A Multi-Center, Pilot Study to Assess the Safety and Efficacy of a Selective Cytopheretic Device (SCD) for the treatment of immunomodulatory dysregulation due to pediatric acute kidney injury (AKI).

Protocol Number:	*SCD-PED-02*
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Signature *Date*

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PROTOCOL SYNOPSIS

Study Title	A Multi-Center, Pilot Study to Assess the Safety and Efficacy of a Selective Cytopheretic Device (SCD) for the treatment of immunomodulatory dysregulation due to pediatric acute kidney injury (AKI).
Study Phase	Pilot Study.
Product Description	The Selective Cytopheretic Device (SCD) is comprised of tubing, connectors, and a synthetic membrane cartridge. The device is connected in series to a commercially available Continuous Renal Replacement Therapy (CRRT) device. Blood from the CRRT circuit is diverted after the CRRT hemofilter through to the extra capillary space (ECS) of the SCD. Blood circulates through this space and it is returned to the patient via the venous return line of the CRRT circuit. Regional citrate anticoagulation is used for the entire CRRT and SCD blood circuits. The SCD[redacted] is a synthetic membrane with the ability to bind activated leukocytes and when used in a continuous renal replacement extracorporeal circuit in the presence of regional citrate anticoagulation modulates inflammation.
Rationale	Our primary hypothesis is that up to ten sequential 24-hour SCD treatments will improve survival in pediatric patients with Acute Kidney Injury (AKI) as compared to CRRT alone (standard of care). Further, SCD therapy will reduce the duration of maintenance dialysis secondary to AKI due to acute tubular necrosis (ATN). ATN is an acute reversible process and if not reversed in three months, it may not be reversed at all. [1, 2] Studies suggest that approximately 10-20% of AKI patients do not recover renal function [3, 4] and, therefore, require chronic dialysis. Importantly, acute kidney injury is a highly lethal condition in critically ill patients. Despite improvements in acute medical care and advances in dialysis therapies, the mortality rate during the past four decades of this condition has not improved. Critically ill patients with AKI in hospital ICU settings have mortality rates of approximately 50%, including pediatric patients [5, 6, 7, 8, 9, 10, 11, 12] AKI promotes a systemic inflammatory response syndrome (SIRS) which results in systemic microvascular damage and, if severe, multi-organ dysfunction. [13, 14] Activated circulating

	leukocytes play a central role in this process. [15] Leukocytes, especially neutrophils, are major contributors to the pathogenesis and progression of many inflammatory disorders, including SIRS, sepsis, ischemia reperfusion injury, and acute respiratory distress syndrome (ARDS). Many therapeutic approaches are under investigation to limit the activation and tissue accumulation of leukocytes at sites of inflammation to minimize tissue destruction and disease progression. [16, 17, 18]
Primary Objective	To evaluate the safety of up to ten consecutive 24 hour SCD treatments in the pediatric AKI population being treated with Continuous Renal Replacement Therapy (CVVH, CVVHD, or CVVHDF) with regional citrate anticoagulation.
Secondary Objective	To assess the effect of SCD treatment on various measures of pediatric patient clinical outcomes and to evaluate the integrity of the SCD device and patient safety in SCD treatments from the time of initiation of therapy to as many as ten consecutive 24-hour SCD treatments.
Study Endpoints	In this Pilot study four domains of interest have been designated. The four domains are: • Patient Safety: Safety Endpoint Measures include adverse events, laboratory safety parameters, vital signs, EKG, ventilation status and hemodynamic variables. • Clinical Efficacy: The Primary Clinical Efficacy Endpoint Measure is in-hospital; 28 and 60-day all-cause mortality. Time to renal recovery and necessity for chronic dialysis up to day 60 will also be determined. Additional efficacy endpoints include time to intensive care unit (ICU) discharge, and time to hospital discharge. • Device Integrity and Performance: The Endpoint Measure is SCD use without failure. This measure will help assess the integrity and performance of the SCD device. • SCD Mode of Action: Exploratory Endpoint Measures will include inflammatory cytokines, cell activation parameters and biomarkers at clinical sites with this capability. The analysis will be performed at a central laboratory.
Assessment of Device Integrity and Performance	Criteria for assessment of device integrity and performance include: • Significant Clotting within the Device as assessed by visual inspection. • Evidence of leakage (i.e., cracking/breakage of a port, connector, hemofilter cartridge or tubing).

	• Any unforeseen malfunction that results in the need for discontinuation.
Study Populations	Pediatric patients with a body weight (BW) between ≥10 and ≤ 20kg receiving care in the Intensive Care Units of each participating hospital will be screened daily and identified for participation in the trial.
Study Design	This is an open-label, multi-center Pilot study.
Approximate Number of Subjects	Up to 10 patients will be enrolled in this study.
Approximate Number of Centers	Up to 7 Clinical Centers in the United States will participate in this study.
Duration of Subject Participation	Each patient will be followed for 60 days following enrollment.
Inclusion Criteria	1. A patient, or legal representative, has informed consent form. 2. Must be receiving medical care in an intensive care unit (e.g., PICU, CICU). 3. Age less than 18 years. 4. Body weight ≥10 and ≤ 20kg. 5. Intent to receive full supportive care through aggressive management utilizing all available therapies for a minimum of 96 hours. 6. Clinical diagnosis of AKI requiring CRRT. AKI is defined as acute kidney injury with any one of the following: • Increase in SCr by ≥0.3 mg/dL (≥26.5 μmol/L) within 48 hours or; • Increase in SCr to ≥1.5 times baseline, which is known or presumed to have occurred within the prior 7 days or; • Urine volume <0.5ml/kg/h for 6 hours 7. At least one non-renal organ failure (defined as receiving mechanical ventilation or at least one vasoactive medication to treat hypotension) OR presence (proven or suspected) of sepsis.
Exclusion Criteria	1. Threshold blood pressure of 80/40 mmHg-- patients with both a systolic blood pressure of less than 80 mmHg and a diastolic blood pressure of less than 40 mmHg. 2. Patients with a solid organ transplant or those with a bone marrow or stem cell transplant in the previous 100 days or who have not engrafted. 3. Acute or chronic use of circulatory support device other than ECMO such as LVADs, RVADs, BIVADs.

	4. Presence of preexisting advanced chronic renal failure (i.e., ESRD) requiring chronic renal replacement therapy prior to this episode of acute kidney injury or with pre-existing chronic kidney disease (CKD) defined as an eGFR<30 ml/min/1.73m^2. Patients who have never seen a pediatric nephrologist will be assumed not to have pre-existing CKD. 5. AKI occurring in the setting of burns, obstructive uropathy, scleroderma renal crisis, atheroembolism, functional or surgical nephrectomy, cyclosporine, or tacrolimus nephrotoxicity. 6. Metastatic malignancy which is actively being treated or may be treated by chemotherapy or radiation during the subsequent three month period after study therapy. 7. Chronic immunosuppression with the exception of corticosteroids up to a dose of 10 mg per day. 8. Known positive HIV or AIDS or COVID-19 9. Current Do Not Attempt Resuscitation (DNAR), Allow Natural Death (AND), or withdrawal of care status, or anticipated change in status within the next 7 days. 10. Patient not expected to survive 28 days because of an irreversible medical condition. (This is not restrictive to AKI, and may include situations such as the presence of irreversible brain damage, untreatable malignancy, inoperable life threatening condition, or any condition to which therapy is regarded as futile by the PI.) 11. Any medical condition that the Investigator thinks may interfere with the study objectives. 12. Treating clinician does not feel it is in the best interest of the patient 13. Platelet count <15,000/mm^3 at time of screening. 14. Concurrent enrollment in another interventional clinical trial. Patients enrolled in clinical trials where only measurements and/or samples are taken (NO TEST DEVICE OR TEST DRUG USED) are allowed to participate. 15. Use of any other investigational drug or device within the previous 30 days. 16. Use of an AN-69 hemofilter membranes
Evaluation Plan	The primary evaluation of this study will be safety and all-cause mortality or dialysis dependency through 60 days post-enrollment. Additional endpoints will be time on CRRT, total ICU and hospital days. Mortality and dialysis dependency rates will be compared to historical controls.

LIST OF ABBREVIATIONS

ACD-A	Anticoagulant Citrate Dextrose- Formula A
AE	Adverse Event
AKI	Acute Kidney Injury
AIDS	Acquired Immunodeficiency Syndrome
AND	Allow Natural Death
ARDS	Acute Respiratory Distress Syndrome
ARF	Acute Renal Failure
ASHD	Atherosclerotic heart Disease
ATN	Acute Tubular Necrosis
BRS	Bradykinin Release Syndrome
BW	Body Weight
CV	Cardio-Vascular disease
CBC	Complete Blood Count
CCC	Clinical Coordinating Center
CCHMC	Cincinnati Children's Hospital Medical Center
CICU	Cardiac Intensive Care Unit
CKD	Chronic Kidney Disease
COVID-19	Coronavirus Disease 2019
CRF	Case Report Form
CRRT	Continuous Renal Replacement Therapy
CTICU	Cardiothoracic Intensive Care Unit
CVVH	Continuous Veno-Venous Hemofiltration
CVVHD	Continuous Veno-Venous Hemodialysis
CVVHDF	Continuous Veno-Venous Hemodiafiltration
DCC	Data Coordinating Center
DNAR	Do Not Attempt Resuscitation
DSMB	Data Safety Monitoring Board
ECMO	Extra-corporeal Membrane Oxygenation
ECS	Extra Capillary Space
ECV	Extracorporeal Circuit Volume
ESRD	End Stage Renal Disease
FDA	Federal Drug Administration
FiO2	Fraction of Inspired Oxygen
GCS	Glasgow Coma Score

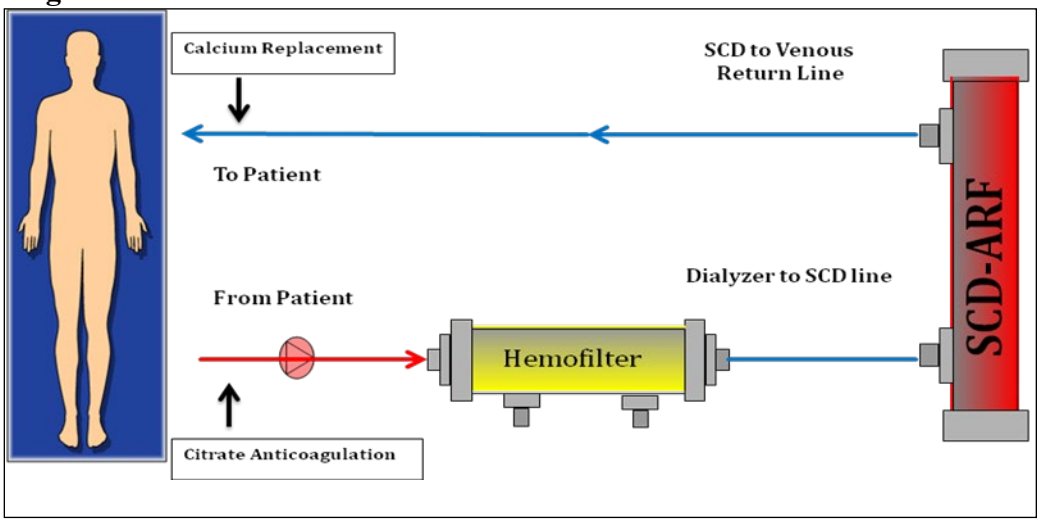
GFR	Glomerular Filtration Rate
HD	Hemodialysis
HIV	Human Immunodeficiency Virus
HRS	Hepatorenal Syndrome
iCa or ionCa	Ionized Calcium
ICU	Intensive Care Unit
IRB	Institutional Review Board
LAR	Legal Authorized Representative
LTFU	Long Term Follow Up
LVAD	Left Ventricular Assist Device
MAP	Mean Arterial Pressure
MICU	Medical Intensive Care Unit
MOF	Multiple Organ Failure
nriCa	Non Recommended Ionized Calcium Range
PEEP	Positive End Expiratory Pressure
pH	Power of Hydrogen
PICU	Pediatric Intensive Care Unit
ppCRRT	Prospective Pediatric CRRT Registry
PRISM	Pediatric Risk of Mortality
RAD	Renal Assist Device
RCA	Regional Citrate Anticoagulation
riCa	Recommended Ionized Calcium Range
SAE	Serious Adverse Event
SaO2	Saturation of Oxygen
SCD	Selective Cytopheretic Device
SCr	Serum Creatinine
SICU	Surgical Intensive Care Unit
SIRS	Systemic Inflammatory Response Syndrome
SOFA	Sequential Organ Failure Assessment
WBC	White Blood Cell
UADE	Unanticipated Adverse Device Effect

1. INTRODUCTION

The Selective Cytopheretic Device (SCD) is comprised of tubing, connectors, and a synthetic membrane cartridge. The device is connected in series to a commercially available Continuous Renal Replacement Therapy (CRRT) device. Blood from the CRRT circuit is diverted after the CRRT hemofilter through to the extra capillary space (ECS) of the SCD. Blood circulates through this space and it is returned to the patient via the venous return line of the CRRT circuit. Regional citrate anticoagulation is used for the entire CRRT and SCD blood circuits.

The SCD[redacted] is a synthetic membrane with the ability to bind activated leukocytes and when used in a continuous renal replacement extracorporeal circuit in the presence of regional citrate anticoagulation modulates inflammation. See **Figure 1**.

Figure 1: Schematic of the CRRT circuit and SCD



1.1 RATIONALE FOR THERAPY

Our primary hypothesis is that up to ten sequential 24-hour SCD treatments will improve survival in patients with Acute Kidney Injury (AKI) as compared to CRRT alone (standard of care). Further, SCD therapy will reduce the duration of maintenance dialysis secondary to AKI due to acute tubular necrosis (ATN). ATN is an acute reversible process and if not reversed in three months, it may not be reversed at all. [1, 2] Studies suggest that approximately 10-20% of AKI patients do not recover renal function [3, 4] and, therefore, require chronic dialysis.

Importantly, acute kidney injury is a highly lethal condition in critically ill patients. Despite improvements in acute medical care and advances in dialysis

therapies, the mortality rate during the past four decades of this condition has not improved. Critically ill patients with AKI in hospital ICU settings have mortality rates of up to 50%, including pediatric patients [5, 6, 7, 8, 9, 10, 11, 12]

AKI promotes a systemic inflammatory response syndrome (SIRS) which results in systemic microvascular damage and, if severe, multi-organ dysfunction. [13, 14] Activated circulating leukocytes play a central role in this process. [15] Leukocytes, especially neutrophils, are major contributors to the pathogenesis and progression of many inflammatory disorders, including SIRS, sepsis, ischemia reperfusion injury, and acute respiratory distress syndrome (ARDS). Many therapeutic approaches are under investigation to limit the activation and tissue accumulation of leukocytes at sites of inflammation to minimize tissue destruction and disease progression. [16, 17, 18] Preclinical studies utilizing a porcine model of septic shock have demonstrated that SCD therapy immunomodulates activated circulating leukocytes. This effect is associated with improvement of multiorgan dysfunction. [19]

1.2 RATIONALE FOR NUMBER OF SCD TREATMENTS FOR UP TO 10 DAYS

In prior studies, 10-35% of patients were still on CRRT at 7 days, including the recently completed SCD-PED-001 pediatric trial. Since the safety profile of the SCD in all the prior clinical studies have demonstrated no device related adverse events, the immunomodulatory effects on circulating white cells will maintain a beneficial response in a subject still requiring CRRT. The understanding of the mechanism of action of the SCD have demonstrated that the low iCa environment in the blood perfusion circuit initiates the natural apoptotic process of the bound neutrophils, thereby suppressing the excessive neutrophil inflammatory activity of the circulating innate immunologic system. The risk of neutropenia and immunosuppression is prevented due to this natural process. In addition, the treatment of four COVID-19 patients with the SCD under expanded access emergency use from 14-17 days had no SCD related adverse events.

In the recently completed SCD-PED-001 pediatric clinical trial, 5 of 14 patients required the full 7 day SCD treatment course, suggesting a subset of patients require more than 7 days of SCD treatment to recover and would benefit for longer duration of SCD treatment.

2. PRIOR CLINICAL EXPERIENCE

2.1 PHASE II RENAL ASSIST DEVICE STUDY (RAD003 IND NUMBER 11077)[20]

2.1.1 STUDY SUMMARY

The poor outcomes in patients with acute kidney injury (AKI) and end-stage renal disease (ESRD) on chronic dialysis are due to immune dysregulation associated with these disorders. Evolving evidence suggests that the kidney, and specifically renal epithelial cells, plays an important role in the immunological response of leukocytes under disease states. Method: In this regard, the development of therapeutic approaches utilizing renal epithelial cells has been tested in preclinical animal models and clinical trials. Results: These approaches have been demonstrated in phase II human trials to improve the survival of intensive care unit patients with AKI and multiorgan failure.

2.1.2 SAFETY RESULTS

All serious adverse events (SAEs) occurring during the first 28 days after randomization were consistent with a seriously ill ICU patient population with AKI receiving CVVH.

2.2 ESRD SAFETY AND BIOINFLAMMATORY ASSAY STUDY

2.2.1 STUDY SUMMARY

A study of the SCD was conducted at the Henry Ford Hospital in Detroit, Michigan entitled “A Phase I/II Trial of a Two-Cartridge Hemodialysis System on Inflammatory Markers in Chronic Hemodialysis Subjects”. This study was designed to determine what effect one treatment with the SCD would have on the reduction of bioinflammatory markers such as cytokines IL-2, IL-6, IL-8 and IL-10 and white cell activation in chronically inflamed End Stage Renal Disease (ESRD). The study enrolled fifteen subjects at one site, with four of the original 15 subjects re-enrolled in what was called “Amendment 2”.

2.2.2 SAFETY RESULTS

Fifteen chronic hemodialysis (HD) subjects with elevated CRP levels were treated for four hours under standard HD with systemic heparin anticoagulation and, at their next dialysis treatment session, received standard HD plus SCD therapy with regional citrate anticoagulation. The 15 subjects have completed the study treatment period and safety data is shown in **Table 1** as follows:

Table 1: Report of Adverse Events - ESRD trial

Adverse Event	Number of Mild Severity Events	Number of Moderate Severity Events
Fever	1	0
Chills	2	0
Headache	1	0
Nausea	3	0
Vomiting	2	1
Diarrhea	1	0
Dizziness	1	1
Visual Disturbances	0	1
Lethargy	0	1
Itching	1	0
Latent TB infection	1	0
Cough	1	0
Left cheek swelling	1	0
Decreased hemoglobin	1	1
Increased CRP level	2	0
Neck swelling cellulites	0	1
Ankle sprain	0	1
Replacement of left dysfunctional IJ catheter for HD	1	0
Dialysis catheter fell off during sleep	0	1
Muscle cramp	0	1
Chest pain	0	1
Clotted extracorporeal system. 350 ml blood loss	0	1
L Upper quadrant pain	0	1
Decreased WBC	0	1

2.3 AKI SAFETY, MORTALITY AND DEVICE INTEGRITY STUDY (CHINA)[21]

2.3.1 STUDY SUMMARY

Despite decades of improvements in the provision of renal replacement therapy, the morbidity and mortality associated with acute kidney injury (AKI) in the intensive care unit (ICU) setting remains extremely high. Much of the morbidity and mortality of this disorder is the consequence of systemic cellular damage that results from immune dysregulation. This was a prospective, single-arm, single-center study designed to evaluate the safety and efficacy of treatment with a Selective Cytopheretic Device (SCD)

on clinical outcomes in AKI requiring renal replacement therapy in the ICU. The subjects enrolled in the trial were compared with historical case-matched controls with respect to age and Sequential Organ Failure Assessment (SOFA) score. The mortality for the case-matched controls was 77.78%, whereas the mortality in the SCD treatment group was 22.22% ($p = 0.027$). Multiple regression analysis identified treatment with SCD as the only significant variable affecting mortality among age, SOFA score, and average change in urine output over the first 7 days during or after treatment. Mean total urine output in the 10 subjects receiving SCD treatment increased from a baseline of approximately 500 ml/d to more than 2,000 ml/d by day 7 of treatment.

2.3.2 SAFETY RESULTS

A total of 12 subjects were enrolled in this study. There were no SAEs reported.

In the 9 subjects analyzed on SCD treatment, no neutropenic events were reported. Mean WBC counts remained normal throughout treatment, with a mild decline noted upon initiation of therapy that was shown to rebound by day 7. No bleeding events were reported. Average platelet counts remained in the functional range (above 50,000) throughout treatment, with a mild decline noted upon initiation of therapy that was shown to plateau by day 4 to an average platelet count of 75,000. A summary of all adverse events (AEs) observed in the 9 subjects on SCD treatment is presented in **Table 2**. These events were not attributed to the device by the investigator, as they commonly occur with CRRT treatment.

Table 2: Adverse event reporting – China Study – n=9 subjects

Adverse Event (n=9 patients)	Number of Mild Severity Events	Number of Moderate Severity Events
Hypercalcemia	6	2
Thrombocytopenia	1	0
Hypocalcemia	0	1
Allergic Reaction	1	0
Hypophosphatemia	2	0
Hypernatremia	1	0

2.4 PILOT STUDY USA ARF-002 - IDE [redacted][22]

2.4.1 STUDY SUMMARY

Acute kidney injury (AKI) is characterized by deterioration in kidney function resulting in multisystem abnormalities. Much of

the morbidity and mortality associated with AKI result from a systemic inflammatory response syndrome (SIRS). This study described herein was a prospective, single-arm, multicenter US study designed to evaluate the safety and efficacy of the Selective Cytopheretic Device (SCD) treatment on AKI requiring continuous renal replacement therapy (CRRT) in the ICU. The study enrolled 35 subjects. The mean age was 56.3 ± 15. With regard to race, 71.4% of the subjects were Caucasian, 22.9% were Black, and 5.7% were Hispanic. Average SOFA score was 11.3 ± 3.6. Death from any cause at Day 60 was 31.4%. Renal recovery, defined as dialysis independence, was observed in all of the surviving subjects at Day 60. The results of this pilot study indicate the potential for a substantial improvement in patient outcomes over standard of care therapy, which is associated with a greater than 50% 60-day mortality in the literature. The SCD warrants further study in scientifically sound, pivotal trial to demonstrate reasonable assurance of safety and effectiveness.

2.4.2 SAFETY RESULTS

A summary of all adverse events (AEs) observed in the 35 subjects is presented in **Table 3**. The AEs observed were those expected for a critically ill patient population with acute renal failure and/or in an ICU setting.

Table 3. Summary of Adverse Events ARF-002

Adverse Events	Number of Subjects with Events N (% of 35)	Number of Events % of 199 (n/199)
Total adverse events	33 (94%)	199
Serious adverse event	23 (66%)	14.1% (28/199)
Unanticipated Adverse Device	0 (0%)	0.0% (0/199)
Relationship to Study*		
Unrelated to study therapy	32 (91%)	93.5% (186/199)
Possibly related to study therapy	8 (23%)	6.0% (12/199)
Probably related to study therapy	0 (0%)	0.0% (0/199)
Definitely related to study	1 (3%)	0.5% (1/199)
Outcome		
Resolved w/ sequelae	14 (40%)	13.6% (27/199)
Resolved w/out sequelae	25 (71%)	64.3% (128/199)
Continuing	11 (31%)	11.1% (22/199)
Death	11 (31%)	5.5% (11/199)
Reported as Unknown	3 (9%)	5.5% (11/199)
Frequency		
Single Episode	29 (83%)	54.3% (108/199)
Intermittent	16 (46%)	21.1% (42/199)
Continuous	16 (46%)	24.1% (48/199)
Reported as Unknown	1 (3%)	0.5% (1/199)

Adverse Events	Number of Subjects with Events N (% of 35)	Number of Events % of 199 (n/199)
Severity		
Mild	21 (60%)	34.7% (69/199)
Moderate	25 (71%)	51.8% (103/199)
Severe	19 (54%)	13.6% (27/199)

*Subjects experienced more than one AE, therefore, numbers do not add up to N=22 (number of subjects experiencing any adverse event).

2.5 PIVOTAL STUDY (PROTOCOL SCD-003) - IDE [redacted] [23]

2.5.1 STUDY SYNOPSIS

Per the SCD-003 Protocol, the primary hypothesis is that up to seven sequential 24-hour SCD treatments will improve survival in patients with Acute Kidney Injury (AKI) as compared to CRRT alone (standard of care). Further, SCD therapy may reduce the duration of maintenance dialysis secondary to AKI due to acute tubular necrosis (ATN). ATN is an acute reversible process and if not reversed in three months, it may not be reversed at all. [1, 2] Studies suggest that approximately 10% of AKI patients do not recover renal function [3, 4] and, therefore, require chronic dialysis. Importantly, acute kidney injury is a highly lethal condition in critically ill patients. Despite improvements in acute medical care and advances in dialysis therapies, the mortality rate during the past four decades of this condition has not improved. Critically ill patients with AKI in hospital ICU settings have mortality rates of 50 to 80%. [5, 6, 7, 8, 9, 10, 11, 12]

The SCD requires regional citrate anticoagulation be used for the entire CRRT and SCD blood circuits. Per SCD-003, Section 5.1: Each participating clinical site is to use their regional citrate anticoagulation protocol for the CRRT and SCD-ARF circuits (Study Arm) and for the CRRT only (Control Arm). The recommended ionized calcium level (measured post SCD-ARF) in the CRRT and SCD-ARF blood circuit should be between 0.25 and 0.40 mmol/L.

2.5.2 SAFETY RESULTS AT INTERIM ANALYSIS

The planned interim analysis was initiated at subject 134 and subject enrollment paused on May 24, 2013, to assess the clinical impact on study endpoints. On September 3, 2013, the Data Safety Monitoring Board (DSMB) convened and reviewed the SCD-003 data. Upon completion of their meeting, they stated that there were no safety concerns with the outcomes presented in the following tables.

Table 4 delineates the Summary of Site-reported Serious Adverse Events (SAEs) using site-reported category and term. None of the SAEs were considered 'definitely' device related per the Principal Investigator.

Table 4: Summary of Site-reported Serious Adverse Events (SAEs).
All Subjects N=132*

	CRRT + SCD N = 69		CRRT Alone N = 63*			Total N =132*	
Category	**Ets**	**Pts % (n/N)**	**Ets**	**Pts % (n/N)**	**Fisher's Exact P-Value**	**Ets**	**Pts % (n/N)**
Total	80	65.2% (45/69)	71	63.5% (40/63)	0.857	151	64.4% (85/132)
Blood and lymphatic system disorders	9	11.6% (8/69)	4	4.8% (3/63)	0.212	13	8.3% (11/132)
Cardiac disorders	15	17.4% (12/69)	11	15.9% (10/63)	1.000	26	16.7% (22/132)
Gastrointestinal disorders	5	5.8% (4/69)	7	9.5% (6/63)	0.518	12	7.6% (10/132)
General disorders and administration site conditions	4	5.8% (4/69)	7	11.1% (7/63)	0.350	11	8.3% (11/132)
Infections and infestations	14	17.4% (12/69)	11	15.9% (10/63)	1.000	25	16.7% (22/132)
Injury, poisoning and procedural complications	1	1.4% (1/69)	0	0.0% (0/63)	1.000	1	0.8% (1/132)
Investigations	0	0.0% (0/69)	1	1.6% (1/63)	0.477	1	0.8% (1/132)
Metabolism and nutrition disorders	2	2.9% (2/69)	2	3.2% (2/63)	1.000	4	3.0% (4/132)
Musculoskeletal and connective tissue disorders	1	1.4% (1/69)	1	1.6% (1/63)	1.000	2	1.5% (2/132)
Nervous system disorders	6	7.2% (5/69)	1	1.6% (1/63)	0.211	7	4.5% (6/132)
Other	2	2.9% (2/69)	6	7.9% (5/63)	0.258	8	5.3% (7/132)
Psychiatric disorders	0	0.0% (0/69)	1	1.6% (1/63)	0.477	1	0.8% (1/132)
Renal and urinary disorders	1	1.4% (1/69)	3	4.8% (3/63)	0.348	4	3.0% (4/132)
Respiratory, thoracic and mediastinal disorders	13	14.5% (10/69)	10	15.9% (10/63)	1.000	23	15.2% (20/132)
Skin and subcutaneous tissue disorders	0	0.0% (0/69)	2	3.2% (2/63)	0.226	2	1.5% (2/132)

Table 4: Summary of Site-reported Serious Adverse Events (SAEs).
All Subjects N=132*

	CRRT + SCD N = 69		CRRT Alone N = 63*			Total N =132*	
Category	**Ets**	**Pts % (n/N)**	**Ets**	**Pts % (n/N)**	**Fisher's Exact P-Value**	**Ets**	**Pts % (n/N)**
Vascular disorders	7	10.1% (7/69)	4	6.3% (4/63)	0.536	11	8.3% (11/132)

*Two subjects enrolled to CRRT alone arm (012-002, 003-015) however not treated.

60 Day Mortality based on the SCD-003 protocol intent to treat is presented in **Table 5**.

Table 5: 60 Day Mortality

60 Day Mortality	CRRT + SCD N= 69	CRRT Alone N= 65	Overall N=134
All Subjects Enrolled	100.0% (69/69)	100.0% (65/65)	100.0% (134/134)
Alive	61% (42/69)	64% (38/59*)	63% (80/128)
Dead	39% (27/69)	36% (21/59*)	38% (48/128)

*Does not include six subjects LTFU (002-003, 011-002, 004-007, 011-004, 013-002, 007-025)

2.5.3 EFFICACY RESULTS AT INTERIM ANALYSIS

As a result of the mortality in Control Arm (CRRT alone) being approximately 10% lower than published levels of 45-50%, further analysis was undertaken. As a result of the analysis, it was discovered that amount of time the subject was maintained in the recommended ionized calcium range (0.25-0.40 mmol/L per SCD-003 protocol) presented a difference in efficacy outcomes. One reason for the deficiency in clinical trial execution to ensure the protocol's riCa target range of ≤0.40 mmol/L be maintained could be attributed to the national shortage of injectable calcium. If the subject did not experience circuit clotting, the PIs emphasis of achieving and continuously maintaining the subject in the recommended ionized calcium range was not consistently adhered to. In addition, the injectable calcium shortage resulted in 9 of the 21 open clinical sites unable to enroll subjects due to low hospital inventories of injectable calcium.

Of the 134 subjects enrolled in the SCD-003 protocol at the time of the interim analysis, 19 SCD subjects (CRRT+SCD) and 31

control subjects (CRRT alone) were maintained in the protocol's recommended range ((≤0.40 mmol/L) ≥90% of the therapy time).

Table 6 details all-cause mortality at day 60 (primary endpoint) of the treated subjects which received the recommended ionized calcium (riCa) and **Table 7** details all-cause mortality at day 60 of the treated subjects which did not receive the recommended ionized calcium (nriCa).

Table 6: 60 Day Mortality of Subjects – Recommended Ionized Calcium Range (riCa)

60 Day Mortality riCa	CRRT + SCD N= 19	CRRT Alone N= 27*	Overall N=46
Alive	84% (16/19)	59% (16/27)	70% (32/46)
Dead	16% (3/19)	41% (11/27)	30% (14/46)
Pearson chi2(1) = 3.2793 Pr = 0.070			

*Three subjects LTFU (004-007, 007-025, 011-004). One subject withdrew consent (013-002).

Table 7: 60 Day Mortality of Subjects – Non Recommended Ionized Calcium Range

60 Day Mortality nriCa	CRRT + SCD N= 50	CRRT Alone N= 32*	Overall N=82
Alive	52% (26/50)	69% (22/32)	59% (48/82)
Dead	48% (24/50)	31% (10/32)	42% (34/82)
Pearson chi2(1) = 2.2555 Pr = 0.133			

*Two subjects LTFU (002-003, 011-002). Two subjects enrolled to CRRT alone arm (012-002, 003-015) however not treated.

A borderline statistically significant difference in 60-day all-cause mortality (primary endpoint) was found in the cohort where the subject was maintained for ≥90% of the treatment in the protocol's recommended ionized calcium (riCa) target range of ≤0.40 mmol/L over those that were maintained ≤90% of the treatment duration.

The secondary endpoints of renal replacement therapy dependency at day 60, mortality at day 28, number of ventilator free days at day 28 and mortality of the sub population of severe septic subjects at day 60 were analyzed. No statistical significance was shown

except for the secondary endpoint of dialysis dependency. Dialysis dependency showed a statistically significant difference between the subjects maintained for ≥90% of the treatment in the protocol's riCa target range of ≤0.40 mmol/L over those at ≤90% of the treatment duration. **See Table 8**.

Table 8: Dialysis Dependency at Day 60 – Recommended Ionized Calcium Range

Dialysis Dependency at Day 60 riCa	**CRRT + SCD**	**CRRT Alone**
N	0/16	4/16
p-value	0.033	

When a composite endpoint of all-cause mortality (primary endpoint) or renal replacement therapy dependency (a secondary endpoint) at day 60 is analyzed, a statistical significance is observed. See **Table 9**.

Table 9: 60 Day Combined End Point of All-Cause Mortality or Dialysis Dependency – Recommended Ionized Calcium Range

60 Day Combined End Point of All-Cause Mortality or Dialysis Dependency -- riCa	**CRRT + SCD N=19**	**CRRT Alone N=27**
	15.8%	55.6%
N	3/19	15/27
p-value	0.007	

2.5.4 CONCLUSION AT INTERIM ANALYSIS

The SCD-003 Protocol included one formal interim effectiveness analysis on the primary endpoint of time to all-cause mortality by 60 days for review by the DSMB. On September 3, 2013, CytoPherx (now SeaStar Medical) received concurrence from the DSMB that they would have recommend continuing the SCD-003 study (with appropriate corrective action to ensure the protocol's riCa target range of <0.40 mmol/L be maintained). However, due to the existing statistical deficit, an increase in sample size to achieve a statistically significant treatment difference on the primary endpoint would have been required. This increase in sample size would be prohibitive for CytoPherx (now SeaStar Medical) to finance.

Rather than continue SCD-003, SeaStar terminated the study and intends to submit to FDA a supplement to IDE [redacted] for

approval of a new clinical trial protocol (no changes to the SCD device) designed to achieve statistical significance of a death or dialysis dependence composite endpoint.

2.6 Clinical Study: SCD-PED-001 (Pilot Safety and Efficacy of SCD treatment in Pediatric Patients with AKI) IDE [redacted]

This was a single treatment arm, open-label multi-center pilot study, grant funded by the FDA, designed to enroll 16 subjects across 4 clinical sites in the United States. The study assessed the safety of the SCD and outcomes in pediatric patients (>15 kg, ≤22 years) with AKI and multiorgan failure receiving CRRT. Sixteen subjects were enrolled between December 2016 and January 2020. In these subjects, the SCD was integrated post CRRT membrane, changed daily, and circuit ionized (i)Ca maintained <0.4 mmol/L. Subjects received SCD treatment for up to 7 days or CRRT discontinuation.

Of the 16 subjects treated in this study, 8 were male and 8 were female. Mean subject age was 12 years (range 4-21yr), weight was 53 kg (range 19-111 kg) and Pediatric Risk of Mortality (PRISM II) score was 7 (range 2-19). Two subjects received ECMO concurrently with CRRT and SCD. The most common ICU diagnosis was shock. Circuit iCa were maintained at <0.4 mmol/L for 90.2% of assessments. Median SCD duration was 6 days (range 1 to 7). Fifteen subjects survived SCD therapy, and 12 subjects survived to ICU discharge. All 12 ICU survivors were dialysis independent at 60 days. No SCD related adverse events were noted. One subject died during treatment as a result of Influenza myocarditis. The 3 subjects who died after SCD treatment was completed but before ICU discharge included one who died as a result of a post op complication and two who died as a result of ECMO complications (intracerebral hemorrhage, withdrawal of care). Further important observations were noted, including the fact that 8 subjects had septic shock or severe pneumonia but only 1 subject death in this group. This 12.5% mortality rate compares to 50% mortality rate in ppCRRT data registry in similar patients with 3 organ failures. Half of the SCD treated subjects in this cohort required CRRT for less than 5 days compared to 9.6 days from the ppCRRT database. This data suggest the SCD is safe in critically ill children who require CRRT. While efficacy claims cannot be made statistically, the 75% survival rate and 100% renal recovery rate in surviving children suggest a favorable benefit to risk ratio.

Over the course of this study, a total of 12 Serious Adverse Events (SAEs) were reported. All 12 of these SAEs were determined by the site PI and confirmed by the PI of the CCC/DCC, to be related to previous medical history or underlying disease processes. Summary of the SAEs from this study are provided in Table 10 below.

Table 10: SAEs by Subject for Study SCD-PED-001

Subject ID	SCD Dates	SAE Date	Description of SAE	Severity	Device Related	Causality to Study	Outcome
CIN-01	NA	12/4/2016	Cardio-respiratory arrest	Severe	No	Unrelated	Resolved w/o Sequelae
CIN-04	5/12/2017 5/15/2017	5/29/2017	Pneumoperitoneum	Severe	No	Unrelated	Resolved w/ Sequelae
CIN-04	5/12/2017 5/15/2017	6/16/2017	Nephrolithiasis	Moderate	No	Unrelated	Resolved w/o Sequelae
ALA-02	7/8/2017 7/14/2017	7/31/2017	Stevens-Johnson Syndrome	Severe	No	Unrelated	Resolved w/o Sequelae
ATL-01	2/14/2018 2/15/2018	2/15/2018	Cardiac Arrest	Severe	No	Unrelated	Death
CIN-07	7/5/2018 7/11/2018	7/8/2018	Junctional Tachycardia	Severe	No	Unrelated	Resolved w/Sequelae
CIN-07	7/5/2018 7/11/2018	7/10/2018	Vascular Graft Occlusion	Moderate	No	Unrelated	Resolved w/o Sequelae
CIN-07	7/5/2018 7/11/2018	7/12/2018	Worsening Respiratory Failure	Severe	No	Unrelated	Death
CIN-09	12/15/2018 12/20/2018	12/18/2018	Cerebral Hemorrhage	Severe	No	Unrelated	Not resolved*
CIN-11	12/4/2019 12/11/2019	1/8/2020	Cardiac Arrest	Severe	No	Unrelated	Resolved w/Sequelae
CIN-12	1/19/2020 1/22/2020	2/24/2020	Pulmonary Hemorrhage	Severe	No	Unrelated	Not resolved*
CIN-12	1/19/2020 1/22/2020	2/24/2020	Adrenal Insufficiency	Severe	No	Unrelated	Not resolved*

*Not resolved events were ongoing at the time of the subject's death but did not cause the subject's death.

No reported adverse event has been considered related to the study device. One incident of leaking from the SCD connectors was reported in February 2018, but no additional reports have been received. Summary adverse event information has is provided in Table 11 and Table 12 below.

Table 11. Summary of Adverse Events for SCD-PED-001

Adverse Events	Number of Subjects with Events N (% of 16)	Number of Events % of 40 (n/40)
Total adverse events	14 (87.5%)	40
Serious adverse events	8 (50%)	30.0% (12/40)
Unanticipated adverse device effect	0 (0%)	0.0% (0/40)
Relationship to Study*		
Unrelated to study therapy	14 (100%)	100.0% (40/40)
Possibly related to study therapy	0 (0%)	0% (0/40)
Probably related to study therapy	0 (0%)	0.0% (0/40)
Definitely related to study therapy	0 (0%)	0.5% (1/40)
Outcome		
Resolved w/ sequelae	3 (7.5%)	7.5% (3/40)
Resolved w/out sequelae	12 (75%)	77.5% (31/40)
Death	2 (12.5%)	5.0% (2/40)
Not Resolved	2 (12.5%)	10.0% (4/40)
Severity		
Mild	5 (31%)	40.0% (16/40)
Moderate	9 (56%)	32.5% (13/40)
Severe	8 (50%)	27.5% (11/40)

Table 12: Summary of Adverse Events for Study SCD-PED-001

	Total Adverse Events	Serious	Device Related Adverse Events	Deaths related to an Adverse Event
Adverse Events	40	10	0	2
Unanticipated Adverse Device Effect	0	0	0	0

2.7 Emergency use of the SCD to treat COVID-19 patients.

SCD treatment for COVID-19 associated ARDS has occurred under the emergency use expanded access mechanism. Four COVID-19 patients have been treated with SCD to date, all at the University of Michigan Hospital. The first 2 patients both had extremely poor prognoses, with hypoxemic respiratory failure on maximal therapy, receiving extracorporeal membrane oxygenation (ECMO). After being placed on SCD therapy, within 12h oxygenation improved for both patients with reduced oxygen requirements and all inflammatory markers were substantively reduced. In the first patient, a remarkable drop was observed in IL-6, decreasing from 231 pg/mL before therapy to 5.65 pg/mL at 30h after the start of SCD therapy [24]. Both patients rapidly improved and were able to be weaned from ECMO, and subsequently released from the hospital alive. The compelling response to SCD therapy in these patients have been recently published [24]. The third patient was chosen for treatment after he continued to deteriorate despite receiving steroids, IL-6 receptor blockade, antivirals, and convalescent plasma treatment. This patient was also receiving CRRT for acute kidney injury. Before SCD therapy, he had a pO2 of 59 on 100% O2 and PEEP 16. Within 6 hours of initiation of SCD treatment, oxygenation improved to pO2 of 117 on 90% O2 improving his pO2/FiO2 ratio from 59 to 130, preventing this patient from requiring ECMO. A fourth patient was treated due to persistent hypoxemic respiratory failure on ECMO after treatment with remdesivir, convalescent plasma, as well as azithromycin and ceftriaxone for superimposed *Staphylococcus aureus* pneumonia. In this patient, inflammatory markers remained persistently elevated during early treatment but had fallen to 22 pg/mL (from 177 pg/mL pre-treatment) for IL-6 and 35 pg/mL (from 134 pg/mL) for IL-10 by the end of SCR treatment on day 12.

No device related SAEs were reported in these patients. Of note, SCD therapy was extended up to 17 days in these patients.

2.8 Ongoing SCD Trials

2.8.1 Investigator Initiated Feasibility Study: "Immunomodulatory Biomimetic Device to Treat Myocardial Stunning in ESRD Patients"

- IDE [redacted], IRB approved
- 10 subjects, single site, one 4 hours SCD treatment in chronic hemodialysis patients with recurrent hemodialysis hypotension due to rapid large volume removal rates.
- SCD treatment vs. control treatment in same subject; myocardial ischemia as measured with Echocardiogram.
- If effective, reduce chronic heart failure progression (5-10% ejection fraction loss annually).

2.8.2 Investigator Initiated Pilot Study to Assess the Safety and Efficacy of a Selective Cytopheretic Device (SCD) to Treat ICU Patients with Acute on Chronic Systolic Heart Failure with Cardiorenal Syndrome Awaiting Left Ventricular Assist Device (LVAD) Implantation or Diuretic Resistant (2 clinical protocols)

- IDE [redacted]; IRB approved
- 10 subjects, single site, non-control trial. Stage 4 CHF patients with worsening renal function from CRS are not eligible for LVAD.
- Daily 6 hour SCD treatment (up to 7 days) to assess improvement in renal function and LV systolic function to become eligible for LVAD implantation.
- Positive efficacy study would be proof of concept of SCD effects to improve LV function and renal function.
- Recent Supplement to include CRS diuretic resistant patients requiring ultrafiltration: 3 treatments during 4 hour UF treatments.

2.8.3 Investigator Initiated Pilot Study to Assess the Safety and Efficacy of a Selective Cytopheretic Device (SCD) to Treat ICU Patients with Acute Kidney Injury (AKI) and Hepatorenal Syndrome (HRS) Type I

- IDE [redacted], IRB approved
- 10 subjects, single site trial in ICU patients with AKI and HRS not on liver transplant list. Prognosis is 80 percent mortality in 90 days.
- Daily 24 hour SCD treatment up to 7 days to assess improvement in renal function and volume removal.

2.8.4 A Multi-Center Pilot Study to Assess the Safety and Efficacy of a Selective Cytopheretic Device (SCD) in Patients Developing Acute Kidney Injury (AKI) or Acute Respiratory Distress Syndrome (ARDS) Associated with COVID-19 Infection (Sponsor: SeaStar Medical)

- IDE [redacted] S34; IRB approved

- 35 subjects, up to 10 clinical sites, treatment arm only, ICU patients with COVID 19 with ARDS and/or AKI requiring CRRT.
- Daily 24 hour SCD treatment up to 10 days to assess safety and efficacy on 60 day mortality and renal recovery.

3. STUDY OBJECTIVES AND ENDPOINTS

3.1 PRIMARY OBJECTIVE

To evaluate the safety of up to ten consecutive 24 hour SCD treatments compared to historic data on in-hospital mortality and on all-cause mortality and dialysis dependency at day 28, and day 60 in the pediatric AKI population being treated with Continuous Renal Replacement Therapy (CVVH, CVVHD, or CVVHDF) with regional citrate anticoagulation.

3.1.1 SECONDARY OBJECTIVE

To assess the effect of SCD treatment on various measures of pediatric patient clinical outcome and to evaluate the integrity of the SCD device and patient safety in SCD treatments from the time of initiation of therapy to as many as ten consecutive 24-hour SCD treatments.

3.2 STUDY ENDPOINT

In this Pilot study four (4) domains of interest have been designated. The four (4) domains are:

1. Patient Safety: Safety Endpoint Measures include adverse events, laboratory safety parameters, vital signs, EKG, ventilation status and hemodynamic variables.
2. Clinical Efficacy: The Primary Clinical Efficacy Endpoint Measure is in-hospital; 28 and 60-day all-cause mortality. Time to renal recovery and necessity for chronic dialysis up to day 60 will also be determined. Additional efficacy endpoints include time to intensive care unit (ICU) discharge, and time to hospital discharge.
3. Device Integrity and Performance: The Endpoint Measure is SCD use without failure. This measure will help assess the integrity and performance of the SCD device.
4. SCD Mode of Action: Exploratory Endpoint Measures will include inflammatory cytokines, cell activation parameters and biomarkers at clinical sites with this capability.

Endpoints are defined as:

ALL-CAUSE MORTALITY:

All-cause mortality is defined as in-hospital, 28-day and 60-day all-cause mortality.

DIALYSIS DEPENDANCY AT DAY 60:
Dialysis dependency at day 60 is defined as patient not receiving any form of intermittent or continuous renal replacement therapy at or within 3 days of 60 days post enrollment in the study with no plans for additional intermittent or continuous renal replacement therapy.

PATIENT SAFETY:
Adverse events, laboratory safety parameters, vital signs, EKG, ventilation status and hemodynamic variables.

DURATION OF RENAL SUPPORT:
The duration of renal support will be defined as the numbers of days from the initiation of renal replacement therapy (start of observation period) to final dialysis treatment. Duration of renal support will be censored if the patient is still dialysis dependent at the time of death or are dependent at day 60.

RECOVERY OF RENAL FUNCTION:
Recovery of renal function will be defined as lack of need for continuous dialysis support on day 28, and will be classified as yes or no at day 28. Subjects who were alive at day 28 will be included in the analysis. Patients who remain dialysis dependent at study completion (day 60) or time of death will be categorized as having no recovery of renal function.

ASSESSMENT OF DEVICE INTEGRITY AND PERFORMANCE
Criteria for assessment of device integrity and performance include:

- Significant Clotting within the Device as assessed by visual inspection
- Evidence of leakage (i.e., cracking/breakage of a port, connector, hemofilter cartridge or tubing).
- Any unforeseen malfunction that results in the need for discontinuation.

This trial is an early safety efficacy study but is not powered for statistical significance. Insights into a comparison of historical controls matched to SCD treated subjects will utilize the ppCRRT Registry to match for: etiology of AKI, co-morbidities, CRRT modality, dose and form of anticoagulant, adverse events. [10]

The ppCRRT Registry has data fields for the primary reason for CRRT, including AKI and its cause, as well as co-morbidities which will be matched for primary organ system involvement. The CRRT modality was evenly distributed between convective and diffusive modality, with 60% of the convective modalities provided as CVVH and 40% as CVVHDF. The anticoagulant protocol will be standardized with ACD-A/$CaCl_2$ and all centers

used the same protocol in ppCRRT Registry as outlined in this study protocol. Thus, the ppCRRT Registry dataset will be able to be matched to our study subject cohort. Also as noted above, subjects will be matched for age, size and PRISM II score.

4. STUDY DESIGN

This is an open-label, multi-center Pilot Study.

5. SELECTION OF PATIENT POPULATION

5.1 STUDY POPULATION

Up to 10 pediatric patients with a clinical diagnosis of AKI requiring CRRT will be enrolled in this study, in up to 7 Clinical Centers in the United States.

Pediatric patients with a body weight (BW) between ≥10 and ≤ 20kg receiving care in the Intensive Care Units of each participating hospital will be screened daily and identified for participation in the trial.

5.2 INCLUSION CRITERIA

1. The patient's parent or legal representative has provided informed consent.
2. Must be receiving medical care in an intensive care unit (e.g., PICU, CICU).
3. Age less than 18 years.
4. Body weight between ≥10 and ≤ 20kg
5. Intent to receive full supportive care through aggressive management utilizing all available therapies for a minimum of 96 hours.
6. Clinical diagnosis of AKI requiring CRRT. AKI is defined as acute kidney injury with any one of the following:
   - Increase in SCr by ≥0.3 mg/dL (≥26.5 μmol/L) within 48 hours or;
   - Increase in SCr to ≥1.5 times baseline, which is known or presumed to have occurred within the prior 7 days or;
   - Urine volume <0.5ml/kg/h for 6 hours
7. At least one non-renal organ failure (defined as receiving mechanical ventilation or at least one vasoactive medication to treat hypotension) OR presence (proven or suspected) of sepsis. Sepsis is defined as at least two of the following criteria in the setting of a probable or documented infection[29]:

Age	1mo-1yr	2-5 yr	6-12 yr	13-17 yr
Tachypnea (breaths/min) or mechanical ventilation not related to underlying neuromuscular disease of the receipt of general anesthesia	> 49	> 22	> 18	> 14
Tachycardia (beats/min)	> 150	> 140	> 130	> 110
Bradycardia if <1 yr (beats/min)	< 109			
Hyper or Hypothermia	> 38.5°C or < 36°C			
WBC ($x10^3/mm^3$)	> 17.5 or < 5	> 15.5 or < 6	> 13.5 or < 4.5	> 11 or < 4.5

5.3 EXCLUSION CRITERIA

1. Threshold blood pressure of 80/40 mmHg-- patients with both a systolic blood pressure of less than 80 mmHg and a diastolic blood pressure of less than 40 mmHg.
2. Patients with a solid organ transplant or those with a bone marrow or stem cell transplant in the previous 100 days or who have not engrafted.
3. Acute or chronic use of circulatory support device, other than ECMO, such as LVADs, RVADs, BIVADs.
4. Presence of preexisting advanced chronic renal failure (i.e., ESRD) requiring chronic renal replacement therapy prior to this episode of acute kidney injury or with pre-existing chronic kidney disease (CKD) defined as a eGFR<30 ml/min/1.73m^2. Patients who have never seen a pediatric nephrologist will be assumed not to have pre-existing CKD.
5. AKI occurring in the setting of burns, obstructive uropathy, scleroderma renal crisis, atheroembolism, functional or surgical nephrectomy, cyclosporine or tacrolimus nephrotoxicity.
6. Metastatic malignancy which is actively being treated or may be treated by chemotherapy or radiation during the subsequent three month period after study therapy.
7. Chronic immunosuppression with the exception of corticosteroids up to a dose of 10 mg per day.
8. Known positive HIV or AIDS or COVID-19
9. Current Do not Attempt Resuscitation (DNAR), Allow Natural Death (AND), or withdrawal of care status, or anticipated change in status within the next 7 days.
10. Patient not expected to survive 28 days because of an irreversible medical condition. (This is not restrictive to AKI, and may include situations such as the presence of irreversible brain damage, untreatable malignancy, inoperable life threatening condition, or any condition to which therapy is regarded as futile by the PI.)

11. Any medical condition that the Investigator thinks may interfere with the study objectives.
12. Treating clinician does not feel it is in the best interest of the patient.
13. Platelet count <15,000/mm^3.
14. Concurrent enrollment in another interventional clinical trial. Patients enrolled in clinical trials where only measurements and or samples are taken (NO TEST DEVICE OR TEST DRUG USED) are allowed to participate.
15. Use of any other investigational drug or device within the previous 30 days.
16. Use of AN-69 hemofilter membranes

6. STUDY ACTIVITIES

6.1 SCREENING PERIOD

The screening period is the time period up to 48 hours before enrollment when a patient is identified, informed consent is obtained, and the patient is evaluated for inclusion in the clinical trial. Parental permission will be obtained from parents and/or legally authorized representatives (LAR) on a written informed consent document. Because of the critically ill nature of the study population, it is expected that most children will not be able to provide assent. Only adverse events directly related to the screening procedures will be reported during this time.

Medical history and test results must be examined to ensure the patient meets all eligibility criteria. Tests that are specific to this protocol (i.e. not standard of care) require the patient or legal representative to have provided written consent for participation.

Any patients that do not meet study criteria, will not be enrolled into the study. These patients will not be counted in the overall study enrollment numbers, but will be listed on the screening log.

Prior to enrollment please contact the study sponsor or designee below to validate that the patient has met all inclusion and exclusion criteria.

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6.2 CRRT PARAMETERS

6.2.1 CITRATE ANTICOAGULATION

Each participating clinical site will use their regional citrate anticoagulation protocol. The clinical site is responsible to achieve and maintain a serum post filter ionized calcium level of <0.40 mmol/L throughout the entire SCD treatment period (day and night). The serum post filter ionized calcium level will be collected from the CRRT Circuit post SCD[redacted]. Examples of regional citrate anticoagulation protocols are included in Appendix D. If the site has difficulty in maintaining post-fiter iCa levels <0.4mM from prior experience with patients in this body weight between 10 and 20 kg body weight, the Sponsor and Lead Investigator will discuss options of citrate protocols to be used in enrolled patients to meet post filter iCa levels <0.4 mM as required during SCD treatment.

See the RCA example protocol in Appendix D.

Clinical sites will utilize the following parameters to alleviate the metabolic derangements associated with regional citrate based anticoagulation:

a) *Alkalosis*: Patients with an arterial pH <7.40 at enrollment will initiate CRRT with solutions containing either 25 or 35 meq/L of base as directed by local standard of care. During the study, if the patient's arterial pH increases to >7.45 OR the patient's serum bicarbonate increases to >35 meq/L, the CRRT solution will be changed to a base concentration of 25 meq/L, OR 0.9% NaCl will be added dialysis fluid to correct the alkalosis per local standard of care.

b) *Citrate lock*: If the patient develops citrate lock, as defined by a serum ionCa <0.95 mmol/L and a total serum Ca >12.5 meq/L, the citrate infusion will be discontinued OR the CRRT clearance will be increased by 50% or per local standard of care, AND CRRT circuit Ca and patient Ca will be checked at least every one hour. For centers that stop citrate, once the patient ionCa is >1.0 mmol/L, the citrate will be restarted at a lower rate determined by the site investigator, and hourly ionCa pairs will be rechecked x 3 to ensure a stable patient ionCa >1 mmol/L and stable circuit ionCa <0.40 mmol/L.

Of importance the clinical plan is to monitor the perfusion circuit iCa levels at least q1h ±30min at the initiation of regional citrate anticoagulation (RCA) until a steady state iCa range is between 0.25 to 0.40 mmol/L. Once established, iCa levels both in the circuit and in the patient will be monitored q6h ±1hr. If the iCa is outside the targeted range or alterations in citrate or calcium infusion rates are made, iCa levels are measured at least q1h ±30min until targeted ranges are once again achieved. Of importance, for efficacy of SCD treatment, iCa levels must be maintained in the recommended range at least 90% of the time, based on a sensitivity analysis showing that the mortality endpoint difference was lower with target iCa 80-85% of total treatment in clinical trial SCD-003.

The protocol example identified in **Appendix D** may be utilized.

6.2.2 BLOOD CIRCUIT VOLUME

The additional blood volume for the SCD is 120 ml. This additional circuit blood volume for the standard SCD in enrolled patients can be handled with blood priming with matched blood similar to the standard practice in neonates. Blood prime will occur if the total extracorporeal circuit volume (ECV) is greater than 10% of the patient's blood volume, based on an estimate of blood volume = 70 ml/kg x patient body weight (kg) [25]. The situation is most commonly encountered in neonatal CRRT, where circuit ECV can represent nearly 50% of patient blood volume, yet the same principles apply for this clinical study. [26]

Hypotension associated with blood priming can occur from transient dilution of vasoactive medications (e.g., norepinephrine), or from exposure to the hyperkalemic, hypocalcemic acidotic nature of blood banked blood, which can cause a bradykinin release syndrome (BRS) when the blood is exposed to the CRRT membrane. The BRS is usually associated with AN-69 membranes that will not be used in this study. Each center has its own blood priming protocol to mitigate the potential for this reaction. However, minimum standards for blood priming in this trial are detailed in **Appendix E** to ensure patient safety. [27]

Patients with AKI requiring CRRT are often hypotensive prior to initiation of CRRT. Initiation of CRRT itself can improve hemodynamics by correcting acidosis and hypocalcemia. Most patients with hemodynamic instability receive vasoactive medications. Since the age range and associated normal blood pressure parameters is wide for this pediatric study, it is difficult to

mandate an age specific lower blood pressure threshold to preclude CRRT initiation, especially if CRRT can address the instability as noted above. However, a threshold BP of 80/40 mmHg-- patients with both a systolic blood pressure of less than 80 mmHg and a diastolic blood pressure of less than 40 mmHg will not be entered into the study.

If a patient develops hypotension or experiences worsening hypotension during CRRT/SCD initiation (defined as a > 10 mmHg decrease in mean arterial pressure (MAP) during the first 15 minutes of CRRT initiation or integration of the SCD into the circuit), the patient, depending on the site PI's judgement, will receive a either a 10 ml/kg crystalloid bolus over 10 minutes or vasoactive medications will either have their dose increased (if already prescribed) or will be initiated. Each center will select the vasoactive medication that is best suited for the clinical indication.

6.2.3 SCD PLACEMENT IN CRRT / ECMO CIRCUIT

Appendix F illustrates the integration of the CRRT circuit and SCD into the ECMO circuit. The CRRT return will be post pump.

Patients who receive CRRT on ECMO almost always develop their AKI, and hence their need for CRRT, after they have been on ECMO for at least 24 hours. Thus, the additional extracorporeal volume (ECV) associated with the CRRT circuit is not a factor, as the blood volume from ECMO is stable. In fact, the contribution of the CRRT circuit is decreased (in terms of percent of ECV) since the ECMO circuit adds to the patient blood volume. The requirement for blood priming will be left to the clinical team treating the patient, as these cases will differ significantly from one another.

The pressure gradients are not an issue with CRRT on ECMO. In the most standard configurations, the CRRT circuit draws blood from the ECMO circuit post-pump and returns blood to the ECMO circuit pre-membrane, which is the safest configuration. The CRRT circuit will therefore see positive pressure from the ECMO circuit, and the current CRRT machines are designed to handle this. The CRRT pumps however, regulate the pressures within the CRRT circuit, so the SCD will see similar pressures compared to a non-ECMO situation.

6.2.4 Concomitant Medications

Since clearance or adsorption of medications have not been completely studied with SCD therapy, dosing of concomitant medications as per other extracorporeal therapies should be employed and evaluated during SCD treatment. In this regard:

- Subjects are permitted to take prescribed medications only.
- Continuously administered intravenous medications should be administered without interruption during SCD therapy
- Essential oral medications should be administered without adjustment of their dosing schedule
- Emergent intermittent intravenous and oral medications will be administered when required, without regard to the timing of SCD therapy
- For antibiotics, subjects will receive standard of care anti-infective dosing according to published schedules adjusted for CRRT [30]. Antibiotic serum levels will also be measured as per standard of care guidelines to ensure effective dosing and avoidance of toxicity.
- For vasopressors, dose adjustments will be made to achieve targeted mean arterial pressure.

A list of critical medications is listed in **Appendix C**. The clinical investigators will be alerted to evaluate dosing schedules for these medications carefully for potential adjustments to achieve desired effects during SCD therapy.

6.3 SCD SCHEDULE OF EVENTS

		SCD TREATMENT PERIOD[1]										POST SCD PERIOD[2]						
	Pre-SCD	Hr 0-24	Hr 24-48	Hr 48-72	Hr 72-96	Hr 96-120	Hr 120-144	Hr 144-168	Hr 168-192	Hr 192-216	Hr 216-240	Hr 0-24	Hr 24-48	Hr 48-72	Hr 72-96	Hr 96-120	Day 28[3]	Day 60[3]
Vital Signs / Physical Assessment																		
ICU Admission Weight	X																	
Temperature, BP, Heart Rate	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		
Daily Fluid Balance and Urine Output	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		
Blood Product Administration	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		
Critical Medications	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		
Physical Exam by Investigator	X											X						
PRISM II Score	X																	
Sepsis Assessment	X																	
Fluid Overload at SCD Initiation	X																	
Microbiology / Culture Data	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		
Concomitant Treatments or Procedures	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		
Laboratory Testing																		
CBC with differential	X	X	X	X	X	X	X	X	X	X	X	X						
Creatinine	X	X	X	X	X	X	X	X	X	X	X	X					X	X
BUN, Na, K, Cl, HCO_3, Ca, Mg, PO_4, iCa	X	X	X	X	X	X	X	X	X	X	X	X						
ALT, AST, Bilirubin, ALP, TP, Albumin, Glucose	X	X	X	X	X	X	X	X	X	X	X							
Blood for Research Biomarkers[4]	X		X		X		X		X		X					X		
Cardiorespiratory Assessment																		
Ventilator Use and Settings	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Arterial Blood Gas[8]	X	X	X	X	X	X	X	X	X	X	X	X						
Vasopressors Administered	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		
ECMO	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Renal Replacement and SCD																		
Post Filter iCa	X[5]	X[6,7]	X[7]	X[7]	X[7]	X[7]	X[7]	X[7]	X[7]	X[7]	X[7]							
SCD Filter Change, Q24Hr ± 4Hrs		X	X	X	X	X	X	X	X	X	X							
CRRT Settings and Other RRT Use	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
CRRT Device and SCD Performance	X	X	X	X	X	X	X	X	X	X	X							
Other – Event Driven																		
Adverse Events	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X[9]	X[9]

[1]SCD Treatment Period lasts 240 hours or until termination criteria are met per Section 7

[2]The Post SCD Period lasts 120 hours or until ICU discharge, whichever comes first

[3]Window for follow up visits is day from SCD initiation +7 days

[4]Blood for research biomarkers should be completed before SCD initiation (or 6 hours prior), as well as 48h, 96h, 144h, 192h, end of SCD, and 120 hours post SCD ±6 hours

[5]Post filter iCa only need recorded in the pre-SCD time period if patient was on CRRT with RCA prior to SCD initiation

[6]Post filter iCa should be obtained Q1Hr± 30min until <0.40 mmol/L for two consecutive measurements within the first 12 hours of SCD

[7]Post filter iCa should be obtained Q1Hr± 30min until post filter iCa level<0.40 mmol/L for two consecutive measurements after any CRRT recirculation or restart, citrate or calcium rate change, and then Q6Hr±1Hr after

[8]An arterial blood gas is only required if the patient is receiving invasive mechanical ventilation and has an arterial line per standard of care

[9]Only SAEs need to be reported on Day 28 and Day 60

6.4 BASELINE PERIOD

The Baseline Period begins at enrollment and ends at the start of the SCD Treatment Period (when the subject starts treatment). The following information will be recorded as close to the start of the SCD Treatment Period as possible. Data from standard of care tests may be used as baseline data, as long as it is captured within the time frames noted.

- **Demographic Data**
  a) Date of birth
  b) Gender
  c) Race/ethnicity
- **Hospitalization Data**
  a) Hospital Admission Date
  b) Hospital Admission Diagnosis
  c) ICU Admission Date
  d) ICU Admission Diagnosis
  e) Medical History
- **Vital Signs/Physical Assessment (within 12 hours prior to the start of SCD Treatment Period unless otherwise indicated)**
  - Temperature
  - Blood Pressure and heart rate
  - Body weight at ICU admission
  - PRISM II Score
  - Physical Exam
  - Urine Output (previous 24 hours)
  - ICU percent fluid overload at CRRT initiation (Defined as [(Fluid In (liters) – Fluid Out (liters))/ICU admission weight (kg)] x 100% where Fluid In and Fluid Out are calculated from ICU admission to CRRT initiation.
- **Clinical Laboratory Tests (within 12 hours prior to the start of SCD Treatment Period)**
  - CBC with differential
  - BUN/Creatinine
  - Na, K, Cl, HCO_3, Ca, Mg, Ionized Ca, PO_4
  - ALT, AST, Bilirubin, ALP, Total Protein, Albumin, Glucose
- **Respiratory**
  - Status – Ventilator support (yes/no; date of intubation)
  - If on Vent: FiO_2
  - If on Vent with an arterial line: Arterial Blood Gas (12 hours prior to start of SCD Treatment Period)
  - If on Vent without an arterial line: Arterial Blood Gas (if available within 12 hours of start of Observation Period per standard of care)
  - ECMO Status (yes/no; date of cannulation)
- **Blood for Research**
  - Blood sample should be collected within 6 hours before the start of the SCD Treatment Period

  - Blood sample will be sent to an outside laboratory for testing of biomarkers. The research laboratory is Innovative BioTherapies located at 650 Avis Dr., Ann Arbor, MI 48108.
- **Other (Event-driven)**
  - Acknowledgement (yes/no) to critical medications, vasopressors, and blood products administered to the patient in the 24 hours prior to start of SCD Treatment Period
  - Adverse Events occurring since enrollment
  - Microbiology/culture data (within the last 72 hr.)

6.5 SCD TREATMENT PERIOD

The SCD Treatment Period begins when the subject starts treatment and will continue up to a maximum of 240 hours from the time of Treatment Start. A subject may begin CRRT therapy and SCD treatment at the same time, which would also mark the beginning of the SCD Treatment Period.

This treatment period has been broken into ten 24 hour time periods. It ends when the subject has reached 240 hours in the treatment period or when the subject meets the termination criteria outlined in **Section 7.2** as determined by the Principal Investigator or Medical Team at the participating Clinical Site.

All tests are scheduled on a 24 hour cycle, or once a day, and can be scheduled to be drawn around each participating site's ICU blood drawing schedule, with the exception of any test that requires a specific draw frequency.

The following information will be obtained for subjects enrolled during the SCD treatment period on a daily basis unless otherwise specified:

- **Vital Signs/Physical Assessment**
  - Temperature
  - Blood Pressure, heart rate
  - Urine Output (previous 24 hours)
  - Net Fluid Balance (previous 24 hours)
- **Clinical Laboratory Tests**
  - CBC with differential
  - BUN, Creatinine
  - Na, K, Cl, HCO_3, Ca, ionized Ca, Mg, PO_4
  - ALT, AST, Bilirubin, ALP, Total Protein, Albumin, Glucose
- **Respiratory**
  - Status – Ventilator support (yes/no; date of intubation or extubation, as applicable)
  - If on Vent: FiO_2
  - If on Vent with an arterial line: Arterial Blood Gas

- If on Vent without an arterial line: Arterial Blood Gas (if completed as standard of care)
- ECMO Status (yes/no; date of cannulation or decannulation as applicable)

- **Blood for Research**
  - Collect at hours 48, 96, 144, 192 and end of SCD treatment (± 6 hours). Blood for biomarkers will be collected as close as possible to the time of SCD scheduled changes in the days required (± 6 hours) if practical and within window.
  - Blood sample will be sent to an outside laboratory (Innovative Therapies) for testing of biomarkers
- **CRRT Parameters (Start at hour 0)**
  - Parameters include modality, blood flow rate, net fluid removal, dialysate or replacement solution flow rate, citrate infusion rate, calcium replacement rate.
  - System performance (circuit survival - event driven)
  - Post filter ionized calcium

Therapy Initiation	Q 1 hr±30min. until post filter iCa level <0.40 mmol/L; for two consecutive measurements within the first 12 hours of SCD then Q 6hr±1hr.
After any Calcium or Citrate rate change	Q 1 hr±30min. until post filter iCa level <0.40 mmol/L; then Q 6hr±1hr.
After any Interruption: Recirculation, Procedure, Restart	Q 1 hr±30min. until post filter iCa level <0.40 mmol/L; then Q 6hr±1hr.

- **SCD Performance (Start at hour 0)**
  - SCD[redacted] performance (event driven)
  - SCD and SCD Blood Tubing Set Change every 24 hour (± 4 hours)

- **Other (Event Driven)**
  - Acknowledgement (yes/no) to critical medications, vasopressors, and blood products administered to the patient
  - Adverse Events
  - Diagnostic/Therapeutic Procedures
  - Microbiology/culture data

6.6 STUDY FOLLOW UP PERIOD

6.6.1 POST SCD TREATMENT PERIOD

This Study Follow Up period has been broken into five 24 hour time periods. It starts when the subject has reached 240 hours in the treatment period or when the subject meets the termination criteria outlined in **Section 7.2**. It ends at 120 hours post SCD or ICU discharge, whichever comes first. Record the following data daily in the Study Follow-up Period unless otherwise noted:

- **Vital Signs/Physical Assessment**
  - Blood pressure, heart rate, temperature
  - Physical Exam (first 24 hours only)
  - Urine Output (from previous 24 hours)
  - Net fluid balance (from previous 24 hours)
- **Clinical Laboratory Tests (first 24 hours only)**
  - CBC with differential
  - BUN, Creatinine
  - Na, K, Cl, HCO_3, Ca, Mg, Ionized Ca, PO_4
- **Blood for Research**
  - Collect at 120 hours post SCD ± 6 hours
  - Blood sample will be sent to an outside laboratory (Innovative Biotherapies) for testing of biomarkers
- **Respiratory**
  - Status – Ventilator support (yes/no)
  - If on Vent: FiO_2
  - If on Vent with an arterial line: Arterial Blood Gas (first 24 hours only)
  - If on Vent without an arterial line: Arterial Blood Gas (if completed as standard of care, first 24 hours only)
  - ECMO Status (yes/no)
- **Renal Replacement Therapy status**
  - RRT status
- **Other (Event Driven)**
  - Microbiology/culture data
  - Adverse Events
  - Acknowledgement (yes/no) to antibacterial agents administered to the patient.
  - Concomitant treatments or procedures

6.6.2 DAY 28 POST ENROLLMENT

This visit may be done via telephone call or office visit. Please record the following information on day 28 (+7 days) following enrollment:

- Presence / date and setting of mechanical ventilation (since last visit)
- Presence / date and settings of renal replacement therapy (since last visit)
- Serious Adverse events (since last visit)

- Serum creatinine (if an office visit takes place)
- ICU discharge date
- Hospital discharge date

6.6.3 DAY 60 POST ENROLLMENT

This visit may be done via telephone call or office visit. Please record the following information on day 60 (+7 days) following enrollment:

- Presence / dates of mechanical ventilation (since last visit)
- Presence / dates of renal replacement therapy (since last visit)
- Serious Adverse events (since last visit)
- Serum creatinine (if an office visit takes place)

7. TREATMENT INTERRUPTIONS – DISCONTINUATION CRITERIA

7.1. TREATMENT DISCONTINUATION CRITERIA

Subjects may be withdrawn from therapy prior to hour 240 for a variety of reasons. When therapy is discontinued prior to hour 240, the follow-up period will immediately begin and data will continue to be collected per protocol.

The Principal Investigator will assign a primary reason for therapy termination utilizing the categories listed in 7.2 and 7.3 below, and if appropriate, one or more subcategories. The Principal Investigator will notify the Sponsor or designee within 24 hours of withdrawal of participation.

7.2. CLINICAL CRITERIA:

1. **Improvement in Clinical Status**
   If a subject's condition improves (e.g. renal condition has improved to the extent that CRRT can be discontinued or hemodynamic status has improved so that the dialysis modality can be changed from CRRT to IHD) treatment may be discontinued before the 240 hour treatment period. The decision to discontinue treatment based upon improvement in clinical status will be made by the Principal Investigator.

2. **CRRT related events**
   Such as inability to maintain vascular access based upon PI's clinical assessment. Includes failure of the CRRT device and/or circuit that requires treatment to be discontinued prior to hour 240 of therapy.

3. **SCD related medical events**
   Such as persistent leukopenia, neutropenia or thrombocytopenia based upon PI's clinical assessment of relationship to SCD.

4. **SCD Failure/Malfunction**
   Any failure of the SCD that requires treatment to be discontinued prior to hour 240 of therapy e.g. clotting or evidence of leakage in the SCD or inability to maintain a patent circuit.

5. **Death or withdrawal of life support**

6. **Regional Citrate Anticoagulation Intolerance**
   All subjects must be able to tolerate and achieve satisfactory regional citrate anticoagulation. Tolerance of regional citrate anticoagulation is defined as achieving:
   - Two (2) consecutive circuit ionized calcium levels of < 0.40 mmol/L (or < 1.6 mg/dL),
   - At least 30 minutes apart, and
   - Within the first 12 hours of SCD initiation.

   If unable to achieve the specific circuit ionized calcium levels within the time frame above, treatment with the SCD must be discontinued. The subject will progress on to the post-SCD follow up period, unless the subject and/or legal guardian withdraws consent for the study.

7. **Hematologic Events**
   - Persistent leucopenia, neutropenia, thrombocytopenia based upon PI's clinical assessment
   - Absolute neutrophil count less than 500 mm3 will be an indicator for therapy termination
   - Platelet count less than 30,000 mm3 after transfusion of 6 units of platelets

8. **Other**
   Any other reason that the Principal Investigator deems appropriate for discontinuation from the SCD Treatment period must be documented.

7.3. NON-CLINICAL CRITERIA:

- **Withdrawal of participation from SCD treatment period**
  Subjects may withdraw their consent to participate in the study observation period at any time. If the subject wishes to remain in the clinical study, the follow-up period will immediately begin at the termination of SCD treatment period. All data will continue to be collected per protocol.

  The Principal Investigator will notify the Sponsor or designee within 24 hours of withdrawal of participation.

- **Withdrawal of consent**

If a subject or legal representative withdraws consent, all protocol related activities will be immediately discontinued. If study therapy (CRRT+SCD) is being administered at the time of withdrawal, normal procedures will be followed for CRRT+SCD therapy discontinuation (please see SCD Operator's Manual). No further study activity will be conducted.

The Principal Investigator will notify the Sponsor or designee within 24 hours of withdrawal of consent.

8. CLINICAL TRIAL TERMINATION CRITERIA

The Sponsor reserves the right to terminate the clinical trial for safety or administrative reasons at any time. If the Sponsor, Principal Investigator, Data Safety Monitoring Board (DSMB), Institutional Review Board (IRB), or Food and Drug Administration (FDA) officials discover conditions during the study indicating that the trial or participation by a clinical site should be discontinued, this action may be taken after appropriate consultation between the Sponsor and the Investigators.

Conditions that may warrant discontinuation of the trial at a specific clinical site may include the following:

- Failure of the Investigator to enroll patients into the trial at an acceptable rate (as defined and agreed upon between the Investigator and the Sponsor).
- Failure of the Investigator to comply with the pertinent FDA regulations.
- Submission of false information by the Investigator from the research facility to the Sponsor or the FDA.
- Failure to achieve and maintain post filter iCa levels of <0.40 mmol/L

Conditions that may warrant discontinuation of the trial may include, but are not limited to, the following:

- Discovery of an unexpected, serious or unacceptable risk to the patients enrolled in the study.
- Decision on the part of the Sponsor to suspend or discontinue testing, evaluation or development of the study product at any time.

9. SAFETY

9.1. ADVERSE EVENTS

An Adverse Event is any sign, symptom, illness, clinically significant abnormal laboratory value or other adverse medical event that appears for the first time or worsens in a subject during this clinical study, regardless of whether or not it is considered related to the device.

Anticipated adverse events that may occur during CRRT include:

- thrombocytopenia
- hypo- or hypernatremia
- hypo- or hyperkalemia
- hypo- or hypercalcemia
- hypo- or hyperglycemia
- other electrolyte imbalance
- air embolism
- hypotension
- hypertension
- hemolysis
- increased oxygenation requirements
- fatigue
- dyspnea
- muscle spasm
- myalgia
- nausea
- vomiting
- fluid imbalance
- leukopenia
- arrhythmias
- hypothermia
- hyperthermia
- lactic acidosis
- temporary decrease in cardiac output or cardiac index
- disruption of skin integrity
- paresthesia
- tetany
- altered mental status
- bleeding
- shock
- infection
- bacteremia
- laryngospasm
- seizure
- death

Anticipated AEs associated with the addition of SCD to CRRT include:

- neutropenia
- thrombocytopenia
- hypotension
- hypo- or hypercalcemia
- alkalosis
- clotting within the device
- leakage due to cracking or breakage

9.2. SERIOUS ADVERSE EVENTS

Any Adverse Event, whether considered study-treatment related or not, which fits any of the criteria below, is considered a serious adverse event (SAE):

- Results in death
- Is life-threatening (meaning that the patient was at risk of death at the time of the event; this does not refer to an event which might have caused death if it had occurred in a more severe form)
- Requires in-patient hospitalization or prolongs the existing hospitalization
- Is a persistent disability/incapacity
- Is a congenital anomaly or birth defect
- Is considered an important medical event by the Principal Investigator (e.g., surgery, return to ICU, emergency procedures)

9.3. UNANTICIPATED ADVERSE DEVICE EFFECT

Unanticipated adverse device effect (UADE) is any serious adverse effect on health or safety, any life-threatening problem or death caused by, or associated with a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the application; or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects. The Sponsor-Investigator will report UADEs to the FDA as they occur (e.g., no later than 10 working days after the Sponsor Investigator first receives notice of the effect), as mandated by 21 CFR 812.150(b)(1).

9.4. ANTICIPATED ADVERSE EVENT

Co-morbidities and symptoms/laboratory/physiological deviations normally associated with pre-existing conditions (e.g., diabetes, ASHD, other CV conditions, pneumonia, dialysis shunt problems, neurologic deficits) are considered “anticipated adverse reactions.” The investigator is required to take special care in differentiating concomitant illness events from those related to the therapy by use of subject history, relationship to treatment time and cartridge integrity, and other characteristics of clinical circumstances present at the time of the adverse experience, including drug interactions of concomitant medications and effects of surgical and/or medical procedures.

Adverse events associated with CRRT or underlying critical illness are also to be considered “anticipated”. Such events include but are not limited to the list in **Section 9.1** above.

Any questions as to the expectedness of an adverse event will be discussed with Drs Yessayan and Goldstein. Final review and assessment will be made by the Sponsor-Investigator, Dr. Yessayan and the DSMB.

9.5. CAUSALITY

All enrolled subjects must have all AEs assessed for causality (probability that the AE may have been caused by the study treatment) by the Principal Investigator. The following definitions for causality assessment will be used in this study:

9.5.1. DEFINITELY RELATED

A clinical event, including a significant change in a laboratory test, that occurs in a plausible time relationship to the SCD treatment or other protocol-required activity, and which cannot be explained by concurrent disease or other drugs, chemicals, or procedures and that follows a clinically reasonable response upon withdrawal of the SCD treatment.

9.5.2. PROBABLY RELATED

A clinical event, including a significant change in a laboratory test, that occurs within a reasonable time sequence in relationship to the SCD treatment or other protocol-required activity that is unlikely to be attributed to concurrent disease, other drugs, chemicals, or procedures, and that follows a clinically reasonable response upon withdrawal of the SCD treatment.

9.5.3. POSSIBLY RELATED

A clinical event, including a significant change in a laboratory test, that occurs within a reasonable time sequence in relationship to the SCD treatment or other protocol-related activity that could also be explained by concurrent disease, drugs, chemicals, or procedures. The clinical course after withdrawal of the SCD treatment may be unclear with respect to the contribution of the SCD treatment to the AE.

9.5.4. UNRELATED

An AE, including a significant change in a laboratory test, that occurs with a temporal relationship to the SCD treatment or protocol-required activity that makes an association with the SCD treatment or study activity improbable, and in which other drugs, procedures or underlying disease(s) provide likely explanation.

9.6. SEVERITY – INTENSITY

The intensity of all adverse events should be evaluated using the following definitions:

9.6.1. MILD

An event that requires minimal clinical treatment or an adverse event requiring monitoring but no intervention or treatment; causes slight discomfort.

9.6.2. MODERATE

An event that requires non-routine intervention, (i.e., a new clinical treatment or diagnostic procedure), administered within an hour of the event; causes annoying discomfort.

9.6.3. SEVERE

An event requiring immediate intervention; causes significant discomfort.

9.7. ADVERSE EVENT REPORTING

Adverse event reporting requirements will be based on the time period in which the adverse event occurs. It is understood that many of the signs and symptoms observed with ICU patients are expected. However, these should still be reported as adverse events if they meet the definitions of the Protocol. A "clinical care" event should therefore be reported as an adverse event if it meets any of the definitions. A group of symptoms that can be combined under one diagnosis, should be reported as a single adverse event. If a group of symptoms cannot be combined under one diagnosis, the symptoms should be reported as separate adverse events. These events will be reported as the number of events per time of exposure to the device.

9.7.1. SCREENING AND ENROLLMENT PERIOD

Only adverse events, serious and not serious, directly related to the screening procedures will be captured during this period. Unrelated clinical adverse events that occur prior to SCD treatment will be considered part of the medical history.

9.7.2. TREATMENT PERIOD THROUGH END OF STUDY

All adverse events that occur from the time of SCD start will be recorded as follows:

- Serious Adverse Events (identified in **Section 9.2**) will be recorded until Day 60 after enrollment, death, or subject withdrawal of study consent, whichever occurs first.
- Non-serious adverse events will be recorded until end of 120 hours after the SCD Treatment Period ends, death, subject withdrawal of study consent or until ICU discharge, whichever occurs first.

Any serious adverse event (regardless of relationship to the

treatment) occurring from the time of enrollment through end of study must be reported to the CCC and DCC at Cincinnati Children's Hospital, within 24 hours of knowledge of the event.

Each clinical trial site will be supplied with written SAE reporting instructions, SAE reporting forms and contact information for reporting of serious adverse events.

For any AE that is ongoing at the time of the initial report, periodic follow-up information will be required until the adverse event is resolved or the patient is no longer in the study, whichever occurs first.

The Investigator is responsible for all adverse events reporting to the Institutional Review Board (IRB) according to the requirements of the IRB and for providing clinical trial monitors with all medical records needed to source-verify the adverse events.

9.8. SPONSOR INVESTIGATOR RESPONSIBILITIES

The Sponsor-Investigator will review documented serious adverse effects and abnormal test findings to determine 1) if the abnormal test finding should be classified as an adverse effect; 2) if there is a reasonable possibility that the adverse effect was caused by the investigational device or, if applicable, other study treatment or diagnostic product(s); and 3) if the adverse effect meets the criteria for a *serious adverse effect*.

In accordance with 21 CFR Part 812.150(a)(1) and (b)(1), the sponsor shall promptly report the results of an evaluation of any serious and unanticipated adverse device effect to FDA, the University of Michigan IRBMED and participating investigators (if any) as soon as possible, but not later than 10 working days after the sponsor first receives notice of the effect. Thereafter, the sponsor shall submit such additional reports concerning the effect as the FDA requests. Complications and non-serious or anticipated adverse events should be documented and tabulated but need not be submitted by the sponsor to the FDA as individual reports.

10. DEVICE ACCOUNTABILITY

All use of the SCD will be under the direct supervision of the principal investigator or his/her designee. The investigational devices will be clearly labeled as investigational use only and have a clearly marked serial number for each device. The receipt date and lot number will be recorded, and package sterility confirmed. The devices will be stored in a secure location with access limited to the study team.

All records of receipt, use, and disposition of the devices will be maintained by the study team. At the completion of the study, there will be a final reconciliation by study personnel of devices shipped, used, and devices remaining. Any discrepancies noted will be investigated, resolved, and documented prior to return or destruction of unused devices.

11. DATA SAFETY MONITORING BOARD

An independent Data Safety Monitoring Board (DSMB) will review safety results over the course of the study on a schedule set by Drs. Yessayan and Goldstein and the DSMB. The DSMB will comply with the FDA Guidance: "Guidance for Clinical Trial Sponsors on the Establishment and Operation of Clinical Trial Data Monitoring Committees". The DSMB will be formed to provide scientific and medical feedback for the study.

The DSMB will focus on the following areas:

- *Performance* – to assess study conduct and compliance
- *Effectiveness* – to assess mortality and dialysis dependency
- *Safety* – to assess the incidence, severity, relationship, and timing of adverse events and to identify safety concerns; and
- *Context* – to assess the study relative to the AKI literature.

The DSMB will reflect the disciplines and medical specialties necessary to interpret the data from the clinical study and to fully evaluate participant safety. The SCD-PED-02 DSMB will be made up of at least 2 medical practitioners with expertise in AKI and critical care medicine. The DSMB will operate under a charter and will identify prospective data display specifications to conduct independent data reviews. The DSMB will make recommendations to the Sponsor to continue the study as planned or to stop the study due to safety. The DSMB will meet to perform safety evaluations and to provide feedback to the Sponsor Investigator at least quarterly. The DSMB reserves the right to request more frequent evaluations.

12. PROTOCOL ANALYSIS / SCIENTIFIC SOUNDNESS

This study derives from the hypothesis that up to ten or less sequential 24 hour SCD treatments will improve survival in patients with AKI requiring CRRT as compared to historical data. Further, SCD therapy may reduce the duration of maintenance dialysis secondary to acute renal failure (ARF) due to acute kidney injury (AKI).

The precise mechanism of action of the SCD is becoming better understood and appears to be an immunomodulatory process which inhibits leukocyte activation, a trigger of systemic inflammatory response syndrome (SIRS) and multi-system organ failure. The modulation of the pro-inflammatory state is believed to also allow recovery of renal function in AKI and other organ failure. The cartridge in

the presence of citrate anticoagulant acts as a selective cytopheretic device to sequester and inhibit potentially damaging circulating leukocytes.

Recent data have demonstrated, that the inflammatory response of neutrophils adhered to a [redacted] membrane in the presence of low iCa and citrate are released with a normalized apoptotic life span, compared to a delayed apoptotic rate promoted in systemic inflammation. Leukocytes, especially neutrophils, are major contributors to the pathogenesis and progression of clinical inflammatory disorders, including SIRS, sepsis, ischemia/reperfusion injury and ARDS. Further data have demonstrated that monocytes with a proinflammatory phenotype, binds more avidly than monocytes with a reparative phenotype to the membrane in low iCa environment. This selective binding results in a less inflammatory circulating monocyte population in disorders of inflammation. The protocol has been carefully constructed with precise AKI diagnostic criteria, CRRT or comparable mode treatment and definition of patient's age, need to tolerate citrate anticoagulation and placement in the ICU during SCD therapy. Subject exclusion criteria are also detailed so as to provide a well characterized and standardized subject population. Observational periods and a complete schedule of events in the protocol delineate specific efficacy measurements as required to test the study hypothesis, including bio-inflammatory assay markers, and WBC activation studies.

Besides the test of the hypothesis the study is focused on observable, empirical and laboratory determinations of subject safety while on the SCD. Previous human trials have been conducted on the SCD device and the protocol contains a review of AE and SAE data from all SCD human studies and findings from those studies contributed to constructing the investigative safety profile. Safety endpoint measures specified in the protocol include adverse events, laboratory safety parameters, vital signs, ventilation status and hemodynamic variables. Device integrity and performance measurements are also defined as are circumstances which demand premature termination of SCD treatment.

Prolonged exposure to the SCD is anticipated to result in superior outcomes, although as noted above, this pilot study is not designed to assess for efficacy. However, we understand that a minimum amount of exposure to the SCD will be required for an appropriate safety analysis and for making any inference regarding efficacy. The ppCRRT experience demonstrated that the majority of patients (201/370) received CRRT for 1 to 7 days and exhibited 65% survival (Symons et al CJASN 2007 Table7).

This trial is an early safety efficacy study but is not powered for statistical significance. Insights into a comparison of historical controls matched to SCD treated subjects will utilize the ppCRRT Registry to match for: etiology of AKI, co-morbidities, CRRT modality, dose and form of anticoagulant, adverse events. [10]

The ppCRRT Registry has data fields for the primary reason for CRRT, including AKI and its cause, as well as co-morbidities which will be matched for primary organ system involvement. The CRRT modality was evenly distributed between convective and diffusive modality, with 60% of the convective modalities provided as CVVH and 40% as CVVHDF. The anticoagulant protocol will be standardized with ACD-A/$CaCl_2$ and all centers used the same protocol in ppCRRT Registry as outlined in this study protocol. Thus, the ppCRRT Registry dataset will be able to be matched to our study subject cohort. Also as noted above, subjects will be matched for age, size and PRISM II score.

The Prospective Pediatric CRRT (ppCRRT) database will be used as a control for the CRRT treatment related factors including: patient age, gender, primary cause of AKI, total treatment duration, CRRT modality (CVVH, CVVHD, CVVHDF), CRRT small solute clearance (ml/1.73m2/hour), baseline estimated GFR (using the original Schwartz formula, which was used in the ppCRRT), urine output in the 24 hours prior to CRRT initiation (ml/kg/hour), percent fluid overload at CRRT initiation based on admission ICU weight, vasoactive medication number at time of CRRT initiation and mean airway pressure at CRRT initiation.

An additional analysis will also evaluate patients who have achieved targeted iCa levels during 90% of treatment time and those who do not.

In summary this protocol meets all criteria necessary for clinical scientific inquiry related to a test of hypothesis in a well monitored environment focused on subject safety during the conduct of the study. Basic statistics will be performed on the data sets along with historical data comparisons and subjected to subset analysis where appropriate. The results will help to guide the design of a future pivotal study of this device.

The rational for therapy, study objectives and endpoints, design, inclusion and exclusion criteria are explicitly defined. Subject safety parameters are well documented including safety reporting, safety observations and assays, procedures for subject withdrawal and study discontinuation criteria along with monitoring device integrity and performance. Lastly, a review of all prior SCD human study safety data is included in the protocol for full disclosure to study investigators and the informed consent document includes a full description of all known and potential risks of the device treatment.

13. DATA COLLECTION – STUDY MONITORING AND AUDIT

The Principal Investigator at each clinical site is responsible for assuring the accuracy and completeness of all study documentation. To assure adequate protection of the rights of human subjects, per 21 CFR §812.40, 812.43 and 812.46, this study will be monitored by the Clinical Coordinating Center (CCC) and the Data Coordinating Center (DCC) at Cincinnati Children's Hospital. Monitoring will be conducted within ICH/GCP Guidelines. Clinical Monitoring Plan to ensure the following:

- The facilities continue to be acceptable.
- The protocol is appropriately followed.
- Any agreed upon changes to the protocol have been approved by the IRB
- Accurate, complete and current records are maintained and available for all patients.
- For each subject. collection of protocol-required data and entry of that data into the CRFs must be completed as soon as reasonably possible following that subject's last day in each study period.
- The information recorded and submitted to the CCC and the DCC is representative of the patient's record and other supporting documentation.
- Accurate, complete and timely adverse event reports for serious adverse events are submitted to the CCC and the DCC.
- The Principal Investigator continues to assume primary responsibility for the study.

The Investigator or designee must, upon request, provide to the CCC and the DCC monitors or FDA Investigator the necessary study records for a thorough review of the study's progress. These records include, but are not limited to, original documents and records, such as hospital and clinic charts, consent forms, and laboratory records. The Principal Investigator is required to notify the Sponsor Investigator immediately of the following:

- Withdrawal of IRB Approval
- Any protocol violations or protocol deviations related to study device use
- Notification that Informed Consent was not obtained or consent was withdrawn
- Any other instance in which the Investigator or Sponsor deems it necessary

Additional visits can be scheduled at the request of the Sponsor-Investigator.

14. INVESTIGATOR'S STATEMENT OF RESPONSIBILITY

By my signature, I confirm that my staff and I have carefully read and understand this protocol and agree to comply with the conduct of the specified therein, In particular we have agreed to:

- Conduct the study according to the protocol, amendments, and study guides.
- Obtain Institutional Review Board approval of the study, any amendments to the study and periodic-re-approval, as required.
- Obtain written consent from each study participant or their legal representative.
- Report all serious adverse events to and to the IRB, as required by the protocol and IRB regulations.
- Assure access by study monitors to original source documents.
- Cooperate fully with CCC and the DCC Monitors, or the Food and Drug Administration (FDA).
- Maintain confidentiality and assure security of confidential documents such as the protocol, consent form, case report form, SCD Operator's Manual, final study reports, manuscript, and/or unpublished data and correspondence.
- Achieve and maintain serum post filter ionized calcium levels of <0.40 mmol/L.

_____ _____

Principal Investigator Signature Date

_____

Printed Name

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APPENDIX A – PEDIATRIC RISK OF MORTALITY (PRISM II) SCORE [28]

ORGAN SYSTEM	MEASURE
Respiration	PaO_2 to FiO_2 Ratio, P_aCO_2, Resp. Rate
Coagulation	Protime, PTT
Liver	Serum Total Bilirubin
Cardiovascular	Systolic BP, Diastolic BP, Heart rate
Central nervous system	Glasgow Coma Score, Pupillary Reflexes
Metabolic	Serum potassium, calcium, glucose, bicarbonate glucose

ORGAN SYSTEM	MEASURE	FINDING	POINTS
Respiration	PaO_2 to FiO_2 Ratio (please see Pulmonary System Conversion Table for Non-ventilated Pts)	200 – 300 mmHg	2
		<200 mmHg	3
	P_aCO_2	51-65 mmHg	1
		>65	5
	Respiratory Rate (breath/min)	51-70	1
		>70 or Apnea	5
Coagulation	Protime or Partial Thromboplastin Time	1.5 x control	2
Liver	Serum Total Bilirubin	>3.5 mg/dL	6
Cardiovascular	Systolic Blood Pressure (mmHg)	150-200 or 65-75	2
		>200 or 50-64	6
	Diastolic Blood Pressure (mmHg)	>110	6
	Heart Rate (beat/min)	>150 or <80	4
Central Nervous System	Glasgow Coma Score	<8	6
	Pupillary Reactions	Unequal or dilated	4
		Fixed and dilated	10
Metabolic	Potassium (mEq/L)	3.0 – 3.5 or 6.5 – 7.5	1
		<3.0 or >7.5	5
	Calcium (mg/dL)	7.0 – 8.0	2
		12.0 – 15.0 or <7.0	6
	Glucose (mg/dL)	40-60 or 250 – 400	8
		<40 or >400	8
	Bicarbonate (mEq/L)	<16 or >32	3

Conventions used for the above table include:

- PaO_2 is in mmHg and FiO_2 in percent from 0.21 to 1.00.

APPENDIX A (Continued)
PEDIATRIC RISK OF MORTALITY (PRISM II) SCORE [28]

GLASGOW COMA SCORE

The Glasgow Coma Score (GCS) is scored between 3 and 15, 3 being the worst and 15 the best. It is composed of the three parameters listed below:

Best Eye Response (4)

1. No eye opening
2. Eye opening to pain
3. Eye opening to verbal command
4. Eyes open spontaneously

Best Verbal Response (5)

1. No verbal response
2. Incomprehensible sounds
3. Inappropriate words
4. Confused
5. Oriented

Best Motor Response (6)

1. No motor response
2. Extension to pain
3. Flexion to pain
4. Withdrawal from pain
5. Localizing pain
6. Obeys commands

A Coma Score of 13 or higher correlates with a mild brain injury, 9 to 12 is a moderate injury and 8 or less a severe brain injury.

APPENDIX A (Continued)
PEDIATRIC RISK OF MORTALITY (PRISM II) SCORE [28]

PULMONARY SYSTEM CONVERSION TABLE

O_2 Saturation Conversion Table		**Conversion Table for FiO_2**
Pulse oximetry O_2 saturation may be used for calculating PaO_2/FiO_2 ratio when ABG not available		When measured on mask or nasal cannula

SaO_2 (%)	Calculated PaO_2
80	44
81	45
82	46
83	47
84	49
85	50
86	52
87	53
88	55
89	57
90	60
91	62
92	65
93	69
94	73
95	79
96	86
97	96
98	112
99	145

Nasal Cannula	
100% O_2 Flow Rate (L/min)	FiO_2 (%)
1	24
2	28
3	32
4	36
5	40
6	44
Oxygen Mask	
100% O_2 Flow Rate (L/min)	FiO_2 (%)
5-6	40
6-7	50
7-8	60
9	90
10	99+
Mask with Reservoir Bag	
100% O_2 Flow Rate (L/min)	FiO_2 (%)
6	60
7	70
8	80

APPENDIX B – CRRT MANAGEMENT

Continuous Renal Replacement will be provided to all subjects enrolled in the SCD-PED-02 Trial using automated equipment with integrated ultrafiltration control.

CRRT Modality:	Continuous
Blood Flow Rate:	Prescribed by Investigator Team – Treating Team
Dialysate Type:	Prescribed by Investigator Team – Treating Team
Dialysate Rate:	Prescribed by Investigator Team – Treating Team
Replacement Fluid Type:	Prescribed by Investigator Team – Treating Team
Ultrafiltration:	Prescribed by Investigator Team – Treating Team
Anticoagulation:	Regional Citrate Anticoagulation
Anticoagulation Prescription:	Prescribed by Investigator Team – Treating Team
CRRT Device	Baxter PrismaFlex or PrisMax
Hemofilter:*	Baxter Prismaflex HF 1000 or HF20
CRRT System Change:	Per participating center protocol
SCD [redacted] Change:	Every 24 hours ± 4 hours and as needed
SCD Blood Tubing Set Change	[redacted] with each SCD change

*Hemofilters can be provided to site by sponsor upon request. The clinical site will agree to use one of the protocol approved hemofilters for therapy.

APPENDIX C – CRITICAL MEDICATION LIST

The following is a list of medication categories (with examples) to be acknowledged (yes/no) in the Case Report Forms if administered to the patient during the SCD-PED-02 Clinical Trial (starting at 24 hours prior to SCD Treatment until 240 hours of treatment or ICU discharge, whichever comes first). In addition, antibacterial use is captured during the Post SCD follow-up period.

ANTIBACTERIALS FOR SYSTEMIC USE

- Ampicillin
- Azithromycin
- Aztreonam
- Bactrim (Trimethoprim/ Sulfamethoxazole)
- Cefadroxil
- Cefalexin
- Cefazolin
- Cefazolin sodium
- Cefepime
- Cefepime hydrochloride
- Cefoxitin
- Ceftazidime
- Ciprofloxacin
- Ciprofloxacin hydrochloride
- Clindamycin
- Daptomycin
- Doripenem
- Erythromycin
- Gentamicin
- Levofloxacin
- Linezolid
- Meropenem
- Metronidazole
- Moxifloxacin
- Nafcillin
- Piperacillin
- Piperacillin-tazobactam
- Synercid (Quinupristin/ Dalfopristin)
- Tigecycline
- Tobramycin
- Vancomycin

ANTIMYCOTICS FOR SYSTEMIC USE

- Amphotericin B liposome
- Caspofungin
- Fluconazole
- Micafungin

ANTITHROMBOTIC AGENTS

- Acetylsalicylic acid
- Argatroban
- Bivalirudin
- Clopidogrel
- Drotrecogin alfa
- Enoxaparin
- Enoxaparin sodium
- Heparin
- Warfarin sodium

APPENDIX D –REGIONAL CITRATE ANTICOAGULATION (RCA) PROTOCOL EXAMPLE

1. **Purpose**: The purpose of the guidelines is to standardize, to the extent possible, the provision of regional citrate anticoagulation to patients who require CRRT. While these guidelines should govern the CRRT prescription provided to most patients, there will be special circumstances (e.g. inborn errors of metabolism, exogenous toxin removal) that will require deviation from these guidelines.

2. **Regional citrate anticoagulation:** All CRRT circuits will receive regional citrate anticoagulation. The initial prescription and calcium level monitoring will be based on the size of patients and the presence, or lack thereof, of significant hepatic dysfunction. **REMEMBER TO ACCOUNT FOR THESE FLUIDS IN THE PATIENT REMOVAL RATE AS THESE ARE EXTERNAL TO THE CRRT MACHINE.**
    a. **Patients > 1 year of age and with normal hepatic function:**
        i. Order ACD-A™ and $CaCl_2$ (8 grams/1 liter NS) to bedside
        ii. Initial ACD-A™ rate = 1.5 x CRRT blood pump rate x min/hour (e.g., f blood pump rate is 100 ml/minute; the ACD-A™ rate is 150 ml/hour). This is to be infused at a stopcock or y-connector on access line.
        iii. Initial $CaCl_2$ rate = 0.6 x blood pump rate x min/hour (e.g., if blood pump rate is 100 ml/minute; the $CaCl_2$ rate is 60 ml/hour).
        iv. Calcium monitoring schedule
            1. Check patient total and ionized calcium prior to CRRT initiation.
            2. Check patient and system ionized calcium 5 minutes after CRRT settings at prescribed rates.
            3. Check patient and system ionized calcium 1 hours after CRRT settings at prescribed rates.
            4. Check patient and system ionized calcium every 6 hours thereafter
            5. Check patient total calcium daily
            6. Check patient and system ionized calcium one hour after any change in ACD-A™ or $CaCl_2$ rate
    b. **Desired Calcium Concentration ranges**
        i. Circuit ionized calcium = 0.25 to <0.40 mmol/L
        ii. Patient ionized calcium = 1.1 to 1.3 mmol/L (a higher level may be requested by ICU/CICU service if clinically indicated)
        iii. Patient systemic calcium = 8 to 12 mg/dL
    c. **Citrate anticoagulation management**

i. Bedside RN to call for patient ionCa < 1 mmol/L or >1.5 mmol/L, or circuit ionCa < 0.25 mmol/L or ≥ 0.40 mmol/L
ii. Circuit ionCa managed with ACD-A™ rate
    1. For circuit ionCa < 0.25 mmol/L, decrease ACD-A™ 10%
    2. For circuit ionCa ≥ 0.40 mmol/L, increase ACD-A™ 10%
iii. Patient ionCa managed by $CaCl_2$ rate
    1. For patient ionCa < 0.9, increase $CaCl_2$ 20%
    2. For patient ionCa 0.9- 1, increase $CaCl_2$ 10%
    3. For patient ion Ca 1.3-1.5, decrease $CaCl_2$ 10%
    4. For patient ion Ca> 1.5, decrease $CaCl_2$ 20%

CITRATE ANTICOAGULATION

Each participating clinical site will use their regional citrate anticoagulation protocol. The clinical site is responsible to achieve and maintain a serum post filter ionized calcium level of <0.40 mmol/L throughout the entire SCD treatment period (day and night). The serum post filter ionized calcium level will be collected from the CRRT Circuit post SCD[redacted]. Examples of regional citrate anticoagulation protocols are included in Appendix D. If the site has difficulty in maintaining post-fiter iCa levels <0.4mM from prior experience with patients in this body weight between 10 and 20 kg body weight, the Sponsor and Lead Investigator will discuss options of citrate protocols to be used in enrolled patients to meet post filter iCa levels <0.4 mM as required during SCD treatment.

If centers are unable to achieve desired targets, the below fixed flow ratios ensure greater than 75% citrate removal. The recommended calcium infusion rates restore total Ca and systemic iCa to normal. The use of low blood flows (QB <80 ml/min) on the Prismaflex or PrisMax yields low return pressure alarms. These alarms are mitigated with a 5-inch long, small diameter extension tubing between the catheter and the CRRT blood circuit return end. Recommended filter is HF20.

Table 1. FIXED FLOW RATIO (FFR) CRRT-RCA Settings

Settings / Weight	Blood QB ml/min R=1	Citrate ACDA ml/hour R=1:3	Dialysate QD mL/hour R=1:30	Post-Dilution QRF mL/hour R=1:10	81.6 $CaCl_2$ in D5/0.9%NS mL/hour R=1:1	Effluent QEFF mL/hour R=1:45
10-20 kg	21	63	630	210	21	≥945

The initial Ca infusion rate is the product of blood flow multiplied by a correction factor based on systemic Hb and albumin for optimized precision **Table 2**. The most common correction factors indicated in blue.

Table 2.: Finding the Initial 81.6 mM $CaCl_2$-infusion rate (ml/h): QB x Correction Factor (Alb, Hb):

Alb / Hb	0.0-0.7 g/dL	0.8-1.2 g/dL	1.3-1.7 g/dL	1.8-2.2 g/dL	2.3-2.7 g/dL	2.8-3.2 g/dL	3.3-3.7 g/dL	3.8-4.2 g/dL	4.3-4.7 g/dL	4.8-5.2 g/dL	5.3-5.7 g/dL
5-5.9 g/dl	0.97	1	1.04	1.08	1.12	1.15	1.19	1.23	1.26	1.3	1.34
6-6.9 g/dl	0.93	0.97	1.01	1.04	1.08	1.12	1.15	1.19	1.22	1.26	1.3
7-7.9 g/dl	0.9	0.93	0.97	1.01	1.04	1.08	1.11	1.15	1.18	1.22	1.26
8-8.9 g/dl	0.86	0.9	0.93	0.97	1	1.04	1.07	1.11	1.14	1.18	1.21
9-9.9 g/dl	0.83	0.86	0.89	0.93	0.96	1	1.03	1.07	1.1	1.13	1.17

10-10.9 g/dl	0.79	0.82	0.86	0.89	0.92	0.96	0.99	1.02	1.05	1.09	1.12
11-11.9 g/dl	0.75	0.78	0.82	0.85	0.88	0.91	0.94	0.98	1.01	1.04	1.07
12-12.9 g/dl	0.73	0.76	0.79	0.82	0.85	0.88	0.91	0.94	0.97	1	1.03
13-13.9 g/dl	0.69	0.72	0.75	0.78	0.8	0.83	0.86	0.89	0.92	0.95	0.98
14-14.9 g/dl	0.65	0.68	0.71	0.73	0.76	0.79	0.82	0.84	0.87	0.9	0.93
15-15.9 g/dl	0.61	0.64	0.66	0.69	0.72	0.74	0.77	0.8	0.82	0.85	0.88
16-16.9 g/dl	0.57	0.6	0.62	0.65	0.67	0.7	0.72	0.75	0.78	0.8	0.83
17-17.9 g/dl	0.53	0.56	0.58	0.61	0.63	0.65	0.68	0.7	0.73	0.75	0.78
18-18.9 g/dl	0.5	0.52	0.54	0.57	0.59	0.61	0.63	0.66	0.68	0.7	0.73
19-19.9 g/dl	0.46	0.48	0.5	0.53	0.55	0.57	0.59	0.61	0.63	0.65	0.68

Adjust the Ca-infusion rate +/-10-20% using systemic iCa levels every 6 hours:
It is expected that the systemic iCa will usually be near GOAL +/-0.1 mM with the initial Ca infusion rate. Some patients may sequester Ca in their tissues (for instance with acute pancreatitis or rhabdomyolysis) or conversely may release Ca from their bones (for instance with hypercalcemia of malignancy). Systemic citrate accumulation to maximum 1.5-2 mM is also possible in severe shock/liver failure. Such patients may require +10-20% adjustments to their initial Ca-infusion rate based on systemic iCa levels as in Table 3. Systemic iCa >1 mM will be achieved with total Ca ≤12 mg/dL with the above settings even in severe liver failure.

Table 3: 81.6 mM $CaCl_2$ Infusion Rate Change Based on Systemic iCa Every 6 hours:

	The patient's ionized calcium level checked every 6 hours: GOAL 1.15 mM				
	Less than <0.95 mmol/L	0.95 – 1.04 mmol/L	**1.05 - 1.25 mmol/L**	1.26 – 1.4 mmol/L	**More than >1.4 mmol/L**
Current Ca-infusion Flow Rate mL/h	**Increase** Rate **+20%**; notify ICU physician	**Increase** Rate **+10%**	**No Change**	**Reduce** Rate **-10%**	**Reduce** Rate **-20%**; notify ICU physician
<12	+2 ml/h	+1 ml/h	No change	-1 ml/h	-2 ml/h
12-<17	+3 ml/h	+1.5 ml/h	No change	-1.5 ml/h	-3 ml/h
17-<22	+4 ml/h	+2 ml/h	No change	-2 ml/h	-4 ml/h
22-<27	+5 ml/h	+2.5 ml/h	No change	-2.5 ml/h	-5 ml/h
27-<32	+6 ml/h	+3 ml/h	No change	-3 ml/h	-6 ml/h
32-<37	+7 ml/h	+3.5 ml/h	No change	-3.5 ml/h	-7 ml/h
37-<42	+8 ml/h	+4 ml/h	No change	-4 ml/h	-8 ml/h
42-<47	+9 ml/h	+4.5 ml/h	No change	-4.5 ml/h	-9 ml/h

47-<52	+10 ml/h	+5 ml/h	No change	-5 ml/h	-10 ml/h
52-<57	+11 ml/h	+5.5 ml/h	No change	-5.5 ml/h	-11 ml/h
57-<62	+12 ml/h	+6 ml/h	No change	-6 ml/h	-12 ml/h
62-<67	+13 ml/h	+6.5 ml/h	No change	-6.5 ml/h	-13 ml/h
67-<72	+14 ml/h	+7 ml/h	No change	-7 ml/h	-14 ml/h
72≤	+13 ml/h	+6.5 ml/h	No change	-6.5 ml/h	-13 ml/h

APPENDIX E – SCD/CRRT BLOOD PRIMING PROTOCOL

The blood volume for the Prismaflex HF1000 is 165 ml and for the SCD is 120 ml, leading to a total extracorporeal circuit volume (ECV) is 285 ml. The blood volume for the Prismaflex HF20 is 58mL leading to a total ECV of 178mL. Blood prime will occur if the ECV total is greater than 10% of the patient's blood volume, based on an estimate of blood volume = 70 ml/kg x patient body weight (kg) [25]. As such, regardless of the use of the HF1000 or HF20 filter, **all circuits will need to be blood primed**, as estimated total blood volume for eligible patients would be 700 – 1400mL, where the ECVs above would account for >10%. The priming volumes for prismaflex and prismax are the same with the same set of filters. The Thermax heater of the Primsax if used will require additional 27 ml.

This additional circuit blood volume can be handled with blood priming with matched blood similar to the standard practice in neonates. The situation is most commonly encountered in neonatal CRRT, where circuit ECV can represent nearly 50% of patient blood volume, yet the same principles apply for this clinical study. [26]

The recommendation for all patients, except when concurrently treated with ECMO:

- A crystalloid primed SCD circuit will be placed in line with a crystalloid primed CRRT circuit. For the initial CRRT/SCD circuit, the entire CRRT + SCD circuit will then be primed with blood per local standard of care prior to initiating CRRT /SCD therapy with the patient. Given the relatively large blood ECV to patient size in this population, the following patient lab parameters should be achieved prior to initiating CRRT/SCD therapy to prevent severe acidosis or hypocalcemia
    - Patient pH of at least 7.20
    - Patient ionized calcium of at least 1.1 mmol/L
- Patient should be given calcium gluconate IV (at least 300 mg) at the start of CRRT/SCD therapy.
    - An equivalent about of IV calcium can be given in a different formulation (ex. 100 mg of IV calcium chloride).
- Once the CRRT+SCD therapy is initiated, patient ionized calcium and pH should be obtained no later than 15 minutes after achievement of final CRRT blood flow and effluent rates.
- For the daily SCD circuit change, one of four options will be available:
    - A completely new CRRT circuit can be used with the SCD circuit with a repeat of the procedure in the paragraph above
    - A circuit to circuit prime can be performed per local standard of care whereby the blood in the previous CRRT + SCD circuit is used to prime a new CRRT + SCD circuit
    - 60 to 120 mL of blood will be rinsed back to the patient. The old SCD and tubing will be removed from the CRRT circuit, and a new crystalloid primed SCD filter and tubing will be connected. The volume that was rinsed back to the patient will be removed over the next 3 hours, unless otherwise instructed by the clinical team

- The circuit will be placed into a saline prime, by rinsing the blood to a waste bag. The old SCD will be removed and a new crystalloid primed SCD will be connected in line post filter. The entire CRRT+SCD circuit will be re-primed with blood, and then connected back to the patient.

APPENDIX F – PLACEMENT OF SCD IN CRRT / ECMO CIRCUIT