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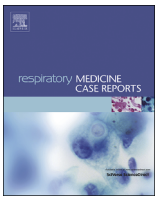

Case Report

A novel extracellular vesicle paradigm for the treatment of COVID-19 induced acute respiratory distress syndrome (ARDS)

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ABSTRACT

Efficacy of mesenchymal stem cells (MSCs) for treatment of acute respiratory distress syndrome (ARDS) suggests bioactive bone marrow MSC extracellular vesicles (BM-MSC EVs) may be effective. A patient with severe COVID-19 associated ARDS who was presumed to expire was treated with a BM-MSC EV preparation (14 doses over two months) as a rescue treatment for refractory COVID ARDS. Near complete reversal of lung inflammation and fibrosis (per computed tomography), near complete restoration of mobility, hospital discharge (3 months) with resumption of normal activities of daily living (one year) and return to work occurred. No adverse events occurred despite repeated dosing of investigational product, highlighting safety of this potential therapy for ARDS.

1. Introduction

Acute respiratory distress syndrome (ARDS) due to the SARS-CoV-2 virus (COVID-19 ARDS) demonstrates the same pathologic changes of diffuse alveolar damage as classic ARDS. ARDS develops in 33%–42 % of hospitalized patients with COVID-19 and in 61–81 % of patients admitted to the intensive care unit (ICU) [1]. Regardless of etiology, ARDS treatment options are limited to lung protective mechanical ventilation (MV) and prone positioning. New, effective treatments are desperately needed to reduce overall patient mortality [2].

Treatment with bone marrow-derived mesenchymal stem cells (BM-MSC) has already shown promise in the treatment of severe COVID-19, COVID-19 related ARDS, other sepsis-based ARDS, and other disease states [3–8]. The repeated observation of reduction in lung injury suggest that BM-MSCs have the potential to treat ARDS. However, specific logistical and infrastructure challenges make it difficult to scale BM-MSCs for widespread distribution and utilization in clinical settings. These include the need for an onsite cGMP or cell therapy pharmacy to deliver fresh cell therapy rather than cryopreserved cell therapy, inability to generate large numbers of doses due to limited starting material, inability to deliver a consistent product due to donor-to-donor variability, and the prohibitively high costs associated with manufacturing. In addition, prothrombotic properties, pulmonary trapping, and alloimmunity limit the use of MSCs in different disease states.

Paracrine effects, rather than *trans*-differentiation across cell types are emerging as the most important aspect of MSC function [9]. Extracellular vesicles (EVs) derived from non-immunogenic BM-MSCs deliver the paracrine effects of the MSCs while providing a sta-

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ble, off the shelf product that can be stored and prepared in any clinical facility regardless of a cGMP facility on site. These scalable, off the shelf properties allow for widespread adoption in the clinic and hospital of a cell derived, regenerative therapeutic (BM-MSC EVs) that delivers the functional aspects of cell therapy.

During the COVID-19 pandemic, we proposed that a BM-MSC EV investigational product (IP) could safely reduce lung injury associated with severe COVID-19 and might also assist in the lung's recovery process. We describe herein the case of a COVID-19 patient with severe respiratory failure and subsequent refractory ARDS requiring prolonged mechanical ventilation who recovered after receiving multiple doses of the IP. This case illustrates the potential for a BM-MSC EV product to manage COVID ARDS and highlights this BM-MSC EV IP as a novel, convenient and potentially effective therapeutic for the treatment of ARDS. A preprint has previously been published by the authors of this case report in April of 2023 [10].

2. Case presentation and treatment protocol

The patient is a 40-year-old female with a history of mild intermittent asthma, diverticulosis, and a remote history of thyroid cancer status post thyroidectomy. The patient has provided consent for this publication. The patient had a prolonged ICU course due to COVID-19 induced ARDS that started four days after delivering her second child via Cesarean section. Treatment for ARDS included lung protective mechanical ventilation with prone positioning, elevated positive end expiratory pressure (PEEP), fluid removal, treatment of secondary infections with intravenous (IV) antibiotics, and supportive care for multisystem organ failure. She also received standard supportive care for COVID ARDS including lung protective ventilation, intermittent neuromuscular blockade to enhance ventilator synchrony, and IV corticosteroids.

After one month of supportive care failed to improve her status and discussions about palliative care options began, Mary Washington Hospital Institutional Review Board approval was obtained on January 20, 2022, and an eIND (IND #28207) was approved by the Food and Drug Administration (FDA) for the administration of 15 mL of IP. ExoFlo™ (IP) is a cGMP manufactured, acellular EV product prepared from a single donor BM-MSC culture that confers the anti-inflammatory and regenerative benefits of BM-MSCs without the aforementioned limitations associated with cell therapy [6,8,11–16]. Before clinical use, the IP is subject to stringent release criteria and a series of potency assays tailored to ARDS to ensure consistency of product across manufacturing runs. IV administration has been evaluated in a phase 1 safety study (n = 24), an expanded access program (n = 103), and a phase 2 randomized, placebo controlled clinical trial (n = 102), designed to evaluate both safety and efficacy in the treatment of COVID-19 associated ARDS (COVID ARDS)., The IP is currently in a phase 3 randomized, placebo controlled trial for all cause ARDS (NCT05354141) [13,14]. The IP was granted a Regenerative Medicine Advanced Therapy (RMAT) designation for the treatment of COVID ARDS by the Food and Drug Administration (FDA) in April 2022. Given the promising safety and efficacy data with intact MSCs and the strong safety results at the time of this case, further evaluation with the IP was justified.

At the time of IP administration, she was sedated with a FiO_2 of 100 % and PEEP of 15cm H2O, and she was receiving norepinephrine to maintain adequate perfusion. She received two 15 mL doses of ExoFlo on Day 1 and Day 2 with slight improvement. She was not considered a candidate for extracorporeal membrane oxygenation (ECMO) due to the prolonged mechanical ventilation. Seventeen days later, she continued having febrile episodes and still required maximum ventilatory support. Her FiO2 on the ventilator was 90 % and her PEEP was at 14cm H2O. Since her lung function had not improved, the FDA approved an additional treatment course of IP. After the second day of her second course of IP treatment, the PEEP and sedation started to be weaned successfully, and she was able to move her lower extremities again during sedation holiday. Due to this marked improvement, she continued the IP treatment course with 15 mL every 24 hours for a total of 5 days. By the fifth dose of IP (9th dose in total), her ventilatory requirements had improved to 55 % FiO2 and a PEEP of 8. The day after her fifth dose, two months from her ARDS diagnosis, she underwent a bedside tracheostomy and percutaneous endoscopic gastrostomy (PEG) tube placement.

Two days following her second round of IP treatment, her sedation continued to be weaned, her urine output began to increase, and she was initiated on oral intake with ice chips. Two weeks following this treatment course, the FDA approved a third treatment course with IP. Following her third course of IP (5 days, 15 mL Q 24 hours) her tracheostomy was downsized, ventilatory support was discontinued, and she achieved 96 % oxygen saturation on trach collar. Fig. 1 illustrates this progress as determined by chest computed tomography (CT) imaging. Fig. 1A shows a CT scan before the second five-day course of IP, and Fig. 1B shows her improved CT scan one month after the second five-day course of IP.

Four days after her third treatment course with IP (14 total doses), she was discharged from the hospital to a rehabilitation facility. She was still regaining strength and remained on oxygen at rest. Eight months after discharge from the hospital, she was able to return to work part time. One year after discharge, she has regained most of her strength. She still has some fine motor deficits in her right hand, and she still requires oxygen with exertion. She has a mild restrictive pattern and mild DLCO reduction on her pulmonary function tests, but her chest CT and overall functional status have both greatly improved. Her most recent CT scan taken one year after discharge from hospital (Fig. 2B, 13 months after the second course of IP) shows improvement in the fibrotic injury appreciated on her earlier chest CTs (Fig. 2A, additional chest CT taken before the second five-day course of IP).

3. Discussion

Clinical Discussion: This case report builds on encouraging safety and efficacy results from a phase 1 trial, and now a randomized, placebo controlled phase 2 clinical trial, that evaluated the BM-MSC EV IP in COVID-19 ARDS [13,14]. Those trial results illustrate the exciting potential for a BM-MSC EV preparation, given in up to two doses, to halt ARDS progression and contribute to recovery of lung function. Prior to this patient's second course of IP, the ICU team was discussing palliative care options with the family and the patient had been made do not resuscitate (DNR), since she was in the severe COVID ARDS category of patients who are often unable

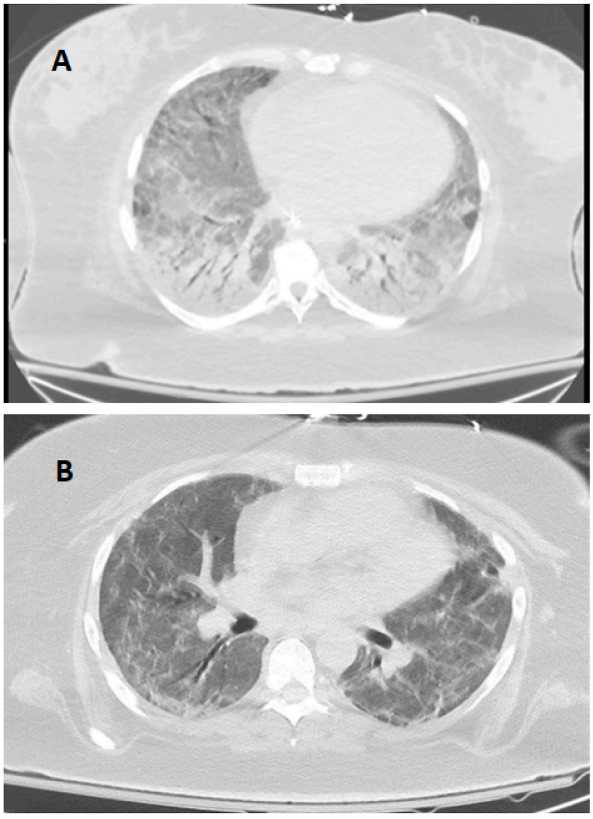

Fig. 1. A. Chest CT imaging before five-day course of IP (February 8, 2022). B. Chest CT showing improvement after five-day course of IP (March 8, 2022).

to recover. After one month of lung protective mechanical ventilation, she still required a PEEP of 15cm H2O and fraction of inspired oxygen of 100 %. Also, prior to IP, her chest CT showed pulmonary fibrosis with acute and chronic inflammation. If this category of patients does recover, they usually remain dependent on substantial amounts of supplemental oxygen. Once she started showing signs of major improvement during her second course of treatment with IP, however, it became apparent that she had a good chance for a meaningful recovery.

Imaging Discussion: Interestingly, we observed significant serial improvement in the chest CTs obtained over the 13 month follow up. This suggests that the IP may also promote tissue repair and regeneration in patients with ARDS. This observation is consistent with the clinical efficacy observed in the IP phase 1 and phase 2 COVID-19 ARDS clinical trials; the phase 2 demonstrated a significant decrease in mortality among patients with COVID-ARDS treated with 2 doses of IP at 15 mL [13,14].

Several studies highlight the potential of MSCs for treating ARDS, but also point to the practical limitations of intact cell therapy. Previously allogeneic adipose MSCs were shown to be safe in a pilot study of a small group of patients with ARDS, although efficacy was not apparent [17]. In 2015, the phase I START trial enrolled 9 patients with moderate-to-severe ARDS and monitored outcomes for 60 days following a single intravenous dose of up to 10 million MSCs/kg of BM-MSCs; no SAEs were observed in the 6 hours post infusion or in the weeks following [8]. The 60 patient START Phase 2a study which delivered a single dose of BM-MSCs failed to show a therapeutic effect, although no treatment related adverse events were reported [6]. Subsequent analysis of bronchoalveolar lavage samples from a subset of the patients in the START trial showed that the single BM-MSC infusion significantly reduced airspace ang-2, IL-6, soluble TNFR-1 and total protein levels compared to placebo control samples; interestingly, no significant difference was observed in plasma samples of the same patients [18]. In a recent study of allogeneic BM-MSCs, seven patients with COVID-19 pulmonary disease experienced improved lung function without any observed adverse effects within 2 h after treatment and did not have delayed hypersensitivity or secondary infections [4]. Within 2 days of treatment, 6 out of 7 patients recovered, and within 3 days, 3 out of 7 patients were discharged from the hospital. Exploratory endpoints were significant for decreased pro-inflammatory cytokine TNF-α and for increased anti-inflammatory IL-10, suggesting that BM-MSCs may halt the COVID-19 cytokine storm. Our case study complements these studies and highlights the potential for redirection of intact cell therapy to a more practical BM-MSC EV therapeutic.

The treatment plan with the IP for this single patient differed from the previous clinical trial protocols in that this patient received a total of 14 doses of 15 mL of IP during her treatment course as compared to up to two doses in the trials. No treatment-related safety issues have arisen in this patient. In fact, one year from her initial treatment, this patient has returned to work and is able to parent her new child. This case study involving 14 doses also illustrates the practicality and ease of use of the EV preparation as compared to the challenges associated with preparing 14 doses of a MSC preparation from a single donor. A single case study like this is limited by

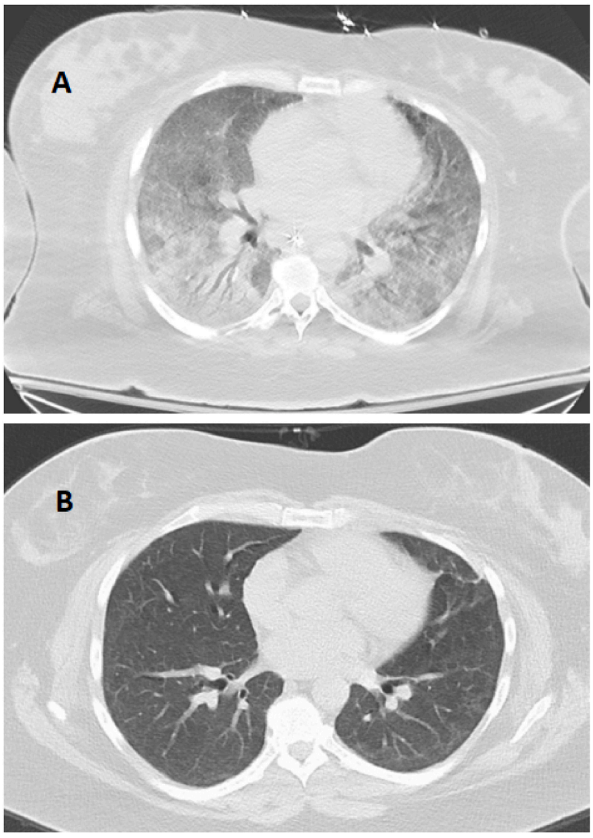

Fig. 2. A. Chest CT before five-day course of IP (February 8, 2022). B. Chest CT one year after discharge from hospital (March 9, 2023).

the lack of a control arm and the inability to make conclusions about statistical significance. Currently a Phase III, randomized, blinded trial is underway, NCT05354141, to further evaluate the safety and efficacy of ExoFlo in patients with any cause of moderate to severe ARDS. A limitation of this treatment will be pregnant patients and patients on extracorporeal support, since they will not be included in the Phase III trial. While our current case report shows safe and effective use in one patient, the Phase III data will be necessary to demonstrate beneficial use to a wider population.

4. Conclusion

- The positive outcome of this case study using a consistent multiple dosing regimen suggests that BM-MSC EVs may be a powerful new therapeutic approach to control ARDS and improve lung function.
- This case study highlights the practical utility of a scalable and off the shelf BM-MSC EV therapeutic with the potential to treat ARDS safely and effectively, without the costs and limitations of an intact mesenchymal stem cell therapy.

Summary declaration

ALL, JTR, AS and VS reported either employment or consultancy with Direct Biologics that provided the investigational product used in this case study.

Data availability

All data is contained within the manuscript.

Funding

Direct Biologics provided the ExoFlo material used in this case study.

CRediT authorship contribution statement

Erik Osborn: Conceptualization, Data curation, Formal analysis, Investigation, Supervision, Validation, Writing – original draft, Writing – review & editing. **John T. Ransom:** Conceptualization, Data curation, Formal analysis, Project administration, Resources, Software, Visualization, Writing – review & editing. **Anastasiya Shulman:** Funding acquisition, Project administra-

tion, Supervision, Validation, Visualization. **Vikram Sengupta:** Conceptualization, Data curation, Methodology, Project administration, Resources. **Mohammed Choudhry:** Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Validation, Visualization. **Ali Hafiz:** Data curation, Formal analysis, Funding acquisition, Investigation, Validation, Visualization, Writing – review & editing. **Jacob Gooden:** Data curation, Formal analysis, Methodology, Resources, Software, Visualization, Writing – review & editing. **Amy L. Lightner:** Funding acquisition, Project administration, Supervision, Validation.

Declaration of competing interest

No conflict except the below.

John Ransom reports a relationship with Direct Biologics that includes: employment. John Ransom reports a relationship with Vivreon Biosciences, LLC that includes: co-owner. John Ransom reports a relationship with Stabilux Biosciences, Inc: Investor and board member.

Anastasiya Shulman reports a relationship with Direct Biologics that includes: employment.

Vik Sengupta reports a relationship with Direct Biologics that includes: consulting.

Amy L. Lightner reports a relationship with Direct Biologics that includes: employment as Medical Director. Amy Lightner reports a relationship with Boomerang Medical that includes: Consultant and Chief Medical Officer.

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