Restrictive versus Liberal Red Blood Cell Transfusion Strategies in Pediatric Acute Respiratory Distress Syndrome

A Study of the Pediatric Acute Lung Injury and Sepsis Investigators (PALISI) Network

Sponsored by:

National Heart, Lung, and Blood Institute
US National Institute of Child Health and Human Development
US National Institutes of Health

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LIST OF ABBREVIATIONS AND ACRONYMS

AE	Adverse Event
ARDS	Acute Respiratory Distress Syndrome
CDC	Centers for Disease Control and Prevention
CFR	Code of Federal Regulations
CI	Confidence Interval
CLIA	Continuous Laboratory Improvement Act
CPQA	Clinical Pharmacology Quality Assurance
DNR	Do-Not-Resuscitate
DO2	Oxygen Delivery
DSMB	Data and Safety Monitoring Board
EAE	Expedited Adverse Event
EC	Ethics Committee
ECMO	Extracorporeal Membrane Oxygenation
EQA	External Quality Assurance
FDA	(United States) Food and Drug Administration
GCP	Good Clinical Practices
Hb	Hemoglobin
HIV	Human Immunodeficiency Virus
ICH	International Conference on Harmonization
ICF	Informed Consent Forms
ICU	Intensive Care Unit
ID	Identification
IL-6	Interleukin-6 Levels
IRB	Institutional Review Board
LDMS	Laboratory Data Management System
LL	Local Laboratory
MedDRA	Medical Dictionary for Regulatory Activities
MODS	Multiple Organ Dysfunction Syndrome
NCLN	National Clinical Laboratory Network
NHLBI	National Heart, Lung, and Blood Institute
NIH	(United States) National Institutes of Health
OI	Oxygenation Index
OSI	Oxygenation Saturation Index
PAERS	PALISI Adverse Experience Reporting System
PALICC	Pediatric Acute Lung Injury Consensus Conference
PALISI	Pediatric Acute Lung Injury and Sepsis Investigators
PARDS	Pediatric Acute Respiratory Distress Syndrome
PEEP	Positive End-Expiratory Pressure
PICU	Pediatric Intensive Care Units
pSMILE	Patient Safety Monitoring and International Laboratory Evaluation
PRISM	Pediatric Risk of Mortality
PRO	Protocol Registration Office

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LIST OF ABBREVIATIONS AND ACRONYMS - CONTINUED

QA	Quality Assurance
QC	Quality Control
RBC	Red Blood Cells
RE	Regulatory Entity
ROC	Regulatory Operations Center
RSC	Regulatory Services Center
SAE	Serious Adverse Event
SUSAR	Suspected Unexpected Serious Adverse Reaction
SOP	Standard Operating Procedures
SSP	Study Specific Procedures
VO2	Oxygen Consumption

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I, the Investigator of Record, agree to conduct this study in full accordance with the provisions of this protocol. I agree to maintain all study documentation for a minimum of three years after submission of the site's final Financial Status Report to the Pediatric Acute Lung Injury and Sepsis Investigators (PALISI) Network, unless otherwise specified by PALISI. Publication of the results of this study will be governed by PALISI policies. Any presentation, abstract, or manuscript will be made available by the investigators to the PALISI Manuscript Review Committee for review prior to submission.

I have read and understand the information in this protocol and will ensure that all associates, colleagues, and employees assisting in the conduct of the study are informed about the obligations incurred by their contribution to the study.

_____
Name of Site Investigator of Record

_____ _____
Signature of Site Investigator of Record Date

Restrictive versus Liberal Red Blood Cell Transfusion Strategies in Pediatric Acute Respiratory Distress Syndrome

SCHEMA

Purpose:

- To evaluate the clinical efficacy of a restrictive RBC transfusion strategy versus standard of care among patients with PARDS

Design:

- Single-blinded, randomized Phase III multi-center clinical trial

Study Population:

- Pediatric patients admitted to the participating PICUs meeting inclusion criteria with PARDS
- Ongoing accrual until target study size is reached

Study Size:

- 668 randomized PICU patients

Treatment Regimen:

- A restrictive transfusion strategy (<7 g/dl)

Study Duration:

- Patient follow-up period of 28 days

Primary Objectives:

- To determine if a restrictive transfusion strategy (<7 g/dl) is superior to standard of care among pediatric patients with PARDS
- To better understand the causal pathways of progressive lung injury and organ dysfunction among PARDS patients by evaluating biomarkers associated with outcomes in PARDS

Secondary Objectives:

- To evaluate if immunocompromised status modifies the relationship between transfusion strategies and mortality, organ function, and IL-6

Study Sites:

- 20 research hospital PICUs across the United States (for patient accrual)
- Partner research lab
- Offsite research staff at University of Washington
- Contracted statistical support for randomization

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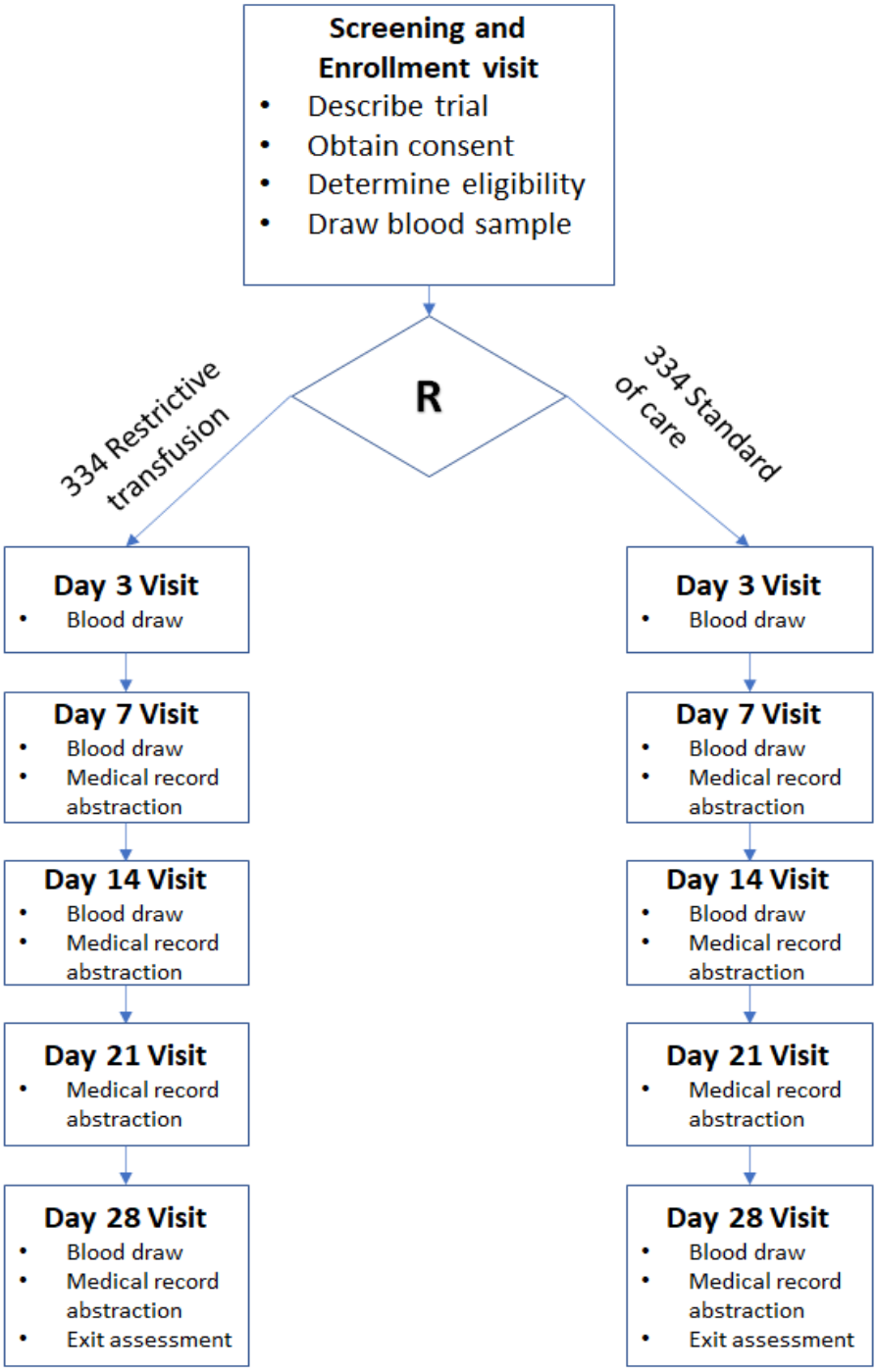
OVERVIEW OF STUDY DESIGN AND RANDOMIZATION SCHEME



1. INTRODUCTION

1.1. Background and Prior Research

Acute respiratory distress syndrome (ARDS) is a diffuse and inflammatory form of acute lung injury characterized clinically by an acute deterioration in pulmonary gas exchange resulting in hypoxemia (1,2). Differences in practice patterns, comorbidities, and outcomes specific to pediatric patients led to a pediatric definition of ARDS in 2015, termed pediatric acute respiratory distress syndrome (PARDS) (3). When this definition was prospectively evaluated among 23,280 patients across 145 international pediatric intensive care units (PICU), PARDS occurred in approximately 3% of PICU patients and in 6% of those receiving invasive mechanical ventilation. However, mortality estimates among pediatric patients with PARDS were high around 15%, and in excess of 30% in those with severe hypoxemia (4). Additionally, the burden of disease among the immunocompromised is significant, with this group representing over 35% of children who die with PARDS (4).

Red blood cells (RBC) are transfused in over 40% of children with PARDS, with the intent to improve oxygen delivery and outcomes (5). However, in some patients, RBC transfusion itself may further impair lung function and worsen outcomes (5–7). Unfortunately, few high-quality data exist to guide RBC transfusion decision-making in critically ill pediatric patients with PARDS. Current guidelines are based on a randomized control trial of hemoglobin-based RBC transfusion strategies in a heterogeneous population of critically ill children that did not demonstrate differences between liberal and transfusion arms. This study has generally been interpreted as supporting a restrictive transfusion threshold of 7 grams/deciliter (g/dl) in critically ill children (8). Notably, this analysis excluded children with hypoxemia and therefore, the impact of restrictive transfusion in the PARDS population is unknown.

There exists no strong data to support the recommendation of a specific hemoglobin transfusion threshold in critically ill pediatric patients with PARDS (9,10). Additionally, the impact of immunocompromised status in supporting any specific hemoglobin thresholds in the PARDS population is not well established.

1.2. Rationale

In critically ill children, the predominant reason to transfuse RBCs is to improve oxygen delivery (DO2) thereby preventing the development of shock by ensuring appropriate tissue oxygenation and increasing oxygen consumption (VO2) (11). While this could be of particular importance in patients with hypoxemia and PARDS, data do not support this hypothesis. In heterogeneous populations of critically ill patients, RBC transfusion has failed to show benefit in systemic oxygen uptake (12). Furthermore, in adults with ARDS, while DO2 increased, there was no increase in VO2 after transfusion (13). In contrast, there is accumulating data regarding the negative consequences of RBC transfusion in critical illness with or without PARDS, with various studies showing increased inflammatory mediator release (14), development of multiple organ dysfunction syndrome (MODS) (15,16), and higher mortality (6,17).

The primary pathologic description of ARDS is a diffuse injury to the alveolar epithelial-endothelial barrier (diffuse alveolar damage) resulting in noncardiogenic pulmonary edema. Infiltration of activated neutrophils into the alveolar space is a pathologic hallmark of ARDS (1,2). Disruption of alveolar fluid clearance and surfactant, apoptosis, and coagulopathy are additional pathobiological mechanisms associated with worse lung injury (1). However, activation of the innate immune system and inflammation are known to be integral to the development and evolution of ARDS (18). Furthermore, dysregulated inflammation can precipitate damage to the lung and other non-pulmonary end organs (19). RBC transfusion may

only serve to drive this dysregulated immune response increasing pulmonary and non-pulmonary morbidity and mortality.

The presence of an inflammatory process such as PARDS can worsen the clinical status of a critically ill child and lead to MODS when a secondary inflammatory insult occurs. There is strong evidence that RBC transfusion generates, propagates, and enhances proinflammatory reactions. Proinflammatory molecules, including bioactive lipids and cytokines contained in plasma-rich blood products, may trigger the inflammatory cascade that drives organ dysfunction (20,21). According to the *two-hit* or *neutrophil priming* hypothesis, patients must have a predisposing factor, which primes their neutrophils; then, the patients' neutrophils are activated in a *second hit* by donor plasma that contains these proinflammatory molecules (22). Leukoreduction, a method by which leukocytes are removed from whole blood, is now standard of care in most hospitals. However, leukoreduction has been shown to decrease, but not completely eliminate the proinflammatory effects of transfusions (23). Furthermore, in vitro data suggest that inflammatory mediators remain active even in leukocyte-reduced RBC units (24).

Lungs with active inflammatory processes, such as PARDS, may be particularly sensitive to further injury due to the proinflammatory phenomenon of RBC transfusion (25). RBC transfusions in those with ARDS and PARDS have been associated with worsening respiratory dysfunction (26), augmenting ventilation-perfusion mismatch (27), prolonged duration of invasive mechanical ventilation (5), and higher mortality (6,28). If PARDS includes a potent inflammatory response and RBC transfusions may exacerbate that inflammatory response as a *second hit*, it is important to delineate which, if any, proinflammatory molecules are central to this pathobiology.

Interleukin-6 (IL-6) has long been implicated in the pathobiology of ARDS. This cytokine is produced by various immune cell types at sites of tissue inflammation (29) and may act as an important proinflammatory mediator for the induction of the acute phase response (30). Plasma and bronchoalveolar lavage levels of IL-6 have been identified as markers of acute lung injury (31,32) and predictive of prolonged invasive mechanical ventilation, organ dysfunction, and morbidity and mortality in ARDS (33–36). In a landmark trial in adult ARDS, the authors convincingly argue that large tidal volumes increase lung 'stretch' which induces disruption of the alveolar-capillary barrier and elaboration of inflammatory mediators. They argue it is these inflammatory mediators that not only worsen lung injury, but also increase risk of non-pulmonary end-organ dysfunction. By randomizing to a lower tidal volume ventilation strategy, the authors demonstrated a 22% relative risk reduction in mortality, decreased non-pulmonary end-organ dysfunction, and decreased IL-6 levels (19).

PARDS has been shown relative to the general PICU population to be more severe and associated with higher mortality in immunocompromised patients (37). The pediatric immunocompromised population with PARDS may be exquisitely sensitive to the adverse consequences of RBC transfusion. RBC transfusion has been noted to increase IL-6 levels in oncology patients potentially providing a mechanism by which immunocompromised children may be harmed by the immunomodulatory properties of transfusions (38). There exists no strong data to support higher hemoglobin threshold in these patients.

Further investigation is required to better characterize the specific balance between benefits and harms associated with RBC transfusion among pediatric populations with PARDS. We will perform a randomized control trial to test our central hypothesis that a restrictive blood transfusion strategy is associated with improved survival, as well as decreased new or progressive organ dysfunction, among pediatric PARDS patients. We will also determine if a restrictive transfusion strategy is most beneficial among the immunocompromised population with PARDS.

2. STUDY OBJECTIVES AND DESIGN

2.1. Primary Objectives

The primary objectives of this study are to:

Aim 1a. To determine if a restrictive transfusion strategy (<7 g/dl) is superior to standard of care among pediatric patients with PARDS.
Hypothesis 1a: A restrictive transfusion strategy will increase 28-day survival and decrease new or progressive organ dysfunction among pediatric patients with PARDS.

Aim 1b. To better understand the causal pathways of progressive lung injury and organ dysfunction among PARDS patients by evaluating biomarkers associated with outcomes in PARDS.
Hypothesis 1b: A restrictive transfusion strategy will be associated with a decrease in interleukin 6 (IL-6) levels from time of randomization (day 0) to day 3, day 7, day 14, and day 28.

2.2. Secondary Objective

The secondary objective of this study is to:

Aim 2. To evaluate if immunocompromised status modifies the relationship between transfusion strategies and mortality, organ function, and IL-6.
Hypothesis 2: A restrictive transfusion strategy will show more benefit in the immunocompromised group relative to the immunocompetent group. Among the immunocompromised group, there will be greater decreases in 28-day mortality, greater reductions in new or progressive organ dysfunction, and greater decreases in IL-6.

2.3. Study Design

We will perform a phase III multicenter randomized clinical trial evaluating a restrictive RBC transfusion strategy versus usual care among pediatric patients with PARDS. We hypothesize that a restrictive RBC transfusion strategy will increase 28-day survival and decrease new or progressive MODS.

Patients admitted to the PICU with acute respiratory failure will be screened for participation. Eligible patients will be intubated and will require positive pressure ventilation, have an oxygenation index (OI) $\geq$ 4 and/or oxygenation saturation index (OSI) $\geq$ 5, and have chest radiograph findings with new infiltrate(s) consistent with non-cardiogenic pulmonary edema at enrollment. The diagnosis of PARDS will be as defined by the Pediatric Acute Lung Injury Consensus Conference (PALICC) criteria (see appendix I).

Randomization will be centralized, with a concurrent stratified randomization scheme based upon center and age group (age groups: patients less than 28 days, patients 28 days to 1 year, and patients 1 year to 18 years of age). Since clinical staff, on-site research coordinators and their assistants will be involved in patient care and treatment assignment at time of randomization, they will be aware of the treatment arm. Additionally, patients and parents/surrogate decision makers will be aware of the treatment group assignment. Members of the Data and Safety Monitoring Board (DSMB) will be unblinded. Lastly, a contracted statistical consulting team will be responsible for generating and maintaining the randomization list. On the other hand, all off-site research team members including the principal investigator, biostatisticians, medical monitors, and data management team members will be blinded to the group assignment.

Attending physicians will follow guidelines for RBC transfusions outlined for each group. The transfusion protocol will be applied up to 28 days of stay in the PICU or until the time of death, whichever occurs first.

Ventilation strategies according to the ARDS Network protocol of low tidal volume will begin within one hour after randomization and continue until day 28 or death, whichever occurs first. Patients will receive low tidal ventilation at 6 milliliters per kg (ml/kg) to target peak inspiratory pressure (or plateau pressure) of less than 30 centimeters (cm) of H2O. Patients will receive positive end-expiratory pressure (PEEP) according to the PEEP-Fio2 table according to the ARDS Network protocol (19). Standard ventilatory strategies will be important to prevent any confounding by ventilatory management that may impact our downstream patient outcomes.

We will randomize each consecutive pediatric patient admitted to the PICU with PARDS as defined by the PALICC criteria to a restrictive transfusion strategy (less than 7 g/dl) or to standard of care. All RBC transfusions will be leukocyte-reduced and as per institutional practice. Leukocyte-reduction is a method by which the red cells are filtered to remove leukocytes and then stored in the usual fashion.

The primary outcomes of interest will be all-cause 28-day mortality and concurrent MODS or progression of MODS following randomization. Organ dysfunction will be determined by the definitions published by Proulx et al. and Goldstein et al to identify daily presence of specific organ dysfunction in order to meet criteria for MODS (39,40) (see appendix II).

New or progressive MODS will be determined by daily screening for dysfunction of the cardiovascular, respiratory, hematologic, renal, neurologic, and gastrointestinal systems in the 28 days following randomization, or before death, whichever occurs first. New or progressive MODS is defined as follows: New MODS is defined as a patient with ≤1 organ dysfunction on day 0 of randomization who subsequently developed ≥2 concurrent organ dysfunctions. Progressive MODS was defined as a patient with existing MODS (≥2 organ dysfunctions) on day 0 of randomization who developed at least one other concurrent organ dysfunction or had worsening of one or more organ dysfunctions. We will specifically describe respiratory dysfunction by a Pao2/Fio2 ratio or Spo2/Fio2 ratio decrease by at least 20% after transfusion, as described previously by Kleiber et al. (26).

The secondary outcome is IL-6 levels. IL-6 levels via blood samples will be obtained from all patients on day of randomization for measurement of baseline plasma IL-6 by immunoassay. Subsequent IL-6 levels will be obtained from all participants on day 3, day 7, day 14, and day 28 following randomization.

We will evaluate if the relationships between transfusion strategies and our primary and secondary outcomes of interest vary by immunocompromised status. The designation ‘immunocompromised’ will be given for patients with an oncologic, immunologic, rheumatologic, or transplant diagnosis and active immunosuppressive chemotherapy, or a congenital immunodeficiency as previously described by Zubrow et al. (5).

3. STUDY POPULATION

Pediatric patients meeting criteria for PARDS and admitted to participating PICU will be eligible for inclusion in this study. Participants will be selected for the study according to the criteria in Section 3.1 and 3.2 and the guidelines in Section 3.4. They will be recruited, screened, and enrolled as described in Section 3.3 below and assigned to a study intervention group as described later in Section 7.4. Issues related to participant retention and withdrawal from the study are described in sections 3.5 and 3.6, respectively.

3.1. Inclusion Criteria

Pediatric patients who meet the following criteria will be included in the study:

- All pediatric patients requiring pediatric intensive care admission for acute respiratory failure requiring invasive mechanical ventilation.
- Ages: newborn to 18 years old
- Meeting criteria for PARDS according to the PALICC definition within the first 7 days after admission to the pediatric intensive care unit

3.2. Exclusion Criteria

Pediatric patients who meet any of the following criteria will be excluded from this study:

- Patients with active bleeding and thus requiring blood product resuscitation
- Patients who meet death by neurological criteria
- Patient with active do-not-resuscitate (DNR) or limitations to care
- Patients requiring veno-venous and veno-arterial extracorporeal membrane oxygenation (ECMO) will be excluded
- Pregnant patients

3.3. Recruitment Process

We acknowledge that the study population is particularly small, and that recruitment into this study would depend on the occurrence of PARDS among pediatric patients and their referrals by treating physicians. Therefore, seeking to minimize the study costs and duration, we plan to continuously recruit patients until the desired sample size is achieved. We will utilize collaborating physicians from the different ICUs in different hospitals and have them screen all eligible patients. We will pilot a screening study before the trial with the objectives of assessing the feasibility of the trial, identifying potential barriers, such as investigators', and collaborating physicians' adherence to the study protocol, estimating the trial duration, drop-out and suspense rates, and collecting preliminary data for sample size calculations for the trial (41). We will take a sample size of 30 patients from 10 sites to estimate accrual rate (42).

Other studies have identified a number of potentially-modifiable barriers to patient recruitment. Those include research team workload and availability, narrow time windows for inclusion, protocols prohibiting co-enrollment, and physician refusals (43). Burns and colleagues have also reported that greater research coordinator experience, site research volume and broader time windows for inclusion were all found to be significant predictors of fewer declined consents. We plan to address those barriers to patient recruitment in our trial by extending the study duration until the desired sample size is collected, and continually providing orientation sessions for the treating doctors at the study sites. As the trial progresses, we also plan to track recruitment efficiency (rate) as a proportion of all eligible patients.

3.4. Co-Enrollment Guidelines

Given the paucity of eligible patients, pediatric patients with PARDS, and seeking to enhance trial recruitment, we will permit co-enrollment of participants in our trial as well as other ongoing trials. Prohibiting co-enrollment has been cited by Brun and colleagues as one reason for poor recruitment (43). Therefore, we believe that allowing co-enrollments would enhance recruitment to our trial. Since our study population is a critical patient one, we do not expect it to be enrolled in other studies. Even if that was the case, we do not have limitations and will not seek to identify specific studies with which we will

permit our trial participants to be enrolled, as long as those studies involve no administration of active drugs or interventions that may potentially alter the exposure-outcome relationship in our trial. For instance, we will permit co-enrollment with studies that involve collection of data (e.g. questionnaires) or tissue samples (e.g. blood). On the other hand, if the other study involves administering blood transfusion as an intervention, co-enrollment will not be permitted. The Principal Investigator will be the one to make the decision on such instances.

3.5. Participant Retention

In order to reduce the number of missed recruitment opportunities, we propose to address the above-mentioned barriers by the following:

- Allowing research team to contact parents to facilitate obtaining consents
- Allowing alternative consent models, e.g., hybrid consent models prioritizing substitute decision makers involvement when available and preemptive consent.
- Choosing hospitals with sufficient documented patients volume and standard medical care that is compatible with both study arms (restricted and liberal transfusion)
- Ensuring that study personnel are trained and willing to conduct the study. It was found that an enthusiastic lead investigator at the site is the most important factor associated with recruitment
- Tracking recruitment by having a trajectory graph of recruitment, that we plan to check regularly to identify and address any challenges, or further motivate recruitment.
- If needed, we may revisit inclusion and exclusion criteria and clear any possible confusion in writing, re-write inclusion and exclusion criteria, increase the recruitment duration, increase budget or simplify the informed consent (41).
- Holding frequent meetings with the research team and collaborating physicians from the different recruitment sites to track recruitment and resolve issues.
- Educating patients about the trial, taking the time to answer their questions, and ensuring that they are well informed before asking for their consent.
- Designing the trial in a way that provides researchers with more time for cases' identification and families with more decision-making time to process information, and consent to participate in the study (44).

With the above strategies, we target to achieve a 95% level of retention.

3.6. Participant Withdrawal

Regardless of the participant retention methods, the consenting party may voluntarily initiate a withdrawal from the study for any reason at any time. Additionally, the protocol can be *temporarily* suspended, at the discretion of the attending physician, during periods of active or clinically significant blood loss, surgical intervention, hemodynamic instability. Physicians will be asked to promptly resume the randomization once the condition of the patient no longer fulfills the suspension criteria. Withdrawal decisions will be made after consultation with the Protocol Chair and the Protocol Biostatistician. Data monitoring and collection will be unchanged during collection of data.

4. STUDY TREATMENT/PRODUCT/INTERVENTION

4.1. Treatment/Product/Intervention Formulation/Content

The intervention will be a "restrictive" transfusion strategy, that is a set hemoglobin threshold of <7 g/dl. As part of the clinical management of PARDS, patients are transfused with the intent of improving oxygen delivery and outcomes. The transfusion strategy is defined by a hemoglobin level of <7 g/dL.

4.2. Treatment/Product/Intervention Regimen(s)

The strategy will be maintained, and each participant will continue on the allocated arm receiving the restricted transfusion strategy throughout the study duration. The timing of RBC transfusion initiation in a patient with PARDS is determined by the attending physician based not only on the Hb levels but also on hemodynamic instability. Either of the RBC transfusion strategies will be applied to patients until (1) 28 days after admission to the hospital, (2) discharge from the ICU, (3) death, whichever comes first.

4.3. Treatment/Product/Intervention Supply and Accountability

We will maintain records of all transfusions received, their frequency and volume throughout each participant's enrollment duration.

4.4. Adherence Assessment

We will particularly monitor adherence to assignment and protocol by following the patients' medical records once they are recruited into the trial. Decisions about blood transfusion will be made by the attending physician based on the hemoglobin (Hb) levels and evidences haemodynamic instability, both of which will be documented in the patients' medical records.

4.5. Toxicity Management

If the patient's clinical condition warrants modification and the clinical decision made by the treating doctor was to administer blood transfusion, then such modifications can be made immediately. This does not imply that the patient is removed from the trial because we will analyze according to the intention-to-treat principle, but we will collect such data for secondary per-protocol and sensitivity analyses.

4.6. Clinical Management of Pregnancy

Since the study population is pediatric patients, this section does not apply to our protocol. If it ever happens that a patient was pregnant, we would consider that an exclusion criterion.

4.7. Concomitant Medications

We acknowledge that most admissions to ICU are not planned so it would be hard to know this information. However, all concomitant medications taken or received by participants will be reported on applicable study case report forms to the best degree possible. Medications used for the treatment of AEs that occur during study participation also will be recorded on applicable study case report forms.

5. STUDY PROCEDURES

An overview of the study visits and procedures schedule is presented in Appendix III. Presented below is additional information on visit-specific study procedures. Detailed instructions to guide and standardize all study procedures across sites will be provided in the study-specific procedures manual.

Study visit location - All study visits will be conducted at the 20 participating hospitals. Enrollment into the study occurs when an eligible patient is admitted to the PICU at a participating hospital. If a patient is discharged prior to the end of follow-up, they will be asked to return to the participating hospital for their exit visit 28 days after randomization.

Enrollment visit - All PARDS patients, as defined by the PALICC, admitted to a participating PICU will be screened by the research coordinator staff for inclusion in the trial. Parents/patients will be counseled regarding the trial and informed consent will be collected from a parent or responsible party prior to enrollment in the study. Eligibility for enrollment in the study will be ascertained after signed informed consent. All eligible patients with signed consent will undergo a blood draw prior to the assignment of a randomized arm. Randomization decisions will be provided to the attending physician. Research coordinator on site will be responsible for completing all forms associated with consent, eligibility, randomization assignment, sample collection labeling, and enrollment visit documentation.

Additional blood draws - Additionally, the research coordinator will be responsible for blood collection from patients after study enrollment on days three, seven, 14, and 28. We expect most patients will continue to be hospitalized for the entire 28-day period after enrollment, however in the case that patients are discharged from the hospital the research coordinator will schedule follow-up visit appointments with the patient's responsible party prior to discharge. Transportation and costs associated with the follow-up visit will be covered by the study budget (appendix IV).

The research coordinator and staff will be responsible for maintaining a stock of specimen labels which will be applied to the paper forms associated with study visits as well as samples collected during blood draws. Short term storage of blood samples will occur on site at the participating PICU. Once enough samples have been collected to fill a single shipment, they will be shipped to the central repository for long term storage.

Ascertainment of other outcome and safety measures - Weekly consultation occurring every 7 days after randomization between the research coordinator and the attending physicians will be coordinated independently by study site. During these meetings, the attending physician will work with the research coordinator, in consultation with study medical monitors if needed, to extract data from medical records and document in study forms. Data extracted will be recorded on a standard set of forms with questions related to adverse events, death outcome, ventilation use, protocol compliance, and organ function. Additionally, there will be forms to collect a general medical overview provided by the attending physician. For patients discharged from the hospital prior to the end of the 28-day period, an exit visit will be scheduled at the time of discharge. This exit visit will require the patient to come back to the hospital where the same set of forms will be filled out and blood will be collected.

6. SAFETY MONITORING AND ADVERSE EVENT REPORTING

6.1. Safety Monitoring

Close cooperation between the Protocol Chair(s), study site Investigator(s), Medical/Program Officer, Protocol Coordinator, Protocol Biostatistician, and other study team members will be necessary in order to monitor participant safety and respond to occurrences of excess mortality in a timely manner. Before

the study begins, the team will decide on a schedule to hold regular conference calls during the period of study implementation, and additional ad hoc calls will be convened if required.

This study is subject to oversight by the DSMB. Planned DSMB reviews will begin with study protocol approval and will be regularly scheduled at 6-month intervals (subject to change based on the expected enrollment windows). Interim analyses are planned when 25%, 50%, and 75% of the total expected mortality events are observed.

The study investigators will appoint two medical monitors for continuous monitoring of all patients' clinical medical records during participants' hospitalization. While participants are enrolled in the study, medical monitors will be required to contact and review each participant's medical records with an attending physician twice a week. Two additional monitors will be appointed by the study investigators for follow-up reporting of all adverse events at monthly intervals for 6 months post discharge. A prespecified threshold will be set for the suspension of study accrual based on an excess of mortality of 3 times higher number of deaths in either arm after 12 deaths have been reached in at least one arm. Study accrual can be stopped at any time by the protocol team after review of medical monitoring reports due to concerns over unacceptable risk.

6.2. Adverse Event Definitions and Reporting Requirements

6.2.1. Adverse Event

An adverse event (AE) is defined as any untoward medical occurrence in a clinical research participant administered an investigational product and which does not necessarily have a causal relationship with the investigational product. As such, an AE can be an unfavorable or unintended sign (including an abnormal laboratory finding, for example), symptom or disease temporally associated with the use of an investigational product, whether or not considered related to the product.

Medical monitors for the study will coordinate with the study centers and their attending physicians to report, document, and code all adverse events experienced during the participants' hospitalization. During any period of hospitalization within 6 months post-intervention, the medical monitors will be responsible for contacting the participants attending physician to go over medical records and code all adverse events. When a participant is discharged from the hospital, a thorough review of all medical records for the duration of the hospitalization will be done.

Ongoing monitoring for serious adverse events will continue for 3 months post-study. At the end of follow-up or early discharge from hospital, study participants will be given a 24-hour telephone number to report any non-life threatening adverse events and directed to emergency services for any life threatening complications. Study staff, in consultation with the two appointed medical monitors, will review all AE reports and code information onto AE reporting forms. These forms will record the Medical Dictionary for Regulatory Activities (MedDRA) code of all AEs, note if the event is serious, its severity, and its potential relationship to the intervention. Additional fields will capture the outcome of the AE, either resolution, hospitalization, death, or other outcome.

All AEs will be graded using the PALISI Table for the Grading Severity of Adult and Pediatric Adverse Experiences (also referred to as the “Toxicity Table”) Table for the Grading Severity of Adult and Pediatric Adverse Experiences (also referred to as the “Toxicity Table”). Investigator’s Brochure, Package Insert, PALISI Drug Risk List, and his/her clinical judgment. These documents are all available at (insert link)

6.2.2. Serious Adverse Event

Serious adverse event (SAE) will be defined per U.S. Code of Federal Regulations (CFR) 312.32 and International Conference on Harmonization (ICH), "Good Clinical Practice: Consolidated Guidance" and "Clinical Safety Data Management: Definitions and Standards for Expedited Reporting", as AE occurring after intervention that:

- Results in death
- Is life-threatening
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect
- Requires inpatient hospitalization or prolongation of existing hospitalization

This includes important medical events that may not be immediately life-threatening or result in death, or hospitalization but may jeopardize the patient or may require intervention to prevent one of the outcomes listed above.

Per ICH SAE definition, hospitalization itself is not an adverse event, but is an outcome of the event. The following types of hospitalization do not require expedited reporting:

- Any admission unrelated to an AE (e.g., for labor/delivery, cosmetic surgery, administrative, or social admission for temporary placement for lack of place to sleep)
- Protocol-specified admission (e.g., for procedure required by protocol)
- Admission for diagnosis or therapy of a condition that existed before receipt of study agent(s) and has not increased in severity or frequency as judged by the clinical investigator

6.2.3. Expedited Adverse Event Reporting

6.2.3.1. Adverse Event Reporting to PALISI

Requirements, definitions and methods for expedited reporting of AEs are outlined in Version 2.0 of the PALISI Manual, which is available on the RSC website at http://rsc.tech-res.com/safetyandpharmacovigilance/.

The PALISI Adverse Experience Reporting System (PAERS), an internet-based reporting system must be used for expedited AE (EAE) reporting to PALISI. In the event of system outages or technical difficulties, expedited AEs may be submitted via the PALISI EAE Form. For questions about PAERS, please contact PALISI-ES at PALISI-ESSupport@nnhlbi.nih.gov or from within the PAERS application itself.

Sites where PAERS has not been implemented will submit expedited AEs by documenting the information on the current PALISI EAE Form. This form is available on the RSC website: http://rsc.tech-res.com/safetyandpharmacovigilance/. For questions about *EAE reporting, please contact the RSC (PALISISRSCSafetyOffice@tech-res.com).*

6.2.3.2. Reporting Requirements for this Study

The SAE EAE Reporting Category, as defined in Version 2.0 of the PALISI EAE Manual, will be used for this study.

In addition to the EAE Reporting Category identified above, other AEs that must be reported in an expedited manner include transfusion specific reactions: 1) blood infection 2) immunomodulatory complications (including but not limited to febrile non-hemolytic transfusion reactions, urticaria, hemolytic transfusion reactions, etc.).

6.2.3.3. Grading Severity of Events

The most current PALISI Table for Grading the Severity of Adult and Pediatric Adverse Events (PALISI AE Grading Table) is used and is available on the RSC website at http://rsc.tech-res.com/safetyandpharmacovigilance/.]

6.2.3.4. Expedited AE Reporting Period

The expedited AE reporting period for this study is 60 days as per the PALISI EAE manual. After the protocol-defined AE reporting period, unless otherwise noted, only suspected unexpected serious adverse reactions as defined in Version 2.0 of the EAE Manual will be reported to PALISI if the study staff become aware of the events on a passive basis (from publicly available information).

7. STATISTICAL CONSIDERATIONS

7.1. Review of Study Design

In this phase III multicenter randomized clinical trial, our objective is to evaluate the clinical efficacy of a restrictive RBC transfusion strategy versus standard of care among patients with PARDS. First, we will attempt to answer our primary research questions by analyzing the possible associations between a restrictive RBC transfusion strategy and 28-day mortality, as well as either new occurrence of MODS or progressive MODS. The analysis regarding 28-day mortality will involve a multiple logistic regression model and a Wald test to perform hypothesis testing. The analysis regarding new or progressive MODS will involve survival curves as well as multiple logistic regression models and a Wald test to perform hypothesis testing. To answer our secondary research questions, we will analyze the association between a restrictive transfusion strategy and IL-6 levels to better understand the causal pathways. We will also compare the effect of a restrictive transfusion strategy on IL-6 levels as well as the outcomes in different immunocompromised groups. Multiple linear regression models will be used in the analyses.

7.2. Endpoints

7.2.1. Primary Endpoints

Consistent with the primary study objective to investigate whether or not a restrictive transfusion strategy is more beneficial than standard of care procedures, the following endpoints will be assessed:

- 28-day mortality
- occurrence of new or progressive MODS after time of randomization, until 28 days post-randomization

28-day mortality will be measured as a binary endpoint, where the patient either died before 28 days post-randomization occurred, or they survived to 28 days post-randomization. If the patient survived, their recorded survival data will be right-censored. If the patient was discharged before 28 days post-randomization, the parent or surrogate decision maker will be contacted by research staff to ascertain the required information. 28-day mortality data will be used to fit a multiple logistic regression model to deduce association between mortality and restrictive RBC transfusion strategy.

Occurrence of new or progressive MODS will be measured as a binary endpoint, where the patient either experienced new or progressive MODS before 28 days post-randomization occurred, or they did not. If the patient did not experience new or progressive MODS during the 28 days post-randomization, their recorded observation will be right-censored. If the patient was discharged before 28 days post-randomization, the parent or surrogate decision maker will be contacted by research staff to ascertain the required information. (Note: in the study population, all patients start at 1 dysfunctional organ, being their lungs). A multiple logistic regression model will be fit to deduce association between occurrence of new or progressive MODS and restrictive RBC transfusion strategy.

When answering the research question involving potential effect modification of immunocompromised status, new multiple logistic regression models will be fit, one for each primary endpoint as described above, where in each new model, immunocompromised status will be included as an effect modifier via an interaction term (immunocompromised status x transfusion strategy). The analyses will involve a likelihood ratio test, where the baseline model used for comparison is the original multiple logistic regression model for the primary endpoint in question (without the effect modifier).

7.2.2. Secondary Endpoints

Consistent with the secondary study objective to evaluate whether a restrictive transfusion strategy is associated with the IL-6 levels, the following endpoint will be assessed:

- IL-6 levels on day 0, day 3, day 7, day 14, and day 28 from randomization

IL-6 level as a surrogate endpoint will be measured as a continuous variable, from blood samples drawn from the day of enrollment, and day 3, day 7, day 14, and 28th day post-randomization. The difference in log-transformed plasma IL-6 level between day 0 and day 3 will be used as the outcome in the multiple linear regression model to deduce association between IL-6 level and restrictive RBC transfusion strategy.

7.3. Accrual, Follow-up, and Sample Size

To power up the primary outcome: the estimated 28-day mortality in patients with PARDS who receive standard of care transfusion is 20%, a 6% absolute risk reduction corresponds to a 70% relative risk.

Then, in our sample size calculation, we have p_0= 0.80 and p_1= 0.86, and hence $1 - p_0$= 0.20 and $1 - p_1$= 0.14. Using a 5% one-sided significance level and 90% power, as well as $\Delta = 0.86 - 0.80 = 0.06$, we have the following:

$$N = \frac{(Z_{0.95}+Z_{0.90})^2}{(\Delta)^2} * [p_1(1-p_1) + p_2 * (1-p_2)] =$$

$$\frac{(1.645+1.282)^2}{(0.86-0.80)^2} * [(0.86 * 0.14) + (0.80 * 0.20)] = 668$$

Then the total sample size is 668 patients.

7.4. Random Assignment / Study Arm Assignment

A stratified randomization scheme is used, where randomization is centralized. A 1:1 (treatment: standard of care) schema is used. Computer-generated randomization lists will be created by a separate organization within each site. These lists include random pairs of study ID numbers and treatment arm, where equal proportions of treatment versus standard of care is achieved. A different list for each stratum will be created. Patients are stratified according to center and age group (< 28 days, 28 days - 364 days, 365 days - 18 years). Stratifying by center and by age group will allow for greater homogeneity among each stratum, as standard of care can vary greatly across different centers. Keeping in mind the need for efficient randomization, randomized allocation of the treatment arm will be achieved as follows: when research staff approach the parent or surrogate decision maker to gather informed consent and then enroll the patient, the research staff will have ready the next available study ID number within the correct stratification group, and the paired study arm. The study arm will then be communicated to the physician and parent/surrogate decision maker as soon as possible.

7.5. Blinding

The following parties are blinded to the group assignment:

- Principal investigator
- Biostatisticians
- Medical monitors
- Data management team members

The following parties are unblinded to the group assignment:

- Clinical staff
- On-site research coordinator(s)
- On-site research assistant(s)
- Patients and parents/surrogate decision makers
- Members of the DSMB
- Contracted statistical consulting team responsible for randomization list

All on-site clinical staff (i.e., doctors and nurses involved with the patient's care) and patients and their parents/surrogate decision makers will be unblinded for ethical reasons, as will the DSMB. On-site research staff (i.e., coordinator(s) and assistant(s)) will be unblinded because they will be the ones responsible for assigning the treatment vs. control arm at the time of randomization, and for abstracting information from medical records. The contracted statistical consulting team responsible for creating the paired study ID numbers and group assignment randomization list will also be unblinded since they are the ones pairing study ID numbers to group assignment.

In order for the principal investigator, biostatisticians, medical monitors, and data management team members to be blinded to the group assignment, the on-site research staff will redact the study arm information before handing off results to the blinded parties.

7.6. Data Analysis

7.6.1. Primary Analyses

Statistical analyses of all outcomes are conducted using the intention-to-treat approach.

To address our scientific question regarding a possible association between 28-day mortality and transfusion strategy, the biostatistician will compute the adjusted odds ratios for treatment effect using multiple logistic regression. The multivariate model will include age, site, and score on the Pediatric Risk of Mortality (PRISM) assessment. The response variable is 28-day mortality (a binary variable), and the predictor of interest is transfusion strategy (i.e. restricted or standard-of-care). The biostatistician will also calculate the 95% confidence interval (CI) for the adjusted odds ratio deduced from the multiple logistic regression model, as well as a p-value.

To address our scientific question regarding a possible association between occurrence of new or progressive MODS and transfusion strategy, the biostatistician will calculate the adjusted odds ratios for treatment effect using multiple logistic regression. The multivariate model will include age, site, score on the PRISM assessment, and 28-day mortality. 28-day mortality is included because it is important to control for death in this model, since death precludes the possibility of developing new or progressive MODS. This means there is a causal relationship between 28-day mortality and occurrence of new or progressive MODS, and hence mortality should be controlled for in the regression model. The response variable is the occurrence of new or progressive MODS (a binary variable), and the predictor of interest is transfusion strategy (i.e. restricted or standard-of-care). The biostatistician will also calculate the 95% CI for the adjusted odds ratio deduced from the multiple logistic regression model, as well as a p-value.

To address our scientific question regarding a possible association between IL-6 levels and transfusion strategy, the biostatistician will fit a multiple linear regression model with the difference in log-transformed plasma IL-6 level between day 0 and day 3 as the outcome and the indicator of a restrictive transfusion strategy as the predictor of interest. The model will also include age, site, and score on the PRISM assessment as covariates. The estimated coefficients along with the 95% confidence interval and the p-value using Wald test will be calculated and reported.

No adjustments of p-values will be made for controlling for multiple hypothesis testing.

7.6.2. Secondary Analyses

To address our scientific question regarding immunocompromised status as being a potential effect modifier for transfusion status and 28-day mortality, a second multiple logistic regression model will be fit that includes the same covariates as described above, but with the addition of an interaction term between immunocompromised status and transfusion strategy. Hypothesis testing will be conducted via a likelihood ratio test, where the previous fitted model will be the restricted model for comparison. The biostatistician will report the adjusted odds ratios of this new model, as well as the 95% CI and p-value. They will also report the statistic and p-value resulting from the likelihood ratio test.

To address our scientific question regarding immunocompromised status as being a potential effect modifier for transfusion status and occurrence of new or progressive MODS, a second multiple logistic regression model will be fit that includes the same covariates as described above, but with the addition of an interaction term between immunocompromised status and transfusion strategy. Hypothesis testing will be conducted via a likelihood ratio test, where the previous fitted model will be the restricted model for comparison. The biostatistician will report the adjusted odds ratios of this new model, as well as the 95% CI and p-value. They will also report the statistic and p-value resulting from the likelihood ratio test.

To address our scientific question regarding immunocompromised status as being a potential effect modifier for transfusion status and IL-6 levels, a second multiple linear regression model will be fitted with the difference in log-transformed plasma IL-6 level between day 0 and day 3 as the outcome. The model will include all covariates from the previous model, with the addition of immunocompromised status and the interaction between immunocompromised status and transfusion strategy. The estimated coefficients along with the 95% confidence interval and the p-value using Wald test will be calculated and reported.

7.6.3. Missing Data

With the targeted level of retention (95%), missing data should not affect the analysis. However, if the targeted level of retention is not achieved, the investigators will need to examine the reasons for missingness, and the biostatistician can choose the proper imputation method to address missing data.

8. HUMAN SUBJECTS CONSIDERATIONS

8.1. Ethical Review

This protocol and the template informed consent form(s) contained in Appendix V — and any subsequent modifications — will be reviewed and approved by the PALISI Protocol Review Committee and PALISI Prevention Science Review Committee with respect to scientific content and compliance with applicable research and human subjects regulations.

The protocol, site-specific informed consent form, participant education and recruitment materials, and other requested documents — and any subsequent modifications — also will be reviewed and approved by the ethical review bodies responsible for oversight of research conducted at the study site.

Subsequent to initial review and approval, the responsible IRBs/ECs will review the protocol at least annually. The Investigator will make safety and progress reports to the IRBs/ECs at least annually, and within three months of study termination or completion. These reports will include the total number of participants enrolled in the study, the number of participants who completed the study, all changes in the research activity, and all unanticipated problems involving risks to human subjects or others. In addition, all open DSMB reports will be provided to the IRBs/ECs. Study sites will submit documentation of continuing review to the PALISI Protocol Registration Office, in accordance with the current PALISI Protocol Registration Policy and Procedure Manual.

8.2. Informed Consent

When the research staff confirms that a patient in the PICU is eligible for study enrollment, a parent or surrogate decision maker will be approached. Written informed consent will be obtained from either a parent or surrogate decision maker for each study participant Each study site is responsible for developing

a study informed consent form for local use, based on the template in Appendix V, that describes the purpose of the study, the procedures to be followed, and the risks and benefits of participation, in accordance with all applicable regulations. The study site also is responsible for translating the template form into local languages, and verifying the accuracy of the translation by performing an independent back-translation.

Literate parents/surrogate decision makers will document their provision of informed consent by signing their informed consent forms. Non-literate parents/surrogate decision makers will be asked to document their informed consent by marking their informed consent forms (e.g., with an X, thumbprint, or other mark) in the presence of a literate third party witness. (Further details regarding PALISI requirements for documenting the informed consent process with both literate and non-literate participants are provided in the PALISI Standard Operating Procedure for Source Documentation.) Any other local IRB/EC requirements for obtaining informed consent from non-literate persons also will be followed.

The parent or surrogate decision maker will be provided with a copy of their informed consent forms if they are willing to receive them.

8.3. Risks

Potential risks of study participation include unforeseen consequences of administering lower transfusion doses than standard of care. As with any transfusion, there are risks associated with the procedure such as allergic reactions and infection. Note that while extremely rare, it is also possible to be exposed to a bloodborne infection, or experience immunomodulatory issues, including an acute immune hemolytic reaction, a delayed hemolytic reaction, or a graft-versus-host disease. However, note that these risks are also associated with standard of care, and hence are not unique to the treatment arm.

Additionally, there are risks associated with blood draws, the most common being bruising. It is also possible to experience pain, redness and/or swelling of the vein, and very rarely, infection. There is also a risk of fainting.

Although study sites will make every effort to protect participant privacy and confidentiality, it is possible that participants' involvement in the study could become known to others.

8.4. Benefits

There may be no direct benefits to participants in this study, however, participants and others may benefit in the future from information learned from this study. Specifically, information learned in this study may lead to the development of safer standard of care procedures when treating pediatric patients with PARDS. This, in turn, could lead to higher survival rates of future pediatric patients with PARDS.

8.5. Incentives

Pending IRB/EC approval, reimbursement is available for travel to study visits if a patient is discharged prior to the 28-day exit.

8.6. Confidentiality

All study-related information will be stored securely at each study site. All participant information will be stored in locked file cabinets in areas with access limited to study staff. All laboratory specimens, reports, study data collection, process, and administrative forms will be identified by a coded number only to maintain participant confidentiality. All local databases will be secured with password-protected access

systems. Forms, lists, logbooks, appointment books, and any other listings that link participant ID numbers to other identifying information will be stored in a separate, locked file in an area with limited access.

Participant's study information will not be released without the written permission of the participant's parent or surrogate decision maker, except as necessary for monitoring by the US Food and Drug Administration, other government and regulatory authorities, and/or site IRBs/ECs.

8.7. Communicable Disease Reporting Requirements

Study staff will comply with all applicable local requirements to report communicable diseases identified among study participants to local health authorities. Participants will be made aware of all reporting requirements during the study informed consent process.

8.8. Study Discontinuation

The study also may be discontinued at any time by NHLBI, and/or site IRBs/ECs.

9. LABORATORY SPECIMENS AND BIOHAZARD CONTAINMENT

9.1. Laboratory Specimens Collection

As described in Section 5, the research coordinator will be responsible for blood collection from patients after study enrollment and maintaining a stock of specimen labels which will be applied to the paper forms associated with study visits as well as samples collected during blood draws. Short term storage of blood samples will occur on site at the participating PICU. Once enough samples have been collected to fill a single shipment, they will be shipped to the central repository for long term storage. There will be no chemistry, and hematology tests conducted at the participating hospitals. Instead, all tests will be conducted at the central, partner research lab. These lab tests will be monitored by Patient Safety Monitoring and International Laboratory Evaluation (pSMILE) and must demonstrate successful participation in the relevant External Quality Assurance (EQA) programs. Each study site (PICU) will adhere to standards of good clinical laboratory practice, and local standard operating procedures for specimen management including proper collection, processing, labeling, transport, and storage of specimens to the research lab. Finally, specimen collection and storage at the PICU will be documented using the Laboratory Data Management System (LDMS) as described in the study-specific procedures manual. All specimens will be shipped in accordance with International Air Transportation Authority specimen shipping regulations. All shipments will be documented using the LDMS as described in the study-specific procedures manual.

9.2. Network Laboratory Specimens

The trial will utilize the National Clinical Laboratory Network's (NCLN's) validated assays following harmonized SOPs while implementing uniform assay workflow, instrumentation, and data analysis. Samples will be shipped to the central lab and a centralized biorepository of specimens using the NCLN's centralized data reporting. The specimens will be tracked using a specimen tracking system, which includes facilities to label specimens with pertinent identifiers within the study context or completely delinked identifiers. Each study site will adhere to standards of good clinical laboratory practice and the Network Laboratory Manual for proper collection, processing, labeling, and transport of specimens to the central lab.

9.3. Quality Control and Quality Assurance Procedures

The clinical sites will document that their clinical laboratories are certified under the Continuous Laboratory Improvement Act of 1988 (CLIA-certified) and/or participate in PALISI sponsored EQA programs. The research coordinator will conduct biweekly visits to each site to assess the implementation of on-site specimen collection and storage quality control (QC) procedures. The research coordinator will follow up directly with site staff to resolve any QC or quality assurance (QA) problems identified through on-site assessments.

All of the assays to be used in this study have been approved for use by the cross-network Clinical Pharmacology Quality Assurance (CPQA) program. The quality of the assays is also continuously evaluated by a twice yearly proficiency testing program administered by the CPQA. Satisfactory scores are required for the laboratory to be able to continue to use assays in support of PALISI studies. The Pharmacology Core Lab will adhere to Good Laboratory Practice for processing all samples. The total number of specimens undergoing QA testing will represent 10% percent of all specimens collected. The principal investigator will select samples for quality assurance testing.

9.3.1. QC for IL-6 as an inflammatory marker

Testing for IL-6 will be part of the chemistry lab. The test is typically available 24 hours a day with turnaround times ranging from 1 to 8 days. The specimen type is blood sample of 1.5 mL, collected as part of routine blood collection in a Lavender (EDTA) top tube, placed on wet ice and sent to for processing. Specimens must be processed within 30 minutes of drawing. Then, specimens are centrifuged at 1,500 for 10 minutes, then plasma aliquot is removed and the sample is frozen immediately before being stored.

9.3.2. QC for serum biomarkers for monitoring severity of MODS

There are multiple serum biomarkers for monitoring MODS and they vary with different organs. Examples include serum blood urea nitrogen (BUN) for monitoring the kidney function, and total serum bilirubin for monitoring the liver function (appendix II). All tests used will be on blood specimens. Specific protocols for collection and storage for each sample type will be followed as well.

9.4. Specimen Storage and Possible Future Research Testing

All blood specimens collected in this study will be stored at least through the end of the study. In addition, study participants (or their caregivers, where appropriate) will be asked to provide written informed consent as part of the study enrollment for their blood specimens to be stored after the end of the study for possible future testing.

9.5. Biohazard Containment

As the transmission of HIV and other blood-borne pathogens can occur through contact with contaminated needles, blood, and blood products, appropriate blood and secretion precautions will be employed by all personnel in the drawing of blood and shipping and handling of all specimens for this

study, as currently recommended by the United States Centers for Disease Control and Prevention. All infectious specimens will be transported in accordance with United States regulations (42 CFR 72).

10. ADMINISTRATIVE PROCEDURES

10.1. Protocol Registration

Prior to implementation of this protocol, and any subsequent full version amendments, each site must have the protocol and the protocol consent form(s) approved, as appropriate, by their local institutional review board (IRB)/ethics committee (EC) and any other applicable regulatory entity (RE). Upon receiving final approval, sites will submit all required protocol registration documents to the PALISI Protocol Registration Office (PALISI PRO) at the Regulatory Services Center (RSC). The PALISI PRO will review the submitted protocol registration packet to ensure that all of the required documents have been received.

INITIAL REGISTRATION LANGUAGE – TO BE INCLUDED IN EVERY PROTOCOL

Site-specific informed consent forms (ICFs) *WILL NOT* be reviewed or approved by the PALISI PRO, and sites will receive an Initial Registration Notification when the PALISI PRO receives a complete registration packet. Receipt of an Initial Registration Notification indicates successful completion of the protocol registration process. Sites will not receive any additional notifications from the PALISI PRO for the initial protocol registration. A copy of the Initial Registration Notification should be retained in the site's regulatory files.

AMENDMENT REGISTRATION LANGUAGE – TO BE INCLUDED IN EVERY PROTOCOL UNLESS SITE SPECIFIC ICFS WILL BE REVIEWED

Upon receiving final IRB/EC and any other applicable RE approval(s) for an amendment, sites should implement the amendment immediately. Sites are required to submit an amendment registration packet to the PALISI PRO at the RSC. The PALISI PRO will review the submitted protocol registration packet to ensure that all the required documents have been received. Site-specific ICF(s) WILL NOT be reviewed and approved by the PALISI PRO and sites will receive an Amendment Registration Notification when the PALISI PRO receives a complete registration packet. A copy of the Amendment Registration Notification should be retained in the site's regulatory files.

For additional information on the protocol registration process and specific documents required for initial and amendment registrations, refer to the current version of the PALISI Protocol Registration Manual.

10.2. Study Activation

Pending successful protocol registration and submission of all required documents, core staff will "activate" the site to begin study operations. Study implementation may not be initiated until a study activation notice is provided to the site.

10.3. Study Coordination

Study implementation will be directed by this protocol as well as the Study Specific Procedures (SSP) manual. The SSP manual — which will contain reference copies of the *Requirements for Source Documentation in PALISI Funded and/or Sponsored Clinical Trials*, as well as the PALISI Manual for

Expedited Reporting of Adverse Events to PALISI, Version 2.0, dates January 2010 and the PALISI Toxicity Tables — will outline procedures for conducting study visits; data and forms processing; AE assessment, management and reporting; dispensing study products and documenting product accountability; and other study operations.

Study case report forms and other study instruments will be developed by the protocol team and PALISI. Data will be transferred to PALISI for data entry, cleaning, reporting and analysis. Quality control reports and queries will be generated and distributed to the study sites on a routine schedule for verification and resolution.

Close coordination between protocol team members will be necessary to track study progress, respond to queries about proper study implementation, and address other issues in a timely manner. Rates of accrual, adherence, follow-up, and AE incidence will be monitored closely by the team as well as the PALISI Study Monitoring Committee. The Protocol Chair, PALISI Medical Officer, Protocol Biostatistician, STudy Monitoring Committee, Project Manager, and Protocol Specialist will address issues related to study eligibility and AE management and reporting as needed to assure consistent case management, documentation, and information-sharing across sites.

10.4. Study Monitoring

On-site study monitoring will be performed in accordance with PALISI policies. Study monitors will visit the site to:

- verify compliance with human subjects and other research regulations and guidelines;
- assess adherence to the study protocol, study-specific procedures manual, and local counseling practices; and
- confirm the quality and accuracy of information collected at the study site and entered into the study database.

Site investigators will allow study monitors to inspect study facilities and documentation (e.g., informed consent forms, clinic and laboratory records, other source documents, case report forms), as well as observe the performance of study procedures. Investigators also will allow inspection of all study-related documentation by authorized representatives of the NHLBI, and US and in-country government and regulatory authorities. A site visit log will be maintained at the study site to document all visits.

10.5. Protocol Compliance

The study will be conducted in full compliance with the protocol. The protocol will not be amended without prior written approval by the Protocol Chair and NHLBI Medical Officer. All protocol amendments must be submitted to and approved by the relevant IRB(s)/EC(s) and the PALISI Regulatory Support Center (RSC) prior to implementing the amendment.

10.6. Investigator's Records

The study site investigator will maintain, and store in a secure manner, complete, accurate, and current study records throughout the study. “The investigator will retain all study records for at least three years after submission of the final Financial Status Report to PALISI, which is due within 90 days after the end of the cooperative agreement with PALISI, unless otherwise specified by PALISI.” Study records include administrative documentation — including protocol registration documents and all reports and correspondence relating to the study — as well as documentation related to each participant screened for

and/or enrolled in the study — including informed consent forms, locator forms, case report forms, notations of all contacts with the participant, and all other source documents

10.7. Use of Information and Publications

Publication of the results of this study will be governed by the PALISI Manual of Operations and policies. Any presentation, abstract, or manuscript will be submitted by the Investigator to the PALISI Manuscript Review Committee, PALISI, and [treatment/product manufacturer] for review prior to submission.

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12. APPENDICES

12.1. Appendix I: Diagnostic Criteria for Pediatric Acute Respiratory Distress Syndrome

Age	Exclude patients with perinatal-related lung disease			
Timing	Within 7 days of known clinical insult			
Origin of edema	Respiratory failure not fully explained by cardiac failure or fluid overload			
Chest imaging	Chest imaging findings of new infiltrate(s) consistent with acute pulmonary parenchymal disease			
Oxygenation	**Noninvasive mechanical ventilation**	**Invasive mechanical ventilation**		
	PARDS (no severity stratification)	Mild	Moderate	Severe
	Full face mask bilevel ventilation or CPAP ≥5 cm H_2O P/F ratio ≤300 S/F ratio ≤264[1]	4≤ OI <8 5≤ OSI <7.5	8≤ OI <16 7.5≤ OSI <12.3	OI ≥16 OSI ≥12.3
Special populations				
Cyanotic heart disease	Standard criteria above for age, timing, origin of edema, and chest imaging with an acute deterioration in oxygenation not explained by underlying cardiac disease.			
Chronic lung disease	Standard criteria above for age, timing, and origin of edema with chest imaging consistent with new infiltrate and acute deterioration in oxygenation from baseline that meet oxygenation criteria above.			
Left ventricular dysfunction	Standard criteria for age, timing, and origin of edema with chest imaging changes consistent with new infiltrate and acute deterioration in oxygenation that meet criteria above not explained by left ventricular dysfunction.			

• **Fig. 48.1** Pediatric acute respiratory distress syndrome (PARDS) definition. Use arterial oxygen partial pressure (Pao_2)–based metric when available. If Pao_2 is not available, wean Fio_2 to maintain peripheral capillary oxygen saturation (Spo_2) ≤97% to calculate oxygen saturation index (OSI; [Fio_2 × mean airway pressure × 100]/Spo_2) or Spo_2:Fio_2 (S/F) ratio. For nonintubated patients treated with supplemental oxygen or nasal modes of noninvasive ventilation, see Fig. 48.2 for at-risk criteria. Acute respiratory distress syndrome severity groups stratified by oxygenation index (OI; [Fio_2 × mean airway pressure × 100]/Pao_2) or OSI should not be applied to children with chronic lung disease who normally receive invasive mechanical ventilation or children with cyanotic congenital heart disease. *CPAP,* continuous positive airway pressure; *P/F,* Pao_2:Fio_2 ratio.

12.2. Appendix II: Criteria for Multi-Organ Dysfunction Syndrome

Cardiovascular dysfunction

Despite administration of isotonic intravenous fluid bolus ≥40 mL/kg in 1 hr

- Decrease in BP (hypotension) <5th percentile for age or systolic BP <2 SD below normal for age[a]

OR

- Need for vasoactive drug to maintain BP in normal range (dopamine >5 μg/kg/min or dobutamine, epinephrine, or norepinephrine at any dose)

OR

- Two of the following
  - Unexplained metabolic acidosis: base deficit >5.0 mEq/L
  - Increased arterial lactate >2 times upper limit of normal
  - Oliguria: urine output <0.5 mL/kg/hr
  - Prolonged capillary refill: >5 secs
  - Core to peripheral temperature gap >3°C

Respiratory[b]

- Pa_{O_2}/F_{IO_2} <300 in absence of cyanotic heart disease or preexisting lung disease

OR

- Pa_{CO_2} >65 torr or 20 mm Hg over baseline Pa_{CO_2}

OR

- Proven need[c] or >50% F_{IO_2} to maintain saturation ≥92%

OR

- Need for nonelective invasive or noninvasive mechanical ventilation[d]

Neurologic

- Glasgow Coma Score ≤11 (57)

OR

- Acute change in mental status with a decrease in Glasgow Coma Score ≥3 points from abnormal baseline

Hematologic

- Platelet count <80,000/mm^3 or a decline of 50% in platelet count from highest value recorded over the past 3 days (for chronic hematology/oncology patients)

OR

- International normalized ratio >2

Renal

- Serum creatinine ≥2 times upper limit of normal for age or 2-fold increase in baseline creatinine

Hepatic

- Total bilirubin ≥4 mg/dL (not applicable for newborn)

OR

- ALT 2 times upper limit of normal for age

BP, blood pressure; ALT, alanine transaminase.

[a]See Table 2; [b]acute respiratory distress syndrome must include a Pa_{O_2}/F_{IO_2} ratio ≤200 mm Hg, bilateral infiltrates, acute onset, and no evidence of left heart failure (Refs. 58 and 59). Acute lung injury is defined identically except the Pa_{O_2}/F_{IO_2} ratio must be ≤300 mm Hg; [c]proven need assumes oxygen requirement was tested by decreasing flow with subsequent increase in flow if required; [d]in postoperative patients, this requirement can be met if the patient has developed an acute inflammatory or infectious process in the lungs that prevents him or her from being extubated.

12.3. Appendix III: Schedule of Study Visits and Procedures

		Procedures					
	Study Visit	**Informed Consent**	**Eligibility form completed**	**Blood Draw**	**Randomization**	**Medical Record abstraction**	**Exit interview**
Enrollment	**Enrollment**	X	X	X	X		
Post-enrollment	**Day 3**			X			
	Day 7			X		X	
	Day 14			X		X	
	Day 21					X	
	Day 28			X			X

12.4. Appendix IV: Budget Calculations

University of Washington

SUMMARY OF PROPOSED COSTS

Period (dates)		Year 1 10/1/2022 Through 9/30/2023	Year 2 10/01/2023 Through 9/30/2024	Year 3 10/01/2023 Through 9/30/2024	Year5 10/01/2023 Through 9/30/2024	Year6 10/01/2023 Through 9/30/2024	Total
DIRECT LABOR - Percent of Effort		$ 123,854	$ 222,937	$ 200,643	$ 206,663	$ 144,664	$ 898,761
FRINGE BENEFITS - Percent of Effort		$ 30,964	$ 55,734	$ 50,161	$ 51,666	$ 36,166	$ 224,690
TOTAL DIRECT LABOR		$ 154,818	$ 278,672	$ 250,804	$ 258,328	$ 180,830	$ 1,123,452
SUBCONTRACTS		$ 532,854	$ 1,065,708	$ 1,097,679	$ 1,053,772	$ 526,886	$ 4,276,899
MTDC		$ 304,818	$ 278,672	$ 250,804	$ 258,328	$ 180,830	$ 1,273,452
TOTAL DIRECT COSTS		$ 837,672	$ 1,344,380	$ 1,348,484	$ 1,312,101	$ 707,716	$ 5,550,351
INDIRECT COSTS ON MTDC	Rate 52%	$ 158,505	$ 144,909	$ 130,418	$ 134,331	$ 94,032	$ 662,195
TOTAL PROPOSED COST		$ 996,177	$ 1,489,289	$ 1,478,902	$ 1,446,431	$ 801,748	$ 6,212,546

MTDC = Modified Total Direct Costs include Total Direct Labor plus $50,000 for each subcontract (first year only)

12.5. Appendix V: Sample Informed Consent Form(s)

PARENTAL/SURROGATE DECISION MAKER PERMISSION FORM
CONSENT FORM: Ages 18 and up

Study Title: Restrictive versus Liberal Red Blood Cell Transfusion Strategies in Pediatric Acute Respiratory Distress Syndrome

A Randomized, Single-Blind, Standard-of-Care-Controlled Trial

Principal Researcher: Colin Sallee, MD

The Research Team:

Name/Degree	Title	Department	Phone Number	Email
Colin Sallee	Research Fellow	Pediatrics	XXX-XXX-XXXX	XXX@uw.edu
Brian Befano	Epidemiologist	Epidemiology	XXX-XXX-XXXX	XXX@uw.edu
Mohamed Albirair	Implementation Scientist	Global Health	XXX-XXX-XXXX	XXX@uw.edu
Niki Petrakos	Biostatistician	Biostatistics	XXX-XXX-XXXX	XXX@uw.edu
Ning Yang	Biostatistician	Biostatistics	XXX-XXX-XXXX	XXX@uw.edu

Clinical Research Center: XXX-XXX-XXXX

If you have questions about your rights as a research study participant, you can call the Institutional Review Board at XXX-XXX-XXXX.
24-hour Emergency Contact Number(s): XXX-XXX-XXXX and ask for the Oncology resident on call.

1. **Researchers' Statement**

   You have the option of having your child or teen join a research study. This is a parental permission form. It provides a summary of the information the research team will discuss with you. If you decide that your child can take part in this study, you would sign this form to confirm your decision. If you sign this form, you will receive a signed copy for your records.

   The word "**you**" in this form refers to your child/teen.

   Feel free to take notes, write questions, or highlight any part of this form.

2. **What you should know about this study:**

   - This form explains what would happen if you join this research study.
   - Please read it carefully. Take as much time as you need.

- Please ask the research team questions about anything that is not clear.
- You can ask questions about the study any time.
- If you choose not to be in the study, if will not affect your care.
- If you say "Yes" now, you can still change your mind later.
- You can quit the study at any time.
- You would not lose benefits or be penalized if you decide not to take part in the study or to quit the study later.

3. What is the goal of this study?

The goal of any research study is to answer questions. We (the research team listed on the front of this form and our staff) are doing this research study to answer the following questions:

- Compared to standard of care, does a restrictive transfusion strategy (<7 g/dl) cause a decrease in 28-day mortality?
- Compared to standard of care, does a restrictive transfusion strategy (<7 g/dl) cause a decrease in the occurrence of multiple organ dysfunction syndrome (MODS)?
- Compared to standard of care, does a restrictive transfusion strategy (<7 g/dl) cause a larger decrease in interleukin 6 (IL-6)?
- Do these differences change depending on the patient's immunocompromised status?

4. Why do I have the option of joining the study?

You have the option to take part in this research study because you have pediatric acute respiratory distress syndrome (PARDS) and have been admitted to the PICU.

5. How many people will take part in this study?

We think that about 30 people will take part in this research study at the University of Washington. 638 people will take part at hospitals around the country.

6. If I agree to join this study, what would I need to do?

If you join the study, you would have 5 blood draws, and potentially a more restrictive threshold for blood transfusion.

These blood draws and more restrictive threshold help us find out if being in this study causes any effects that are important to know about. Medical charts will be used to check on the safety of people in the study. We also use them to learn if an experimental treatment is helping or not.

Explanation of Research Tests or Procedures:

The tests that would be done include:

- Blood draws

Research Study Visits:

Visit	Procedures	Location	How much time the visit will take
Day 0	Blood draw	Clinical Research Center (PICU)	5 minutes
Day 3	Blood draw	Clinical Research Center (PICU)	5 minutes

Day 7	Blood draw	Clinical Research Center (PICU)	5 minutes
Day 14	Blood draw	Clinical Research Center (PICU)	5 minutes
Day 28	Blood draw	Clinical Research Center (PICU)	5 minutes

7. How long will I be in the study?

If you choose to take part in all the study visits, you would be in the study for 28 days.

If you join the study, you can decide to stop at any time for any reason. If you decided to stop, you would need to talk with Dr. Befano so you leave the study in a safe way.

8. What are the potential harms or risks if I join this study?

There are potential harms or risks if you take part in this study. Some are common and some are rare. These are described below.

Potential Harms and Discomforts (from the most common to more rare):

- Bruising
- Light-headedness
- Nausea
- Fatigue
- Weakness
- Allergic reactions
- Fever
- Blood infection
- Immunomodulatory complications (including but not limited to febrile non-hemolytic transfusion reactions, urticaria, hemolytic transfusion reactions)
- Bio-chemical complications (e.g., in electrolytes)
- Death

A Data Safety Monitoring Board will review the information from this research study. This board is made of a group of experts. They are responsible for looking at how people in the research study are doing. If you take part, we would tell you about any new information we learn that might affect your health or your willingness to stay in the study.

9. What are the potential benefits if I join this study?

Potential Benefits for You:
Being in this study might benefit you in the following ways:

- You may have less inflammation and a better chance of survival.
- You may feel better.

Potential Benefits for Others:
We hope to use information we get from this study to benefit others who have PARDS.

10. What other options do I have?

If you choose not to be in this study, you can:

- Continue standard treatment for PARDS.

Please talk to your doctor or the research team about these options.

11. How would you keep my information confidential?

If you take part, we will make every effort to keep your information confidential. We will store all of your research records in locked cabinets and secure computer files. We will not put your name on any research data. Instead, we will label your information with a study number. The master list that links a person's name to their study number is stored in a locked cabinet or on a secure computer file.

If results of this research are published, we would not use information that identifies you.

We would only use your information for research. These are some reasons that we may need to share the information you give us with others:

- If it's required by law.
- If we think you or someone else could be harmed.
- Sponsors, government agencies, or research staff sometimes look at forms like this and other study records. They do this to make sure the research is done safely and legally. Anyone who reviews study records would keep your information confidential.
    - Agencies or sponsors that may look at study records include:
        - The US Food and Drug Administration (FDA)
        - Hospital Auditors
        - Government Agencies
        - Others responsible for watching over the safety, effectiveness, and conduct of the research.

If you join this study, we would put information about this study in your medical record. We do this because the research study involves patient care.

We would keep your results until December 31, 2050.

12. Would it cost me money to be in the study?

If you take part in this study, there would be no cost to you and no cost to your insurance company.

13. What if I were injured because I joined this study?

If you were injured as the direct result of this research study, the University of Washington would provide treatment. We would refer you for treatment if needed.

You would NOT need to pay for this treatment and neither would your insurance company. This is the only compensation offered for study-related injuries. It is important that you tell the

Principal Researcher Dr. Sallee if you think that you have been injured because of taking part in this study. You can call him at XXX-XXX-XXXX.

14. Would I be paid if I join this study?

You will not be paid for enrolling in this study. However, you will be reimbursed for any travel expenses for blood draws if you are discharged prior to 28 days after randomization.

15. Who do I contact if I have problems, questions, or want more info?

If I have questions or would like to know about…	You can call…	At…
• Emergencies • General study questions • Research-related injuries • Any research concerns or complaints	Dr. Collin Sallee	Phone: XXX-XXX-XXXX Pager: XXX-XXX-XXXX
• Emergencies • General study questions • Research-related injuries • Any research concerns or complaints	Dr. Brian Befano	Phone: XXX-XXX-XXXX
• Your rights as a research participant	Institutional Review Board This is a group of scientists and community members who make sure research meet legal and ethical standards.	Phone: XXX-XXX-XXXX

More information:
A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by U.S. Law. This website will not include information that can identify you. At most, the website will include a summary of the results. You can search this website at any time.

16. If I join the study, can I stop?

Yes. Taking part in research is always a choice. If you decide to be in the study, you can change your mind at any time. We ask that you tell Dr. Befano. To reach him, please call XXX-XXX-XXXX.

If you choose to leave the study, it will not affect your care at the University of Washington. You will not lose any benefits or be penalized if you choose to leave the study.

17. What would my signature on this form mean?

Your signature on this form would mean:

- The research study was explained to you.
- You had a chance to ask all the questions you have at this time. All your questions have been answered in a way that is clear.
- You understand that the persons listed on this form will answer any other questions you may have about the study or your rights as a research study participant.
- **You have rights as a research participant. We will tell you about new information or changes to the study that may affect your health or your willingness to stay in the study.**
- By signing this consent form, you do not give up any of your legal rights. The researcher(s) or sponsor(s) are not relieved of any liability they may have.
    - You agree to have your child take part in this research study.

Please Note: If the person taking part in this research study is a foster child or a ward of the state, then please tell the researcher or their staff.

_____

Printed Name of Research Participant

_____

Signature of Research Participant (required if 14 years or older)

_____ _____

Date *Time*

_____

Printed Name of Parent or Surrogate Decision Maker

Signature of Parent or Surrogate Decision Maker

Date *Time*

18. Researcher's Signature

I have fully explained the research study described by this form. I have answered the participant and/or parent/guardian's questions and will answer any future questions to the best of my ability. I will tell the family and/or the person taking part in this research of any changes in the procedures or in the possible harms/possible benefits of the study that may affect their health or their willingness to stay in the study.

Printed Name of Researcher Obtaining Parental Permission or Consent

Signature of Researcher Obtaining Parental Permission or Consent

Date *Time*

19. Interpreter Information

_____

Printed Name of Interpreter during initial presentation of study

_____

Signature of Interpreter during initial presentation of study

_____ _____

Date *Time*

20. Witness Information for Short Form Use

14.1 Witness Statement
I have been present during the verbal presentation of this research study.

_____

Printed Name of Witness

_____

Signature of Witness

_____ _____

Date *Time*

Original form to:
Research Team File

Copies to:
Parents/Surrogate Decision Makers
Medical Records

Research Support, Non-U.S. Gov't. *Jama*. Nov 15 2016;316(19):2025-2035. doi:10.1001/jama.2016.9185
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