indication-based transfusion protocol. Unless otherwise specified, data for endpoints will be collected from randomization to ECMO decannulation or 30 days post-randomization, whichever is earliest.

- Daily first recorded value from day-time ECMO specialist assessment each day) non-invasive venous O2 saturation measured from venous limb of ECMO circuit
- Total volume of blood products administered (RBC: both PRBC and whole blood, cryoprecipitate, plasma, platelets)
- Number of donor exposures
- Blood stream infection during ECMO based on a positive blood culture obtained from the participant or ECMO circuit[1]
- Daily AM (first reported) serum creatinine and total daily 24h urine output during ECMO
- Daily determination of acute kidney injury > stage 2, using KDIGO definition[21]
- Transfusion related: Allergic reactions using Centers for Disease Control (CDC) definitions[2]
- Proportion of ECMO days meeting moderate – severe bleeding
- Death or recannulation for ECMO within 48 hours and 72 hours following decannulation
- ECMO circuit component replacement (oxygenator, pump, or circuit)
- Moderate or Severe Hemolysis (defined as plasma free Hb: Moderate > 50 – 100 mg/dL; severe > 100 mg /dL sustained in two consecutive measurements) using daily measured plasma free hemoglobin values at trial sites measuring plasma free hemoglobin as standard for care (SOC). For sites not performing plasma free

hemoglobin other measures of hemolysis if performed as SOC such as lactate dehydrogenase (LDH) will be collected. Absence of these lab values, both plasma free hemoglobin and LDH, will not constitute protocol violation.

- 30-day, in-hospital, and 1 year mortality after randomization
- Duration of ECMO support in hours
- Duration of mechanical ventilation post-randomization in hours for survivors to ICU discharge
- Occurrence of electroencephalographic evidence of seizure prior to hospital discharge or within 90 d post randomization, whichever is earliest
- Occurrence of brain infarction, intracranial hemorrhage, or ischemic injury (composite) confirmed using head ultrasound and Computed Tomography (CT) during ECMO
- Occurrence of brain infarction, intracranial hemorrhage, or ischemic injury (composite) confirmed using head ultrasound, CT, or Magnetic Resonance Imaging (MRI) prior to hospital discharge or within 90 d post randomization, whichever is earliest
- Pediatric Overall Performance (POPC) and Pediatric Cerebral Performance Category (PCPC) scales at hospital discharge, 3, 6, 9, 12 months post-randomization
- Functional Status Score assessed at hospital discharge, 3, 6, 9, and 12 months following randomization

3.3.2: Secondary Resource Utilization Endpoints

- ICU length of stay in survivors
- Number of days not in the ICU at 60 days post-randomization
- Hospital length of stay in survivors
- Number of days not in hospital at 90 days post-randomization
- Discharge location: home, other local hospital, or rehabilitation facility

3.3.3. Secondary Neurodevelopment Endpoints

- Adaptive Functioning - measured by the **Adaptive Behavior Assessment System-3 (ABAS-3)**. The ABAS-3 is a parent completed standardized questionnaire that assesses adaptive skills in children aged birth- 21 years. Outcomes of ECMO survivors using ABAS-3 have been previously described. Composite scores for overall adaptive functioning (General Adaptive Composite, GAC), Conceptual, Social and Practical domain (mean ± standard deviation, normal range 100 ± 15) as well as nine subscales (mean ± standard deviation, normal range 10 ± 3) will be reported[13, 22].
- Behavior - measured by the **Child Behavior Checklist (CBCL):** The CBCL is a parent-completed checklist assessing emotional and behavioral problems. It has strong psychometric properties, including high test–retest reliability [13, 23, 24]. The CBCL has different versions for young children (ages 1.5 to 5 years) and older children (ages 6 to 18 years). Ratings on these items yield standardized T scores on several empirically based syndromes and DSM-oriented scales within each version by age and gender. The CBCL has been used in prior ECMO studies. Standardized T scores on several empirically based syndromes and DSM-oriented scales within each version by age and gender will be reported.

- Health-related quality of life – measured by the **Pediatric Quality of Life Inventory 4.0 (PedsQL 4.0):** Pediatric Health Related Quality of Life will be measured using the Pediatric Quality of Life Inventory 4.0 (PedsQL 4.0) Core Scales, at 9 months post-randomization. PedsQL 4.0 is a parent reported age-specific instrument that is designed to assess quality of life in both healthy and acute or chronically ill children[25]. The PedsQL 4.0 instrument provides a general quality of life assessment that addresses physical, emotional, social, and school functioning for children 2 to 18 years old. The PedsQL Infant Core Scales will be used for participants 1 month to < 2 years of age at time of assessment. In addition, a cardiac disease-specific module will be used to assess quality of life specific to children with cardiac disease, which is estimated to be 40% of the trial cohort[14].

4 Participant Selection and Withdrawal

4.1 Inclusion Criteria (all must be met):

1. Age < 6 year at ECMO cannulation
2. Veno-arterial (VA) mode of ECMO
3. First ECMO run during the index hospitalization

4.2 Exclusion Criteria (any single criterion met at time of screening excludes the patient):

1. Gestationally-corrected age < 37 weeks
2. Veno-venous (VV) mode of ECMO
3. Patients initially started on VV-ECMO and then transitioned to VA ECMO
4. ECMO used for procedural support (ECMO deployed and decannulated in procedural area with no ICU ECMO care) or ECMO duration expected to be < 24 h
5. Limitation of care in-place or being discussed
6. Congenital bleeding disorders
7. Hemoglobinopathies
8. Primary Residence outside the country of enrollment.
9. Concurrent participation in a separate interventional trial that has the potential to impact neurodevelopmental status of the patient (note that observational non-interventional studies do not qualify the patient for exclusion)
10. Patients cannulated for ECMO at a non-trial center and transferred to a trial site
11. Randomization not possible within 36 hours following ECMO cannulation (e.g., due to staffing or delays related to communication with participant family)"

4.3 Participant Recruitment and Screening

Children undergoing VA-ECMO cannulation at participating centers will be eligible for participation in *TITRE*. All young children (age < 6 y) supported with ECMO at the participating centers will be screened by the site research coordinator for eligibility for participation in *TITRE*. If the patient meets eligibility criteria, the inpatient care team staff physician caring for the patient will be contacted for approval to approach the family. Following inpatient team approval, a member of the site *TITRE* research team will approach the parents/Legal Adult Representative (LAR) to introduce the study, provide printed documents with information about the research study, and assess interest in study participation. The site PI or Co-PI for the *TITRE* research team

(research coordinator or research clinician) will review understanding of the nature of the research and consent process, and obtain informed consent from the parent/LAR prior to randomization, which occurs at <30 hours post-cannulation. If there is physician refusal to approach the family of a trial-eligible patient, then the physician's declination will be recorded as the reason for non-consent.

At each stage of the recruitment, screening, and study follow-up, it will be emphasized that participation in the study is entirely voluntary and the parent/LAR can withdraw consent to participate any time. Ages for assent and consent may vary slightly by study site according to local practices and customs but will be standardized at a given site by the Institutional Review Board (IRB) or Research Ethics Board/Committee (REB).

4.4 Early Withdrawal of Participants

4.4.1 Permanent Withdrawal from Study Intervention

Withdrawal from study intervention is the permanent discontinuation of allocated study intervention (Indication-based RBC transfusion) before the last potential exposure (i.e., pre-ECMO decannulation or 30 days post- randomization). This may occur if there is a clinical indication, physician preference, or family/LAR request for withdrawal from the assigned treatment strategy. However, the participant will remain in the trial and complete all study visits and requirements. Withdrawal from study intervention with continued participation in the trial is preferable to withdrawal from the trial (see Section 4.4.2), in order to ensure complete data for the primary, intention-to-treat, analysis of the trial. For cases where, parent(s) or LAR(s) request that the study intervention strategy be permanently discontinued, it will be up to the treating physician to make a clinical decision on care subsequent to the formal withdrawal request. It is likely that the patient would be transitioned back to standard care according to treating institutional guidelines.

4.4.2 Withdrawal from Trial

Withdrawal from the trial is the discontinuation of study participation before meeting the last scheduled data collection time point. Every effort to minimize the number of participants who withdraw from the trial will be made. However, if such withdrawal occurs at the request of the participant, family/LAR, or physician, all data collected prior to withdrawal from the study will remain in the study database for use in analysis; however, further or new data collection will not continue after the withdrawal date. It should be noted that study withdrawal is not required if parent(s) or LAR(s) requests discontinuation of study intervention (see Section 4.4.1).

Reasons for withdrawal from the study may include: (1) Parent(s), or LAR (S) elect to withdraw consent from all future study activities, including follow-up; (3) the Clinical Site Principal Investigator (PI) determines that it is in the best interest of the patient to be withdrawn or (4) the Research Monitor withdraws the participant from the trial (**see Section 7.4.2**). Every effort will be made to retain all participants in the trial and have all study visits completed, even if withdrawal from study intervention is indicated. The patient is considered to be "lost to follow-up" if multiple attempts to reestablish contact with the participant have failed for more than 18 months following the last successful contact. Management of RBC transfusion for study participants who withdraw from the trial will be transitioned to management protocols at each site.

4.4.3 Data Collection and Follow-up for Withdrawn Participants

For participants who withdraw prematurely from the study, every reasonable effort will be made to consent the patient to obtain basic survival data (30-day and 1-year survival) throughout the protocol's pre-defined follow-up period.

5 Study Intervention

This trial tests a RBC transfusion strategy and does not involve administration of protocol-mandated study medications. Care of the ECMO patients including but not limited to use of mechanical ventilation, inotrope support, sedation and analgesia, and anticoagulation will be in accordance with established institutional guidelines and will not be specified by the trial protocol. Storage and administration of RBC and blood products will also be governed by institutional protocols.

5.1 Study Arms

Participants will be assigned to one of two study transfusion strategy arms (see Figure 6-1) in a ratio of 1:1 at Visit 1 after they are confirmed to meet all eligibility criteria and provide informed consent. The two arms are:

- Center-specific threshold-based RBC transfusion strategy
- Indication-based RBC transfusion strategy, defined as:

Participants randomized to center-specific threshold will use currently existing guidelines for threshold-based transfusion in their center. For those randomized to the indication-based arm, when the center-specific threshold RBC transfusion is met, RBC transfusion will be provided if any one of the following criteria is met:

1. *Moderate or Severe <u>Bleeding</u>* defined as any one of [26]:

   a. causing hemodynamic instability defined as increase in heart rate or decrease in systolic or mean blood pressure > 20% from baseline or age based normal values;

   b. quantifiable bleeding of > 5 ml/kg/h for > 1 h;

   d. requiring surgical or procedural intervention;

   e. volume administration to maintain pump preload while bleeding;

   f. requiring temporary cessation of anticoagulation or use of a reversal agent

   g. requiring administration of hemostatic agents (e.g., ε aminocaproic acid or factor VIIa) [27]

   h. decrease in Hb/HCT > 10% of baseline. Baseline will be defined using one of the following: Pre- ECMO Hb/HCT, Hb/HCT measured within 12 h prior to onset of bleeding, or normative center-specific values of Hb/HCT.

OR

2. *<u>Reduced tissue DO_2,</u>* defined as blood lactate level ≥ 5 mmol/L, or blood lactate > 3 mmol/L and increasing on 2 consecutive measurements at least 2 hours apart after optimizing ECMO support

OR

3. *<u>Hb/HCT Threshold Criteria,</u>*

a. Hb < 10 g/dL or HCT <30%: if age ≤28 days at randomization or if patient has single ventricle anatomy and is age <1 year at randomization;

b. Hb < 8 g/dL or HCT ≤ 25%: for all other patients.

Additional Considerations:

1. Clinicians may defer RBC transfusion in participants with lactic acidosis as defined in the indication-based arm if the participant has adequate tissue oxygen delivery based on their clinical assessment. For example, if the serum lactate is decreasing with ECMO support but laboratory measurements remain above > 5 mmol/L or delayed clearance of lactate in the presence of hepatic dysfunction 5 mmol/L, or if the Hb/HCT is already in the center-specific threshold range for RBC transfusion (e.g., Lactate is > 5 mmol/L but is already above the center's transfusion threshold). Also, see Appendix C for suggestion on clinical assessment of tissue oxygen delivery.

2. Sites can use Hb < 8 g/dL or HCT ≤ 25% for all patients including neonates (age ≤ 28 d) and single ventricle anatomy aged less than 1 year based on their current local practice guidelines.

Single ventricle anatomy: Single ventricle anatomy is defined using the National Library of Medicine [28]: A single ventricle or univentricular heart is one in which one ventricle is severely underdeveloped, or a ventricular septal wall did not form. Through various mechanisms, the anomalous structure typically results in the mixing of oxygenated and deoxygenated blood. Occurrences are generally caused by genetic factors, though environmental factors are known to promote malformation. Key univentricular variations and typical features:

Hypoplastic left heart syndrome (HLHS): The left ventricle, mitral valve, aortic valve, and aorta are underdeveloped.

Tricuspid atresia: The tricuspid valve fails to develop, leading to an underdeveloped right ventricle.

Ebstein anomaly: Abnormal development of tricuspid valve leaflets causes right ventricular atrialization. The anomaly is associated with various cardiac structural abnormalities, including pulmonary valve pathologies, septal defects, and electrical conduction lesions.

Double outlet right ventricle: The aorta and the pulmonary artery exit from the right ventricle, leaving the left ventricle underdeveloped.

Double inlet left ventricle: Both atria connect to the left ventricle, resulting in an underdeveloped right ventricle.

Atrioventricular canal defect: An atrial or ventricular septal defect forms large enough to make a functionally single ventricle.

See **Appendix A** for additional specifications related to Bleeding definitions. See **Appendix B** for specifications related to optimizing ECMO support. See **Appendix C** for specifications regarding assessment of cardiac output in patients for lactic acidosis.

Additional RBC transfusion considerations for both study arms:

- Volume of RBC transfused in the two arms is not specified by this study, and is at the discretion of the treating team based on local clinical practice.
- RBC transfusion and Hb or HCT for ECMO weaning and decannulation are not specified by the study and will be at the discretion of the treating team based on local clinical practice. Typically, for ECMO weaning trials RBC transfusion is administered starting 24 hours prior to a weaning trial. Details of RBC transfusion for the purposes of weaning will be recorded. RBC transfusion for purposes of weaning off ECMO will not be classified as a protocol deviation.
- Patient who fail a trial of weaning will return to the assigned treatment (center-specific threshold or indication-based RBC transfusion) at the conclusion of the weaning trial.
- Patients recannulated for ECMO ≤ 36 h after ECMO decannulation will return to the assigned treatment arm after recannulation. Those cannulated > 36 h after ECMO decannulation are not required not continue on the assigned treatment arm as this will be a 2nd ECMO run. Data collection and neurodevelopment will proceed per study protocol.
- RBC transfusion after ECMO decannulation is not specified by this protocol as is at the discretion of the treating team and institutional guidelines.

Management of TITRE treatment assignments during interventional and surgical procedures:

The TITRE treatment assignment may be suspended for up to 24 hours for the conduct of interventional or surgical procedures. This time period can start immediately pre-procedure if patient HCT/Hb optimization is necessary, and can be continued for a 24 hour time period covering post-procedural care. This suspension must be documented in the electronic case report form. If participant stability is achieved prior to conclusion of the 24 hour time period, the TITRE treatment assignment should be resumed as soon as possible, at the discretion of the treating physician.

5.2 Compliance with Trial Intervention (Red Cell Units)

Transfusion of RBCs will be directed by the site PI for each participant randomized, i.e., to the indication-based and center-specific threshold-based arms. RBC units will be collected, prepared and stored as per the local blood bank policy and procedures of each participating site. Consent for and administration of RBC transfusion to patients will be conducted in compliance with the local transfusion administration policy for each site. Compliance with all RBC transfusions during ECMO will be assessed by routine monitoring of transfusion records, electronic medical records, bleeding events, and laboratory data. Each investigator will also be asked to certify data regarding product collection, preparation, storage, volume, indication, and timing of transfusion, and compliance with randomization arm. Monitoring for non-compliance with the prescribed transfusion strategy will be conducted, to minimize crossover to the other treatment arm. It should be noted that RBC transfusion administered to optimize Hb/HCT in the 24 h prior to decannulation from ECMO for subjects randomized to either arm will not represent non-compliance.

5.3 Method for Assigning Participants to Treatment Groups

Once informed consent is obtained, randomization will occur 18-30 h after ECMO deployment. Bleeding events requiring RBC transfusion are very common within 24 hours after ECMO deployment. Furthermore, for patients in whom ECMO is deployed using a clear prime, RBC transfusion to mitigate low HCT related hemodilution is usually completed within a few hours after ECMO deployment. Thus, *randomization to study treatment at 18 – 30 h after ECMO deployment ensures that bleeding and events and hemodilution related to ECMO cannulation are adequately managed prior to randomization*. However, bleeding events requiring transfusion are expected to occur, albeit at a lower frequency, throughout the ECMO run. These events will be managed with RBC transfusion as needed.

Study participants will be randomly assigned in a 1:1 ratio to the indication-based or center-specific threshold-based RBC transfusion strategy arms using secure web-based randomization that is integrated into the database management system. A total of 228 participants will be randomized to one the two arms (114 participants per group). The stratified randomization scheme is described in Section 7.6.1.

5.4 Blinding

Medical providers, including physicians, nurses, and ECMO specialists, involved in the care of the trial participant will not be blinded to the RBC transfusion strategy to which the participant is randomized during the course of ECMO. However, personnel involved in neurodevelopmental assessment at 12 months post-randomization will be blinded to the randomized RBC transfusion strategy. Research coordinators performing the virtual follow-up visits to administer the PCPC, POPC and FSS will not be blinded, but will utilize a standardized script to minimize bias. Members of the Endpoint Adjudication Committee will also be blinded to RBC transfusion strategy. Blinding of participant's parents, family or LAR is not possible, because of their ability to access and review participant's electronic medical records and participation in daily medical rounds with ICU providers during and after ECMO support. Every effort to maintain a state of equipoise will be undertaken, as the superiority of one RBC transfusion strategy over the other tested in TITRE has not been established.

6. Study Procedures

6.1 Overall Study Design

The overall study flow is outlined in **Figure 6-1** and the indication-based RBC transfusion protocol is displayed in **Figure 6-2.** Study visits are outlined in **Table 6-1**. Study participants are critically ill children closely followed in-hospital units, including but not limited to in-patient ward, intensive care unit, and emergency departments, and will follow standard clinical management as deemed appropriate by their treating teams. Decisions regarding deployment of ECMO will be at the discretion of the treating team and will not be specified by the protocol.

Treatment of primary disease, location of ECMO cannulation and care prior to participant enrollment are not specified by this protocol. Treatment prior to consent for study participation will not influence qualification for study participation if an eligible participant meets screening inclusion/exclusion criteria.

All ECMO patients at trial sites will be screened for meeting study inclusion criteria after deployment. If the patient meets inclusion criteria, and does not have any exclusion criteria, parent

(s) or LAR(s) will be approached for study participation.t. If, after learning about the study, the family elects to participate, consent will be obtained from parent (s)/LAR(s) as appropriate during this visit. Study randomization will occur once eligibility criteria have been met and written informed consent has been obtained at 18 – 30 h post-ECMO deployment. Consented participants who are unable to be randomized within the trial randomization window of 18 – 30 h will be allowed to participate in the study if randomization is possible within 36 h after ECMO deployment. Patients randomized > 30 but ≤ 36 h will be considered as a minor protocol violation. Participants will be excluded if randomization is not possible ≤ 36 h.

Figure 6-1: Schematic Representation of *ITITRE* Trial and Primary Clinical Endpoint

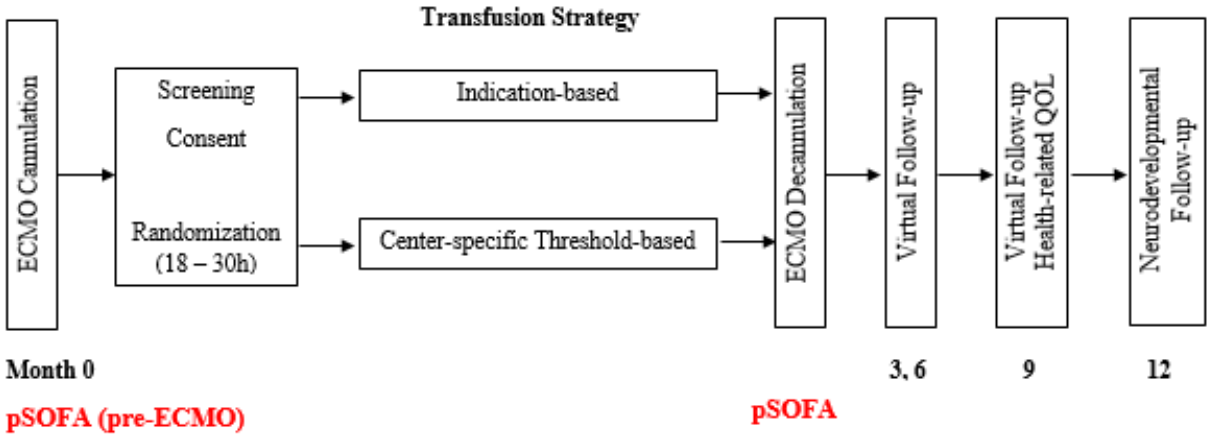
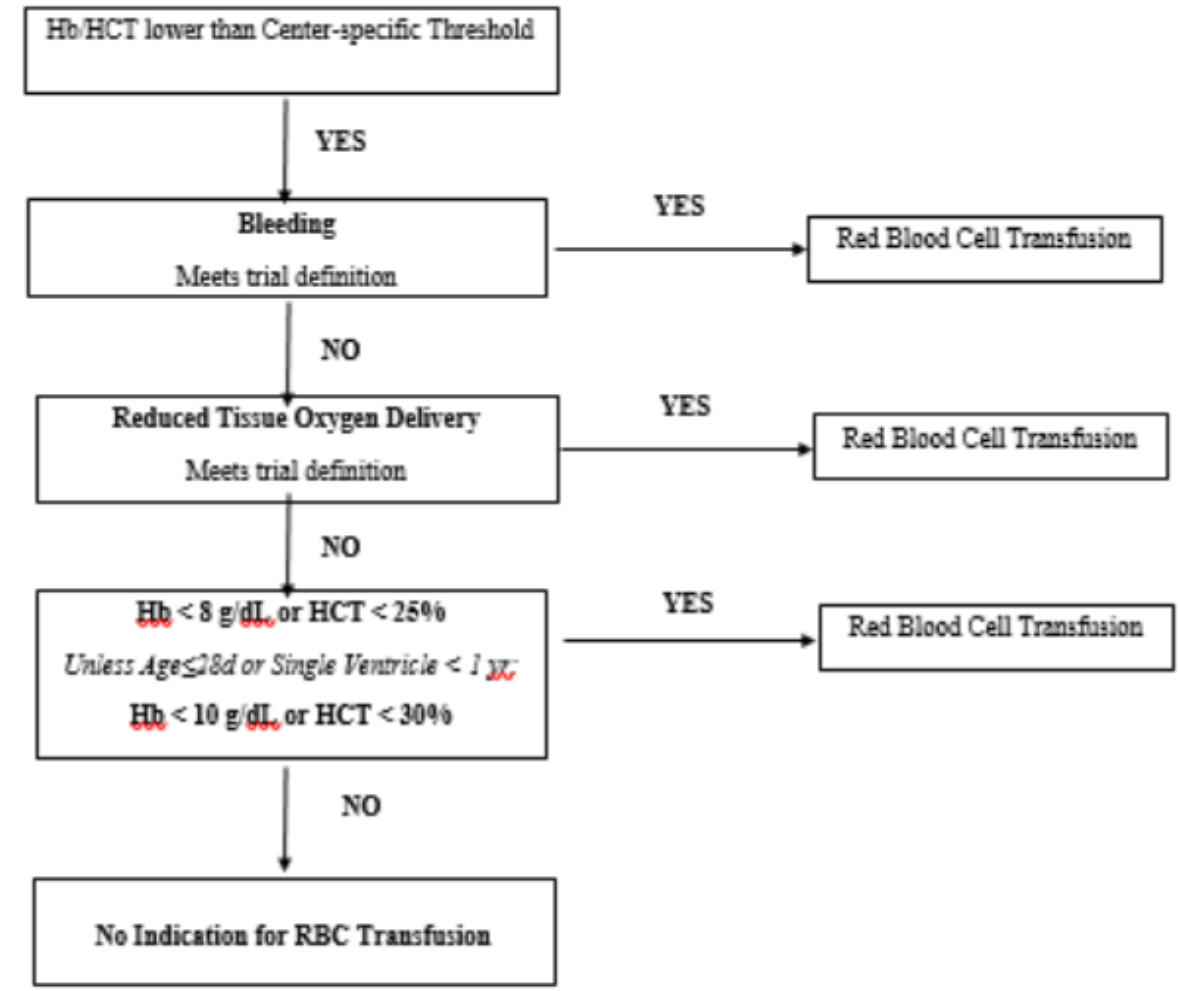


Figure 6-2: Indication-Based Transfusion Protocol



Genetics and other biomarkers of organ injury are expected to play a critical role influencing resilience to ECMO and other clinical outcomes [48-51]. For example, susceptibility to cerebral or cardiac ischemia reperfusion injury in response to surgery or hemodynamic insults may be influenced by common genetic variants or genetic polymorphisms in the pathways that regulate inflammation, thrombosis, vascular reactivity, and oxidative stress. A biorepository of participant DNA, serum, and urine will be a key resource to help elucidate determinants of response to the stress of ECMO in this high-risk population. The specimen collection time points are shown in Table 6-3. Specimen amounts are detailed in Section 9.7.2.

Table 6-3. *TITRE* Schedule of Visits and Measurements

	ECMO				Follow-up Post-Randomization				
Activity/ Measurement	**Cann-ulation**	**Day 1**	**Day 2+**	**Decann-ulation**	**Hosp D/C**	**3 mo ±1 mo**	**6 mo ±1 mo**	**9 mo ±1 mo**	**12 mo ±2 mo**
Screening	X								
Consent		0-30h							
Randomization		0-30h							
Begin assigned transfusion strategy		18-30h							
Endpoint pSOFA	X			X					
8 AM SVO_2		X	X	X					
RBC volume	X	X	X	X	X				
Donor exposure		X	X	X	X				
Infection, Bleeding	X	X	X	X					
Renal function	X	X	X	X					
Brain Radiology		X	X	X	X				
POPC/PCPC scale	X				X	X	X	X	X
FSS	X				X	X	X	X	X
Study Compliance Measures	X	X	X	X					
Adverse Events	X	X	X	X	X	X	X	X	X
Virtual check-in						X	X	X	
QOL								X	
Neurodevelopment Assessment									X
Biobank (Blood, Urine)	X			X					
Ancillary Studies	O	O	O	O	O	O	O	O	O

pSOFA: Pediatric Organ Dysfunction Assessment; SVO_2; systemic venous oxygen saturation from ECMO circuit; QOL: quality of life; POPC: Pediatric Overall Performance; PCPC: Pediatric Cerebral Performance; FSS: Functional Status Score; O: optional

6.2 Visits

6.2.1 Visit 0: Screening and Informed Consent: Timing: After ECMO deployment

After a patient is placed on ECMO at the participating trial sites, they will be screened to see if they meet inclusion/exclusion criteria. A trial eligibility form will be competed at this time. If a patient meets inclusion criteria and does not have any exclusion criteria then the research coordinator at the site will approach the care team to seek permission to approach the family for informed consent. Once permission is granted, the research coordinator/site PI will approach the family to explain the details of the study and complete the informed consent process. Informed consent to participate in the biobank requires separate consent in the section on biobanking in the Informed Consent form. If informed consent for participation in biobank is obtained, blood will be drawn (5 ml for participants under age 2 years and 10 ml if ≥ 2 years) and 1 ounce (25-30 ml) of urine (if patient is capable) will be collected from the participant. Only if informed consent occurs outside of business hours, this specimen collection may be delayed for up to 48 hours. Please see biobank processing for instruction regarding processing and storing specimens.

6.2.2 Visit 1: Randomization and Pre-ECMO pSOFA score: Timing: <30 hours after ECMO deployment

Randomization will occur <30 hours after ECMO deployment. During this visit, the medical record will be reviewed to collect participant and ECMO information. This will include, but not limited to, participant age at ECMO deployment, height, weight, diagnosis, hemodynamic support pre-ECMO, RBC transfusion volume, and laboratory studies on the day of ECMO deployment. Pre-ECMO (using vital signs and laboratory values 24 h prior to ECMO cannulation) and post-randomization (vital signs and laboratory values from randomization to 24 h post-randomization) pSOFA scores will be collected at this visit. Additionally, the POPC and PCPC scales, and FSS scores will also be collected using a scripted form, within one month of the randomization. If not feasible to collect these data via the scripted form (e.g., patient dies), this information will instead be obtained from medical records. Daily medical progress notes, and laboratory testing relevant to the endpoint events will be de-identified and entered into the study database for endpoint adjudication.

6.2.3 Visit 2: Daily ECMO care: Timing: Daily from Day 2 of ECMO until day of ECMO decannulation or 29th day post-randomization, whichever is earliest.

During these visits daily clinical and ECMO information will be collected. Additionally, daily volume of RBC and blood products transfused and reasons for RBC transfusion will be collected. RBC unit storage solution for every RBC transfusion administered during ECMO will be collected. There will be daily screening and capture of information related to vital signs, laboratory values, blood stream infection, renal function, acute kidney injury using the KDIGO definition, and review of head ultrasound, CT scan, or EEG reports for neurologic events. These visits will also include assessment of adherence to the allocated treatment arm and reasons for non-adherence will be collected. Additionally, participant charts will be screened for complications and serious adverse events (SAE). Daily medical progress notes, and laboratory testing relevant to the SAE events will be de-identified and entered into the study database for SAE adjudication.

6.2.4 Visit 3: Trial Primary endpoint: Timing: 12 – 36 h post-ECMO decannulation. If a required data point is not available during this period, medical records available up to 48 hours after decannulation will be used. For participants remaining on ECMO at 30 days post-randomization,

the timing is instead 0 – 24 hours after the participant's 30th day post-randomization. During this visit the post-ECMO decannulation pSOFA score will be collected along with clinical and ECMO related variables, RBC and blood product transfusion volume and laboratory studies. Daily medical progress notes, and laboratory testing relevant to the endpoint events will be de-identified and entered into the study database for endpoint adjudication. These visits will also include assessment of adherence to the allocated treatment arm and reasons for non-adherence will be collected. Participant charts will be screened for complications and SAEs and daily medical progress notes, and laboratory testing relevant to the SAE events will be de-identified and entered into the study database for AE adjudication. For subjects decannulated from ECMO, data on successful decannulation, defined as survival or no need for ECMO recannulation within 36 hours after decannulation, will be collected. Additionally, blood will be drawn (5 ml for participants under age 2 years and 10 ml if ≥2 years) and 1 ounce (25-30 ml) of urine (if patient is capable) will be collected from the participant. Note: Biobank specimens will only be collected for participants who consented to this aspect of the study. Only if decannulation occurs outside of business hours, this specimen collection may be delayed for up to 48 hours.

6.2.5 Visit 4: Hospital Discharge: Timing: day of discharge from hospital to home or rehabilitation facility.

During this visit medical records and laboratory tests for study information related to complications and SAEs, RBC and blood product transfusion volume, number of donor exposures, and discharge information (home or rehabilitation facility) will be collected. Neurological imaging data including MRI, CT scan or ultrasound for neurologic complications will be collected. The POPC and PCPC scales and FSS scores will also be collected using a scripted form within two weeks of the discharge date. If not feasible to collect these data via the scripted form (e.g., patient dies), this information will instead be obtained from medical records. Daily medical progress notes and laboratory testing relevant to the SAE events will be de-identified and entered into the study database for SAE adjudication.

6.2.6 Visit 5: 3 month post-randomization follow-up: Timing 3 ± 1 month.

During this visit we will collect follow-up information on general health, readmission to hospital for care or procedures. We will use a virtual communication platform for this visit and use a scripted form to conduct the visit. If the participant is still hospitalized, then we will complete the visit in-person. These will include the PCPC and POPC scales and FSS scores. If not feasible to collect these data via the scripted form (e.g., family cannot be reached), this information will instead be obtained from medical records.

6.2.7: Visit 6: 6 month post-randomization follow-up: Timing 6 ± 1 month

During this visit we will collect follow-up information on general health, readmission to hospital for care or procedures. We will use a virtual communication platform for this visit and use a scripted form to conduct the visit. If the participant is still hospitalized, then we will complete the visit in-person. These will include the PCPC and POPC scales and FSS scores. If not feasible to collect these data via the scripted form (e.g., family cannot be reached), this information will instead be obtained from medical records.

6.2.8: Visit 7: 9 month post-randomization follow-up: Timing 9 ± 1 month

During this visit we will deploy and complete the health-related quality of life PedsQL 4.0 instrument (core scales) to assess quality of life, function and well-being. For children 1 m to 2

years of age at assessment we will use the PedsQL Infant Core Scales. This will be deployed electronically or mailed paper forms based on prior arrangements with parents/LARs of study participant. Additionally, the POPC and PCPC scales and FSS score will be collected. If the participant is still hospitalized, then we will complete the visit in-person. If not feasible to collect the POPC, PCPC, and FSS data via the scripted form (e.g., family cannot be reached), this information will instead be obtained from medical records.

6.2.9: Visit 8: 12 month neurodevelopmental end-point: Timing 12 ± 2 months

This in-person visit is to evaluate the patient for the primary neurodevelopmental end point. During this visit age appropriate instruments (BSID-4, WPPSI-IV) will be used to conduct detailed neurodevelopmental follow-up. Furthermore assessment for information on secondary neurodevelopmental endpoints (ABAS-3, CBCL) will be administered to accompanying parent/LAR of study participant to evaluate adaptive skills and cognition. Additionally, the POPC and PCPC scales and FSS score will be collected.

7 Safety and Adverse Events

Study participants will be carefully monitored to protect individual human participants enrolled in the trial and to provide a complete and accurate assessment of the safety profile of the RBC transfusion strategy. Because adverse events are common in all patients supported with ECMO, selected adverse events called Serious Adverse Events (SAEs) will be used for monitoring safety in the trial. All other adverse events will be called complications. Complications will be collected from the time of consent until hospital discharge and will be reported at the time of hospital discharge. SAEs will be collected from the time of consent until 30 post-study completion and will be reported within 24 hours after occurrence to the DCC (See Sections 7.1.3-7.1.5 on reporting instructions). Participants withdrawing consent for study participation will be monitored until the time of consent withdrawal. The definition of SAEs and reporting procedures are summarized below.

7.1 Definitions

7.1.1 Serious Adverse Event

A serious adverse event (SAE) includes any event that results in any of the following outcomes

- Death
- Life-threatening i.e., the participant was at immediate risk of death from the event as it occurred in the opinion of the Investigator. It does not refer to an event, which hypothetically might have caused death if more severe.
- Persistent or significant incapacity/disability
- Requires hospitalization or prolongs hospitalization (Note: a pre-scheduled hospitalization is not considered an SAE nor are hospital stays less than 12 hours in duration). Hospitalization unrelated to the SAE is excluded (e.g. social hospitalization such as a parent/family unable to find transportation during the night).
- Congenital anomaly/birth defect (not applicable for this trial)
- Any other medically important event that, in the opinion of the clinical site PI, jeopardizes the participant and may require medical or surgical intervention to prevent one the outcomes listed above Examples of other medically significant events include

allergic bronchospasm requiring intensive treatment in an emergency department or home, laboratory abnormalities that results in hospitalization.

Because many typical clinical events occurring in critically ill children supported by ECMO meet these criteria, **only the following six sentinel events will be defined as Serious Adverse Events for the purposes of this trial**:

1. Death
2. Cardiopulmonary resuscitation (CPR) during ECMO
3. Intracranial hemorrhage defined as intraparenchymal, subdural, subarachanoid, or ≥ grade 2 intraventricular hemorrhage by radiological imaging during ECMO and up to hospital discharge.
4. Cerebrovascular accident (CVA) confirmed with imaging during or after ECMO and up to hospital discharge.
5. ECMO component change (oxygenator, pump or circuit) that leads to deterioration in patient condition
6. Air emboli in the patient that is associated with the ECMO circuit, which requires circuit or patient intervention

In addition, any other event that in the opinion of a site investigator is considered to be a Serious Adverse Event, that cannot be reported as a complication may be classified as such, and will then adhere to the rules for expedited reported described in 7.3.2.

Of note, elective and planned hospitalizations will not be reported as serious adverse events.

7.1.2 Complications

If a clinical event or finding does not meet the definition of SAE defined in Section 7.1.1, the event will be classified as a Complication, using the TITRE Complications Code List available in the Trial Manual of Operations and will be reported as a complication rather than as an SAE (see Table 7-5). The Complication will be recorded according to the date of the first presenting symptom.

Events that are continuing will be identified once as a complication. If the complication resolves and recurs as an independent event, only then should it be reported again as a new serious adverse event, or as a new complication using the Complications Code List.

Any medical condition or abnormal laboratory value present at enrollment that remains unchanged or improves will *not* be reported as a complication. However, worsening of a medical condition that was present at enrollment will be considered a new event and reported as a complication.

Certain clinical findings will <u>not</u> be considered Complications and do not require reporting, <u>unless</u> they result in an intervention or treatment. These include but are not limited to the following conditions that are common in patients receiving ECMO support:

a) Horner's Syndrome

b) Musculoskeletal pain
c) Gastroesophageal reflux disease (GERD)
d) Vocal cord paralysis
e) Diaphragmatic paralysis
f) Feeding difficulties or failure to thrive

7.1.3 Expected Events

An event is considered expected if it is known to be associated with the underlying disease process causing cardiorespiratory failure or with a study treatment that the participant is receiving. For this protocol, some of the RBC transfusion-related and ECMO-related expected events are listed in **Table 7-1** and **Table 7-2**, respectively. Tables 7-1 and 7-2 should not be considered exhaustive.

- If a qualifying SAE is associated with one of the conditions in Tables 7-1 and 7-2 as its underlying cause, then the SAE should be classified as Expected.
- The conditions noted in Tables 7-1 and 7-2 are also included in the Complications Code List. This list is documented in the Trial Manual of Operations.

Table 7-1. Adverse Events Known to be associated with Transfusion of Blood Products

Blood Product	Expected AEs		
Packed red blood cells	• Acute lung injury • Acute respiratory distress • Allergic reaction • Alloimmunization (Antibody development) • Bronchospasm • Chills • Delayed serologic transfusion reaction • Disseminated intravascular coagulation	• Edema • Epistaxis • Hematuria • Hives • Hypoxemia • Infection • Jaundice • Maculopapular rash	• Pain • Pruritis • Purpura • Tachypnea • Thrombocytopenia
Platelets	• Acute lung injury • Acute respiratory distress • Allergic reaction • Alloimmunization (Antibody development) • Bronchospasm • Chills • Cyanosis • Delayed serologic transfusion reaction • Disseminated intravascular coagulation	• Edema • Epistaxis • Hematuria • Hives • Hypoxemia • Infection • Jaundice • Maculopapular rash	• Pain • Pruritis • Purpura • Tachypnea • Thrombocytopenia
Plasma (FFP and/or FP-24)	• Acute lung injury • Acute respiratory distress	• Edema • Epistaxis	• Pain • Pruritis

	• Allergic reaction • Alloimmunization (Antibody development) • Bronchospasm • Chills • Cyanosis • Delayed serologic transfusion reaction • Disseminated intravascular coagulation	• Hematuria • Hives • Hypoxemia • Infection • Jaundice • Maculopapular rash	• Purpura • Tachypnea • Thrombocytopenia

Table 7-2. Adverse Events Known to be Associated with ECMO

ECMO	Expected AEs
	• Mechanical o Membrane Lung Failure o Blood Pump Failure o Circuit tubing/Raceway rupture o Circuit or component change o Cannula malposition o Accidental decannulation o Temperature regulation device malfunction o Clots in the circuit o Clots in circuit components (hemofilter, oxygenator, pump) o Air embolism/Air entrainment in circuit • Bleeding o Gastrointestinal bleeding o Cannula site bleeding o Surgical site bleeding o Mediastinal bleeding o Pulmonary Hemorrhage o Bleeding related to anticoagulant use • Central Nervous System Complications o Stroke o Hypoxic Ischemic encephalopathy o Intracranial Hemorrhage o Seizures o Brain Death o Sedative or Analgesic medication dependence • Renal o Acute Kidney injury o Renal Failure with or without need for renal replacement therapy o Hematuria

	• Cardiovascular
	  ○ Cardiopulmonary resuscitation during ECMO
	  ○ Cardiac Arrhythmia
	  ○ Cardiac Tamponade (blood or other)
	  ○ Hypotension
	  ○ Lactic acidosis
	• Respiratory
	  ○ Pneumothorax
	  ○ Ventilator Induced Lung Injury
	• Hepatic
	  ○ Liver Failure
	  ○ Hyperbilirubinemia
	• Hematology
	  ○ Hemolysis
	• Limb/Vascular Injury
	  ○ Limb ischemia or amputation from thromboembolism
	  ○ Limb Ischemia or amputation from vascular occlusion from cannulation
	  ○ Compartment syndrome
	  ○ Blood vessel injury as a result of cannulation
	• Infection
	  ○ Pneumonia
	  ○ Respiratory tract infection
	  ○ Cannulation site infection
	  ○ Blood stream infection
	  ○ Sepsis
	  ○ Catheter associated blood stream infection (CLABSI)
	  ○ Urinary tract infection
	  ○ Hospital acquired infection in any site
	  ○ Cannulation site wound infection
	• Skin
	  ○ Pressure Injury and ulceration
	• Musculoskeletal
	  ○ Joint contractures
	  ○ Mobility disorders and muscle weakness
	  ○ Peripheral nerve injury or peripheral neuritis

7.1.3 Serious Adverse Event Reporting Period

The overall study period during which SAEs must be reported is defined as the period from the time the participant, parent, guardian, and/or LAR sign the Informed Consent Form and dating to 30 days after study completion, termination, or withdrawal. See Section 7.1.1. for the time frames during which specific SAEs must be reported.

7.1.4 Preexisting Condition

According to the tailored definition of SAE for this trial, preexisting conditions should not be reported as SAEs, unless the event is one that in the opinion of the site investigator should be reported. If such an event is a preexisting condition, it should be reported as an SAE only if the frequency, intensity, or the character of the condition worsens during the study period. Of note, at screening, any clinically significant abnormality should be recorded as a preexisting condition.

7.1.5 Post-Study Adverse Event

The local site PI should follow all unresolved SAEs until the events are resolved, the participant is lost to follow-up, or the SAE is otherwise explained.

The local site PI should notify the DCC of any death occurring at any time after a participant has discontinued or terminated study participation that may reasonably be related to the study intervention.

7.1.6 Severity Criteria (Grade 1-5)

The six sentinel SAEs being reported in this study (see Section 7.1.1) by definition all meet the definition of grade 4 or 5 according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 5.0 (CTCAE v5.0) shown in **Table 7-3**.

Table 7-3. Definition of Adverse Event Grades

Grade (severity)	Definition
Grade 1 (Mild AE)	Experiences which are usually transient, requiring no special treatment, and not interfering with the participant's daily activities
Grade 2 (Moderate AE)	Experiences which introduce some level of inconvenience or concern to the participant, and which may interfere with daily activities, but are usually ameliorated by simple therapeutic measures
Grade 3 (Severe AE)	Experiences which are unacceptable or intolerable, significantly interrupt the participant's usual daily activity, and require systemic drug therapy or other treatment
Grade 4 (Life-threatening or disabling AE)	Experiences which cause the participant to be in imminent danger of death
Grade 5 (Death related to AE)	Experiences which result in participant death

7.1.7 Causality (Attribution)

The sentinel events listed in Section 7.1. will have causality assigned by the site PI and the study Medical Monitor. The site clinical PI is to assess the causal relation (i.e., whether there is a reasonable possibility that the study intervention caused the event) of any reported SAE using the definitions shown in **Table 7-4**.

Table 7-4. Definition of Adverse Event Relatedness to Study Treatment

Relatedness	Definition
Not Related	Another cause of the AE is more plausible; a temporal sequence cannot be established with the onset of the AE and administration of the investigational product; or, a causal relationship is considered biologically implausible
Unlikely Related	The current knowledge or information about the AE indicates that a relationship to the investigational product is unlikely
Possibly Related	There is a clinically plausible time sequence between onset of the AE and administration of the investigational product, but the AE could also be attributed to concurrent or underlying disease, or the use of other drugs or procedures. Possibly related should be used when the investigational product is one of several biologically plausible AE causes.
Related	The AE is clearly related to use of the investigational product.

7.2 Recording of Serious Adverse Events and Complications

All SAEs occurring after the participant, parent, guardian, and/or LAR has signed the Informed Consent Form (ICF) must be fully recorded in the participant's hospital record and electronic case report form (eCRF) from the time of ICF signing and dating to 30 days after study completion, termination, or withdrawal. At each contact with the participant, the local site PI must seek information on SAEs and Complications by specific questioning and, as appropriate, by examination. Information on all SAEs should be recorded immediately in the source document, and also in the appropriate eCRF. Documentation must be supported by an entry in the participant's file.

Each event should be described in detail along with start and stop dates, seriousness, severity, relationship to investigational product, action taken and outcome. The clinical course of each event should be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause. SAEs that are still ongoing at the end of the study period must be followed up to determine the final outcome. Any SAE that occurs after the study period and is considered to be possibly related to the study treatment or study participation should be recorded and reported immediately.

7.3 Reporting of Serious Adverse Events and Unanticipated Problems

7.3.1 Local Site PI reporting: notifying the Data Coordinating Center (DCC)

SAEs and unanticipated problems (UP) must be reported to the DCC and CCC at Boston Children's Hospital within 24 hours of the local site PI's awareness of the event and include all SAEs and UPs that occur from the time of ICF signing and dating to 30 days after study completion. To report such events, an AE form must be completed electronically by the local site PI and submitted to the DCC within 24 hours. The local site PI will keep a copy of this SAE information on file at the study site. SAEs must also be reported to the study clinical research associate (CRA), medical monitor, designee of the study CRA, or designee of the medical monitor

by phone and/or e-mail with confirmation of receipt. Table 7-5 summarizes reporting timelines for local site PIs.

Table 7-5. Reporting Timeline for Clinical Centers to Notify DCC

Classification	Reporting Timeframe
Serious	Within 24 hours of first knowledge of the event
Complications	Submitted on case report forms according to study visit/contact or at hospital discharge

Within the following 48 hours, the local site PI must provide further information on the SAE in the form of a written narrative to the DCC. This should include a copy of the completed Serious Adverse Event (SAE) form and any other diagnostic information that will assist the understanding of the event. Significant new information on ongoing SAEs should be provided promptly to the DCC. The Medical Monitor or her designee will review all SAEs post-randomization within 2 working days of the report receipt in order to assign a final determination on expectedness and relatedness to the study intervention (RBC transfusion).

All SAEs that have not resolved by the end of the study, or that have not resolved upon discontinuation of the participant's participation in the study, must be followed until any of the following occurs:

- The event resolves
- The event stabilizes
- The event returns to ≤ CTCAE grade 1
- The event can be attributed to agents other than the RBC transfusion strategy or to factors unrelated to study conduct
- It becomes unlikely that any additional information can be obtained (participant or health care practitioner refusal to provide additional information, lost to follow up after demonstration of due diligence with follow-up efforts)

All deaths should be reported with the primary cause of death as the SAE term, rather than death itself, which is typically the outcome of the event. Any death occurring within 30 days of study completion must be reported as an SAE. If the local site PI is in doubt about the applicable reporting obligations, he/she should consult with the study CRA or medical monitor at the DCC.

The medical monitor or designee will assess each SAE to determine whether it is unexpected according to the ICF, Protocol Document, RBC unit information or insert, medication package insert, or commonly used drug database (i.e. Micromedex, Lexi-Comp).

7.3.2 Expedited Reporting Requirement for Serious Adverse Events

All SAEs that are related to the study intervention (RBC transfusion) and either (1) unanticipated (Suspected Unexpected Serious Adverse Reaction - SUSAR) or (2) fatal or life threatening will be reported by the DCC to the IRB/REB, the funding agency (U.S. Department of Defense) and the Chair of the Data and Safety Monitoring Board (DSMB) within 7 calendar days of report receipt by the DCC. Expedited reporting is not required for other AEs. Table 7-6 summarizes the reporting timelines for all events by the DCC to regulatory bodies.

Table 7-6. Reporting Timeline for the DCC to Regulatory Bodies

Classification	Unexpectedness	Relatedness*	Reporting Timeframe
Serious: Fatal life-threatening	Unexpected	Related	Within 7 calendar days of report receipt to IRB, DSMB, and Health Canada
Serious: Fatal life-threatening	Unexpected or Expected	Unrelated	Semi-annually to DSMB; Annually to DoD and IRB
Serious (Suspected Adverse Reaction)	Unexpected	Related	Within 15 calendar days of report receipt to IRB and DSMB
Serious	Unexpected or Expected	Unrelated	Semi-annually to DSMB ; Annually to DoD and IRB

*Unrelated= Not related or unlikely to be related

Related= Possibly related or definitely related

7.3.2.1 Reporting to Regulatory Agencies – Institutional Review Board

Guidelines for reporting of SAEs to the IRB are described in Section 7.3.2 and Table 7-6.

If a local site is not ceding authority to the central IRB, then the local site PI is responsible for complying with their local IRB/REB's reporting requirements. Copies of each report and documentation of IRB/REB notification and receipt will be kept in the local site PI's study file. A central IRB located at Boston Children's Hospital will be used for the majority of sites in this trial.

7.3.2.2 Reporting to Local Site PIs

For any site that has not ceded authority to the central IRB, the DCC shall submit to the local site PI the determination regarding safety concerns or lack thereof from the DSMB after each DSMB meeting (or at the direction of the DSMB Chair with respect to UPs reviewed on an expedited basis). The local site PI shall provide to his/her IRB/REB the DSMB summary.

7.4 Medical Monitoring

It is the responsibility of each the local site PI to oversee the safety of the study at his/her site. This safety monitoring will include careful assessment and appropriate reporting of AEs as noted above, as well as the construction and implementation of a site data and safety-monitoring plan (see Section 9 Auditing, Monitoring and Inspecting). Medical monitoring will include a regular assessment of the number and type of SAEs that occur in patients at the local PI's site.

7.4.1 Independent Data and Safety Monitoring Board

An independent DSMB will monitor the data for this study. The DSMB is composed of experts in critical care, transfusion medicine, neurology, biostatistics and clinical trial design, and bioethics. The DSMB will review trial data on a periodic basis, but not less than twice per year. The DSMB report will include information on accrual rate, data quality and data completeness, overall and by center, and protocol compliance. It will also include safety endpoint data and

narratives by treatment arm. In addition, the DSMB will regularly review serious AEs and protocol deviations associated with the research to ensure the protection of human participants. The DSMB is assisted by a Medical Research Monitor in reviewing SAEs (see Section 7.4.2). The DCC Medical Monitor will serve as the Sponsor's designee for determining causality and expectedness of all SAEs. Results of the DSMB deliberations will be communicated to the IRB/REB and the appropriate regulatory authorities at the time of continuing review, or in an expedited fashion, according to the timelines in Table 7-6.

7.4.2 Department of Defense Mandated Independent Research Monitor

The Research Monitor, Melania Bembea, MD, is responsible to oversee the safety of the research and report observations/findings to the IRB/REB or a designated institutional official. The Research Monitor will review all SAEs and unanticipated problems involving risks to participants or others associated with the protocol. The independent determination of the Medical Monitor is the finding that will be presented to the DSMB in semiannual reporting. The independent report of any SAE that is determined to be unexpected and possibly related to study treatment will also be submitted to the IRB/REB within 7 days of submission of the full case file to the Medical Monitor. The Research Monitor may discuss the research protocol with the investigators; shall have authority to stop a research protocol in progress, remove individual human participants from a research protocol, and take whatever steps are necessary to protect the safety and well-being of human participants until the IRB/REB can assess the monitor's report; and shall have the responsibility to promptly report their observations and findings to the IRB/REB or other designated official and the U.S. Army Medical Research and Materiel Command Office of Research Protections, Human Research Protection Office.

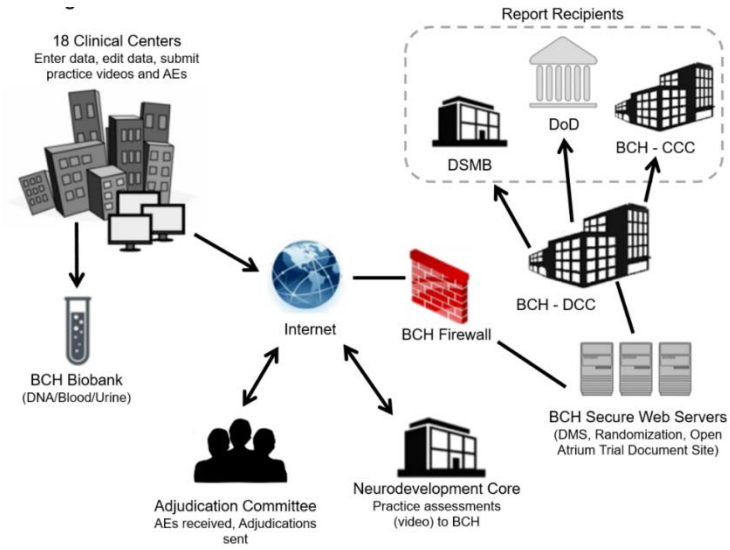
8 Data Management and Statistics

8.1 Data Management Plan

8.3.1 Database Management System (DMS) Capabilities

All trial data will be entered electronically into OpenClinica, a secure, version-controlled, web-based electronic data capture and DMS that supports regulatory trials to ensure Good Clinical Practice (GCP). The system permits direct, paperless data entry from the study sites, with central data storage at the DCC. It will have a password expiration period of 3-6 months in order to ensure enhanced security protection of sensitive data.

Figure 8-1. Research Data Flow



The data management system will provide real-time reporting to the DoD, CCC and study sites, as well information to users on protocol compliance and upcoming study visits. It allows for role-appropriate access, and provides audit trails for data entry, exports, and reports. Trial data will be entered using participant identification number; neither patient name nor medical record number will be submitted to the DCC. The link of name and participant ID number will reside only at the study site. Biospecimens will also be assigned a unique ID number that will be linked to the participant ID number. **Figure 8-1** depicts research data flow.

8.3.2 Data Recovery

The study database is backed up nightly. At any point in time, BCH retains in storage 7 contiguous days of backup, followed by 4 weekly backups and 13 monthly backups. The servers for long-term storage are located at BCH in a fully power and network-redundant commercial data center. In addition, the database management system retains an audit trail of every action occurring within the system and this audit trail history is captured within each database backup.

8.3.3 Database Lock

For key deliverables requiring analysis of the trial data, an export of the entire database into SAS datasets will be performed at such periodic intervals (e.g., semiannual DSMB reports) in order to have a frozen dataset from which all results will be generated. At trial end, a final lock and export will occur after all data queries are resolved. The final trial results will be prepared from this locked dataset. The study database will be retained at the DCC for 5 years after trial end. The exported raw SAS datasets and the analytic datasets will be maintained at the DCC indefinitely.

8.4 Statistical Plan

8.4.1 Randomization Scheme

Randomization will be stratified according to two binary factors, for a total of 4 strata:

1) patient age: neonate (≤28 days) vs. non-neonate; and

2) patient diagnosis (single ventricle disease vs. other diagnosis). See Section 5.1 for definition of single ventricle disease.

Randomization will also be performed within study site. Participants will be randomly assigned in a 1:1 ratio within strata to the center-specific threshold-based vs. indication-based management arms, using secure web-based randomization. Participant randomization will require prior entry of eligibility data and confirmation that written informed consent has been obtained. A minimization algorithm will be used to ensure balanced allocations across the two treatment groups

within each stratum within site. In addition, there will be dynamic balancing of treatment assignment within study sites. In case of randomization system/internet failure at the centers, the randomization assignment will be provided to the site by the DCC, where the master list according to stratum is securely maintained.

8.4.2 Power and Sample Size

Aim 1, Primary Endpoint: The primary trial endpoint is the change in pSOFA score from randomization (ECMO initiation) to ECMO decannulation (or 30d post-randomization, whichever is earlier). Our null hypothesis is that there is no difference in the mean change in pSOFA score between the two treatment arms. The alternative hypothesis is that the mean change scores for the two arms will differ. With the following assumptions and a two-sided, 0.05 level test:

- 85% power
- Standard deviation of pSOFA change score = 4.2
- 10% crossover from Indication-based Strategy to Center-specific threshold-based Mgmt.
- 5% crossover from Center-specific threshold-based Mgmt. to Indication-based Strategy
- 2% inflation for interim look for efficacy

The target sample size is N=228 patients (114/group) to have 85% power to detect a mean difference of 2.0 in pSOFA change scores. The SD range, and the mean difference, is based on estimates from the change in a similar organ dysfunction score, MODS [52]. This sample size will also provide 85% power to detect a mean group difference of 2.5 even if the SD of Δ is 5.2 (24% higher). Furthermore, the analytic approach for the primary trial hypothesis will be analysis of covariance, with adjustment for baseline pSOFA score, which will increase power if correlation exists between pSOFA at ECMO initiation and decannulation.

Aim 1, Secondary Endpoint: For in-hospital mortality, a secondary endpoint, with a target N of 228 (222 prior to inflation), there is 85% power to detect a difference of 55% (i.e., the expected rate in the center-specific threshold-based group) vs. 35%, incorporating the assumptions regarding treatment crossover noted above. This effect size is very similar to that observed in the study by O'Halloran et al [10]. Hence, a total sample size of 228 provides high power to detect clinically important treatment group differences, in both organ dysfunction and mortality.

Aim 2, Primary Endpoint: For Aim 2 (Neurodevelopment), it is recognized that mortality during ECMO support, will be high. Hence a 45% mortality rate is assumed, yielding an available sample size at one year of N=126. For the neurodevelopmental instruments that apply to all/nearly all ages in this survivor cohort (e.g. ABAS, CBCL, and combined standardized scores for neurodevelopment), with 63 per group (under the null hypothesis of no survival difference), there is 85% power to detect a 0.54 SD difference between treatment arms. For the Bayley-4 and the WPPSI-IV, if the scaled scores are analyzed separately (approximately 60% of survivors), then there will be n=38 per group and 85% power to detect a 0.7 SD mean difference.

8.4.3 Analysis Plan

8.4.3.1 General Approach

Statistical analyses for *TITRE* will be performed in two stages: Stage 1: Analyses conducted during the trial, in aggregate (both treatment arms combined), to identify outliers, patterns of missing data, and any unusual differences by study site. Stage 2: Analyses performed at trial end, described in Sections 7.6.3.3 – 7.6.3.7.

Patients will be randomized to center-specific threshold-based vs. indication-based management transfusion strategy and analyzed on an intention-to-treat (ITT) basis. That is, all randomized participants will be included in the analysis regardless of loss to follow-up/death and that all participants will be analyzed according to their randomized assignment. Secondary-approach efficacy analyses will also be conducted, assigning participants to treatment actually received (per-protocol), and with examination of group differences as a function of estimated compliance with the transfusion strategy (compliance to be defined fully in the Statistical Analysis Plan [SAP]).

8.4.3.2 Study Population Definitions

The Study Populations are defined as follows:

(1) Intention-to-Treat (ITT) Population: All patients who are randomized who were assessed at least once after receipt of at least one RBC transfusion.
(2) Modified ITT Population: The subset of patients in the ITT Population who were not found post-randomization to have failed any trial eligibility criterion that was measured prior to randomization (i.e., excludes ineligible patients who were randomized in error).
(3) Per-Protocol Population: The subset of patients in the Modified ITT Population who: a) do not have major deviations from the protocol; and b) have complete data through the time of ECMO decannulation. Major protocol deviations will be pre-specified prior to the start of the trial.
(4) As Treated Population: All randomized patients who underwent RBC transfusion.

Primary efficacy analyses (for all endpoints) will be conducted on data from the ITT population, with participants remaining in their assigned treatment group regardless of actual treatment received.

Secondary efficacy analyses (for the Aim 1 and Aim 2 primary endpoints) will be conducted on data from the modified ITT population and the Per-Protocol population, with participants remaining in their assigned treatment group regardless of actual treatment received.

Tertiary efficacy analyses (for the Aim 1 and Aim 2 primary endpoints) will be conducted on the As Treated Population, with participants classified into the group corresponding to treatment actually received (i.e., non-ITT), defined using a compliance measure described in the SAP. This population may exclude some participants who do not meet the compliance criterion for either of the 2 trial treatments.

8.4.3.3 Aim 1 Endpoints Analysis Plan

Clinical: For the primary endpoint, we wish to test the superiority hypothesis that, compared with standard threshold-based management, improvement in organ dysfunction (change in pSOFA score) will be greater using an indication-based RBC transfusion protocol during ECMO. Of note, the pSOFA rubric assigns a maximum score of 24 if the patient dies while supported by ECMO; furthermore, all pSOFA components are clinically collected; hence very little missing outcome data are anticipated. Nevertheless, in concert with a primary ITT approach to ensure analysis of the entire cohort, multiple imputation will be used for the primary outcome for any participants who have insufficient clinical data to calculate a pSOFA score. Using the resultant dataset, the primary analytic approach for the baseline-adjusted change score comparison will be analysis of covariance; our preliminary data indicate that the change score is reasonably normally distributed and further, the sample is sufficiently large for the central limit theorem to apply.

The secondary analytic approach for the primary endpoint will be a fully covariate-adjusted regression, to control for any factors known to be highly associated with poor outcome, such as a single ventricle diagnosis.

For the other continuous endpoints, i.e., daily mixed venous O_2 saturation and (log-transformed) duration of ventilation, linear regression and Student's t-test will be used. Poisson regression will be utilized to compare number of donor exposures, accounting for length of ICU stay. A Fisher exact test will be performed to estimate treatment effect on 30-day and in-hospital mortality, with logistic regression applied when incorporating pre-specified subgroup factor interaction testing, rather than linear regression that will be used for the continuous endpoints. Exploratory analyses for the clinical endpoints will utilize generalized linear models with the appropriate link, to identify correlates and risk factors for poor outcome, that may be more important than or independent of transfusion strategy. Bootstrapping [53, 54] will be performed in these analyses to ensure reliability of parameter estimates and variable selection for final models.

Resource Utilization: For the primary approach, ICU and hospital lengths of stay will be compared between treatment arms using Student's t-test applied to log-transformed stay. A log-rank test will also be performed to compare distributions of time to discharge. These analyses will be conditional on survival to discharge.

8.4.3.4 Aim 2 Endpoints Analysis Plan

Neurodevelopment: Primary treatment group comparisons for the Bayley-4 (Cognitive, Language and Motor standard scores) and WIPPSI-IV (Weschler Verbal Reasoning; Visual Spatial, Working Memory; Full Scale IQ score) at one year post-randomization will be performed using a t-test after appropriate normalizing transformation, as needed, since the distributions are often left-skewed. Because there are important modifiers of neurodevelopment, although it is expected that these will be balanced between treatment arms due to randomization, secondary modeling will be performed using linear regression or median regression adjusting for covariates, such as maternal education level, to achieve the most efficient treatment group comparison. Tests of interaction will also be performed to determine whether the treatment group difference, if one exists, is specific to any select patient subgroups (see candidate list of pre-specified factors in Section 7.6.3.6). Similar analytic approaches will be applied to the results of the ABAS-3, CBCL, and PedsQL scores, and respective domain scores associated with specific skill areas. Of note, the analyses will utilize age-normed scores expressed in standardized units; hence age-specific instruments for a particular questionnaire (e.g., PedsQL) can be analyzed in aggregate using the full cohort of participants who completed the instrument. There are additional, secondary analyses of neurodevelopment and health-related QOL that extend beyond our primary hypothesis of treatment group (transfusion-strategy) difference, to be described in the SAP. These include:

- predictive regression modeling to identify risk factors and Classification and Regression Tree (CART) to identify predictor thresholds that discriminate patient subgroups at low and high risk of below-normal performance [55];
- incorporation of genetic findings from ancillary studies that utilize the extracted DNA per protocol; and
- use of statistical learning approaches for validation of trial cohort models by applying the models to cohorts in independent databases from other clinical centers or registries [56].

8.4.3.5 Interim Analyses

Interim analyses will be for safety; and one look at efficacy (change in pSOFA) for potential early stopping. The DSMB will also be provided with summaries of Hb/HCT level of the two treatment arms after a prescribed number of enrollments (if requested) and in regular reports.
Safety: The adverse and serious AE rates will be estimated by treatment arm and presented to the DSMB at least twice annually. The rates by arm will be presented overall for each group as well as by treatment-relatedness. The counts of AEs that lead to study treatment discontinuation as well as those resulting in death will be presented. Safety laboratory findings by study visit will also be compared by treatment arm.

Early stopping: For efficacy, one interim look by treatment arm is planned at 60% information, using an O'Brien-Fleming or Haybittle-Peto stopping boundary [57]. This guideline will be presented to the DSMB for confirmation prior to the first randomization. No stopping boundary for futility is planned. Statistical information for the primary endpoint will be equivalent to the number of ECMO decannulations.

8.4.3.6 Subgroup Analysis

The following pre-specified subgroups defined at the time of randomization will be utilized in final analyses:

- Males vs. Females
- Surgery vs. no surgery prior to ECMO
- Pre-existing neurological abnormality/injury vs. None (to be defined)
- CHD vs. No CHD; and: Complex CHD (to be defined) vs. Other/No CHD
- ECMO indication: Cardiac vs. Respiratory
- Neonate status: Age ≤28 vs. >28 days
- Hispanic vs. Non-Hispanic
- Race/ethnicity: White, Non-Hispanic vs. All Others
- Race/ethnicity: White vs. Black vs. Asian vs Other
- Single ventricle diagnosis vs. Other

A test of the interaction of Treatment Assignment x Subgroup factor will be conducted to determine whether there is differential treatment effectiveness according to patient subgroup. As is expected with trials of relatively rare conditions, there is more limited power to detect differential effects by subgroup; hence, any test with $p<0.10$ will be considered to be potential evidence of interaction. Confidence intervals will be reported for all treatment effect estimates within subgroup.

8.4.3.7 Missing Data and Sensitivity Analyses

Aim 1: Very little missing data are expected for the primary endpoint, change in pSOFA score, particularly because deaths are assigned a score of 24. Further, the primary ITT analysis will impute a zero score (no change) for the rare patient with missing clinical information. However, if death occurs very rapidly after randomization, there will be low information to assess the relative efficacy of the two transfusion strategies. For this reason, a sensitivity analysis that excludes participants who do not survive to Day 3 post-randomization will be performed for all key trial endpoints. This comparison will be informative with respect to whether the primary analysis model results are robust.

Aim 2: For Aim 2 (Neurodevelopment), it is possible that amongst the survivors, some may not undergo the neurodevelopmental assessment or may have an incomplete assessment. These data not collected could be missing at random, or not (patient too ill to return, patient doing well with no motivation to return). To assess the generalizability of trial results, the characteristics of the

one-year survivors completing and not completing the assessment will be compared. Two sensitivity analyses that will be performed are: imputing a mean healthy normal score for those without assessments; and imputing a score that is 2 SD below normal. If the treatment effect estimate is relatively unchanged, then these analyses will provide evidence towards the robustness of the findings of the primary analysis performed for Aim 2.

These approaches, as well as other sensitivity analyses and more detail regarding the primary and secondary (e.g., per-protocol; multivariable adjustments) analytic approaches for the key trial endpoints, will be fully described in the SAP.

8.5 Publication of Study Results

Neither the complete nor any part of the results of the study carried out under this protocol, nor any of the information provided by the sponsor for the purposes of performing the study, will be published or passed on to any third party without the consent of the study sponsor and publications committee. Any investigator involved with this study is obligated to provide the sponsor with complete test results and all data derived from the study.

9 Data Handling and Record Keeping

9.3 Confidentiality

Information about study participants will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed participant authorization informing the participant of the following:

- What protected health information (PHI) will be collected from participants in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research participant to revoke their authorization for use of their PHI.

In the event that a participant revokes authorization to collect or use PHI, the Investigator, by regulation, retains the ability to use all information collected prior to the revocation of participant authorization. For participants that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (i.e. that the participant is alive) at the end of their scheduled study period.

Participant medical records may be reviewed and research staff may contact participants up to 5 years after the study ends for the last patient participating (approximately 2032). This activity is explicitly stated in the informed consent form.

9.3.1 Identifiers

Each patient screened for possible entry to the trial will be identified with a unique study identification (ID) number. The leading digits will represent the study site, and the remaining digits will serve as the patient sequence number within site. No patient names, initials, addresses, or medical record numbers will leave the enrolling site as part of the research data. Biospecimens will also be assigned a unique ID number that will be linked to the participant ID number.

9.3.2 Study Records

The maintenance of confidentiality of study records and data is of paramount importance and the procedures are described in detail in the trial informed consent form. Study data will be kept in locked cabinets or areas at the study site. The link between patient name and study ID number

will be stored in a separate location. Published reports will include study data only in aggregate. While identifying information will not leave the local institution, other persons such as members of the DCC to conduct a site visit and data audit and members of the DoD will have permission to review study records. A statement of this access will be present in the informed consent form.

9.3.3 Sharing of Individual Study Results

All clinical data collected in this study are based on routine clinical care associated with ECMO support. Therefore, there are no clinical study results that the patient's physician will not already have access to, to share and to use for the patient's clinical management. The parent-reported health-related quality of life and behavior assessment instruments are not be part of routine clinical care; but since these are self-administered instruments, the participant is already aware of the information.

9.3.4 Data Reporting

Data will be reported in aggregate form for semi-annual DMSB reports and trial manuscripts at trial end. Listings of any individual events, such as AEs, will be identified by study ID number.

9.4 Source Documents

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Examples of these original documents, and data records include: hospital records, clinical and office charts, pathology reports, laboratory notes, memoranda, participants' diaries or evaluation checklists, pharmacy dispensing records, RBC and blood product information documents, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, digitized imaging data, x-rays, participant files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

9.5 Electronic Case Report Forms

The study eCRF is the primary data collection instrument for the study. All data requested on the eCRF must be recorded. All missing data must be explained. eCRFs will be completed electronically for the study and will have automatic data verification in place to decrease the likelihood of incomplete or inaccurate data.

9.6 Records Retention

The Sponsor and/or the designated Data Coordinating Center, will maintain study records for a period of 7 years following the end of the award.

9.7 Quality Assurance/Quality Control (QA/QC) Plan for Study Data

9.7.1 QA/QC of Trial Data

Quality assurance (QA) processes are proactive to ensure, with high likelihood, that data will be collected accurately and consistently. QA of trial data will employ several approaches, including but not limited to: a requirement for sites to perform a test randomization to ensure that real-time randomizations will be executed smoothly; pilot testing of CRFs to identify complex data fields that require adaptation for clinical relevance and accessibility; an online Trial Manual of Operations prepared by the DCC with detailed instructions for data collection by CRF and by data

field, where needed; and real-time validations of submitted data. QC of trial data will be achieved through site monitoring, where the monitor will conduct a comparison of source documentation to submitted data; and with ongoing statistical analysis to identify outliers and/or unusual patterns in the data.

9.7.2 Laboratory Evaluations

All the measurements in the proposed study are part of routine clinical care. Blood and urine samples will be collected for a biobank. There will be collection at two time points.

At each time point:

- Blood will be drawn (5 ml for participants under age 2 years and 10 ml if ≥2 years) and
- One ounce (25-30 ml) of urine (if patient is capable) will be collected from the participant.

Thus:

- The total blood volume to be collected will not exceed 10 ml for participants under age 2 years and 20 ml if ≥2 years.
- The total urine volume to be collected will not exceed 60 ml.

Participants and/or LARs may decide whether or not to participate in the biobank. Specimens will be labeled with the participant's study ID number and the Biobank Manual of Operations will specify the storage and shipping requirements necessary to ensure viable specimens that maintain the required level of confidentiality. Specimens will be processed at the participating center and shipped to the Boston Children's Hospital (BCH) Biobank for storage and availability for future investigations.

10 Study Monitoring, Auditing, and Inspecting

10.3 Study Monitoring Plan

This study will be monitored according to Good Clinical Practice (GCP) standards. The Investigator will allocate adequate time for such monitoring activities. The Investigator will also ensure that the monitor or other compliance or quality assurance reviewer is given access to all the above noted study-related documents and study related facilities (e.g. pharmacy, blood bank, diagnostic laboratory, etc.), and has adequate space to conduct the monitoring visit.

10.4 Auditing and Inspecting

The Investigator will permit study-related monitoring, audits, and inspections by the IRB/REB, the Sponsor, government regulatory bodies, and Medical center compliance and quality assurance groups of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The Investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, blood bank, diagnostic laboratory, etc.). Participation as an Investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable medical center compliance and quality assurance offices.

Site performance will be monitored analytically with respect to enrollment, protocol violations, and data quality. Site monitoring via an in-person audit by the monitor overseen by the DCC will also occur once for each site during the course of the trial, after the site has enrolled at least 4

patients. The audit will include review of regulatory documentation and screening practices as well as a data audit. For-cause site monitoring will also be conducted if warranted, with the DCC PI and/or a trial co-chair traveling to the site to meet with the local study team. A corrective action plan (CAP) will be deployed to facilitate improvement with respect performance issues.

11 Ethical Considerations

This study is to be conducted according to US and international standards of Good Clinical Practice (International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted independent IRB or REB, in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the IRB/REB concerning the conduct of the study will be made in writing to the Investigator and a copy of this decision will be provided to the Human Research Protections Office (HRPO) of the Sponsor before commencement of this study.

All participants and/or their LAR for this study will be provided an informed consent form describing this study and providing sufficient information for participants to make an informed decision about their participation in this study. The template consent form will be modified to meet local regulatory requirements and submitted with the protocol for review and approval by the IRB/REB for the study. The formal consent of a participant or their LAR, using the IRB/REB-approved consent form, must be obtained before that participant undergoes any study procedure. Signatures on the consent form will be required by the, parent, guardian or LAR, and the Investigator-designated research professional obtaining the consent. Because all participants will be under six years of age at enrollment, no assent will be obtained. Furthermore, no TITRE participant will reach the age of majority (18 years old at most clinical sites) during the course of the study, hence no reconsent procedures are required.

12 Study Finances

12.3 Funding Source

The study will be funded by a Fiscal Year 2021 Peer Reviewed Medical Research Program Clinical Trial Award sponsored by the Department of Defense office of the Congressionally Directed Medical Research Programs (PR212162).

12.4 Conflict of Interest

Any investigator who has a conflict of interest with this study (patent ownership, royalties, or financial gain greater than the minimum allowable by their institution, etc.) must have the conflict reviewed by a properly constituted Conflict of Interest Committee and a Committee-sanctioned conflict management plan must be put in place. This plan(s) will be reviewed and approved by the Funding Sponsor prior to participation in the study by the investigator(s).

13 Publication Plan

Neither the complete nor any part of the results of the study carried out under this protocol, nor any of the information provided by DCC or DoD for the purposes of performing the study, will be published or passed on to any third party without the consent of the TITRE Trial Publications and Presentations Committee.

APPENDIX A

BLEEDING DEFINITIONS

(Adapted from Nellis M et al. *Crit Care Med* 2019) (27)

Bleeding requiring RBC transfusion for purposes of this study will be defined as one of:

a. causing hemodynamic instability defined as increase in heart rate or decreased systolic or mean blood pressure > 20% from baseline;
b. quantifiable bleeding (chest drain, other drains, or soaked dressings) of > 5ml/kg/h for > 1 h;
c. decrease in Hb/HCT > 10% of baseline,
d. requiring surgical or procedural intervention;
e. volume administration to maintain pump preload; and
f. requiring administration of hemostatic agents (e.g., ε aminocaproic acid or factor VIIa)

Mild bleeding defined as

a). Bleeding for cannula or another superficial site that stops with application of direct compression;

b). Quantifiable bleeding (chest drain, other drains or soaked dressings) < 1ml/kg/h and not requiring volume administration to manage preload to the ECMO pump;

c). Blood from Nasogastric tube, endotracheal tube, and hematuria with < 20% change in Hb or HCT

Will <u>not</u> meet definition for RBC transfusion for bleeding indications.

APPENDIX B

OPTIMIZATION OF ECMO SUPPORT

Bedside management of ECMO support will not be standardized across the *TITRE* Trial sites and will be at the discretion of the treating physician and guided by institutional protocols. This Appendix serves as a guide to optimizing VA ECMO support for interested participating sites.

a. Optimal ECMO flow = 100 – 150 ml/kg/min or CI of 2.5 – 3 L/min/m

b. Measure adequacy of ECMO support using reduction in serum lactate and improvement in end-organ function

c. Where possible, based on patient physiology and ECMO duration allowing native ejection can be beneficial

d. Optimizing ECMO support:

i. Ensure that venous and arterial cannula are adequately positioned using radiography, echocardiography, or ultrasound imaging

ii. Ensure sizes of venous and arterial cannulas are appropriate for patient weight, and are adequate for targeted support.

iii. Ensure no thoracic/cardiac tamponade (pneumothorax, pleural effusion, hemothorax by radiology or ultrasound and pericardial effusion by echocardiography or ultrasound)

iv. Ensure adequate preload to ECMO pump using servo pressures

v. Optimize pump settings:

a. *Centrifugal pump*

1. Increase RPM slowly to achieve targeted flows

2. If mean arterial pressure is high consider limiting the use of vasoconstrictors or inotrope support and consider vasodilator infusions to reduce pump afterload

3. If arterial to venous (A-V) shunt is present ensure that patient flow is optimized

b. *Roller Pump*

1. Increase RPM to achieve targeted flow

2. Ensure pump occlusion is correctly set per institutional standards

c. Optimizing Perfusion pressure or Mean Arterial pressure (MAP)

i. Mean arterial pressure (MAP) should be measured using an intra-arterial catheter

ii. For patients with MAP > 20% expected or targeted MAP: consider use of vasodilator infusions to achieve MAP goal

iii. For patients with MAP < 20% expected or target MAP, on optimal ECMO flows, optimize preload and consider using vasoconstrictor or inotrope infusion

APPENDIX C

GUIDANCE FOR ASSESSING ADEQUATE CARDIAC OUTPUT IN PATIENTS WITH LACTIC ACIDOSIS

A participant can be considered as having adequate cardiac output:

1. Serum lactate is decreasing over > 3 consecutive measurements measured 2 hours apart and meets all of the following (A – D):
   A. Heart Rate and Mean BP are the expected range
   B. Decreasing vasoactive support
   C. Urine output > 1ml/kg/h
   D. No volume requirements in the absence of bleeding
   E. Optional: For sites that routinely use Near Infrared Spectroscopy: NIRS measurements: Cerebral > 50% and Renal > 75% of peripheral arterial SaO_2 or arterial SaO_2 measured from blood gas by Co-oximetry

References:

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2. Ulate KP, Yanay O, Jeffries H, Baden H, Di Gennaro JL, Zimmerman J. An Elevated Low Cardiac Output Syndrome Score Is Associated With Morbidity in Infants After Congenital Heart Surgery. Pediatr Crit Care Med. 2017 Jan;18(1):26-33