Check for updates

Regenerative Medicine and Immunomodulatory Therapy: Insights From the Kidney, Heart, Brain, and Lung

Christopher J. Pino[1], Angela J. Westover[1], Kimberly A. Johnston[1], Deborah A. Buffington[1] and H. David Humes[1,2,3]

[1]Innovative BioTherapies, Inc., Ann Arbor, Michigan, USA; [2]Department of Internal Medicine, University of Michigan Medical School, Ann Arbor, Michigan, USA; and [3]CytoPherx, Inc., Ann Arbor, Michigan, USA

Regenerative medicine was initially focused on tissue engineering to replace damaged tissues and organs with constructs derived from cells and biomaterials. More recently, this field of inquiry has expanded into exciting areas of translational medicine modulating the body's own endogenous processes, to prevent tissue damage in organs and to repair and regenerate these damaged tissues. This review will focus on recent insights derived from studies in which the manipulation of the innate immunologic system may diminish acute kidney injury and enhance renal repair and recovery without the progression to chronic kidney disease and renal failure. The manner in which these interventions may improve acute and chronic organ dysfunction, including the heart, brain, and lung, will also be reviewed.

As a primer to this review, which will focus on a novel immunomodulatory therapy using a selective cytopheretic device (SCD) in a variety of studies to treat various etiologies of acute and chronic organ dysfunction, the reader is referred to recent comprehensive reviews on the classical tissue engineering approach to renal replacement function.[1–3] In brief, the tissue engineering approach uses biological components (proteins, cells, tissues) as well as degradable or nondegradable biomaterials such as polymers as a support matrix or delivery vehicles to reconstruct damaged tissue in the body. In this review, a cell-processing approach will be predominantly featured that leverages the integral role of the immune system in the body's innate repair and regenerative mechanisms, which is modulated by an extracorporeal device to avoid abnormal pathology. Immunomodulation via continuous leukocyte processing from an extracorporeal circuit with a biomimetic membrane is shown to affect neutrophils and monocytes, altering the progression of inflammatory diseases, alleviating tissue damage caused in disease states without treatment, and enhancing repair dependent upon regenerative and

remodeling processes. In this regard, the immunomodulation approach in regenerative medicine is vastly different from the tissue engineering approach, where damage to tissue can be limited to avoid abnormal pathology, and normal repair processes can be augmented rather than having to engineer and recapitulate tissue structure and function.

Role of Neutrophils in Acute Tissue/Organ Injury—Kidney, Brain, Heart, and Lung

Inflammation plays a central role in the development of acute and solid organ dysfunction. It is the excessive dysregulated leukocyte inflammatory processes in many acute and chronic disease states that lead to tissue damage, which results in organ/tissue dysfunction. In an example of acute systemic inflammation, systemic inflammatory response syndrome (SIRS) evolves into multiple organ dysfunction syndrome (MODS) due to excessive inflammation promoted by both activated circulating leukocytes and activated microvascular endothelial cells of solid organs. The interaction of activated leukocytes and activated endothelium results in poor tissue perfusion, with ischemic consequences to vital organs. The interaction of activated neutrophils and endothelium also leads to increased vascular permeability with fluid leakage from the intravascular space to tissue interstitium with resulting hypovolemia, hypotension, and cardiovascular instability. In the

kidney, sequestration and aggregation of neutrophils in the peritubular capillaries and infiltration into interstitial spaces of the kidney can lead to the necrosis of proximal tubule cells, promote acute kidney injury (AKI), and, if substantive, acute renal failure (ARF). In the heart, this can lead to increases in interstitial fluid and death of cardiac myocytes. In the lungs, this leads to edematous lung tissue and decreased gas exchange, which can cause hypoxia and cell death. In the brain, local tissue injury, most dramatically exemplified by intracerebral hemorrhage/hemorrhagic stroke (ICH) is aggravated by the body's acute inflammatory response due to local tissue injury rather than systemic processes. Local tissue injury promotes tissue release of cytokines and chemokines to attract leukocytes to the area of damage and to digest and remodel injured tissue for repair and return of function. The degree of tissue damage after initial insult is potentiated by excessive inflammatory response of the circulating innate immunologic system.[4,5] Controlling these maladaptive responses may ameliorate the degree of tissue injury and dysfunction.

Role of Monocytes/Macrophages on Chronic Organ Dysfunction—Kidney, Heart, Brain, and Liver

The monocyte/macrophage component of the innate immunological system is critical in the host response to invading pathogens and tissue repair and remodeling after tissue injury.[6,7] The optimization of these processes is dependent upon a finely regulated and coordinated response of the monocyte/macrophage system. A balanced monocyte/macrophage response, both in phenotype and in timing, is necessary for optimal host defense and tissue repair.[8] Monocytes are produced in the bone marrow and are continuously released into the circulation, constituting approximately 5% to 10% of the circulating leukocyte pool in humans.[8] Human circulating monocytes are not a homogeneous population. Three subsets of monocytes have been identified and are based upon the expression of cell surface markers, CD14 (lipopolysaccharide [LPS] co-receptor) and CD16 (Fc γ R111). Within the population of monocytes, the majority are the classical subset with high CD14 but no CD16 expression (CD14^{hi}CD16$^-$), with the minority population further subdivided into the intermediate subset (CD14^{hi}CD16$^+$) and the nonclassical subset (CD14^{low}CD16^{++}). The classical and intermediate monocytes have the ability for phagocytosis and production of inflammatory effectors, similar to Ly6c^{hi} mouse monocytes. The nonclassical monocytes have a patrolling anti-inflammatory and reparative role similar to the Ly6c^{low} mouse monocytes.[8] The monocyte/macrophage system exists in at least 2 distinct phenotypes of differentiation: pro-inflammatory and anti-inflammatory.[9,10] Upon inflammatory signals, promoted by infection or tissue injury, circulating monocytes infiltrate tissue and differentiate into the M1 (inflammatory) or M2 (anti-inflammatory, reparative) macrophage phenotype. The M1 macrophage is usually the initial responder to coordinate and accentuate the pro-inflammatory response to destroy invading pathogens and to digest cellular and tissue debris. The M2 macrophage becomes more prominent later in this process, to repair and remodel damaged tissue promoted by this vigorous inflammatory process.

Various chronic organ dysfunction disorders have been associated with chronic inflammation. Chronic heart failure (CHF), chronic kidney disease (CKD), and type 2 diabetes mellitus (T2D) have been shown to have an increase in pro-inflammatory CD14^{hi} monocytes compared to those in normal controls.[11–16] In fact, an increase in CD14^{hi} monocytes in these chronic disease states correlate with worse clinical outcomes.[11,12,15,17–19] The development of atherosclerosis in the general and CKD population, as well as in end-stage renal disease (ESRD) patients on chronic hemodialysis (HD), are associated with M1 macrophage phenotype.[18,20,21] The circulating CD14^{hi} monocyte subset increases as renal function declines, with higher numbers of CD14^{hi} monocytes predicting adverse cardiovascular outcomes in ESRD patients undergoing chronic HD.[12,18,20] Pro-inflammatory (CD14^{hi}) monocytes are also increased in T2D and correlate with progression to diabetic nephropathy and uremia.[13,16,17,22] Furthermore, a higher level than normal of circulating inflammatory monocytes has been shown to result in worse clinical outcomes in both acute myocardial and brain injury.[23–25] A persistent pro-inflammatory response also characterizes progressive organ dysfunction in CHF, CKD, and obesity-related T2D.[11,15–18] The recognition of this relationship between the chronic pro-inflammatory monocyte/macrophage subtypes and disease progression have identified the monocyte/macrophage system as a therapeutic target for altering the clinical progression of chronic inflammatory disorders. Accordingly, a treatment that shifts the circulating monocyte pool from CD14^{hi} to CD14^{low} phenotype may have a clinical benefit to ameliorate the progression of various chronic inflammatory disorders.

Selective Cytopheretic Device Therapy and Acute Kidney Injury
Initial Clinical Insight and Proposed SCD Mechanism of Action

Selective cytopheretic device therapy (SCD$_{Rx}$) to treat inflammatory disorders originated from the clinical

evaluation of a tissue-engineered renal assist device (RAD)[26] containing adult human renal epithelial cells as a component of a bioartificial kidney. In the RAD phase IIa clinical study, subsets of patients were treated with a cell containing RAD or a sham (non-–renal cell containing) RAD cartridge.[27] The phase IIb study was a randomized, controlled, blinded, multi-center study in intensive care unit (ICU) patients with ARF secondary to AKI undergoing continuous renal replacement therapy (CRRT). The clinical study was suspended after an interim analysis due to an unanticipated high survival rate of the sham device arm. In retrospective analysis of the sham control groups, the improved survival rate was demonstrated in the presence of regional citrate anticoagulation (RCA) when compared to systemic heparin anticoagulation.[28] Subjects were divided into the following 4 groups: RAD with citrate anticoagulation; sham device with citrate anticoagulation; RAD with heparin anticoagulation; and sham device with heparin anticoagulation. The 28-day survival rate in the heparin sham patient group was 50% versus 75% in the citrate sham group (n = 12 for each treatment arm), and the 90-day survival rate was 25% (heparin) versus 67% (citrate). The baseline demographics for the 2 subsets were comparable, with similar sequential organ failure assessment (SOFA) scores (13.4 ± 1.1 vs. 12.2 ± 0.9), organ failure number (4.17 ± 0.46 vs. 3.93 ± 0.36), and incidence of sepsis (58% vs. 58%) for the citrate versus heparin sham groups, respectively.[28] This clinical result, although unexpected, was consistent with a potential clinical benefit of the fiber-based device without cultured renal cells (RAD sham), when used with RCA, which later became known as SDD therapy (SCD$_{Rx}$) (Figure 1).

The therapeutic benefit afforded by this combination of a device and a pharmacological agent (citrate) on a systemic clinical disorder can be better understood from the following: (i) Microscopy of the sham cartridges (future SCD) after patient treatment demonstrated adherent leukocytes on the outer surface of the membranes of the cartridge along the blood flow path.[27] The attached leukocytes were dominated by neutrophils and monocytes (Figure 2), which preferentially adhere, compared to other leukocytes such as lymphocytes, eosinophils, and basophils.[29] The ability of leukocytes to adhere to the outer walls of the hollow fiber membranes rather than the inner walls, which is the conventional blood flow path for renal dialysis/hemofiltration applications, was due to the shear forces of blood flow. The shear stress of blood along the outer wall of the membrane was near capillary force of <1 dyne/cm^2 compared to the shear stress of 100 dyne/cm^2 for blood flowing

along the conventional luminal surface of the hollow fiber membranes. (ii) RCA lowers the ionized calcium (iCa) in blood within the circuit to <0.4 mM, a level that inhibits the coagulation system, has an inhibitory effect on leukocyte and platelet activation,[29,30] and also affects the calcium-dependent selectin- and integrin-mediated interactions between leukocytes and the membrane.[31,32] Extravasation of neutrophils and monocytes from the systemic circulation into tissues is a highly regulated process. In a low–shear force environment like that found in capillaries or created within the SCD, neutrophils and monocytes roll along surfaces and are slowed via selectin binding followed by integrin-mediated, firm adhesion before diapedesis.[31]

An *in vitro* blood study using flow chambers to visualize leukocyte interactions with fiber materials suggests that leukocytes roll, then transiently adhere to fibers, are retained for a significant time period[29] (referred to as sequestration), and are then released. Binding selectivity in the SCD is increased in the low-iCa environment, where calcium-dependent selectin rolling, integrin binding, and downstream conformational changes of attached cells are inhibited.[33] Neutrophils[34,35] and monocytes[36,37] mobilize intracellular stores of CD11b, or CD11R3, the porcine analogue of human CD11b,[38] to the cell surface as they become (primed) activated. Measurement of CD11b, allows for real-time measurement of systemic acute neutrophil (priming) and monocyte activation. In addition, monocyte populations are heterogeneous in their expression of CD11b,[39] with CD14^{hi}CD16$^-$ being the highest and CD14^{low}CD16$^+$ being the lowest (Figure 3). The selectivity of binding of the highest-activated leukocytes has been repeatedly observed in preclinical animal models in which systemic CD11b levels decrease through the treatment course.[28,29,40,41] This effect was measured directly in a clinical trial by comparing the CD11b mean fluorescence intensity of the circulating cells in the peripheral blood to those directly associated with the SCD.[42] These results, when taken together,[28,29,40–43] suggest an SCD mechanism of action with a simultaneous, combination effect to transiently sequester activated circulating neutrophils and monocytes, with enhanced selectivity for inflammatory leukocytes, which alters the overall activation of bound and processed leukocytes. Clinical efficacy in AKI/multiple organ dysfunction (MOD) may be due to sequestration and immunomodulation of leukocytes in the SCD, which appears to block the inflammatory sequence associated with accumulation and aggregation of leukocytes in the peritubular capillaries and to reduce infiltration into interstitial spaces, which, when unchecked, promotes kidney injury following SIRS.
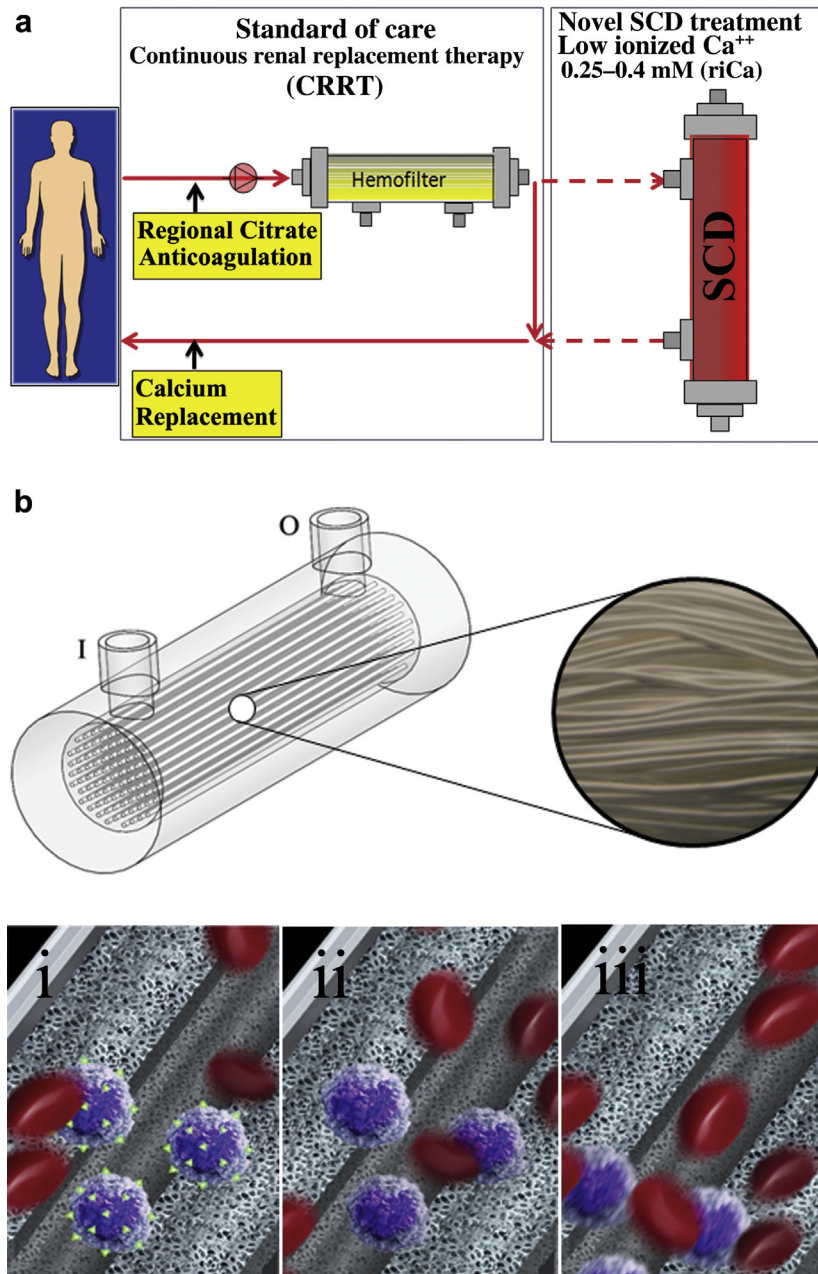
Figure 1. Schematic representation of (a) selective cytopheretic device therapy (SCD$_{Rx}$) and (bi–iii) current understanding of the mechanism of action (MoA) of the SCD, which involves leukocyte (LE)/fiber interactions. (i) Binding of activated LE (purple) with mobilized surface integrins (green); (ii) "reset" LE; (iii) release of immunomodulated LE. Erythrocytes are depicted in all panels as red.

SCD Clinical Treatment in AKI

SCD Phase I/II: ARF Safety, Mortality, and Device Integrity Study Performed Outside the United States.

The initial clinical study of the SCD was completed at the Huashan Hospital in Shanghai, China.[44] This prospective, single-arm, single-center study was designed to evaluate the safety and efficacy of SCD$_{Rx}$ on clinical outcomes in AKI requiring CRRT in the ICU. The patients enrolled in the trial were compared with historical case-matched controls with respect to age and SOFA score. The mortality for the case-matched controls was 7 of 9 or 78%, whereas the mortality in the SCD$_{Rx}$ group was 2 of 9 or 22% ($P < 0.027$). Multiple regression analysis identified treatment with SCD as the only significant variable affecting mortality among age, SOFA score, and average change in urine output over the first 7 days during or after treatment. Mean total urine output in the 9 SCD$_{Rx}$ subjects increased from a baseline of ~500 to >2000 ml/d by day 7 of treatment. Total white blood cell (WBC) counts also declined with SCD$_{Rx}$, but not to leukopenic levels. Reported serious adverse events (SAEs) were consistent with the targeted patient group and did not result in any clinical sequelae.
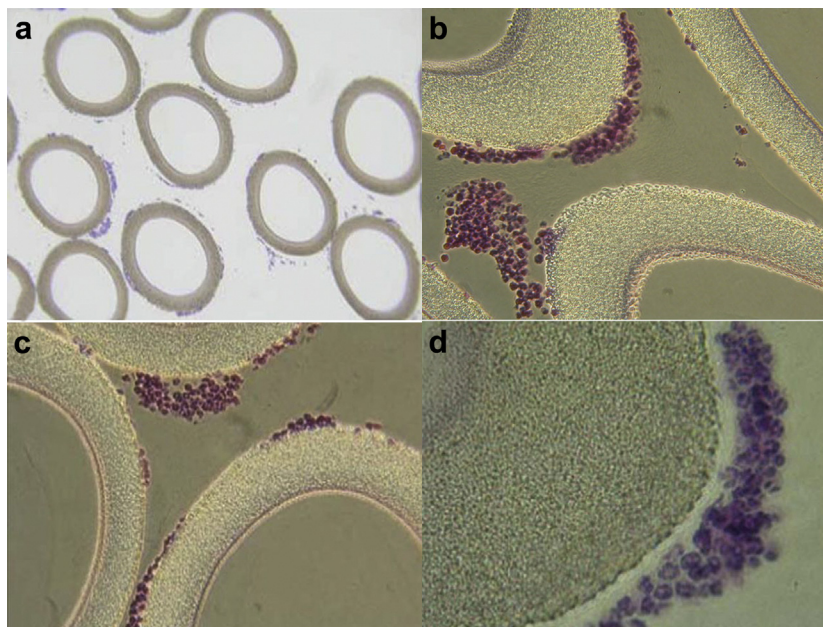
Figure 2. Micrographs of the sham, acellular cartridges as part of the regional citrate anticoagulation arm of the Renal Assist Device (RAD) clinical trial. Patient treatment demonstrated adherent leukocytes (LE) on the outer surface of the membranes of the cartridge along the blood flow path within the extracorporeal circuit, which translated into patient benefit. This was the basis for the treatment now referred to as SCD_{Rx}. (a–d) Light micrographs stained with hematoxylin and eosin. Low-power micrograph showing adherent cells around each fiber (a, original magnification ×160). (b,c) Higher-power micrographs showing clustering of bound LE (b and c, original magnification ×400). (d) High-power micrograph displaying predominance of NE and MO in the adherent cell clusters (original magnification ×1600). MO, monocyte; NE, neutrophil.

SCD Phase II: Pilot Prospective Multi Center US Study (Investigational Device Exemption G090189; Protocol ARF-002). A prospective, single-arm, multicenter, US study was designed to evaluate the safety and efficacy of SCD_{Rx} on AKI requiring CRRT in the ICU. The study enrolled 35 subjects.[45] The mean age was 56 ± 15 years. The average SOFA score was 11.3 ± 3.6. Death from any cause at day 60 was 31%. Renal recovery, defined as dialysis independence, was observed in all surviving subjects at day 60. The results of this pilot study indicate the potential for a substantial improvement in patient outcomes over standard-of-care therapy, which is associated with a greater than 50% 60-day mortality in the literature.[45] There were no SCD-associated SAEs.

SCD Phase III: A Multicenter, Randomized, Controlled, Pivotal Study to Assess the Safety and Efficacy of an SCD in Patients With AKI (Investigational Device Exemption G090189, Protocol SCD-003). The primary objective of this study was to determine whether CRRT+SCD_{Rx}, compared to CRRT alone, would result in a clinically relevant and statistically significant improvement in all-cause mortality through day 60.[46] Secondary objectives included assessment of renal replacement therapy (RRT) dependency at day 60, mortality at day 28, number of ventilator free days (VFD) at day 28, and mortality at day 60 in the subset of patients with severe sepsis. This was a 2-arm, randomized, open-label, controlled,

multicenter pivotal study that enrolled 134 patients at 21 US medical centers. The ICU AKI patients of each participating hospital were randomized to treatment undergoing CRRT or CRRT+SCD_{Rx}. Each participating clinical site used its established RCA protocol for the CRRT+SCD circuits (study arm) and for the CRRT-only (control arm). The recommended iCa (riCa) level (measured after SCD) in the CRRT and SCD circuit was specified to be between 0.25 and 0.4 mmol/l.

During the second quarter of the enrollment period, a national calcium shortage occurred in the United States from FDA-related quality manufacturing issues of the major US supplier. Due to reliance of the SCD on a narrow intracircuit iCa range for functional efficacy and the concern that patients randomized to SCD_{Rx} were not getting effective therapy, the interim analysis was performed early after enrollment of 134 patients. Enrollment was paused on 24 May 2013, to assess the clinical impact of the calcium shortage on study endpoints. The shortage of calcium infusion solutions resulted in a tendency to minimize citrate infusion rates. Accordingly, iCa levels within the blood circuit tended to be above the riCa of 0.25 to 0.40 mmol/l. Subsequently, the injectable calcium shortage resulted in 9 of the 21 open clinical sites being unable to enroll patients because of low hospital inventories of injectable calcium, contributing to the early termination of the study. Of the 134 patients in the analysis, 69 received CRRT alone and 65 received SCD_{Rx}.
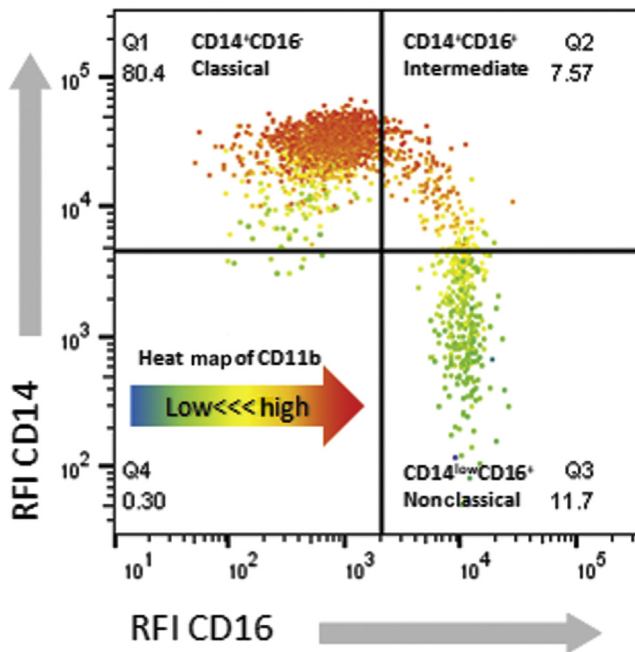
Figure 3. Human monocytes can be classified by CD14 and CD16 expression into classical (Q1: CD14$^+$CD16$^-$), intermediate (Q2: CD14$^+$CD16$^+$), and nonclassical (Q3: CD14^{low}CD16$^+$) subsets using flow-cytometric techniques. Representative cytometric analysis of systemic human blood is shown, with CD14 and CD16 expression displayed as a dot plot of relative fluorescence intensity (RFI). The intensity of CD11b expression of each event is heat mapped according to the arrow in Q4 (blue indicates lowest and red indicates highest CD11b RFI). The subsets have differential CD11b expression according to phenotype, contributing to integrin-dependent selective cytopheretic device selectivity for pro-inflammatory, classical, and intermediate MO. MO, monocytes.

No significant differences were noted between the control and treatment groups in baseline characteristics. No statistically significant difference was found between the treated and control patients, with a 60-day mortality of 39% (27/69) and 36% (21/59), respectively, with 6 patients lost to follow-up. The amount of time that the patients in both the control and treatment groups were maintained in the riCa range (0.25−0.40 mmol/l), as specified in the study protocol, was substantially lower than expected due to the injectable calcium shortage. Of the 134 patients enrolled at the time of the interim analysis, 19 SCD patients and 31 control patients were maintained at riCa for greater than or equal to 90% of the therapy time. Furthermore, none of the SAE were considered device related per the principal investigator. Comparison of these subgroups of patients revealed that 60-day mortality was 16% (3/19) in the SCD group compared to 41% (11/27) in the control group ($P = 0.11$). Dialysis dependency showed a borderline statistically significant difference between the SCD versus control patients maintained for greater than 90% of the treatment in the protocol's riCa target range with values of 0% (0/16) and 25% (4/16), respectively

($P = 0.10$). When the riCa SCD and control subgroups were compared for a composite index of 60-day mortality and dialysis dependency, the percentage in SCD subjects was 16% versus 58% in the control subjects ($P < 0.01$). When the riCa subpopulation was considered, a statistically significant difference was detected in several parameters: log urine output substantially increased, and WBC and neutrophil counts diminished in the SCD group versus the control group over time.

The observation that, in those patients who had the riCa level greater than 90% of the time of SCD$_{Rx}$, mortality improved from 41% to 16%, is clinically compelling. In addition, the observation, both in the pilot SCD-002 trial and in this SCD-003 study reported here, that no patient receiving appropriate SCD$_{Rx}$ was dialysis dependent at day 60 is also compelling. Previous large, prospective clinical studies in AKI with MOD had a greater than 20% incidence of dialysis dependency of patients followed up for 60 or more days.[47,48] The effect of SCD$_{Rx}$ to modulate excessive leukocyte activation most likely plays a critical role in the recovery of renal function after a substantive AKI event. The relationship of ongoing inflammation in the kidney after AKI and chronic progressive kidney disease and dialysis dependency has been demonstrated.[49,50] In this patient population, immunomodulation by SCD$_{Rx}$ appears to positively promote kidney healing as evidenced by the lack of dialysis dependency at day 60. In addition, improvement in overall mortality may suggest improved immune balance that persists through the late SIRS process to ameliorate the compensatory anti-inflammatory response that follows the excessive systemic pro-inflammatory state in AKI and MOD.[51] Furthermore, the significant decrease in WBC and neutrophil counts, as well as the improvement in urine output over time, corroborates findings in the mechanistic and pilot studies previously published.[29,44,45,52]

SCD Phase II: Pilot Pediatric Patients With AKI. Similar to the adult AKI clinical trial, a 5-center US study of the SCD in a critically ill pediatric population (>20 kg, age up to 22 years) with AKI and MOD receiving CRRT as part of standard of care has been initiated and is ongoing under the FDA-approved investigational device exemption (IDE) G150179. Mortality rates in pediatric patients with AKI and MOD requiring CRRT have historically approached 50%.[53−55] In this pilot-study clinical trial, pediatric patients have received SCD$_{Rx}$ for up to 7 days or when CRRT is discontinued, whichever comes first. Results to date are as follows: 6 pediatric patients (3 female and 3 male) have been enrolled and have completed SCD$_{Rx}$. Patient age ranged from 6.5 to 17.5 years, with a Pediatric Risk of Mortality score

(PRISM II) range of 2 to 14. Admission diagnoses were as follows: 1 severe rhabdomyolysis (case study presented below), 3 septic shock, 1 shigatoxin-associated hemolytic-uremic syndrome, and 1 community-acquired pneumonia. Patients received 3 (n = 2), 4 (n = 1), 6 (n = 1), or 7 (n = 2) days of SCD_{Rx}. All 6 patients survived and were off CRRT at the time of hospital discharge. No SCD-related SAEs were recorded.[56]

A case study of the first pediatric patient treated with SCD has been published, which describes the treatment course of an 11-year-old female patient with an uncommon reaction to anesthesia (propofol) during an elective surgery that resulted in MOD: severe AKF requiring CRRT, acute liver failure, acute respiratory failure requiring mechanical ventilation, and hematologic failure from disseminated intravascular coagulation and rhabdomyolysis with oliguria.[42] After 24 hours of SCD_{Rx}, her liver injury and hematologic failure improved. After 4 days of therapy, her lung function improved, resulting in extubation. After 7 days of SCD_{Rx}, her kidney function improved, resulting ultimately in complete recovery of kidney function not requiring dialysis treatment. The patient was discharged from hospital with normal renal function.[42]

This technology has been tested in several different clinical trials, as summarized in Table 1, which includes trials during the original development of the RAD, in which SCD_{Rx} emerged, and in 4 trials using the SCD in adult ICU patients with AKI requiring CRRT[28,44,45] and a pediatric trial.[42] These trials have demonstrated an excellent safety profile and suggestive efficacy impact. At the 28-day survival point, MOF ICU patients treated with the SCD, on average, had a full-day decrease in ventilator dependency. A

supplemental IDE is currently FDA approved for a prospective, randomized, multicenter 175 patient clinical trial with a new primary composite endpoint of 60-day mortality or dialysis dependency. The analysis clearly demonstrated clinical efficacy without safety issues, providing substantive support to move this technology to other patient populations.

SCD_{Rx} and ESRD in HD patients

Patients with ESRD on chronic HD suffer accelerated morbidity and mortality rates due to cardiovascular disease and infections. Chronic inflammation plays a critical role in these poor outcomes. The activated monocyte has become a prime therapeutic target to modulate this inflammatory process. SCD_{Rx} was evaluated to assess its effects on the circulating monocyte pool. A pilot trial was undertaken in 15 ESRD patients on HD with C-reactive protein (CRP) levels greater than 5 mg/dl (SCD Phase I/II ESRD Safety and Bioinflammatory Assay Study, additional data added to the original submission of IDE G090189). In this study, ESRD patients were treated with one 4-hour session of SCD therapy, and an excellent safety profile was observed with no decline in leukocyte or platelet counts. The effect of SCD_{Rx} on monocyte phenotypes in these patients was determined on peripheral blood monocytes by using flow cytometry. SCD_{Rx} promoted a significant shift ($P < 0.013$) in monocyte phenotype, from predominantly $CD14^{hi}$-expressing monocytes at baseline/pre-SCD_{Rx} to $CD14^{low}$-expressing monocytes post-SCD_{Rx}.[43] In a subset of patients (n = 7) presenting with T2D, this persistent decline in monocyte CD14 expression was sustained for 2 weeks after therapy.[43] These results demonstrate that SCD_{Rx} has the potential to modulate the chronic pro-inflammatory state in ESRD patients.

SCD_{Rx} in Preclinical Large-Animal Models of Acute Myocardial Infarction and Chronic Heart Failure

Ischemia/reperfusion injury (IRI), characterized by a vigorous inflammatory response immediately post-reperfusion via molecular signals generated by injured endothelium and cardiomyocytes, results in increased injury from leukocyte infiltration into the peri-infarct zone. This response becomes important in healing necessary to re-establish cardiac performance, but is excessive and maladaptive.

SCD_{Rx} was evaluated in a canine model of IRI, based on left circumflex coronary artery occlusion. Acute myocardial infarction was induced for 3 hours, and SCD_{Rx} was established using an extracorporeal blood circuit with RCA, 30 minutes before reperfusion and continued for up to an additional 3 hours afterward.

Table 1. SCD_{Rx} clinical application history

RAD phase I/II	ARF and MOF receiving CVVH, 10 patients
RAD phase IIa	ARF and MOF receiving CVVH, 58 patients[27]
RAD phase IIb	ARF and MOF receiving CVVH, Citrate Arm comparable to SCD_{Rx}, 24 patients receiving Acellular Sham, 12 with heparin, 12 with RCA[28]
SCD phase I/II	ARF and MOF receiving CVVH, 9 patients (China)[44]
SCD phase II	Protocol ARF-002, 35 patients[45]
SCD phase III	Protocol SCD-003, 134 patients[46]
SCD phase III	FDA/IDE approved, adult AKI, 175 patients
SCD phase II	FDA/IDE approved, pediatric AKI, 16 patients[42]
SCD phase I/II	Safety and bioinflammatory assay ESRD study, 15 patients[43]

AKI, acute kidney injury; ARF, acute renal failure; CVVH, continuous veno-venous hemofiltration; ESRD, end-stage renal disease; FDA, US Food and Drug Administration; IDE, investigational device exemption; MOF, multiple organ failure; SCD, selective cytopheretic device; SCD_{Rx}, selective cytopheretic device therapy.

Systemic inflammation was monitored by CD11b expression of leukocyte populations by using flow cytometry, and leukocyte infiltration was evaluated by histology. Systemic cytokine and cardiac injury marker troponin−I (cTn-I) levels were assayed. Left ventricular (LV) function, infarct sizem and edema were evaluated in treated animals (n = 3) and compared to control animals (n = 4). SCD$_{Rx}$ resulted in a 50% reduction of infarct size (19.2 ± 2.7 vs. 10.2 ± 4.5% of LV volume respectively, $P < 0.05$) (Figure 4), less edema (LV wall thickening) and 10 times lower cTn-I levels.[57] Leukocyte infiltration was prominent in the peri-infarct zone of controls but was negligible in the SCD group.

The SCD immunomodulatory platform therapy was also evaluated in a canine chronic heart failure (CHF) model induced by chronic trauma via multiple sequential intracoronary embolizations with microspheres.[58] Dogs were administered either three 6-hour therapy sessions over 1 week (n = 7) or no treatment (n = 5). Data from an interim analysis demonstrated that LV ejection fraction (EF) increased substantially in the treatment group, from 33.6 ± 1.3 (n = 5) to 43.3 ± 2.5 (n = 5; 6−48 hours posttherapy) and 37.0 ± 0% (n = 2; 4 weeks

posttherapy), reflective of 29% and 10% increases, respectively.[59] In the no treatment group (n = 5), EF% did not change. This effect was not due to a decline in systemic vascular resistance, which was similar in both groups. Ventriculograms demonstrated that SCD$_{Rx}$ converted viable but noncontracting myocardium to contracting myocardium. This benefit was maintained throughout the 4-week posttherapy follow-up period (Figure 4). Of relevance to the immunomodulatory impact of SCD$_{Rx}$, elevated peritoneal macrophage absolute number, which is associated with CHF,[60] was lower in SCD$_{Rx}$ dogs compared to the no-treatment dogs. In addition, the macrophage phenotype shifted from pro-inflammatory M1 seen in the no-treatment dogs to the reparative/anti-inflammatory M2 phenotype seen in SCD$_{Rx}$ dogs (unpublished results). Modulation of peritoneal macrophages demonstrates that the SCD$_{Rx}$ impact is not only organ specific but also affords the benefit of systemic immunomodulation. In summary, in this CHF model, SCD$_{Rx}$ improved myocardial contractility and modulated chronic inflammation, demonstrating strong support of SCD$_{Rx}$ as an innovative treatment approach to treat CHF.
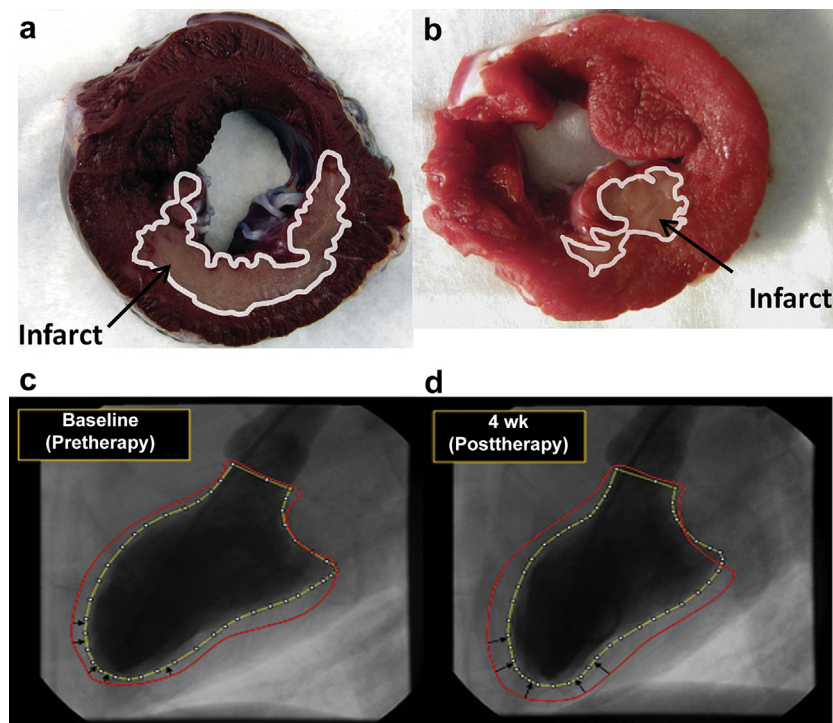

Figure 4. (a,b) Area of the infarcted heart was evaluated using 2,3,5-triphenyltetrazolium chloride. Red indicates viable tissue; white (with corresponding outline trace) indicates irreversibly injured tissue. Uneven discoloration (darkening) of the hearts is due to residual Evans blue dye injected to identify the area at risk for infarct. Evaluation of all cross sections indicated that (b) selective cytopheretic device therapy (SCD$_{Rx}$) afforded a significantly reduced infarct size compared to (a) untreated controls ($P < 0.05$). For the chronic heart failure (CHF) model, ventriculograms of CHF canine heart: (c) baseline week 0 before SCD therapy (wk0 pre-Rx) and (d) week 4 after SCD therapy (wk4 post Rx). Red line depicts the border of the left ventricular diastolic silhouette (most relaxed state during filling); yellow line depicts the border of the left ventricular systolic image (most contracted state), demonstrating improved contractility (black arrows) of the left ventricle after SCD$_{Rx}$.

SCD$_{Rx}$ in Preclinical Large-Animal Models of Traumatic Brain Injury and Intracranial Hemorrhage

Inflammation has recently been recognized as a central contributor to the pathobiology of stroke and traumatic brain injury (TBI), indicating that therapies that target inflammation may provide a new approach to the treatment of the acute phase of this disease process, particularly for intracranial hemorrhage (ICH), as there are currently no interventive therapies for this subset of stroke patients. Pilot preclinical studies were initiated to determine the SCD effect on the acute inflammatory cascade in ICH.

The acute effects of SCD$_{Rx}$ were evaluated in a porcine model of ICH, for which thrombin was used as the inflammatory nidus. ICH was induced simultaneously with SCD$_{Rx}$ by using an extracorporeal blood circuit with RCA. Therapy continued for 24 additional hours, during which systemic inflammation was monitored by assay of systemic cytokines. Platelet activation and CD11R3 expression of leukocyte populations were determined using flow cytometry. Postmortem coronal sections of frozen brain tissue were evaluated for leukocyte infiltration, and neuronal injury was evaluated by histology. SCD effects on lesion size and brain edema were evaluated in treated animals (n = 3) and compared to a contemporaneous control. SCD$_{Rx}$ resulted in a reduction in edema (Figure 5), reduced expression of neuronal injury markers, and reduced leukocyte infiltration as evaluated in animals sacrificed at 24 hours after ictus.[61] Pilot studies demonstrate that immunomodulation with the SCD represents a novel therapy that has the potential to improve outcomes associated with ICH.

SCD$_{Rx}$ in a Preclinical Large-Animal Model of Acute Lung Injury

Acute lung injury (ALI) is included within the clinical definition of acute respiratory distress syndrome (ARDS).[62] ARDS affects more than 190,000 Americans annually.[63] Mortality rates range from 26% to 58% and despite advances in supportive care; no pathophysiologically driven therapeutic intervention for ARDS is currently available.[64] ALI results from direct (pulmonary) and indirect (extrapulmonary) injury to the lungs. Pneumonia, aspiration, pulmonary contusion, inhalation injury, and fat emboli from fractured long bones constitute direct causes of ALI, whereas indirect lung injury resulting from SIRS is observed with polytrauma requiring multiple transfusions, severe sepsis, and burns. Sepsis remains the most common cause of ARDS, with 46% of cases triggered by primary pulmonary disorders.

Aside from the use of lung-protective ventilator strategies or extracorporeal membrane oxygenation (ECMO), which are purely supportive of gas exchange while attempting to limit further lung injury, there are no pathogenesis-directed therapies for ARDS, leaving a vast unmet medical need. Patients with lung injury more commonly than not encounter more than "one hit" modulating the immunological response to injury by increasing duration and amplitude of the inflammatory response. In fact, many second "hits" occur after proinflammatory responses (e.g., SIRS) have waned, and patients manifest compensatory anti-inflammatory responses (CARS) with suppressed immunity and diminished resistance to infection. This scenario seemingly places patients at risk for manifesting clinically significant ARDS and MODS.[65] Leukocytes are major contributors to the pathogenesis and progression of the inflammatory response and have been implicated in the pathogenesis of ARDS. Sequestration and infiltration in lung tissue progresses to diminish pulmonary gas exchange and to exacerbate disruption of the alveolar capillary membrane.[66] Therapeutic strategies to block inflammation are expected to decrease morbidity/mortality by limiting activity and tissue accumulation of leukocytes at sites of inflammation.[67,68]

Immunomodulatory effects of SCD$_{Rx}$ during ARDS have been investigated in a pilot study using a porcine model of acid-induced ALI (Humes HD, Buffington DA, Transportable renal replacement therapy for battlefield applications DoD/TARTRC, 2011–2013 [proposal application]). In brief, pigs were anesthetized and ventilated when 0.4N HCl was delivered via a tracheal catheter. Pa:FiO$_2$ decreased to <300 in all pigs, which were then allocated to untreated or SCD$_{Rx}$ cohorts and followed up for 6 hours. Pulmonary vascular resistance was lower in SCD$_{Rx}$ pigs without a concomitant decrease in systemic vascular resistance. The extent of leukocyte infiltration into the lungs at 6 hours postinjury was reduced as determined by immunohistochemical staining. Using the number of CD11R3$^+$ events normalized for total cells identified through 4′,6-diamidino-2-phenylindole (DAPI) staining, the extent of this reduction in leukocyte infiltration was quantitatively evaluated with ImageJ software (National Institutes of Health, Bethesda, MD). Leukocyte infiltration was reduced in the SCD pigs across all major lung lobes. This reduction in leukocyte infiltration into lung tissue has also been observed when assessing SCD$_{Rx}$ in a porcine model of septic shock—associated MODS.[29] Fewer neutrophils were recovered in the bronchioalveolar lavage fluid of SCD$_{Rx}$ versus untreated ALI pigs. In the clinical setting, the concentration of neutrophils collected in bronchioalveolar lavage fluid has been shown to correlate with the
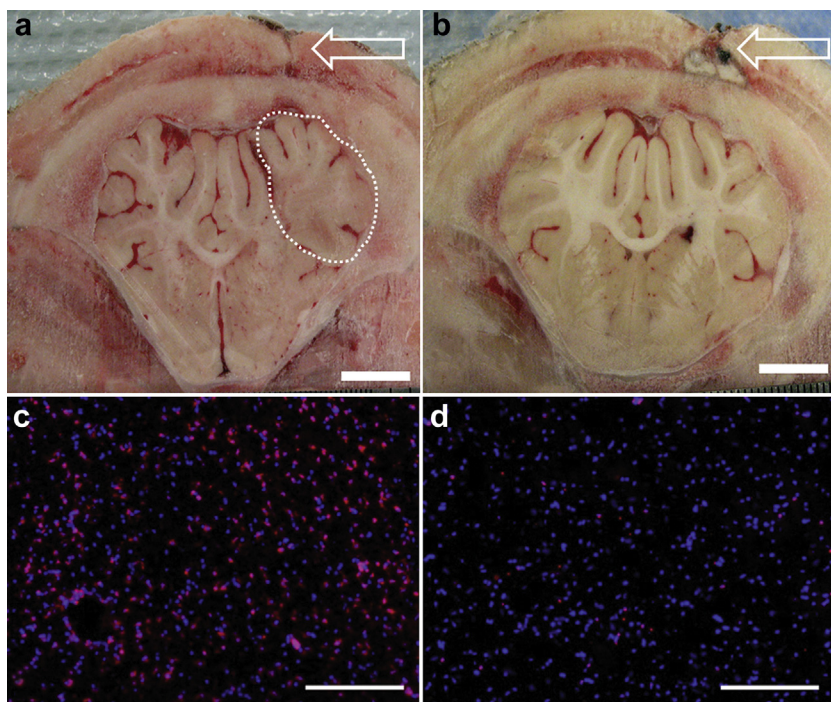
Figure 5. (a,b) Coronal brain sections are shown at the site of thrombin injection (arrows in a and b). Area of damage (demarcated by the dotted line) (a) can be identified by the lack of defined subcortical white matter due to swelling (edema) and is clearly evident in the (a) brain of the untreated control pig, but not in the (b) brain of a representative selective cytopheretic device therapy (SCD$_{Rx}$) animal. Bar = 1 cm. (c,d) Leukocytes (LE) normally not present in brain tissue migrate into sites of injury causing further damage. LE, identified by immunohistochemistry using a CD11R3-specific antibody (red), is more prevalent in the (c) untreated control animal, indicating that (d) SCD$_{Rx}$ can limit damage from ICH. Nuclei of all cells are counterstained with DAPI (blue). Bar = 100 μm. SCD, selective cytopheretic device.

severity and outcome of ARDS.[69,70] Accordingly, the significant decrease in neutrophil extravasation could possibly lead to improved long-term lung function. The impact of SCD$_{Rx}$ on lung function has been demonstrated by an increase in ventilator-free days at 28 days during a SCD phase III clinical trial.[46] Preclinical evaluation of SCD$_{Rx}$ as a treatment for ALI in a porcine model of trauma with sepsis is currently being conducted (Humes HD, Assessment of a therapeutic device for treatment of acute lung injury using a combat-relevant porcine model. DoD/PRMRP, 2016–2019 [proposal application]).

SCD$_{Rx}$ in a Preclinical Large-Animal Model of Type 2 Diabetes Mellitus

T2D is a complex disease whereby insulin resistance is a critical pathophysiological disorder. Obesity is associated with tissue inflammation, which is now recognized as a critical etiology of insulin resistance.[71–74] Circulating WBC counts, including absolute neutrophil and monocyte counts, are elevated in diabetic patients compared to nondiabetic patients.[75–77] Not only do these cells of the innate immunologic system increase in absolute number, but they also exist in a persistently activated state.[78–81] It is clear that recruitment of circulatory monocytes to form tissue macrophages within

adipose tissue is the initiating event in obesity-induced inflammation and insulin resistance.[74] The internal environment of adipose tissue favors the M1 pro-inflammatory phenotype of adipocyte tissue macrophage, resulting in tissue inflammation and insulin resistance. Pro-inflammatory cytokines, produced by adipocyte tissue macrophages and other cells, have been shown to promote insulin resistance in a paracrine and endocrine fashion.[82] Interventions with anti-inflammatory action therefore have beneficial effects to improve insulin sensitivity.

To evaluate the effect of SCD$_{Rx}$ on insulin resistance in a preclinical model of T2D, an Ossabaw miniature swine model of metabolic syndrome was used. When fed an excess-calorie atherogenic diet over several months, Ossabaw swine develop at least 5 of the 6 criteria for metabolic syndrome, including primary insulin resistance, obesity with significant visceral adipose expansion, hypertriglyceridemia and increased low-density lipoprotein:high-density lipoprotein cholesterol, mild hypertension, and coronary artery disease.[83–86] Treatment with the SCD in this porcine model demonstrated a decline in circulating neutrophil activation parameters and monocyte counts.[41] These changes were associated with improvements in insulin resistance as determined by i.v. glucose tolerance

testing.[41] Improvements were also reflected in lowering of homeostatic model assessment of insulin resistance (HOMA-IR) scores for up to 2 weeks after SCD$_{Rx}$.[41]

Summary

Increasingly, clinicians have recognized the critical role that the immune system plays in the response after organ injury, and the dire ramifications of excessive, dysregulated inflammation in both acute and chronic disease states, as well as the resulting impact on solid organ function. This review article has examined a number of the peer-reviewed publications, as well as work in progress, to elucidate the mechanisms of action for a novel immunomodulatory therapy, SCD$_{Rx}$, to treat various inflammatory disease indications. This demonstrates a growing body of clinical and preclinical support for immunomodulatory interventions.

DISCLOSURE

ACKNOWLEDGMENTS

SUPPLEMENTARY MATERIAL

Definitions of Abbreviations.
Supplementary material is linked to the online version of the paper at www.kireports.org.

REFERENCES

1. Buffington DA, Westover AJ, Johnston KA, Humes HD. The bioartificial kidney. *Transl Res*. 2014;163:342–351.

2. Pino CJ, Humes HD. Stem cell technology for the treatment of acute and chronic renal failure. *Transl Res*. 2010;156:161–168.

3. Pino CJ, Yevzlin AS, Tumlin J, Humes HD. Cell-based strategies for the treatment of kidney dysfunction: a review. *Blood Purif*. 2012;34:117–123.

4. Vinten-Johansen J. Involvement of neutrophils in the pathogenesis of lethal myocardial reperfusion injury. *Cardiovasc Res*. 2004;61:481–497.

5. Iadecola C, Anrather J. The immunology of stroke: from mechanisms to translation. *Nat Med*. 2011;17:796–808.

6. Mills CD. M1 and M2 macrophages: oracles of health and disease. *Crit Rev Immunol*. 2012;32:463–488.

7. Mills CD, Kincaid K, Alt JM, et al. M-1/M-2 macrophages and the Th1/Th2 paradigm. *J Immunol*. 2000;164:6166–6173.

8. Italiani P, Boraschi D. From monocytes to M1/M2 macrophages: phenotypical vs. functional differentiation. *Front Immunol*. 2014;5:514.

9. Gordon S, Taylor PR. Monocyte and macrophage heterogeneity. *Nat Rev Immunol*. 2005;5:953–964.

10. Lumeng CN, Bodzin JL, Saltiel AR. Obesity induces a phenotypic switch in adipose tissue macrophage polarization. *J Clin Invest*. 2007;117:175–184.

11. Barisione C, Garibaldi S, Ghigliotti G, et al. CD14CD16 monocyte subset levels in heart failure patients. *Dis Markers*. 2010;28:115–124.

12. Nockher WA, Scherberich JE. Expanded CD14+ CD16+ monocyte subpopulation in patients with acute and chronic infections undergoing hemodialysis. *Infect Immun*. 1998;66: 2782–2790.

13. Patino R, Ibarra J, Rodriguez A, et al. Circulating monocytes in patients with diabetes mellitus, arterial disease, and increased CD14 expression. *Am J Cardiol*. 2000;85:1288–1291.

14. Satoh N, Shimatsu A, Himeno A, et al. Unbalanced M1/M2 phenotype of peripheral blood monocytes in obese diabetic patients: effect of pioglitazone. *Diabetes Care*. 2010;33:e7.

15. Wrigley BJ, Shantsila E, Tapp LD, Lip GY. CD14++CD16+ monocytes in patients with acute ischaemic heart failure. *Eur J Clin Invest*. 2013;43:121–130.

16. Yang M, Gan H, Shen Q, et al. Proinflammatory CD14+CD16+ monocytes are associated with microinflammation in patients with type 2 diabetes mellitus and diabetic nephropathy uremia. *Inflammation*. 2012;35:388–396.

17. Fadini GP, de Kreutzenberg SV, Boscaro E, et al. An unbalanced monocyte polarisation in peripheral blood and bone marrow of patients with type 2 diabetes has an impact on microangiopathy. *Diabetologia*. 2013;56:1856–1866.

18. Rogacev KS, Seiler S, Zawada AM, et al. CD14++CD16+ monocytes and cardiovascular outcome in patients with chronic kidney disease. *Eur Heart J*. 2011;32:84–92.

19. Rogacev KS, Zawada AM, Emrich I, et al. Lower Apo A-I and lower HDL-C levels are associated with higher intermediate CD14++CD16+ monocyte counts that predict cardiovascular events in chronic kidney disease. *Arterioscler Thromb Vasc Biol*. 2014;34:2120–2127.

20. Heine GH, Ulrich C, Seibert E, et al. CD14(++)CD16+ monocytes but not total monocyte numbers predict cardiovascular events in dialysis patients. *Kidney Int*. 2008;73: 622–629.

21. Woollard KJ, Geissmann F. Monocytes in atherosclerosis: subsets and functions. *Nat Rev Cardiol*. 2010;7:77–86.

22. Cipolletta C, Ryan KE, Hanna EV, Trimble ER. Activation of peripheral blood CD14+ monocytes occurs in diabetes. *Diabetes*. 2005;54:2779–2786.

23. Tsujioka H, Imanishi T, Ikejima H, et al. Impact of heterogeneity of human peripheral blood monocyte subsets on

myocardial salvage in patients with primary acute myocardial infarction. *J Am Coll Cardiol*. 2009;54:130–138.

24. Courties G, Moskowitz MA, Nahrendorf M. The innate immune system after ischemic injury: lessons to be learned from the heart and brain. *JAMA Neurol*. 2014;71: 233–236.

25. Urra X, Villamor N, Amaro S, et al. Monocyte subtypes predict clinical course and prognosis in human stroke. *J Cereb Blood Flow Metab*. 2009;29:994–1002.

26. Humes HD, MacKay SM, Funke AJ, Buffington DA. Tissue engineering of a bioartificial renal tubule assist device: in vitro transport and metabolic characteristics. *Kidney Int*. 1999;55:2502–2514.

27. Tumlin J, Wali R, Williams W, et al. Efficacy and safety of renal tubule cell therapy for acute renal failure. *J Am Soc Nephrol*. 2008;19:1034–1040.

28. Humes HD, Sobota JT, Ding F, Song JH. A selective cytopheretic inhibitory device to treat the immunological dysregulation of acute and chronic renal failure. *Blood Purif*. 2010;29:183–190.

29. Ding F, Song JH, Jung JY, et al. A biomimetic membrane device that modulates the excessive inflammatory response to sepsis. *PLoS One*. 2011;6:e18584.

30. Morabito S, Pistolesi V, Tritapepe L, Fiaccadori E. Regional citrate anticoagulation for RRTs in critically ill patients with AKI. *Clin J Am Soc Nephrol*. 2014;9:2173–2188.

31. Ley K. The role of selectins in inflammation and disease. *Trends Mol Med*. 2003;9:263–268.

32. Zelensky AN, Gready JE. The C-type lectin-like domain superfamily. *FEBS J*. 2005;272:6179–6217.

33. Schaff UY, Dixit N, Procyk E, et al. Orai1 regulates intracellular calcium, arrest, and shape polarization during neutrophil recruitment in shear flow. *Blood*. 2010;115: 657–666.

34. Hamblin A, Taylor M, Bernhagen J, et al. A method of preparing blood leucocytes for flow cytometry which prevents upregulation of leucocyte integrins. *J Immunol Methods*. 1992;146:219–228.

35. Finn A, Rebuck N. Measurement of adhesion molecule expression on neutrophils and fixation. *J Immunol Methods*. 1994;171:267–270.

36. Lundahl J, Hallden G, Skold CM. Human blood monocytes, but not alveolar macrophages, reveal increased CD11b/CD18 expression and adhesion properties upon receptor-dependent activation. *Eur Respir J*. 1996;9:1188–1194.

37. Fontes ML, Mathew JP, Rinder HM, Zelterman D, Smith BR, Rinder CS. Atrial fibrillation after cardiac surgery/cardiopulmonary bypass is associated with monocyte activation. *Anesth Analg*. 2005;101:17–23.

38. Dominguez J, Alvarez B, Alonso F, et al. Workshop studies on monoclonal antibodies in the myeloid panel with CD11 specificity. *Vet Immunol Immunopathol*. 2001;80:111–119.

39. Wong KL, Yeap WH, Tai JJ, et al. The three human monocyte subsets: implications for health and disease. *Immunol Res*. 2012;53:41–57.

40. Pino CJ, Lou L, Smith PL, et al. A selective cytopheretic inhibitory device for use during cardiopulmonary bypass surgery. *Perfusion*. 2012;27:311–319.

41. Westover AJ, Johnston KA, Buffington DA, Humes HD. An immunomodulatory device improves insulin resistance in obese porcine model of metabolic syndrome. *J Diabetes Res*. 2016;2016:3486727.

42. Selewski DT, Goldstein SL, Fraser E, et al. Immunomodulatory Device Therapy in a Pediatric Patient with Acute Kidney Injury and Multiorgan Dysfunction. *Kidney International Reports*. 2017.

43. Szamosfalvi B, Westover A, Buffington D, et al. Immunomodulatory device promotes a shift of circulating monocytes to a less inflammatory phenotype in chronic hemodialysis patients. *ASAIO J*. 2016;62:623–630.

44. Ding F, Yevzlin AS, Xu ZY, et al. The effects of a novel therapeutic device on acute kidney injury outcomes in the intensive care unit: a pilot study. *ASAIO J*. 2011;57:426–432.

45. Tumlin JA, Chawla L, Tolwani AJ, et al. The effect of the selective cytopheretic device on acute kidney injury outcomes in the intensive care unit: a multicenter pilot study. *Semin Dial*. 2013;26:616–623.

46. Tumlin JA, Galphin CM, Tolwani AJ, et al. A multi-center, randomized, controlled, pivotal study to assess the safety and efficacy of a selective cytopheretic device in patients with acute kidney injury. *PLoS One*. 2015;10:e0132482.

47. Bellomo R, Cass A, Cole L, et al. Intensity of continuous renal-replacement therapy in critically ill patients. *N Engl J Med*. 2009;361:1627–1638.

48. Palevsky PM, Zhang JH, O'Connor TZ, et al. Intensity of renal support in critically ill patients with acute kidney injury. *N Engl J Med*. 2008;359:7–20.

49. Jang HR, Rabb H. The innate immune response in ischemic acute kidney injury. *Clin Immunol*. 2009;130:41–50.

50. Kinsey GR, Li L, Okusa MD. Inflammation in acute kidney injury. *Nephron Exp Nephrol*. 2008;109:e102–e107.

51. Ward NS, Casserly B, Ayala A. The compensatory anti-inflammatory response syndrome (CARS) in critically ill patients. *Clin Chest Med*. 2008;29:617–625, viii.

52. Ayub K, Hallett MB. Ca2+ influx shutdown during neutrophil apoptosis: importance and possible mechanism. *Immunology*. 2004;111:8–12.

53. Symons JM, Chua AN, Somers MJ, et al. Demographic characteristics of pediatric continuous renal replacement therapy: a report of the prospective pediatric continuous renal replacement therapy registry. *Clin J Am Soc Nephrol*. 2007;2:732–738.

54. Modem V, Thompson M, Gollhofer D, et al. Timing of continuous renal replacement therapy and mortality in critically ill children. *Crit Care Med*. 2014;42:943–953.

55. Goldstein SL, Somers MJ, Baum MA, et al. Pediatric patients with multi-organ dysfunction syndrome receiving continuous renal replacement therapy. *Kidney Int*. 2005;67:653–658.

56. Goldstein S, Selewski D, Asskenazi D, et al. Multicenter evaluation of the selective cytopheteric device (SCD) in critically ill children requiring CRRT: report from the first 4 patients. *J Am Soc Nephrol*. 2017;28:422.

57. Humes HD, Buffington DA, Westover AJ, et al. Immunomodulation with a selective cytopheretic device (SCD) reduces myocardial infarct (MI) size in a canine model of ischemia-reperfusion injury (IRI). Poster presented at: American Society of Nephrology: Renal Week 2013; Atlanta, GA.

58. Sabbah HN, Stein PD, Kono T, et al. A canine model of chronic heart failure produced by multiple sequential coronary microembolizations. *Am J Physiol*. 1991;260:H1379–H1384.

59. Humes HD, Buffington DA, Westover AJ. Immunomodulatory therapy (Rx) demonstrates sustained improvement in myocardial contractility in a canine model of chronic heart failure (CHF). Poster presented at: American Society of Nephrology: Renal Week 2016; Chicago, IL.

60. Batista ML Jr., Santos RV, Cunha LM, et al. Changes in the pro-inflammatory cytokine production and peritoneal macrophage function in rats with chronic heart failure. *Cytokine*. 2006;34:284–290.

61. Humes HD, Buffington DA, Westover AJ, et al. Immunomodulation with a selective cytopheretic device (SCD) reduces injury in a pig model of intracranial hemorrhage (ICH). Poster presented at: American Society of Nephrology: Renal Week 2013; Atlanta, GA.

62. Thompson BT, Moss M. A new definition for the acute respiratory distress syndrome. *Semin Respir Crit Care Med*. 2013;34:441–447.

63. American Lung Association. Lung Disease Data Report 2008. Available at: http://action.lung.org/site/DocServer/lung-disease-data-2008-report.pdf. Accessed December 21, 2017.

64. Modrykamien AM, Gupta P. The acute respiratory distress syndrome. *Proc [Bayl Univ Med Cent]*. 2015;28: 163–171.

65. Mannick JA, Rodrick ML, Lederer JA. The immunologic response to injury. *J Am Coll Surg*. 2001;193:237–244.

66. Ware LB, Matthay MA. The acute respiratory distress syndrome. *N Engl J Med*. 2000;342:1334–1349.

67. Bolling KS, Halldorsson A, Allen BS, et al. Prevention of the hypoxic reoxygenation injury with the use of a leukocyte-depleting filter. *J Thorac Cardiovasc Surg*. 1997;113: 1081–1089; discussion 1089–1090.

68. Gu YJ, de Vries AJ, Boonstra PW, van Oeveren W. Leukocyte depletion results in improved lung function and reduced inflammatory response after cardiac surgery. *J Thorac Cardiovasc Surg*. 1996;112:494–500.

69. Parsons PE, Fowler AA, Hyers TM, Henson PM. Chemotactic activity in bronchoalveolar lavage fluid from patients with adult respiratory distress syndrome. *Am Rev Respir Dis*. 1985;132:490–493.

70. Steinberg KP, Milberg JA, Martin TR, et al. Evolution of bronchoalveolar cell populations in the adult respiratory distress syndrome. *Am J Respir Crit Care Med*. 1994;150:113–122.

71. Kahn SE, Hull RL, Utzschneider KM. Mechanisms linking obesity to insulin resistance and type 2 diabetes. *Nature*. 2006;444:840–846.

72. Odegaard JI, Chawla A. Pleiotropic actions of insulin resistance and inflammation in metabolic homeostasis. *Science*. 2013;339:172–177.

73. Olefsky JM, Glass CK. Macrophages, inflammation, and insulin resistance. *Annu Rev Physiol*. 2010;72:219–246.

74. Osborn O, Olefsky JM. The cellular and signaling networks linking the immune system and metabolism in disease. *Nat Med*. 2012;18:363–374.

75. Chung FM, Tsai JC, Chang DM, et al. Peripheral total and differential leukocyte count in diabetic nephropathy: the relationship of plasma leptin to leukocytosis. *Diabetes Care*. 2005;28:1710–1717.

76. Shim WS, Kim HJ, Kang ES, et al. The association of total and differential white blood cell count with metabolic syndrome in type 2 diabetic patients. *Diabetes Res Clin Pract*. 2006;73: 284–291.

77. Tsai JC, Sheu SH, Chiu HC, et al. Association of peripheral total and differential leukocyte counts with metabolic syndrome and risk of ischemic cardiovascular diseases in patients with type 2 diabetes mellitus. *Diabetes Metab Res Rev*. 2007;23:111–118.

78. Nijhuis J, Rensen SS, Slaats Y, et al. Neutrophil activation in morbid obesity, chronic activation of acute inflammation. *Obesity [Silver Spring]*. 2009;17:2014–2018.

79. Schroder S, Palinski W, Schmid-Schonbein GW. Activated monocytes and granulocytes, capillary nonperfusion, and neovascularization in diabetic retinopathy. *Am J Pathol*. 1991;139:81–100.

80. van Oostrom AJ, van Wijk JP, Sijmonsma TP, et al. Increased expression of activation markers on monocytes and neutrophils in type 2 diabetes. *Neth J Med*. 2004;62: 320–325.

81. Wierusz-Wysocka B, Wysocki H, Siekierka H, et al. Evidence of polymorphonuclear neutrophils (PMN) activation in patients with insulin-dependent diabetes mellitus. *J Leukoc Biol*. 1987;42:519–523.

82. Lee YS, Li P, Huh JY, et al. Inflammation is necessary for long-term but not short-term high-fat diet-induced insulin resistance. *Diabetes*. 2011;60:2474–2483.

83. Dyson MC, Alloosh M, Vuchetich JP, et al. Components of metabolic syndrome and coronary artery disease in female Ossabaw swine fed excess atherogenic diet. *Comp Med*. 2006;56:35–45.

84. Neeb ZP, Edwards JM, Alloosh M, et al. Metabolic syndrome and coronary artery disease in Ossabaw compared with Yucatan swine. *Comp Med*. 2010;60:300–315.

85. Spurlock ME, Gabler NK. The development of porcine models of obesity and the metabolic syndrome. *J Nutr*. 2008;138: 397–402.

86. Sturek M, Tune JD, M. A. Ossabaw Island miniature swine: metabolic syndrome and cardiovascular assessment. In: Swindle MM, Smith MM, eds. *In Swine in the Laboratory: Surgery, Anesthesia, Imaging, and Experimental Techniques, Third Edition*. Boca Raton, FL: CRC Press; 2015:451–465.