CYTOSORB® REDUCTION OF FREE HEMOGLOBIN/ACUTE KIDNEY INJURY (AKI) DURING CARDIAC SURGERY

REFRESH II

Sponsor:	CytoSorbents Corporation 7 Deer Park Drive, Suite K Monmouth Junction, NJ 08852 Phone: (732) 329-8885
Study Product:	CytoSorb® Device, 300 mL
IDE # (if applicable):	G140256
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Investigational Sites/PIs	List maintained in the Trial Master File
Clinical Vendors	A list of clinical vendors utilized in this study is maintained in the Trial Master File
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This clinical investigation is performed in accordance with applicable guidelines, standards and regulations.

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Investigator's Statement and Signature

Investigator approval:

I have read this protocol and the Investigator Brochure and agree that the documents contain all the necessary information required to conduct the study, and I agree to conduct it as described. I understand that this study will not be initiated without appropriate IRB approval and that the administrative requirements of the governing body will be fully complied with.

Investigator's Signature	Date
Investigator's Printed Name	
Site Name	Site #

1. SYNOPSIS

Sponsor	CytoSorbents Corporation
Title	***CytoSorb® REduction of FREe Hemoglobin/Acute Kidney Injury (AKI) during Cardiac Surgery (REFRESH II Trial)***
Study device	CytoSorb® is a sorbent-filled hemoperfusion cartridge that is designed to reduce plasma free hemoglobin (pfHb) and other inflammatory mediators from blood that are generated during cardiopulmonary bypass in cardiac surgery.
Purpose of study	To evaluate the safety and performance of the CytoSorb® device to decrease the incidence or severity of acute kidney injury (AKI) as defined by Kidney Disease Improving Global Outcomes (KDIGO) clinical practice guideline definition of acute kidney injury when used intraoperatively with cardiopulmonary bypass (CPB) in subjects undergoing cardiac surgery. The objective of using CytoSorb® treatment in this setting is to provide clinically meaningful improvements in renal function by mitigation of intraoperative injury by removal of nephrotoxic agents such as pfHb and complement.
Study design	Prospective, multi-center, randomized, pivotal double-blinded clinical study. Subjects will be randomized in a 1:1 ratio to either standard of care (SOC) alone or standard of care plus treatment with the CytoSorb® device, with or without HCA. The number of patients with and without HCA will be assessed in the interim analysis. Interim analyses will be carried out for potential (NON-BINDING) early stopping for an efficacy or futility conclusion. An option to increase sample size (SSR, i.e., Sample Size Re-estimation) will be considered. SSR is based on the second interim analysis (IA) results using the conditional probability (CP) to estimate the sample size necessary to boost the CP to 88%, assuming the observed response proportions are the TRUE underlying proportions. To assure proper startup of the sites we will employ a roll in approach for up to the first two enrolled and treated subjects from each site for training. The roll in subjects will be treated with the CytoSorb® device. The data will be used for safety analysis only.

Study Population	The population evaluated for effectiveness in the treatment group will consist of a primary sub-population and a secondary sub-population. • The primary sub-population includes all subjects enrolled and randomized to the treatment group who are undergoing valve replacement with another procedure (e.g., CABG and valve replacement) *without* Hypothermic Circulatory Arrest (HCA) • The secondary sub-population consists of all surgical subjects undergoing aortic reconstructions with or without other procedures (e.g., CABG, valve replacement) *with* HCA
Sample Size	Up to 420 subjects will be enrolled and randomized at a 1:1 ratio at up to 40 investigational sites in the United States. A roll-in cohort of up to 80 additional subjects may be enrolled in the study and assigned to treatment with CytoSorb device.
Duration of participation for each patient	Primary, secondary, and exploratory endpoints will be evaluated on the day of cardiac surgery up to Day Seven (7) post-cardiac surgery or through hospital discharge, whichever occurs first. Subjects will be followed through 30-day follow-up visit to fully evaluate safety (AEs will be followed through study exit and device-related SAEs will be followed until a return to a stable state as determined by the investigator).
Patient treatment	Two devices, configured into a parallel circuit are placed between the oxygenator and the venous reservoir and run as a bypass shunt to the main blood flow in a standard heart-lung machine. The first device will be introduced at t=0 (start of CPB). The second device will be introduced at t=90 minutes after the start of CPB.
Study Objectives:	To evaluate the safety and performance of the CytoSorb® device to decrease the incidence or severity of AKI in the first 48 hours after surgery when used intraoperatively with cardiopulmonary bypass (CPB) in subjects undergoing cardiac surgery. The study hypothesis is that, for patients at increased risk for AKI based upon use of the Cleveland Clinic Score for stratification of the risk of cardiac surgery associated AKI (CSA-AKI), the intra-operative usage of the CytoSorb® device in addition to the standard of care in a bypass circuit during cardiopulmonary bypass will decrease the incidence or severity of AKI per the Kidney Disease Improving Global Outcomes (KDIGO) clinical practice guideline definition of acute kidney injury.
Primary Effectiveness Endpoint	The primary effectiveness endpoint is the incidence or severity of AKI in the first 48 hours after surgery when used intraoperatively with cardiopulmonary bypass (CPB) in subjects undergoing cardiac surgery as

	defined by the KDIGO clinical practice guideline through 48 hours post-cardiac surgery. Assessment of serum creatinine and hourly measurements of urine output will be recorded for the first 48 h following admission to the ICU. The intended primary effectiveness sub-population includes all subjects enrolled and randomized to the treatment group who are undergoing valve replacement with another procedure (e.g., CABG, valve replacement) *without* Hypothermic Circulatory Arrest (HCA). Balance between patients undergoing cardiac surgery with and without HCA will be evaluated during the interim analysis and the primary sub-population confirmed.
Primary Safety Endpoint	The primary safety endpoint of this trial is to evaluate the product safety profile through the assessment of device related adverse events during the study period.
Secondary Effectiveness Endpoints	Secondary effectiveness endpoints include the following: • Change from baseline (defined as the start of CPB) in pfHb assessed hourly for the duration of CPB and at the end of CPB; • Change from baseline (defined as start of CPB) in Complement 3a (C3a) assessed hourly for the duration of CPB and at the end of CPB; • Severity of AKI through hospital discharge or post-surgical Day 7, whichever is sooner: • Incidence of AKI through hospital discharge or post-surgical Day 7, whichever is sooner: • Change from baseline (defined as pre-procedure) in serum creatinine through hospital discharge or post-surgical Day 7, whichever is sooner; Additionally, the following pharmacoeconomic endpoints are included: • Duration of hospital stay, post-surgery • Duration of ICU stay, post-surgery • Duration of renal replacement therapy, post-surgery • Duration of vasopressor use, post-surgery • Duration of ventilator use, post-surgery
Secondary Safety Endpoints	Secondary safety endpoints include the following: • Incidence and severity of procedure-related adverse events, through 30-days post-cardiac surgery. • Incidence of Serious device-related adverse events

	• Incidence and severity of unanticipated adverse device effects (UADEs) • Change from baseline (pre-procedure) in vital signs (blood pressure, pulse and temperature) • Change from baseline (pre-procedure) in safety laboratory assessments (hematology, coagulation and comprehensive metabolic panel) pre- and post-surgery through Day 7 or hospital discharge, whichever occurs first, • Intra-cardiac surgery arterial blood gas measurements. • Post-surgical care unit assessments (SOFA, vasopressor requirements, blood product transfusions, ventilation requirements, requirement for mechanical support, and renal replacement requirements) through hospital discharge or post-surgical Day 7, whichever occurs first.
Exploratory Effectiveness Endpoints	The following known inflammatory mediators and predictive markers of kidney injury will be evaluated either during cardiac surgery or post-cardiac surgery. During cardiac surgery: • Change from baseline (defined as start of CPB) in Complement 5a (C5a) and inflammatory marker panel assessed hourly during CPB and at the end of CPB. Post-cardiac surgery days 1-3: • Change from baseline (defined as start of CPB) in C5a, inflammatory mediator panel, plasma kidney injury markers HMGB1 and Cystatin C, urine kidney injury markers NGAL, TIMP2, and IGFBP7 on post-surgical Days 1 through 3
Inclusion Criteria	1. Male or female and ≥18 years of age. 2. Scheduled for non-emergent surgical procedures requiring CPB with full sternotomy: a. Any combination of heart valve replacement or valve repair and another procedure (e.g., CABG) *without* HCA; coronary artery bypass graft (CABG) alone excluded OR b. Aortic reconstruction alone *with* HCA OR c. Aortic reconstruction with any combination of valve repair or valve replacement or CABG (but not CABG alone) *with* HCA 3. Estimated glomerular filtration rate (eGFR) ≥ 30 ml/min/1.73m^2 and at least ONE of the following additional risk factors for Cardiac Surgery Associated[1] (CSA)-AKI:

	a. Age ≥ 75 years b. Previous cardiac surgery with sternotomy; c. Documented New York Heart Association (NYHA) Class III or IV heart failure within 1 year prior to surgery; d. Left ventricular ejection fraction (LVEF) ≤ 35% by invasive or noninvasive diagnostic cardiac imaging - echocardiography, nuclear imaging, computed tomography, magnetic resonance imaging or angiography performed within 90 days prior to surgery (if LVEF ≤ 35% by any invasive or noninvasive imaging procedure, patient meets the risk factor); e. Insulin-requiring diabetes; f. Non-insulin-requiring diabetes AND the presence of ≥ +2 proteinuria on urinalysis (medical history or dipstick); g. Pre-operative anemia (hemoglobin < 11g/dl) h. BMI ≥ 30 kg/m2
Exclusion Criteria	1. Weight < 99 lbs. or > 383 lbs.; 2. The presence of AKI (KDIGO criteria) at the time of screening; 3. eGFR < 30 ml/min/1.73m^2; 4. Prior organ transplantation; 5. Dialysis dependence; 6. Cardiogenic shock or hemodynamic instability within 48 hours prior to surgery as defined by a systolic BP <80 mm Hg and pulse >120 beats per minute (bpm) and requirement for inotropes or vasopressors or other mechanical devices such as intra-aortic balloon counter-pulsation (IABP); or extracorporeal membrane oxygenation (ECMO). Short term vasopressor support is allowed during induction; however, ongoing requirement for vasopressor support to the time initiation of CPB would be excluded; 7. Requirement for any of the following within seven (7) days prior to cardiac surgery: a. defibrillator or permanent pacemaker, b. mechanical ventilation, c. intra-aortic balloon counter-pulsation (IABP), d. left ventricular assist device (LVAD), e. other forms of mechanical circulatory support (MCS) such as extracorporeal membrane oxygenation (ECMO);

	8. Cardiopulmonary resuscitation within 14 days prior to cardiac surgery; 9. Known or history of inadequately treated cancer within the past 5 years. Inadequately treated cancer is defined as the continued presence or recurrence of cancer; 10. Known or suspected sepsis at time of screening or within the 30 days prior to cardiac surgery; 11. Known or suspected glomerulonephritis or interstitial nephritis at time of screening; 12. Untreated endocarditis; however, successfully treated endocarditis, defined as having completed a full course of antibiotics with no evidence of recurrence of infection, is acceptable; 13. Other current active infection requiring antibiotic treatment including Hepatitis B (HBV), Hepatitis C (HCV) or human immunodeficiency virus (HIV) infections. Documented inactive HBV and HCV are allowed as well as HIV infections treated with a stable antiviral regimen for a minimum of 30 days prior to the date of surgery; 14. Any other clinically relevant finding or medical history that, based upon the determination of the Investigator, may introduce additional risk to the patient or confound the results of the study; 15. Use of stress dosed steroids at the time of surgery, immunosuppressant medications to prevent transplant rejection or immunosuppressant chemotherapeutic agents; 16. Presence of acute or chronic pancreatitis; 17. Patients known to have Child-Pugh Class A Liver disease or with alanine aminotransferase (ALT) or aspartate aminotransferase (AST) > 2 times the Upper Limit of Normal (ULN); 18. Any congenital coagulation disorder; 19. Pregnancy or lactation; 20. Treatment with an investigational drug or device within 60 days prior to surgery; 21. Inability to comply with the requirements of the study protocol; 22. Subject has a platelet count of < 50,000 /μL.

Table 1: Definitions

Arterial Blood Gas Assessments	Intra-procedure, while patient is on CPB Post-Procedure, only if arterial line is in place
Enrolled	A patient who has provided informed consent to participate in the trial is considered to be enrolled.
Roll-in	The first two enrolled and treated patients at each site used for training purposes. Roll in patients are treated with the CytoSorb device and their data used for safety analysis only.
Randomized	A patient who has been randomly assigned (according to a validated randomization schedule) in a 1:1 ratio to either standard of care (SOC) or SOC with CytoSorb device.
Intra-Cardiac Procedure	During CPB, while on pump
Post-Cardiac Procedure	Following end of CPB
Pre-Procedure	Day of the surgery but before induction of general anesthesia, exclusive of sedation.
Screening	Time-period to determine eligibility, not more than 14 days prior to day of cardiac surgery. When screening laboratory assessments are obtained on the day of surgery, they may be substituted for the pre-procedure laboratory assessments
Baseline	The start of CPB is defined as baseline for pfHb, C3a, C5a, inflammatory mediator panel, and markers of kidney injury (plasma and urine markers) endpoints. Pre-procedure is defined as baseline for all other endpoints.
Start Of CPB	Pump on
End Of CPB	Pump off, just prior to protamine reversal (± 5 minutes)
Post-Surgery Care Unit	Coronary Care Unit, Intensive Care Unit, Step Down Unit
Post-Surgery Care Unit Assessments and Labs	Day 1 assessments start at or after midnight post-cardiac surgery. Assessments and lab blood draw times should be approximately the same time each day (see Schedule of Assessments for specific assessments and labs, as well as approved windows).

Table 2: Abbreviations

Abbreviation/Term	Definition
ABG	Arterial blood gas
ADL	Activities of Daily Living
AE	Adverse Event
AKI	Acute kidney injury
ALT	Alanine aminotransferase
APTT	Activated partial thromboplastin time
ARDS	Acute respiratory distress syndrome
AST	Aspartate aminotransferase
AUC	Area under the concentration by time curve
BP	Blood pressure
C3a	Serum complement component C3a
C5a	Serum complement component C5a
CABG	Coronary artery bypass graft
CFR	Code of Federal Regulations
CKD	Chronic kidney damage
CPB	Cardiopulmonary bypass
CRF	Case Report Form
CRO	Contract research organization
CRP	C-reactive protein
DMC	Data monitoring committee
DMP	Data management plan
ECG	Electrocardiogram
ECMO	Extracorporeal membrane oxygenation
eCRF	Electronic case report form
EDC	Electronic data capture
ESRD	End-stage renal disease
FDA	US Food and Drug Administration
FIO_2	Fraction of inspired oxygen
GCP	Good Clinical Practice
HB	Hemoglobin
HCA	Hypothermic Circulatory Arrest
HLM	Heart-lung machine
HMGB1	High mobility group box protein 1

Abbreviation/Term	Definition
HR	Heart rate
IABP	Intra-aortic balloon
ICH	International Conference on Harmonization
ICU	Intensive care unit
IDE	Investigational Device Exemption
IGFBP7	Insulin-like growth factor binding protein 7
INFG	Interferon Gamma
IL-2	Interleukin 2
IL-4	Interleukin 4
IL-5	Interleukin 5
IL-6	Interleukin 6
IL-10	Interleukin 10
IL-12	Interleukin 12
INR	International normalized ratio
IRB	Institutional Review Board
KDIGO	Kidney Disease: Improving Global Outcome group
NGAL	Neutrophil gelatinase-associated lipocalin
NYHA	New York Heart Association
OUS	Outside of the US
$paC0_2$	Partial pressure of carbon dioxide
paO_2	Partial pressure of oxygen
PEEP	Positive end-expiratory pressure
pfHb	Plasma free hemoglobin
PT	Prothrombin time
RBC	Red blood cell
SAE	Serious adverse event
SCr	Serum creatinine
SOC	System organ class
SOFA	Sequential Organ Failure Assessment Score
SOP	Standard operating procedure
TIMP-2	Tissue inhibitors of metalloproteinases
TNF-α	Tumor necrosis factor alpha
UADE	Unanticipated adverse device effect

Abbreviation/Term	Definition
US	United States
VAD	Ventricular assist device
WBC	White Blood Cell

2. INTRODUCTION

Cardiac surgery requiring cardiopulmonary bypass (CPB) has been routine in clinical practice for over 50 years and numerous technological advancements have taken place over this time. This procedure has been lifesaving, by allowing many complex cardiac surgical procedures to take place safely. However, despite advances in perfusion, anesthetic and surgical techniques, CPB still frequently evokes inflammatory and hemolytic reactions that may lead to worsening of renal function and postoperative morbidity. The incidence of renal damage has been estimated between 10-25% with little improvement despite extensive investigation. Furthermore this rate is higher for patients with significant co-morbidities such as diabetes or congestive heart failure[3].

The pathogenesis of cardiovascular surgery associated renal injury is multifactorial, including CPB associated intravascular hemolysis (resulting in the generation of pfHb), exposure of blood to CPB surfaces during surgery with initiation of the early phase inflammatory response via contact activation[4] and an acute rise in inflammatory mediators during the procedure including: complement activation, the coagulation system, leukocytes, cytokines, chemokines, and adhesion molecules. Multiple factors including temperature variation and oxygenation may further escalate the immune response. This combination of processes was described as the Systemic Inflammatory Response Syndrome (SIRS) and is associated with frequent complications of renal failure[5,6].

Mitigation of the deleterious effects of renal injury therefore requires a multimodal strategy aimed at decreasing circulating factors likely to exacerbate tissue injury during the period of heightened risk, i.e., during CPB. Two well characterized etiologies of worsening renal function are the generation of pfHb and activated complement during CPB.

Circulating levels of pfHb are normally low; however, hemolysis increases dramatically during CPB surgery, resulting in increased pfHb. The extent of hemolysis during CPB surgery is directly correlated with both the length of the CPB procedure (> 3 hours) and the complexity of cardiac surgery[7]. Red blood cells (RBCs) can hemolyze during CPB due to factors such as negative pressure cardiotomy suction, blood material interactions, increases in foreign surface to-blood volume ratios, shear stress due to high flow rates through artificial blood circuits, and others[8].

The mechanisms whereby hemoglobin is thought to induce renal injury or dysfunction include (a) tubular obstruction caused by heme protein precipitation, (b) ischemic injury resulting from increased renal vascular resistance and vasoconstriction, and (c) heme iron-driven oxidative injury[9,10]. By overwhelming the macrophages associated uptake of Haptoglobin-Hb complexes high levels of pfHb may lead to inflammation and endothelial, tissue, and organ injury[11-13]. Hemoglobin first reacts with endogenous compounds to form oxygen radicals that induce pro-oxidant damage[14,15]. This damage is known to promote a pro-inflammatory cascade and cellular interactions through pathways involving activation, generation, or expression of thrombin, complement, neutrophils, cell-adhesion molecules, mast cells, and multiple inflammatory mediators, including the release of cytokines, IL-1β, IL-6, IL-8, and TNF-α 5[16]. These mediators

have been shown to be associated with the development of postoperative SIRS, and major post-operative complications[17-19].

Hemolysis, resulting in release of free hemoglobin (pfHb), has been directly correlated with induction of kidney injury[16,18]. In one study reported in the literature, peak pfHb levels ≥ 64.5 mg/dL held a significant diagnostic value for the prediction of acute kidney injury (AKI)[20]. The complications of AKI may require increased length of stay and ICU time, prolonged mechanical ventilation, increased risk of nosocomial infections, or may increase the risk for systemic inflammatory response syndrome (SIRS) and potential progression to organ failure[21]. Kubota et al. noted a protective effect of haptoglobin infusion intraoperatively to reduce the incidence of post-operative AKI, indicating that the uptake of Haptoglobin-Hb complex by macrophages may be protective against the tissue injury associated with pfHb[12]. Because of this, the removal of pfHb during CPB has the potential to significantly reduce the risk of hemolysis-induced organ injury[8].

Activation of the complement alternative pathway also occurs during CPB[20] and examination of clinical samples and preclinical models has shown that activation of the complement system is a critical cause of acute kidney injury[20]. Eculizumab, an anti-C5 monoclonal antibody inducing terminal complement blockade, showed reduced complement activation, inflammation, endothelial damage, thrombosis, and renal injury markers in an exploratory study of atypical hemolytic uremic syndrome (aHUS)[22]. Precedent for complement inhibition was also established using soluble human complement receptor type 1 (sCR1) and heparin bound CPB circuits[23].

By directly causing tissue injury in the post-operative SIRS response, and through adverse physiologic changes, pfHb is thought to be a significant contributor in the development of multiple organ failure (MOF) in many patients following cardiac surgery. These complications may require increased length of stay and ICU time, prolonged mechanical ventilation, increased risk of nosocomial infections, or may increase the risk for systemic inflammatory response syndrome (SIRS) and potential progression to organ failure[24]. The renal toxicity associated with the generation by cardiac surgery of both pfHb and inflammatory mediators (activated compliment cascade and inflammatory cytokines) suggests that an intra-operative reduction of these elements may reduce the potential for post-operative complications, improve clinical outcomes and reduce the risk of renal injury[25].

Despite ongoing efforts to mitigate the effects of CV surgery, only limited progress has been made in altering the prevalence of surgically associated renal injury as assessed by the clinically accepted definitions of acute kidney injury (AKI) and in long term morbidity associated with decreased renal function. A potential reason may be the failure to prevent the loss of renal functional reserve occurring below the threshold of creatinine elevation that defines AKI. Ishani et al[21] linked the magnitude of sCr increase post cardiac surgery to increasing rates of chronic kidney damage (CKD), progression of kidney disease and death. Similarly, even minimal increases in sCr were associated with adverse long-term outcomes Duque-Sosa[26] and Lassnigg, et al observed that even minimal (0.1 mg/dl) increases in sCr, in the immediate postoperative period, were associated

with significantly increased mortality, providing an independent risk factor in predicting outcome after cardiac surgery[27].

2.1 Summary of Previous Clinical Experience Informing Study Design:

CytoSorbents conducted REFRESH I, a prospective, randomized, controlled safety trial enrolling a small heterogeneous population, at eight major U.S. cardiac surgery centers to evaluate the safety and feasibility of CytoSorb®, a blood purification technology designed as an adjunctive therapy to remove inflammatory mediators (i.e., pfHb, activated complement and cytokines) in high risk cardiac surgery patients aged 18-80 years of age, where cardiopulmonary bypass (CPB) was expected to exceed 3 hours. Subject assessments were performed until subjects were discharged from the ICU. Subjects were monitored for AEs through 30 days after the procedure.

This trial achieved the primary safety endpoint of the study with a favorable adjudication of all serious adverse events. There were no significant differences in the rates of adverse events (AEs) or serious adverse events (SAEs) between the two groups, including 30-day mortality, and no unanticipated adverse device effects (UADEs) were reported. Three subjects (1 Control; 2 Treated) died of AEs that were considered by the Independent Physician Adjudicator to be not related to the device. Most AEs were related to the pre-existing conditions or to surgical procedures. AEs specifically related to the investigational device were infrequent (i.e., involving 2 [8.7%] subjects in the Treated arm) and in both instances the subjects recovered without sequelae.

A detailed description of the REFRESH I study results along with subsequent safety analyses of REFRESH I data; safety information derived from clinical applications other than CPB; and supporting preclinical assessments may be found in the Investigator Brochure. The investigator should read and understand the Investigator Brochure and use it in conjunction with this protocol in the evaluation of risk and benefit for patients being considered for enrollment into this study.

2.2 REFRESH II Study design:

The REFRESH II study is designed to assess the intraoperative use of the CytoSorb® device in patients with an increased risk of acute renal failure following open heart surgery[3] to decrease the circulating presence of nephrotoxic agents (such as pfHb, cytokines, and activated compliment during CPB) in conjunction with standard of care treatment. The study aims to determine whether the use of the CytoSorb® device can decrease the incidence or severity of AKI in the 48 hours immediately following surgery. This will be evaluated in a patient population that has been enriched for increased risk of cardiac surgery associated AKI and for procedures associated with a higher risk for pfHb generation.

In this study, creatinine and urine output collection will be used to identify acute kidney injury (AKI) as defined by the KDIGO clinical practice guidelines. This identification will be augmented through the use of additional indicators of renal damage and dysfunction, including assays for urinary TIMP2 and IGFBP-7 and serum elevations in HMGB1 and Cystatin C[28].

The CytoSorb® device that will be utilized in this randomized and blinded trial is based on size adsorption filtration. Each CytoSorb® adsorber cartridge contains micro-porous, polystyrene bead technology. Earlier in vitro and in vivo studies have shown this to correlate with the removal of endogenous mediators of inflammation and pfHb. This product, approved under a CE mark in Europe and other countries has been used in over 25,000 critical care cases, including about 6,000 cardiac surgery cases. It is believed that the removal of these renal-toxic substances from patient blood during surgery will lead to an overall reduction in kidney dysfunction among the patients who assigned to the treatment group relative to those who will be on standard of care alone.

3. INVESTIGATIONAL DEVICE DESCRIPTION

3.1 Investigational Device Description

CytoSorb® is a sorbent-filled hemoperfusion cartridge that is designed to reduce plasma free hemoglobin and other inflammatory mediators from blood that are generated during cardiopulmonary bypass in cardiac surgery. The device is placed into a parallel bypass circuit (between the oxygenator and the venous reservoir) in a standard heart-lung machine.

CytoSorb® consists of a cylinder and end-cap assembly filled with biocompatible porous polymer beads (**Figure 1**). At either end of the cylinder, a fine mesh screen is placed to retain the polymer beads within the device. Each end-cap has a standard dialysis connector, which is compatible with standard dialysis tubing. The polymer beads are composed of a divinylbenzene/polyvinyl pyrrolidone co-polymer, where each bead has hundreds of thousands of tightly controlled pores and channels that are generated via suspension polymerization. These pores and channels, in turn, enable the porous polymer beads to remove middle molecular weight substances based on pore capture (size) and surface adsorption.

The CytoSorb® device is designed for use in an extracorporeal circuit. The device can be connected on a standard heart-lung machine in a parallel configuration with the CPB circuit. The target flow rate through the device is intended to be approximately 300-400 mL/min which is about 10-20% of a typical flow rate encountered in CPB. In this study, two parallel CytoSorb® cartridges will be attached to the bypass circuit, and they are connected between the oxygenator and cardiotomy reservoir as shown in the **Figure 2** below and separate from the main blood flow to the patient. These devices will be used as such; for the first 90 minutes of CPB, blood will be directed through the first of the two devices. If and when CPB continues for more than 90 minutes, the second CytoSorb® device will then be opened to the perfusion circuit. Blood flow through the CytoSorb® circuit is controlled via an adjustable roller clamp distal to the cartridges and monitored via ultrasonic flow detection (a Transonic, or equivalent) flow monitor will be supplied) to maintain a target blood flow of ~800 mL/min with both devices, or ~400 mL/min with the single device. A minimum flow of ~300 ml/min through the single device, or ~600 ml/min through the complete circuit (both devices) is acceptable. Once the hemoperfusion session is

complete, remaining blood in the circuit goes back to the patient, if appropriate per hospital protocol.

In addition to the CytoSorb® device, two off-the-shelf disposable connector and tubing sets supplied from Molded Products based on their commercial Y-Blood line set CYT800-C (both covered by 510k K003712) will be used to connect the CytoSorb® devices to the oxygenator and venous reservoir. A standard roller clamp and disposable hemostats will also be provided.

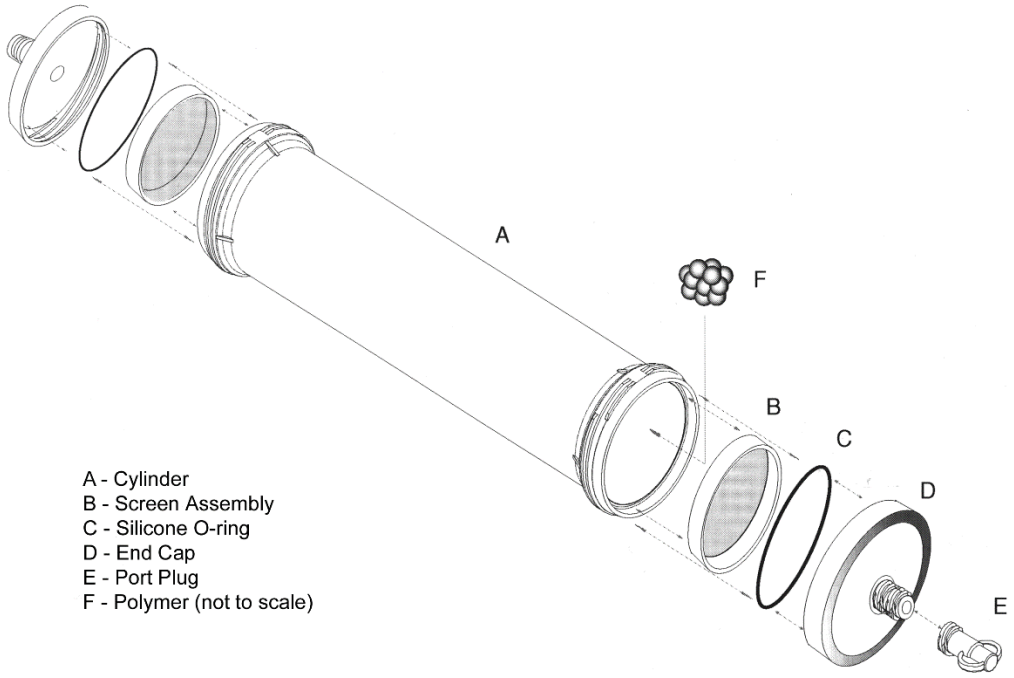

Figure 1: CytoSorb® Device

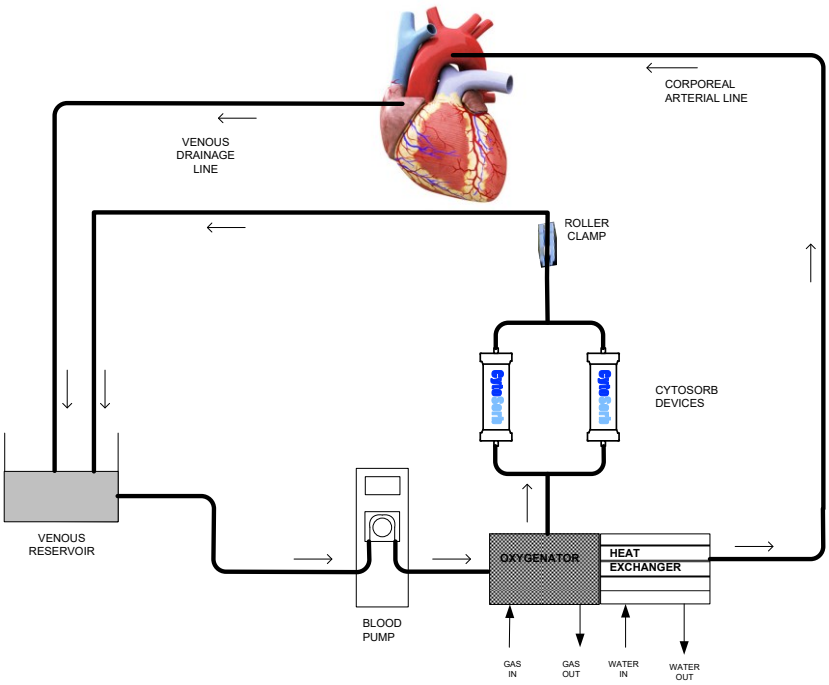

Figure 2: CytoSorb® 300 mL Device on CPB Circuit

3.2 Investigational Device Identification

The CytoSorb® 300mL device will be used in this study. It is anticipated that two CytoSorb® devices (in parallel) will be used for each subject randomized to the treatment arm.

3.3 Investigational Device Accountability

Each CytoSorb® device will be assigned lot numbers and/or unique serial numbers. Access to investigational devices shall be controlled and the investigational devices shall be used only in the clinical investigation and according to the clinical investigation plan. The sponsor will keep records to document the physical location of all investigational devices from shipment of investigational devices to the investigation sites until return or disposal. The Principal Investigator or an authorized designee shall keep records documenting the receipt, use, return and/or disposal of the investigational devices, which shall include:

- Date of receipt;
- Identification of each investigational device (lot number/serial number);
- Expiry date;
- Date of use;
- Study identification;
- Date on which the investigational device was returned, if applicable, and;
- Date of return of unused, expired or malfunctioning Investigational Intended Use/Current Indication for Use

3.4 The intended use of the investigational device(s).

The proposed investigative use of CytoSorb® device is in an extracorporeal circuit for the removal of free hemoglobin as an adjunct to the standard of care in subjects undergoing cardiac surgery requiring CPB and will only be used as specified by this clinical protocol.

3.5 Investigational Device Procedure(s)/Training

The CytoSorb® device is designed for use in a standard heart-lung machine CPB circuit with an arterial pump. In this study, as described in the Instructions for Use (IFU), up-to two CytoSorb® cartridges in a parallel configuration will be in a bypass circuit connected between the oxygenator and cardiotomy reservoir as shown in **Figure 2**, and separate from the main blood flow to the subject. Blood flow through this circuit is controlled via an adjustable roller clamp distal to the cartridges and monitored via ultrasonic flow detection to maintain flow. A minimum blood flow of ~ 300 ml through the single device, or ~ 600 ml through the paired devices together is acceptable.

The circuit containing the CytoSorb® devices will be connected prior to the start of the procedure. Blood flow through the first CytoSorb® device will commence when subject goes on CPB. Flow through the second CytoSorb® device will commence at 90 minutes from the start of CPB (at this time both CytoSorb® devices will have blood flowing through them). Use of the CytoSorb® devices is stopped at the end of CPB. Once the CPB procedure is complete the hemoperfusion session will be ended, the circuit is flushed with saline, with the return of blood in the circuit back to the subject, per the hospital protocol.

CytoSorb® device training for the device users involved in this study will occur prior to CytoSorb® device use and will be documented in a training log.

3.6 Blinding

Blinding to treatment assignment, as implemented in this study will apply only to select study personnel and for specific durations. Blinding of the surgical staff to treatment assignment is implemented in this study starting at the point of randomization and extending through the duration of the entire study. This blinding is implemented to minimize bias in surgical care decisions that may be introduced by knowledge of treatment assignment. The potential for introduction of bias is most relevant during the surgical procedure where knowledge of treatment assignment and prior clinical experience with the investigational device may impact clinical decisions such as frequency of platelet or whole blood infusion and assessment of adverse events that occur during surgery by medical staff responsible for administering these treatments.

To minimize bias both subjects and designated site study personnel (e.g. Surgeon, Anesthesiologist, OR staff) involved in OR activities will be blinded to the treatment assignment. The study coordinator may remain unblinded, however extreme caution should be exercised by the study coordinator to prevent inadvertently revealing treatment assignment to individuals making clinical decisions on subject care.

The unblinded team at the site (Perfusionist and study coordinator) will be supplied with a barrier to separate the perfusion area from the surgical staff view. A Field Clinical Engineer (FCE), as designated by the Sponsor, may be present to assist with set up of the blind and provide guidance during the procedure.

3.6.1 Maintaining the Study Blind

The following describes the measures being taken to maintain the study blind.

a) **Subjects:** Subjects will not be told their randomization assignment by any site study personnel involved in the trial and will receive the same care throughout the trial.

b) **Randomization Assignment:** Each site will randomize subjects using the electronic data base. After the appropriate information is entered into the electronic case report form (eCRF), the randomization function will be activated, and the assignment made (Standard of Care or Standard of Care with CytoSorb® Device). This randomization process is performed by the unblinded team within 5 business day prior to the procedure, including the day of the procedure.

c) **Site Study Personnel:** There are two designated teams at each site:
- Blinded: These individuals remain blinded from the point of randomization through the duration of the study, inclusive of all subjects enrolled at the site.
  - Surgical team
  - Anesthesiologist
- Unblinded: These individuals will be aware of treatment assignment and will refrain from revealing it to any other study staff for the duration of the study, inclusive of all subjects enrolled at the site.
  - Perfusionist
  - Study Coordinator
  - FCE

The **Blinded Team** is responsible for administering all pre-, intra- and post-procedure subject testing and care as designated by the protocol.

The **Unblinded Team** is responsible for those activities and documentation related to the procedure, including the selection of the device (sham or CytoSorb®) used at time of procedure, and recording the procedure and device study data in the eCRFs and device accountability log. The Sponsor will work with each individual site to determine the documentation needed in the subjects' medical records (e.g. procedure notes) to protect the study blind. The Study Coordinator on this team remains unblinded to the subject treatment assignment and is particularly responsible for:

1. Obtaining randomization code from EDC system within 5 business days prior to procedure including day of procedure

2. Ensuring appropriate device cartridges are available and in OR suite on procedure day
3. Completing Device Accountability Log
4. EDC entry for all data pertaining to treatment assignment and procedure information
5. EDC entry for any AEs associated with the use of the device or procedure that are assessed and any additional EDC entry
6. Scheduling routine follow-up care/visits for all study subjects
7. Performing follow-up visits

3.6.2 Unblinding Procedure

It is the intention of this study to unblind both the subject and the site study personnel at the completion of the study. This will occur **after** all necessary 30-day follow-up assessments are completed and all the data have been collected and verified.

If the subject develops a condition within the 30-day follow-up window, the subject and team will be unblinded only if doing so is necessary to make informed decisions about clinical care. To the extent possible, the surgical team will remain blinded throughout the duration of the study.

Any subject that is unblinded prematurely for any reason shall continue to be followed in accordance with the protocol evaluations outlined. A subject is NOT automatically considered to be withdrawn if unblinding occurs earlier than anticipated.

If the site believes there is reason to unblind either themselves or the subject (significant change in subject's health status related to the possible use of the device, withdrawal or discontinuation of study participation), the site will contact the Sponsor to discuss the subject's circumstances, at which time the Sponsor, if in agreement with the site's request, will approve release of the blinding information by the unblinded Sponsor designee.

4. STUDY JUSTIFICATION

Cardiac surgery requiring cardiopulmonary bypass (CPB) has been in clinical practice for over 50 years. This procedure has been lifesaving by allowing many complex cardiac surgical procedures to take place safely. Despite advances however, CPB still frequently evokes inflammatory reactions that lead to postoperative morbidity, organ dysfunction and worsening of renal function. The inflammatory reaction underlying the pathophysiology of these conditions originates from exposure of blood to CPB surfaces during surgery with initiation of the early phase inflammatory response via contact activation[4]. Two specific complications associated with inflammation arising from CPB that pose significant clinical burden in post-operative patient care are the risk of worsening renal function following cardiac surgery and the potential for hemodynamic instability (vasoplegia).

Worsening renal function following cardiovascular surgery is a frequent complication and is associated with significant patient morbidity, complication of post-operative management and mortality[20,25]. Its severity and chronicity have most commonly been assessed using definitions of Acute Kidney Injury (AKI) and Chronic Kidney Disease (CKD) (Kidney Disease: Improving Global Outcome group - KDIGO 2012). A retrospective examination of in-hospital mortality and length of hospital stay (LOS) in 39,369 broad surgical patients[29] showed that AKI was associated with a fivefold higher mortality and five days longer LOS. AKI is a common post-operative complication occurring in 5-30% of patients following cardiac surgery[30], and is associated with a more complicated clinical course and with an excessive mortality of up to 80%[27]. The long-term mortality risks associated with AKI after cardiac surgery remain high for 10 years after the procedure, irrespective of other risk factors, even in patients that showed complete renal recovery[31]. However, patients not meeting the formal definition of AKI may have already suffered a substantial loss of renal functional reserve; even minimal increases in serum creatinine in the immediate post-operative period >0.0 relative to a patient's pre-operative baseline value are associated with significantly increased mortality and provide an independent risk factor in predicting outcome after cardiac surgery[27]. Ishani et al[21] linked the magnitude of sCr increase post cardiac surgery to increasing rates of CKD, progression of kidney disease and death. Importantly, even minimal increases in sCr were associated with adverse long-term outcomes. Duque-Sosa[26] assessed changes in creatinine as *percent* increase from baseline to peak creatinine levels after cardiac surgery [none (<0%), class I (1%-24%), Class II (25%-49%), Class III (50%99%), or Class IV (>100%)]. This is consistent with the observation of an independent association between even mild AKI and increased morbidity and mortality. Thus, avoiding the development of the initial injury represents a clinically important objective and while the origins of worsening renal function are multifactorial, significant contributors include the increases in levels of inflammatory cytokines and circulatory pfHb.

Vasoplegic Syndrome is characterized by "severe persistent low systemic vascular resistance, hypotension, decreased filling pressure, normal or elevated cardiac output, and tachycardia". CPB induced inflammation is associated with activation of the complement alternative pathway and this can lead to organ dysfunction and vasoplegic syndrome[32]. This amplification of the inflammatory cascade is considered directly associated with the incidence of vasoplegia[33] commonly occurring during CPB along with the SIRS, leading to significant increases in tumor necrosis factor alpha (TNF-α) and IL-6, pro-inflammatory cytokines, has been observed in some patients undergoing CABG with CPB and may play a significant role in vasoplegia[34].

Mitigation of the deleterious effects of renal injury therefore requires a multimodal strategy aimed at decreasing factors likely to exacerbate tissue injury during the period of heightened risk, i.e., during CPB.

Two well characterized etiologies of worsening renal function are the generation of pfHb and activated complement during CPB. The extent of hemolysis during CPB surgery is directly correlated with both the length of the CPB procedure (> 3 hours) and the complexity of cardiac

surgery[7]. Red blood cells (RBCs) can hemolyze during CPB due to factors such as negative pressure cardiotomy suction, blood to material interactions, increases in foreign surface to-blood volume ratios, shear stress due to high flow rates through artificial blood circuits, and others[8].

Hemoglobin is thought to induce worsening renal function through: (a) tubular obstruction caused by heme protein precipitation, (b) ischemic injury resulting from increased renal vascular resistance and vasoconstriction, and (c) heme iron-driven oxidative injury[9,10], leading to inflammation and endothelial, tissue, and organ injury[11,13]. This promotes a pro-inflammatory cascade involving activation, generation, or expression of thrombin, complement, neutrophils, cell-adhesion molecules, mast cells, and multiple inflammatory mediators and includes the release of cytokines, IL-1β, IL-6, IL-8, and TNF-α 5,[16]. Plasma free hemoglobin also accounts for a significant portion of the damaging oxidative stress response that can damage other organs following cardiac surgery[35]. By directly causing tissue injury, in the post-operative SIRS response and through adverse physiologic changes, pfHb is thought to be a significant contributor in the development of multiple organ dysfunction syndrome (MODS) and multiple organ failure (MOF) in many patients following cardiac surgery.

Complement activation within the injured kidney is a proximal trigger of many downstream inflammatory events within the renal parenchyma that exacerbate injury to the kidney[36]. Targeted complement inhibition has become a potential strategy for preventing renal ischemia-reperfusion injury, end-organ damage and fibrosis in surgery and hemodialysis with both clinical and preclinical models suggesting that expression of C3aR and C5aR on both renal and circulating leukocytes contributes to the pathogenesis of renal ischemia-reperfusion injury, as well as a role for C3a and C5a in contributing to the pathogenesis of renal ischemia reperfusion[37].

Given that pfHb is a known offender causing organ damage in both kidneys and lungs, data suggestive of organ function preservation by intraoperative use of CytoSorb® would be of potential clinical benefit[18,40] post cardiac surgery. It has been demonstrated in published studies that the risk of AKI increases with the length of CPB and that post-procedure AKI is associated with increases in pfHb (Billings 2016; Windsant 2010)[25,35]. As discussed earlier, AKI was based solely on the serum creatinine criteria. While none of the resulting Odds Ratios (OR) for developing AKI are significantly different from 1, the observed OR (0.260) for patients with a valve replacement suggests there may be a benefit associated with CytoSorb® treatment. This could be the result of the lower increase in pfHb during CPB observed for the valve replacement patients in REFRESH I. Other studies have shown a direct link between development of AKI and chronic kidney disease (CKD) which is independently associated with higher mortality.

Plasma free hemoglobin also accounts for a significant portion of the damaging oxidative stress response that can damage other organs following cardiac surgery[35]. These complications may require increased length of stay and ICU time, prolonged mechanical ventilation, increased risk of nosocomial infections, or may increase the risk for systemic inflammatory response syndrome (SIRS) and potential progression to organ failure[24]. Because of this, the removal of free

hemoglobin during CPB has the potential to significantly reduce the risk of hemolysis-induced organ injury[25].

Furthermore, the REFRESH I study (see Investigator Brochure) showed that there was a trend for an increased risk of AKI associated with larger increases in TIMP-2 x IGFBP-7 from baseline (p=0.5822). It has been shown that Urine TIMP-2 x IGFBP-7 [(ng/mL)^2/1000] is associated with the incidence of AKI.[41] A greater increase in Urine TIMP-2/creatinine ratio was seen in the Control arm compared to the Treatment arm suggestive of a possible protective effect of treatment with CytoSorb®. A recent study demonstrated that significantly ($p<0.001$) higher levels of these markers were observed 4 hours after ICU admission in patients who developed AKI than those whom did not develop AKI.[42] These markers remained flat or decreased in patients without AKI, and started to elevate by the time of ICU admission in those that developed AKI. A reduction in pfHb and other inflammatory mediators could potentially be another mechanism by which CytoSorb® could decrease the risk of AKI in post cardiac surgery patients.

Consistent with the effect of device, treatment in the REFRESH I study (see Investigator Brochure) also showed a strong trend in decreasing activated complement C3a and C5a during surgery as well as other inflammatory markers. This trend was shown in a previous CytoSorb® study by Born in 2014[24]. Research has shown that the complement system has a role in inducing ischemia reperfusion injury in post cardiac surgery patients[43].

The REFRESH I Clinical Study (see Investigator Brochure), using CytoSorb®, showed considerable reductions in both C3a and C5a complement components in subjects undergoing elective valve replacement and other cardiac surgeries, relative to control patients.

This proposed pivotal study will assess whether the CytoSorb® Device (a non-pyrogenic, sterile, single-use device designed to remove plasma free hemoglobin and other inflammatory mediators) can decrease the incidence or severity of AKI, as defined by KDIGO, when used intraoperatively with CPB in subjects undergoing cardiac surgery. An assessment of renal function characteristics will be made during post-cardiac surgery days 1-2 immediately following surgery and will examine use of an adjunctive therapy for decreasing cardiovascular surgery renal injury leveraging the established features of the CytoSorb® hemoadsorptive device, specifically through adsorption of inflammatory mediators including pfHb and complement.

The primary objective of the study outlined in this protocol will be to examine the ability of treatment with CytoSorb® to produce a decrease in the proportion of subjects with AKI. As secondary endpoints, this study will assess whether treatment with CytoSorb® can decrease the incidence of patients with worsening renal function as reflected by an increase in serum creatinine relative to their pre-surgical baseline, and the ability of CytoSorb® to remove significant amounts of pfHb and alternative complement pathway inflammatory mediators (C3a, C5a) during cardiac surgery requiring CPB. The demonstration of effective removal of hemoglobin and activated compliment will be assessed for their relationship to a reduction in post-operative complications and preservation of kidney function.

The REFRESH I study indicated that the valve replacement sub-population showed the strongest trend for a device mediated reduction of pfHb concentration (see Investigator Brochure). An analysis of the primary subgroup, patients undergoing valve replacement with another procedure, relative to the entire study population will be conducted to confirm this during the run-in phase of this adaptive trial.

In REFRESH I two CytoSorb® devices were introduced in parallel 1 hour after the start of CPB. In REFRESH II, we are proposing to introduce the devices sequentially with the first device starting at T=0 (start of CPB) and the second device starting at T= 90 minutes after start of CPB. Reduction of pfHb for both the 1 hour delayed introduction of CytoSorb® (REFRESH I procedure) and the sequential introduction of CytoSorb® (REFRESH II procedure) at time 0 and 90 minutes after start of CPB has been modeled in *in-vitro* testing (see Investigator Brochure). In both the 1 hour delayed CytoSorb® introduction post start of CPB (REFRESH I procedure), and the sequential introduction of CytoSorb® at time 0 and 90 minutes (REFRESH II procedure) after start of CPB, both device configurations perform equivalently with regard to pfHb reduction. In both experiments, hemoglobin (Hb) was infused over the duration of the experiment; therefore, any difference between the control and treatment groups demonstrates the effect of CytoSorb® to remove pfHb. The result of the *in-vitro* testing demonstrates that the CytoSorb® device can effectively remove hemoglobin from blood. Initiation of hemofiltration at the start of CPB will allow for the adsorption of potentially nephrotoxic inflammatory mediators from the onset of bypass.

5. POTENTIAL RISK/BENEFIT

5.1 Potential Investigational Device Benefit

The observed and potential benefits of using CytoSorb® device intraoperatively during CPB in cardiac surgery:

- Reduction of free hemoglobin generated as a result of the CPB procedure
- Reduced inflammatory burden generated during the CPB procedure via reduction in C3a and C5a
- Potentially, a decrease in worsening renal function in subjects at significant risk
- Potentially, a reduction in vasopressor requirements
- Possible decrease in the amount of time on a mechanical ventilator
- Possible decrease in hospital stay, post CPB, including time in ICU
- Reduced risk of systemic inflammatory response syndrome (SIRS)

5.2 Investigational Device Residual Risks

A risk analysis was performed per ISO 14971, Medical devices -- *Application of risk management to medical devices, to determine the risks associated with CytoSorb®*. The risk profile associated with the CytoSorb® is expected to be minimal and consistent with other extracorporeal treatments currently in clinical use, such as dialysis. These risks include:

- Removal of drugs and hormones
- Allergic response to device materials
- Reduction in platelet count
- Reduction in white blood cell count
- Reduction in proteins (albumin, total protein) which may increase with CPB duration
- Reductions in albumin may result in a reduction in serum calcium
- Coagulation within device
- Infection
- Blood loss
- Hypothermia and chills
- Hemolysis
- Device leakage
- Circuit leakage

- Death

5.3 Risk Mitigation/Risk Benefit Rationale

To evaluate device related risk, a risk analysis was performed in accordance with the harmonized International standard EN ISO 14971:2012 *"Medical devices - Application of risk management to medical devices"*. The results of the analysis indicate that all potential device-related hazards have been reduced to an acceptable level. Mechanical and performance concerns have been reduced by extensive human, animal, and bench testing. The device materials have been shown to be biocompatible and acceptable for human use through ISO10993 testing and prior human clinical studies of the device for removal of beta-2 microglobulin in end-stage renal disease subjects and removal of cytokines in the treatment of sepsis. The CytoSorb® 300-ml Device has CE Mark approval as an extracorporeal cytokine filter and is believed to pose little or no additional risks relative to commercialized blood filter and dialysis devices.

All attempts will be made to minimize the clinical risks associated with the procedure. Adherence to protocol will be stressed by CytoSorbents' personnel during in-service training. All subjects will be monitored per routine standard of care. Based upon a re-analysis of data from the REFRESH I study (see Investigator Brochure) decreases in serum albumin and total serum calcium may be observed in the study population. Therefore, serum albumin, total serum calcium, and ionized calcium will be assessed pre-procedure; hourly for the duration of CPB; at the end of CPB; at admission to the post-surgical care unit and during post-operative Day 1. Administration of fluids (i.e. crystalloids) will be recorded pre-procedure, during the surgical procedure and following surgery through the end of the first post-operative day. Likewise, the supplementation of albumin and calcium will be recorded during the surgical period and following surgery through the end of the first post-operative day.

Treatment with CytoSorb® was well tolerated in the REFRESH I trial (see Investigator Brochure). This trial achieved the primary safety endpoint of the study with a favorable adjudication of all serious adverse events. There were no significant differences in the rates of adverse events (AEs) or serious adverse events (SAEs) between the two groups, including 30-day mortality, and there were no unanticipated adverse device effects (UADEs) reported.

AEs specifically related to the investigational device were infrequent (i.e., involving 2 [8.7%] subjects in the Treated arm with thrombocytopenia) and in both instances the subjects recovered without sequelae. The most frequent SAEs (occurring in ≥ 2 subjects in either treatment arm) were atrial fibrillation, thrombocytopenia, coagulopathy, anemia, anemia postoperative, pleural effusion, hypotension, malnutrition, and acute kidney injury. There were no appreciable differences in the frequency of SAEs between treatment arms.

Additionally, the Data Monitoring Committee (DMC) will be reviewing safety information on an ongoing basis, and this will also provide an additional level of risk mitigation.

6. INVESTIGATIONAL DESIGN

6.1 Study Objective(s)

6.1.1 Primary Objective(s)

To evaluate the safety and performance of the CytoSorb® device to decrease the incidence or severity of AKI in the first 48 hours after surgery when used intraoperatively with cardiopulmonary bypass (CPB) in subjects undergoing cardiac surgery. The study hypothesis is that, for patients at increased risk for AKI based upon use of the Cleveland Clinic Score for stratification of the risk of cardiac surgery associated AKI (CSA-AKI), the intraoperative usage of the CytoSorb® device in addition to the standard of care in a bypass circuit during cardiopulmonary bypass will decrease the incidence or severity of AKI per the Kidney Disease Improving Global Outcomes (KDIGO) clinical practice guideline definition of acute kidney injury.

The study intent is that the intraoperative use of the CytoSorb® device, in conjunction with a standard of care CPB circuit will decrease the incidence or severity of AKI per the Kidney Disease Improving Global Outcomes (KDIGO) clinical practice guidelines definition of acute kidney injury as compared to a standard of care control population during the first 48 hours post cardiac surgery.

6.2 Study Design

This will be a prospective, multi-center, randomized, pivotal, double-blinded clinical study. Subjects will be randomized in a 1:1 ratio to either standard of care (SOC) alone or standard of care and treatment with the CytoSorb® device, with or without HCA. Number of patients with and without HCA will be assessed in the interim analysis

Interim analyses will be carried out for potential (NON-BINDING) early stopping for an efficacy or futility conclusion.

An option to increase sample size (SSR, i.e., Sample Size Re-estimation) will be considered. SSR is based on the second interim analysis results using conditional probability (CP) to estimate the sample size necessary to boost the CP to 88%, assuming the observed response proportions are the TRUE underlying proportions.

To assure proper startup of the sites we will employ a roll in approach for up to the first two enrolled subjects from each site for training. All subjects enrolled as roll-in cases are treated with CytoSorb® device and are followed per protocol. The roll-in cases will be analyzed for safety as outlined in Section 11.1.1.

6.3 Study Outcomes

6.3.1 Primary Safety Endpoint

The primary safety endpoint of this trial is to evaluate the product safety profile through the assessment of device related adverse events during the study period.

6.3.2 Primary Effectiveness Endpoint(s)

The primary effectiveness endpoint is the incidence or severity of AKI in the first 48 hours after surgery when used intraoperatively with cardiopulmonary bypass (CPB) in subjects undergoing cardiac surgery as defined by the KDIGO clinical practice guideline through 48 hours post-cardiac surgery. Assessment of serum creatinine and hourly measurements of urine output will be recorded for the first 48 hours following admission to the ICU. The intended primary effectiveness sub-population includes all subjects enrolled and randomized to the treatment group who are undergoing valve replacement with another procedure (e.g., CABG, valve replacement) *without* Hypothermic Circulatory Arrest (HCA). Balance between patients undergoing cardiac surgery with and without HCA will be evaluated during the interim analysis and the primary sub-population confirmed.

6.3.3 Secondary Safety Endpoints

- Incidence and severity of procedure-related adverse events, through 30-days post-cardiac surgery.
- Incidence of Serious device-related adverse events
- Incidence and severity of unanticipated adverse device effects (UADEs)
- Change from baseline (pre-procedure) in vital signs (blood pressure, pulse and temperature)
- Change from baseline (pre-procedure) in safety laboratory assessments (hematology with differential, coagulation panel, and comprehensive metabolic panel) pre- and post-cardiac surgery through Day 7 or hospital discharge, whichever occurs first,
- Intra-cardiac surgery arterial blood gas measurements.
- Post-surgical Care Unit assessments (SOFA, vasopressor requirements, blood product transfusions, ventilation requirements, requirement for mechanical support, and renal replacement requirements) through hospital discharge or post-surgical Day 7, whichever occurs first.

6.3.4 Secondary Effectiveness Endpoint(s)

Secondary effectiveness endpoints include the following:

- Change from baseline (defined as the start of CPB) in pfHb assessed hourly for the duration of CPB and at the end of CPB;
- Change from baseline (defined as start of CPB) in Complement 3a (C3a) assessed hourly for the duration of CPB and at the end of CPB;
- Severity of AKI through hospital discharge or post-surgical Day 7, whichever is sooner
- Incidence of AKI through hospital discharge or post-surgical Day 7, whichever is sooner
- Change from baseline (defined as pre-procedure) in serum creatinine through hospital discharge or post-surgical Day 7, whichever is sooner;

Additionally, the following pharmacoeconomic endpoints are included:

- Duration of hospital stay, post-surgery
- Duration of ICU stay, post-surgery
- Duration of renal replacement therapy, post-surgery
- Duration of vasopressor use, post-surgery
- Duration of ventilator use, post-surgery

6.3.5 Exploratory Effectiveness Endpoints

The following known inflammatory mediators and predictive markers of kidney injury will be evaluated either during cardiac surgery or post-cardiac surgery.

During cardiac surgery:

- Change from baseline (defined as start of CPB) in C5a and inflammatory marker panel assessed hour during CPB an at the end of CPB

Post-cardiac surgery days 1-3:

- Change from baseline (defined as start of CPB) in C5a, inflammatory mediator panel, plasma kidney injury markers HMGB1 and Cystatin C and urine kidney injury markers NGAL, TIMP2, and IGFBP7 on post-surgical Days 1 through 3.

6.4 Subjects

Overview of subject population to include baseline characteristics and factors such as age, gender and race. Include # of subjects required to be included in the clinical investigation.

6.4.1 Inclusion Criteria

1. Male or female and ≥18 years of age.

2. Schedule for non-emergent surgical procedures requiring CPB with full sternotomy:
    a. Any combination of heart valve replacement or valve repair and another procedure (e.g., CABG) *without* HCA, coronary artery bypass graft (CABG) alone excluded OR
    b. Aortic reconstruction alone *with* HCA OR
    c. Aortic reconstruction with any combination of valve repair or valve replacement or CABG (but not CABG alone) *with* HCA

3. Estimated glomerular filtration rate (eGFR) ≥ 30 ml/min/1.73m^2 and at least ONE of the following additional risk factors for Cardiac Surgery Associated[3] (CSA)-AKI:
    a. Age ≥ 75 years
    b. Previous cardiac surgery with sternotomy;
    c. Documented New York Heart Association (NYHA) Class III or IV heart failure within 1 year prior to surgery;
    d. Left ventricular ejection fraction (LVEF) ≤ 35% by invasive or noninvasive diagnostic cardiac imaging - echocardiography, nuclear imaging, computed tomography, magnetic resonance imaging or angiography performed within 90 days prior to surgery. (If LVEF ≤ 35% by any invasive or noninvasive imaging procedure, patient meets the risk factor.)
    e. Insulin-requiring diabetes;
    f. Non-insulin-requiring diabetes AND the presence of ≥ +2 proteinuria on urinalysis (medical history or dipstick);
    g. Preoperative anemia (hemoglobin < 11g/dl)
    h. BMI ≥ 30 kg/m^2

6.4.2 Exclusion Criteria

1. Weight < 99 lbs. or > 383 lbs.;
2. The presence of AKI (KDIGO criteria) at the time of screening;
3. eGFR < 30 ml/min/1.73m^2;
4. Prior organ transplantation;
5. Dialysis-dependence;
6. Cardiogenic shock or hemodynamic instability within 48 hours prior to surgery as defined by a systolic BP <80 mm Hg and pulse >120 beats per minute (bpm) and requirement for inotropes or vasopressors or other mechanical devices such as intra-

aortic balloon counter-pulsation (IABP); or extracorporeal membrane oxygenation (ECMO). Short term vasopressor support is allowed during induction; however, ongoing requirement for vasopressor support to the time initiation of CPB would be excluded;

7. Requirement for any of the following within seven (7) days prior to cardiac surgery:
   a. defibrillator or permanent pacemaker,
   b. mechanical ventilation,
   c. intra-aortic balloon counter-pulsation (IABP),
   d. left ventricular assist device (LVAD),
   e. other forms of mechanical circulatory support (MCS) such as (ECMO);
8. Cardiopulmonary resuscitation within 14 days prior to cardiac surgery;
9. Known or history of inadequately treated cancer within the past 5 years. Inadequately treated cancer is defined as the continued presence or recurrence of cancer;
10. Known or suspected sepsis at time of screening or within the 30 days prior to cardiac surgery;
11. Known or suspected glomerulonephritis or interstitial nephritis at time of screening;
12. Untreated endocarditis; however, successfully treated endocarditis, defined as having completed a full course of antibiotics with no evidence of recurrence of infection, is acceptable;
13. Other current active infection requiring antibiotic treatment including Hepatitis B (HBV), Hepatitis C (HCV) or human immunodeficiency virus (HIV) infections. Documented inactive HVB and HCV are allowed as well as HIV infections treated with a stable antiviral regimen for a minimum of 30 days prior to the date of surgery;
14. Any other clinically relevant finding or medical history that, based upon the determination of the Investigator, may introduce additional risk to the patient or confound the results of the study;
15. Use of stress dosed steroids at the time of surgery, immunosuppressant medications to prevent transplant rejection or immunosuppressant chemotherapeutic agents;
16. Presence of acute or chronic pancreatitis;
17. Patients know to have Child-Pugh Class A Liver disease or with alanine aminotransferase (ALT) or aspartate aminotransferase (AST) > 2 times the Upper Limit of Normal (ULN);

18. Any congenital coagulation disorder;
19. Pregnancy or lactation;
20. Treatment with an investigational drug or device within 60 days prior to surgery;
21. Inability to comply with the requirements of the study protocol;
22. Subject has a platelet count of < 50,000 / µL;

6.4.3 Informed Consent & Enrollment Procedures

Informed consent shall be obtained in writing from the subject or their legally authorized representative and the process shall be documented before any procedure specific to the clinical investigation is applied to the subject, except when special circumstances described in Section 14.3 (Informed Consent Process) apply. If during the course of the study assessments, a subject is found not to be eligible for inclusion in the study, the subject or their representative should be notified and the reason for ineligibility documented on the screening log/form.

The general process for obtaining informed consent shall:

- Ensure that the principal investigator or his/her authorized designee conducts the informed consent process;
- Include all aspects of the clinical investigation that are relevant to the subject's decision to participate throughout the clinical investigation;
- Avoid any coercion or undue improper influence on, or inducement of, the subject to participate;
- Not waive or appear to waive the subject's legal rights;
- Use native non-technical language that is understandable to the subject;
- Provide ample time for the subject to read and understand the informed consent form and to consider participation in the clinical investigation;
- Include personally dated signatures of the subject and the principal investigator or an authorized designee responsible for conducting the informed consent process;
- Provide the subject with a copy of the signed and dated informed consent form and any other written information; and,
- Ensure important new information is provided to new and existing subjects throughout the clinical investigation.

6.4.4 Subject Withdrawal/Discontinuation

A study subject will be discontinued from participation in the study if:

- The investigator feels that the subject can no longer fully comply with the requirements of the study or if any of the study procedures would not be in the best interest of the subject.

- The subject is lost to follow up. A subject will be considered "lost to follow-up" and terminated from the study when all of the following criteria have been met:
  - Failure to complete the 30-day follow up telephone call without due cause; and
  - Documentation of three unsuccessful attempts to contact the subject via telephone and by certified mail.
- The subject wishes to withdraw their consent for participation in the study.

7. FIELD CLINICAL ENGINEERS

Support by a Field Clinical Engineer (FCE), as designated by the Sponsor, may be offered to assist with set up of the blind and provide guidance during the procedure. In instances where the Sponsor designated FCE has previously observed set-up of the investigational device and has found the unblinded perfusionist(s) skilled and competent to manage study procedures, a waiver of FCE assistance may be granted. All such waivers of FCE assistance will be documented and retained in the study file.

The FCE will observe and proctor the unblinded Perfusionist as he or she assembles the CytoSorb® cartridges (2) and peripheral FDA approved components as specified in the Instructions for Use. The unblinded Perfusionist is responsible for the final hookup to the hospital perfusion apparatus.

The CytoSorb® devices will be blinded by placement behind a barrier. When the device is being used, the unblinded Perfusionist will ensure proper flow through the CytoSorb® device.

Assembly of the blind space may be required in advance should other OR personnel be in the work space during set up to preserve knowledge of treatment allocation. The FCE or unblinded Perfusionist ensures the blind is maintained prior to, throughout and post-surgery.

8. STUDY ASSESSMENTS AND PROCEDURES

An outline of study assessments, procedures, labs and associated time windows at each visit is given in **Table 3** and **Table 4.** Assessments and procedures that are indicated as Standard of Care (SOC) are optional and should be recorded if the assessment or procedure is SOC for that institution. Failure to record an assessment/procedure that is listed as SOC will not be a violation of protocol. Required assessments and procedures (those not designated as SOC) not completed or completed outside of specified windows will be recorded as protocol deviation (see Section 13.2). Definitions of analysis populations and descriptions of statistical analyses of endpoints is briefly described in Section 11 and detailed in the Statistical Analysis Plan (SAP) for the study.

8.1 AKI Definition, Severity Grading, and Duration

Acute kidney injury (AKI) is defined in the KDIGO guidelines as meeting any of the following:

- Increase in serum creatinine by ≥0.3 mg/dL (≥26.5 µmol/L) within 48 h or;

- Increase in serum creatinine to ≥1.5 times baseline, which is known or presumed to have occurred within the prior 7 days or;
- Urine volume < 0.5 ml/kg/h for 6 hours

For the purposes of this study, the 48-h referenced in the first part of the definition will be the first 48 h following admission to the ICU (or equivalent post-surgical placement). Serum creatinine and urine volume (urine output) will be recorded through post-surgical Day 7 or hospital discharge, whichever comes sooner.

Staging of AKI for severity assessment will use the criteria summarized in the table below. Staging of AKI may be based on either change in serum creatinine OR changes in urine output.

Stage	Serum Creatinine	Urine Output
1	1.5 to 1.9-times baseline or ≥0.3 mg/dL (≥26.5 µmol/L) increase	< 0.5 mL/kg/h for 6-12 h
2	2.0-2.9 times baseline	< 0.5 mL/kg/h for ≥ 12 h
3	3.0 times baseline or SCr ≥4.0 mg/dl (≥353.6 µmol/L) or Initiation of renal replacement therapy	< 0.3 mL/kg/h for ≥ 24 h or Anuria for ≥ 12 h

Duration of AKI will be assessed through post-surgical Day 7 or hospital discharge whichever comes sooner. Duration of AKI is measured as the number of days from the point where the patient meets the criteria of AKI per the KDIGO definition until the point where the definition is no longer met.

8.2 Recording of urine output

Urine output is recorded at the following intervals:

- Total urine volume from the start of surgery to the end of CPB
- Total urine volume from the end of CPB to admission to the ICU
- Urine volume is recorded each hour for the first 48 h following admission to the ICU. The urine volume is recorded each hour even if the urinary catheter is removed before the full 48 h have elapsed.
- After the 48 h from ICU admission has elapsed, collect urine at the following intervals:
    - If the urinary catheter is still inserted, record urine volume every 3 h
    - If the urinary catheter has been removed, record urine volume every 8 h

8.3 Subject Screening

All subjects scheduled to undergo CPB surgery will be assessed for the potential inclusion in the study by a research physician, research nurse, or designated study staff. A pre-Screening Log will be used to maintain a cumulative log of all assessed subjects at the site. Should a subject not meet eligibility, the reason for non-eligibility will be recorded on the pre-Screening Log.

Medicare patients who meet the study eligibility criteria will be enrolled into the study. The results of this study are expected to be highly generalizable to the Medicare population.

8.4 Screening Visit

Written informed consent will be obtained from the subject (or legally acceptable representative on behalf of the subject) before any study-specific procedures are performed. (Note: Consent by a legally acceptable representative is allowed where applicable, and is in accordance with local laws, regulations, and ethics committee policy.) Subjects are considered enrolled once they sign the informed consent. Screening laboratory samples may be obtained up to 14 days prior to the date of surgery, so that results are available for review at the visit.

Patients are qualified for study participation during the screening period by confirmation of meeting all the inclusion criteria and none of the exclusion criteria. Evaluation of inclusion criterion 2 is based upon the intended surgical procedure during the screening period. During the pre-procedure and intra-operative periods, the intended procedure may change due to factors that could not be adequately determined or anticipated in advance of the procedures. These day-of-surgery changes to intended procedures may result in completion of a procedure that does not align with the eligibility criteria as detailed in inclusion 2. Since eligibility is assessed based on the intended procedure during the screening period, a change on the operative day is not considered a protocol deviation. Patients who have changes to their intended procedures on the day of surgery are study subjects and should progress through the study as detailed in the protocol. These subjects will be included in the Intent-to-Treat analysis.

The following procedures will be performed, and results recorded in the electronic Case Report Forms (eCRF):

- The investigator / designee will solicit a written informed consent from the subject (or legally acceptable representative on behalf of the subject) for study participation.
- Eligibility based on inclusion / exclusion criteria will be assessed.
- Medical history will be reviewed relative to the inclusion/exclusion criteria.
- Demographic details (age, gender, and race).
- Prior and concomitant medications taken during the past 60 days inclusive of any IV contrast agents administered within 48 hours of the procedure
- Complete physical examination
- Vital signs (HR, BP, Temp.) after 5 minutes supine
- Height and weight

- Echocardiogram for ejection fraction (if an acceptable method of determining LVEF (**Section 6.4.1 Inclusion 3d**) has not been performed within 90 days of enrollment)
- NYHA Class
- eGFR
- Urine or serum pregnancy test (women of child-bearing age without permanent birth control within 7 days prior to the surgery).
- Blood samples will be drawn for the following assessments:
    - Hematology
    - Coagulation panel
    - Comprehensive metabolic panel (must include total bilirubin and sodium)
    - Serum creatinine

8.5 Procedure Assessments

8.5.1 Subject Randomization

Subjects will be prospectively randomized. Randomization must occur prior to the start of CPB surgery, but only after the subject provides written informed consent and subject eligibility criteria is confirmed. Randomization should be performed within 5 days of start of the CPB surgery to minimize incidence of dropout prior to cardiac surgery and to allow adequate time for scheduling with the FCE. Subjects will be randomly assigned in a one to one (1:1) ratio to either the control arm (SOC) or treatment arm (SOC + CytoSorb® device). To ensure even distribution of subjects receiving treatment and control, with and without Hypothermic Circulatory Arrest (HCA) during procedure, the randomization will be stratified by study center and the intended use of HCA as a surgical technique during procedure.

Neither the investigational sites nor the Sponsor will have access to the randomization schedules, and subjects will not be told about their assignment prior to the surgery. A copy of the randomization assignment will be maintained in the subject's file. If the subject withdraws consent prior to the surgery, the reason for failure to treat as randomization will be recorded in the subject's study records. Safety data will not be collected for subjects who drop out prior to surgery.

8.5.2 Pre-Procedure

The following procedures will be performed, and results recorded in the eCRF. When patient screening is completed on the day of surgery, procedures/assessments that are listed to be completed again at pre-procedure do not need to be duplicated.

- Eligibility based on inclusion / exclusion criteria will be confirmed. Subjects who meet all inclusion and exclusion criteria will be randomized to either the control arm or to the treatment arm;
- Vital signs;

- Weight
- FiO_2 (if patient is intubated on mechanical ventilatory support)
- Sequential Organ Failure Assessment (SOFA) score will be assessed before induction of anesthesia;
- Initiate recording of fluids administration (e.g. crystalloids)
- Initiate recording of supplementation with calcium and/or albumin
- Blood samples will be drawn for the following assessments before induction of anesthesia:
  - Hematology;
  - Coagulation panel;
  - Comprehensive metabolic panel;
  - ABG (per SOC);
  - Ionized calcium
  - ACT (per SOC);
  - Serum creatinine;
  - Complement C3a and C5a;
  - Inflammatory mediator panel includes Interleukin 2 (IL2), Interleukin 4 (IL4), Interleukin 5 (IL5), Interleukin 6 (IL6), Interleukin 10 (IL10), Interleukin 12 (IL12), Interferon Gamma (INFG), Tumor Necrosis Factor alpha (TNFA);
  - Kidney injury markers – urine: urine neutrophil gelatinase-associated lipocalin (NGAL), insulin growth factor binding protein 7 (IGFBP7), tissue inhibitor of metalloproteinases 2 (TIMP2);
  - Kidney injury markers – plasma: high mobility group box 1 protein (HMGB1) and Cystatin C;
  - Plasma free hemoglobin (pfHb);

8.5.3 Procedure (CPB Surgery)

8.5.3.1 Treatment Arm

Subjects randomized to the treatment arm will receive standard of care bypass plus treatment with one CytoSorb® device at the beginning of CPB. If and when CPB continues for more than 90 minutes of bypass time, a second (parallel configuration) device will be opened to the bypass circuit. Treatment with the CytoSorb® device(s) will continue until the end of CPB. Blood flow is to be maintained continuously through the CytoSorb® devices.

As per the instructions for use (IFU), prior to hemoperfusion, prime and flush the circuit with saline or crystalloid solution and prevent any air from entering into the system and clamp

lines. During this phase, gently tap the CytoSorb® device to liberate any trapped air. Also, for a short period, the pump speed can be increased to allow for clear identification of any possible leaks at the increased pressure/flow rate. Hemoperfusion with CytoSorb® will begin at the commencement of CPB flow to the subject.

CytoSorb® treatment flow rates will be controlled utilizing a roller clamp placed just beyond the junction of the outlet-side Y-tube. The flow rate will be measured using a Sponsor provided Transonic (or equivalent) ultrasonic flow meter and probe, also placed just beyond the junction of the outlet-side Y-tube. The blood flow rate of the device will be adjusted to yield an initial target reading of ~400 ml/min (minimum above 300 ml/min is acceptable) measured beyond the junction of the outlet-side Y-tube. If/when CPB goes beyond 90 minutes and the second, parallel CytoSorb unit is brought into play 90 minutes into CPB by opening its C-clamp, total flow should be adjusted to yield a target flow rate of ~800 ml/min (minimum of 600 ml/min is acceptable), as measured beyond the junction of the outflow "Y" tube. Because of the parallel nature of the circuit, when using both CytoSorb units, this will equate to a target flow rate of ~400 ml/min through each device, with an acceptable lowest flow rate of 300 ml/min per device.

Throughout the treatment session, monitoring the CytoSorb device for blood leaks, pressure spikes or clots is essential. Any adverse device events and/or device observations must be recorded by indicating the location of leaks or by recording the size of clots present.

Assessments during procedure:

At the start of CPB:

- Blood product transfusions;
- Record CytoSorb start time;
- Operational notes (surgery/CPB start time, clamp times);
- Flow rate in CytoSorb circuit;
- Adverse events;
- Administration of fluids will be collected;
- Supplementation with Calcium or Albumin will be recorded;
- Concomitant medications will be collected, as needed;
- FiO_2
- Blood samples will be drawn for the following assessments:
  - ABG;
  - Ionized calcium;

- Total serum calcium;
- Serum albumin;
- ACT (per SOC);
- Complement C3a and C5a;
- Inflammatory mediator panel;
- Plasma free hemoglobin (pfHb);

Hourly while on CPB:

- Vasopressor requirements;
- Administration of fluids will be collected;
- Supplementation with Calcium or Albumin will be recorded;
- Flow rate in CytoSorb circuit (record flow rates every 30 minutes during treatment with CytoSorb device(s));
- Blood product transfusions;
- Blood samples will be drawn for the following assessments:
  - ABG;
  - Ionized calcium;
  - Total serum calcium;
  - Serum albumin;
  - ACT (per SOC);
  - Complement C3a and C5a;
  - Inflammatory mediators;
  - Plasma free hemoglobin (pfHb);

90 minutes from start of CPB:

- Record the start time of the second CytoSorb device and stop time;
- Blood samples will be drawn for the following assessments:
  - Complement C3a and C5a;
  - Inflammatory mediators;
  - Plasma free hemoglobin (pfHb);

End of CPB (i.e. pump shut off, prior to protamine):

- Vital signs;

- Vasopressor requirements;
- Administration of fluids will be collected;
- Supplementation with Calcium or Albumin will be recorded;
- Record the stop time of the CytoSorb device(s);
- Blood product transfusions;
- Record type of surgical procedure(s);
- Operational notes (surgery/CPB stop time, clamp times);
- Urine output;
- Adverse events;
- Concomitant medications will be collected as needed;
- FiO_2
- Blood samples will be drawn for the following assessments:
  - Hematology;
  - Coagulation panel;
  - Comprehensive metabolic panel;
  - ABG;
  - Ionized calcium;
  - ACT (per SOC);
  - Serum creatinine;
  - Complement C3a and C5a ;
  - Inflammatory mediator panel ;
  - Plasma free hemoglobin (pfHb) ;

*8.5.3.2 **Control Arm***

Subjects randomized to the control arm will receive standard of care bypass at the beginning of CPB.

The following procedures will be performed, and results recorded in the eCRF:

- Eligibility based on inclusion / exclusion criteria will be confirmed. Subjects who meet all inclusion and exclusion criteria will be randomized to either the control arm or to the treatment arm

- Vital signs
- Sequential Organ Failure Assessment (SOFA) score will be assessed before induction of anesthesia;
- Initiate recording of fluids administration (e.g. crystalloids)
- Initiate recording of supplementation with calcium and/or albumin
- Blood samples will be drawn for the following assessments:
  - Hematology
  - Coagulation panel
  - Comprehensive metabolic panel
  - ABG
  - Ionized Calcium
  - ACT (per SOC)
  - Serum creatinine
  - Complement C3a and C5a
  - Inflammatory mediator panel
  - Kidney injury markers – urine: urine NGAL, IGFBP7, TIMP2);
  - Kidney injury markers – plasma: HMGB1 and Cystatin C;
  - Plasma free hemoglobin (pfHb);

Assessments during procedure:

At the start of CPB:

- Blood product transfusions
- Operational notes (surgery/CPB start time, clamp times)
- Administration of fluids will be collected
- Supplementation with Calcium or Albumin will be recorded
- Adverse events
- Concomitant medications will be collected as needed
- FiO_2
- Blood samples will be drawn for the following assessments:
  - ABG

- Ionized calcium
- Total serum calcium
- Serum albumin
- ACT (per SOC)
- Complement C3a and C5a
- Inflammatory mediator panel
- Plasma free hemoglobin (pfHb)

Hourly while on CPB:

- Vasopressor requirements
- Administration of fluids will be collected
- Supplementation with Calcium or Albumin will be recorded
- Blood product transfusions
- Blood samples will be drawn for the following assessments:
  - ABG
  - Ionized calcium
  - Total serum calcium
  - Serum albumin
  - ACT (per SOC)
  - Complement C3a and C5a
  - Inflammatory mediator panels
  - Plasma free hemoglobin (pfHb)

90 minutes from start of CPB:

- Blood samples will be drawn for the following assessments:
  - Complement C3a and C5a
  - Inflammatory mediator panels
  - Plasma free hemoglobin (pfHb)

End of CPB (i.e. pump shut off, prior to protamine):

- Vital signs

- Vasopressor requirements
- Administration of fluids will be collected
- Supplementation with Calcium or Albumin will be recorded
- Blood product transfusions
- Record type of surgical procedure(s)
- Operational notes (surgery/CPB stop time, clamp times)
- Urine output
- Adverse events
- Concomitant medications will be collected as needed
- FiO_2
- Blood samples will be drawn for the following assessments:
  - Hematology
  - Coagulation panel
  - Comprehensive metabolic panel
  - ABG
  - Ionized Calcium
  - ACT (per SOC)
  - Serum creatinine
  - Complement C3a and C5a
  - Inflammatory mediator panels
  - Plasma free hemoglobin (pfHb)

8.5.4 Sampling during CPB

Subject blood samples to be drawn from an arterial line during CPB procedure by appropriate medical staff per institutional guidance (i.e., anesthesiologist or perfusionist).

8.5.5 Sampling End of CPB (i.e. pump shut off, post protamine)

Subject blood samples will be drawn by appropriate medical staff (i.e. anesthesiologist or perfusionist) per institutional guidelines, post-cardiac surgery. Note that samples for hematology and coagulation assessments should be drawn after the administration of protamine at a timepoint consistent with the standard of care for the institution.

8.5.6 Sample Collection and Storage

Instructions on the process for the collection and storage of the blood and urine samples are provided in the Study Laboratory Manual.

8.6 Post-Surgical Care Unit (i.e., CCU, ICU)

Admission to Post-Surgical Care Unit through 7 days or hospital discharge, whichever occurs first

8.6.1 Admission to Post-Surgical Care Unit (Treatment and Control Groups)

The following assessments will be performed within one hour after admission to the post-surgical care unit.

- Vital signs
- Weight
- FiO_2 (if the patient is intubated on mechanical ventilatory support)
- SOFA Score
- Record vasopressor requirements
- Blood product transfusions
- Record need for ventilation and mechanical support
- Record need for renal replacement
- Record urine output each hour for the first 48 h after surgery even if catheter is removed before 48 h
- Adverse events
- Concomitant medications will be collected as needed
- Administration of fluids will be collected
- Supplementation with Calcium or Albumin will be recorded
- Blood samples will be drawn for the following assessments:
    - Hematology
    - Coagulation panel
    - Comprehensive metabolic panel
    - ABG (per SOC)
    - Ionized calcium
    - ACT (per SOC)

- Serum creatinine
- Complement C3a and C5a
- Inflammatory mediator panel Kidney injury markers (plasma): HMGB1, Cystatin C:
- Kidney injury markers (urine): NGAL, IGFBP7, TIMP2);
- Plasma free hemoglobin (pfHb)

8.6.2 Post-Surgical Days 1-3

- Vital signs
- Weight
- FiO_2 (if the patient is intubated on mechanical ventilatory support)
- SOFA Score
- Record vasopressor requirements
- Blood product transfusions
- Record need for ventilation and mechanical support
- Record need for renal replacement
- Record urine output each hour for the first 48 h after surgery even if the catheter is removed before 48 h
- Adverse events
- Concomitant medications will be collected as needed
- Administration of fluids will be collected (Day 1 only required, SOC thereafter)
- Supplementation with Calcium or Albumin will be recorded (Day 1 only required, SOC thereafter)
- Blood samples will be drawn for the following assessments:
  - Hematology
  - Coagulation panel (per SOC)
  - Comprehensive metabolic panel
  - Serum creatinine
  - Complement C3a and C5a
  - ABG (per SOC)

- Ionized Calcium
- Inflammatory mediator panel
- Kidney injury markers (plasma): HMGB1, Cystatin C
- Kidney injury markers (urine): NGAL, TIMP2, IGFBP7)

8.6.3 Post-Surgical Day 4 through 7

(or hospital discharge, whichever occurs first) (Treatment and Control Groups)

The following assessments will be performed daily starting on post-surgical day 4 through day 7, or until hospital discharge, whichever occurs first:

- Vital signs
- Weight
- FiO_2 (if the patient is intubated on mechanical ventilatory support)
- Record vasopressor requirements
- Blood product transfusions
- Record need for ventilation and mechanical support
- Record need for renal replacement
- Record urine output
- Adverse events
- Concomitant medications will be collected as needed
- Administration of fluids will be collected
- Supplementation with Calcium or Albumin will be recorded
- Blood samples will be drawn for the following assessments:
  - Hematology (per SOC)
  - Coagulation panel (per SOC)
  - Comprehensive metabolic panel (per SOC)
  - Serum creatinine

8.6.4 Hospital Discharge

The following information with be collected at the time of hospital discharge:

- SOFA Score

- Record need for ventilation and mechanical support
- Record need for renal replacement
- Record urine output
- Hospital discharge date (to calculate duration of hospital stay)
- Adverse events
- Contact information where subject is going if less than 7 days post-cardiac surgery
- Pertinent concomitant medications
- Hematology
- Coagulation panel
- Comprehensive Metabolic Panel
- Serum Creatinine

8.7 Day 30 Post-Cardiac Surgery

The subjects will be contacted via telephone at 30 days post-cardiac surgery for assessment of the following since hospital discharge:

- Blood product transfusions
- Adverse events

Table 3: Schedule of Assessments

Event	Screening[1]	Procedure (CPB/Surgery)				Post-Surgery period			Discharge	Follow-up
		Pre-Procedure *(before induction of general anesthesia)*	**Start of CPB** *(±5 minutes)*	**Hourly while on CPB** *(±5 minutes)*	**End CPB** *(post protamine)*	**Admission to Post-Surgery Care unit** *(+1 hour)*	**Daily for Post day 1-3 Post-Cardiac Surgery or Hospital Discharge** *(whichever is sooner)*[2,5]	**Day 4-7 Post-Cardiac Surgery**	**Hospital Discharge**	**30 Day Follow-up** *(±5 days)*
Informed Consent	X									
Demographics, Height	X									
Weight	X	X				X	X	X		
Medical History	X									
Prior Medications (taken within 60 days of Screening Visit)	X									
Concomitant Medications – inclusive of IV contrast media administered within 48 h of procedure		As Needed								
Inclusion/Exclusion Criteria (w/in 14 days of surgery)	X									
Inclusion/Exclusion Review		X								
Vital Signs (HR, BP, Temp)[3]	X	X			X	X	X	X		
New York Heart Association (NYHA)	X									
Echocardiogram (Ejection Fraction)[6]	X									
Estimated Glomerular Filtration Rate (eGFR)	X									
Physical Examination	X									
SOFA Score (includes FiO2, level of hypotension assessment, evaluation of		X[7]				X[7]	X[7]		X[7]	

Event	Screening[1]	Procedure (CPB/Surgery)				Post-Surgery period			Discharge	Follow-up
		Pre-Procedure ***(before induction of general anesthesia)***	**Start of CPB** ***(±5 minutes)***	**Hourly while on CPB** ***(±5 minutes)***	**End CPB** ***(post protamine)***	**Admission to Post-Surgery Care unit** ***(+1 hour)***	**Daily for Post day 1-3 Post-Cardiac Surgery or Hospital Discharge** ***(whichever is sooner)[2,5]***	**Day 4-7 Post-Cardiac Surgery**	**Hospital Discharge**	**30 Day Follow-up** ***(±5 days)***
mechanical ventilation and vasopressor requirement, MAP, Glasgow Coma Score)[7]										
Randomization		X[4]								
Vasopressor Requirements[8]				X	X	X	X	X	X[9]	
FiO_2 (if patient is intubated on mechanical ventilatory support)		X	X		X	X	X	X		
Start/Stop Times of CytoSorb Treatment & Flow rate every 30 minutes *(Treatment arm only)*			X	X	X					
Blood Product Transfusions[10]			X	X	X	X	X	X	X	X
Ventilation & Mechanical support Requirements						X	X	X	X	
Renal Replacement Requirements						X	X	X	X	
Type of Surgical Procedure(s)					X					
Administration of fluids is recorded[11]		X----------						----------X		
Supplementation with Ca and/or Albumin[11]		X----------						----------X		
Operational notes (Surgery start & stop times, clamp times)			X		X					
Adverse Events/SAEs		As Needed (from time ICF is signed and subject is enrolled until study exit)								

[1] Within 14 days of surgery. If Screening is completed on the day of surgery, duplicate pre-procedure assessments/procedures do not need to be repeated.

[2] Daily, preferably at the same time each day.

[3] After approximately 5 minutes supine

[4] Randomization can be performed within 5 days prior to the cardiac surgery, but only after informed consent has been obtained.

[5] A minimum of 12 hours from time of admission to Post-Surgery Care Unit to the start of collection of data for daily is required.
[6] Echocardiogram for ejection fraction if an acceptable method (Section 6.4.1, Inclusion 3d) has not been completed within 90 days.
[7]SOFA score will be assessed pre-procedure (before induction of anesthesia or sedation); on admission to post-surgical care; daily for 3 days post-procedure; and at Day 7 post-procedure or discharge, whichever occurs first). For post-surgical SOFA assessments, the lowest mean arterial pressure (MAP) from the day is used in the SOFA determination.
[8]Vasopressor requirements: the maximum vasopressor dosage will be recorded each day (maximum dose over 1 hour).
[9] Vasopressors will be collected through day 7 if subject is still hospitalized.
[10] Blood transfusions will be collected through 30 days post cardiac surgery. Transfusion type (e.g. whole blood, packed red cells, platelets) will be recorded along with the volume administered
[11] Administration of fluids (crystalline) and supplementation with calcium and/or albumin begins at pre-procedure and will continue through the end of post-surgical Day 1. All administration and supplementation will be recorded. Following Day 1, administration of fluids and supplementation will be recorded as SOC until the point where the treating physician discontinues collection.

Table 4: Schedule of Laboratory Assessments

Event	Screening[1]	Procedure (CPB/Surgery)					Post-surgical period			Discharge	Follow Up
		Pre-procedure Day of surgery but before induction of anesthesia	Start of CPB (±5 min)	Hourly while on CPB (±5 min)	90 minutes after Start of CBP Start of Device # 2) (±5 min)	End CPB[14] Off pump and post protamine	Admission to post-surgical care unit (+ 1 h)	Post-surgical Days 1-3	Post-surgical Days 4-7 or discharge, whichever is sooner	Hospital Discharge	30-day Follow up (±5 days)
Pregnancy Test[4]	X										
Hematology [5, 8]	X	X[2]				X	X	X	SOC	X	
Coagulation [5,8]	X	X[2]				X	X	SOC	SOC	X	
Comprehensive Metabolic Panel [5, 9]	X	X[2]				X	X	X	SOC	X	
Serum Creatinine [3,15]	X	X[2]				X	X	X	X	X	
Complement C3a & C5a		X	X	X		X	X	X			
Arterial Blood Gases[13] (includes PaO_2, $PaCO_2$, pH, HCO_3)		SOC	SOC	SOC		SOC	SOC	SOC			
Ionized Calcium, Total Serum Calcium, Serum Albumin		X	X	X		X	X	X			
Activated Clotting Time (ACT)[10]		SOC	SOC	SOC		SOC	SOC				

Event	Screening[1]	Procedure (CPB/Surgery)					Post-surgical period			Discharge	Follow Up
		Pre-procedure Day of surgery but before induction of anesthesia	Start of CPB (±5 min)	Hourly while on CPB (±5 min)	90 minutes after Start of CBP Start of Device # 2) (±5 min)	End CPB[14] Off pump and post protamine	Admission to post-surgical care unit (+ 1 h)	Post-surgical Days 1-3	Post-surgical Days 4-7 or discharge, whichever is sooner	Hospital Discharge	30-day Follow up (±5 days)
Inflammatory Mediators[11]		X	X	X	X	X	X	X			
Kidney Injury Marker-Plasma HMGB1, Cystatin C		X					X	X			
Kidney Injury Markers (Urine NGAL, TIMP2 & IGBFP7		X					X	X			
Plasma Free Hemoglobin (pfHb)[5]		X	X	X	X	X	X				
Urine Output						X	X[6]	X[6]	X[6]	X	X

[1] Within 14 days of surgery.

[2] If Screening is completed on the day of surgery, duplicate pre-procedure assessments/procedures do not need to be repeated.

[3] Daily for each post-surgical day.

[4] Women of child-bearing age without permanent birth control will be evaluated with a urine or serum pregnancy test within 7 days prior to CPB procedure.

[5] Samples to be drawn from an arterial line during cardiopulmonary bypass procedure. (End CPB sample to be drawn by appropriate medical staff per institutional guidance (i.e., anesthesiologist or perfusionist) t after CytoSorb Device is stopped at end of CPB)

[6] Urine output will be recorded hourly for the first 48 hours even if the urinary catheter is removed before 48 h after surgery. After 48 hours, urine output will be measured every 3 hours while the catheter is in place. Once the catheter is removed, urine output shall be recorded every 8 hours for 7 days or through hospital discharge, whichever occurs first.

[7] Pre-procedure blood samples will be drawn prior to induction of anesthesia.

[8] Hematology includes: Hemoglobin, WBC w/differential, Platelets and Hematocrit. Coagulation panel includes (Activated Partial Thromboplastin Time (APTT), Prothrombin Time/International Normalized Ratio (PT/INR), and fibrinogen)

[9] Comprehensive metabolic panel must include sodium and total bilirubin.
[10] Only required as per site's standard of care.
[11] Inflammatory mediators panel includes Interleukin 2 (IL2), Interleukin 4 (IL4), Interleukin 5 (IL5), Interleukin 6 (IL6), Interleukin 10 (IL10), Interleukin 12 (IL12S), Interferon Gamma (INFG), Tumor Necrosis Factor alpha (TNFA)
[13] Values will be collected for the study as long as the patient is intubated on mechanical ventilatory support. If the patient is not intubated on mechanical ventilatory support, they are not required for the study.
[14] End of CPB sample is drawn by appropriate medical staff per institutional guidelines (i.e., anesthesiologist or perfusionist) after pump shut off, per institution SOC for drawing coagulation panel post administration of Protamine.
[15] All creatinine values for assessment of AKI will be taken from local lab draws (CMP). At time of lab sample preparations, a backup creatinine sample will be drawn and prepared and sent to Central Laboratory for archiving only. In event of missed Creatinine value, the archive sample will be run by Central Lab.

8.8 Safety/Device Assessment

8.8.1 Adverse Events

Throughout the study, the Investigator or designee will determine adverse event (AE) occurrences. An adverse event is any untoward medical occurrence (signs, symptoms, abnormal laboratory findings) in a subject regardless of relationship to the device or procedure. Each adverse event is considered to be either anticipated or unanticipated as described below. AEs occurring after the initiation of treatment (start of CPB) are considered to be treatment emergent. The site is required to report the classes of Adverse Events that occur in the study, described below.

Any AEs and remedial action required will be recorded in the patient's source data. The nature, time of onset, duration, and severity will be documented, together with an Investigator's (or designee's) opinion of the relationship to investigational device. Since the incidence and severity of AKI, as defined by KDIGO clinical practice guidelines, are the primary endpoints of the study, the presence of AKI should not be reported as an AE in the trial.

If an AE occurs, appropriate diagnostic and therapeutic measures are to be taken. Follow-up evaluations of the patient are to be performed until the patient recovers or until the clinical Investigator considers the situation to be no longer clinically significant.

Adverse events are monitored and registered on the AE form of the eCRF at each visit. Whenever possible, a specific diagnosis should be entered as the AE term. Outcomes and severity of the AE are not to be recorded as the AE term. In absence of a specific diagnosis, an individual AE form has to be filled in for each sign or symptom. Changes from baseline laboratory values which are abnormal values and that are classified as clinically significant by the investigator must be recorded as AEs.

Persistent AEs will be entered once in the eCRF until they are resolved or if a new event has to be documented due to deterioration. These AEs will be carefully monitored. If an AE is still not resolved at the end of the investigation, this will be documented as ongoing.

For recurrent AEs (i.e., AEs of the same nature, but with a different date of onset), an individual AE form has to be completed for each of them.

Adverse events will be assessed for a relationship to each of the following and relationship classified as unrelated, possible, probable, definite or unknown:

Investigational Device Related Adverse Event: An adverse event which, in the judgment of the Investigator, results as a consequence of the investigational device.

Surgical Procedure Related Adverse Event: An adverse event which, in the judgment of the Investigator, results as a consequence of the subject's surgical procedure and is not specific to the investigational device used.

Non-Investigational Device Related Adverse Event: An adverse event which, in the judgment of the Investigator, it is reasonable to believe that the event is associated with an accessory device used during the surgical procedure and is not specific to the investigational device use.

Adverse events will also be evaluated for severity based on the following definitions:

Grade 1	Mild:	Asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
Grade 2	Moderate:	Minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental Activities of Daily Living (ADL)*.
Grade 3	Severe:	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL**.
Grade 4* **	Life-threatening:	Life-threatening consequences; urgent intervention indicated.
Grade 5* **	Death:	Death related to AE

*Instrumental ADL refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.
**Self-care ADL refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.
***Adverse events that are assigned a severity of life-threatening or death will be recorded as Serious Adverse Events.

8.8.2 Serious Adverse Events

A serious adverse event (SAE) is any untoward medical occurrence that meets one or more of the following criteria:

- Led to death
- Led to a serious deterioration in the health of the subject that:
  - Resulted in a life-threatening illness or injury
  - Resulted in a permanent impairment of a body structure or body function
  - Required in-patient hospitalization or prolongation of existing hospitalization
  - Resulted in medical or surgical intervention to prevent permanent impairment to body structure or body function
- Led to fetal distress, fetal death, or congenital abnormality or birth defect.

8.8.3 Anticipated Adverse Events/Device Related Adverse Events

A variety of complications are expected to occur in subjects undergoing CPB and cardiac surgery, regardless of whether or not CytoSorb is used. These may include, but are not limited to:

Serious, but less common, risks:

- Bleeding during or after the surgery including bleeding in relation to anticoagulation
- Blood clots that can cause heart attack, stroke, or lung problems
- Heparin-induced thrombocytopenia
- Bypass induced thrombocytopenia
- Reduction in platelets
- Infection, including pneumonia
- Organ injury including:
  - Respiratory damage causing breathing problems or failure to wean from the ventilator
  - Cardiac dysrhythmias/arrhythmias
  - Kidney injury
  - Cognitive impairment
- Death

Less serious, but more common risks and adverse events:

- Low-blood pressure
- Nausea and/or vomiting
- Chills
- Allergic response to device materials
- Reduction in platelet count
- Reduction in white blood cell count
- Reduction in proteins (albumin, total protein)
- Coagulation within device
- Heparin-induced thrombocytopenia

Potential Adverse Device Events Related to CytoSorb:

- Removal of drugs and hormones
- Allergic response to device materials
- Reduction in platelet count
- Reduction in white blood cell count
- Reduction in proteins (albumin, total protein) which may increase with CPB duration.
- Reductions in albumin may result in a reduction in serum calcium

- Coagulation within device
- Infection
- Blood loss
- Hypothermia and chills
- Hemolysis
- Device leakage
- Circuit leakage
- Death

8.8.4 Unanticipated Adverse Device Effect

An unanticipated adverse device effect (UADE) is any serious adverse effect on health or safety or any life-threatening problem or death caused by, or associated with the device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan or application (including a supplementary plan or application), or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects.

8.8.5 Device Malfunction/Observations

If there is a device malfunction or other observation with the CytoSorb device, the Investigator (or designee) is required to complete the Device Observation eCRF to notify the Sponsor immediately and indicate if the observation resulted in an adverse event and indicate if complications are related to the device, procedure or underlying disease.

In the event of a suspected observation or device problem, the device shall be returned to the Sponsor for analysis. Instructions for returning the investigational device are included in the Study Reference Manual.

If the Investigator cannot determine the cause of the event, it should be classified as unknown.

8.9 Reporting Procedures

8.9.1 Investigator Reporting

The Investigator at each participating center is ultimately responsible for reporting adverse events to the Sponsor or its designee. The information to be reported on the adverse event should include the start date of the adverse event, treatment, resolution, and assessment of both the seriousness and the relationship to the investigational device. The Investigator (or designee) is required to complete the adverse event eCRF at each study visit if an adverse event occurs. One adverse event eCRF must be completed for each adverse event. Investigators must report SAEs/UADEs to the Sponsor within 24 hours of investigational site knowledge of event

occurrence by entering data into the eCRF. The Investigator should report all serious adverse events to the IRB as required.

If there is a device malfunction or other observation, the Device Observation CRF requires the Investigator to notify the Sponsor within 24 hours, indicate if the observation resulted in an adverse event and indicate if complications are related to the device, procedure or underlying disease.

In the event of a suspected observation or device problem, the device shall be returned to the Sponsor for analysis. Instructions for returning the investigational device are included in the Study Reference Manual.

8.9.2 Sponsor Reporting

All adverse events will be reported to the regulatory authority/FDA at least annually.

9. STUDY OVERSIGHT

9.1 Clinical Events Committee

An independent group of physicians that are not involved in the clinical investigations will act as the Clinical Events Committee (CEC) under the direction of the CRO. The CEC will be responsible for the review and validation of reported potential ENDPOINT adverse events (i.e. all device/procedure related SAEs) that occur within 30 days post procedure per the CEC Charter. Policies and procedures governing the work of the CEC will be provided in a CEC Charter. The CEC Charter will be developed prior to the start of study enrollment.

9.2 Data Monitoring Committee (DMC)

The study will utilize a Data Monitoring Committee (DMC) which will be comprised of three (3) total members (2 physicians and a biostatistician) with relevant clinical/medical experience with the product and/or indication/disease under clinical investigation. The primary role of the DMC is to oversee the safety of subjects enrolled into the study. The membership of the committee will be independent from the investigative sites and study sponsor in order to reduce any potential for perception of bias and to remain free from potential conflicts of interest. In order to oversee the safety of subjects as they are enrolled into the study, the DMC will establish a charter including a mission statement, operating procedures, and proposed monitoring criteria for the study, including any required interim analysis time points for assessing safety and proposed study stopping rules, if appropriate. The DMC will meet to ratify their operational charter within a reasonable timeframe relative to the enrollment of the first few subjects entering into the study. Written recommendations from the DMC will be provided to the study sponsor.

9.3 Central Laboratory

A central laboratory shall be utilized to analyze some of the blood samples, and some of the urine samples collected in this study. The sample size and shipping requirements are provided to the sites in the Study Laboratory Manual. All sites will be trained on the collection, preparation, and shipping requirements for central laboratory samples.

10. STUDY MONITORING

The CRO will monitor and manage the data for the investigational study on behalf of the Sponsor, CytoSorbents, Inc. Some co-monitoring and remote data checking will also be done by Sponsor. A study specific monitoring plan will be created to ensure protocol compliance and applicable regulatory requirements.

Clinical monitors will verify subject data and ensure compliance is consistent with Good Clinical Practice (GCP), clinical protocol and other study requirements, according to the guidelines per the Monitoring Plan, set forth in CROs monitoring Standard Operating Procedures (SOPs), and 21 CRF Part 812.

10.1 Monitor Training

The Sponsor and/or designated monitors will be trained appropriately to monitor study progress including but not limited to the protocol and eCRFs, and per the Monitoring Plan.

10.2 Site/Investigator Training

The Sponsor and CRO shall be jointly responsible for providing training to the Investigator and appropriate clinical site personnel on the following topics:

- Protocol
- EDC/Database operations
- Central Laboratory Requirements
- CytoSorb® Device

10.3 Site Monitoring

Completed eCRFs will be verified by the monitor both at the investigational sites and remotely at regular intervals throughout the study. The Investigator will allow the monitor and/or representative of the Sponsor, and any regulatory body to review and inspect the study files, subject eCRFs, subject medical records and other related study documents as required.

All eCRFs will be reviewed for completeness and clarity. Missing or unclear data will be investigated by the monitor and will be retrieved, clarified and entered by study personnel as necessary throughout the study. CytoSorbents, Inc., or their authorized representative may request additional documentation from the Investigator such as physician procedure notes or physician written summaries when adverse events are observed and reported.

10.4 Regulatory Agency Inspection

In the event that an Investigator is contacted by a regulatory agency regarding this study, the Investigator will notify the Sponsor or its designee immediately. The Investigator and research coordinator must be available to respond to reasonable requests and queries made during the inspection process. The Investigator must provide the Sponsor or designee with copies of all correspondence that may affect the review of the current trial (e.g., Form FDA 483, Inspectional Observations and Warning Letters). The Sponsor may provide needed assistance in responding to regulatory audits.

11. STATISTICAL CONSIDERATIONS

General statistical considerations: Descriptive statistics will be the mean, standard deviation, median, minimum and maximum for continuous variables and count and percent of patients for categorical variables. Statistical analyses will be carried out using SAS Version 9.4 or later and/or the R software language.

11.1 Analysis Populations

Intent-to-Treat (ITT): All randomized patients.

Modified Intent-to-Treat (mITT): All randomized patients who meet all inclusion criteria through the completion of the surgical procedure. There are two mITT analysis sub-populations defined as follows:

- The primary sub-population includes all subjects enrolled and randomized to the treatment group who are undergoing valve replacement with another procedure (e.g., CABG, valve replacement) *without* Hypothermic Circulatory Arrest (HCA). This is the primary population for efficacy analyses.
- The secondary sub-population consists of all surgical subjects enrolled and randomized to the treatment group who are undergoing aortic reconstructions with or without other procedures (e.g., CABG, valve replacement) *with* HCA. This is a secondary population for efficacy analyses.

The primary analyses on the primary efficacy endpoint will be performed on these subpopulations. Subjects will be analyzed according to the treatment to which they were randomized.

Safety: Comprised of all roll-in and randomized subjects in whom treatment was attempted. Safety analysis will be performed on this population; it will also be performed separately on the above mITT populations. Patients will be summarized according to the actual treatment received.

Per Protocol (PP): Defined as the subset of patients in the mITT sub-population who had no major protocol violations. Efficacy analyses on the two PP sub-populations will be considered secondary. Patients will be analyzed by actual treatment received.

Roll in patients are not randomized and are only included in the safety population.

11.1.1 Safety and Tolerability Evaluations:

The following analyses will be conducted on the Safety population and the two mITT sub-populations.

Continuous safety data will be summarized with descriptive statistics (arithmetic mean, standard deviation [SD], median, minimum, and maximum) by treatment group. Categorical safety data will be summarized with frequency counts and percent of patients by treatment group. Adverse events (AEs) will be coded using the most current Medical Dictionary for Regulatory Activities (MedDRA®) available. A by-participant AE data listing, including verbatim term, preferred term, system organ class, treatment, severity, and relationship to study device will be provided. The number and percent of participants experiencing procedure and device related AEs and serious AEs will be summarized by treatment group, system organ class, and preferred term.

Laboratory evaluations and vital signs assessments will be summarized by treatment group and protocol specified collection time point. A summary of change-from-baseline at each protocol specified time-point by treatment group will also be presented. "Shift Tables" will be provided to summarize count and percent of patients with changes from baseline normal range status (below, within, above) to on/post-treatment normal range status.

Further details regarding presentation and analysis of safety data will be detailed in the statistical analysis plan (SAP).

11.1.2 Effectiveness evaluations:

The primary endpoint will be assessed in each modified intent-to-treat (mITT) population in step-wise fashion beginning with the primary sub-population. If a significant beneficial effect is met for CytoSorb over control at a one-sided overall 0.025 level of significance, then the study will be considered to be a primary endpoint statistical success on this sub-population and testing will proceed to the secondary sub-population. Otherwise the study will be considered to have statistically failed the primary endpoint.

We have selected to use a modified intent-to-treat population (mITT) population to avoid the consequence of including patients that were excluded from the study after randomization due to administrative reasons.

Use of the mITT population as defined is not anticipated to result in bias or informative censoring of the data.

The primary endpoint (rank order presence and severity of AKI) will be compared between treatments with Wilcoxon Rank Sum test. Continuous endpoints will be assessed via ANCOVA with baseline value as covariate. Binary endpoints will be assessed via unconditional exact binomial test. The primary endpoint will be summarized by subgroup factors, e.g., those who receive IV contrast within 48 hours prior to surgery versus those who do not. Additionally, subgroup-by-treatment interaction may be tested via generalized ordered categorical model and/or for binary combinations of outcomes (e.g., presence / absence of any AKI, and/or severe AKI) by logistic regression. Details will be in the SAP.

11.2 Statistical Power and Sample Size Determination

The REFRESH II study hypothesis is that, for patients at increased risk for AKI based upon use of the Cleveland Clinic Score for stratification of the risk of cardiac surgery associated AKI (CSA-AKI), the intra-operative usage of the CytoSorb device in addition to the standard of care in a bypass circuit during cardiopulmonary bypass will decrease the overall incidence and/or proportion of acute kidney injury (AKI) by stage as defined by the Kidney Disease Improving Global Outcomes (KDIGO) clinical practice guideline definition of acute kidney injury. The AKI Stage is scored as 0, 1, 2 or 3. AKI Stage of 0 indicates no AKI, and AKI stages 1, 2 and 3 indicate mild, moderate, and severe AKI, respectively. Success will be defined by demonstrating a shift in distribution toward overall less severe AKI or fewer incidences of AKI in the CytoSorb population.

The distribution of AKI Stage (0, 1, 2, 3) expected in the control group was estimated from prior data. Sample trial outcomes for which power was computed are summarized in **Error! Reference s ource not found.** below for total N=267 patients in the primary analysis subgroup ("without HCA", randomized 1:1). The proportion of "without HCA" patients will be at least 2/3 of the total sample size 400. 420 patients will be enrolled to account for an expected 5% premature withdrawal rate. Power was computed for comparing binomial proportions of patients with AKI Stage 1, 2, or 3 (or i.e., any AKI); proportions with AKI Stage 2 or 3; and for an ordered categorical rank test comparing the distribution of AKI Stages 0, 1, 2, 3 between treatments. Since it is uncertain whether CytoSorb will lower the overall AKI rate or that of Stages 2 and 3, the ordered categorical test will be primary, i.e., the primary null and alternative hypotheses are

- H0: $\pi 00 = \pi 01$, $\pi 10 = \pi 11$, $\pi 20 = \pi 21$, $\pi 30 = \pi 31$
- H1: $\{\pi 30 \geq \pi 31$ and/or $(\pi 20 + \pi 30) \geq (\pi 21 + \pi 31)$ and/or $(\pi 10 + \pi 20 + \pi 30) \geq (\pi 11 + \pi 21 + \pi 31)\}$ AND one or more of the above is strictly ">"

where $\pi i0$ is the proportion of control patients in AKI level i (i=0, 1, 2 and 3) and where $\pi i1$ is the proportion of CytoSorb patients in level i. Note that level i=0 is no AKI. The test of the null

hypothesis will be carried out at an overall one-sided 0.025 level of significance using a one-sided Wilcoxon Rank Sum test, taking into account the interim analyses as described below.

11.2.1 Interim Analyses

Specifically, since the treatment difference could be larger or smaller than expected, interim analyses will be carried out for potential (NON-BINDING) early stopping for an efficacy or futility conclusion.

Note that the BLINDED combined AKI incidence rate will be monitored throughout the trial to inform on enrichment with patients at higher risk of AKI so as to yield sufficient AKI events to improve the expected precision of the treatment comparison.

Table 5: Overall Power for the ordinal outcome, the Any AKI dichotomous outcome (labeled Stages 1,2,3 below), and the Moderate/Severe AKI dichotomous outcome (labeled Stages 2,3 below).

		Percent of Patients with Indicated AKI Stage					
AKI Stage -->		**0= no AKI**	**Stage 1**	**Stage 2**	**Stage 3**	**Stages 1,2,3**	**Stages 2,3**
Example 1 (total N=267)	**CytoSorb**	36%	53%	10%	1%	64%	11%
	Control**	24%	47%	26%	3%	76%	29%
power* -->		0.93				0.57	0.96
Example 2 (total N=267)	**CytoSorb**	44%	40%	14%	2%	56%	16%
	Control**	24%	47%	26%	3%	76%	29%
power* -->		0.96				0.94	0.72
* power to yield statistically significant (alpha=0.025 1-sided) difference between treatments with total N "without HCA" patients randomized 1:1							

Note that the dichotomous outcomes will be considered secondary. The Table 5 power calculations do not consider the planned interim analyses. They are presented for reference, and to demonstrate the rationale for the primary analysis method based on the ordinal outcome. Note that for example 1, had the primary endpoint been the dichotomous outcome any AKI, power would have been only 57%. For example, 2, had the primary endpoint been the dichotomous outcome moderate/severe AKI, power would have been only 72%. For both examples 1 and 2, power is over 90% via the ordinal outcome.

Simulations were carried out for a Group Sequential Design (GSD) planned for Total N=267 "without HCA" patients (randomized 1:1), with Interim Analyses (IA) after AKI status (Stage 0,1,2,3) is determined for 138 (IA1 at ½ total N) and N=200 (IA2 at ¾ total N). NON-BINDING early stopping for efficacy is considered if p<0.001 at IA1 or IA2; and NON-BINDING early futility stopping is considered if the Conditional Power (CP) is less than 5%. The final analysis requires p<0.0247 1-sided in order to control the overall type 1 error at 0.025 1-sided.

NOTE: CP is the probability of a statistically significant (alpha=0.0247 1-sided) at the final analysis (N=267) if the TRUE underlying between-treatment difference is equal to that observed at the IA. These p-value boundaries for efficacy conclusions control the overall type 1 error at 0.025 1-sided.

11.2.1.1 Biomarker Interim Review

A review of biomarker results will be completed at the time of the interim analysis. If insufficient data exist to enable detection of treatment effect, then the number of patients providing biomarkers may be increased. Methods used to determine the required biomarker sample size increase along with the revised number for patients will be detailed in an amendment to the protocol following the analysis.

11.2.2 Sample Size Re-estimation

An option to increase sample size (SSR, i.e., Sample Size Re-estimation) is also considered. SSR is based on the IA2 results using CP to estimate the sample size necessary to boost the CP to 88%, assuming the observed response proportions are the TRUE underlying proportions. CP is the probability of a significant result at final analysis assuming the observed response proportions are the TRUE underlying ones. The design works as follows at the IA:

- If CP<5%, the trial MAY be stopped for futility
- If p<0.001 1-sided, the trial MAY be stopped for an early efficacy conclusion
- If 5% ≤ CP < 50% (unfavorable zone), the trial continues to the planned N=267
- If 50% ≤ CP < 88% (promising zone), sample size is increased to that required to boost CP to 88%
    - If that sample size exceeds N=333, the trial continues to that N=333
- If CP ≥ 88% and p>0.001 1-sided (favorable zone), the trial continues to N=267 subjects, as originally planned

This is the "promising zone" sample size re-estimation (SSR) design of Mehta & Pocock (2011)[44], which uses the work of Chen, DeMets, Lan (2004)[45] to justify preservation of type 1 error for the final analysis based on the combined data from the entire trial. The power and performance characteristics based on 10,000 simulations of this design are summarized in Table 6 below for 6 TRUE underlying scenarios and associated analyses based on either the binary endpoint of

proportion of patients with AKI or the corresponding ordered categorical test, each via normal approximation. [NOTE: power and design performance characteristics were computed via Cytel's EAST software, which uses normal approximation for GSD calculations and adaptive design simulations. The actual treatment group comparisons on the primary endpoint will be using the Wilcoxon Rank Sum test using the GSD stopping boundaries mentioned above; details to be specified in the SAP; treatment group comparisons on the dichotomous endpoints will be carried out using the chi-square test.] Effect Size (mean difference divided by SD) was computed for the two categorical distributions in the table above. The binary endpoint differences also correspond to those of AKI Stages 1, 2, 3 and AKI Stages 2, 3 in Table 5 above. Note that the ordered categorical test preserves the power at over 80% for both distributions, whereas the power for binary rate differences less than 0.15 is much less than 80%.

For the Scenario A in the table below, the statistical design described above was simulated 10,000 times with responses generated from TRUE underlying response rate 0.76 for control and 0.61 for CytoSorb. 92% of those simulations yielded a resultant p-value at one of the 3 analysis (IA1, IA2, or final analysis) that was lower than the boundary for that analysis ($p<0.001$ 1-sided for IA1 and IA2, and $p<0.0247$ for the final analysis). If there was no efficacy conclusion at IA1 and IA2 and the IA2 result was "promising" (i.e., $50\% \leq CP < 88\%$), then the sample size was increased to boost CP toward 88%, resulting in power to 97% for those IA2 outcomes. The average “without HCA” sample size across the 10K simulations was 221, and 320 for the subset of simulations that yielded a CP in the promising zone. 3% of the simulations yielded a futility result at IA1, 1% at IA2, and 4% at the final analysis. 25% of simulations yielded $p<0.001$ 1-sided at IA1 and 22% at IA2; 46% of simulations yielded $p<0.0247$ 1-sided at the final analysis. 6% of the simulations proceeded past IA2 with IA2 result in the "unfavorable" zone ($5\% \leq CP < 50\%$); 12% in the "promising" zone ($50\% \leq CP < 88\%$); and 32% in the "favorable" zone ($CP \geq 88\%$ and IA2 p-value > 0.001 1-sided). Those proportions of outcomes represent the estimated probabilities of those outcomes for the actual trial.

Scenarios A, B, C, and D represent GSD+SSR design options based on the proportions of patients with AKI (A & B corresponding to Example 1 in Table 5) or with severe AKI (C and D corresponding to Example 2 in Table 6); Scenarios E and F (corresponding to the ordered categorical examples 1 and 2 in Table 6) represent GSD+SSR design options based on the ordinal AKI Stage 0,1,2,3 endpoint. All scenarios were assessed via normal approximation to those respective distributions as listed below in Table 6.

Table 6: Performance Characteristics for Group Sequential Design with Sample Size Re-Estimation Option for binary and ordinal AKI endpoint

Scenario-->	A	B	C	D	E	F
TRUE underlying AKI rate control	**0.76**	**0.76**	**0.29**	**0.29**		
TRUE underlying AKI rate CytoSorb	**0.56**	**0.64**	**0.11**	**0.16**		
TRUE underlying Effect Size (ordinal)					**0.39**	**0.42**
overall power (alpha=0.025 1-sided)	0.92	0.55	0.96	0.70	0.88	0.92
power if IA2 promising	0.97	0.83	0.97	0.87	0.93	0.95
average sample size	221	237	215	243	227	219
average sample size (if IA2 promising)	320	323	318	322	324	324
Probability of "futility" at IA1	0.03	0.19	0.01	0.11	0.05	0.03
Probability of "futility" at IA2	0.01	0.07	<0.01	0.05	0.02	0.01
Probability of "futility" at Final Analysis	0.04	0.19	0.02	0.14	0.05	0.04
Probability of "efficacy" at IA1	0.25	0.05	0.28	0.08	0.21	0.26
Probability of no "efficacy" at IA1 but efficacy IA2	0.22	0.06	0.25	0.10	0.19	0.22
Probability "efficacy" at Final Analysis	0.46	0.44	0.43	0.53	0.48	0.44
Probability IA2 unfavorable	0.06	0.21	0.06	0.16	0.09	0.07
Probability IA2 promising	0.12	0.18	0.09	0.19	0.14	0.12
Probability of no “efficacy” at IA2 but IA2 favorable	0.32	0.24	0.31	0.31	0.30	0.29

11.3 Study Success

An individual study subject will be defined as a success if during the 48 hours following surgery, they do not receive a diagnosis of AKI by the KDIGO Guidelines. In contrast, a patient whose clinical parameters result in a diagnosis of AKI will be classified according to AKI stage. Study success will be defined by demonstration of a statistically significant shift in distribution of AKI Stages 0, 1, 2, 3 toward less severe and/or no AKI with CytoSorb in comparison to standard of care.

11.4 Treatment of Missing or Spurious Data

Multiple imputation methodology will be used for missing data imputation; details will be in the SAP.

11.5 Disposition and Baseline Characteristics

Number and percent of patients discontinuing prematurely from the study will be provided by treatment group and by reason discontinued. All data for background and demographic variables will be listed by treatment group and subject. Summary statistics will be provided by treatment group. Relevant medical history, current medical conditions, results of laboratory screens, and any

other relevant information will be summarized by treatment group and listed by treatment group and subject

12. DATA HANDLING AND RECORD KEEPING

Each participating site will maintain appropriate medical and research records for this trial, in compliance with ICH E6 Section 4.9 and regulatory and institutional requirements for the protection of confidentiality of subjects. As part of participating in a manufacturer-sponsored study, each site will permit authorized representatives of the sponsor(s), the sponsor's designee, and regulatory agencies to examine (and when required by applicable law, to copy) clinical records for the purposes of quality assurance reviews, audits, and evaluation of the study safety and progress.

Source data are all information, original records of clinical findings, observations, or other activities in a study necessary for the reconstruction and evaluation of the trial.

The Investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported. All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data.

12.1 Data Management Procedures

Electronic Case Report Forms (eCRFs) will be used to collect all subject data during the course of the study, which are part of a database that meets 21 CFR Part 11 requirements. eCRFs must be fully completed for each subject and electronically signed by the Investigator when complete.

Federal Regulations and Good Clinical Practice Guidelines require that Investigators maintain information in the study subject's medical records that corroborate data collected on the eCRFs. In order to comply with these regulatory requirements, the following information should be maintained:

- Medical history/physical condition of the study subject before involvement in the study sufficient to verify protocol entry criteria.
- Dated and signed notes on the day of entry into the study including the study Investigator, study name, subject number assigned and a statement that consent was obtained.
- Dated and signed notes from each study subject visit with reference to the CRFs for further information, if appropriate (for specific results of procedures and exams).
- Information related to adverse events.
- Study subject's condition upon completion of or withdrawal from the study.
- Discharge summaries/procedure reports.

12.2 Data Retention

Maintenance of study records should be maintained for a period of two (2) years after the latter of two occurrences:

1. The date when the investigation is terminated or completed, or
2. The date that the records are no longer needed to support a premarket approval (PMA).

These documents should be retained for a longer period, however if required by local regulations. No records will be destroyed without the written consent of the sponsor, if applicable. It is the responsibility of the Sponsor to inform the Investigator when these documents no longer need to be retained.

12.3 Investigator Records

Investigators will maintain complete, accurate and current study records. Investigator records shall include the following materials:

- **Correspondence**: All significant correspondence with another investigator, an IRB, the sponsor, a monitor, or FDA, including required reports.
- **Subject Records**: Signed informed consent forms, copies of supporting documents (laboratory reports, reports of diagnostic tests, medical records, etc.) and records of exposure of each subject to the device. Informed consent must comply with FDA regulations (21 CFR, part 50).
- **Clinical Investigational Plan/Protocol**: A current copy of the Clinical Study Protocol and amendments including Instructions for Use of the CytoSorb® and copies of the eCRFs.
- **Institutional Review Board (IRB) Information**: All information pertaining to IRB review and approval of this clinical study including a copy of the IRB letter approving the clinical study, a blank informed consent form approved by the IRB, and certification from the IRB Chairman that the IRB complies with FDA regulations (21CFR, Part 56), and that the IRB approved the clinical study protocol based on the Report of Prior Investigations.
- **Investigator Agreements**: Copies of the signed Investigator, Sub-Investigator Agreements with accompanying curriculum vitae. Signed Protocol pages.
- Study documents including Investigational Device Accountability logs (including device shipment list/packing lists), Screening and Enrollment Logs, Site Visit Log,
- **Other**: Any other records that may be required by applicable state or federal laws.

12.4 Investigator Reports

The Investigator will prepare and submit the following reports:

Table 7. Responsibilities for Preparing and Submitting Reports

Type of Report	Prepared by Investigator for	Time of Notification
Enrollment Notification eCRF	Sponsor	Within 24 hours of the subject signing the ICF
Completion of eCRFs	Sponsor	Within 14 working days
Serious/ Unanticipated Adverse Event eCRF	Sponsor and IRB (as required)	Within 24 hours of knowledge or as required by IRB
Adverse Event (device related or not) eCRF	Sponsor and IRB (as required)	Within 10 working days or as required by IRB
Device malfunction or device observation eCRF	Sponsor	Within 24 hours of knowledge
Subject Death	Sponsor and IRB (as required)	Within 24 hours of knowledge
Subject Withdrawal	Sponsor	Within 24 hours of knowledge
Withdrawal of IRB or FDA Approval	Sponsor	Within 24 hours of knowledge
Protocol Deviations due to medical emergencies	Sponsor and IRB	Within 24 hours of occurrence or knowledge
Protocol Deviations	Sponsor and IRB (as required)	Within 5 business days of knowledge
Informed Consent Not Obtained	Sponsor and IRB	Within 24 hours of knowledge
Final study report (site)	Sponsor and IRB	At time of study closure at site
Final summary report	Sponsor, IRB and FDA	Within six months of study completion

13. QUALITY CONTROL AND ASSURANCE

13.1 Site and Investigator Selection

The Sponsor selects qualified investigators with appropriate experience at health care facilities with adequate resources to participate in this study. The investigational sites will be selected using combined current assessments of site and investigator qualifications.

13.2 Protocol Deviations

An Investigator is not allowed to deviate from the Protocol without the prior written approval of the Sponsor, or where there are concerns of patient safety. Under emergency circumstances, deviations from the Protocol to protect the rights, safety and well-being of human subjects may proceed without prior approval of the sponsor and the IRB. Such deviations shall be documented and reported to the sponsor and the IRB (as required) as soon as possible.

A protocol deviation is a failure to comply with the requirements specified within this clinical study protocol. Examples of protocol deviations may include enrollment of a study subject who does not meet all of the inclusion/exclusion criteria specified in the protocol, visits performed outside of the protocol specified visit window, and missed study visits. Each investigator shall conduct this clinical study in accordance with this clinical study protocol, regulatory body regulations, Good Clinical Practices, and any conditions of approval imposed by their IRB.

All deviations are reviewed and assessed for their impact on subject safety by the Sponsor or designee. The PI and study staff are responsible for knowing and adhering to their IRB reporting requirements.

The protocol deviations for this protocol consist of, but not limited to the following:

- Failure to obtain subject's informed consent prior to any study-related activities;
- Failure to conduct protocol required clinical follow-ups;
- Failure to conduct protocol required clinical follow-ups within time windows; and,
- Failure to report serious adverse events according to protocol requirements.

In the event of any deviation from the protocol, the Investigator will be notified of the site's non-compliance. Corrective actions will be required, if necessary. Continued protocol deviations despite re-education of the study site personnel or persistent protocol deviation may result in termination of the site's study participation. Subjects enrolled at these sites will continue to be followed per the clinical protocol.

13.3 Protocol Deviation Process

Investigators must report protocol deviations to the Sponsor within five working days of investigational site knowledge of the deviation by entering data into the Case Report Form. Any

protocol deviations that affect the rights, safety or well-being of the subject or the scientific integrity of the clinical investigation, including those which occur under emergency circumstances must be reported within 24 hours to the Sponsor and IRB if required by the IRB.

13.4 Corrective/Preventive Action

The Sponsor reserves the right to terminate an investigational site from the study for any of the following reasons:

- Repeated failure to complete Electronic Case Report Forms
- Failure to obtain Informed Consent
- Failure to report Serious Adverse Events within 24 hours of knowledge
- Loss of or unaccountable investigational device inventory
- Repeated protocol violations
- Failure to enroll an adequate number of subjects

14. ETHICS/PROTECTION OF HUMAN SUBJECTS

14.1 Statements of Compliance

This clinical investigation shall be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki, and 21 CFR Part 812, 50, 54, 56.

The clinical investigation shall not begin until the required approval from the IRB has been obtained, if appropriate.

Any additional requirements imposed by the IRB, or regulatory authority shall be followed.

14.2 Institutional Review Board (IRB)

Each participating institution must provide for the review and approval of this protocol and the associated informed consent documents and recruitment material by an appropriate IRB. Any amendments to the protocol or consent materials must also be approved before they are placed into use.

14.3 Informed Consent Process

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout an individual's study participation. Extensive discussion of risks and possible benefits of this therapy will be provided to the subjects and their families. Consent forms describing in detail the study interventions/products, study procedures, and risks are given to the subject and written documentation of informed consent is required prior to starting

intervention/ administering study product. Consent forms will be IRB-approved, and the subject will be asked to read and review the document. Upon reviewing the document, the Investigator will explain the research study to the subject and answer any questions that may arise. The subject will sign an informed consent document prior to any procedures being done specifically for the study. The subject should have the opportunity to discuss the study with their surrogates or think about it prior to agreeing to participate, unless study timeframes do not allow for such discussions. The subjects may withdraw consent at any time throughout the course of the trial. A copy of the informed consent document will be given to the subjects for their records. The rights and welfare of the subjects will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

When informed consent of the subject is not possible because of the subject's medical condition, the informed consent of the legally authorized representative, if present, shall be requested.

If informed consent is not obtained prior to any research procedures being conducted, the investigative site must notify the Sponsor and its IRB within 24 hours of knowledge of the incident.

14.3.1 Subjects Unable to Read or Write

Informed consent shall be obtained through a supervised oral process if the patient or legally authorized representative is unable to read or write. An independent witness shall be present throughout the process. The written informed consent form and any other information shall be read aloud and explained to the prospective patient or his/her legally authorized representative and, whenever possible, shall sign and personally date the informed consent form. The witness also signs and personally dated the informed consent form attesting that the information was accurately explained, and that informed consent was freely given.

14.3.2 New Information

If new information becomes available, that can significantly affect a patient's future health and medical care, which information shall be provided to the patient(s) affected in written form. If relevant, all affected subjects shall be asked to confirm their continuing informed consent in writing.

14.4 Patient Confidentiality

Confidentiality of data shall be observed by all parties involved at all times throughout the clinical investigation. All data shall be secured against unauthorized access.

The privacy of each subject and confidentiality of his/her information shall be preserved in reports and when publishing any data. The Principal Investigator or institution shall provide direct access to source data during and after the clinical investigation for monitoring, audits, IRB review and regulatory authority inspections. As required, the Principal Investigator or institution shall obtain

permission for direct access to source documents from the subject, hospital administration and national regulatory authorities before starting the clinical investigation.

15. PROTOCOL AMENDMENTS

The Protocol, CRFs, ICF and other patient information, or other clinical investigation documents shall be amended as needed throughout the clinical investigation, and a justification statement shall be included with each amended section of the document. Proposed amendments to the Protocol shall be agreed upon between the Sponsor and Principal Investigator, or the coordinating investigator. The amendments to the Protocol and the subject's informed consent form shall be notified to, or approved by, the IRB and regulatory authorities as required. For non-substantial changes [e.g. minor logistical or administrative changes, change of monitor(s), telephone numbers, renewal of insurance] not affecting the rights, safety and well-being of human subjects or not related to the clinical investigation objectives or endpoints, a simple notification to the IRB and, where appropriate, regulatory authorities can be sufficient. The version number and date of amendments shall be documented.

16. TERMINATION OF STUDY OR STUDY SITE PARTICIPATION

The Sponsor may terminate the study at any time. If the study is terminated prior to the completion of expected enrollment for any reason, all participating centers will be notified within five working days. All subjects already enrolled will continue to be followed for the planned course of study described in this protocol. The study shall be terminated following the final follow-up visit of the last enrolled patient.

The Sponsor reserves the right to terminate study site participation and remove appropriate study materials at any time. Specific instances that may precipitate such termination include but are not limited to the following:

- Failure to meet minimum patient enrollment requirements
- Failure to comply with protocol specified procedures and documentation
- Failure to comply with Good Clinical Practice

The site Investigator may also discontinue study participation with suitably written notice to the Sponsor.

17. PUBLICATION POLICY

REFRESH II Study Publication Policy

CytoSorbents manages its clinical studies in an ethical and rigorously scientific manner, working with leading experts in the field, to clearly and publicly demonstrate the benefits, risks, and value of the CytoSorb Device to caregivers and study patients alike. We accept the obligation to facilitate publication of medically important clinical data in a timely, objective, accurate, and balanced manner, regardless of the outcome of this trial. To ensure that an accurate record of the study data is presented to the public, CytoSorbents understands the need to allow sufficient time for careful preparation, analysis, interpretation, and review of study data and reports prior to their dissemination.

17.1 Method and Timing of Release of Prespecified Clinical Endpoint Results from the REFRESH II Clinical Study

- Safety and Efficacy Data generated for all prespecified primary and secondary endpoints, shall be compiled, analyzed, reviewed, and published in the following manner:
- Per Section 12.4 of the REFRESH II Protocol, the REFRESH II Principal Investigator shall compile and disseminate a clinical summary report that shall be disseminated to CytoSorbents (as Sponsor), all participating local and central IRBs, and the FDA. Data from this Summary Report shall also be published on the ClinicalTrials.Gov website simultaneously.
- Following database closure, a study publication shall be submitted to a peer reviewed journal within 18 months of database closure. In the event that the study is terminated early, a publication of this data will be submitted within 12 months of database closure.

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