Supplemental Appendix A. SCD Formulation and Treatment Methods

The SCD is an extracorporeal cartridge containing polysulfone hollow fibers with blood flow directed to the side ports of the cartridge with closed end caps (Supplemental Figure 1). Blood flow directed to the side ports enters the extra-capillary space of the device so that blood flows along the extraluminal side of the membranes with shear stress approaching capillary shear. This low shear stress allows activated leukocytes to adhere to the outer surface of the hollow fiber membranes while they traverse along the blood flow path. The SCD treatment requires regional citrate anticoagulation (RCA) to maintain at all times the ionized calcium (iCa) concentration in the blood circuit between 0.25 to 0.4 mmol/L. This set up allows continuous cell processing of circulating neutrophils and monocytes to a less proinflammatory phenotype, thereby tempering the hyperinflammatory state of the effector cells of the innate immunologic system which are central to promoting the cytokine storm and subsequent tissue damage.

The SCD-2.5 was supplied by SeaStar Medical. This SCD has 2.5 M² blood contacting surface area. The SCD sustains the same blood flow settings of the CRRT circuit. To attain its immunomodulatory effect, the SCD requires a rigorously controlled low ionized calcium (iCa) concentration between 0.25 and 0.4 mM in the extracorporeal blood circuit achieved with regional citrate anticoagulation (RCA) and dialysate/replacement fluid with no calcium. As required in RCA protocol, replacement calcium is infused into the blood circuit exiting the SCD so that blood returning to the patient maintains a normal calcium level in the patient. A CRRT protocol was utilized with weight-based dialysate/replacement fluid rates and fixed citrate-to-blood flow ratio, as opposed to a titration approach. Personalized dosing for calcium supplementation based on precalculated effluent calcium losses is also utilized to (1) achieve adequate circuit ionized calcium (iCa) (<0.4 mm/L) for any hematocrit level and hence plasma flow and (2) keep systemic citrate levels <2 mmol/L irrespective of body citrate metabolism and eliminate the risk of clinically significant hypocalcemia.
This low iCa is necessary to maintain anticoagulation within the circuit as well to provide the immunomodulation activity of the device. The low iCa environment within the extra-capillary space of the device promotes the selective binding of the most activated circulating neutrophils and monocytes in the blood due to the calcium requirement of cell surface molecules, including CD62L and CD11b/CD18 integrin, of these leukocytes to bind to surfaces \((2,3)\). The more activated the cells, the more binding molecules are expressed on the cell surface, thereby increasing the density of these binding sites on the cell and more intense binding to the membranes. The leukocytes bind for minutes to hours on the membranes due to the low shear stress along the blood path within the SCD. Prolonged exposure of these cells in the low iCa environment within the SCD results in release of these cells back to the patient with a less proinflammatory phenotype resulting in a dampening of the overall activated state of the circulating population of neutrophils and monocytes. For neutrophils, recent data demonstrates that cell processing of neutrophils within the device results in the initiation of the apoptotic program of the bound neutrophils as has been reported in low iCa environments \((4,5)\) with release back to the circulation where the normal clearance of apoptotic neutrophils occur via phagocytosis and digestion by macrophages within the bone marrow, spleen, and liver \((6)\). For monocytes, cells released from the SCD back into the circulation shifts the circulating pool of monocytes to a less inflammatory phenotype \((7)\). This continuous leukocyte processing results in the removal of the more proinflammatory leukocytes from the circulation and release of less inflammatory neutrophils and monocytes which are quickly removed from the circulation via normal homeostatic processes. This continuous process during a systemic inflammatory state results in an immunomodulatory effect to temper the excessive, dysregulated hyper-inflamed state and allows tissue repair and recovery.
Supplemental Appendix B. Materials and Methods for Flow Cytometry and Biomarker Analysis

Cytokines and biomarkers IL-1b, IL-6, IL-8, IL-10, IL-17A, TNFα, MCP-1, IL-23, N-Gal, IL-2, TNF-R1, VEGF-R1, IL-15, and S100A8/A9 were analyzed with commercial ProcartaPlex Panels (ThermoFisher) and read using a Luminex by the University of Michigan’s Cancer Center Immunology Cores. BNP (Invitrogen EHPRONPPB), C5a (Invitrogen BMS2088), ST2 (R&D DST200), and cTnT (ElabScienceE-EL-0646) were run following manufacturer’s directions and read using an M5 plate reader and SoftMAX software (Molecular Devices). For cytometric analysis, erythrocytes were lysed immediately with ammonium chloride buffer, and cells washed in cold PBS with 0.2% EDTA and adjusted to 10^6 cells per test. Live Dead Aqua (ThermoFisher L-34974) was added to exclude labeling artifacts caused by dead cells and followed by incubation with fluorochrome-conjugated antibodies. For monocyte phenotyping, antibodies targeting human CD91 (clone: A2MR-a2), CD14 (clone: TÜK4), CD16 (clone: 3G8), HLADR (clone: TU36), CC192 (clone: MEM-166) and CD197 (clone:150503) were used to determine changes in the circulating monocyte population. CD91 is useful as a pan monocyte marker (8) CD14 and CD16 allow for identification of classical, intermediate and non-classical phenotypes (9). HLADR is shed from the surface of monocyte as they become anergic to stimulatory signals (10) and assists with the identification of CD16+ intermediate (HLADRhig) and non-classical (HLADRLow) monocytes (11). CD192 and CD197 correspond to the C-C motif chemokine receptors CCR2 and CCR7. CCR2 and CCR7 expression was increased on all monocyte subsets in CKD subjects compared with controls (12). Neutrophils were evaluated using anti-human CD66b (Clone:913542), CD10 (Clone: H10a), CD184 (CXCR4, Clone: 12G5), CD62L (Clone: DREG-56) and CD33 (CloneWM53). CD33 is expressed on immature NE (13) and CD10 is expressed on mature neutrophils and may provide a quantitative analysis of bone marrow neutrophil release (14,15). CD184 is expressed on aging NE marked for apoptosis (16).
can be used as a measure of neutrophil aging and turnover (17). CD16 and CD62L have been used to define immunologically distinct subpopulations of neutrophils in response to inflammatory signals (18) and CD62L sheds upon binding of neutrophils to endothelium. Neutrophils (19,20) and monocytes (21,22) mobilize intracellular stores of CD11b to the cell surface as they become (primed) activated. Blood cells treated with a saturating amount of anti-human CD11b (clone ICRF44 and CBRM 1/5) detects all extracellular CD11b and the integrin only in the open/activated conformation respectively, allowing a real-time measurement of systemic acute neutrophil priming and activation (23). Similar to CD11b, carcinoembryonic antigen related cell adhesion molecule 8, CD66b is translocated to the surface of neutrophils with stimulation from inflammatory mediators and released in soluble form after degranulation of secondary granules (24,25). It is also useful as a pan neutrophil marker. SCD cytometric data was acquired with an Attune flow cytometer (ThermoFisher) and analyzed using FlowJo software. Panel compensations were auto-generated by Attune software using lot matched antibody labeled ARC Amine Beads or ABC compensation control beads (ThermoFisher) where appropriate. Antibody panels, manufacturer and channels used, are shown in Supplemental Table 2.

Supplemental Appendix C. Flow Cytometry and Cytometric Analysis

Cytometric Analysis. To correlate the clinical outcomes of SCD treatment and leukocyte parameters, flow cytometry was undertaken to see changes in cell surface markers of circulating and SCD bound neutrophils and monocytes before, during and after SCD treatments. Elution of the post treatment SCDs on days 1,3,5,7,9 demonstrated 9.36±1.85 (SD) x 10^9 (64% neutrophils, 24% monocytes) leukocytes were bound to the devices.

As seen in Figure 3, cytometric analysis demonstrated that the SCD bound the more activated circulating neutrophils with fold increases in the MFI of CD11b and CD66b of 2.08 and 2.94-fold (p<1x10^-6 and p< 7x10^-4), respectively, of the eluted cells from the SCD compared to
circulating neutrophils. The side scatter area of circulating CD66b neutrophils significantly declined (p<0.008) from a level of 80 x 10³ at baseline to 46 x 10³ at the end of treatment indicating a significant decrease in neutrophil granularity. The dramatic decline in MFI of CD62L (L-selectin) of the SCD associated neutrophils compared to circulating cells (p<1x10⁻⁷ reflected the binding events occurring on the SCD membranes. L-selectin is shed from neutrophils upon attachment to endothelium and surfaces (26).

For monocytes, SCD bound the most activated circulating monocytes with CD11b and CD14 MFIs increasing 2.38 (p<1x10⁻¹¹) and 2.33-fold (p<5x10⁻⁹), respectively. Of interest, the MFI of circulating CD192 monocytes increased on average 1.93-fold during SCD treatment compared to baseline levels (p<0.002). The cell surface expression of monocyte CD192 was also measured in circulating and SCD bound monocytes; CD192 is also known as CCR2, the receptor for monocyte chemotactic protein (MCP)-1 (27). This chemokine ligand/receptor interaction critically modulates human monocyte adhesion and migration. Tissue release of MCP-1 provides a gradient to attract monocytes to areas of tissue inflammation and injury. The measurement of these surface markers demonstrated that SCD treatment resulted in an increase in CD192 MFI of the circulating monocyte pool. These changes may reflect important declines in the migration of CD192 expressing monocytes out of the circulation due to reduction of MCP-1 plasma levels with SCD treatment observed in this study (27) (see Figure 4).
**Supplemental Table 1. Inclusion and Exclusion Criteria**

| Inclusion Criteria                                                                                     | Exclusion Criteria                                                                                       |
|--------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------|
| 1. A patient, or legal authorized representative (LAR), has provided informed consent with a signed     | 1. Cardiovascular instability that precludes initiation of renal replacement therapy.                    |
| informed consent form.                                                                                 | 2. Irreversible brain damage based on available historical and clinical information.                     |
| 2. Positive COVID-19 test                                                                              | 3. Presence of any solid organ transplant at any time.                                                   |
| 3. Must be receiving medical care in an intensive care nursing situation (e.g., ICU, MICU, SICU, CTICU, | 4. Patients with stem cell transplant in the previous 100 days or who have not engrafted.                |
| Trauma).                                                                                                | 5. Acute or chronic use of circulatory support device other than ECMO such as LVADs, RVADs, BIVADs.     |
| 4. Age 18 to 80 years.                                                                                | 6. Metastatic malignancy which is actively being treated or may be treated with chemotherapy or radiation|
| 5. Males and females (females of child-bearing potential who are not pregnant (confirmed by a negative   | during the subsequent three month period after study treatment.                                         |
| serum pregnancy test) and not lactating if recently post-partum).                                      | 7. Chronic immunosuppression defined as >20 mg prednisone qd alone without other immunosuppressant      |
| 6. Intent to deliver full supportive care through aggressive management utilizing all available therapies for | medications (ie cyclophosphamide, azathioprine, methotrexate, rituximab, mycophenolate, cyclosporine). |
| a minimum of 96 hours.                                                                                 | 8. Patient is moribund or chronically debilitated for whom full supportive care is not indicated.       |
| 7. Platelet count >30,000/mm³ at Screening                                                            |                                                                                                         |
| 8. Clinical diagnosis of AKI requiring CRRT or ARDS:                                                   |                                                                                                         |
|                                                       |                                                                                                         |
| AKI (the sudden loss of the ability of the kidneys to excrete wastes, concentrate urine and conserve  |
| electolytes) is defined as meeting one of the following criteria:                                       |                                                                                                         |
| • Increase in SCr by ≥0.3 mg/dL (≥26.5 μmol/L) within 48 hours or;                                     |                                                                                                         |
| • Increase in SCr to ≥1.5 times baseline, which is known or presumed to have occurred within the      |                                                                                                         |
| prior 7 days or;                                                                                       |                                                                                                         |
| • Urine volume <0.5ml/kg/h for 6 hours                                                                 |                                                                                                         |
| OR                                                                                                     |                                                                                                         |
| Severe respiratory insufficiency as defined as:                                                        |                                                                                                         |
| (1). Endotracheal mechanical ventilation and                                                           |                                                                                                         |
| (2). Presence of all the following conditions for > 72 hours:                                          |                                                                                                         |
| i. PaO2/FIO2 < 150 with PEEP > 5 cm H2O for > 30 minutes.                                              |                                                                                                         |
| ii. Bilateral opacities not fully explained by effusion, lobar/lung collapse, or nodules              |                                                                                                         |
| iii. Respiratory failure not fully explained by cardiac failure or fluid overload                       |                                                                                                         |
| Respiratory insufficiency as defined in (1) and (2) above must have developed within 14 days of a      |                                                                                                         |
| positive COVID-19 test.                                                                               |                                                                                                         |
9. Concurrent enrollment in another interventional clinical trial. Patients enrolled in observational studies (NO TEST DEVICE OR DRUG USED) are allowed to participate.

10. Any reason the Investigator deems exclusionary.
Supplemental Table 2. Materials for Flow Cytometry

### Systemic Blood Panel 1: Monocytes

| Target | Channel and label | Vendor | Catalog# | Clone |
|--------|-------------------|--------|----------|-------|
| CD11b  | BL1 FITC          | BioRAD | MCA551F  | ICRF44|
| CD91   | BL2 PE            | ThermoFisher | 12-0919-42 | A2MR-a2|
| CD14   | BL3 PERCP Cy5.5*  | BioRAD | MCA1568  | Tuk4  |
| CD192  | RL1 Alexa Fluor® 647 | BD Biosciences | 558406 | 48607 |
| CD16   | RL3 APCH7         | BD Biosciences | 560195 | 3G8   |
| CD197  | VL1 BD OptiBuild™421 | BioLegend | 353208 | Go43H7|
| LIVE/DEAD | VL2 fixable Aqua | ThermoFisher | L34966 | NA    |
| HLA-DR | VL3 BD OptiBuild™605, | BD Biosciences | 562845 | TU36   |

* Antibody conjugated using LNK142PERCPCY5.5 Kit (Bio-Rad).

### Systemic Blood Panel 2: Neutrophils

| Target | Channel | Vendor | Catalog# | Clone |
|--------|---------|--------|----------|-------|
| CD11b  | BL1 FITC | BioRAD | MCA551F  | ICRF44|
| CD11b activated | BL2 PE | BioLegend | 301406 | CBRM1/5|
| CD10   | BL3 PERCPCy5.5 | BD Biosciences | 563508 | HI10a |
| CD62L  | BL4 PE CY7 | BD Biosciences | 565535 | DREG-56|
| CD33   | RL2 Alexa Fluor® 700 | BioLegend | 303436 | WM53 |
| CD16   | RL3 APCH7 | BD Biosciences | 560195 | 3G8   |
| CD66b  | VL1 BD OptiBuild™421 | BD Biosciences | 562940 | G10F5 |
| LIVE/DEAD | VL2 fixable Aqua | ThermoFisher | L34966 | NA |
| CD184  | VL4 BD OptiBuild™711 | BD Biosciences | 740799 | 12G5 |

B, R, and VL# refer to the Blue Red and Violet laser channels of the Attune Cytometer.
Supplemental Table 3. Serious Adverse Events in Covid Patients

| Serious Adverse Events                                      | Number |
|-------------------------------------------------------------|--------|
| Blood and lymphatic system disorders                        | 1      |
| Cardiac disorders                                           | 9      |
| Gastrointestinal disorders                                  | 1      |
| General disorders and administration site conditions        | 3      |
| Hepatobiliary disorders                                     | 2      |
| Infections and infestations                                 | 22     |
| Injury, poisoning and procedural complications               | 0      |
| Investigations                                              | 0      |
| Metabolism and nutrition disorders                          | 1      |
| Musculoskeletal and connective tissue disorders             | 0      |
| Nervous system disorders                                    | 0      |
| Other                                                       | 0      |
| Psychiatric disorders                                       | 0      |
| Renal and urinary disorders                                 | 0      |
| Respiratory, thoracic and mediastinal disorders             | 8      |
| Skin and subcutaneous tissue disorders                      | 0      |
| Vascular disorders                                          | 3      |
| **Total**                                                   | **50** |
Supplemental Figure 1. SCD Extracorporeal Blood Circuit and Leukocyte Processing.

Schematic representation of (A) selective cytopheretic device treatment circuit, (B) SCD cartridge with blood inlet (I) and blood outlet (O) ports directing blood along the outer membranes of the hollow fibers (blowup) and (C) current understanding of the mechanism of action (MoA) of the SCD, which involves leukocyte (LE)/fiber interactions: (a) Binding of activated LE (purple) with mobilized surface binding molecules (green); (b) "reset" LE; (c) release of immunomodulated LE. Erythrocytes are depicted in all panels as red.
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