Study Protocol

Version 1.4 – April 22, 2024

NCT05796557

1. Table of Contents

2. Abstract

Background	Due to coagulopathy and thrombocytopenia induced by hemodilution and the extracorporeal circuit itself, children supported by extracorporeal membrane oxygenation (ECMO) are at significant risk of bleeding. To prevent bleeding, pediatric intensivists often prescribe prophylactic platelet transfusions. However, in observational studies, prophylactic platelet transfusions to children on ECMO have been independently associated with increased thrombosis, mortality, and paradoxically, increased bleeding. Guidelines to direct platelet transfusions in this patient population are limited by the lack of evidence and therefore based on expert opinion alone. Given the significant associated risks, it is crucial to provide evidence to guide clinicians. ECSTATIC is designed to address the lack of evidence on platelet transfusion strategies in pediatric patients on ECMO, through a randomized controlled trial (RCT), comparing two prophylactic transfusion approaches in non-bleeding children. The proposed pilot trial is innovative in that it is focused on children supported by ECMO, a population in whom transfusion strategies have never been tested previously; it involves the largest separation between the two arms of any platelet transfusion trial conducted in the past; and it involves two newly developed definitions of bleeding and thrombosis particularly applicable to children supported by ECMO. The R34 pilot trial will provide necessary and sufficient information to proceed with the definitive ECSTATIC RCT to evaluate the impact of a low platelet transfusion threshold on the clinical outcomes in children on ECMO. ECSTATIC has the potential to optimize efficacy, to reduce platelet transfusion exposure and to decrease mortality and morbidity of these extremely ill neonates and children.
Design	Pilot randomized controlled trial
Population	We will include critically ill children (0 to <18 years of age), admitted to a participating PICU/NICU/CICU, on ECMO, and who have either no bleeding or minimal bleeding within 24 hours of cannulation. We will exclude children with 1) post conception age < 37 weeks at time of screening; 2) underlying oncologic diagnosis or recipient of bone marrow transplant in the last year; 3) congenital bleeding disorder or thrombocytopenia; 4) pregnant or admitted post-partum (within 6 weeks after giving birth); 5) decision to withdraw or withhold some critical care or interventions; and 6) known objection to blood transfusions; or 7) on ECMO for > 24 hours at time of enrollment. Fifty patients will be enrolled in the pilot trial in ten sites (9 in the United States and 1 in Israel). Enrolled means consented and randomized.

Interventions	Comparator: Higher prophylactic platelet threshold strategy: Patients randomized to this arm will be transfused as soon as the platelet count is < 90 x 10^9 cells/L. Intervention: Lower prophylactic platelet threshold strategy: Patients randomized to this arm will be transfused as soon as the platelet count is < 50 x 10^9 cells/L.
Allocation	Subjects will be randomized in a 1:1 ratio to either arm. Subjects will be stratified by age (≤28 days vs >28 days).
Outcome Measures	Primary Study Outcome: Pre-transfusion platelet count (supplemented with total volume of platelet transfusions, in mL/kg/day and in mL/kg/run); primary safety outcome: progression to severe bleeding, severe thrombosis, and/or all-cause mortality. Secondary Endpoints: Observed values of estimated separation in mean pre-transfusion platelet counts; eligibility rate relative to predicted rate; enrollment of eligible patients percentage; adjudication rate of progression to severe bleeding/thrombotic outcomes; approach for consent percentage; consent percentage; transfusion compliance rate; transfusion strategy suspension percentage; withdrawal and or lost-to-follow-up percentage; violation of local non-study-specific circulatory support protocol percentage. Tertiary Outcome: A novel composite outcome of severe bleeding and/or severe thrombosis (supplemented with component outcomes of progression to severe bleeding, progression to severe thrombosis, and progression to both severe bleeding and severe thrombotic thrombosis.
Statistical Design and Power	For this pilot trial, enrolling 50 patients (25 per arm) would lead to approximately 81% power to detect a difference in mean platelet levels at the time of transfusion, assuming an autocorrelation between platelet levels at the time of transfusion of 0.3, a difference between the pre-transfusion platelet counts of 30, and a standard deviation of the pre-transfusion platelet count of 40.
Subject Participation Duration	Subjects will be enrolled over a 24-month period (quarter 3 to 10 of the study). Each patient will be followed daily until 24 hours past the treatment strategy's expiration (i.e., until at most day 22 after randomization). Mortality will be measured at day 28 (+/- 4 day window) and day 90 (+/- 10 day window).

3. Study Teams

A. Co-Principal Investigators

Marianne Nellis, MD, MS
Division of Pediatric Critical Care Medicine
Weill Cornell Medicine
525 East 68th St, M-512
New York, NY 10065
man9026@med.cornell.edu

Oliver Karam, MD, PhD
Section of Pediatric Critical Care Medicine
Yale School of Medicine
333 Cedar Street
New Haven, CT 06520-8064
oliver.karam@yale.edu

B. Executive Committee

Jennifer Muszynski, MD
Nationwide Children's Hospital
Columbus, OH
Jennifer.Muszynski@nationwidechildrens.org

Philip Spinella, MD
University of Pittsburgh
Pittsburgh, PA
spinella@pitt.edu

Marisa Tucci, MD
Sainte Justine Hospital
Montreal, QC, Canada
marisa.tucci@gmail.com

Jacques Lacroix, MD
Sainte Justine Hospital
Montreal, QC, Canada
jlacroix052@gmail.com

Simon Stanworth, MD
John Radcliffe Hospital
Oxford, UK
simon.stanworth@nhsbt.nhs.uk

Paul Clarke, MD
Norfolk and Norwich University Hospital
Norwich, UK
paul.clarke@nnuh.nhs.uk

Scott Weiss, MD, MSCE
Nemours Children's Hospital
Wilmington, DE
scott.weiss@nemours.org

C. Statisticians and Data Coordinating Center

Bradley Barney, PhD
Statistician
University of Utah
Salt Lake City, UT
bradley.barney@hsc.utah.edu

Eun Hea Unsicker
Coordinator
Trials Innovation Network
University of Utah
Salt Lake City, UT
eunhea.unsicker@hsc.utah.edu

Chella Palmer
Statistician
University of Utah
Salt Lake City, UT
Chella.palmer@hsc.utah.edu

D. Participating Institutions and Principal Investigators

Jesse Bain, DO
Children's Hospital of Richmond
Richmond, VA
jesse.bain@vcuhealth.org

Jamie Furlong-Dillard, MD
Norton Children's Hospital
Louisville, KY
jamie.furlong-dillard@louisville.edu

Umesh Joashi, MBBS
Komansky Children's Hospital of NY Presbyterian
New York, NY
ucj4001@med.cornell.edu

Eva Cheung, MD
Morgan Stanley Children's Hospital of NY Presbyterian
New York, NY
ec2335@cumc.columbia.edu

Garrett Coles, MD
Golisano Children's Hospital
Rochester, NY
garrett_coles@urmc.rochester.edu

Aditya Badheka, MBBS, MS
University of Iowa Health Care
Iowa City, IA
aditya-badheka@uiowa.edu

Robert Niebler, MD
Children's Hospital of Wisconsin
Milwaukee, WI
rniebler@mcw.edu

Matthew Paden, MD
Children's Healthcare of Atlanta – Emory
Atlanta, GA
matthew.paden@choa.org

Caroline Ozment, MD
Duke University School of Medicine
Durham, NC
caroline.ozment@duke.edu

Ofer Schiller, MD
Schneider Children's Medical Center
Petach Tikvah, Israel
schillero@clalit.org.il

4. Abbreviations

BASIC	Bleeding Assessment Scale in Critically Ill Children
CICU	Cardiac intensive care unit
DCC	Data Coordinating Center
DSMB	Data Safety Monitoring Board
ECMO	Extracorporeal membrane oxygenation
ECPR	Extracorporeal cardiopulmonary resuscitation
ECSTATIC	ECMO hemostatic transfusions in children
EFIC	Exception from informed consent
IRB	Institutional Review Board
NHLBI	National Heart, Lung, and Blood Institute
NICU	Neonatal intensive care unit
PICU	Pediatric intensive care unit
RCT	Randomized controlled trial
TEG	Thromboelastometry
V-A	Veno-arterial
V-V	Veno-venous

5. Introduction

A. Background

The ultimate goal of our program is to improve outcomes in pediatric patients on extracorporeal membrane oxygenation (ECMO). We propose a randomized controlled trial (RCT), comparing two prophylactic platelet transfusion approaches in non-bleeding children, to identify if a low platelet threshold transfusion strategy is non-inferior to a high platelet threshold transfusion strategy. Prior to conducting the definitive trial, we propose to undertake a pilot trial to establish the degree of separation between intervention arms, the consenting process, and the adjudication of outcomes.

Pediatric ECMO

Over 3,600 neonates and children are supported worldwide each year by ECMO, with an increase of 5-10% in the number of cases each year (1,2). ECMO serves as cardiopulmonary support in critically ill patients with severe, refractory respiratory or heart failure. In addition, extracorporeal cardiopulmonary resuscitation (ECPR) can be employed during cardiac arrest. The system includes an extracorporeal circuit (tubing) with large cannula draining blood from the body, an external pump draining systemic venous blood towards a membrane allowing gas exchange (oxygenation and CO_2 removal) and recirculating warmed blood into a main vessel. In veno-arterial (V-A) ECMO, blood is drained from a large vein and returned to an artery, supporting both the heart and lungs, whereas in veno-venous (V-V) ECMO, the blood is drained from a vein and returned to a vein, thereby providing primarily pulmonary support. This implies that central vessels (internal jugular vein or femoral vein, and aorta or femoral artery) are cannulated, either percutaneously or by surgical cut-down. Cannulation sites depend on the patient age, weight and clinical context. Central ECMO via median sternotomy and exposure of cardiac structures is a preferred option when used immediately after cardiopulmonary bypass during cardiac surgery. The ECMO circuit is typically primed with red blood cells and plasma or whole blood in infants and small children and may be primed with isotonic fluid in older children and adults.

While ECMO may be lifesaving, bleeding is a common complication due to extracorporeal circuit-induced coagulopathy and platelet dysfunction due to exposure of blood to the ECMO circuit (3–5)**.** Given the risk of thrombosis, children are also placed on anticoagulation which may contribute to the risk of bleeding. In a cohort of over 500 infants and children supported by ECMO, 70% had significant bleeding (as defined by the Extracorporeal Life Support Organization) by day 10 and bleeding events were independently associated with time to death (OR 1.75, 95% CI 1.20-2.55) (6). Furthermore, the amount of quantifiable bleeding (as measured from chest tube output) was associated with mortality in a dose-dependent fashion (7).

Patients can develop either thrombocytopenia and/or platelet dysfunction while supported by ECMO. Studies of neonates have shown drops in platelet counts ranging from 25-60% shortly after cannulation (3,8), whereas pediatric patients, with a larger baseline circulating blood volume and less hemodilution, have been reported to drop their platelet count by approximately 30% (9). Few studies have examined platelet dysfunction in children on ECMO. In a case series of 10 neonates on ECMO for respiratory failure, platelet aggregation studies 15 minutes after the initiation of ECMO demonstrated a 46% mean decrease in the response to collagen from baseline, and a significantly reduced response to ristocetin and to adenosine 5'-diphosphate (8).

In a retrospective study of 24 children who had thromboelastography (TEG) testing performed during ECMO support (10), severe qualitative platelet dysfunction was more commonly seen for adenosine diphosphate (ADP)-mediated aggregation (92%) compared to arachidonic acid (AA)-mediated aggregation (75%). In addition, ADP-mediated percent of platelet aggregation was significantly lower than AA-mediated platelet aggregation (15% vs. 49%, p<0.001). However, no significant changes in aggregation were seen for bleeding versus non-bleeding patients. In general, platelet function testing is limited in neonates and children due to the blood volume required, as well as lack of standardization for pediatric norms (11).

Epidemiology of Platelet Transfusions

To prevent bleeding and its associated outcomes, clinicians frequently prescribe platelet transfusions, to enhance hemostasis. In the same cohort of 514 children on ECMO, children received platelet transfusions on over two-thirds of all ECMO days (Figure 1) (12).

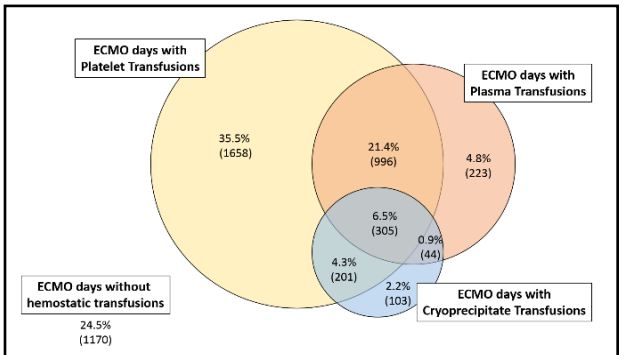


Figure 1: Venn diagram of the ECMO days with plasma, platelet, and cryoprecipitate transfusions. [Karam et al. Epidemiology of Hemostatic Transfusions in Children Supported by Extracorporeal Membrane Oxygenation. Crit Care Med 2020]

As compared to other critically ill children, pediatric patients on ECMO receive a large proportion of platelet transfusions. In an epidemiologic study of critically ill children receiving platelet transfusions on ECMO, each child received an average of 92 mL/kg of platelet transfusions over the course of their ECMO support (13). Ninety-seven percent of patients received at least one platelet transfusion during their ECMO course (6,9). The transfusion of platelets, as well as other blood components, can lead to microthrombi in the ECMO circuit (14), as well as clinically relevant thrombotic complications in the patient. In the cohort of 514 children on ECMO, 33% had a significant thrombotic event by day 10 (Figure 2) (6).

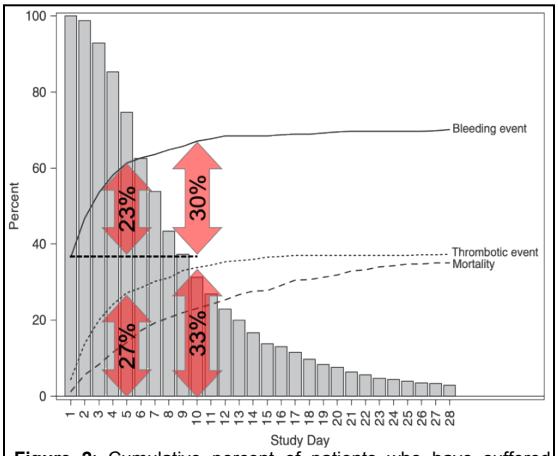


Figure 2: Cumulative percent of patients who have suffered mortality, bleeding events, or thrombotic events by day of extracorporeal membrane oxygenation support. Over the first 10 days, 30% of patients who are not bleeding on day 1 will have a bleeding event, and 33% of the patients will have a thrombotic events. [Dalton et al. AMJCCM 2017]

Clinicians have very little evidence-based guidance as to the indications for the prescription of prophylactic platelet transfusions for children on ECMO. ECSTATIC seeks to provide evidence to guide prophylactic platelet transfusion strategies for children requiring ECMO support. Currently, both ELSO (15) and the Association for the Advancement of Blood & Biotherapies (AABB) (16) have recommended platelet transfusion thresholds of 80-100x10^9/L, but both groups acknowledge the recommendations are based on expert opinion and little evidence. Observational data show no association between platelet count and subsequent bleeding, both in adults and children (17). Transfusion protocols are largely based on anecdotal institutional experience (18). There is significant heterogeneity in transfusion practices on ECMO (19). In a recent international survey of pediatric

ECMO centers, platelet transfusions were prescribed at a median (IQR) total platelet count threshold 72 (54-88) x10^9/L for non-bleeding patients (13).

Outcomes Associated with Platelet Transfusions

The decision to use prophylactic platelet transfusions as a hemostatic agent must be balanced with the fact that platelet transfusions are associated with significant morbidity and mortality, including bleeding and thrombosis. In critically ill children receiving platelet transfusions, each additional dose (10mL/kg) was independently associated with a 2% increase in mortality (20). The data from the only available randomized controlled trial (RCT) focused on pediatric platelet transfusion strategies, conducted in preterm infants, showed that a high platelet threshold for transfusion led to a 1.5-fold increase in the odds of major bleeding or death (95%CI 1.06-2.32) (21).

In observational studies, adjusting for minimum lactate within 48 hours of ECMO initiation, ECPR, renal failure, new neurologic event on ECMO, preterm neonatal age, and a diagnosis of congenital diaphragmatic hernia, platelet transfusions have been independently associated with increased bleeding, thrombosis and mortality in children supported by ECMO (9). The association between platelet transfusion and both 90-day and 1-year mortality has also been confirmed in adults on VA ECMO (22).

Mechanisms of Poor Outcomes

The mechanisms of the observed morbidities and mortalities associated with platelet transfusions are poorly understood. Animal models of thrombocytopenia have suggested that it is not the low platelet count itself that increases the risk of bleeding, but rather the low platelet count in an inflamed environment (23,24). Platelet transfusions have been noted to be pro-inflammatory through a variety of mechanisms. Platelets themselves can synthesize inflammatory mediators and release them through interactions with endothelial cells and immune cells (25). Platelets release biological response modifiers during storage that include oxygenated moieties of membrane lipids (26), microparticles (27), serotonin, histamine and ADP/ATP (28), all of which can induce further inflammation upon transfusion. It is therefore plausible, in an attempt to correct thrombocytopenia, the platelet transfusion itself actually worsens inflammation and can lead, counterintuitively, to increased bleeding and thrombosis.

Summary

There clearly remain knowledge gaps in both the optimal strategy to transfuse platelets to prevent bleeding in children supported by ECMO, as well as mechanisms to target to prevent the associated morbidities. Studies investigating these questions are urgently needed (29,30) to test the efficacy of such strategies. If the proposed aims of this pilot study are achieved, we will be able to undertake a full trial which will provide a better understanding of how to transfuse platelets prophylactically to children supported by ECMO. This contribution will be significant because it ultimately will influence transfusion medicine guidelines and reduce the morbidity associated with these therapies.

B. Preliminary Studies

We conducted a large, point prevalence study of platelet transfusions in critically ill children (20). Upon adjusted analysis, total administered platelet dose was independently associated with increased ICU mortality (odds ratio for each additional 1 mL/kg platelets transfused 1.002; 95% CI 1.001-1.003; p=0.005). We examined only those children within the cohort who were supported by ECMO (13); seventy-nine percent (71/90) of the platelet transfusions were given for prophylaxis in non-bleeding patients. The median (IQR) total platelet count prior to transfusion was 72 (54-88) $x10^9$/L and did not vary based on bleeding versus non-bleeding indications (according to local assessment). Through the course of their admission, children supported by ECMO received a total median (IQR) dose of 92 (42-239) mL/kg of platelet transfusions. Institutional protocols varied.

We also examined the use of platelet transfusions in children supported by ECMO in the Bleeding and Thrombosis on ECMO (BATE) dataset (12), and showed that platelets were transfused on 68% of ECMO days. Platelet transfusion dose was independently associated with chest-tube output (p<0.001), other bleeding requiring red blood cell transfusion as defined by the authors (p=0.03), and daily set platelet goal (p=0.009), but not with total platelet count (p=0.75). These results mirrored other published associations for children supported by ECMO (9) and further justified our proposed RCT.

C. Justification for the study

Justifications to undertake a randomized controlled trial (RCT) to address platelet transfusion in children on ECMO include the following:

- Many potential confounders that preclude from inferring causation from observational studies, thus only an RCT can evaluate the outcomes associated with platelet transfusion strategies.
- Pediatric ECMO is associated with an in-hospital mortality rate of approximately 50% - the highest mortality associated with any standard of care pediatric treatment (1). Since platelet transfusions are independently associated with an increased morbidity and mortality, in both critically ill adults (31) and children (13), a low platelet threshold transfusion strategy could improve outcomes.
- In addition, the currently available observational data suggest that platelet transfusions are associated with an increased risk of both bleeding and thrombosis (9) which may be related to the inflammation induced by platelet transfusions.
- Despite the large volume of platelet transfusions that they receive, the current platelet transfusion recommendations for children supported by ECMO are based solely on expert opinion (1).
- There is a huge knowledge gap (29,30) and no high-quality evidence to guide platelet transfusion practice in these children supported by ECMO (15).
- There is clearly equipoise with respect to platelet transfusion in pediatric ECMO patients (29).

In summary, platelet transfusions are widely used in children on ECMO (12) with little evidence to guide practitioners. There is no evidence that platelet transfusions are associated with improved outcomes; some data even suggest that platelet transfusion may be harmful (9). There is a need for a greater understanding of appropriate platelet transfusion strategies in these vulnerable children.

D. Hypotheses and Aims

This protocol pertains to the pilot clinical trial, which will evaluate the feasibility of randomizing a **LOW** platelet threshold transfusion strategy, in which a platelet count of < $50x10^9$/L will trigger a platelet transfusion, compared to a **HIGH** platelet threshold transfusion strategy, in which a platelet count of < $90x10^9$/L will trigger a platelet transfusion (Figure 3). We hypothesize that, in non-bleeding children on ECMO, a low prophylactic platelet threshold transfusion strategy is non-inferior to a high platelet threshold transfusion strategy, specifically related to bleeding or thrombotic complications, and this hypothesis is intended as a candidate primary hypothesis of the full RCT.

The objective of this pilot trial is to determine the feasibility of the full RCT through the following:

- **Aim 1** is to demonstrate a **separation between the LOWER and HIGHER platelet threshold transfusion arms**. Specifically, we will demonstrate a difference in the average pre-transfusion platelet count between the two arms from randomization to when the treatment strategy was permanently discontinued. In addition, we will estimate the difference in average daily platelet dose during the same period, which we anticipate will be a difference of at least 20% between the groups randomized to each threshold.
- **Aim 2** is to demonstrate the **ability for observed endpoints to satisfy various prespecified feasibility thresholds**. Specifically, there are 10 different criteria, and we will show that at least 5 of the 10 thresholds are satisfied by the estimated endpoints.

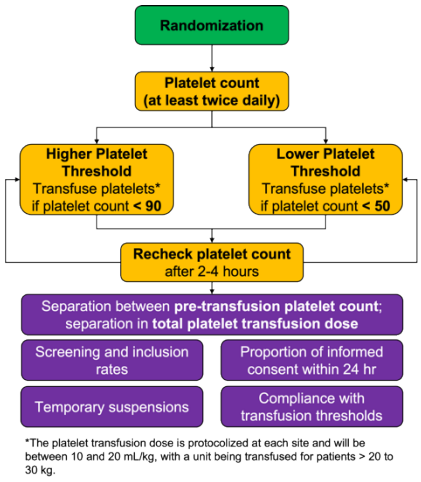


Figure 3: Design of the pilot RCT. In addition to the listed outcomes, we will also collect epidemiological information on progression to severe bleeding and/or clotting, and we will further assess safety by considering mortality and adverse events.

- **Aim 3** is to provide additional information on the epidemiology of a novel composite outcome of progression to severe bleeding and/or severe thrombosis, to incorporate in the design of the full RCT. Specifically, we will demonstrate that the estimated rate of adjudication by an independent panel is more than 90% of the enrolled subjects for whom the outcome was reported by the site as having occurred.

We anticipate that if this pilot study should demonstrate feasibility of the larger study, the information gathered in the larger trial will provide the rationale for an evidence-based and rational strategy for platelet transfusions in pediatric ECMO patients, with the hope of reducing transfusions, as well as preventing additional complications.

E. Innovation

Our proposal is innovative in numerous ways:

- **Focus on children supported by ECMO**. Children supported by ECMO receive a disproportionately large number of transfusions and these cumulative transfusions have been associated with mortality, bleeding and thrombosis. The R34 pilot trial will be the first to assess

feasibility for consenting and enrolling subjects into a multicenter RCT of children on ECMO. The ability to enroll after regular informed consent will be relevant for any future ECMO trial.

- The **pragmatic design** will allow the results to be applicable across a variety of ECMO centers and relevant for any ECMO trial.
- **Large separation in transfusion strategies**. To ensure the outcomes can be caused by the intervention, it is important that the two interventions are separated. To date, limited trials of platelet transfusion threshold strategies in pediatrics have compared 50 to 25 x10^9 cell/L (21) in premature neonates and in adolescents and adults receiving chemotherapy have compared 20 to 10 x10^9 cell/L (32). The sites participating in this pilot have all agreed to randomize to a much wider separation in transfusion strategies between arms (90 vs 50 x 10^9 cell/L). These thresholds are based on the 25th and 75th percentile of real-life transfusion thresholds (13), to ensure equipoise similar to other transfusion studies (33).
- **Novel approach to associated outcomes**. Platelet transfusions are routinely prescribed to prevent bleeding. However, platelet transfusions, due to their inflammatory nature, may actually worsen both bleeding and thrombosis, based on the results of the RCT in neonates and the observations in children on ECMO. We propose a novel composite outcome that will address both bleeding and thrombosis.

6. Patient Eligibility

A. Anticipated Cohort

Our aim is to enroll consecutive non-bleeding or minimally bleeding critically ill children on ECMO, who are at risk of progression to severe bleeding and/or to severe thrombosis, and at risk of receiving platelet transfusions.

Among the children on ECMO who are not bleeding on Day 1, 30% will be bleeding by Day 10 (Figure 2, page 11) (6). Furthermore, by day 10, 33% of patients will have experienced a thrombotic complication. Finally, 66% of those patients will receive at least one platelet transfusion on any given day on ECMO.

B. Criteria to Identify Participating Sites

The following criteria were used to identify participating sites in the pilot study:

1) Offering a pediatric ECMO program
2) Unanimous agreement to participate from surgical and medical practitioners (NICU, PICU, and/or CICU) and ECMO program leadership
3) Unanimous agreement to follow the PEACE anticoagulation, transfusion and hemostatic agent replacement guidelines
4) Well-established track record of excellence in clinical trials participation.

For this pilot study, there is no need for our results to be generalizable to non-study PICU groups, in terms of clinical trial experience and geographic and demographic scope. However, we will cap the number of enrolled patients per center to 10, to avoid over-representation of large centers.

In addition, as the future large RCT may require participation of **international sites**, we will include one international site in the pilot to provide some evidence on the feasibility of the trial outside of the US.

The ten participating sites are (alphabetical order): Children's Healthcare of Atlanta – Emory, Atlanta, GA; Children's Hospital of Richmond, Richmond, VA; Children's Hospital of Wisconsin, Milwaukee, WI; Duke University School of Medicine, Durham, NC; Golisano Children's Hospital, Rochester, NY; Norton Children's Hospital, Louisville, KY; Komansky Children's Hospital of New York Presbyterian, New York, NY; Morgan Stanley Children's Hospital of New York Presbyterian, New York, NY; Schneider Children's Medical Center, Petach Tikvah, Israel; and University of Iowa Health Care, Iowa City, IA.

C. Eligibility

Children will be eligible if admitted to a participating tertiary care PICU, NICU or CICU for whom the decision to cannulate for ECMO support has been made.

D. Inclusion Criteria

Critically ill children (0 to <18 years of age), admitted to a participating PICU/NICU/CICU, on ECMO, and who have either **no bleeding or minimal bleeding**, according to the BASIC definition (appendix B) (34) **within 24 hours of cannulation** (any of these criteria define minimal bleeding):

- streaks of blood in endotracheal tube or during suctioning only
- streaks of blood in nasogastric tube
- macroscopic hematuria
- subcutaneous bleeding (including hematoma and petechiae) < 5 cm in diameter
- quantifiable bleeding < 1 mL/kg/hr (e.g., chest tube). Since the objective is to capture bleeding, chest tube output can be higher than 1 mL/kg/hr, provided the output is serosanguinous and the site investigator judges the bleeding portion of the chest tube output is < 1 mL/kg/hr.
- bloody dressings required to be changed no more often than every 6hr, or weighing no more than 1 mL/kg/hr if weighed, due to slow saturation

This definition, which was developed by a large consortium of experts, including ECMO specialists, has been validated with substantial inter-rater reliability: the free marginal kappa between two observers is 0.74 (95% CI, 0.57–0.91) (34).

If a patient experiences **more than minimal bleeding** after ECMO cannulation, they can be enrolled once bleeding meets criteria for minimal bleeding, with the condition that the bleeding resolves within the first 24 hours of ECMO initiation.

E. Exclusion Criteria

To ensure our trial is as **pragmatic** as possible, we have as few exclusion criteria as possible:

1) post conception age < 37 weeks at time of screening
2) underlying oncologic diagnosis (defined as receipt of chemotherapy or radiation in the last six months) or recipient of bone marrow transplant in the last year

3) congenital bleeding disorder or congenital thrombocytopenia
4) pregnant or admitted post-partum (within 6 weeks of giving birth)
5) decision to withdraw or withhold some critical care or interventions
6) known objection to blood transfusions
7) on ECMO for > 24 hours at time of enrollment

Inclusion criteria and exclusion criteria will be ascertained by research staff in conjunction with the pediatric critical care clinical team. While we recognize that neonates (< 28 days of life) have distinctly different coagulation systems as compared to older children (35), they make up at least half of all children supported by ECMO (6) and therefore, strategies for prophylactic platelet transfusion must be understood in this group as well. Randomization will be stratified by age (see below).

7. Consent

A. Informed Consent

All critically ill children cannulated for ECMO in a participating center will be screened on a 24 hour and 7 days a week basis. The ECMO team will be educated on this trial and will call the study team for each patient who is being approached for consent for ECMO or who is on ECMO at the time of PICU admission. To detect any potential biases, a de-identified screening log will be maintained at each site to record the number of eligible patients, the number of patients eligible for consent not randomized, the number of platelet transfusions between cannulation and consenting process (and if those transfusions were prophylactic or therapeutic), if the patient experienced bleeding between cannulation and enrollment, and the reason for their exclusion. This should allow detection of any selection bias.

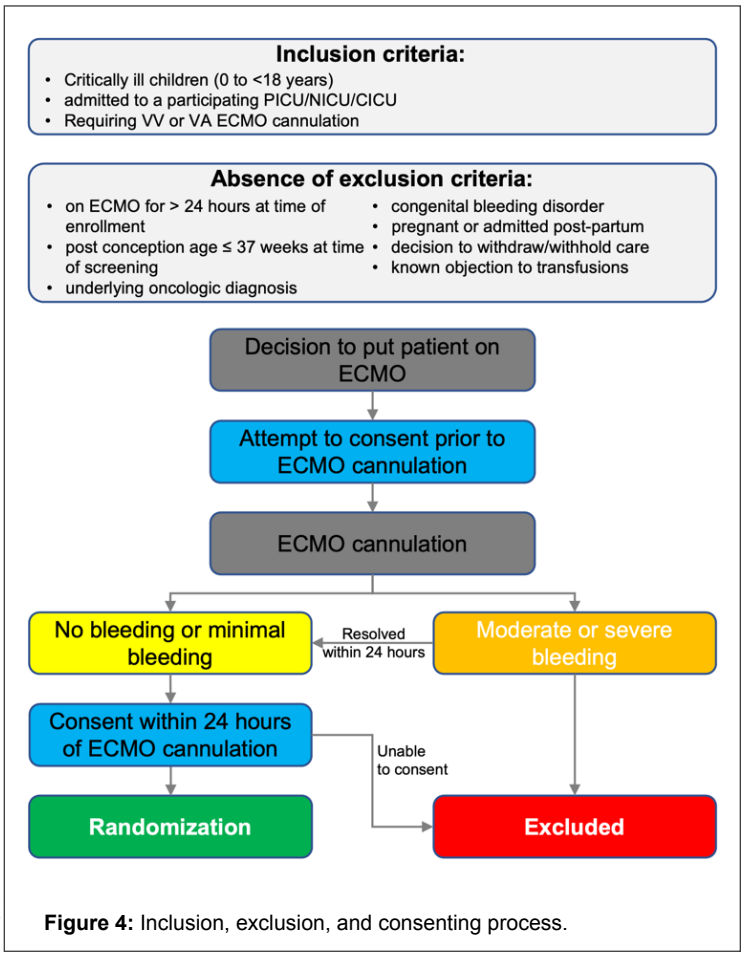


Figure 4: Inclusion, exclusion, and consenting process.

When the patient is expected to require ECMO within the next few hours, we will attempt to obtain consent prior to ECMO cannulation. However, we recognize that some parents might not able to make an informed choice in a situation where

they fear for their child's life and where they might feel coerced (36–39).Therefore, we will approach the parents for consent as soon as possible, but at least within 24 hours of cannulation.

The screening, inclusion and exclusion, and consent process is shown in Figure 4.

Of note, for patients who will turn 18 years of age before the 28- and/or 90-day follow up call, we will provide the parents with a separate consent form for follow-up phone calls, at the time of ICU discharge, as well as a pre-stamped envelope. Once the patient turns 18 years of age, we will ask him to sign the consent form to allow us to be recontacted and mail it back to the site PI. The site PI or coordinator will call the patient before the 28- or 90-day evaluation, and remind them to mail the consent form. Once the research team has received the consent form, they will set up the 28- and/or 90-day follow-up evaluation call.

8. Study Design

A. Intervention

Subjects will be **randomized** in a 1:1 ratio to either a lower or a higher platelet prophylactic transfusion strategy (Figure 3, page 14). All platelet units will be prepared in accordance to existing national standards (40).

We chose platelet count as the basis for transfusion since 100% of sites in our study rely on this number, rather than platelet function or viscoelastic testing, to make transfusion decisions [9]. Platelet counts will be assayed at least twice daily.

To ensure **clinical equipoise,** we chose platelet transfusion thresholds that are based on observational data, similar to the thresholds chosen for red blood cell transfusion studies (33). The 25th and 75th percentile of real-life transfusion thresholds were used as a basis for the trial's thresholds to avoid extremes and promote equipoise.

In our original epidemiologic study from 2017-2018, among non-bleeding children on ECMO, the 25th percentile of platelet count before transfusion was 54 x10^{9} cell/L, whereas the 75th percentile was 88 x10^{9} cell/L (13).

In addition, we performed two additional studies to assess clinical equipoise: an analysis of contemporary data from the Recipient Epidemiology and Donor Evaluation Study (REDS) IV-P and a survey of ECMO providers at the participating sites.

We conducted a retrospective multicenter study from four academic children's hospitals, participating in the REDS-IV-P study between April 2019 and July 2022. All children (0 to 18 years old) supported with ECMO and transfused platelets were included. Transfusion doses were categorized as lower (≤ 75th percentile, i.e. ≤ 24 mL/kg) and higher doses (> 75th percentile, i.e. > 24 mL/kg), as a surrogate marker for bleeding status. Three hundred ten children, median age 6.4 months (IQR 25 days-5.4 years) and median body weight 6.6 kg (IQR 3.7-18.0) on ECMO received 3437 platelet transfusions. Of these, 2578 were lower doses and 859 were higher doses. Among the former, the median pre-transfusion platelet count was 72 x 10^9/L (IQR 56-94) in neonates (<28 days) and 71 x 10^9/L (IQR 48-102) in older infants and children. Among the latter, the median pre-transfusion platelet count was 113 x 10^9/L (IQR 72-166) in neonates and 112 x 10^9/L (IQR 66-177) in older infants and children (Figure 5). The median time between the platelet count and the platelet transfusion was 2.6 hours (IQR 1.3-4.7). There was no association between the patient's age and pre-transfusion platelet count (p=0.86), but there was a significant difference between the four sites (p=0.01).

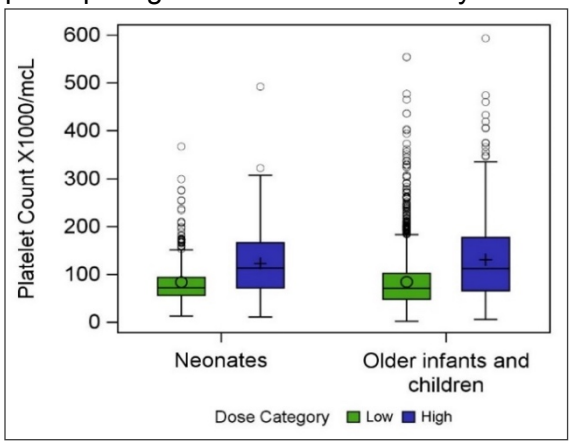


Figure 5. Pretransfusion platelet thresholds from REDS-IVP

We surveyed ECMO providers at all the participating sites. The overall response rate was 56% (114/204). 66% (68/103) of the responders were pediatric intensivists while 37% (38/103) were pediatric cardiac intensivists. 26% (27/103) had 10 to 14 years of experience, while 45% (46/103) had more than 15 years of experience.

When looking at the overall platelet transfusion thresholds, the median was 70 (IQR 50-99). There were differences between the various scenarios. The median platelet threshold was 50 for non-bleeding children, 70 for non-bleeding neonates, 75 for minimally bleeding children,

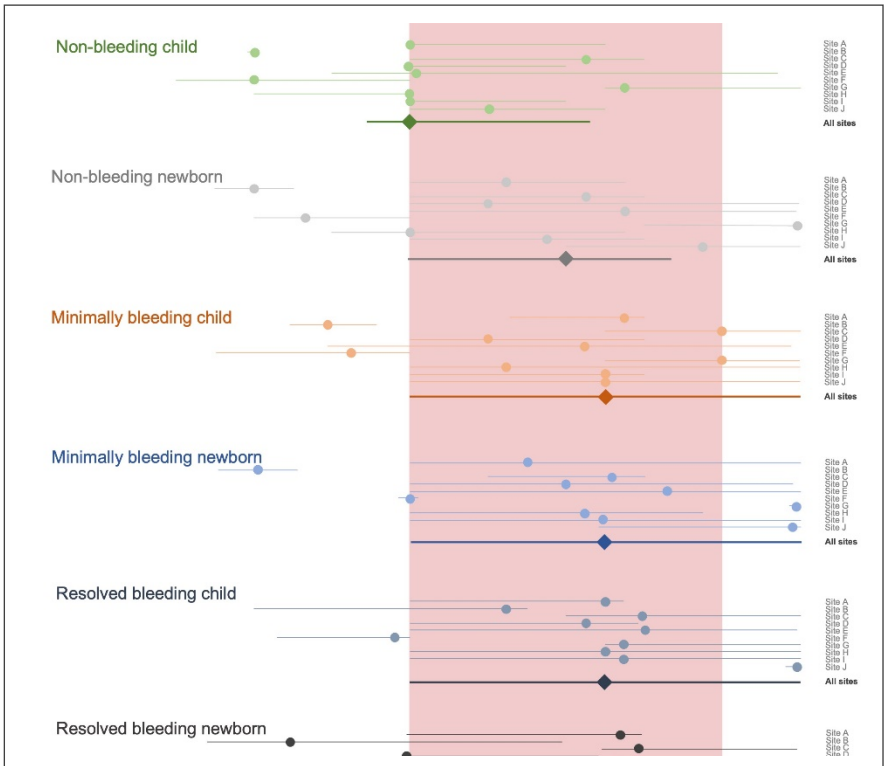


Figure 6. Platelet transfusion thresholds from survey of participating centers. There are six different scenarios, and each site's responses are displayed, with the median (circle) and the interquartile range (line). The averaged responses per scenario are summarized by the diamonds and bold lines. The pink zone indicates the ECSTASTIC transfusion thresholds.

75 for minimally bleeding neonates, 75 for resolved bleeding in children, and 80 for resolved bleeding in neonates. As shown in Figure 6, there was a wide heterogeneity among sites as well as within sites: each line representing the median (circle) and IQR (line) of one site. 59% (61/103) of the responders were uncertain or very uncertain about the level of evidence, 33% (34/103) were neutral, and 8% (8/103) were certain or very certain.

Given the evidence presented above from three studies, we will use transfusion thresholds of **90 (higher platelet threshold arm) vs 50 x10^9/L (lower platelet threshold arm).**

Patients should not be transfused above the upper transfusion thresholds, i.e., patients should not be transfused for a platelet count ≥ 90 x10^9 cell/L in the higher platelet threshold arm and ≥ 50 x10^9 cell/L in the lower platelet threshold arm. Patients must be transfused below the transfusion thresholds, i.e., patients should be transfused for a platelet count < 90 x10^9 cell/L in the higher platelet threshold arm and < 50 x10^9 cell/L in the lower platelet threshold arm. The transfusion should occur within 12 hours of the sample collection time for the platelet count laboratory test. If a platelet count in either direction of the threshold has a platelet count within the next 12 hours in the opposite direction of the threshold (e.g., went from below threshold to not below threshold) without an intermediate platelet transfusion, the earlier platelet count's implied action is superseded by the most recent platelet count to allow sites the flexibility to obtain a confirmatory platelet count in instances where the earlier count is considered unreliable. Specific criteria for temporary suspensions are detailed below.

The **transfusion dose is intended to be 10-20 mL/kg** of apheresis platelets (whole blood-derived platelets may be used if apheresis platelets are not available), which is the median platelet dose reported in two large observational studies (6,20), though adjustments to the dosing to be in line with local implementations are permitted so long as dosing is not differentially applied between assigned transfusion strategies. Though a range of dosing is provided, all doses given at each site will be the same across both arms of the study and will be in line with local guidelines. For patients more than 20-30 kg (per site guidelines), one unit of platelets will be transfused.

For the sites in which the information is available (currently 5 out of the 10 participating sites), the total platelet count (per mL of transfusate) that each patient receives from each transfusion will be recorded. For sites in whom this exact information is not known, we will record if they receive "low-yield" platelet transfusions in which the platelet count is < 3 x 10^{11} platelets. In addition, we will collect information about irradiation, pathogen reduction and ABO compatibility of the platelet product.

A **platelet count must be drawn two to four hours** after the end of the platelet transfusion. **Another platelet transfusion** should be given if the platelet count drops again under the threshold of the RCT arm to which the participant was allocated.

B. Safety

Although ELSO guidelines suggest keeping the platelet count > 100 x10^9 cell/L in neonates (vs 80 in older children) (41), there is currently no evidence to suggest neonates are at higher risk of bleeding than older children, at a similar platelet count.

Additional analyses from 514 pediatric ECMO runs, comparing bleeding in neonates versus infants at particular platelet counts, as shown in Figure 7, are reassuring (unpublished analysis, from the BATE dataset (6)). The **proportion of children experiencing bleeding is stable throughout the range of platelet counts**, which suggests that lower platelet counts are not associated with increased proportion of bleeding. Furthermore, **neonates seem to have an overall lower proportion of bleeding** than older children.

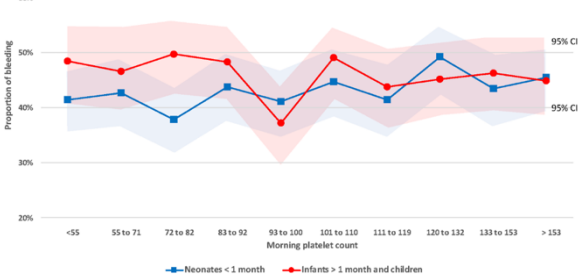


Figure 7: Proportion of bleeding according to morning platelet count, by age (neonates versus older infants and children) [unpublished analysis from Dalton et al, AJRCCM 2017]

In addition, thrombocytopenia was not a risk factor for bleeding on ECMO, either from chest tubes or other sites (p=0.82) (7).

C. Co-Interventions

As this is an **open-label** trial, we need to ensure co-interventions that may be associated to the primary clinical outcome in the larger trial (progression to severe bleeding, severe thrombosis or both) are managed identically in both arms, to avoid potential interactions and biases (e.g., providers managing patients in the low platelet threshold arm might be more prone to prescribe a lower dose of heparin). However, as the **pragmatic design** is important for the **generalizability of the future results**, independently of co-interventions, and as there is variability in the anticoagulation (42) and plasma transfusion strategies on ECMO (13), Therefore, we will be **imposing the adherence to the new international guidelines** (Pediatric ECMO Anticoagulation Initiative, to be published in 2024).

In addition, all participating sites will have agreed to **follow their own local guidelines**:

1. **Plasma transfusions** (which coagulation test is to be measured, how frequently is it measured, what is the threshold for plasma transfusion, what is the dose of plasma)
2. **Anticoagulation** (what is the heparin bolus and starting dose, what is the primary test used to adapt heparin, what values will prompt a change in heparin and by how much, when to recheck the anticoagulation test)
3. **Cryoprecipitate or fibrinogen concentrate** (when to measure fibrinogen level or perform viscoelastic testing, what level triggers administration of cryoprecipitate or fibrinogen concentrate, what is the dose, and when to recheck the fibrinogen level)
4. **Antithrombin concentrate** (when to measure antithrombin level, what level triggers administration of antithrombin concentrate or plasma transfusion, what is the dose, and when to recheck the antithrombin level)
5. **Priming of circuit** (with plasma, saline, platelets, standard RBC units and/or whole blood)

Data on these co-interventions will be collected daily and compliance with the local guidelines will be measured, so that the effect of any imbalances on the primary outcome can be examined after the trial is complete.

Other co-interventions, such as ECMO flow management, vasopressors, ventilation, red blood cell transfusion, sedation, and the use of hypothermia, will **not be protocolized**, due to the

pragmatic nature of the trial. In addition, the use of other hemostatic agents, such as tranexamic acid, aminocaproic acid, DDAVP, and von Willebrand factor will not be protocolized as these are typically used in patients with active bleeding and not to prevent bleeding (7). However, all these co-interventions will be recorded.

D. Compliance Measures

Patients in the lower platelet threshold arm of the study will be considered adherent to protocol if 90% or more of pre-transfusion platelet counts are < 50 $x10^9$ cell/L, and likewise patients in the higher platelet threshold arm of the study will be considered adherent to protocol if 90% or more of pre-transfusion platelet counts are < 90 $x10^9$ cell/L.

Should a center have more than 10% of their patients with non-compliant transfusions, we will work with the primary investigator to understand the barriers to following the trial's protocol, and may provide additional measures, such as team education.

In addition to the aforementioned compliance measures, the sixth feasibility criterion pertains to a combined metric of transfusion protocol compliance that considers platelet counts and transfusions. Details of this combined metric are provided in Appendix F.

Measures to Ensure Compliance

Trial conduct depends upon administration of platelet transfusions according to the appropriate transfusion threshold to patients in the two study arms. Study procedures will ensure timeliness and accuracy for platelet transfusion prescriptions. Such measures include:

1) recruitment and engagement of ECMO directors as co-site-investigators, as well as inclusion of ECMO expertise on all important trial committees
2) development of institute-specific protocols
3) in-person and/or virtual meetings prior to initiation of the trial at every participating site
4) giving lectures and/or grand rounds to all participating centers
5) twice daily monitoring of the platelet levels of all patients on ECMO
6) Poster at the patient's headboard with the platelet transfusion threshold

In similar trials, the adherence was 97% in the PlaNet-2 trial (21), whereas it was 92% in a low platelet threshold transfusion trial in oncology patients (PLADO trial) (32). Whereas neither of these studies were in ECMO patients, they occurred in the critically ill who were at high risk for bleeding. As such, they demonstrated clinical equipoise for randomization to two separate platelet transfusion thresholds.

Given that ECMO programs protocolize nearly every aspect of a patient's care, we believe ECMO centers are the ideal environment to conduct the study.

E. Randomization

An independent biostatistician will generate the randomization scheme based upon instructions from the study statistician. The randomization process will consist of a computer-generated random listing of treatment allocation using a pre-established algorithm.

Local study teams will contact the REDCap randomization module when patients are eligible for enrollment. Patient characteristics that establish eligibility will be confirmed.

Time zero will be the time that the patient is randomized to one arm of the study within the web randomization system. A site-specific back-up randomization envelope will be available for one use per site in the event that one is emergently needed. Only the study statisticians and designate at the Data Coordinating Center (DCC) will have knowledge of the randomization codes.

Allocation will be a **1:1 ratio**.

Subjects will be **stratified by age** (≤28 days vs >28 days) because it is infeasible to stratify by more than one variable given the sample size. We prioritized this variable since most of the safety concerns are around lower transfusion strategies in infants. Other variables considered for stratification in the future large trial will be type of ECMO support (V-A vs V-V) and site.

Patients will not be stratified based on the severity of the disease, as all scores predicting outcome on ECMO use data from the first few days of the ECMO run (43–45). In addition, scores that evaluate severity at admission (PRISM III and PIM2) are not validated nor calibrated in ECMO patients (46,47). In the pilot, we will neither stratify according to the indication (medical vs post-surgical), as this would significantly increase the sample size. However, this may be explored in regression models and sensitivity analyses.

Research personnel at each site and PICU caregivers will not have access to the randomization schedule. Moreover, in order to **conceal randomization**, 3 sizes of **blocks permutation** will be used on a random sequence: 2, 4 and 6 subjects per block.

F. Blinding

This is an open-label trial, since the **transfusion strategy cannot be blinded** from the clinical team, as they must order the transfusions based on the platelet count. The research team (Marianne Nellis, Oliver Karam, and members of the steering committee) will be blinded to treatment allocation until the end of the analysis. Since the statisticians will need to report to the Data Safety Monitoring Board, they will not be blinded.

G. Treatment Period and Follow Up

The intervention will be implemented immediately upon randomization and maintained in effect until four possible events (whichever occurs first):

1) progression to severe bleeding, according to the BASIC definition, and/or severe clotting, according to the NHLBI consensus definition, outside of temporary suspensions
2) decannulation from ECMO (or ECMO flow stopped, even if cannulas left in place)
3) twenty-one days post-randomization (as the progression to new bleeding and new thrombosis has plateaued by day 21 and 95% of the patients will be off ECMO by then (6))
4) or temporary suspension lasting more than 24 hours.
5) Withdrawal from the study, either by the attending physician or the parents.

The mortality follow-up will be at 28 (+/- 4 days) and 90 (+/- 10) days after randomization.

H. Temporary suspension of Individual Patients

The protocol will allow for temporary suspensions, i.e., transfusion at a higher platelet count threshold than allowed for the randomization arm, if the clinical situation warrants immediate transfusion for certain conditions including: 1) chest-tube insertion; 2) surgical intervention while on ECMO (such as tracheostomy or repair of congenital diaphragmatic hernia); 3) during ECMO circuit change; or 4) in preparation for decannulation. In addition, while patients will be monitored for severe bleeding and/or severe clotting events during this time, they will not have the transfusion strategy permanently discontinued if they develop such an event during the temporary suspension, provided that the severe bleeding/clotting has resolved within the 24 hours of the temporary suspension. If a patient continues to have severe bleeding or severe clotting past the 24 hours of the temporary suspension, the intervention is stopped.

Adherence to the trial protocol must be resumed as soon as possible once these events are controlled or completed. Data monitoring and collection will be unchanged during suspension (including pre-transfusion platelet counts and platelet transfusion volumes), but data on length and justification of suspension will also be collected. Suspensions less than 24 hours will not be considered a breach of adherence to the protocol. After 24 hours of temporary suspension, the patient will have all assigned treatment strategy implications lifted (i.e., no restrictions on the range at which any subsequent platelet transfusions may occur) but will continue to be followed for ascertainment of safety and study outcomes. Temporary suspensions will only necessitate exclusion from analyses for one of the secondary endpoints (#6: proportion of compliant platelet transfusion actions), as explicitly noted in Section 9.B. The safety outcomes will still be followed but will be analyzed as a sub-group (e.g., severe bleeding during temporary suspensions).

9. Outcomes

The objective for the **pilot study** will be to assess the **feasibility** of the full RCT.

A. Primary Outcome

The primary objective is to assess the separation between the higher and lower platelet threshold arms. Indeed, for a transfusion trial to be successful, there has to be a clinically meaningful separation between the transfusion strategies, to be able to imply causality for the outcome. Since the higher platelet threshold is <90 $x10^9$/L and the lower platelet threshold is < $50x10^9$/L, we expect the **separation of the pre-transfusion platelet count (primary outcome) to be at least 30 $x10^9$/L**. To assess the separation between both arms, we will collect the pre-transfusion platelet count before each transfusion event.

In addition, we will collect the total daily platelet transfusion dose for a patient while the patient is on ECMO. The average daily dose (in ml/kg/day) will be computed by the research team, by dividing the total platelet transfusion volume by the patient's weight at admission and the number of days on ECMO.

These outcomes will be measured outside of temporary suspensions; that is, transfusions occurring during a temporary suspension are ignored from these analyses.

The primary *safety* outcome, which is assessed in conjunction with the early trial stopping rule, is occurrence of any of the following from the time of randomization (enrollment) to 24 hours after the transfusion strategy threshold was permanently discontinued: progression from no bleeding or minimal bleeding to severe bleeding, according to the BASIC definition (34); severe thrombotic event, according to the NHLBI Hemostasis Clinical Trial Outcomes Working Group (need for circuit change, need for thrombectomy, ischemic stroke, or distal thromboembolism, as diagnosed clinically) (48); death (i.e., all-cause mortality). The primary safety outcome ignores severe bleeding/severe clotting that begins during a period of temporary suspension, but does not ignore deaths that occur during a period of temporary suspension.

The adjudication committee will evaluate patients who are deemed by site investigators to meet this endpoint (severe bleeding or clotting) in batches of 5 patients. The adjudicators will rotate, which will help prevent bias and ensure that all adjudicators have an equal opportunity to contribute to the study. We will report the inter-rater reliability (Kappa) at the end of the study. The committee will make determinations for whether bleeding was the primary cause of the worsening clinical outcome or if clotting was severe. Specifically, the sites will complete the severe bleeding/clotting daily worksheet (see Appendix E) on a daily basis. If a severe bleeding event, or clotting event, the event will be recorded in the database (and the intervention will stop for that participant since this safety outcome has been reached). Once five new patients have any of these events reported, the adjudication committee will review the events and report if they agree with the determination of the outcome within 72 hours of their review or if they need additional information from the site PI. Patient enrollment will continue during their reviews.

B. Secondary Outcomes

Our second objective is to demonstrate the ability for observed endpoints to satisfy various prespecified feasibility thresholds. The information the pilot trial will provide is both necessary and sufficient for the implementation of the full clinical trial. Based on PALISI and BloodNet's suggestions, we will seek further funding and progress to the full trial if at least 5 of the following 10 criteria are met:

1. Estimated separation in mean pre-transfusion platelet count is > 30 x10^9 cell/L;
2. Eligibility rate is > 50% of the predicted eligibility rate of 7.9 patients per site per year;
3. Proportion of patients whose parents were approached for consent within 24 hours of cannulation (number of patients approached / number of patients eligible for consent) > 90%;
4. Proportion of patients for whom we were able to obtain informed consent (number of patients consented / number of patients approached) > 50%;
5. Randomization of consented patients is > 66%;
6. Proportion of compliant platelet transfusion actions (examples of noncompliance are transfusion given at or above threshold or without any platelet count in the previous 12 hours, excluding transfusions given during a temporary suspension, or transfusion not given within 12 hours of a platelet count below threshold) > 90% (see Appendix F for details);
7. Proportion of patients with one or more temporary suspensions not due to preparation for decannulation < 10%;

8. Proportion of patients who withdraw from the study and/or are lost to follow-up (i.e., withdraw and/or are missing 90-day mortality assessment) < 6%;
9. Proportion of patients with non-platelet transfusion protocol violations (violation of site-specific management protocol for any aspect besides platelet transfusion threshold compliance) < 5%;
10. First instance of progression to severe bleeding or thrombotic outcomes can be adjudicated in > 90% of the patients for whom such an outcome was reported by the site.

Similar to pre-transfusion platelet counts, overall platelet transfusion doses, and progression to severe bleeding and/or clotting, feasibility outcomes 1, 6, and 9, are to be analyzed outside of temporary suspensions.

C. Tertiary Outcomes

Our tertiary outcome is to demonstrate the ability for an adjudication committee to determine the severity of bleeding and/or thrombotic outcomes.

This is similar to the primary safety outcome, except that it does not include mortality in the definition. That is, progression to severe bleeding or a severe clotting event must occur, whether or not there is aa all-cause death. We will demonstrate that the recorded data is sufficient for the adjudication of severe bleeding, severe thrombus or both in 90% of enrolled subjects with this outcome reported by the site.

Justification of BASIC and NHLBI definitions of bleeding and thrombosis:

We believe that the BASIC (34) (Table 1) and NHLBI definitions (48) of bleeding and thrombosis provide the most relevant descriptions as they relate to children supported by ECMO. Importantly, central nervous system involvement, a frequent site of bleeding on ECMO, may be captured by a change in the neurologic variables of the PELOD-2 score (both the Glasgow Coma Scale and the pupillary reactions are in the PELOD-2 score) which is incorporated in the BASIC definition. In order to ensure that neurologic variables are not missed (since the majority of patients will be deeply sedated and paralyzed), we will also consider any new and/or expanding intracranial hemorrhage as severe bleeding and collect information regarding the location of the bleeding.

Importantly, several of the organ dysfunction criteria of the PELOD-2 score may be normalized by the ECMO circuit (blood pressure, PaO_2/FiO_2, pCO_2), which might lead to an underestimation of the organ failure. Severe bleeding is likely to cause a change in these variables (such as hypotension or worsening respiratory function), which might be mitigated by changes in ECMO support. Therefore, we will not only record the PELOD-2 variables but also the potential increased ECMO support. Although we recognize some limitations to the PELOD-2 score on ECMO, similar scores, such as the adult SOFA score, have been used to

Table 1: Severe Bleeding Definition [27] (at least one of the following criteria)
1) bleeding that leads to at least one organ dysfunction, using PELOD-2 score criteria of organ dysfunction
2) bleeding that leads to hemodynamic instability, defined as an increase in heart rate by > 20% from baseline or a decrease in mean arterial pressure by > 20% from baseline (i.e., prior to bleeding event)
3) bleeding leading to a drop in hemoglobin > 20% within 24 hr
4) quantifiable bleeding ≥ 5 mL/kg/hr for ≥ 1hr (e.g., chest tube)
5) intraspinal bleeding leading to loss of neurologic function below the lesion, nontraumatic intra-articular bleeding leading to decreased range of movement, or intraocular bleeding leading to impaired vision
6) new and/or expanding intracranial hemorrhage

report organ failure on ECMO (49,50). Therefore, in the absence of a validated score to evaluate organ failure on ECMO, it seems justified to use the validated PELOD-2 score contained within the BASIC definition.

Of note, we will not use the ELSO definition for bleeding as the primary clinical outcome of the full trial, as it considers bleeding only if the patient requires RBC transfusions or interventions ("hemorrhagic complications requiring packed red blood cell (PRBC) or whole blood transfusion (>20 ml/kg/calendar day of PRBCs or >3 U PRBCs/calendar day in neonates and pediatrics and > 3U PRBCs/calendar day in adults) or other intervention such as surgical or endoscopic intervention") (51). Indeed, this definition will lead to potential biases and increased heterogeneity, because of the high variability of the indications to transfuse and/or to intervene surgically (52). Similarly, the recently developed NHLBI bleeding definition is also tied to interventions (including transfusions and changes in anticoagulation) (48). However, we will measure the ELSO definition as a secondary outcome.

Severe thrombotic events will be defined according to the NHLBI Consensus Conference (48) as circuit clot leading to circuit change, need for thrombectomy, ischemic stroke, or distal thromboembolism.

Bleeding and/or Thrombotic Outcome Adjudication

ECSTATIC is an open-label trial, but the bleeding and/or thrombosis outcome will be adjudicated by an independent central committee, blinded to the allocation group. This committee will be composed of three members, a pediatric cardiothoracic surgeon, a pediatric cardiac intensivist and a neonatologist, all of whom have significant experience caring for children on ECMO. The members of the adjudication committee will not be associated with any of the enrolling sites, nor members of the research consortiums supporting this trial. The adjudication committee will determine if the bleeding was the primary cause of organ dysfunction, hemodynamic instability, acute anemia, loss of spinal function, articular range of motion, or impaired vision, as per the definition of severe bleeding (34), or if clotting was severe. The members of the adjudicating committee will not receive any information on allocation and on the platelet counts of participants. Two members of the adjudicating committee will review the same information that the Data Coordinating Center will provide (please see Appendix E). They will determine independently if there is a cause-effect relationship between the bleeding episode and one or many of the outcomes listed above. In case of discordance between the two members, a third reviewer will be asked to state what they think, using the same information.

The information that will be given to the adjudicators include: description of the bleeding or clotting episode (when it starts, its duration, the volume of blood loss, etc.), description of intervention (allocation will be kept blinded as much as possible) and of co-interventions (including procedural sedation, boluses of medications with hemodynamic or vasodilatory effects, initiation of CRRT, etc.), and description of outcomes (when the adverse event appears, what adverse events, etc.). The adjudication will be done in batches of 5 subjects at a time with the primary safety outcome (per site report), as described above. The adjudications will be made within 72 hours of the start of the review. Patient enrollment will not be paused during the time of their review. In addition, the adjudication committee will report their assessment of the outcomes immediately prior to the

DSMB meetings that are during the enrollment phase so that the most up to date safety data can be provided to the DSMB.

D. Other Outcomes

Although many adult studies evaluate mortality, which is a more objective outcome, there are many other variables that will directly influence mortality on ECMO, in addition to platelet transfusions (6,9). However, mortality will be one of the secondary outcomes of the full trial. Mortality at 28 and 90 days will be assessed by phone calls to the families in the case that patients have been discharged at those times.

In addition, we will collect the proportion of serious adverse events, which will include transfusion-related reactions (including hyperkalemia, allergic reactions, transfusion-related acute lung injury, transfusion-associated circulatory overload and nosocomial infections), and ECMO-related complications not already captured in the outcomes (including seizures, need for neurosurgical intervention, cardiac arrhythmias, tamponade, pneumothorax, compartment syndrome, fasciotomy and amputation). Please see 11C for details on expected vs unexpected serious adverse events.

We will also collect the platelet processing of each unit received (i.e., apheresed versus whole blood derived, platelet number per mL of transfusate, ABO compatibility, irradiation, volume reduction, pathogen reduction, and storage age). For the sites in which the information is available (currently 5 out of the 10 participating sites), the total platelet count (per mL of transfusate) that each patient receives from each transfusion will be recorded. For sites in whom this exact information is not known, we will record if they receive “low-yield” platelet transfusions in which the platelet count is $< 3 \times 10^{11}$ platelets.

Additional data to be collected are:

- Hemorrhagic complications according to the ELSO definition (51)
- Thrombotic complications according to the ELSO definition (51)
- Blood product exposure, defined as the total number of transfusions (red blood cell, plasma, platelets, cryoprecipitate, fibrinogen concentrate) and other hemostatic therapies (activated Factor VII, tranexamic acid, aminocaproic acid, DDAVP, and von Willebrand factor, etc.)
- Re-explorations and other surgical procedures to treat bleeding
- Duration of ECMO, defined as 90-day ECMO-free days (e.g., if a patient is decannulated after 7 days, the 90-day ECMO-free days is 83 days; whereas if a patient dies on ECMO after 7 days, the 90-day ECMO-free days is 0 days)
- Length of PICU stay, defined as the 90-day PICU-free days
- Functional outcome, defined as the discharge Pediatric Overall Performance Category (POPC) and Pediatric Cerebral Performance Category (PCPC) (53) which have been validated in infants and older children and have been used to measure outcomes in infants, neonates and children on ECMO (54) (Appendix C).
- Mortality on ECMO, within 24 hours of the treatment strategy’s expiration, at 28 days, and at 90 days.

We will therefore be collecting six of the eight core outcome measures for ECMO research recommended by Hodgson et al (we will not be collecting the health-related quality of life and ability to return to work in the pilot trial) (55). Of note, neurodevelopment at one year is beyond the scope of the pilot trial, but will be assessed as an ancillary study of the full RCT.

E. Application of Results to the Implementation of the Full Trial

- The information gained from the pilot will help to identify revisions to the plans for the full RCT.
- We will optimize and document the ability to adjudicate a *bleeding and/or thrombotic outcomes* using both the BASIC and NHLBI developed definitions.
- *Eligibility numbers and enrollment rates* will inform the number of centers, size of centers (high volume versus low volume), and location of centers (US-only versus international) required to accrue the required sample size for the subsequent ECSTATIC RCT.
- We will document the ability to enroll patients through standard consent, which will allow us to determine if an Exception From Informed Consent (EFIC) will be needed for the larger RCT. This finding will also contribute to the selection of sites for the larger RCT as some countries have no provision for EFIC.
- *Protocol deviations*, should they occur in the pilot trial, will provide opportunities to refine key protocol elements, including reasons for protocol suspension, in order to maximize protocol adherence and minimize risk of bias in the subsequent ECSTATIC RCT.
- We will document and classify *temporary suspensions* to allow for refinement in the subsequent RCT.

10. Sample Size and Statistical Analysis

A. Sample Size

Salient assumptions to permit power estimation include the number of observed transfusion days per subject, the within-subject dependence structure, the within-treatment variability, and the difference in means between treatments. Previous data, albeit not with an enforced low platelet threshold transfusion strategy, were used to inform the first three assumptions. It was assumed the distribution of the number of transfusion days per subject will mimic historical data (e.g., approximately 10%-20% will have no transfusions). It was assumed a compound symmetry covariance structure will adequately account for within-subject dependence; such a structure is induced by a mixed effects model with subject-specific random intercepts. Fitting a model with such a covariance structure to historical data [6] yielded an estimated autocorrelation slightly above 0.2; to be conservative the power estimation assumed ρ=0.3 (6). The assumed within-treatment standard deviation, 40×10^9/L, resembles historical data but is anticipated to be a conservative estimate for the trial.

Although the difference in platelet strategy thresholds is 40×10^9/L (i.e., 90 $\times 10^9$/L vs 50 $\times 10^9$/L), this full effect will not be realized for the mean difference because the strategies depend on platelet counts which are not continuously observed, and as such, by the time of measurement a patient may have a platelet count considerably below the threshold. Patients with a platelet count

under 50 x10^9/L would be transfused under either strategy. Therefore, a more realistic separation for the difference in means between strategies is 30 x10^9/L.

Assuming pre-transfusion platelet levels will be approximately normally distributed with a difference in means of 30 x10^9/L, then with **50 patients (25 per arm)** enrolled there would be approximately **81% power** to detect a difference in mean platelet levels at the time of transfusion. Power may be higher due to conservative assumptions.

The design of the ***full RCT***, which will test the non-inferiority of a low platelet threshold strategy, will be informed by the separation in pre-transfusion platelet counts in both arms, median number of transfusions in each group, proportion of patients who develop the primary safety outcome (progression to severe bleeding and/or severe thrombotic event and/or study-related death) or tertiary outcome (progression to severe bleeding and/or severe thrombotic event), proportion of patients who require temporary suspension, proportion of patients with non-platelet transfusion protocol violations, and the consent rate.

B. Planned Recruitment Rate

There is a wide heterogeneity in the number of ECMO runs per center and per year. The sum of the eligible patients from the ten centers who have committed to participate in the pilot trial is 79 patients per year. We therefore would require an enrollment rate of 32% to recruit all of the patients within 24 months [50 patients / (2 years x 79 patients per year) = 0.32]. As a result, we are very confident we will complete the pilot trial within 3 years.

All the participating sites have at least 10 ECMO runs per year (the larger sites have up to 50 a year). Therefore, we anticipate that even the smaller sites should be able to enroll 5 patients over 18 months. However, should the enrollment be slower than expected, we will lift the cap on the larger sites (currently set at 10 subjects).

C. Statistical Analysis

For the *pilot study*, the analysis of the primary outcome measure will be assessing the *pre-transfusion platelet count* to describe the separation between the two platelet transfusion strategies, while accounting for intra-subject correlation. For each arm, we will estimate the mean pre-transfusion platelet count throughout the ECMO runs. This primary outcome will be measured for each transfusion and represent the latest available collected platelet count that is before the transfusion began. In the event that there was no preceding platelet count since a previous transfusion, the outcome for that transfusion will be regarded as missing. Participants who do not have a non-missing value for this outcome (e.g., they had no transfusions) will not be included in the analysis of the primary outcome. Because we anticipate intra-subject correlation when there are multiple transfusions for a patient, we will fit a linear model to pre-transfusion platelet counts with a fixed effect for assigned treatment (higher threshold vs. lower threshold). We will account for repeated measures by considering three within-subject covariance structures with constant variances: compound symmetry; the continuous analog of an autoregressive order 1 process, also known as CAR(1); and a hybrid of compound symmetry and CAR(1) achieved by including random subject effects and CAR(1) correlations in the within-subject errors. We will use restricted maximum likelihood (REML) for model estimation and use the model with the lowest Akaike Information Criterion (AIC) to select the best covariance structure. Once the covariance structure

is determined, the Kenward-Roger calculation for denominator degrees of freedom (df) will be applied in the F-test (equivalently, for the df in the t-test) of the treatment strategy's model coefficient. The test of the null hypothesis that the mean pre-transfusion platelet count is the same for each treatment arm vs the alternative that the higher threshold has a higher mean will be considered statistically significant if and only if the one-sided p-value is less than 0.025. In addition, a two-sided 95% confidence interval for the difference in means will be reported along with estimates of each treatment's mean and the difference in means, with all inferences coming from the repeated measures model described above.

In addition, we will measure the effect of the days on ECMO, by providing summaries of the daily platelet counts for each specific ECMO day with ≥ 5 patients still on ECMO, independent of receiving a platelet transfusion. This will allow evaluating trends over time and a possible attenuation of the separation over time.

Furthermore, we will also estimate the difference in the *median platelet transfusion dose* (in mL/kg/run) between the lower and higher platelet threshold strategy arms, and the median daily platelet transfusion dose (in mL/kg/day) to account for different lengths of ECMO runs. These outcomes will use two-sided nonparametric percentile bootstrap 95% confidence intervals, based on a minimum of 10,000 bootstrap replicates, for the difference in medians because by construction for each of these outcomes, there is only one outcome per person. As such, repeated measures modeling is not warranted.

Finally, we will also report the *screening and inclusion rates*, the proportion of patients who were *approached* within 24 hours of cannulation, the proportion of patients for whom we will be able to obtain *informed consent*, the number of *platelet transfusions prior to randomization*, the proportion of compliance with *transfusion thresholds*, number of *temporary suspensions*, and the ability to record data and *adjudicate bleeding and/or clotting outcomes*. In short, we will provide summaries of outcomes, with special emphasis for these summaries on the ten feasibility criteria of Aim 2.

Unless stated otherwise, the outcomes mentioned throughout the protocol are not intended to be tested for significance in this trial, but rather will be summarized descriptively (e.g., frequency and percentage for categorical variables, means and standard deviations or medians and interquartile ranges for continuous variables).

Subgroup analyses will be considered exploratory and will be interpreted with care given the expected small sample sizes. For all Aim 1 outcomes, we will perform two subgroup analyses, one to evaluate the effect of V-V vs V-A ECMO, the other to evaluate neonates vs infants and older children (≤ vs. > 28 days of age). For the Aim 1 model, the subgroup analyses are intended to be based on reapplying the model with the same covariance structure already identified in the overall analysis to the subset of participants from a particular subgroup (where subgroups are defined based on ECMO type or age). However, if estimation is deemed too unwieldy for some subsets, an alternative approach will be to use all participants in fitting one model but include ECMO type or age as covariates in the model. For all secondary outcomes, we will perform two subgroup summaries, one to evaluate the effect of V-V vs V-A ECMO, the other to evaluate neonates vs infants and older children (≤ vs. > 28 days of age).

While the primary safety outcome will be tested for a difference in the proportion of patients in the safety population (i.e., those randomized, even in the very unlikely event that an unconsented

patient was randomized), other safety outcomes, including all-cause mortality and adverse event occurrences, will be summarized, overall and by treatment assignment, for the safety population.

Statistical Support

This proposal, and the study's statistical analyses, were supported by the Trial Innovation Network. The statistician involved in the design and sample size calculation is Bradley Barney, PhD, School of Medicine, University of Utah.

Interim Analysis

There are two classes of interim analyses: a set of inferential statistical analyses for a formal interim stopping rule; and largely descriptive summaries focusing on data and safety monitoring used in reports prepared for the DSMB. The statistical testing underlying the formal stopping rule will be conducted when every new batch of five site-reported primary safety outcomes has been adjudicated. After every 5 patients who are thought to have the primary safety outcome of severe bleeding or thrombotic event or all-cause mortality, we will conduct a two-sided Fisher's exact test using an alpha threshold of 0.0333. This p-value threshold was chosen so that, accounting for the varying number of interim tests that may be conducted, the overall type I error rate is close to 0.05. Each test will include those with a known outcome at the time the adjudication process is completed (even if for a participant enrolled after the fifth new reported event), with participants for whom the outcome has not yet been determined being excluded until the outcome has been determined. We will test the null hypothesis that the two treatment strategies have the same expected proportion of patients who will experience the primary safety outcome. If a test is significant, we will pause the trial for safety reasons. The DSMB will evaluate the risk and make a recommendation to stop or continue the study to the NIH.

A DSMB report with safety and data completion metrics will be prepared and distributed to the DSMB for every DSMB meeting during the enrollment phase. Scheduled DSMB meetings are intended to be held twice during the study's enrollment period: approximately 8 weeks after the tenth participant randomization, and approximately 8 weeks after the 25th participant randomization, to allow time for outcome assessment and report preparation.

Since the adjudication committee will be reviewing the major safety concerns (severe bleeding, and/or clotting) for every patient with site-reported major safety concerns and because there are formal tests when a set of five additional patients has a primary safety outcome per site report, there will be no need to pause enrollment during the interim analysisThe study will be paused if there is evidence of harm, as noted earlier, i.e., a statistically significant difference in the proportion of patients progressing to severe bleeding, severe thrombosis or all-cause mortality using $p \leq 0.0333$. If such a pause occurs, the study might then be stopped after the NHLBI, DSMB, and PIs have the opportunity to discuss findings that led to the automatic pause. In addition, serious adverse events related to the intervention will be evaluated and reported in real time (within 48 hours of occurrence) by the Medical Monitor and reported to the DSMB.

Note that the primary safety outcome will also be tested for a difference in arms after all participants have been enrolled, again using the 0.0333 threshold to be declared statistically significant, unless a previous test was already significant at this threshold. However, this is not considered an interim analysis because it is after all participants were enrolled.

Many factors may influence exactly what the stopping rule thresholds are in the trial, such as primary safety outcomes that are overruled by the adjudication panel (expected to be uncommon), the underlying event rate, and the balance of randomization to treatment arms throughout the trial.

An example of how the rule translates to go/no-go thresholds under one particular set of assumptions is provided. Assuming that at every interim look, there have been equal numbers randomized to each treatment, and that at every interim look, half of the total number of enrolled patients have experienced the primary safety outcome (though the numbers can differ by arm), then there would be tests after 10, 20, 30, 40, and all 50 participants were enrolled, so long as there has not been a previous test that was significant. For any given test to have a two-sided p-value ≤ 0.0333, the primary safety outcome rates would have to be as follows:

- With n=10 participants, 0/5 (0%) or 5/5 (100%) in the lower threshold arm
- With n=20 participants, either ≤2 (≤20%) or ≥8 (≥80%) out of 10 in the lower threshold arm
- With n=30 participants, either ≤4 (≤26.7%) or ≥11 (≥73%) out of 15 in the lower threshold arm
- With n=40 participants, either ≤6 (≤30%) or ≥14 (≥70%) out of 20 in the lower threshold arm
- With n=50 participants, either ≤8 (≤32%) or ≥17 (≥68%) out of 25 in the lower threshold arm

D. Limitations with Strategies to Overcome

- Sites may not be able to maintain clinical equipoise throughout the study period, as the study is not blinded. To compensate for this, prior to the study initiation, we will present the study protocol to leaders in the ECMO team at each site, including general surgeons, cardiothoracic surgeons, anesthesiologists, neonatologists, and pediatric intensivists to ensure compliance. We will review compliance for each site at monthly meetings and at the interim analysis and provide further education at each site as needed. Moreover, we will highlight the fact that the full ECSTATIC study will be a non-inferiority RCT, which underlines that we expect to find no statistically significant difference in the outcomes of the patients who will participate in the large study.
- The proportion of platelets transfused during temporary suspensions may dilute the separation between platelet exposure between the two arms. For this reason, we have limited the number of reasons for temporary suspensions. We will also analyze the impact of suspensions on the platelet count separation.
- Different methods can be used to obtain platelet products: 13% of platelet transfusions given to ECMO patients are whole blood derived - either by platelet-rich plasma (USA and UK) or the buffy-coat method (Europe and Canada), and 87% are obtained by apheresis (single-donor) (13). The volume of a whole blood derived platelet unit is about 50 mL while that of an apheresis platelet unit ranges from 200 to 300 mL. We will not be able to control the methods of processing platelets at each site. However, processing details of each platelet transfusion will be recorded. At least five of the ten participating sites will be reporting the number of platelets per mL of transfusate received so that we can describe the dose of platelets

transfused and consider controlling for this in the larger trial. Though it is not feasible in the pilot due to cost, we will consider assaying the transfusates themselves in the larger trial to determine the actual platelet count transfused.

- It will be possible to consider the pilot ECSTATIC trial as a vanguard phase of the full RCT and to merge their data to the data of the patients enrolled in the full trial if no significant changes are made to the design of the full RCT. On the other hand, merging patients of the pilot and the full trials would be inappropriate if significant changes are made (e.g., the threshold platelet count is changed). In that case, we will combine the data of the pilot and of the full RCT by performing a meta-analysis.

11. Study Monitoring and Study Organization

Approval for use of this protocol by the Human Studies Committee (HSC) must be obtained in accordance with the institutional assurance policies of the U.S. Department of Health and Human Services. Institutional Review Board (IRB) approval of the ECSTATIC protocol and consent forms will be required prior to patient participation on the trial. The single IRB at BRANY will coordinate IRB approvals at each site within the United States. All reasonable measures will be taken to protect the confidentiality and identity of the patient and patient's records according to State and Federal laws. Patient identity will not be revealed in any publication.

A. Study Monitoring

Data will be collected at PICU entry (e.g., PRISM and PELOD-2 scores, co-morbidity, coagulation evaluation, etc.), at ECMO cannulation, at randomization, daily while patient is on ECMO, at ECMO end, and at PICU discharge. Data will be collected at least on a daily basis from ECMO initiation to ECMO end. Mortality (28-day and 90-day) is the only data that would be collected after PICU discharge. The application of the RCT protocol (lower or higher platelet threshold strategy) will end at the earliest of any of the following five events: (1) progression to severe bleeding, according to the BASIC definition, and/or severe clotting, and/or death; (2) decannulation from ECMO; (3) Twenty-one days post-randomization (as the progression to new bleeding and new thrombosis has plateaued by day 21 and 95% of the patients will be off ECMO by then); 4) temporary suspension lasting more than 24 hours; or 5) study withdrawal.

Data management will include an audit trail, a security system, query functionality and quality control done according to US CFRs. Data management will be performed at the Trial Innovation Network at the University of Utah under the supervision of the study statistician.

Data will be entered on site in the web-based eCRF. For validation purposes only, double data entry will be used for the first two patients enrolled at each site. During the validation phase, CRF and entries will be considered adequate if the frequency of discordance is lower than 2% in the eCRF.

The data coordinating center (DCC, see below) will be responsible for data quality assurance done through eCRF (via regular data extraction) and queries.

B. Data Safety Monitoring Board

There are two principal mechanisms to achieve the mandate of protecting participant safety and being proper stewards of study data for the ECSTATIC Trial. The first is the oversight of a Data Safety and Monitoring Board (DSMB) to assess participant safety and the integrity of the study. The second is the proven collection of processes employed by the University of Utah Data Coordinating Center (DCC) to collect, protect, and analyze study data.

DSMB

The purpose of the DSMB is to advise the Federal funding agency (NHLBI) and the ECSTATIC Principal Investigators (Drs. Karam and Nellis) regarding the continuing safety of study subjects and the continuing validity and scientific merit of the study. The DSMB will be a panel of specialists chosen by the NHLBI with input from the PIs. The panel will include a pediatric intensivist, two pediatric hematologists, a bioethicist, and a statistician. One member, directed by the NHLBI, will chair the group. Any conflicts of interest of the members of the DSMB will be declared and reviewed by the executive secretary and the NHLBI ethics officer prior to their first meeting.

The DSMB is responsible for monitoring accrual of study subjects, adherence to the protocol, assessments of data quality, performance of individual clinical sites, review of serious adverse events and other subject safety issues. The DCC will provide reports relating to these topics to DSMB members prior to the interim DSMB meeting(s). It is anticipated that the DSMB will meet four times: (1) prior to patient enrollment, (2) approximately 8 weeks after 10 patients are enrolled, (3) approximately 8 weeks after 25 patients are enrolled and (4) at trial completion. The DSMB and/or Medical Monitor will have the final say in determining if there is a need for more frequent interim analyses and/or need for more frequent meetings.

As described above, the DSMB will meet prior to the start of the ECSTATIC Trial to review the protocol prior to implementation. The NHLBI executive secretary will draft the DSMB charter, which will be reviewed by the DSMB for approval. The charter will include responsibilities of the DSMB, definitions of a meeting quorum, information about meeting logistics and frequency, and an outline of report contents the DSMB will be given prior to the biannual meetings. In addition, they will define event triggers that would call for an unscheduled review, stopping procedures that are consistent with the protocol, unmasking (unblinding), and voting procedures. After the DSMB has approved its charter and the final protocol, the Data Coordinating Center will send this information to the single Institutional Review Board (sIRB) at BRANY. If the sIRB has changes they would like made to the study protocol and/or consent, the changes will be sent to the NHLBI and DSMB for review and approval. Certification of IRB approvals will be sent to the NHLBI Grants Management Officer (GMO) before enrollment may begin at each site. In addition, after the start of the trial, any amendments to the protocol and/or consent will be submitted to the NHLBI and DSMB for approval prior to submission to the sIRB. Finally, should the sIRB request any additional study actions, such as holding enrollment, requiring additional monitoring, etc, the co-PIs (Drs. Nellis and Karam) will notify the DSMB and the NHLBI within 24 hours, by email.

DSMB meetings to evaluate study protocols, prior to study implementation, may be open or closed according to the decision of the DSMB members. We suggest that pre-enrollment meetings be open to members of the ECSTATIC investigative team. After enrollment commences, we suggest DSMB meetings consist of both open and closed sessions. Open sessions would allow for inclusive attendance in order to facilitate the review and appropriate alterations of the protocol in response to DSMB concerns. Any especially sensitive information, such as interim review of trial data by treatment arm, will be limited to the closed session. Attendance at the closed session will be for DSMB membership, DCC biostatisticians, and possibly administrative personnel supporting DSMB members (e.g, the DSMB executive secretary and others) and/or Medical Monitor.

The DSMB can recommend whether or not to terminate enrollment in ECSTATIC because of potential safety concerns or study feasibility issues. In addition, they can recommend modifications to the trial. We proposed two interim DSMB meetings: (1) approximately 8 weeks after 10 patients are enrolled, (2) approximately 8 weeks after 25 patients are enrolled. The focus of the interim analysis will be on patient safety and enrollment characteristics. DSMB members must be satisfied that the timeliness, completeness, and accuracy of the data submitted to them for review are sufficient for evaluation of the safety and welfare of study participants. The interim analyses may show overall rates of adverse events in the open session, but summaries by treatment arm would be restricted to the closed session. Formal interim analysis for efficacy or futility of the primary outcome, separation in pre-transfusion platelet count, is not recommended. Our preference for not conducting an efficacy analysis mid-trial is in part because of the small sample size, but also because the primary outcome is relevant to the design of the future study, but not to the safety of the pilot. Patient safety is of paramount importance.

As per NHLBI practices, the NHLBI ES is responsible for preparation and transmission of the formal DSMB minutes to the Director of the applicable Division within 14 calendar days of each meeting or call. The NHLBI program office will prepare a Summary Report of Board Recommendations and submit it to primary study investigators(s) and DCC within 30 calendar days of each meeting, if the DSMB does not identify any safety or other protocol-related concerns. If the DSMB does identify concerns, the NHLBI staff will distribute, as soon as feasible, preferably within 7 calendar days of the DSMB meeting. Primary study investigators(s) or DCC will forward the Summary Report to each participating research site. In the unlikely event that the DSMB recommends emergent cessation of enrollment in ECSTATIC because of safety concerns, this communication will be made as soon as possible upon the conclusion of the closed session. If the NHLBI concur with this recommendation, the DCC will notify all ECSTATIC clinical sites to cease enrollment immediately.

Monitoring

Adverse Events: Each site PI will be responsible, in coordination with their local site research coordinators, to monitor the enrolled patients for adverse events on a daily basis. The adverse events will be reported to the PIs and DSMB accordingly as described below and as follows.

An Independent Medical Monitor will be appointed by the investigators to review and monitor all serious and unexpected adverse events in real time and perform a periodic review of patient safety. Any serious adverse events that are considered by the site to be possibly or probably related to the study intervention will be reported to the Medical Monitor within 24 hours of the

event. For each of these serious adverse events, the site investigator will provide sufficient medical history and clinical details for a safety assessment to be made with regard to continuation of the trial. The Medical Monitor will review the event with the site within 24 hours and determine if the event is related to the intervention and if it should immediately trigger DSMB review. The Independent Safety Monitor will be a physician with clinical expertise in ECMO with no real or perceived conflicts of interest. The Independent Safety Monitor will not be part of the DSMB, but the DSMB may review these reports or consult with the monitor as needed.

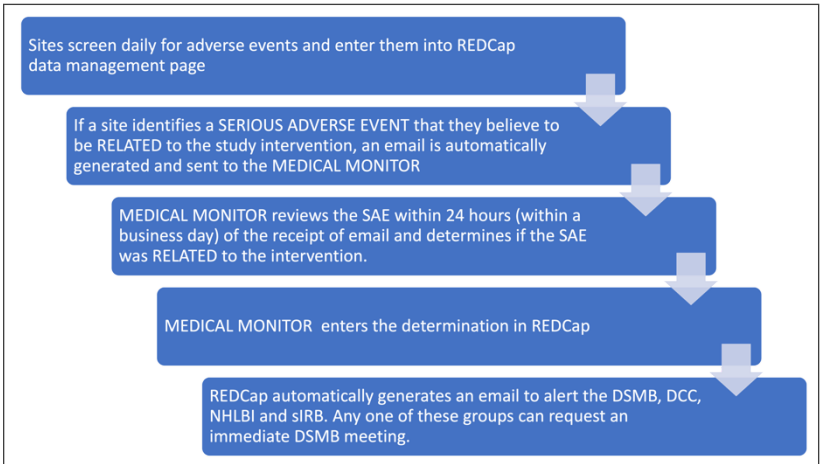


Figure 8: Serious safety event and medical monitor's reporting process

Data Quality: The DCC at the University of Utah will be responsible for monitoring the quality of data entry in real-time as described below.

DCC Features and Safeguards

The DCC in the Department of Pediatrics at the University of Utah School of Medicine provides data coordination and management services for a variety of national research networks. Anchoring these services is a state-of-the-art, energy efficient data center. The DCC currently manages over 125 terabytes (TB) of data and supports more than 3500 users around the country and provides a secure, reliable, enterprise-wide infrastructure for delivering mission critical DCC systems and services.

The DCC employs the following utilities and practices to ensure the safety and security of data stored at the facility.

- The DCC's electrical power system contains an uninterruptible power supply (UPS) with diesel backup generator.
- The DCC is protected with an FM-200 backed fire suppression system.
- Incremental backups of data occur Monday through Friday. A full data backup occurs weekly. Full backups are sent off-site on a weekly basis to a secure secondary location.
- On premise security guards monitor and enforce access control 24 hours a day, 7 days a week, 365 days a year.
- The DCC and external building access points are monitored with video surveillance and entry is restricted by card access and layered security measures and controls.
- Direct access to data center machines is only available while physically on premise or via a VPN client.
- All network traffic is monitored for intrusion attempts.
- All DCC personnel have completed Human Subjects Protection and Health Information Portability and Accountability Act (HIPAA) education.

- The DCC requires all users to sign specific agreements concerning security, confidentiality, and use of our information systems before access is provided.

Data management efforts extend beyond collecting and securely storing data. To promote the collection of valid data, the DCC uses an in-house application named QueryManager to support a variety of studies. Rules are built to identify certain types of potentially problematic data. Clinical sites will be sent queries in real time when data for participants at their site violate these rules, and these queries are tracked until the problem is automatically resolved (e.g., by correcting aberrant data entries) or manually resolved (e.g., confirmation received from the site that the original data were correct). Critical derived variables and analyses reported in DSMB reports, abstracts or manuscripts are routinely dual-programmed or code-reviewed by a second statistician. These safeguards instill confidence that the collected data and reported results are accurate.

Adverse Events

Definition of an Adverse Event
An adverse event (AE) is an unfavorable occurrence in a study subject that begins or worsens during the study (from the time of randomization through study completion). The reporting of an adverse event does not imply a causal relationship between the event and study participation. Determination of the relationship between the event and study drug or a study mandated procedure must be made by the Site Investigator. All adverse events will be categorized as being possibly, probably, or not related to study intervention. For any unexpected serious adverse events that are considered by the site to be possibly or probably related to the study intervention, a report will be made to the Medical Monitor within 24 hours of the event. The Medical Monitor will review the event with the site within 24 hours and determine if the event is related and if it should immediately trigger DSMB review (automatic emails send by REDCap).

Clinical Adverse Events
Children requiring support from extracorporeal membrane oxygenation (ECMO) are at the extreme of critical illness and therefore we expect a wide range of adverse events, including organ dysfunction, hospital-acquired infections, and death (which occurs in up to 50% of all children on ECMO), to happen as part of the routine clinical care. Adverse events to be collected will include, but not be limited to, transfusion-related reactions (including hyperkalemia, allergic reactions, transfusion-related acute lung injury, transfusion-associated circulatory overload and nosocomial infections), and ECMO-related complications not already captured in the outcomes (including seizures, need for neurosurgical intervention, cardiac arrhythmias, tamponade, pneumothorax, compartment syndrome, fasciotomy and amputation). The occurrence of adverse events will be monitored by the site PI or the site coordinator on a daily basis from the time of enrollment until 24 hours after when the transfusion strategy was permanently discontinued; strategy discontinuation is initiated by five possible events (whichever occurs first): 1) progression to severe bleeding, according to the BASIC definition, and/or severe clotting, and/or death; 2) decannulation from ECMO; 3) Twenty-one days post-randomization (as the progression to new bleeding and new thrombosis has plateaued by day 21 and 95% of the patients will be off ECMO by then); 4) temporary suspension lasting more than 24 hours; or 5) study withdrawal. If AEs are identified, the AEs will be reported as they arise as described below and then will be followed through to resolution or hospital discharge, whichever comes first.

Severity of AEs will be defined as follows:

- No severity: mild event that does not impact the expected clinical course.
- Moderate: moderate event that leads to prolonged time on ECMO, prolonged length of stay or additional procedures.
- Severe: severe event that leads to long-term sequelae or death.

A distinction will be drawn between *serious* and *severe* AEs. A severe AE is a major event of its type. A severe AE does not necessarily need to be considered serious. For example, nausea which persists for several hours may be considered severe nausea, but would not be an SAE. On the other hand, a stroke that results in only a limited degree of disability may be considered a mild stroke, but would be an SAE.

Relatedness

The site investigator must determine if an AE is "probably related", "possibly related", or "not related" to the platelet transfusion strategy. Determinations will be based on the biologic plausibility, the temporal relationship, and the presence or absence of an alternative explanation for an AE.

- *Probably Related:* The event follows a reasonable temporal sequence from the time of beginning the assigned study intervention, and cannot be reasonably explained by other factors such as the subject's clinical state, therapeutic interventions, or concomitant drugs administered to the subject.
- *Possibly Related:* The event follows compatible temporal sequence from the time of beginning the assigned study intervention, but could have been produced by other factors such as the subject's clinical state, therapeutic interventions, or concomitant drugs administered to the subject.
- *Not Related:* The event is clearly related to other factors, such as the subject's clinical state, therapeutic interventions, or concomitant drugs administered to the subject.

Expectedness

All adverse events, including serious adverse events, will be evaluated as to whether their occurrence was expected or unexpected. An adverse event is considered expected if it is known to be associated with ECMO, underlying medical conditions of the subject, is directly related to study outcome, or is otherwise mentioned in the protocol, informed consent, investigator brochure, or other study documents.

Adverse event reporting

The period for collecting adverse events is from the time of randomization through 24 hours past the time when the transfusion strategy was last applicable. AEs that are not SAEs will only be reported if judged by the investigators to be at least possibly related to the transfusion strategy. SAEs will be reported regardless of whether these are judged to be related to the transfusion strategy, with unexpected (unanticipated), serious (including all deaths), and related SAEs reported promptly as noted in Figure 9. The Medical Monitor will notify by email the DSMB, NHLBI, and PIs within 24 hours of being notified by the site investigator (using REDCap's automatically-generated emails).

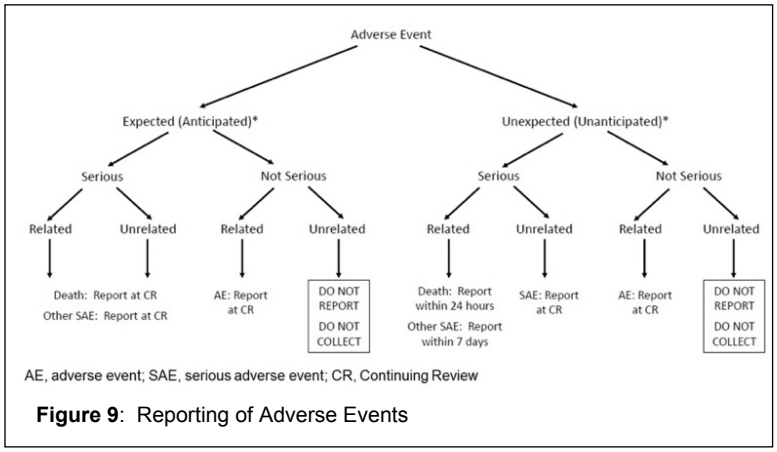


Figure 9: Reporting of Adverse Events

Of note, deaths occurring after ICU transfer or discharge will be reported within 24 hours of it coming to the attention of either of the co-primary investigators (e.g. a patient might die 38 days after the end of the ECMO run, in another institution; in which case, the co-PIs will only be made aware at the 90-day follow-up call).

C. Study Organization

Study primary investigators

Drs. Karam and Nellis (study primary investigators) will have overall responsibility for the project. They will ensure the smooth operation of all committees and facilitate communication between committee members. They will have the final approval of all reports and scientific publications emanating from the study. The study primary investigators will preside over all Executive and Steering Committee meetings. They will also appoint a study Vice-Chair at its first meeting who will assume all responsibilities for the Chairs in their absence.

Executive Committee

The voting members of the Executive Committee will consist of Oliver Karam, Marianne Nellis, Marisa Tucci, Jacques Lacroix, Philip Spinella, Jennifer Muszynski, Scott Weiss, Simon Stanworth and Paul Clarke.

The Executive Committee will oversee all aspects of the ECSTATIC trial including but not limited to:

1. Will ensure that the study is conducted in accordance to the protocol.
2. Will closely monitor the progress of the study.
3. Will monitor study expenses.
4. Will develop or modify policies and instructions regarding daily operations of the trial.
5. Will oversee analysis of the results.
6. Will be responsible for the writing of all publications pertaining to the trial.

7. May develop modifications to the design, execution and analysis of the trial, if applicable.
8. Will have ultimate responsibility for data management and quality assurance, with the help of the staff of the Data Coordinating Center
9. Will receive reports from the DSMB.
10. Will report all safety concerns encountered to the Steering Committee and the Data and Safety Monitoring Board.
11. Will approve all ancillary research and/or sub-group analyses involving study participants proposed by principal investigators or by outsider researchers.

It is expected that one member of the Executive Committee will present a study progress report at all official meetings of the PALISI Network, which holds 2 meetings per year.

The Executive Committee may wish to delegate some responsibilities to other subcommittees such as a writing committee, a quality assurance committee, etc. The Executive Committee will convene regularly (on Zoom) according to a pre-defined schedule and according to the need to discuss any significant issues that may arise. A quorum will require at least three members.

Steering Committee

The voting members of the Steering Committee will consist of the members of the Executive Committee, and each of the site primary investigators from the participating sites. Non-voting members of the Steering Committee will make use of expertise in the fields of pediatric critical care medicine, transfusion medicine, data management, statistics, and finances/administration.

The Steering Committee will convene at least once a year once the trial has begun.

The Steering Committee will have the overall responsibility for the design, execution, analysis and publication of results of the ECSTATIC Trial including but not limited to:

1. implementation of all major policy changes made by the Executive Committee
2. reporting all potential safety problems encountered by the Executive Committee
3. review of accrual patterns
4. reporting randomization and data collection procedures problems as required
5. discussion of all other concerns of any member of the Committee.

Writing Committee

The Writing Committee will reflect trial participation, preserving the writing/authorship roles of the primary study leaders, if they so desire. Broad distribution of authorship among Study Investigators is encouraged. The Writing Committee will be chaired by the study primary investigators. All other principal investigators and co-investigators may be members of the Writing Committee, as determined by the Executive Committee.

The Writing Committee is responsible for the following:

- Review of proposed abstracts, conference presentations and publications, and any discussion necessary to clarify issues concerned with the preparation and publication of manuscripts, such as overlap and data availability.

- Forwarding the detailed plan for the proposed publication to the Executive Committee for review (to ensure accurate representation of trial results and adherence to trial policies) and final approval within 4 weeks.
- All publications must be approved by the Scientific Committee of BloodNet as well as the PALISI Network before it is submitted to a scientific journal; they must also be published on behalf of BloodNet and the PALISI Network.
- Ensure that publication, authorship criteria and rules with respect to conflict of interest are adhered to by the authors.
- Proper acknowledgment of the funding agency is required in all publications
- Periodically provide a listing of the status of submitted manuscripts to the Executive Committee.

The Writing Committee will convene by teleconference as necessary to address and prepare proposed publications.

Data Coordinating Center

The Data Coordinating Center (DCC) in the Department of Pediatrics at the University of Utah School of Medicine will provide data coordination and management services. Anchoring these services is a state-of-the-art, energy efficient data center. The DCC currently manages over 125 terabytes (TB) of data and supports more than 3500 users around the country and provides a secure, reliable, enterprise-wide infrastructure for delivering mission critical DCC systems and services.

The DCC employs the following utilities and practices to ensure the safety and security of data stored at the facility.

- The DCC's electrical power system contains an uninterruptible power supply (UPS) with diesel backup generator.
- The DCC is protected with an FM-200 backed fire suppression system.
- Incremental backups of data occur Monday through Friday. A full data backup occurs weekly. Full backups are sent off-site on a weekly basis to a secure secondary location.
- On premise security guards monitor and enforce access control 24 hours a day, 7 days a week, 365 days a year.
- The DCC and external building access points are monitored with video surveillance and entry is restricted by card access and layered security measures and controls.
- Direct access to data center machines is only available while physically on premise or via a VPN client.
- All network traffic is monitored for intrusion attempts.
- All DCC personnel have completed Human Subjects Protection and Health Information Portability and Accountability Act (HIPAA) education.
- The DCC requires all users to sign specific agreements concerning security, confidentiality, and use of our information systems before access is provided.

Data management efforts extend beyond collecting and securely storing data. To promote the collection of valid data, the DCC uses an in-house application named Query Manager to support a variety of studies. Rules are built to identify certain types of potentially problematic data. Clinical sites are sent queries when data for participants at their site violate these rules, and these queries

are tracked until the problem is automatically resolved (e.g., by correcting aberrant data entries) or manually resolved (e.g., confirmation received from the site that the original data were correct). Critical derived variables and analyses reported in DSMB reports, abstracts or manuscripts are routinely dual-programmed or code-reviewed by a second statistician. These safeguards instill confidence that the collected data and reported results are accurate.

D. Study Timeline

The study will be conducted over three years (Table 2).

The first six months will be primarily spent on the IRB submission, database development, and site on-boarding and training.

The following two years will be spent on patient enrollment. Due to the support of the Data Coordinating Center at the University of Utah, data entry will be monitored in real-time and time for data cleaning will not be required at the end of the trial.

The second half of the third year will be spent on data analysis and manuscript preparation.

Table 2: Timeline

	Year 1				Year 2				Year 3			
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
IRB submission												
Database development												
Site onboarding												
Patient enrollment												
Data validation												
Analysis												
Manuscript preparation												

12. References

1. Extracorporeal Life Support Organization (ELSO) [Internet]. [cited 2022 Aug 1]. Available from: https://www.elso.org/
2. Barbaro RP, Odetola FO, Kidwell KM, Paden ML, Bartlett RH, Davis MM, et al. Association of Hospital-Level Volume of Extracorporeal Membrane Oxygenation Cases and Mortality. Analysis of the Extracorporeal Life Support Organization Registry. Am J Respir Crit Care Med. 2015 Apr 15;191(8):894–901.
3. Cheung PY, Sawicki G, Salas E, Etches PC, Schulz R, Radomski MW. The mechanisms of platelet dysfunction during extracorporeal membrane oxygenation in critically ill neonates. Crit Care Med. 2000 Jul;28(7):2584–90.
4. Nair P, Hoechter DJ, Buscher H, Venkatesh K, Whittam S, Joseph J, et al. Prospective Observational Study of Hemostatic Alterations During Adult Extracorporeal Membrane Oxygenation (ECMO) Using Point-of-Care Thromboelastometry and Platelet Aggregometry. J Cardiothorac Vasc Anesth. 2015 Apr;29(2):288–96.

5. Peek GJ, Firmin RK. The inflammatory and coagulative response to prolonged extracorporeal membrane oxygenation. ASAIO J Am Soc Artif Intern Organs 1992. 1999 Aug;45(4):250–63.
6. Dalton HJ, Reeder R, Garcia-Filion P, Holubkov R, Berg RA, Zuppa A, et al. Factors Associated with Bleeding and Thrombosis in Children Receiving Extracorporeal Membrane Oxygenation. Am J Respir Crit Care Med. 2017 Sep 15;196(6):762–71.
7. Nellis ME, Dalton H, Karam O. Quantifiable Bleeding in Children Supported by Extracorporeal Membrane Oxygenation and Outcome: Crit Care Med. 2019 Nov;47(11):e886–92.
8. Robinson TM, Kickler TS, Walker LK, Ness P, Bell W. Effect of extracorporeal membrane oxygenation on platelets in newborns. Crit Care Med. 1993 Jul;21(7):1029–34.
9. Cashen K, Dalton H, Reeder RW, Saini A, Zuppa AF, Shanley TP, et al. Platelet Transfusion Practice and Related Outcomes in Pediatric Extracorporeal Membrane Oxygenation. Pediatr Crit Care Med. 2020 Feb;21(2):178–85.
10. Saini A, Hartman ME, Gage BF, Said A, Gazit AZ, Eghtesady P, et al. Incidence of Platelet Dysfunction by Thromboelastography-Platelet Mapping in Children Supported with ECMO: A Pilot Retrospective Study. Front Pediatr. 2015;3:116.
11. Hvas AM, Favaloro EJ. Platelet function testing in pediatric patients. Expert Rev Hematol. 2017 Apr 3;10(4):281–8.
12. Karam O, Goel R, Dalton H, Nellis ME. Epidemiology of Hemostatic Transfusions in Children Supported by Extracorporeal Membrane Oxygenation. Crit Care Med. 2020 Aug;48(8):e698–705.
13. Nellis ME, Saini A, Spinella PC, Davis PJ, Steiner ME, Tucci M, et al. Pediatric Plasma and Platelet Transfusions on Extracorporeal Membrane Oxygenation: A Subgroup Analysis of Two Large International Point-Prevalence Studies and the Role of Local Guidelines*. Pediatr Crit Care Med. 2020 Mar;21(3):267–75.
14. Bochsen L, Johansson PI, Kristensen AT, Daugaard G, Ostrowski SR. The influence of platelets, plasma and red blood cells on functional haemostatic assays: Blood Coagul Fibrinolysis. 2011 Apr;22(3):167–75.
15. ELSO Anticoagulation Guidelines 2014 [Internet]. [cited 2020 May 4]. Available from: https://www.elso.org/Portals/0/Files/elsoanticoagulationguideline8-2014-table-contents.pdf
16. Fung MK, Eder A, Spitalnik SL, Westhoff CM, American Association of Blood Banks. Technical manual. 2017.
17. Fong KM, Au SY, Ng GWY, Leung AKH. Bleeding, thrombosis and transfusion in patients on ECMO: A retrospective study in a tertiary center in Hong Kong. Int J Artif Organs. 2020 Oct 17;039139882096558.
18. Northrop MS, Sidonio RF, Phillips SE, Smith AH, Daphne HC, Pietsch JB, et al. The Use of an Extracorporeal Membrane Oxygenation Anticoagulation Laboratory Protocol Is Associated With Decreased Blood Product Use, Decreased Hemorrhagic Complications, and Increased Circuit Life*: Pediatr Crit Care Med. 2015 Jan;16(1):66–74.
19. Muszynski JA, Reeder RW, Hall MW, Berg RA, Shanley TP, Newth CJL, et al. RBC Transfusion Practice in Pediatric Extracorporeal Membrane Oxygenation Support: Crit Care Med. 2018 Jun;46(6):e552–9.
20. Nellis ME, Karam O, Mauer E, Cushing MM, Davis PJ, Steiner ME, et al. Platelet Transfusion Practices in Critically Ill Children. Crit Care Med. 2018 Aug;46(8):1309–17.
21. Curley A, Stanworth SJ, Willoughby K, Fustolo-Gunnink SF, Venkatesh V, Hudson C, et al. Randomized Trial of Platelet-Transfusion Thresholds in Neonates. N Engl J Med. 2019 Jan 17;380(3):242–51.

22. Esper SA, Wallisch WJ, Ryan J, Sanchez P, Sciortino C, Murray H, et al. Platelet transfusion is associated with 90-day and 1-year mortality for adult patients requiring veno-arterial extracorporeal membrane oxygenation. Vox Sang. 2021 Apr;116(4):440–50.
23. Goerge T, Ho-Tin-Noe B, Carbo C, Benarafa C, Remold-O'Donnell E, Zhao BQ, et al. Inflammation induces hemorrhage in thrombocytopenia. 2008;111(10):7.
24. Ho-Tin-Noé B, Boulaftali Y, Camerer E. Platelets and vascular integrity: how platelets prevent bleeding in inflammation. Blood. 2018 Jan 18;131(3):277–88.
25. Aiolfi R, Sitia G. Chronic hepatitis B: role of anti-platelet therapy in inflammation control. Cell Mol Immunol. 2015 May;12(3):264–8.
26. Sonego G, Abonnenc M, Tissot JD, Prudent M, Lion N. Redox Proteomics and Platelet Activation: Understanding the Redox Proteome to Improve Platelet Quality for Transfusion. Int J Mol Sci. 2017 Feb 11;18(2):387.
27. Saito S, Nollet KE, Ngoma AM, Ono T, Ohto H. Platelet-, leucocyte- and red cell-derived microparticles in stored whole blood, with and without leucofiltration, with and without ionising radiation. Blood Transfus [Internet]. 2018 Mar 1 [cited 2020 Sep 4]; Available from: http://doi.org/10.2450/2016.0108-16
28. Ambrosio AL, Di Pietro SM. Storage pool diseases illuminate platelet dense granule biogenesis. Platelets. 2017 Feb 17;28(2):138–46.
29. MacLaren G, Monagle P. Platelet Transfusion During Extracorporeal Membrane Oxygenation: Possible Harm, Ongoing Uncertainty*. Pediatr Crit Care Med. 2020 Feb;21(2):208–9.
30. Biagas KV, Muratore CS. Plasma and Platelet Utilization in Children on Extracorporeal Membrane Oxygenation: A Call for Targeted Anticoagulation*. Pediatr Crit Care Med. 2020 Mar;21(3):296–7.
31. Ming Y, Liu J, Zhang F, Chen C, Zhou L, Du L, et al. Transfusion of Red Blood Cells, Fresh Frozen Plasma, or Platelets Is Associated With Mortality and Infection After Cardiac Surgery in a Dose-Dependent Manner: Anesth Analg. 2020 Feb;130(2):488–97.
32. Josephson CD, Granger S, Assmann SF, Castillejo MI, Strauss RG, Slichter SJ, et al. Bleeding risks are higher in children versus adults given prophylactic platelet transfusions for treatment-induced hypoproliferative thrombocytopenia. Blood. 2012 Jul 26;120(4):748–60.
33. Lacroix J, Hébert PC, Hutchison JS, Hume HA, Tucci M, Ducruet T, et al. Transfusion strategies for patients in pediatric intensive care units. N Engl J Med. 2007 Apr 19;356(16):1609–19.
34. Nellis ME, Tucci M, Lacroix J, Spinella PC, Haque KD, Stock A, et al. Bleeding Assessment Scale in Critically Ill Children (BASIC): Physician-Driven Diagnostic Criteria for Bleeding Severity. Crit Care Med. 2019 Dec;47(12):1766–72.
35. Pal S, Curley A, Stanworth SJ. Interpretation of clotting tests in the neonate. Arch Dis Child - Fetal Neonatal Ed. 2015 May;100(3):F270–4.
36. van Stuijvenberg M, Suur MH, de Vos S, Tjiang GCH, Steyerberg EW, Derksen-Lubsen G, et al. Informed consent, parental awareness, and reasons for participating in a randomised controlled study. Arch Dis Child. 1998 Aug 1;79(2):120–5.
37. Tait AR, Voepel-Lewis T, Siewert M, Malviya S. Factors that influence parents' decisions to consent to their child's participation in clinical anesthesia research. Anesth Analg. 1998 Jan;86(1):50–3.
38. Murtha TD, Faustino EVS. Is "Informed Consent" Truly Informed?*. Pediatr Crit Care Med. 2020 Jun;21(6):589–90.
39. Gertsman S, O'Hearn K, Gibson J, Menon K. Parental Understanding of Research Consent Forms in the PICU: A Pilot Study*. Pediatr Crit Care Med. 2020 Jun;21(6):526–34.

40. FDA Standards for Human Blood and Blood Products - Platelets [Internet]. Electronic Code of Federal Regulations. Available from: https://www.ecfr.gov/cgi-bin/text-idx?SID=5d10701242f6aa41e680efeaef1d712c&mc=true&node=sp21.7.640.c&rgn=div6
41. Extracorporeal Life Support Organization (ELSO). Guidelines for Neonatal Respiratory Failure [Internet]. 2017. Available from: https://www.elso.org/Portals/0/ELSOGuidelinesNeonatalRespiratoryFailurev1_4_1.pdf
42. Bembea MM, Schwartz JM, Shah N, Colantuoni E, Lehmann CU, Kickler T, et al. Anticoagulation Monitoring during Pediatric Extracorporeal Membrane Oxygenation. ASAIO J. 2013 Jan;59(1):63–8.
43. Barbaro RP, Boonstra PS, Paden ML, Roberts LA, Annich GM, Bartlett RH, et al. Development and validation of the pediatric risk estimate score for children using extracorporeal respiratory support (Ped-RESCUERS). Intensive Care Med. 2016 May;42(5):879–88.
44. Şık G, Demirbuğa A, Annayev A, Temur B, Aydın S, Demir Hİ, et al. Clinical and laboratory predictors of survival for pediatric patients on non-postcardiotomy extracorporeal membrane oxygenation (ecmo). Turk J Pediatr. 2020;62(1):24.
45. Bokman CL, Tashiro J, Perez EA, Lasko DS, Sola JE. Determinants of survival and resource utilization for pediatric extracorporeal membrane oxygenation in the United States 1997–2009. J Pediatr Surg. 2015 May;50(5):809–14.
46. Pollack MM, Patel KM, Ruttimann UE. PRISM III: An updated Pediatric Risk of Mortality score. Crit Care Med. 1996 May;24(5):743–52.
47. Slater A, Shann F, Pearson G, for the PIM Study Group. PIM2: a revised version of the Paediatric Index of Mortality. Intensive Care Med. 2003 Feb;29(2):278–85.
48. Spinella PC, El Kassar N, Cap AP, Kindzelski AL, Almond CS, Barkun A, et al. Recommended primary outcomes for clinical trials evaluating hemostatic blood products and agents in patients with bleeding: Proceedings of a National Heart Lung and Blood Institute and US Department of Defense Consensus Conference. J Trauma Acute Care Surg. 2021 Aug 1;91(2S Suppl 2):S19–25.
49. Akin S, Caliskan K, Soliman O, Muslem R, Guven G, van Thiel RJ, et al. A novel mortality risk score predicting intensive care mortality in cardiogenic shock patients treated with veno-arterial extracorporeal membrane oxygenation. J Crit Care. 2020 Feb;55:35–41.
50. Muller G, Flecher E, Lebreton G, Luyt CE, Trouillet JL, Bréchot N, et al. The ENCOURAGE mortality risk score and analysis of long-term outcomes after VA-ECMO for acute myocardial infarction with cardiogenic shock. Intensive Care Med. 2016 Mar;42(3):370–8.
51. ELSO Database Definitions [Internet]. [cited 2020 Apr 27]. Available from: https://www.elso.org/Portals/0/Files/PDF/ELSO%20Database%20Definitions%202018-2-1.pdf
52. Karam O, Nellis ME, Zantek ND, Lacroix J, Faustino EVS, BloodNet and the Pediatric Acute Lung Injury and Sepsis Investigators (PALISI) Network. Criteria for Clinically Relevant Bleeding in Critically Ill Children: An International Survey. Pediatr Crit Care Med J Soc Crit Care Med World Fed Pediatr Intensive Crit Care Soc. 2019;20(3):e137–44.
53. Fiser DH. Assessing the outcome of pediatric intensive care. J Pediatr. 1992 Jul;121(1):68–74.
54. Pollack MM, Holubkov R, Funai T, Clark A, Moler F, Shanley T, et al. Relationship Between the Functional Status Scale and the Pediatric Overall Performance Category and Pediatric Cerebral Performance Category Scales. JAMA Pediatr. 2014 Jul 1;168(7):671.
55. Hodgson CL, Burrell AJC, Engeler DM, Pellegrino VA, Brodie D, Fan E. Core Outcome Measures for Research in Critically Ill Patients Receiving Extracorporeal Membrane Oxygenation for Acute Respiratory or Cardiac Failure: An International, Multidisciplinary, Modified Delphi Consensus Study*. Crit Care Med. 2019 Nov;47(11):1557–63.

56. Faes C, Molenberghs G, Aerts M, Verbeke G, Kenward MG. The Effective Sample Size and an Alternative Small-Sample Degrees-of-Freedom Method. Am Stat. 2009 Nov;63(4):389–99.
57. Chan AW, Tetzlaff JM, Gotzsche PC, Altman DG, Mann H, Berlin JA, et al. SPIRIT 2013 explanation and elaboration: guidance for protocols of clinical trials. BMJ. 2013 Jan 9;346(jan08 15):e7586–e7586.
58. Loudon K, Treweek S, Sullivan F, Donnan P, Thorpe KE, Zwarenstein M. The PRECIS-2 tool: designing trials that are fit for purpose. BMJ. 2015 May 8;350(may08 1):h2147–h2147.

13. Appendices

A. Proposal for future large RCT

Aims

The primary research aim for the future RCT will be to demonstrate the non-inferiority of a lower platelet threshold transfusion strategy, as compared to a higher platelet threshold strategy, in non-bleeding children on ECMO.

Anticipated Cohort

Our aim is to enroll non-bleeding or minimally bleeding children on ECMO, at risk of receiving platelet transfusions.

Design

This will be a multicenter, non-inferiority, randomized, controlled, investigator-initiated, open-label, trial with blinded adjudication of the outcomes (Figure 10). We will take into account the recommendations of SPIRIT to elaborate our final version of the protocol for the full ECSTATIC RCT (57). We will use the PRECIS-2 tool to estimate the pragmatic or explanatory nature of the trial (58).

Site Eligibility

PICUs will be considered eligible to participate in the full ECSTATIC RCT only if there is an ECMO program in the site.

Site Inclusion Criteria: Pediatric and neonatal ICUs, including cardiac PICUs, with at least a mean of 5 ECMO runs per year.

Site Exclusion Criteria: PICUs will be excluded if 1) a consensus from all physicians working in the PICU to apply the research protocol is not reached, and/or 2) if there is no local scientific infrastructure that can support the participation in a large multicenter RCT.

Patient Eligibility

Children will be eligible if admitted to a participating tertiary care PICU, NICU or CICU for whom the decision to cannulate for ECMO support has been made.

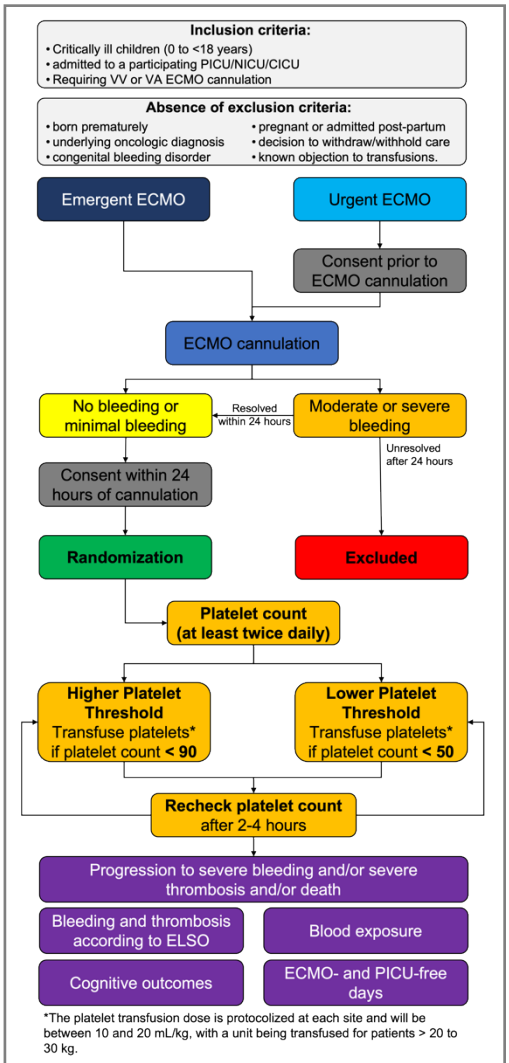


Figure 10: Overall proposal of the future RCT

Inclusion Criteria: Critically ill children (0 to <18 years of age), admitted to a participating PICU/NICU/CICU, on **ECMO for less than 24 hours**, and who have either **no bleeding or minimal bleeding**. If a patient experiences **more than minimal bleeding** after ECMO

cannulation, they can be enrolled once bleeding meets criteria for no/minimal bleeding, with the condition that the moderate or severe bleeding resolves within the first 24 hours of ECMO initiation.

Exclusion Criteria

To ensure our trial is as **pragmatic** as possible, we have as few exclusion criteria as possible:

1) post conception age < 37 weeks at time of screening
2) underlying oncologic diagnosis (defined as receipt of chemotherapy or radiation in the last six months) or recipient of bone marrow transplant in the last year
3) congenital bleeding disorder or congenital thrombocytopenia
4) pregnant or admitted post-partum (within 6 weeks of giving birth)
5) decision to withdraw or withhold some critical care or interventions
6) known objection to blood transfusions
7) on ECMO for > 24 hours at time of enrollment

Inclusion criteria and exclusion criteria will be ascertained by pediatric critical care clinical or research staff.

Intervention

Subjects will be randomized in a **1:1 ratio** to either a lower platelet threshold or a higher platelet threshold prophylactic transfusion strategy.

Subjects in the **lower platelet threshold** arm can be transfused only if their platelet count is below 50 $x10^9$ cell/L, whereas subjects in the **higher platelet threshold** arm should be transfused if their platelet count is below 90 $x10^9$ cell/L.

The transfusion **dose** is intended to be 10-20 ml/kg but with some allowance based on local policies as long as dosing is not differentially applied by treatment arm. For patients > 30 kg, the dose will be one unit.

This intervention will be maintained in effect until five possible events that will signal permanent discontinuation, whichever occurs first: 1) death, or progression to moderate or severe bleeding or severe thrombosis, as diagnosed by the attending physician (i.e., primary safety outcome); 2) decannulation from ECMO; 3) 21 days elapse post-randomization, 4) temporary suspension lasting more than 24 hours, or 5) study withdrawal.

Co-interventions

As this is an **open-label** trial, we need to ensure co-interventions that may be associated to the primary clinical outcome are managed identically in both arms, to avoid potential interactions and biases. However, as the **pragmatic design** is important for the **generalizability of the future results**, we will be **imposing the adherence to the Pediatric ECMO Anti-Coagulation CollaborativE (PEACE) recommendations**. Of note, none of the centers have anticoagulation protocols that are dependent on the platelet count. Data on co-interventions (plasma transfusion strategy, anticoagulation strategy, cryoprecipitate or fibrinogen concentrate, antithrombin concentrate, and priming of circuit) will be collected daily and compliance with the guidelines will be measured, so that the effect of any imbalances on the primary outcome can be examined after the trial is complete. **Other co-interventions**, such as ECMO flow management, vasopressors, ventilation, red blood cell transfusion, sedation, and the use of hypothermia, will **not be protocolized**, due to the pragmatic nature of the trial. In addition, the use of other hemostatic agents, such as tranexamic acid, aminocaproic acid, DDAVP, and von Willebrand factor will not be protocolized as these are typically used in patients with active bleeding and not to prevent bleeding. However, all these co-interventions will be recorded.

Randomization

Allocation will be a **1:1 ratio**. Only the study statistician and designate at the DCC will have knowledge of the randomization codes. Subjects will be **stratified by site** (as there might be a site effect, due to the co-interventions mentioned above) **and by age** (≤28 days vs >28 days), which is of principal interest to keep balanced between arms. Patients will not be stratified based on the severity of the disease or indication (medical vs post-surgical), as this would significantly increase the sample size. Moreover, in order to **conceal randomization**, 3 sizes of **blocks permutation** will be used on a random sequence: 2, 4 and 6 patients per block.

Concealment of randomization

Research personnel at each site and PICU caregivers will not have access to the randomization schedule.

Blinding

This is an open-label trial, since the **transfusion strategy cannot be blinded** from the clinical team, as they must order the transfusions based on the platelet count. The research team, including the adjudicators, will be blinded to treatment allocation, until the end of the analysis.

Temporary suspension

The protocol will allow for temporary suspensions, i.e., transfusion at a higher platelet count threshold than allowed for the randomization arm, if the clinical situation warrants immediate transfusion for certain conditions including: 1) chest-tube insertion; 2) surgical intervention while on ECMO (such as tracheostomy or repair of congenital diaphragmatic hernia); 3) during ECMO circuit change; or 4) in preparation for decannulation. Adherence to the trial protocol must be **resumed as soon as possible** once these events are controlled or completed. Data monitoring and collection will be unchanged during suspension, but data on length and justification of suspension will also be collected. Suspensions less than 24 hours will not be considered a breach of adherence to the protocol. After 24 hours of temporary suspension, the patient will have all assigned treatment strategy implications lifted (i.e., no restrictions on the range at which any subsequent platelet transfusions may occur) but will continue to be followed for ascertainment of safety and study outcomes. Suspended patients will be included in the intention-to-treat analysis.

Primary Outcome

The primary outcome will be the proportion of patients who progress from no bleeding or minimal bleeding to severe bleeding and/or severe thrombosis, ignoring events that begin during a period of temporary suspension.

Using the BASIC definition plus one additional element, **severe bleeding** will be defined as:

1) Bleeding that leads to at least one organ dysfunction, using PELOD-2 score criteria of organ dysfunction
2) Bleeding that leads to hemodynamic instability, defined as an increase in HR by > 20% from baseline or a decrease in BP by > 20% from baseline (i.e., prior to the bleeding event)
3) Bleeding leading to a drop in hemoglobin > 20% within 24hr
4) Quantifiable bleeding ≥ 5 mL/kg/hr for ≥ 1hr (e.g., chest tube)
5) New and/or expanding intracranial hemorrhage on CT scan or head ultrasound
6) Intraspinal bleeding leading to loss of neurologic function below the lesion, nontraumatic intra-articular bleeding leading to decreased range of movement, or intraocular bleeding leading to impaired vision.

Severe thrombotic events will be defined according to the NHLBI Consensus Conference as circuit clot leading to circuit change, need for thrombectomy, ischemic stroke, or distal thromboembolism.

As this is an open-label trial, the primary outcome will be **adjudicated** by an independent central committee, blinded to the allocation group. This committee, composed of three members, will determine if the bleeding was the primary cause of organ dysfunction, hemodynamic instability, acute anemia, loss of spinal function, articular range of motion, or impaired vision.

Secondary Outcomes

The secondary outcomes will be:

1) hemorrhagic complications according to the ELSO definition
2) thrombotic complications according to the ELSO definition
3) blood product exposure, defined as the total number of transfusions (red blood cell, plasma, platelets, cryoprecipitate)
4) duration of ECMO, defined as 90-day ECMO-free days
5) length of stay, defined as the 90-day PICU-free days
6) functional outcome at hospital discharge, defined as the discharge Pediatric Overall Performance Category (POPC) and Pediatric Cerebral Performance Category (PCPC)
7) all-cause mortality on ECMO, at 28 days, and at 90 days.

Sample Size

Preliminary data from the BATE study suggests about 65% of the non-bleeding patients will experience severe bleeding, severe clotting, and/or death (6). Each ml/kg of platelet transfusion was independently associated with 1% increased odds of bleeding and 5% increased odds of mortality. Considering the median dose is 92 ml/kg per ECMO run and a low platelet threshold transfusion strategy is expected to decrease blood exposure by at least 20%, the effect of the low platelet threshold strategy may be a 17% relative decrease in the odds of bleeding [(1/1.01) odds ratio for each 1 ml/kg reduction and median reduction of 20% x 92 ml/kg), i.e., an absolute risk reduction in percentages from 65% to approximately 60.7%. These results, which are extrapolated from observational data where no transfusion strategies were enforced, **need to be confirmed in the pilot trial**.

Non-inferiority margins of safety of 5% and 10% (33) have previously been used in transfusion medicine. With a margin of 5% and based on these very uncertain data, if there is a true relative difference in favor of the low platelet threshold strategy of 6.6% (i.e., 65% vs 60.7% progression to severe bleeding and/or severe clotting), and we anticipate a 5% drop-out rate (similar to what was observed in previous studies), using an alpha of 0.025 and a 90% power, **the sample size to enroll for the test of non-inferiority would be 1238 patients (619 per arm)**, The primary outcome would be ascertained for an expected 588 per arm. We will calculate the 95% confidence intervals (95% CI) around the absolute risk reduction in the proportion of patients progressing to severe bleeding and conclude non-inferiority if the upper limit of the 95% CI is lower than 5%. For example, if the proportion of patients who progress to the primary outcome is 376/588 (64%) in the higher platelet threshold group and 359/588 (61%) in the lower platelet threshold group, the absolute risk reduction would be 3%, but the 95% CI would be -9% to 3%. In this example, although superiority would not have been demonstrated (p=0.34), we could conclude to a non-

inferiority as the upper limit of the absolute risk reductions (+3%) is below the +5% pre-defined limit of the margin of safety.

If non-inferiority were not demonstrated, experts believe such trials still provide important information, since we will be able to report multiple secondary and tertiary outcomes, such as blood product exposure and lengths of ECMO or stay. Such results would still be impactful, as a potential decrease in blood product exposure or in lengths of care would be clinically meaningful.

In terms of feasibility, enrolling 1238 patients in 30 centers who would each enroll 10 subjects by year, would require just over 4 years.

Statistical Analyses

The analysis of the primary outcome measure will be conducted on an **intention to treat** (ITT) basis for all patients randomized in ECSTATIC. The principal analysis, i.e., the influence of platelet transfusion strategy ("higher platelet threshold" vs. "lower platelet threshold") on the primary outcome (progression to severe bleeding and/or severe clotting, ignoring such events that begin during a period of temporary suspension), will be done using an unadjusted z-test (with continuity correction) of non-inferiority, comparing the proportion of patients who progress to the primary outcome after randomization in both groups. The principal effect measure will be an unadjusted absolute risk reduction (ARR) with a 95% CI for the primary comparison.

Secondary analyses of the primary composite outcome will include logistic regression modeling to further elucidate the measure of effect while adjusting for known prognostic risk factors. For adjusted models, risk factors such as site, age, co-morbid illnesses, type of ECMO, indication to ECMO, and severity of illness scores will be added based on clinical (not statistical) rationale. Continuous risk factors (e.g., PELOD-2, number of transfusions) will be entered into the models as a continuous measure to improve statistical efficiency. Regression diagnostics will be performed on all models. We will also compare Kaplan-Meier curves using a log rank test followed by proportional hazards modeling for liberation from ECMO alive, 28- and 90-day mortality, and time to the composite outcome: these analyses will compare the length of time between randomization and appearance of those outcomes.

We will also do a **per-protocol** analysis using an unadjusted absolute risk reduction with 95% CI on the effect of "higher platelet threshold" vs. "lower platelet threshold" transfusion strategies, but such per-protocol analysis will be done only for the primary outcome. We will perform the following **pre-specified subgroup analyses**: types of ECMO (V-V vs V-A), indication to ECMO (medical vs surgical), age (< 7 days, 7 to 28 days, 29 to 365 days, > 365 days), location of the patients (NICU, PICU, CICU), and indication for ECMO (cardiac, respiratory, and ECMO cardiopulmonary resuscitation).

We will also perform some sensitivity analysis pertaining to the primary outcome. For example, we will repeat statistical analyses, adding lost to follow-up patients, using best-case and worst-case scenario.

B. Bleeding Assessment Scale in Critically Ill Children (BASIC) Score

Any of the following criteria define **severe bleeding**:

- Bleeding that leads to at least one organ dysfunction, using PELOD-2 score criteria, within 24 hours of the previous assessment (if there is no previous assessment, the results are presumed to be normal). The organ dysfunction should be associated with the bleeding, in absence of other causes.
- Bleeding that leads to hemodynamic instability, defined as an increase in heart rate (HR) by > 20% from baseline or a decrease in blood pressure (BP) by > 20% from baseline (i.e., prior to bleeding event). The hemodynamic instability should be associated with the bleeding, in absence of other causes.
- Bleeding leading to a drop in Hb >20% within 24 hours. The drop in Hb should be associated with the bleeding, in absence of other causes.
- Quantifiable bleeding ≥ 5 ml/kg/hr for ≥ 1 hour (eg. chest tube, drain).
- Intraspinal bleeding leading to loss of neurologic function below the lesion, non-traumatic intra-articular bleeding leading to decreased range of movement, or intraocular bleeding leading to impaired vision.
- New and/or expanding intracranial hemorrhage (not in the original BASIC definition, but added as explained earlier)

All of the following criteria must be present to define **moderate bleeding**:

- Bleeding more than minimal bleeding but without criteria for severe bleeding.
- Bleeding not leading to organ dysfunction, as measured by the PELOD-2 score.
- Bleeding not leading to hemodynamic instability, i.e. change in HR > 20% or BP < 20% from baseline (i.e., prior to bleeding event).
- Bleeding leading to a drop in Hb ≤ 20%.
- Quantifiable bleeding ≥ 1 ml/kg/hr but < 5 ml/kg/hr for ≥ 1 hour (e.g. chest tube, drain).

Any of the following criteria define **minimal bleeding**:

- Streaks of blood in endotracheal tube (ETT) or during suctioning only.
- Streaks of blood in nasogastric (NG) tube.
- Macroscopic hematuria, or less than or equal to 1+ RBCs present on urine dipstick if available.
- Subcutaneous bleeding (including hematoma and petechiae) < 5 cm (2 in) in diameter.
- Quantifiable bleeding < 1 ml/kg/hr (e.g. chest tube, drain). Since the objective is to capture bleeding, chest tube output can be higher than 1 mL/kg/hr provided the output is serosanguinous and the site investigator judges the bleeding portion of the chest tube output is < 1mL/kg/hr.
- Bloody dressings required to be changed not more often than each 6 hours, or weighing no more than 1mL/kg/hr if weighed, due to slow saturation.

Progressive bleeding is bleeding that either progresses to a higher severity category (e.g. from minimal to moderate bleeding, or from moderate to severe bleeding) or to a higher number of criteria within the same category (e.g. hemodynamic instability progressing to organ failure, or streaks of blood in the ETT and subsequent slightly blood-tinged urine).

Fatal Bleeding is bleeding that is the direct cause leading to death.

C. NHLBI Definition of Severe Thrombotic Complications

Circuit clot leading to circuit change
Need for thrombectomy
Ischemic stroke
Distal thromboembolism

D. PCPC and POPC scores

Pediatric Cerebral Performance Category (PCPC) Scale (53)		
Score	**Category**	**Description**
1	**Normal**	At age-appropriate level; school-age child attends regular school
2	**Mild disability**	Conscious, alert, able to interact at age-appropriate level; regular school, but grades perhaps not age-appropriate, possibility of mild neurologic deficit
3	**Moderate disability**	Conscious, age-appropriate independent activities of daily life; special education classroom and/or learning deficit present
4	**Severe disability**	Conscious, dependent in others for daily support because of impaired brain function
5	**Coma or vegetative state**	Any degree of coma, unaware, even if awake in appearance, without interaction with the environment; no evidence of cortex function; possibility for some reflexive response, spontaneous eye-opening, sleep-wake cycles
6	**Brain death/death**	Brain death, death

Pediatric Overall Performance Category (POPC) Scale (53)		
Score	**Category**	**Description**
1	**Good overall performance**	PCPC 1; healthy, alert, and capable of normal activities of daily life
2	**Mild overall disability**	PCPC 2; possibility of minor physical problem that is still compatible with normal life
3	**Moderate overall disability**	PCPC 3; possibility of moderate disability from non cerebral systems dysfunction alone or with cerebral dysfunction; performs independent activities of daily life but disabled for competitive performance at school
4	**Severe overall disability**	PCPC 4; possibility of severe disability from non cerebral systems dysfunction alone or with cerebral dysfunction; conscious but dependent on others for activities of daily living support
5	**Coma or vegetative state**	Any degree of coma, unaware, even if awake in appearance, without interaction with the environment; no evidence of cortex function; possibility for some reflexive response, spontaneous eye-opening, sleep-wake cycles
6	**Brain death/death**	Brain death, death

E. Worksheet for Site Assessment of Severe Bleeding/Clotting Events

Severe Bleeding and/or Severe Clotting Evaluation Form

Severe clotting:

Description of the clotting event:

_____

_____

Circuit clot leading to circuit change:	❍yes	❍no
Clot that required thrombectomy:	❍yes	❍no
Ischemic stroke:	❍yes	❍no
Clot that caused distal thromboembolism:	❍yes	❍no

Severe bleeding:

Description of the bleeding event:

_____

_____

1) Bleeding that leads to at least one **organ dysfunction**, using PELOD-2 score criteria of organ dysfunction

- Previous day's PELOD-2 score: ___ (bit.ly/pelod2)
- Bleeding day's PELOD-2 score: ___
- Change in PELOD-2 score (Bleeding-Previous): ___
- Change in PELOD-2 score associated with the bleeding, **in absence of other causes** (e.g. natural progression of the underlying condition, suboptimal ECMO support, etc.): ❍yes ❍no
- **Was there a bleeding event that led to organ dysfunction? ❍yes ❍no**

2) Bleeding that leads to **hemodynamic instability**, defined as an increase in heart rate by > 20% from baseline or a decrease in mean arterial pressure by > 20% from baseline (i.e., prior to bleeding event)

- Baseline heart rate: ___/min
- Heart rate during bleeding event: ___/min
- Change in heart rate (100% x (bleeding-baseline)/baseline): ___%
- Change in heart rate associated with the bleeding, in absence of other causes (e.g. medication, CRRT, etc.): ❍yes ❍no
- Baseline mean arterial pressure: ___
- Mean arterial pressure during bleeding event: ___
- Change in mean arterial pressure (bleeding-baseline/baseline): ___%

- Change in mean arterial pressure associated with the bleeding, **in absence of other causes** (e.g. medication, CRRT, etc.): ❍yes ❍no
- **Was there a bleeding event that led to hemodynamic instability? ❍yes ❍no**

3) Bleeding leading to a drop in **hemoglobin** > 20% within 24 hr

- Baseline hemoglobin: ___
- Hemoglobin during bleeding event: ___
- Change in hemoglobin (100% x (bleeding-baseline)/baseline): ___%
- Change in hemoglobin associated with the bleeding, **in absence of other causes** (e.g. hemodilution due to boluses of saline, fluid overload, etc.): ❍yes ❍no
- **Was there a bleeding event that led to a drop in hemoglobin by > 20%? ❍yes ❍no**

4) Quantifiable bleeding **≥ 5 mL/kg/hr for ≥ 1hr** (e.g., chest tube)

- Quantifiable bleeding: ___ mL/kg/hr
- Quantifiable volume from chest-tube or drain believed to be bleeding (i.e. not serosanguinous): ❍yes ❍no
- **Was there a quantifiable bleeding ≥ 5 mL/kg/hr for ≥ 1hr? ❍yes ❍no**

5) **Intraspinal** bleeding leading to loss of neurologic function below the lesion, nontraumatic **intra-articular** bleeding leading to decreased range of movement, or **intraocular** bleeding leading to impaired vision

- Intraspinal bleeding leading to loss of neurologic function below the lesion: ❍yes ❍no
- Intra-articular bleeding leading to decreased range of movement: ❍yes ❍no
- Intraocular bleeding leading to impaired vision: ❍yes ❍no
- **Was there a bleeding event that led to intraspinal, intra-articular, or intraocular bleeding? ❍yes ❍no**

6) New and/or expanding intracranial hemorrhage as evidenced by CT, head ultrasound or MRI

❍yes ❍no

Epidural	❍yes	❍no
Subdural	❍yes	❍no
Intraventricular	❍yes	❍no
Intraparenchymal	❍yes	❍no

F. Platelet Transfusion Strategy Adherence (Compliance)

The sixth feasibility criterion's outcome is defined for a combination of platelet-count level and platelet-transfusion-level observations. Adherence is indicated by having an appropriately dosed platelet transfusion within 12 hours after a below-threshold platelet count, and by not having any platelet transfusion when there is no below-threshold platelet count. In general, there is one adherence/non-adherence determination per platelet count (although some are excluded, and some additional determinations can apply when a transfusion occurs without any platelet count in the previous 12 hours).

		Platelet Transfusion		
		Yes	**No**	**Excluded transfusion(s) was the only transfusion within 12 hours after platelet count[a]**
Platelet count below threshold	**Yes**	Adherence[b,c]	Non-adherence[c]	*Adherence*
	No	Non-adherence[b]	Adherence	*ignored*
	Excluded platelet count[d]	Non-adherence[b]	*ignored*	*Ignored*

[a] Platelet transfusions are excluded if any of the following apply: transfusion start time before randomization or after the time the strategy was permanently terminated; transfusion start time during a period of temporary suspension

[b] The transfusion is considered non-adherent if an included (i.e., not excluded) platelet transfusion did not have a below-threshold platelet count as the most recent platelet count, or if there was an absence of a platelet count within 12 hours prior to the transfusion start time, or the most-recent platelet count was at or above the threshold.

[c] The platelet count is considered non-adherent if an included (i.e., not excluded) platelet count was below the threshold and there was no platelet transfusion (not known to be at an inappropriate dose) within 12 hours after the platelet count.

[d] Platelet counts are excluded if any of the following apply: was more than 12 hours pre-randomization; was after the time the strategy was permanently terminated; was prior to randomization, and before randomization had a transfusion and/or another pre-randomization platelet count; was a below-threshold count but within 12 hours after the blood sample was collected, and prior to a subsequent platelet transfusion, there was either a platelet count at or above the threshold or the transfusion strategy was permanently terminated; was a platelet count at or above the threshold but within 12 hours and prior to a blood transfusion, there was either a below-threshold platelet count or the transfusion strategy was permanently terminated.

Calculation: Adherence % = 100% * (Adherences)/(Adherences + Non-adherences)

Feasibility Criterion 6 Threshold: Adherence > 90%

Statistical Analysis Plan

Protocol Title: ECMO Hemostatic Transfusions in Children

Protocol Version and Date: 1.3.1; July 14, 2023 (BRANY IRB approved July 19, 2023)

SAP Authors: Bradley J. Barney, PhD (with reuse of protocol and DCC template language)

SAP Version and Date: 1.0; November 9, 2023

CONFIDENTIAL

Approvals:

Approved By:

Name	Title	Date
DocuSigned by: Oliver Kuram D5066330F4F748A...	Sutdy PI	11/10/2023
DocuSigned by: Marianne Mellis A891D9374C814C7...	Study PI	11/10/2023
DocuSigned by: Bradley J. Barney 3C3E8D23B98F4BB...	Co-I, Statistician	11/10/2023

Contents

Abbreviations

Abbreviation	Definition
AIC	Akaike Information Criterion
APPROACHED	Approached Population; see Section 4.2
BP	Blood Pressure
CAR(1)	Continuous analog of first-order AutoRegressive process
CONSENTED	Consented Population; see Section 4.2
CRF	Case Report Form
CS	Compound Symmetry
CT	Computerized Tomography
DCC	Data Coordinating Center
df	degrees of freedom
DSMB	Data and Safety Monitoring Board
ECMO	ExtraCorporeal Membrane Oxygenation
eCRF	Electronic Case Report Form
ECSTATIC	ECMO Hemostatic Transfusions in Chldren
ELIGIBLE	Eligible Population; see Section 4.2
ENROLLED	Enrolled Population; see Section 4.2
gm	gram(s)
hr	hour(s)
HR	Heart Rate
ITT	Intention-To-Treat
kg	kilogram(s)
L	liter
mL	milliliter
NHLBI	National Heart, Lung, and Blood Institute
PICU	Pediatric Intensive Care Unit
REDCap	Research Electronic Data Capture
REML	Restricted Maximum Likelihood (also known as Residual Maximum Likelihood)
(S)AE	(Serious) Adverse Event
SAFETY	Safety Population
SAP	Statistical Analysis Plan
SCREENING	Screening Population; see Section 4.2
SE	Secondary Endpoint (SE1=Secondary Endpoint #1, etc.)
V-A	Veno-Arterial
vs	versus
V-V	Veno-Venous

1 PREFACE

1.1 Purpose of SAP

This Statistical Analysis Plan (SAP) describes the planned analysis and reporting for the protocol: ECMO Hemostatic Transfusions in Children.

The structure and content of this SAP provides sufficient detail to meet the requirements and standards set by the Data Coordinating Center (DCC).

1.2 Auxiliary/Other Documents

The following documents were reviewed in preparation of this SAP:

- Protocol: ECMO Hemostatic Transfusions in Children.
- Case Report Forms (CRFs) for the ECSTATIC protocol

The reader of this SAP is encouraged to read the protocol for details on the conduct of this study, and the operational aspects of clinical assessments.

The purpose of this SAP is to outline the planned analyses to be completed for the ECSTATIC trial. The planned analyses identified in this SAP will be included in future study abstracts and manuscripts. Also, exploratory analyses not necessarily identified in this SAP may be performed. Any post hoc, or unplanned, analyses not explicitly identified in this SAP will be clearly identified as such in any published reports from this study.

It is possible that, due to updates or identification of errors in specific statistical software discussed in this SAP, the exact technical specifications for carrying out a given analysis may be modified. This is considered acceptable as long as the original, prespecified statistical analytic approach is completely followed in the revised technical specifications.

2 STUDY OBJECTIVES AND OUTCOMES

2.1 Study Objectives

2.1.1 Primary Objective(s)

The primary objectives of the ECSTATIC trial are:

- Aim 1: to demonstrate a separation between the LOWER and HIGHER platelet threshold transfusion arms. Specifically, we will demonstrate a difference in the average pre-transfusion platelet count between the two arms from randomization to when the treatment strategy was permanently discontinued. In addition, we will estimate the difference in average daily platelet dose during the same period, which we anticipate will be a difference of at least 20% between the groups randomized to each threshold.
- Aim 2: to demonstrate the ability for observed endpoints to satisfy various prespecified feasibility thresholds. Specifically, there are 10 different criteria and we will show that at least 5 of the 10 thresholds are satisfied by the estimated endpoints.
- Aim 3: to provide additional information on the epidemiology of a novel composite outcome of progression to severe bleeding and/or severe thrombosis, to incorporate in the design of the full RCT. Specifically, we will demonstrate that the estimated rate of adjudication by an independent panel is more than 90% of the enrolled subjects for whom the outcome was reported by the site as having occurred.

2.2 Study Outcomes

2.2.1 Primary Outcome(s)

The **primary outcome for Aim 1**, hereafter *primary outcome*, is the platelet count immediately (or as recently as available) before a transfusion. Note that this outcome is defined for each transfusion, and as such, participants may contribute multiple values.

Though not considered primary outcomes, there are two outcomes that are especially related to the primary outcome which are of additional interest: the total platelet transfusion dose while on ECMO in mL/kg/run, and the transfusion dose in mL/kg/hr (or its redundant value, in mL/kg/day, obtained by multiplying the dose in mL/kg/hr by 24). Refer to Section 5.5 for details of the mL/kg/run and mL/kg/hr calculations.

For safety monitoring, there is a **primary safety outcome**, (occurrence of progression to severe bleeding, severe thrombosis, or death, in the acute follow-up period more precisely defined in Section 2.2.5). To avoid confusion, any reference to the primary safety outcome contains the qualifier "safety."

2.2.2 Secondary Outcome(s)

The secondary endpoints (SEs) are: observed values of estimated separation in mean pre-transfusion platelet counts; eligibility rate relative to predicted rate; approach for consent percentage; consent percentage; randomization of consented patients percentage; transfusion compliance rate; transfusion strategy suspension percentage; withdrawal and or lost-

to-follow-up percentage; violation of local non-study-specific circulatory support protocol percentage; and adjudication rate of progression to severe bleeding/thrombotic outcomes.

Each endpoint is based on an underlying outcome and has an associated feasibility threshold. **To support a larger trial, at least five out of ten feasibility criteria must be met.** The underlying outcomes and thresholds for these 10 secondary endpoints (SE1–SE10) criteria are defined in the next paragraphs.

(SE1) Estimated separation in pre-transfusion platelet counts

- Outcome: Uses the primary outcome, pre-transfusion platelet count, defined in Section 2.2.1
- Endpoint: Model-based estimated effect of transfusion strategy; see Section 7.2 for modeling details
- Feasibility threshold: Model-based estimated difference $> 30 \times 10^9$ cells/L

(SE2) Eligibility rate relative to predicted eligibility rate

- Outcome: For each patient screened (see definition in Section 4.2.1), it will be determined whether the patient was indicated in the study database as meeting all study eligibility criteria, did not meet all study eligibility criteria, or whether screening was indeterminate
- Endpoint: The observed eligibility rate as a percentage of the predicted eligibility rate. That is,

$$100\% \times \frac{\text{observed eligibility rate}}{\text{predicted eligibility rate}},$$

  where the predicted eligibility rate is 7.9 patients per site per year, and the observed eligibility rate per site per year will be calculated as (# in ELIGIBLE population)/(total # of site-years enrollment was open). The ELIGIBLE population is defined in Section 4.2.3. For each site, the enrollment duration is the number of calendar days from the site being activated for enrollment to the site being closed to enrollment, inclusive, divided by 365.25 (approximate # of days per year)
- Feasibility threshold: $> 50\%$

(SE3) Approach for consent percentage

- Outcome: For each patient in the ELIGIBLE population (see Section 4.2.3), whether the patient was in the APPROACHED population (approached for study participation within 24 hours of cannulation; see also Section 4.2.4)

- Endpoint: 100% × (# in APPROACHED population) / (# in ELIGIBLE population)
- Feasibility threshold: > 90%

(SE4) Consent percentage

- Outcome: For each patient in the APPROACHED population (see Section 4.2.4), whether the person was consented to study participation (see also Section 4.2.5 for a description of the CONSENTED population)
- Endpoint: 100% × (# in CONSENTED population) / (# in APPROACHED population)
- Feasibility threshold: > 50%

(SE5) Randomization of consented patients percentage

- Outcome: For each patient in the CONSENTED population, whether the person was in the ITT population (i.e., consented and randomized; equivalently, in the ENROLLED population; see Section 4.2.6)
- Endpoint: 100% × (# in ITT population) / (# in CONSENTED population)
- Feasibility threshold: > 66% (previous data indicate approximately one-third of patients bleed within 24 hours of cannulation)

(SE6) Transfusion compliance rate

- Outcome: This is a transfusion-level outcome for the ITT population, such that some patients could have multiple outcomes and others could have no outcomes. Any transfusions that were performed under a period of temporary suspension will be ignored. Among the remaining transfusions performed between randomization and when the treatment strategy was permanently discontinued, the transfusion will be considered compliant if and only if (a) the most recent nonmissing pre-transfusion platelet count (obtained no more than 12 hours before the transfusion, and not obtained prior to an earlier transfusion) was below the assigned threshold AND (b) the site principal investigators agree the transfusion dose was the proper dose in use at the site (e.g., 10 mL/kg if that is the local guideline and the same guideline applies to each arm of the study); if the pre-transfusion platelet count is not available, it will be assumed that the transfusion was not compliant. Also, if a transfusion was indicated per the threshold (i.e., the platelet count was below the threshold) but no transfusion was given within 12 hours, this will also be considered as an outcome and automatically be regarded as noncompliance

- Endpoint: 100% × (# of compliant transfusions not during a temporary suspension)/ (# of transfusions not during a temporary suspension + number of transfusions that should have been given but were not)
- Feasibility threshold: >90%

(SE7) Transfusion strategy suspension (not due to preparation for decannulation) percentage

- Outcome: For each patient in the ITT population (see Section 4.2.6), whether the patient had at least one temporary suspension of the transfusion threshold not due to preparation for decannulation before the permanent discontinuation of the treatment strategy. A temporary suspension of the transfusion threshold will not require a transfusion in excess of the threshold to have been given during the suspension period for it to be counted as a suspension
- Endpoint: 100% × (# in ITT population with at least one temporary suspension of the transfusion threshold not due to preparation for decannulation) / (# in ITT population)
- Feasibility threshold: < 10%

(SE8) Withdrawal and/or lost-to-follow-up percentage

- Outcome: For each patient in the ITT population, whether the patient was withdrawn from study participation by 90 days post-randomization and/or had an unknown mortality status for the timepoint of 90 days after randomization. The 90-day vital status will be considered known if the 90-day communication for mortality assessment is completed within ±10 days, inclusive, of 90 days after randomization, or if the patient was known to have already died (e.g., died while on ECMO) such that the 90-day follow-up mortality assessment is unnecessary
- Endpoint: 100% × (# in ITT population who were withdrawn from the study by 90 days after randomization or had unknown 90-day mortality) / (# in ITT population)
- Feasibility threshold: < 6%

(SE9) Percentage with any violations of local non-study-specific circulatory support protocols

- Outcome: For each patient in the ITT population, whether the patient had any reported instances of non-adherence to the site's non-study-specific circulatory support strategies. For this outcome, any instances of platelet transfusions above the randomized threshold are immaterial; on the other hand, for example, if the anticoagulation

strategy in place at the site was not followed, this is considered a protocol violation for this outcome. The site PIs will determine whether or not local protocols were followed

- Endpoint: 100% × (# of ITT patients with any reported violations of local non-study-specific circulatory support protocols)/(# of ITT patients)
- Feasibility threshold: $< 5\%$

(SE10) Adjudication percentage of progression to severe bleeding/thrombotic outcomes

- Outcome: This is defined for each patient in the ITT population (Section 4.2.6) for whom there was any site-reported instance of progression to severe bleeding and/or thrombosis in the acute follow-up period (the **acute follow-up period** consists of the time from randomization through 24 hours past when the transfusion strategy was last applicable). Because any site-reported severe bleeding or severe prognosis is cause to terminate use of the transfusion strategy for that patient, we focus on whether the initial such event can be adjudicated, whether or not additional distinct events were later observed. The patient's outcome is defined as adjudicated if the adjudication panel reached a decision for the initial site-reported event (confirming or overturning the site-level decision for the initial event), and defined as not adjudicated if the panel was unable to reach either a confirmation or overturning decision for the reported event even after requesting additional information from the site
- Endpoint: 100% × (# of ITT patients with site-reported severe bleeding/thrombotic outcomes for which the adjudication panel was able to adjudicate the patient's first such reported outcome)/(# of ITT patients with site-reported severe bleeding/thrombotic outcomes)
- Feasibility threshold: $> 90\%$

2.2.3 Tertiary Outcome(s)

The tertiary outcome is whether the adjudication committee was able to reach an adjudication decision for progression to severe bleeding and/or a severe thrombotic event. Note that the outcome of progression to severe bleeding and/or severe thrombosis is similar to the primary safety outcome, but the primary safety outcome also includes mortality while the tertiary outcome disregards mortality as a sufficient condition (though it is anticipated that mortality may have already involved a severe bleeding and/or thrombotic event, making the distinction less impactful). This patient-level outcome will be defined for those who have a site-reported progression to severe bleeding and/or severe thrombosis and will use each such patient's first reported instance of a progression to severe bleeding or severe thrombosis. Note that this tertiary outcome is the same as defined in SE10.

2.2.4 Additional Outcome(s)

Additional outcomes mentioned in the protocol include the following, which include most of the measures recommended in [1].

- Hemorrhagic complications according to the ELSO definition
- Thrombotic complications according to the ELSO definition
- Blood product exposure, defined as the total number of transfusions (red blood cell, plasma, platelets, cryoprecipitate, fibrinogen concentrate) and other hemostatic therapies (activated Factor VII, tranexamic acid, aminocaproic acid, DDAVP, and von Willebrand factor, etc.)
- Re-explorations and other surgical procedures to treat bleeding
- Duration of ECMO, defined as 90-day ECMO-free days (e.g., if a patient is decannulated after 7 days, the 90-day ECMO-free days is 83 days; whereas if a patient dies on ECMO after 7 days, the 90-day ECMO-free days is 0 days)
  - This is defined based on calendar days for days 1–90, with day 1 being the day of randomization and day 90 being 89 calendar days after randomization, and because every randomized patient will be on ECMO on day 1, the range for this outcome is 0–89
- Length of Pediatric Intensive Care Unit (PICU) stay, defined as the 90-day PICU-free days (as with ECMO-free days, using calendar days and with possible range of 0–89)
- Discharge Pediatric Overall Performance Category (POPC)
- Discharge Pediatric Cerebral Performance Category (PCPC)
- Mortality on ECMO
- Mortality within 24 hours of the treatment strategy being permanently discontinued
- Mortality at 28 days (obtained on or after 28 days after randomization, unless known death before)
- Mortality at 90 days (obtained on or after 80 days after randomization, unless known death before)
- Number of temporary suspensions of the transfusion strategy

2.2.5 Safety Outcome(s)

Many safety outcomes are obtained during the *acute follow-up period*, defined as the period beginning with randomization and ending 24 hours after the permanent discontinuation of the transfusion strategy. The transfusion strategy for a randomized patient is permanently discontinued at the earliest of any of the following five events: (1) per site report, progression to severe bleeding, according to the BASIC definition, and/or severe clotting, and/or death; (2) decannulation from ECMO; (3) twenty-one days post-randomization (as the progression to new bleeding and new thrombosis has plateaued by day 21 and ≈95% of the patients will be off ECMO by then); 4) temporary suspension lasting more than 24 hours; or 5) study withdrawal.

The primary safety outcome is defined at the patient level for all patients in the ITT population. It is defined as the occurrence of any of the following during the acute follow-up period: progression to severe bleeding (defined below); progression to severe thrombosis (defined below); death. The outcome is initially site-reported but then is to be adjudicated by the adjudication panel. If the panel determines the outcome criteria were not met, the outcome will be considered not to have occurred. Otherwise, if the panel confirms the criteria were met, or if the panel is unable to reach a determination, the primary safety outcome will be considered to have occurred.

Using the BASIC definition [2], **severe bleeding** will be defined as:

1. Bleeding that leads to at least one organ dysfunction, using PELOD-2 score criteria of organ dysfunction
2. Bleeding that leads to hemodynamic instability, defined as an increase in heart rate (HR) by $> 20\%$ from baseline or a decrease in blood pressure (BP) by $> 20\%$ from baseline (i.e., prior to the bleeding event)
3. Bleeding leading to a drop in hemoglobin $> 20\%$ within 24hr
4. Quantifiable bleeding ≥ 5 mL/kg/hr for $\geq$ 1hr (e.g., chest tube)
5. Intracranial hemorrhage on computerized tomography (CT) scan or head ultrasound
6. Intraspinal bleeding leading to loss of neurologic function below the lesion, nontraumatic intra-articular bleeding leading to decreased range of movement, or intraocular bleeding leading to impaired vision.

Severe thrombotic events will be defined according to the NHLBI Hemostasis Clinical Trial Outcomes Working Group as any of the following, as diagnosed clinically: circuit clot requiring circuit change; need for thrombectomy; ischemic stroke; or distal thromboembolism. [3]

Other patient-level safety outcomes include:

- the occurrence of any adverse event (AE) that is non-serious but at least possibly related to the platelet transfusion strategy
- the occurrence of any serious adverse event (SAE)
- the occurrence of any SAE, or of any non-serious AE at least possibly related to the platelet transfusion strategy (i.e., either of the two outcomes mentioned immediately above)
- the occurrence of any serious adverse event at least possibly related to the platelet transfusion strategy.

As with the primary safety outcome, the period during which new adverse events are to be identified for subsequent entry in the study database is the acute follow-up phase from randomization through 24 hours after the transfusion strategy was permanently discontinued. Note that adverse events are recorded if they are non-serious but deemed at least possibly related to the study intervention (transfusion strategy), or if they are serious regardless of study-relatedness.

3 STUDY DESIGN AND METHODS

3.1 Overall Study Design

The ECSTATIC trial is a two-arm, 1:1 randomized controlled trial, to compare lower (50×10^9 cell/L) and higher (90×10^9 cell/L) platelet thresholds for transfusions in children on ECMO. Eligible and consented participants are to have transfusions managed using the randomly assigned strategy from the time of their randomization until one of five prespecified criteria are met which permanently discontinues the strategy, with allowances for temporary suspension of the assigned protocol as described in the study protocol. Because this is a feasibility trial, analyses focus on the ability to demonstrate adherence to the protocol as captured by separation in the average pre-transfusion platelet counts corresponding to transfusions (Aim 1) and on summaries of ten criteria taken as indicators of the feasibility to conduct a larger trial designed to assess efficacy of the transfusion threshold strategy (Aim 2). The larger trial is proposed to use a novel composite outcome of progression to severe bleeding or severe prognosis (see SE10 in Section 2.2.2 or Section 2.2.3), and added attention is given to the adjudication of this outcome (Aim 3).

3.2 Method of Treatment Assignment and Randomization

This study's randomization scheme is 1:1 allocation to the lower or higher transfusion threshold. Randomization will use random block sizes of 2, 4, or 6, and be stratified by age (≤ 28

days or > 28 days). The randomization sequences will be generated using R version 4.2.1 or later, and the randomization sequences will be loaded to the study's Research Electronic Data Capture (REDCap) database.

3.2.1 Delivery of Randomization and Emergency Backup

When a research coordinator or other designated personnel is enrolling a participant, the study team member will use the randomization module in REDCap to make the treatment assignment. In case of emergency because REDCap is not functioning, each site will be provided one backup randomization envelope containing a treatment assignment. It is very unlikely REDCap will not be properly functioning at the time a patient is enrolled, and therefore one backup envelope per site should be more than sufficient.

3.2.2 Handling of Incorrect Randomization in Study Analyses and Reports

We assume that misrandomization will be rare for this trial. For intention-to-treat analyses, the participants will be analyzed according to the assigned treatment arm, even if it was later determined that the incorrect randomization stratum (defined by age) was used.

3.3 Treatment Masking (Blinding)

The clinical team will be unblinded because they need to know the strategy to which they are to adhere. The Data Coordinating Center (DCC), including the statistical team, will also be unblinded. Otherwise, the steering committee will remain blinded except as needed at their own sites to provided clinical care for participants they are treating. The trial's Data and Safety Monitoring Board (DSMB) will not be blinded.

3.4 Study Intervention Compliance

At the transfusion level, a transfusion is considered to be compliant if it was below the assigned threshold (with the count obtained less than 12 hours previously and not obtained before a prior transfusion) and at the dose level to be used at the local site; it is considered to be non-compliant otherwise (see also SE6 in Section 2.2.2). As a supplementary measure of study compliance, we will distinguish between transfusions that occurred during a period of temporary suspension and those that did not occur during such a period. At the individual level, the person is considered as having been managed in compliance with the assigned treatment if and only if there were no non-compliant transfusions given, ignoring any transfusions given during a period of temporary suspension. Any missed transfusions (i.e., platelet counts below the threshold for which there were no transfusions within 12 hours of the platelet count) are considered as noncompliance.

An additional measure of compliance is whether the patient was treated according to institutional protocols for aspects of circulatory support other than the platelet transfusion strategy (e.g., per the site's anticoagulation strategy). The determination of whether local protocols were followed will be made by the study PIs (Drs. Nellis and Karam). See also SE9 in Section 2.2.2.

4 STUDY SUBJECTS AND ANALYSIS POPULATIONS

4.1 Eligibility

This study is among children on ECMO. The exact eligibility criteria are specified in the study protocol.

4.2 Populations for Analyses

4.2.1 Screening Population

The SCREENING population is all patients on ECMO under age 18.

4.2.2 Inclusion Population

The INCLUSION population is all patients who meet all study inclusion criteria and were entered into the study database.

4.2.3 Eligible Population

The ELIGIBLE population is the subset of the INCLUSION population who were determined to meet all eligibility criteria. Note that this will include participants who provided consent but were later found to be ineligible. For the purposes of evaluating the exclusion criterion "on ECMO for $>$ 24 hours at time of enrollment," an unconsented patient who otherwise satisifies all eligibility criteria will be considered eligible if the person would have been eligible to be randomized within 24 hours of cannulation, had the patient been consented. Furthermore, the exclusion criterion "decision to withdraw or withhold some critical care or interventions" will be assumed to not apply unless such a decision was known to be in place (e.g., a participant cannot withdraw if the participant was never consented, but for purposes of determining missed eligibles the person would be counted as eligibile if all other criteria would indicate eligibility).

4.2.4 Approached Population

The APPROACHED population is the subset of the ELIGIBLE population for whom the parents were approached for consent within 24 hours of cannulation.

4.2.5 Consented Population

The CONSENTED population is the subset of the ELIGIBLE population for whom informed consent for study participation was provided.

4.2.6 Intention-to-Treat Population

The Intention-to-Treat (ITT) population is the subset of the CONSENTED population who were randomized to one of the study's two transfusion thresholds. For the ITT population, the treatment arm is the arm to which the participant was assigned, even if this strategy was not adhered to or there was a randomization error because the wrong randomization stratum was used. The ITT population will equivalently be regarded as the ENROLLED population. Note that a participant who consented and was randomized but was later found to be ineligible will still be considered part of the ITT population for analysis and safety assessment purposes. Although we note that the primary analysis of the primary outcome requires being in the ITT population as a necessary condition, not all ITT participants will necessarily contribute to the primary analysis because the primary outcome is a transfusion-level outcome and as such is not applicable if an ITT participant had no transfusions during the acute follow-up period. (Additionally, note that missing primary outcomes from ITT participantes with transfusions will not be imputed.)

4.2.7 Safety Population

The SAFETY population is comprised of all patients in the ITT population. In the unlikely event that a participant is randomized but did not have informed consent provided, this person will be added to the SAFETY population. All individuals in the SAFETY population will be analyzed according to the assigned treatment arm. This population will be used for adverse event summaries and for the primary safety outcome's interim and end-of-study analyses.

5 GENERAL ISSUES FOR STATISTICAL ANALYSES

5.1 Analysis Software

Analysis will be performed using SAS® Software version 9.4 or later when feasible. Other software packages, including R and StatXact®, may be used for particular specialized procedures.

5.2 Methods for Withdrawals, Missing Data, and Outliers

Participants who withdraw from the study intervention should continue to be followed for assessment of safety outcomes, if allowed. However, outcomes such as the primary outcome of pre-transfusion platelet counts and adherence to study intervention will be ignored from analyses beginning at the time of the withdrawal.

Because the outcomes generally relate to adherence or outcomes that should have no missingness (e.g., whether or not a patient consented), it is not expected that missing data will be problematic for the main analyses. There is naturally some protection against detrimental effects due to missing data for model-based analyses that incorporate random effects or other forms of within-subject correlation, such as for the primary outcome.

Outliers may affect the primary analysis because the primary analysis of Aim 1 is mean-based. Pre-transfusion platelet counts that appear to be outliers may be queried to ascertain if the values were correctly recorded. If it is determined that the values were not correctly recorded, they will be corrected if the correct values are available. If it is determined the values were not correctly recorded but the correct values are unavailable, the platelet counts will be regarded as missing for the primary Aim 1 analysis of separation in mean platelet counts.

5.3 Multiple Comparisons and Multiplicity

The first objective has one primary analysis: separation in the average pre-transfusion platelet counts between treatment strategies. There is an associated one-sided test to be conducted at $\alpha = 0.025$. This test provides the key p-value for purposes of the Aim 1 feasibility assessment.

Other tests of the feasibility, including the one-sided test for separation in the average total volumes of transfusions, are considered supplementary or exploratory and as such will not

be adjusted for multiple comparisons.

The early stopping rule for safety allows multiple tests and as such will account for multiplicity as described in Section 6.

5.4 Planned Subgroups, Interactions, and Covariates

Subgroups defined by the randomization strata ($\leq$ 28 days vs $>$ 28 days) will be considered. The intended sample size does not facilitate precise subgroup inference, so subgroup analyses will be considered as supplementary and interpreted carefully. We do not plan to allow for interactions in exploratory models given there is limited information for estimation of even main effects. ECMO type (Veno-Venous [V-V] vs Veno-Arterial [V-A]) may be used as a covariate in some exploratory analyses, and site may be included as a random effect in some exploratory analyses.

5.5 Derived and Computed Variables

Derived variables needed for analysis will be defined in a corresponding data dictionary. Most variables of particular importance were already defined. Select variables of particular importance are defined in the remainder of this subsection.

Additional outcomes: transfusion volumes in mL/kg/run and mL/kg/day. These are the total platelet transfusion doses while on ECMO in mL/kg/run, and the transfusion dose in mL/kg/hr (or its redundant value, in mL/kg/day, obtained by multiplying the dose in mL/kg/hr by 24). When determining the total mL/kg of the platelet transfusions, all doses given from randomization until the permanent discontinuation of the transfusion strategy will be counted, whether or not the dose was transfused during a temporary suspension of the assigned strategy. The total transfusion dose measured in mL/kg/run is simply the mL/kg during this acute follow-up phase, whereas the dose in mL/kg/day is the total dose in mL/kg divided by the number of hours from randomization until permanent strategy discontinuation.

5.6 Independent Review

All statistical analyses for primary reporting of trial results will be independently verified through dual programming and/or code review. Two statisticians will each program all datasets and trial results analyses for the DSMB reports and the primary manuscript and the results will be compared.

6 INTERIM ANALYSES

6.1 Frequency of and Timepoints for Interim Analysis

There are two classes of interim analyses: a set of inferential statistical analyses for a formal interim stopping rule; and largely descriptive summaries focusing on data and safety monitoring used in reports prepared for the DSMB. The statistical testing underlying the formal stopping rule will be conducted when every new batch of five additional participants for whom has the site reported the primary safety outcome occurred has undergone the adjudication process. Details of this testing are provided in Section 6.4.

A DSMB report with safety and data completion metrics will be prepared and distributed to the DSMB for every DSMB meeting during enrollment. Scheduled DSMB meetings are intended to be held twice during the study: approximately 8 weeks after the tenth participant randomization, and approximately 8 weeks after the $25th$ participant randomization.

6.2 Stopping Rules for Interim Efficacy Analysis

This study is not intended to provide definitive efficacy assessments, even at study conclusion. As such, early stopping for efficacy is not a consideration.

6.3 Futility Monitoring in the Interim Analysis

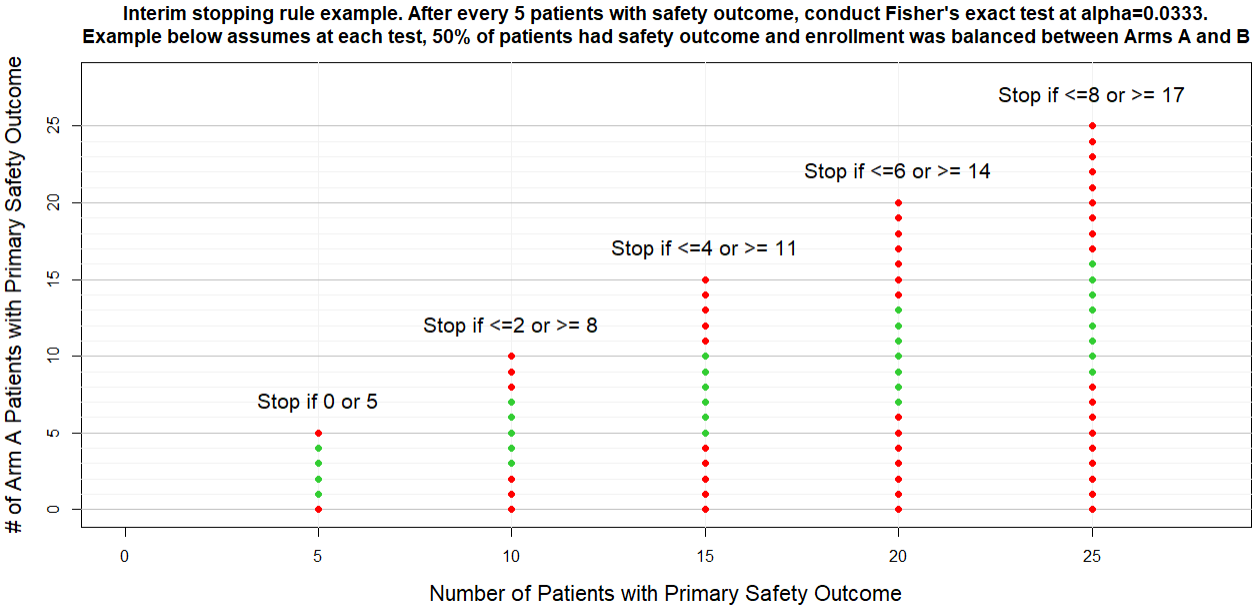
Because the study is not intended to provide definitive efficacy assessments, early stopping for the futility of demonstrating efficacy is also not a consideration.

6.4 Early Trial Termination for Safety

Tests of the primary safety outcome will be conducted from the SAFETY population (see Section 4.2.7). Per the DSMB guidance, we increased the planned frequency of comparing the primary safety outcome between treatment arms for the purpose of applying early trial termination criteria. Instead of one interim analysis after 25 enrollments, testing for a difference between treatment arms in the proportion of enrolled patients experiencing the primary safety outcome, a group sequential approach will be taken.

After the adjudication of every 5 new patients who were reported by the site to have had the primary safety outcome, we will conduct a two-sided Fisher's exact test using an alpha threshold of 0.0333.[1] Each test will include those with a known outcome at the time the

[1]This p-value threshold was chosen so that, accounting for the varying number of interim tests that may be conducted, the overall type I error rate is close to 0.05. The SAP's Appendix contains a detailed motivation for the 0.0333 threshold at each interim look.



adjudication process is completed (even if for a participant enrolled after the fifth new reported event), with participants for whom the outcome has not yet been determined being excluded until the outcome has been determined. We will test the null hypothesis that the two treatment strategies have the same expected proportion of patients who will experience the primary safety outcome. If a test is significant, we will stop the trial for safety reasons.

Many factors may influence exactly what the stopping rule thresholds are in the trial, such as primary safety outcomes that are overruled by the adjudication panel (expected to be uncommon), the underlying event rate, and the balance of randomization to treatment arms throughout the trial.

Figure 6.4 is an illustrative example of how the rule translates to go/no-go thresholds under one particular set of assumptions:

- At every interim look, there have been equal numbers randomized to each treatment.
- At every interim look, half of the total number of enrolled patients have experienced the primary safety outcome (though the numbers can differ by arm)

Note that the primary safety outcome will also be tested for a difference in arms after all participants have been enrolled, again using the 0.0333 threshold to be declared statistically significant, unless a previous test was already significant at this threshold. However, this is not considered an interim analysis because it is after all participants were enrolled.

6.5 Subgroups in the Interim Analysis

Interim DSMB reports will provide information overall (and by treatment group for the closed session). In addition, select summaries (e.g., enrollment rates) may be provided by site to possibly inform training or monitoring efforts.

6.6 Blinding in the Interim Analysis

Interim DSMB reports will only show information by treatment arm in the closed session report. The closed session reports will be made available to unblinded DCC statisticians and DSMB members but not to clinical staff or study investigators.

7 PLANNED ANALYSES

7.1 Description of Participant Characteristics

Summaries of enrolled participants will be provided for baseline characteristics such as, but not necessarily limited to, the following:

- age
- sex
- race/ethnicity
- number of platelet transfusions between cannulation and consenting process and if those transfusions were prophylactic or therapeutic (i.e., to treat active bleeding)
- bleeding between cannulation and enrollment

Summaries of nominal variables will be comprised of frequencies and percentages. Summaries of (quasi-)continuous variables will be comprised of means and standard deviations, and/or medians, first quartiles, and third quartiles, as deemed appropriate. Summaries of ordinal variables may use frequencies with percentages, means with standard deviations, or medians with first and third quartiles, as deemed most suitable.

7.2 Primary Outcome Analysis

For the pilot study, the analysis of the primary outcome measure will be assessing the pre-transfusion platelet count to describe the separation between the two platelet transfusion strategies, while accounting for intra-subject correlation. For each arm, we will estimate the mean pre-transfusion platelet count from randomization to the permanent discontinuation of the treatment strategy. This primary outcome will be measured for each transfusion and represent the latest available collected platelet count that is before the transfusion

began and not before a prior transfusion. In the event that there was no preceding platelet count since a previous transfusion, the outcome for that transfusion will be regarded as missing. Participants who do not have a nonmissing value for this outcome (e.g., they had no transfusions) will not be included in the analysis of the primary outcome. Thus, the primary outcome uses all transfusions with non-missing pre-transfusion platelet counts during the period between randomization and the final termination of the transfusion strategy being in effect (whether or not during a temporary suspension) from all ITT patients with at least one such transfusion having a non-missing pre-transfusion platelet count.

7.2.1 Primary Analysis for Aim 1

The first objective is based on the primary outcome of platelet counts before transfusions. Because we anticipate intra-subject correlation when there are multiple transfusions for a participant, we will fit a linear model to pre-transfusion platelet counts with a fixed effect for assigned treatment (Higher threshold, with tmt=1, vs Lower threshold, with tmt=0), such that the outcome's expected value is represented by $\beta_0 + \beta_{tmt}$ for the higher threshold and β_0 for the lower threshold. We will account for repeated measures by considering three covariance structures: compound symmetry (CS), continuous analog of autoregressive order 1, also known as CAR(1), and a hybrid of CS and CAR(1) achieved by including random subject effects and CAR(1) correlations in the within-subject errors. We will use restricted maximum likelihood (REML) for model estimation and use the model with the lowest Akaike Information Criterion (AIC) to select the best covariance structure. Once the covariance structure is determined, the Kenward-Roger calculation for denominator degrees of freedom (df) will be applied in the F-test (equivalently, for the df in the t-test) of the treatment strategy's model coefficient. The one-sided p-value of the null hypothesis $\beta_{tmt} = 0$ vs $\beta_{tmt} > 0$ will be considered statistically significant if and only if this p-value < 0.025. In addition, a two-sided 95% confidence interval for β_{tmt} will be reported. Likewise, estimated means for the lower threshold and the higher threshold arms will be reported as $\hat{\beta}_0$ and $\hat{\beta}_0 + \hat{\beta}_{tmt}$, respectively.

7.2.2 Additional Analyses

We will measure the effect of the days on ECMO, by providing summaries of the daily platelet counts for each specific ECMO day with ≥ 5 patients still on ECMO, independent of receiving a platelet transfusion. This will allow evaluating trends over time and a possible attenuation of the separation over time. The raw summaries for such days will be reported. As another analysis we will fit a linear model with a main linear effect for study day, a main effect for assigned treatment, a treatment by time interaction effect, and the best-fitting covariance structure, per AIC, among compound symmetry, CAR(1), the CS/CAR(1) hybrid, and subject-specific random intercepts and random time slopes. A two-df Wald test with Kenward-Roger denominator df and REML estimation will be conducted for the null

hypothesis that both the treatment main effect and the treatment by time interaction effect are 0. If this test is rejected at $\alpha = 0.05$, the interaction effect will be separately tested with a two-sided alternative at $\alpha = 0.05$. Then, if the 2-df test was significant but the 1-df test of the interaction effect was not significant, the main effect of treatment will be tested against a two-sided alternative at $\alpha = 0.05$.

Furthermore, we will also estimate the difference in the median platelet transfusion dose (in mL/kg/run) between the lower and higher platelet threshold strategy arms, and the median daily platelet transfusion dose (in mL/kg/day, i.e., $24 \times$ mL/kg/hr) to account for different durations of treatment strategies. These outcomes will use two-sided nonparametric percentile bootstrap 95% confidence intervals, based on a minimum of 10,000 bootstrap replicates, for the difference in medians because by construction for each of these outcomes, there is only one outcome per person. As such, repeated measures modeling is not warranted.

7.3 Secondary Outcomes Analyses (Analyses for Aim 2)

7.3.1 Primary Analysis for Aim 2

The first Aim 2 feasibility criterion, SE1, will be determined by whether the estimated treatment effect from the model in Section 7.2.1 is $> 30 \times 10^9$cells/L. The other nine Aim 2 feasibility criteria, SE2–SE10, are simple yes/no criteria which do not require statistical modeling: simply a comparison of the estimated endpoint with the prespecified threshold.

For each criterion, the associated trial quantity will be reported along with the indication of whether the criterion was met. The number of criteria met will also be reported.

7.4 Tertiary Outcome Analysis

The tertiary outcome is whether the adjudication committee was able to reach an adjudication decision for the outcome of progression to severe bleeding or thrombotic event for all ITT patients for whom such progression was reported by the site. This outcome will be summarized by frequency and percentage, overall and by assigned treatment arm.

7.5 Additional Outcome Analyses

As noted in the protocol, additional analyses will be conducted to assist with planning a future large trial. Though it is not intended that these planning analyses be definitive given the modest sample size in ECSTATIC, descriptive summaries of the additional outcomes will be calculated overall and by assigned treatment arm. If there are suggested differences in safety-related outcomes between assigned arms, the study procedures and eligibility criteria for the larger trial might be revised. Given the nature of these additional outcome analyses

in the ECSTATIC trial itself, it is not necessary to formally test for differences in the additional outcomes, as the precision of estimation would likely be insufficient to overcome issues associated with the multiplicity of testing.

7.6 Technical Approaches for Subgroup Analyses

Subgroup analyses will be considered exploratory and will be interpreted with care given the expected small sample sizes. For all Aim 1 outcomes, we will perform two subgroup analyses, one to evaluate the effect of V-V vs V-A ECMO, the other to evaluate neonates vs infants and older children ($\leq$ vs $>$ 28 days of age). For the Aim 1 model, the subgroup analyses are intended to be based on reapplying the model with the same covariance structure already identified in the overall analysis to the subset of participants from a particular subgroup (where subgroups are defined based on ECMO type or age). However, if estimation is deemed too unwieldy for some subsets, an alternative approach will be to use all participants in fitting one model but include ECMO type or age as covariates in the model. For the primary outcome, a subgroup analysis by site would be expected to have very small sample sizes that would make statistical testing suspect, and so adjustment for site as a random effect would be considered in a supplementary analysis in place of subgroup analyses. Because of a heightened risk that the model may not converge when including subject effects *and* site effects, adjustment for site is not incorporated in the primary analysis.

For all secondary outcomes, we will perform two subgroup summaries, one to evaluate the effect of V-V vs V-A ECMO, the other to evaluate neonates vs infants and older children ($\leq$ vs $>$ 28 days of age).

For outcomes that do not use hypothesis testing, descriptive summaries by site might be reported, though they would require cautious interpretation.

7.7 Safety Analyses

The number and percentage of individuals with any reported adverse events will be summarized at the end of the trial, overall and by treatment arm, for the SAFETY population. In addition to possible end-of-trial DSMB reporting, the interim DSMB reports will summarize adverse event rates, overall for the open session DSMB reports and also by assigned treatment arm for the closed DSMB session.

End-of-study safety analyses will summarize descriptively each safety outcome. The only planned safety hypothesis is for the primary safety outcome, which will be tested throughout the study using the group-sequential approach described in Section 6.4, and if the null hypothesis of no difference in proportion of patients experiencing the primary safety outcome

was not previously rejected, the test will be conducted after all patients have had their outcome determined, again using a nominal alpha threshold of 0.0333 for the two-sided Fisher's exact test so that the overall type I error rate for all sequential tests of the primary safety outcome is approximately 0.05. (See also the Appendix.)

8 SAMPLE SIZE DETERMINATION

The trial has two primary types of formal statistical inferences: testing for separation in the primary outcome of Aim 1—pre-transfusion platelet counts—between transfusions with non-missing pre-transfusion platelet counts from ITT participants in the two study arms at the end of the study, and ongoing testing for differences in the proportions of SAFETY participants with the primary safety outcome between arms for an interim safety stopping rule and/or end-of-study evaluation. While the study is powered for the Aim 1 primary outcome, it is not intended to be powered for a difference in the primary safety outcome in the ECSTATIC trial.

Salient assumptions to permit power estimation include the number of observed transfusion days per subject, the within-subject dependence structure, the within-treatment variability, and the difference in means between treatments. Previous data, albeit not with an enforced low platelet threshold transfusion strategy, were used to inform the first three assumptions. It was assumed the distribution of the number of transfusion days per subject will mimic historical data (e.g., approximately 10%-20% will have no transfusions). It was assumed a compound symmetry covariance structure will adequately account for within-subject dependence; such a structure is induced by a mixed effects model with subject-specific random intercepts. Fitting a model with such a covariance structure to historical data [4] yielded an estimated autocorrelation slightly above 0.2; to be conservative the power estimation assumed $\rho = 0.3$. The assumed within-treatment standard deviation, $40 \times 10^9/L$, resembles historical data but is anticipated to be a conservative estimate for the trial.

Although the difference in platelet strategy thresholds is $40 \times 10^9/L$ (i.e., $90 \times 10^9/L$ vs $50 \times 10^9/L$), this full effect will not be realized for the mean difference because the strategies depend on platelet counts which are not continuously observed, and as such, by the time of measurement a patient may have a platelet count considerably below the threshold. Patients with a platelet count under $50 \times 10^9/L$ would be transfused under either strategy. Therefore, a more realistic separation for the difference in means between strategies is $30 \times 10^9/L$.

Assuming pre-transfusion platelet levels will be approximately normally distributed with a difference in means of $30 \times 10^9/L$, then with 50 patients (25 per arm) enrolled (and thus an estimated effective sample size of 30 statistically independent observations per arm when

accounting for patients with no transfusions, patients with multiple transfusions, and the assumed within-subject dependence in the outcome) there would be approximately 81% power to detect a difference in mean platelet levels at the time of transfusion.Power may be higher due to conservative assumptions.

9 References

References

[1] Hodgson CL, Burrell AJC, Engeler DM, Pellegrino VA, Brodie D, Fan E. Core Outcome Measures for Research in Critically Ill Patients Receiving Extracorporeal Membrane Oxygenation for Acute Respiratory or Cardiac Failure: An International, Multidisciplinary, Modified Delphi Consensus Study*, *Crit Care Med*, 2019 Nov; 47(11): 1557–63.

[2] Nellis ME, Tucci M, Lacroix J, Spinella PC, Haque KD, Stock A, et al. Bleeding Assessment Scale in Critically Ill Children (BASIC): Physician-Driven Diagnostic Criteria for Bleeding Severity. *Crit Care Med*, 2019 Dec; 47(12): 1766–72.

[3] Spinella PC, El Kassar N, Cap AP, Kindzelski AL, Almond CS, Barkun A, et al. Recommended primary outcomes for clinical trials evaluating hemostatic blood products and agents in patients with bleeding: Proceedings of a National Heart Lung and Blood Institute and US Department of Defense Consensus Conference. *J Trauma Acute Care Surg*, 2021 Aug 1; 91(2S Suppl 2): S19–25.

[4] Dalton HJ, Reeder R, Garcia-Filion P, Holubkov R, Berg RA, Zuppa A, et al. Factors Associated with Bleeding and Thrombosis in Children Receiving Extracorporeal Membrane Oxygenation. *Am J Respir Crit Care Med*, 2017 Sep 15; 196(6): 762–71.

Appendix: INTERIM STOPPING RULE OPERATING CHARACTERISTICS

There are two reasons the trial could be stopped early for safety reasons. One is based on the prerogative of the DSMB, based on their review of accumulating trial data in DSMB reports, to recommend the study be terminated; the NHLBI could then enforce such a recommendation, if they were to support the recommendation. Such an action is unpredictable and is not accounted for in examining the operating characteristics of the second reason the trial could be stopped early: a significant observed difference between assigned treatment arms in the proportion of patients experiencing the primary safety outcome. It is this formal, prespecified stopping rule that is examined in the remainder of the appendix.

A Interim stopping rule considerations

The interim stopping rule was calibrated based on several considerations:

Frequency of inferential interim analyses. Because it is feasible for the adjudication panel to review site-reported primary safety outcomes in batches of 5 new patients with this outcome being reported by the enrolling site, with adjudication decisions received within 48 hours of the batch being provided to the panel, the interim stopping rule analyses will be conducted on the same schedule: as soon as the most recent batch of 5 new patients with adjudication decisions are available from the panel.

Ability to detect a difference. A variety of methods have been proposed to conduct sequential tests while preserving a desired type I error rate. Two popular approaches are the O'Brien-Fleming and Pocock approaches. The O'Brien-Fleming approach requires very strong evidence against the null hypothesis initially, but reduces the strength of evidence required with subsequent tests to preserve more alpha for the end-of-study comparison. The Pocock approach uses the same test statistic thresholds (equivalently, the same p-value thresholds) at each pre-specified look and therefore has greater ability to stop the trial earlier when there are underlying differences in the outcome between treatment arms. The Pocock approach will be used to give early analyses more statistical power to detect a difference.

Small-sample inference. Rather than use the standard-normal approximation for the distribution of the test statistic comparing proportions in the two arms, we will use Fisher's exact test because the normal approximation is not justified for dichotomous data with very small sample sizes. Analyses could be as early as when there are 5 participants.

Randomization balance. Although *a priori* randomization will have the same expected number allocated to each treatment at any given instant, the observed balance at any given point may differ. We propagate variability in the observed allocations via simulation to achieve a more realistic assessment of the interim analysis's operating characteristics, such as power and type I error rate.

Desired type I error rate. We aim to have a 5% type I error rate.

B Determining the appropriate p-value threshold for each interim stopping rule analysis

There are standard power calculations available for, say, use of group-sequential testing with Pocock significance thresholds, a test statistic with a standard normal distribution, and a prespecified and (often equally spaced) fixed number of interim analysis timepoints. However, to accommodate the use of Fisher's exact test (relaxing the dubious assumption of a standard normal test statistic), unknown number of interim analyses, and variability in randomization balance, we applied a custom but straightforward Monte Carlo simulation to determine what p-value threshold would be appropriate to yield a 5% type I error rate.

Because of the nature of patients on ECMO, the eligible population is at high risk of experiencing the primary safety outcome. However, a precise estimate of the percentage of enrolled participants who will experience the primary safety outcome is unknown. We considered rates from 20% to 80% to calibrate the threshold for having approximately 5% overall type I error given the multiplicity of testing (and variability in how many tests may be performed).

Monte Carlo simulation with random numbers of events was used to estimate a suitable nominal alpha for each test, and 0.0333 worked well: average overall type I error rate for all tests—including a test at trial completion—when averaging Monte Carlo results over the range $p_A = p_B = p$ such that $p \in (0.20, 0.21, 0.22, \ldots, 0.80)$ is approximately 5.0%, based on 2000 simulations per value of p to have adequate precision. The procedure can be conservative if the proportion of individuals with the primary safety outcome is < 0.30, which is not surprising because then fewer tests would be expected to be conducted. However, because the type I error rate varies with the true p, it was deemed preferable to have the test be conservative in situations where there are fewer events while allowing the type I error rate to be slightly above 5% when events are more common, as the latter indicates more participants would be expected to have the primary safety outcome.

R code for simulating this process is included below, as well as a plot of the estimated type I error rates as a function of p (Figure 1).

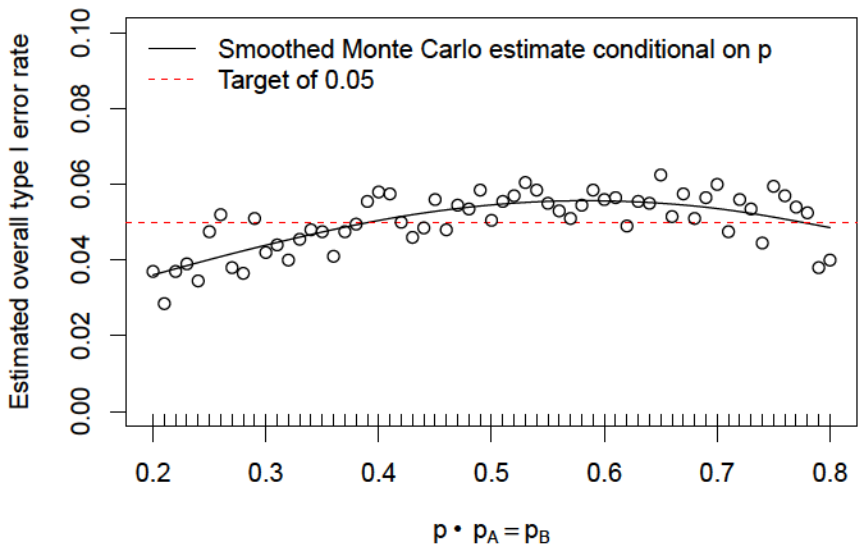


Figure 1: Estimated overall type I error rate when using 2,000 simulated trials for each $p \in 0.20, 0.21, \ldots, 0.80$ and conducting each interim test and end-of-study test using the threshold $p - value \leq 0.0333$. Although the estimated type I error rate varies over p, when marginalized over these values of p, the type I error rate was estimated to be 0.0502, extremely close to the desired 0.05 level.

```
simPocock <- function(p1,p2,totalN,alpha, nsims=1e3){
  whenSig <- rep(NA, nsims)
  nTests <- rep(NA, nsims)
  tmtvec <- 1+rbinom(totalN*nsims, 1, 0.5)
  tmt <- matrix(tmtvec, byrow=FALSE, nrow=totalN, ncol=nsims)
  yvec <- rbinom(nsims*totalN, 1, c(p1,p2)[tmtvec])
  y <- matrix(yvec, byrow=FALSE, nrow=totalN, ncol=nsims)
  for (k in 1:nsims){
    # find where we reach 5 more events, but also guarantee we look at study end
    templooks <- (1:totalN)[diff(cumsum(c(0,y[,k])))==1 & cumsum(y[,k])%%5 == 0]
    Ns.for.looks <- unique(c(templooks[templooks>=5], totalN))

    #print(Ns.for.looks)
    j <- 0
    while (j < length(Ns.for.looks) & is.na(whenSig[k])){
      j <- j+1
      if (fisher.test(matrix(
        c(sum(y[1:(Ns.for.looks[j]),k]==0 & tmt[1:(Ns.for.looks[j]), k]==1),
          sum(y[1:(Ns.for.looks[j]),k]==1 & tmt[1:(Ns.for.looks[j]), k]==1),
          sum(y[1:(Ns.for.looks[j]),k]==0 & tmt[1:(Ns.for.looks[j]), k]==2),
          sum(y[1:(Ns.for.looks[j]),k]==1 & tmt[1:(Ns.for.looks[j]), k]==2)
        ),
        nrow=2, ncol=2))$p.value <= alpha) {
        whenSig[k] <- Ns.for.looks[j]
      }
    }
    nTests[k] <- j
  }
  return(list(nTests=nTests, whenSig=whenSig, estPower = mean(!is.na(whenSig))))
}

pseq <- seq(0.2, 0.8, by=0.01)
powseq <- rep(NA, length(pseq))
set.seed(13250604)
for (j in 1:length(pseq)){
  print(pseq[j])
  powseq[j] <- simPocock( p1=pseq[j], p2=pseq[j], totalN=50, nsims=2e3,
                          alpha=0.0333)$estPower
}
library(mgcv)
```

```
print(paste0("Estimated overall type I error rate when using",
             " alpha=0.0333 threshold for each test, marginalized",
             " average over p in {0.20, 0.21, ",
             "..., 0.80}, is ", round(mean(powseq),4)))
plot(gam(powseq~s(pseq)), shift=mean(powseq), se=FALSE, ylim=c(0,0.10),
     xlab=expression(p %==% p[A]==p[B]),
     ylab="Estimated overall type I error rate",
     main="")
abline(h=0.05, lty=2, col="red")
points(pseq, powseq)
legend("topleft", c("Smoothed Monte Carlo estimate conditional on p",
                    "Target of 0.05"),
       lty=c(1,2), col=c("black", "red"), bty="n")
```

DocuSign

Certificate Of Completion

Envelope Id: C7ACC8F93DDB4B268D887D140023D59E
Status: Completed
Subject: Complete with DocuSign: SAP_ECSTATIC_v1_0_2023Nov9.pdf
Source Envelope:
Document Pages: 32
Signatures: 3
Certificate Pages: 5
Initials: 0
AutoNav: Enabled
EnvelopeId Stamping: Disabled
Time Zone: (UTC-08:00) Pacific Time (US & Canada)

Envelope Originator:
EUNHEA UNSICKER
102 South 200 East, Suite 110
Salt Lake City, UT 84111
u0794209@utah.edu
IP Address: 155.100.47.3

Record Tracking

Status: Original
11/10/2023 7:27:59 AM
Holder: EUNHEA UNSICKER
u0794209@utah.edu
Location: DocuSign

Signer Events	Signature	Timestamp
Oliver Karam oliver.karam@yale.edu Security Level: Email, Account Authentication (None)	DocuSigned by: Oliver Karam D5066330F4F748A... Signature Adoption: Pre-selected Style Using IP Address: 130.132.173.39	Sent: 11/10/2023 7:48:35 AM Viewed: 11/10/2023 7:58:34 AM Signed: 11/10/2023 7:58:56 AM
Electronic Record and Signature Disclosure: Accepted: 11/10/2023 7:58:34 AM ID: 373997c6-7e8e-4f4d-ac95-1676615a8176		
Marianne Nellis man9026@med.cornell.edu Security Level: Email, Account Authentication (None)	DocuSigned by: Marianne Nellis A891D9374C814C7... Signature Adoption: Pre-selected Style Using IP Address: 77.137.76.89	Sent: 11/10/2023 7:58:57 AM Viewed: 11/10/2023 9:52:49 AM Signed: 11/10/2023 9:53:13 AM
Electronic Record and Signature Disclosure: Accepted: 11/10/2023 9:52:49 AM ID: b8e00940-801c-4e0f-bfd5-533a46054298		
Bradley J. Barney bradley.barney@hsc.utah.edu Security Level: Email, Account Authentication (None)	DocuSigned by: Bradley J. Barney 3C3E6D23B98F4BB... Signature Adoption: Pre-selected Style Using IP Address: 155.100.47.0	Sent: 11/10/2023 9:53:14 AM Viewed: 11/10/2023 9:56:18 AM Signed: 11/10/2023 9:58:39 AM
Electronic Record and Signature Disclosure: Accepted: 11/10/2023 9:56:18 AM ID: dbd19e36-62a1-41df-b591-513ee59c9e77		

In Person Signer Events	Signature	Timestamp

Editor Delivery Events	Status	Timestamp

Agent Delivery Events	Status	Timestamp

Intermediary Delivery Events	Status	Timestamp

Certified Delivery Events	Status	Timestamp

Carbon Copy Events	Status	Timestamp

Witness Events	Signature	Timestamp

Notary Events	Signature	Timestamp

Envelope Summary Events	Status	Timestamps
Envelope Sent	Hashed/Encrypted	11/10/2023 7:48:35 AM
Certified Delivered	Security Checked	11/10/2023 9:56:18 AM
Signing Complete	Security Checked	11/10/2023 9:58:39 AM
Completed	Security Checked	11/10/2023 9:58:39 AM

Payment Events	Status	Timestamps

Electronic Record and Signature Disclosure

ELECTRONIC RECORD AND SIGNATURE DISCLOSURE

From time to time, University of Utah (we, us or Company) may be required by law to provide to you certain written notices or disclosures. Described below are the terms and conditions for providing to you such notices and disclosures electronically through the DocuSign system. Please read the information below carefully and thoroughly, and if you can access this information electronically to your satisfaction and agree to this Electronic Record and Signature Disclosure (ERSD), please confirm your agreement by selecting the check-box next to 'I agree to use electronic records and signatures' before clicking 'CONTINUE' within the DocuSign system.

Getting paper copies

At any time, you may request from us a paper copy of any record provided or made available electronically to you by us. You will have the ability to download and print documents we send to you through the DocuSign system during and immediately after the signing session and, if you elect to create a DocuSign account, you may access the documents for a limited period of time (usually 30 days) after such documents are first sent to you. After such time, if you wish for us to send you paper copies of any such documents from our office to you, you will be charged a $0.00 per-page fee. You may request delivery of such paper copies from us by following the procedure described below.

Withdrawing your consent

If you decide to receive notices and disclosures from us electronically, you may at any time change your mind and tell us that thereafter you want to receive required notices and disclosures only in paper format. How you must inform us of your decision to receive future notices and disclosure in paper format and withdraw your consent to receive notices and disclosures electronically is described below.

Consequences of changing your mind

If you elect to receive required notices and disclosures only in paper format, it will slow the speed at which we can complete certain steps in transactions with you and delivering services to you because we will need first to send the required notices or disclosures to you in paper format, and then wait until we receive back from you your acknowledgment of your receipt of such paper notices or disclosures. Further, you will no longer be able to use the DocuSign system to receive required notices and consents electronically from us or to sign electronically documents from us.

All notices and disclosures will be sent to you electronically

Unless you tell us otherwise in accordance with the procedures described herein, we will provide electronically to you through the DocuSign system all required notices, disclosures, authorizations, acknowledgements, and other documents that are required to be provided or made available to you during the course of our relationship with you. To reduce the chance of you inadvertently not receiving any notice or disclosure, we prefer to provide all of the required notices and disclosures to you by the same method and to the same address that you have given us. Thus, you can receive all the disclosures and notices electronically or in paper format through the paper mail delivery system. If you do not agree with this process, please let us know as described below. Please also see the paragraph immediately above that describes the consequences of your electing not to receive delivery of the notices and disclosures electronically from us.

How to contact University of Utah :

You may contact us to let us know of your changes as to how we may contact you electronically, to request paper copies of certain information from us, and to withdraw your prior consent to receive notices and disclosures electronically as follows:
To contact us by email send messages to: DocuSignAdmin@utah.edu

To advise University of Utah of your new email address

To let us know of a change in your email address where we should send notices and disclosures electronically to you, you must send an email message to us at DocuSignAdmin@utah.edu and in the body of such request you must state: your previous email address, your new email address. We do not require any other information from you to change your email address.

If you created a DocuSign account, you may update it with your new email address through your account preferences.

To request paper copies from University of Utah

To request delivery from us of paper copies of the notices and disclosures previously provided by us to you electronically, you must send us an email to DocuSignAdmin@utah.edu and in the body of such request you must state your email address, full name, mailing address, and telephone number. We will bill you for any fees at that time, if any.

To withdraw your consent with University of Utah

To inform us that you no longer wish to receive future notices and disclosures in electronic format you may:

i. decline to sign a document from within your signing session, and on the subsequent page, select the check-box indicating you wish to withdraw your consent, or you may;

ii. send us an email to DocuSignAdmin@utah.edu and in the body of such request you must state your email, full name, mailing address, and telephone number. We do not need any other information from you to withdraw consent.. The consequences of your withdrawing consent for online documents will be that transactions may take a longer time to process..

Required hardware and software

The minimum system requirements for using the DocuSign system may change over time. The current system requirements are found here: https://support.docusign.com/guides/signer-guide-signing-system-requirements.

Acknowledging your access and consent to receive and sign documents electronically

To confirm to us that you can access this information electronically, which will be similar to other electronic notices and disclosures that we will provide to you, please confirm that you have read this ERSD, and (i) that you are able to print on paper or electronically save this ERSD for your future reference and access; or (ii) that you are able to email this ERSD to an email address where you will be able to print on paper or save it for your future reference and access. Further, if you consent to receiving notices and disclosures exclusively in electronic format as described herein, then select the check-box next to 'I agree to use electronic records and signatures' before clicking 'CONTINUE' within the DocuSign system.

By selecting the check-box next to 'I agree to use electronic records and signatures', you confirm that:

- You can access and read this Electronic Record and Signature Disclosure; and
- You can print on paper this Electronic Record and Signature Disclosure, or save or send this Electronic Record and Disclosure to a location where you can print it, for future reference and access; and
- Until or unless you notify University of Utah as described above, you consent to receive exclusively through electronic means all notices, disclosures, authorizations, acknowledgements, and other documents that are required to be provided or made available to you by University of Utah during the course of your relationship with University of Utah .