Stem Cells and Development

DOI: 10.1089/scd.2020.0080


# Exosomes Derived from Bone Marrow Mesenchymal Stem Cells as Treatment for Severe COVID-19

Vikram Sengupta, MD[1], Sascha Sengupta, MD[2], Angel Lazo, Jr., MD[3], Peter Woods, DO[4]; Anna Nolan, MD[5], Nicholas Bremer, MD[6]

[1]Department of Internal Medicine, NYU Langone Medical Center, 530 1st Avenue, New York, NY 10016; T: 212-803-3339; F: 646-768-8600; vikram.sengupta@nyulangone.org

[2]Department of Anesthesiology, Mount Sinai Hospital-Brooklyn, 3201 Kings Highway, Brooklyn, NY 11234; T: 718-951-3073; F: 678-487-5357; sascha.qian@mountsinai.rog

[3]Department of Internal Medicine, Englewood Hospital, 350 Engle St, Englewood, NJ 07631; T: 201-520-7474; F: 201-864-1485; angel.lazo@ehmchealth.org

[4]Department of Internal Medicine, CarePoint Health System—Christ Hospital, 176 Palisade Avenue, Jersey City, NJ 07306; T: 201-795-8200; F: 201-795-8201; peter.woods@carepointhealth.org

[5]Department of Internal Medicine, NYU Langone Medical Center, 530 1st Avenue, New York, NY 10016; T: 212-263-7283; F: 646-501-6783; anna.nolan@med.nyu.org

[6]Department of Anesthesiology, NYU Langone Medical Center, 530 1st Avenue, New York, NY 10016; T: 304-347-6120; F: 304-347-6126; nick.bremer@centerforpainrelief.com

**Corresponding Author:**

Vikram Sengupta, MD

Clinical Assistant Professor

Department of Internal Medicine

NYU Grossman School of Medicine

530 1st Avenue

New York, NY 10016

C: 607-227-6731

F: 646-768-8600

vikram.sengupta@nyulangone.org

Stem Cells and Development

Exosomes Derived From Bone Marrow Mesenchymal Stem Cells as Treatment for Severe COVID-19 (DOI: 10.1089/scd.2020.0080)



**ABSTRACT**

This prospective, non-randomized, open-label, cohort study addresses the safety and efficacy of exosomes (ExoFlo$^{TM}$) derived from allogeneic bone marrow mesenchymal stem cells as treatment for severe COVID-19. During April 2020, ExoFlo was provided to 24 SARS-CoV-2 PCR positive patients at a single hospital center, all of whom met criteria for severe COVID-19 as well as moderate to severe Acute Respiratory Distress Syndrome (ARDS). Patients received a single 15 mL intravenous dose of ExoFlo and were evaluated for both safety and efficacy day 1-14 post-treatment. All safety endpoints were met with no adverse events observed within 72 hours of ExoFlo administration. A survival rate of 83% was observed. 17/24 (71%) of the patients recovered; 3/24 (13%) remained critically ill though stable; 4/24 (16%) expired for reasons unrelated to the treatment. Overall, after one treatment, patients' clinical status and oxygenation improved with an average PaO2/FiO2 ratio increase of 192% ($p < 0.001$). Laboratory values revealed significant improvements in absolute neutrophil count [mean reduction 32% [(p-value < 0.001)] and lymphopenia with average CD3+, CD4+ and CD8+ lymphocyte counts increasing by 46% ($p < 0.05$), 45% ($p < 0.05$), and 46% ($p < 0.001$), respectively. Likewise, acute phase reactants declined, with mean C-reactive protein (CRP), Ferritin, and D-dimer reduction of 77% ($p < .001$), 43% ($p < .001$), 42% ($p <0.05$), respectively. In conclusion, due to its safety profile, capacity to restore oxygenation, downregulate cytokine storm, and reconstitute immunity, ExoFlo is a promising therapeutic candidate for severe COVID-19. Future RCTs are needed to determine ExoFlo therapeutic potential.

Stem Cells and Development
Exosomes Derived From Bone Marrow Mesenchymal Stem Cells as Treatment for Severe COVID-19 (DOI: 10.1089/scd.2020.0080)



## INTRODUCTION

COVID-19, the disease caused by the SARS-CoV-2 coronavirus, has rapidly expanded into a global pandemic. Due to the explosion of cases, concerns regarding resource limitations, and emerging understanding of how best to treat COVID-19, hospitals have developed increasing thresholds for hospital admission as well as mechanical ventilation [1]. Before the pandemic, patients presenting with fever, dyspnea, and hypoxia, and meeting criteria for moderate to severe acute respiratory distress syndrome (ARDS), would typically be intubated. However, these patients are now first maintained with noninvasive supplemental O2 and other optimization measures like proning, with endotracheal intubation being delayed as long as possible. This group of patients holds particular interest for this study, as early intervention could substantially reduce progression to hypoxic respiratory failure requiring mechanical ventilation, a clinical event associated with mortality rates estimated as high as 67-94% [2-4].

Trials for experimental single target agents, including antivirals, antibiotics, and biologics, like Remdesivir, Hydroxychloroquine, and Tocilizumab, respectively, have yielded mixed outcomes with some associated with significant morbidity and mortality [5-8]. Other options for prevention and treatment include vaccination and convalescent plasma, both of which require stable viral epitopes for their efficacy. But much like HIV, the SARS-CoV-2 RNA virus mutates rapidly and directly suppresses host T-cell function, which may ultimately render these therapies ineffective [9]. Clinically, this has been borne out with frequent presentations of multiorgan failure in the setting of immunodeficiency even in previously healthy individuals.

Central to COVID-19 disease progression is the development of cytokine storm, which is thought to be sustained and amplified by evolving parallel processes: (1) the activation of macrophages and other antigen presenting cells (APC), alerting lymphocytes to the presence of the virus, (2) viral RNA replication within host cells, activating synthesis of proinflammatory factors, and (3) viral invasion of lymphocytes, eliciting lymphocyte apoptosis and facilitating ongoing immune evasion [10, 11]. The complex pathophysiology suggests that severe COVID-19 is more amenable to treatment with a pleiotropic agent rather than a single target agent.

While allogeneic bone marrow mesenchymal stem cell (bmMSC) transplantation has shown promise, with trials currently underway, this technology is limited by safety, cell survivability, scalability, and regulatory issues that make it an impractical option to meet the needs of millions of infected patients worldwide [12-14]. However, bone marrow derived exosomes, a crucial set of signaling nanovesicles secreted by bmMSCs, are a novel, multitargeted, next generation biologic agent that could be the key to downregulating the cytokine storm and to reversing the suppression of host antiviral defenses that characteristics of COVID-19 [15]. Containing a panoply of chemokines, growth factors, mRNA and microRNA with anti-inflammatory, regenerative, and immunomodulatory functions, exosomes are the paracrine and endocrine mediators that confer bmMSCs with their healing properties, a fact which taken together with their superior safety profile, stability, and scalability, make exosomes a tantalizing, practical, and yet unexplored treatment option for COVID-19 [15-18]. Multiple preclinical studies have shown favorable therapeutic effects of bone marrow derived exosomes delivered intravenously in animal models of acute lung injury (ALI), ARDS, asthma, and other inflammatory diseases, with analyses revealing reduced alveolar inflammation, enhanced edema clearance, restoration of leaky epithelial membranes and other sequelae of cytokine storm [19-24].

ExoFlo, a bmMSC derived exosome agent produced under current good manufacturing (cGMP) standards in a cGMP facility, certified and inspected by the FDA, meeting key standards for safety, tissue traceability and comprehensive instructions for use, was administered intravenously to 24 patients with SARS-CoV-2 associated ARDS who were clinically deteriorating. The objectives were to evaluate, following a single dose of intravenous ExoFlo, for safety including infusion reactions and any adverse events as well as efficacy including overall status as evidenced by disposition, oxygenation as evidenced by partial pressure of arterial oxygen to fraction of inspired oxygen ratio (PaO2/FiO2) and oxygen support requirements, degree of inflammation and immunocompetence, as evidenced by levels of CRP, D-dimer, Ferritin and cell counts of neutrophils and T-lymphocytes.

Stem Cells and Development
Exosomes Derived From Bone Marrow Mesenchymal Stem Cells as Treatment for Severe COVID-19 (DOI: 10.1089/scd.2020.0080)



## METHODS

Patients were enrolled in a prospective, non-blinded, non-randomized primary safety trial at a single hospital center from April 8-28, 2020. All COVID-19 patients admitted to hospital, meeting acceptance criteria were offered the therapeutic intervention. Inclusion criteria included age 18-85, positive result on SARS-CoV-2 PCR (treatment may be initiated prior to PCR confirmation if known exposure to COVID-19 positive contact but ultimately, result has to be positive), and presentation of fever and/or dyspnea for more than 72 hours, overall clinical deterioration as evidenced by downtrending PaO2/FiO2 ratio. All patients were already initiated on Hydroxychloroquine and Azithromycin in the ED or as an outpatient, which was the institutional and local practice in early to mid-April 2020. Exclusion criteria included pregnancy, severe pre-existing cardiopulmonary, renal, hepatic, and hematologic disease, immunodeficiency secondary to other viruses, severe metabolic disturbances (pH < 7.3), and evidence of irreversible coagulopathy (e.g. frequently occluded vascular access) or disseminated intravascular coagulation (e.g. profuse bleeding from endotracheal tube, lines, and foley).

Written informed consent for receiving treatment derived from allogeneic stem cells was obtained after the initial discussion with the patient or health care proxy. Initial screening included a review of past medical history, physical examination, vitals during hospitalization, pertinent labs and studies, and applicable objective parameters pertaining to critical care support, e.g. ventilator settings, vasopressors, inotropes, and temporary dialysis requirements. 51 patients were considered for eligibility; 27 patients who met acceptance criteria were enrolled into the following three study cohorts (Fig. 1):

Cohort A (N=2): COVID-19 outpatients with fever and dyspnea with objective vitals of RR ≥ 20 and/or SpO2 < 94% on RA. One patient, who was presumed COVID-19 positive, was excluded after the pending COVID-19 test returned negative.

Cohort B (N=21): COVID-19 inpatients with hypoxemia (as defined by SpO2 ≤ 90% on RA or patients that require supplemental oxygen to maintain SpO2 ≥ 94%, who require non-invasive oxygen support, which includes the following modalities: Nasal Cannula (NC), Non-Rebreather, Non-Invasive Positive Pressure Ventilation (NIPPV) such as Bi-Level Positive Airway Pressure (BI-PAP), and High Flow Nasal Cannula (HFNC) Oxygen. One patient was excluded due to Influenza A co-infection.

Cohort C (N=4): Intubated COVID-19 patients with hypoxic respiratory failure on mechanical ventilation. One patient was excluded due to an IV malfunction during administration of ExoFlo.

**Administration Dose & Route**

15 ml of ExoFlo was added to 100 ml of normal saline and administered intravenously over 60 minutes.

**Assessments**

Prior to infusion, on the day of treatment, baseline testing was performed for the following parameters: SARS-CoV-2 PCR; BMP; CBC; PT/INR; LFT; ESR; CRP; Ferritin; D-Dimer; T-lymphocyte panel; Mycoplasma IgM; Legionella Ag; Strep Pneumoniae Ag; Influenza A/B; Urinalysis/Urine Culture; Blood Culture; HgbA1c; Blood Type & Screen; Chest X-ray; EKG. Vital signs were monitored T=5, T=10, T=15, T=30, T=45, and T=60 minutes following infusion initiation, then hourly for the first 6 hours post infusion, every 3-4 hours thereafter per hospital standards. For inpatients, lab collection and direct clinical evaluation were performed on the day of treatment before the infusion and repeated day 1-14 post treatment or until the final day of hospitalization, with flow cytometry data collected for the first 5 days after receiving ExoFlo. For outpatients, lab collection and direct clinical evaluation were performed on the day of treatment before the infusion and repeated daily until recovery.

**Study Oversight**

The study protocol was reviewed and approved by Christ Hospital's institutional review board (approval number IRB 2020.01) under emergency compassionate use rules for immediate enrollment. Written informed consent was obtained for all patients in accordance with local regulations. The program was designed and conducted by the primary and co-investigators who collected the data, monitored the conduct of the program, and performed the statistical analysis. All authors had access to the data and assumed responsibility for the integrity and completeness of the reported data. The IRB protocol was prepared by the primary investigator. All adverse outcomes were reviewed by an independent data safety monitoring board (DSMB).

**Statistical Analysis**

Assessment of pre- and post-treatment datasets were performed using paired-t test analysis on GraphPad Prism 8.0. No additional correlative analysis or multivariate analyses were performed. No sample-size calculations were performed. All COVID-19 patients admitted to hospital, meeting acceptance criteria were offered the therapeutic intervention. The analysis population included all patients who received their first dose of ExoFlo before April 14, 2020, and for whom clinical data for at least 1 subsequent day were available.

**RESULTS**

**Baseline Patient Characteristics**

Baseline demographic and clinical patients' characteristics are reported in Table 1. Seventeen males (age range: 45-84) and ten females (age range: 29-75) were enrolled from April 6 to April 13$^{th}$, 2020. Of the 27 patients enrolled and treated, three were excluded for the following: 1 – thrice negative for COVID-19 PCR test; 1- influenza-A co-infection; 1- IV malfunction. Percentages of patients by race were 30% Caucasian, 63% Hispanic, and 7% Asian. Patients with preexisting conditions comprised 93% of the population. Pre-diabetic and type 2 diabetic patients comprised 86% of the population while hypertension comprised 44.4%.

**Safety**

No infusion reaction or adverse events were observed in any cohort within the first 72 hours. No adverse events were attributable to administration of ExoFlo. Adverse events in Table 2 included worsening hypoxic respiratory failure requiring intubation (N=4), pulmonary embolism (N=1), acute renal failure (N=3), and expiration (N=4)—all events occurring > 72 hours following treatment in 7 patients, which were evaluated by the DSMB to be reasonably attributable to COVID-19 progression or to a clear, temporally correlated provoking stimulus.

**Overall Clinical Outcome**

The survival rate in the study was 83%. 71% of the patients (17/24) recovered and/or were discharged from the hospital following a mean of 5.6 days after intravenous ExoFlo administration. 16% of the patients (4/24) expired. 13% of the patients (3/24) remained critically ill, requiring mechanical ventilation and intensive care.

**Oxygenation**

Oxygenation was assessed by calculating partial pressure of arterial oxygen (PaO2) to fraction of inspired oxygen (FiO2) as well as tracking oxygen support requirement at baseline, on day of treatment, and day 1-14 after administration of the ExoFlo. 80% of patients (20/24) exhibited improved PaO2/FiO2 ratio within 3 days of treatment. The mean increase of PaO2/FiO2 from baseline to day 14 post ExoFlo or final day of hospitalization was 191% ($p<.001$) and correlated with the reduced requirement of oxygen support as shown in Figure 2. Optimal responders exhibited PaO2/FiO2 of 200 mmHg by day 3 following ExoFlo, which was a strong predictor of hospital discharge; suboptimal responders were noted to exhibit slight improvement of PaO2/FiO2 but not above 200 mmHg by day 3.

**Laboratory Data**

Significant reductions in levels of the acute phase reactants CRP, Ferritin and D-Dimer are shown in Figure 3. The mean reduction of CRP was 77%; the mean reduction of ferritin was 43%; and the mean reduction of D-dimer was 42% between baseline and values measured on day 5 post treatment. There were statistically significant reductions in absolute neutrophil count and statistically significant increases in absolute lymphocyte count including subsets staining positive for CD3+, CD4+, and CD8+ on flow cytometry when comparing baseline to day 5 post ExoFlo treatment.

**DISCUSSION**

This prospective, open-label trial on the treatment of COVID-19 demonstrated that the bone-marrow derived product, ExoFlo, can be administered safely via intravenous infusion. The study met all of its primary endpoints. All patients were administered ExoFlo without any infusion reaction. There were no adverse effects in the immediate (less than 6 hours), intermediate (less than 24 hours) or delayed (less than 72 hours) period. All adverse events occurring more than 72 hours following administration of ExoFlo were reviewed by an independent DSMB and concluded to be unrelated to the therapeutic intervention.

All patients in cohort B met criteria for moderate to severe ARDS; due to their downtrending PaO2/FiO2 ratio, these patients were expected to require mechanical

Stem Cells and Development
Exosomes Derived From Bone Marrow Mesenchymal Stem Cells as Treatment for Severe COVID-19 (DOI: 10.1089/scd.2020.0080)



ventilation within 12-24 hours prior to the therapeutic intervention. Only 25% (4/20) in cohort B progressed to mechanical ventilation, a critical event associated with significantly higher morbidity and mortality. Considering that mortality rates are estimated as high as 60-79% in patients requiring noninvasive oxygen support [25, 26], our preliminary findings suggest that ExoFlo may be a preventative measure against progression to invasive oxygen support and mechanical ventilation though further studies with RCTs are warranted to prove efficacy. 75% of cohort B (16/20) recovered, as evidenced by discharge from the hospital, demonstrating a profound reversal of disease progression and suggesting that the optimal time to administer ExoFlo is early in the cytokine storm. Overall, treatment with ExoFlo was associated with an 83% survival rate and a significant improvement in oxygenation as evidenced by a mean increase of 191% in PaO2/FiO2 ratio ($p < 0.001$) as well as reduced oxygen support requirements within 48-72 hours. Improved PaO2/FiO2 ratio above 200 mmHg by day 3 post-treatment was strongly predictive of eventual hospital discharge and recovery.

Interestingly, even among suboptimal responders, all clinical parameters including oxygenation and inflammatory markers showed an initial favorable response to ExoFlo, effects that peaked at day 3-4, suggesting a redose at day 3 post-ExoFlo may be warranted. This is consistent with preclinical observations that circulating proteases may inactivate exosomal products, rendering a time dependent effect in a subset of patients [15]. The significantly improved neutrophilia and lymphopenia including increased CD3+, CD4+ and CD8+ T lymphocytes in addition to the reduction in acute phase reactants following ExoFlo administration suggest that one main therapeutic mechanism of action may be modulation of immune dysfunction.

Overall, the strengths of this study include minimal selection bias in addition to absence of financial sponsorship as the study was prepared, designed, and implemented by independent clinicians. The primary weaknesses of this study are the absence of randomization, blinding, and the limited sample size. Furthermore, only one exosomal product, ExoFlo, was studied. Due to the heterogeneity and complexity of exosomal products, the favorable preliminary data on safety and efficacy of ExoFlo cannot be interpreted as a class effect. Notably, bmMSC derived exosomes were selected for this

study over perinatally derived exosomes (placental, amniotic, or umbilical) because of the greater abundance of peer-reviewed research characterizing and confirming the safety profile of bmMSC derived exosomes [24, 25, 27].

This is the first known clinical study to date using bmMSC derived exosomes as treatment for any disease in an inpatient setting. Despite supporting evidence in medical literature, the clinical use of regenerative medicine has been limited to the outpatient setting in part due to cognitive biases and lack of understanding among physicians, institutions, and regulatory agencies. This study demonstrated profound reversal of hypoxia in patients hospitalized with severe COVID-19 following only a single intravenous dose of bone marrow derived exosomes, with no adverse effects attributable to the treatment. Ultimately, the application of bone-marrow derived exosomes may extend far beyond SARS-CoV-2 ARDS or COVID19, spanning a myriad of inflammatory disease states, including classic ARDS, chronic obstructive pulmonary disease, sepsis, autoimmune disease, and cancer [26-34]. Further clinical studies are warranted to investigate safety and efficacy.

**ACKNOWLEDGEMENTS**

The authors would like to acknowledge Vikas Mukesh Shah, DO and Kory A. Imbrescia, DO for their assistance in the conduct of the study.

**AUTHOR DISCLOSURE STATEMENT**

No competing financial interests exist for any investigators or assistants involved in this study.

**REFERENCES**

Gattinoni L, S Coppola, M Cressoni, M Busana and D Chiumello. (2020) COVID-19 Does Not Lead to a "Typical" Acute Respiratory Distress Syndrome. Am J Respir Crit Care Med. Advance online publication. https://www.icnarc.org/DataServices/Attachments/Download/f48efee2-d38b-ea11-9125-00505601089b

CNARC report on COVID-19 in critical care. (2020). Intensive Care National Audit Research Centre.

Yang X, Y Yu, J Xu, H Shu, J Xia, H Liu, Y Wu, L Zhang, Z Yu, M Fang, T Yu, Y Wang, S Pan, X Zou, S Yuan and Y Shang. (2020). Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. *The Lancet.* Respiratory medicine S2213-2600(20)30079-5.

Richardson S, JS Hirsch, M Narasimhan, JM Crawford, T McGinn, KW Davidson, DP Barnaby, LB Becker, JD Chelico, SL Cohen, J Cookingham, K Coppa, MA Diefenbach, AJ Dominello, J Duer-Hefele, L Falzon, J Gitlin, N Hajizadeh, TG Harvin and TP Zanos. (2020). Presenting Characteristics, Comorbidities, and Outcomes Among 5700 Patients Hospitalized With COVID-19 in the New York City Area. JAMA. Advance online publication. https://doi.org/10.1001/jama.2020.6775

Grein J, N Ohmagari, D Shin, G Diaz, E Asperges, A Castagna, T Feldt, G Green, ML Green, FX Lescure, E Nicastri, R Oda, K Yo, E Quiros-Roldan, A Studemeister, J Redinski, S Ahmed, J Bernett, D Chelliah, D Chen and T Flanigan. (2020). Compassionate Use of Remdesivir for Patients with Severe Covid-19. NEJM. Advance online publication. https://doi.org/10.1056/NEJMoa2007016

Wang Y, D Zhang, G Du, R Du, J Zhao, Y Jin, S Fu, L Gao, Z Cheng, Q Lu, Y Hu, G Luo, K Wang, Y Lu, H Li, S Wang, S Ruan, C Yang, C Mei, Y Wang and C Wang. (2020). Remdesivir in adults with severe COVID-19: a randomised, double-blind, placebo-controlled, multicentre trial. Lancet (UK). Advance online publication. https://doi.org/10.1016/S0140-6736(20)31022-9

Mahévas M, VT Tran, M Roumier, A Chabrol, R Paule, C Guillaud, S Gallien, R Lepeule, TA Szwebel, X Lescure, F Schlemmer, M Matignon, M Khellaf and N Costedoat-

Chalumeau. (2020). No evidence of clinical efficacy of hydroxychloroquine in patients hospitalised for COVID-19 infection and requiring oxygen: results of a study using routinely collected data to emulate a target trial. medRxiv. Online publication. https://doi.org/10.1101/2020.04.10.20060699

Alzghari SK and VS Acuña. (2020). Supportive Treatment with Tocilizumab for COVID-19: A Systematic Review. Journal of Clinical Virology 127:104380.

Wang X, W Xu, G Hu, S Xia, Z Sun, Z Liu, Y Xie, R Zhang, S Jiang and L Lu. (2020). SARS-CoV-2 infects T lymphocytes through its spike protein-mediated membrane fusion. Cellular and Molecular Immunology 1–3. Advance online publication. https://doi.org/10.1038/s41423-020-0424-9

Ye Q, B Wang and J Mao. (2020). The pathogenesis and treatment of the `Cytokine Storm' in COVID-19. Journal of infection S0163-4453(20)30165-1. Advance online publication. https://doi.org/10.1016/j.jinf.2020.03.037

Qin C, L Zhou, Z Hu, S Zhang, S Yang, Y Tao, C Xie, K Ma, K Shang, W Wang and DS Tian. (2020). Dysregulation of immune response in patients with COVID-19 in Wuhan, China. Clinical infectious diseases: an official publication of the Infectious Diseases Society of America. Online publication. https://doi.org/10.1093/cid/ciaa248

Leng Z, R Zhu, W Hou, Y Feng, Y Yang, Q Han, G Shan, F Meng, D Du, S Wang, J Fan, W Wang, L Deng, H Shi, H Li, Z Hu, F Zhang, J Gao, H Liu, X Li and RC Zhao. (2020). Transplantation of ACE2- Mesenchymal Stem Cells Improves the Outcome of Patients with COVID-19 Pneumonia. Aging and disease 11(2), 216–228.

Liang B, J Chen, T Li, H Wu, W Yang, Y Li, J Li, C Yu, F Nie, Z Ma, Z Yang, M Yang, P Nie, Y Gao, C Qian and M Hu. (2020). Clinical Remission of a Critically Ill COVID-19 Patient Treated by Human Umbilical Cord Mesenchymal Stem Cells. ChinaXiv. Online publication. http://chinaxiv.org/abs/202002.00084

Metcalfe SM. (2020). Mesenchymal stem cells and management of COVID-19 pneumonia. Medicine in Drug Discovery 5:100019.

Hessvik NP and A Llorente. (2018). Current knowledge on exosome biogenesis and release. Cellular and Molecular Life Sciences 75(2): 193–208.

Yu B, X Zhang and X Li. (2014). Exosomes Derived from Mesenchymal Stem Cells. International Journal of Molecular Sciences 15:4142–4157.

De Jong OG, BW Van Balkom, RM Schiffelers, CV Bouten and MC Verhaar. (2014). Extracellular vesicles: potential roles in regenerative medicine. Frontiers in immunology 5: 608.

Alipoor SD, E Mortaz, J Garssen, M Movassaghi, M Mirsaeidi and IM Adcock. (2016). Exosomes and Exosomal miRNA in Respiratory Diseases. Mediators of inflammation 5628404.

Katsha A, S Ohkouchi, H Xin, M Kanehira, R Sun, T Nukiwa and Y Saijo. (2011). Paracrine Factors of Multipotent Stromal Cells Ameliorate Lung Injury in an Elastase-induced Emphysema Model. Molecular Therapy 19:196–203.

Lee JH, J Park and JW Lee. (2019). Therapeutic use of mesenchymal stem cell-derived extracellular vesicles in acute lung injury. Transfusion 59(S1): 876–883.

Zhu YG, XM Feng, J Abbott, XH Fang, Q Hao, A Monsel, JM Qu, MA Matthay and JW Lee. (2014). Human mesenchymal stem cell microvesicles for treatment of Escherichia coli endotoxin-induced acute lung injury in mice. Stem Cells (USA) 32(1): 116–125.

Tang X, L Shi, A Monsel, X Li, H Zhu, Y Zhu and J Qu. (2017). Mesenchymal Stem Cell Microvesicles Attenuate Acute Lung Injury in Mice Partly Mediated by Ang-1 mRNA. Stem Cells 35:1849–1859.

Morrison TJ, MV Jackson, EK Cunningham, A Kissenpfennig, DF McAuley, CM O'Kane and AD Krasnodembskaya. (2017). Mesenchymal Stromal Cells Modulate Macrophages in Clinically Relevant Lung Injury Models by Extracellular Vesicle Mitochondrial Transfer. American Journal of Respiratory and Critical Care Medicine 196(10): 1275–1286.

Wang M, Q Yuan and L Xie. (2018). Mesenchymal Stem Cell-Based Immunomodulation: Properties and Clinical Application. Stem Cells International 3057624.

Yang X, Y Yu and J Xu. (2020). Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. The Lancet Respiratory Medicine 8(5): 475-481.

Peng F, L Tu, Y Yang, P Hu, R Wang, Q Hu, F Cao, T Jiang, J Sun, G Xu, C Chang. (2020). Management and Treatment of COVID-19: The Chinese Experience. The Canadian Journal of Cardiology. Advance online publication. https://doi.org/10.1016/j.cjca.2020.04.010

Hicok K, T Vangsness and M Dordevic. (2020). Exosome Origins: Why the Cell Source Matters. Stem Cells Regenerative Medicine 4(1): 1-4.

Wilson JG, KD Liu, H Zhuo, L Caballero, M McMillan, X Fang, K Cosgrove, R Vojnik, CS Calfee, JW Lee, AJ Rogers, J Levitt, J Wiener-Kronish, EK Bajwa, A Leavitt, D McKenna, BT Thompson and MA Matthay. (2015). Mesenchymal stem (stromal) cells for treatment of ARDS: a phase 1 clinical trial. The Lancet Respiratory Medicine 3(1): 24–32.

Lai R, F Arslan, M Lee, N Sze, A Choo, T Chen, M Salto-Tellez, L Timmers, C Lee, R El Oakley, G Pasterkamp, D De Kleijn and S Lim . (2010). Exosome secreted by MSC reduces myocardial ischemia/reperfusion injury. Stem Cell Research 4:214–222.

Huang L, W Ma, Y Ma, D Feng, H Chen and B Cai. (2015). Exosomes in mesenchymal stem cells, a new therapeutic strategy for cardiovascular diseases? International Journal of Biological Sciences 11(2): 238–245.

Shao M, Q Xu, Z Wu, Y Chen, Y Shu, X Cao, M Chen, B Zhang, Y Zhou, R Yao, Y Shi and H Bu. (2020). Exosomes derived from human umbilical cord mesenchymal stem cells ameliorate IL-6-induced acute liver injury through miR-455-3p. Stem Cell Research & Therapy 11(1): 37.

Porro C, S Lepore, T Trotta, S Castellani, L Ratclif, A Battaglino, S Di Gioia, MC Martínez, M Conese and AB Maffione. (2010). Isolation and characterization of microparticles in sputum from cystic fibrosis patients. Respiratory Research 11: 94.

Anderson MR, F Kashanchi and S Jacobson. (2016). Exosomes in Viral Disease. Neurotherapeutics, The Journal of the American Society for Experimental Neurotherapeutics 13(3): 535–546.

Eirin A, XY Zhu, AS Puranik, H Tang, KA McGurren, AJ van Wijnen, A Lerman and LO Lerman. (2017). Mesenchymal stem cell-derived extracellular vesicles attenuate kidney inflammation. Kidney International 92(1): 114–124.

Lanza F, M Dominici, M Govoni, S Moretti, D Campioni, R Corte, A Latorraca, A Tieghi, B Castagnari, F Trotta and G Castoldi. (2000). Prolonged remission state of refractory adult onset Stills disease following CD34-selected autologous peripheral blood stem cell transplantation. Bone Marrow Transplantation 25:1307–1310.

**Table 1**—Clinical Demographics. All participants met criteria for moderate to severe ARDS based on clinical presentation, acute onset, noncardiogenic etiology, and PaO2/FiO2 ratio <= 200 mmHg. 86% of the patients in the study had either T2DM or pre-T2DM. Note: Although the early stages of the COVID-19 associated viral pneumonia may not be entirely consistent with Acute Respiratory Distress Syndrome in many patients, PaO2/FiO2 ratio remains a vital oxygenation metric.

| Baseline Demographics and Pre-Treatment Conditions of Enrolled Participants | | | | | |
|---|---|---|---|---|---|
| | | Total (N=27) | Cohort A (N = 2) | Cohort B (N = 21) | Cohort C (N = 4) |
| Age | Range (Median) | 29-84 (59) | 49-84 | 45-75 (62) | 29-66 (54) |
| | < 50 | 8 | 1 | 6 | 1 |
| | 50 to < 70 | 14 | - | 11 | 3 |
| | ≥ 70 | 5 | 1 | 4 | 0 |
| Gender | Male | 17 | 2 | 14 | 1 |
| | Female | 10 | 0 | 7 | 3 |
| Body Mass Index | Weight(Kg)/Height(m)^2 | 29.7 | 28.5 | 29 | 34.3 |
| O2 Support Category | Mechanical Ventilation | 2 | 0 | 0 | 2 |
| | BIPAP | 2 | 0 | 2 | 0 |
| | High Flow Oxygen (HFNC) | 5 | - | 4 | 1* |
| | Non-Rebreather (NRB) | 10 | 0 | 11 | 0 |
| | Nasal Cannula (NC) | 4 | 1 | 3 | - |



Stem Cells and Development
Exosomes Derived From Bone Marrow Mesenchymal Stem Cells as Treatment for Severe COVID-19 (DOI: 10.1089/scd.2020.0080)

| | | | | | |
|---|---|---|---|---|---|
| | Room Air | 1 | 1 | - | - |
| Illness Prior to Treatment | Duration (Days) | 15 | 6.5 | 16 | 11.3 |
| Illness Prior to Admission | Duration (Days) | 8.5 | N/A | 9.6 | 1.7 |
| Pre-existing Comorbidities | Pre-T2DM | 3 | 0 | 1 | 2 |
| | T2DM | 20 | 1 | 18 | 1 |
| | Hypertension | 12 | 1 | 10 | 1 |
| | Hyperlipidemia | 5 | 1 | 4 | 0 |
| | Any Condition | 25 | 2 | 20 | 3 |
| Stage of ARDS (PaO2/FiO2) | Mild (200 to <=300) | 1 | 1 | 0 | 0 |
| | Moderate (100 to <=200) | 11 | 1 | 10 | 0 |
| | Severe (<100) | 13 | 0 | 9 | 4 |



Stem Cells and Development
Exosomes Derived From Bone Marrow Mesenchymal Stem Cells as Treatment for Severe COVID-19 (DOI: 10.1089/scd.2020.0080)

**Table 2—**Adverse Event Log. All adverse events were reviewed by an independent DSMB. None of the adverse events were attributable to the therapeutic intervention.

| All Adverse Events Including Outcomes Not Likely Associated with Treatment | | | | |
|---|---|---|---|---|
| **PostTx Day** | **Cohort A (N=2)** | **Cohort B (N=21)** | **Cohort C (N=4)** | **Total Events** |
| **0-1** | 0 | 0 | 0 | Pulmonary Embolus N=1 |
| **2** | 0 | 0 | 0 | Respiratory Failure N=4 |
| **3** | 0 | 0 | 0 | Acute Renal Failure N=3 |
| **4** | 0 | Expiration (N=1) | 0 | Expiration N=4 |
| **5** | 0 | Respiratory Failure (N=1) | Expiration (N=1) | |
| **6** | 0 | Respiratory Failure (N=2) | 0 | |
| **7** | 0 | 0 | 0 | |
| **8** | 0 | 0 | 0 | |
| **9** | 0 | Acute Renal Failure (N=1) | 0 | |
| **10** | 0 | Respiratory Failure (N=1) | 0 | |
| **11** | 0 | Acute Renal Failure (N=1) | 0 | |
| **12** | 0 | Expiration (N=1), Pulmonary Embolus (N=1) | 0 | |
| **13** | 0 | Acute Renal Failure (N=1) | Expiration (N=1) | |
| **14** | 0 | 0 | 0 | |

## FIGURE LEGEND

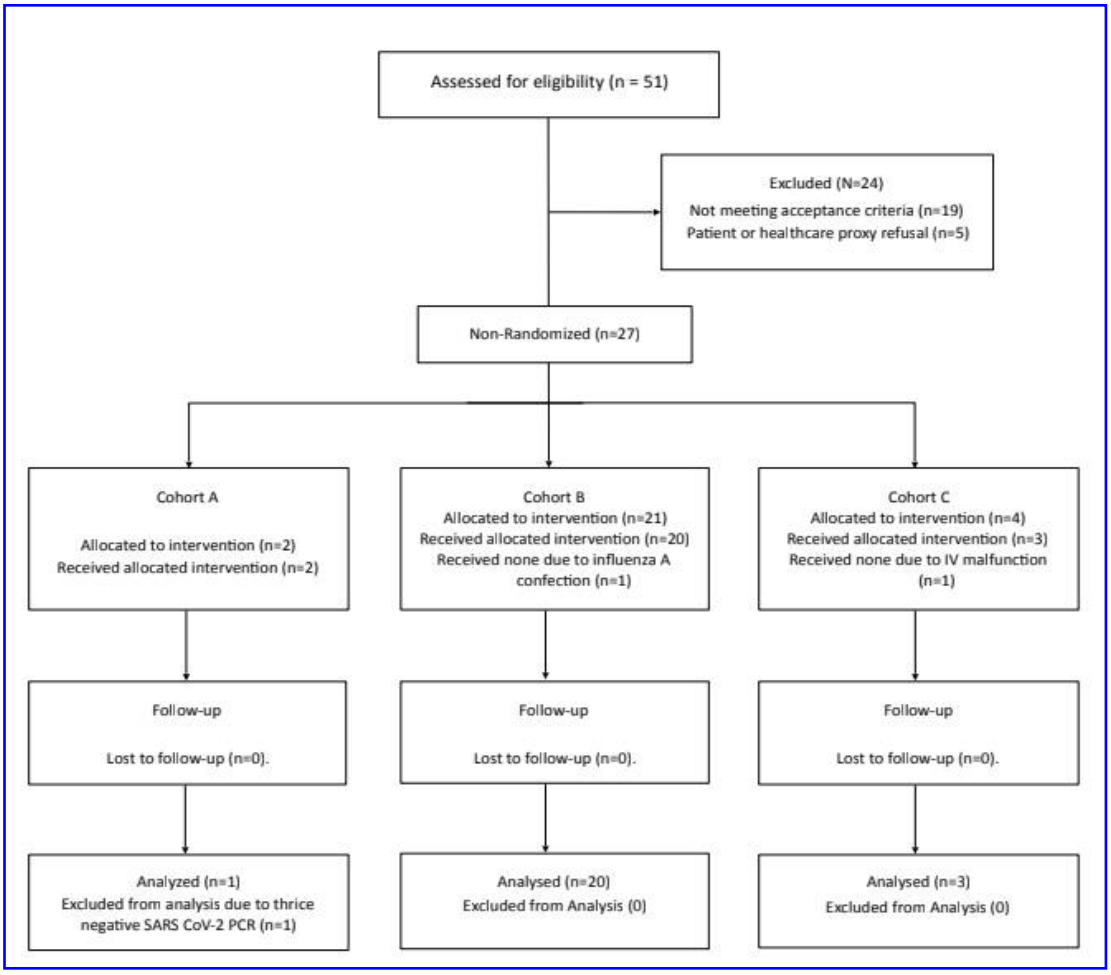


**Figure 1**—Consort Diagram for Study Enrollment, Allocation of Intervention, Follow-up, and Analysis



Stem Cells and Development
Exosomes Derived From Bone Marrow Mesenchymal Stem Cells as Treatment for Severe COVID-19 (DOI: 10.1089/scd.2020.0080)

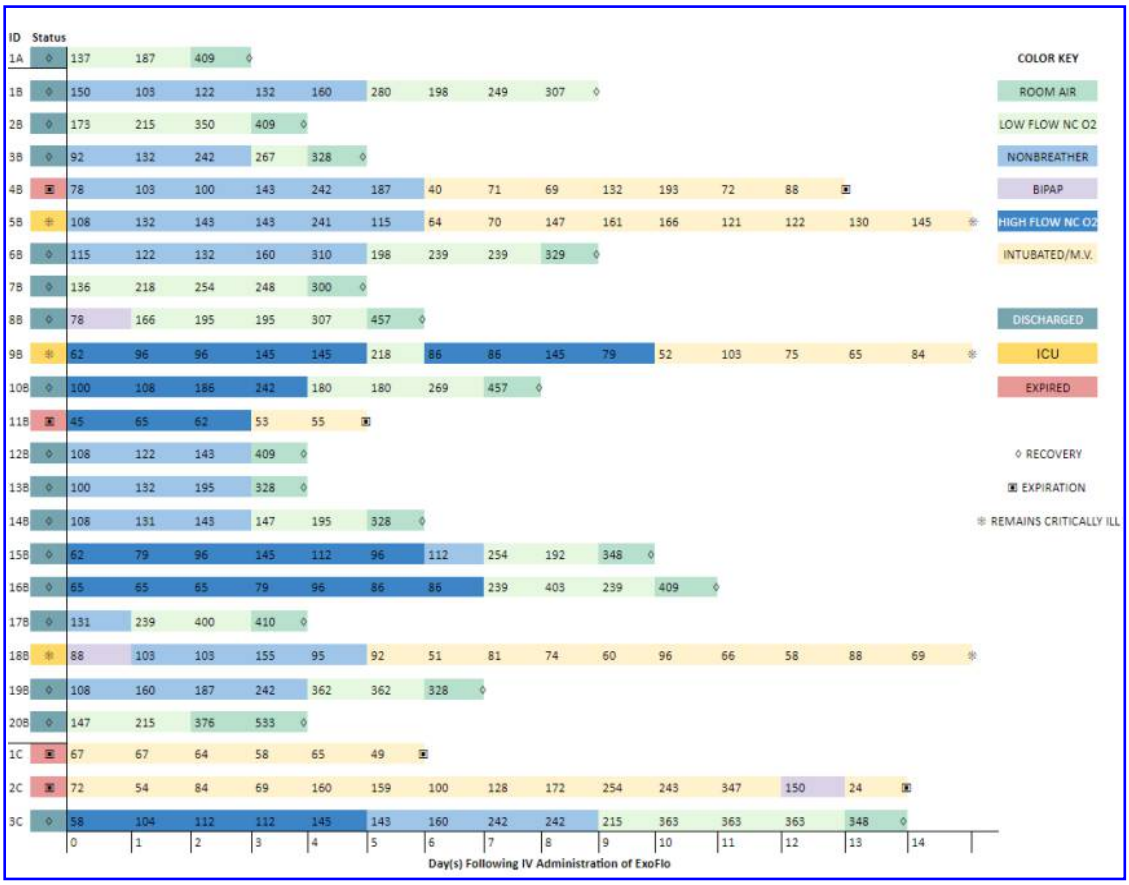


**Figure 2—**Disposition or Final Study Clinical Status, Partial Pressure of Arterial Oxygen (PaO2) to Fraction of Inspired Oxygen (FiO2) Ratio, in addition to Oxygen Requirement Prior to and After Administration of ExoFlo on Day 1-14. Average PaO2/FiO2 ratio increase was 191% ($p < .001$) comparing baseline to 14 days following treatment or final known value.



Stem Cells and Development
Exosomes Derived From Bone Marrow Mesenchymal Stem Cells as Treatment for Severe COVID-19 (DOI: 10.1089/scd.2020.0080)

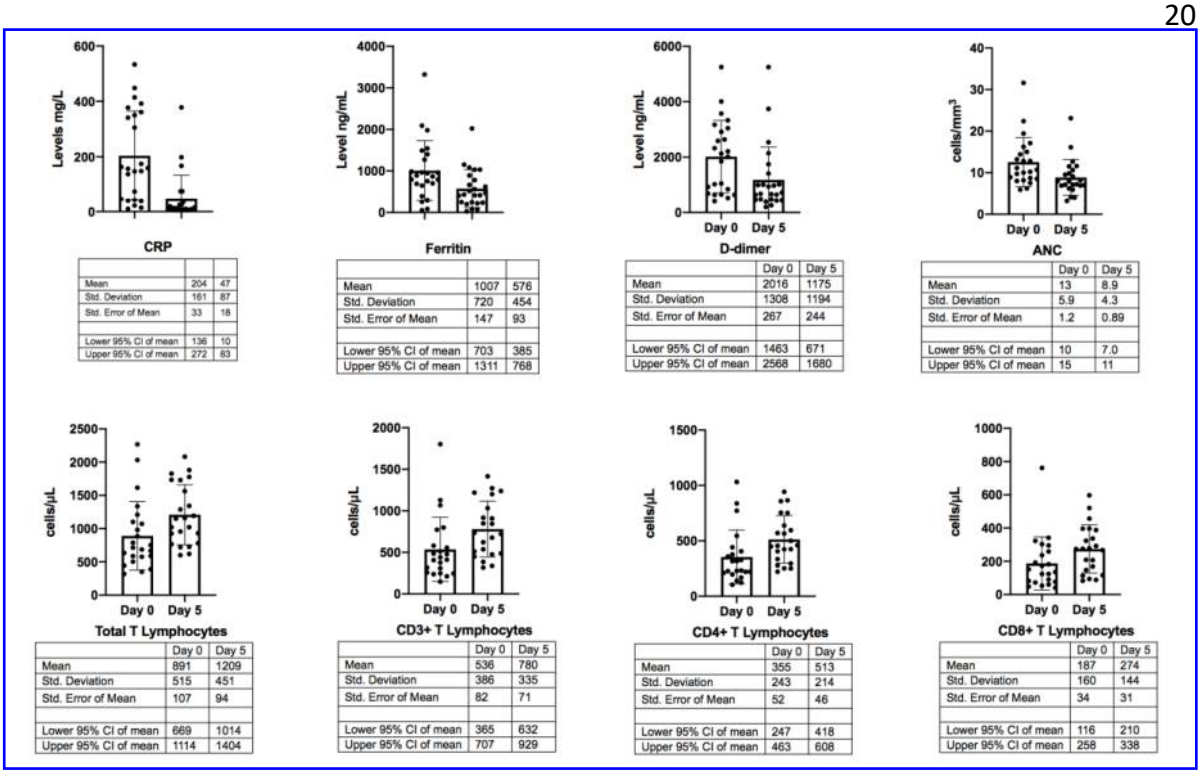


**Figure 3—**Acute Phase Reactants (CRP, Ferritin, and D-dimer) and Immune Cell Populations on Day of Treatment Prior to IV Administration of ExoFlo and on Day 5 Post Treatment. Mean reductions of CRP, Ferritin, and D-dimer reductions were 77% ($p < .001$), 43% ($p < .001$), 42% ($p < 0.05$), respectively. Mean reduction of ANC was 32% ($P < .001$); total lymphocyte count increased by 36% ($P < 0.05$) with CD3+, CD4+, CD8+ T lymphocytes increased by 46 % ($P < 0.05$), 45% ($P < 0.05$), and 46% ($P < 0.001$) respectively.