




## Feasibility, Safety, and Tolerance of Mesenchymal Stem Cell Therapy for Obstructive Chronic Lung Allograft Dysfunction

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**Key Words.** Bronchiolitis obliterans syndrome • Chronic lung allograft dysfunction • Lung transplantation • Mesenchymal stem cells

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### ABSTRACT

Feasibility, tolerance, and safety of intravenous infusions of allogeneic mesenchymal stem cell (MSC) therapy in lung transplant recipients with bronchiolitis obliterans syndrome (BOS) are not well established. MSCs were manufactured, cryopreserved, transported to our facility, thawed, and infused into nine recipients with moderate BOS (average drop in forced expiratory volume in 1 second was  $56.8\% \pm 3.2\%$  from post-transplant peak) who were refractory to standard therapy and not candidates for retransplant. Cells were viable and sterile prior to infusion. Patients received a single infusion of either 1 ( $n = 3$ ), 2 ( $n = 3$ ), or 4 ( $n = 3$ ) million MSCs per kg. Patients were medically evaluated before; during; and at 24 hours, 1 week, and 1 month after infusion for evidence of infusion-related adverse events and tolerance of therapy. Vital signs, pulmonary function test results, Borg Dyspnea Index, and routine laboratory data were recorded. Vital signs and  $O_2$  saturation did not significantly change during or up to 2 hours after MSC infusion. There were no significant changes in gas exchange variables, pulmonary function test results, or laboratory values at 1, 7, and 30 days postinfusion compared with preinfusion values. Infusion of MSCs in patients with BOS was feasible, safe, and well tolerated and did not produce any significant adverse changes in clinical, functional, or laboratory variables during or up to 30 days after infusion. Manufacturing, transport, and administration of intravenous, allogeneic bone marrow-derived MSCs in doses from 1 to 4 million MSCs per kg is safe in lung transplant recipients with BOS. *STEM CELLS TRANSLATIONAL MEDICINE* 2018;7:161–167

### SIGNIFICANCE STATEMENT

Results of this study show that it is feasible and safe to provide cell therapy with intravenous infusion of bone marrow-derived mesenchymal stem cells to lung transplant recipients with moderate obstructive chronic lung allograft dysfunction, warranting future studies to assess the effectiveness of this therapy for management of acute or chronic graft dysfunction.

### INTRODUCTION

Lung transplant patients with treatment refractory moderate (grade 2) bronchiolitis obliterans syndrome (BOS) who do not qualify for a second transplant are at high risk of mortality or extreme disability. BOS is a subtype of chronic lung allograft dysfunction (CLAD) and is defined by progressive decline in forced expiratory volume in 1 second ( $FEV_1$ ) following the highest  $FEV_1$  achieved post-transplant, not explained by acute rejection, acute infection, or stenotic airways [1]. The largest study, from the International Society for Heart and Lung Transplant registry, reports that by 5 years after lung transplant, 45% of recipients develop BOS [2]. The mortality rate associated with BOS ranges from 25% to 56%; the risk

increases with time after diagnosis [3]. Although a variety of therapies have been tried for BOS, there is no standardized therapeutic protocol. Large doses of steroids, enhanced immunosuppression, azithromycin, statin administration, antireflux medical therapy or surgical fundoplication, photopheresis, and other therapies have been tried with variable success. BOS is the most common indication for retransplantation, although this is a controversial alternative with increased mortality [4]. One review examined the outcome of 230 cases of retransplantation performed in 47 centers between 1985 and 1996 [5]. The review showed 1-year survival was only 47%.

Mesenchymal stem cells have long been known to regulate the cellular immune system. They suppress effector T cells through production of

**Table 1.** Inclusion and exclusion criteria**Inclusion criteria**

- a. Age range: 18–75 years.
- b. Sex: male or female.
- c. Patient must have diagnosis of treatment refractory BO/BOS, must have had either an inadequate or lost response (recurrence or persistence of symptoms) to a drug in each of the following three treatment categories within the past 24 months or documented intolerance to a drug in that category at any time: (a) enhanced steroid therapy; (b) enhanced immunosuppression; (c) trial/failure to other therapies like azithromycin, anti-reflux therapy, statin agents, and/or use of alternate immunosuppressive agents like Thymoglobulin<sup>a</sup>, methotrexate, or Rapamune<sup>b</sup>.
- d. Patient must have adequate renal function; calculated creatinine clearance >30 ml/min.
- e. Patient must be available for all specified assessments at the study site through the completion of the study.
- f. Patient must provide written informed consent.

**Exclusion criteria**

- a. Patients with clinically significant illness with manifestations of significant organ dysfunction, which in the judgment of the principal or coinvestigator would render the patient unlikely to tolerate the MSC infusion or complete the study.
- b. Evidence or history of malignancy.
- c. Evidence or history of autoimmune disorders independent of BO/BOS.
- d. Pregnant or breast-feeding.
- e. Positive screening for HIV, hepatitis B, and hepatitis C.
- f. Evidence of liver dysfunction; total bilirubin >1.65 mg/dL, ALT >275 units per L, and AST >240 units per L.
- g. Evidence of significant cardiac dysfunction.
- h. Septicemia with high fever and hemodynamic instability.
- i. History of CMV pneumonitis.
- j. Patients who received any experimental therapy (drug or biologic) for any indication within 3 months of the study enrollment.
- k. Patient qualifies for retransplantation.

<sup>a</sup>Anti-thymocyte globulin [rabbit]; Sanofi, Paris, France, <http://www.sanofi.us/1/us/en/index.jsp>.

<sup>b</sup>Sirolimus; Pfizer, New York, NY, <https://www.pfizer.com>.

Abbreviations: ALT, alanine transaminase; AST, aspartate transaminase; BO/BOS, bronchiolitis obliterans/bronchiolitis obliterans syndrome; CMV, cytomegalovirus; MSC, mesenchymal stem cell.

interleukin-10, transforming growth factor- $\alpha$ , inducible nitric oxide synthase, heme oxygenase-1, prostaglandin E2, and indolamine 2,3-dioxygenase and generation and differentiation of dendritic cells [6]. They are thought to shift immune response toward anti-inflammatory, tolerogenic phenotype (a shift from T helper type 1 to T helper type 2 immune response) as reviewed by Kode et al. [7].

In vivo models have demonstrated that MSCs have the capacity to engraft into lung tissue. Multiple studies indicate that intravenous administration of MSC therapy will preferentially engraft in the lung in the presence of an inflammatory process, which occurs in BOS and pulmonary fibrosis [8, 9]. Once implanted, MSCs are capable of interacting with the surrounding microenvironment and are believed to facilitate regeneration of neighboring tissue by secreting various factors and renewing biologic functions, such as the immune system, or act directly to support and revive cell function and repair damage [8]. Human trials that utilize MSCs to treat disease conditions such as graft-versus-host disease and chronic obstructive pulmonary disease (COPD) have similar pathology to BOS and have shown good tolerance with no significant adverse events [10, 11]. Intravenously infused cells are naturally trapped in the lung with few cells reaching systemic circulation, a phenomenon called pulmonary first-pass effect [12], which provides easy access of therapeutic cells to the pathology site. Infused MSCs may participate in repair of the native lung, thereby inducing immune-modulatory effect in the transplanted lung. Infusion of human MSCs in doses as high as  $5.6 \times 10^6$  MSCs per kg were not associated with any documentable adverse events in a rat model of lung injury induced by bleomycin [13].

In this pilot study, we evaluated the safety and feasibility of administering allogeneic bone marrow-derived MSCs with the intent of inducing remission of moderate treatment-refractory BOS. Lung transplant recipients who had progressed to moderate BOS, despite maximal medical therapy, and who did not qualify for a retransplantation, received intravenous infusion of allogeneic MSCs. Safety of the therapy was evaluated, including tolerance of

MSC infusion and absence of notable cardiopulmonary compromise. Feasibility was also evaluated, including ease of recruitment and practical issues of transporting, preparing, and infusing MSCs.

**MATERIALS AND METHODS****Patient Population**

Study patients were recruited from the clinical practice of the lung transplant program at Mayo Clinic in Jacksonville, Florida. Inclusion and exclusion criteria are listed in Table 1. This study was approved by the Mayo Clinic Institutional Review Board (ID 14-000025), conducted under IND 15807 from the U.S. Food and Drug Administration (FDA), and registered at ClinicalTrials.gov, NCT02181712.

**MSC Manufacturing**

MSC manufacturing was performed by Waisman Biomanufacturing at the University of Wisconsin-Madison, Madison, Wisconsin. Clinical grade bone marrow was purchased from Lonza (Walkersville, MD, <https://www.lonza.com>). Bone marrow was harvested from a male, 18–45-year-old, blood type O, healthy donor who had undergone physical examination and had completed a standardized Donor Health History Questionnaire. Communicable disease testing was performed by a laboratory that is registered with the FDA to perform testing in accordance with Clinical Laboratory Improvement Amendments of 1988 and 42 code of federal regulation (CFR) 493. Testing was performed that included the following: HIV-1/2 antibody, HIV-1 nucleic acid test (NAT), hepatitis B virus (HBV) surface antigen, HBV core antibody, HBV NAT, hepatitis C virus (HCV) antibody, HCV NAT, human T-cell lymphotropic virus types I/II antibody, cytomegalovirus total antibody, West Nile virus NAT, rapid plasma reagin (RPR), and fluorescent treponemal antibody absorption (tested only if RPR is reactive). Donor eligibility determination was performed by the Lonza medical director after

full medical evaluation of the donor and review of the donor history questionnaire and the infectious disease test results. Allogeneic bone marrow-derived MSCs from this single healthy donor were manufactured using standard manual tissue culture flasks with Minimal Essential Medium Alpha Medium (15-012-CV; Mediatech, Inc., Manassas, VA, <https://www.cellgro.com>) and 9.8% HyClone Characterized Fetal Bovine Serum, U.S. Origin (SH30071.031 IR; GE Healthcare, Chicago, IL, <https://www.gehealthcare.com>). Cryopreserved bags of the final product were stored in vapor base liquid nitrogen at less than  $-130^{\circ}\text{C}$  [13]. MSCs were shipped from the manufacturing facility using qualified vapor shippers with calibrated temperature logger to the Human Cell Therapy Laboratory at our facility, where they remained frozen and stored in vapor-phase liquid nitrogen. Each bag contained  $5 \times 10^6$  MSCs per mL in 2.5% dimethyl sulfoxide (total of 20 mL). The final product plus an additional eight aliquots (5 mL each) were cryopreserved in a similar bag for Quality Control Testing on the final product. Testing performed is disclosed in an appendix table (Supporting Information Table 1).

### Transporting MSC

Shipping studies were performed to verify transporting cryopreserved MSCs between the manufacturing facility (Waisman Biomanufacturing at the University of Wisconsin-Madison) and the clinical site (Mayo Clinic in Jacksonville, Florida) did not adversely impact MSC quality. It was discovered that some of the cell product bags were broken, prompting a change in thawing protocol. Because of the risk of MSC clumping and sedimentation during infusion, especially in the high-dose group, a pilot experiment was performed, which led to adjustment and determination of the final cell concentration with least clumping and sedimentation. Similarly, dimethyl sulfoxide content was adjusted to a minimum while maintaining maximal post-thaw viability. To ensure stability of the MSCs during infusion, pre- and postinfusion viability assessments were performed. Cells were thawed and diluted following an approved Standard Operating Procedure. The thawed, diluted cells were held at room temperature and samples were removed each hour over a 4-hour period and assessed for recovery, clumping, and viability (Trypan Blue). MSC viability was  $>88\%$  for all time points in all replicate studies. Studies were repeated with the thaw and viable cell count performed at the Mayo Clinic Cell Therapy Laboratory (Jacksonville, FL) with a viability  $>84\%$ .

### Preparation of MSCs Prior to Infusion

On infusion day, the frozen MSCs underwent a standard protocol for thawing and infusion. Each bag was then diluted fivefold with Plasma-Lyte (100 mL; Baxter Healthcare Corporation, Deerfield, IL, <https://www.baxter.com>) to yield the final dimethyl sulfoxide concentration of 0.5%. One million to four million MSCs per kg were infused intravenously as outlined in Table 2. Three milliliters of the final product was saved for cell count, viability, and bacterial and fungal culture evaluation.

### MSC Infusion on Patients with BOS

Infusion of MSCs was performed in the clinical apheresis unit. MSCs were infused at a rate of 2–3 mL/minute during the first 15 minutes, with the option to be adjusted up to 5 mL/minute if tolerated. Patients were monitored for any adverse reaction. Infusion toxicity was evaluated by continuously monitoring the patient's vital signs, electrocardiogram, and  $\text{O}_2$  saturation by pulse oximetry before, during, and up to 2 hours after MSC infusion. The dose of

MSC infusion was chosen using an incremental protocol: the initial three patients assigned to group 1 received an infusion dose of  $1 \times 10^6$  MSCs per kg; three patients assigned to group 2 received an infusion dose of  $2 \times 10^6$  MSCs per kg; and the final three patients in group 3 received an infusion dose of  $4 \times 10^6$  MSCs per kg.

### Monitoring

Laboratory data (hemoglobin; white blood cell and platelet counts; and blood urea nitrogen, serum creatinine, and glucose levels) were collected for all patients prior to infusion (1–7 days) and on days 1, 7, and 30 after infusion. Arterial blood gases were obtained prior to infusion and on days 7 and 30 after infusion. Spirometric values were obtained within 7 days prior to infusion, just before infusion, and on days 1, 7, and 30 after infusion.

### Statistical Analysis

Individual variables were investigated across time using a one-factor repeated measures analysis of variance. Significant differences among time points were investigated, adjusting for multiple comparisons using the Student-Newman-Kruels test. All statistical results were completed using SAS version 9.4 (SAS Institute Inc., Cary, NC, [https://www.sas.com/en\\_us/home.html](https://www.sas.com/en_us/home.html)).

## RESULTS

### Patient Population

Nine patients (seven men, two women) who were recipients of double lung transplant ( $n = 5$ ) or single lung transplant ( $n = 4$ ) were included. Their advanced age ( $69 \pm 5$  years) was likely influenced by the study design excluding younger patients who could qualify for retransplant. Their mean  $\text{FEV}_1$  prior to MSC infusion was  $56.8\% \pm 3.2\%$  (range 53%–62%) from the peak achieved after transplant, corresponding to a  $43.1\% \pm 3.4\%$  drop (range 38%–47% drop) consistent with moderate BOS. Indications for single lung transplant were COPD (3) and idiopathic pulmonary fibrosis (1). Indications for double lung transplant were idiopathic pulmonary fibrosis (2), COPD (1), primary ciliary dyskinesia (1) and one patient retransplanted for BOS. These patients had survived an average of  $7.5 \pm 3$  years after transplant and had been clinically diagnosed with moderate BOS a median of 5 years after transplant. They were all on triple immunosuppressive therapy: prednisone, mycophenolate (6) or azathioprine (3), and tacrolimus (8) or cyclosporine (1). They had all received enhanced immunosuppressive therapy with steroid boluses (9), methotrexate (5), Rapamune (sirolimus; Pfizer, New York City, NY, <https://www.pfizer.com>; 2) or Thymoglobulin (anti-thymocyte globulin [rabbit]; Sanofi, Paris, France, <http://www.sanofi.us/l/us/en/index.jsp>; 1). One patient had received a retransplant for BOS. They were all receiving azithromycin, proton pump inhibitors, and statin therapy. These data are available in an appendix table (Supporting Information Table 2).

### Feasibility of MSC Therapy in Patients with Moderate to Severe Transplant-Related BOS

The process of freezing, transporting, and thawing MSCs did not affect their viability:  $92.4\% \pm 2.6\%$  were viable prior to infusion and  $93.0\% \pm 3.28\%$  were found to be viable at the end of infusion (Table 3). Group 1 received an average of 1.09 million MSCs per kg, group 2 an average of 2.50 million MSCs per kg, and group 3 an average of 3.93 million MSCs per kg. The median volume of

Table 2. Population studied (n = 9)

Group	Age, years	Sex	Diagnosis	Transplant type	Survival after transplant, years	Time from transplant to BOS-2, years	FEV <sub>1</sub> : percent of baseline <sup>a</sup>	FVC, L	FVC, %	FEV <sub>1</sub> , L	FEV <sub>1</sub> , %	FEV <sub>1</sub> FVC	FEF <sub>25-75</sub> , L	FEF <sub>25-75</sub> , %
Group 1	75	F	COPD	Single (R)	10.1	5.5	53%	1.78	59	1.09	46	0.60	0.3	23
	75	M	COPD	Single (R)	6.4	4.6	62%	2.19	50	1.16	35	0.53	0.4	16
	70	M	IPF	Single (R)	10.7	9.0	59%	2.29	60	1.58	54	0.69	0.9	34
Group 2	63	F	BOS	Double	4.7	3.4	59%	1.88	76	1.50	75	0.79	1.3	64
	74	M	COPD	Single (L)	12.5	5.9	56%	1.68	44	1.13	38	0.67	0.5	20
Group 3	71	M	PCD	Double	8.0	6.0	53%	4.91	102	2.70	74	0.55	1.1	37
	73	M	COPD	Double	6.6	2.4	60%	2.95	78	2.14	73	0.73	1.4	52
	59	M	IPF	Double	2.5	1.4	53%	2.18	44	1.55	40	0.71	1	29
Total patient population <sup>b</sup>	61	M	IPF	Double	6.0	5.0	56%	3.32	67	1.68	45	0.51	0.7	23
	Average	69.0			7.5	4.8	57%	2.58	64.4	1.61	53.33	0.64	0.84	33.11
	STDEV	6.3			3.2	2.2	3%	1.03	18.8	0.5	16.4	0.1	0.4	15.9
Median	71			6.6	5.0	56%	2.19	60.0	1.55	46	0.67	0.90	29	
Max	75			12.5	9.0	62%	4.91	102.0	2.70	75	0.79	1.40	64	
Min	59			2.5	1.4	53%	1.68	44.0	1.09	35	0.51	0.30	16	

<sup>a</sup>FEV<sub>1</sub>: percent of baseline; represents the percent of baseline, using baseline as the highest FEV<sub>1</sub> value achieved after transplant.

<sup>b</sup>Of the 9 patients, 7 were male and 2 were female. Diagnoses included COPD (4), IPF (3), PCD (1), and BOS (1). Five of the included patients were recipients of a double lung transplant, and 4 were recipients of a single lung transplant.

Abbreviations: BOS, bronchiolitis obliterans syndrome; COPD, chronic obstructive pulmonary disease; F, female; FEF<sub>25-75</sub>, forced expiratory flow at 25%–75%; FEV<sub>1</sub>, forced expiratory volume in 1 second; FVC, forced vital capacity; FVC/FEV<sub>1</sub>, forced vital capacity to forced expiratory volume in 1 second ratio; IPF, idiopathic pulmonary fibrosis; M, male; Max, maximum; Min, minimum; PCD, primary ciliary dyskinesia; STDEV, standard deviation.

**Table 3.** Cell count, viability, and sterility of infused MSCs in nine patients

Group	Patient	Weight, kg	Cell count, millions per kg	Volume infused, mL	Cells infused, millions per kg	Preinfusion viability, %	Postinfusion viability, %	Culture
1	1	101.7	134	125	1.34	94.5	95.8	Negative
	2	94.7	104	97	1.08	91.6	92.4	Negative
	3	97.1	84	97	0.84	94.2	94.5	Negative
2	4	61.5	161	150	2.62	97.4	97.4	Negative
	5	84.8	212	197	2.46	91.6	92.8	Negative
	6	98.1	230	197	2.31	92.2	97.2	Negative
3	7	89.5	352	397	3.9	90.4	88.6	Negative
	8	98.5	336	397	3.39	92.2	90.4	Negative
	9	96.8	440	397	4.51	87.6	88.2	Negative
Total patient population								
	Mean	91	228	228	2.49	92.4	93.0	
	SD	12.3	123.3	131.6	1.3	2.8	3.5	
	Median	96.8	212	197	2.46	92.2	92.8	All negative
	Maximum	101.7	440	397	4.51	97.4	97.4	
	Minimum	61.5	84	97	0.84	87.6	88.2	

Abbreviations: MSC, mesenchymal stem cell; SD, standard deviation.

**Table 4.** Variables measured before and after mesenchymal stem cell infusion

Variable	Preinfusion, mean $\pm$ SD	0.5 hours after infusion, mean $\pm$ SD	1 hour after infusion, mean $\pm$ SD	2 hours after infusion, mean $\pm$ SD	<i>p</i> value
Heart rate, bpm	75 $\pm$ 15	73 $\pm$ 15	72 $\pm$ 16	73 $\pm$ 16	.53
BP mean, mmHg	102 $\pm$ 13	98 $\pm$ 15	105 $\pm$ 9	107 $\pm$ 11	.09
Respiratory rate, mmHg	20 $\pm$ 4	20 $\pm$ 3	19 $\pm$ 5	19 $\pm$ 4	.30
FiO <sub>2</sub> , mmHg	0.22 $\pm$ 0.03	0.22 $\pm$ 0.03	0.22 $\pm$ 0.03	0.22 $\pm$ 0.03	—
SaO <sub>2</sub> , mmHg	96 $\pm$ 2	94 $\pm$ 2	95 $\pm$ 3	95 $\pm$ 3	.13
Borg Dyspnea Index	0.70 $\pm$ 1.30	0.70 $\pm$ 1.10	0.70 $\pm$ 1.10	0.70 $\pm$ 1.10	.41

Abbreviations: —, no data; BP mean, mean arterial blood pressure; bpm, beats per minute; FiO<sub>2</sub>, fraction of inspired oxygen; SaO<sub>2</sub>, percent saturation of oxygen in arterial blood; SD, standard deviation.

infused product was 197 mL (range, 97–397 mL). All post-thaw sterility cultures were negative (Table 3).

### Safety and Tolerance of MSC Infusion

Patients were clinically evaluated for evidence of acute or delayed infusion-related adverse events. Vital signs, pulmonary function test results, and Borg Dyspnea Index recorded before and after infusion of MSCs are summarized in Table 4. No significant changes were observed in heart rate, mean blood pressure, respiratory rate, O<sub>2</sub> saturation, or perceived dyspnea at 30, 60, and 120 minutes after MSC infusion compared with baseline values (individual changes in each patient are available in appendix tables (Supporting Information Tables (3–6))).

Pulmonary function test results and gas exchange variables are shown in Table 5. There were no significant alterations in pulmonary function test results or variables reflecting ventilation and oxygenation at 24 hours, 1 week, and 1 month after MSC infusion compared with preinfusion variables.

Table 6 displays the results of blood work obtained before and after MSC infusion at 1 day, 1 week, and 1 month after infusion. There were no significant changes observed for hemoglobin; white blood cell and platelet counts; or blood urea nitrogen, creatinine,

or glucose levels compared with those values obtained before MSC infusion.

### DISCUSSION

Immunomodulation induced by MSC therapy in solid organ transplant recipients has only recently been explored [14]. Intravenous administration of autologous bone marrow-derived MSC (two separate infusions of 1 million cells per kg) has been given to kidney transplant recipients showing histologic evidence of acute rejection, without serious adverse events reported [15].

Tan et al. [16] reported use of bone marrow-derived autologous MSCs as a replacement of antibody induction for patients with end-stage renal disease undergoing kidney transplantation from a living related donor. Patients received 1–2 million MSCs per kg at kidney perfusion and 2 weeks later with standard or reduced calcineurin inhibitors compared with a control group. Use of autologous MSCs resulted in lower incidence of acute rejection, reduced opportunistic infections, and better renal function at 1 year. This group reported lower incidence of adverse events for these patients compared with controls [16].

Given the wide array of possible immunomodulatory and anti-inflammatory properties of MSCs, it is appealing to consider their

**Table 5.** Pulmonary function tests and gas exchange variables before and after stem cell therapy

Test	7 days preinfusion	Infusion day	1 day after infusion	7 days after infusion	30 days after infusion	p value
FVC (L)	2.60 ± 1.05	2.58 ± 1.03	2.54 ± 0.98	2.56 ± 0.95	2.62 ± 1.01	.64
FEV <sub>1</sub> (L)	1.62 ± 0.51	1.61 ± 0.52	1.60 ± 0.30	1.64 ± 0.47	1.66 ± 0.47	.73
FVC/FEV <sub>1</sub>	0.63 ± 0.11	0.64 ± 0.10	0.64 ± 0.10	0.69 ± 0.11	0.66 ± 0.12	.11
FEF <sub>25–75</sub> (L/s)	0.98 ± 0.45	0.84 ± 0.39	0.89 ± 0.39	0.94 ± 0.42	0.97 ± 0.45	.11
FiO <sub>2</sub>	0.22 ± 0.03	—	—	0.22 ± 0.03	0.22 ± 0.03	—
pH	7.41 ± 0.03	—	—	7.41 ± 0.02	7.42 ± 0.02	.80
PaCO <sub>2</sub> , mmHg	39.10 ± 3.90	—	—	39 ± 4.90	38.30 ± 3.20	.34
PaO <sub>2</sub> , mmHg	80.20 ± 8.60	—	—	81.30 ± 10.70	79.10 ± 10.50	.92
SaO <sub>2</sub> , %	94.90 ± 1.30	—	—	94.90 ± 2.00	94.50 ± 1.70	.75
PaO <sub>2</sub> /FiO <sub>2</sub>	372 ± 67	—	—	373 ± 58	365 ± 73	.99

Abbreviations: —, no data; FEF<sub>25–75</sub>, forced expiratory flow at 25%–75%; FEV<sub>1</sub>, forced expiratory volume in 1 second; FiO<sub>2</sub>, fraction of inspired oxygen; FVC, forced vital capacity; FVC/FEV<sub>1</sub>, forced vital capacity to forced expiratory volume in 1 second ratio; PaCO<sub>2</sub>, partial pressure of carbon dioxide in arterial blood; PaO<sub>2</sub>, partial pressure of oxygen in arterial blood; PaO<sub>2</sub>/FiO<sub>2</sub>, partial pressure of oxygen in arterial blood to fraction of inspired oxygen ratio; SaO<sub>2</sub>, percent saturation of oxygen in arterial blood.

**Table 6.** Results of blood work measured before and after mesenchymal stem cell infusion

Test	Preinfusion	1 day after infusion	7 days after infusion	30 days after infusion	p value
Hemoglobin, g/dL	13.2 ± 1.2	13.1 ± 1.3	13.1 ± 1.3	13.2 ± 1.5	.90
WBC count, ×10 <sup>9</sup> /L	6.5 ± 2	6.1 ± 2.0	6.8 ± 1.9	6.4 ± 3	.38
Platelet count, ×10 <sup>9</sup> /L	173 ± 41	172 ± 41	179 ± 40	176 ± 38	.53
BUN, mg/dL	24 ± 9	21 ± 9	21 ± 9	23 ± 9	.24
Creatinine, mg/dL	1.3 ± 0.3	1.3 ± 0.3	1.3 ± 0.3	1.4 ± 0.3	.26
Glucose, mg/dL	132 ± 31	134 ± 32	127 ± 31	141 ± 33	.75

Abbreviations: BUN, blood urea nitrogen; WBC, white blood cell.

use to prevent primary graft dysfunction and prevent or treat acute or chronic rejection. In a study by McAuley et al. [17], human lungs considered unsuitable for lung transplantation, found to have evidence of abnormal alveolar fluid clearance, placed in an ex vivo lung perfusion system were randomized to receive ex vivo ventilation and perfusion alone versus addition of intravenous injection of allogeneic, bone marrow-derived MSCs (5 million cells per kg) added to the perfusate. Alveolar fluid clearance was increased to a normal level in lungs receiving MSCs, suggesting that this therapy can enhance the resolution of pulmonary edema in human lungs deemed unsuitable for transplantation [17].

More recently, Chambers et al. [18] reported results of a phase I trial in which bone marrow-derived MSCs from 5 donors were infused into 10 patients with either BOS grade 1 and added risk factors or BOS grade 2 or 3 showing rapid decline in FEV<sub>1</sub> despite medical therapy. Their protocol infused 2 million MSCs per kg twice a week for 2 weeks for a total of 8 million MSCs per kg. They reported only minor and transient fall in mean arterial pressure and O<sub>2</sub> saturation during infusion of MSCs, with rapid recovery and no other clinically significant adverse events. Two of their patients died from progressive BOS at 152 and 270 days after the final infusion of MSCs, but the investigators did not believe these deaths were related to MSC infusion. They observed a reduction in the rate of decline in FEV<sub>1</sub> following MSC infusion, concluding that infusion of MSCs was feasible and safe in patients with advanced CLAD [18]. Our study also used bone marrow-derived MSCs with the primary purpose to improve lung function, or at least arrest the rate of decline in lung function, in patients with progressive BOS refractory to medical therapy. Our group was less diverse than those in

the study from Chambers et al., as only patients with moderate BOS (grade 2) were included and our patients received only a single infusion of MSCs from a single, healthy donor. Our patients were of advanced age, likely from excluding younger patients who could qualify for retransplant, and they were long-term survivors after transplant, who had developed moderate BOS a median of 5 years after transplant (range, 1.4–9 years). Our study shows that it is feasible to manufacture bone marrow-derived MSCs, cryopreserve them, and transport them long distances for clinical administration, and these cells remain viable and sterile prior to infusion. Our study indicates that the intravenous infusion of bone marrow-derived MSCs is safe, well tolerated, and produces no alteration in vital signs, gas exchange, pulmonary function test variables, or changes in routine blood work values. The long-term effects in lung function and gas exchange resulting from this cell therapy intervention are reported elsewhere [19].

Although this study was designed to treat patients with obstructive CLAD refractory to medical therapy, knowing that this therapy is feasible and safe presents the opportunity for future studies into MSC infusion for management of acute graft failure or early administration of either autologous or allogeneic MSCs after transplant to prevent severe primary graft dysfunction, which is known to be associated with early mortality and late morbidity. Because of the immunomodulatory properties of MSCs, administration of MSCs early after transplant could help reduce the need for heavy immunosuppressive therapy, thus decreasing the consequent high incidence of infectious complications and multiple adverse events associated with administration of calcineurin inhibitors and corticosteroids.

Based on recent advances in ex vivo lung perfusion technology [17] and the results of MSC infusion reported here and by Chambers et al. [18], further studies into combining these therapies may be warranted. Donor lungs could be placed on ex vivo lung perfusion prior to transplantation, to infuse them with previously cultured autologous or allogeneic bone marrow-derived MSCs, aiming to immunomodulate the donor lungs to reduce the incidence of primary graft dysfunction. Future and larger phase II studies will be required to confirm our preliminary findings to better define the role of cell therapy as a potential early immunomodulatory or rescue therapy for lung transplant recipients with acute or chronic rejection.

## CONCLUSION

The results of our study suggest it is safe and feasible to provide cell therapy with intravenous infusion of bone marrow-derived MSCs to lung transplant recipients with moderate obstructive CLAD, warranting future studies to assess the effectiveness of this therapy for management of acute or chronic graft dysfunction.

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## AUTHOR CONTRIBUTIONS

C.A.K. and A.C.Z.: conception and design, financial support, administrative support, provision of study material or patients, collection and/or assembly of data, data analysis and interpretation, manuscript writing, final approval of manuscript; T.A.G.: conception and design, manuscript writing, final approval of manuscript; D.O.H.: collection and/or assembly of data, data analysis and interpretation, final approval of manuscript; D.H. and J.M.C.: conception and design, provision of study material or patients, manuscript writing, final approval of manuscript

## DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

D.J.H. is an employee of Cellular Dynamics International. J.M.C. is an employee of AxoGen Inc. The other authors indicated no potential conflicts of interest.

## REFERENCES

- 1 Estenne M, Maurer JR, Boehler A et al. Bronchiolitis obliterans syndrome 2001: An update of the diagnostic criteria. *J Heart Lung Transplant* 2002;21:297–310.
- 2 Trulock EP, Christie JD, Edwards LB et al. Registry of the International Society for Heart and Lung Transplantation: Twenty-fourth official adult lung and heart-lung transplantation report-2007. *J Heart Lung Transplant* 2007;26:782–795.
- 3 Keller CA, Cagle PT, Brown RW et al. Bronchiolitis obliterans in recipients of single, double, and heart-lung transplantation. *Chest* 1995;107:973–980.
- 4 Osaki S, Maloney JD, Meyer KC et al. Redo lung transplantation for acute and chronic lung allograft failure: Long-term follow-up in a single center. *Eur J Cardiothorac Surg* 2008;34:1191–1197.
- 5 Novick RJ, Stitt LW, Al-Kattan K et al. Pulmonary retransplantation: Predictors of graft function and survival in 230 patients. *Pulmonary Retransplant Registry. Ann Thorac Surg* 1998;65:227–234.
- 6 Ren G, Zhang L, Zhao X et al. Mesenchymal stem cell-mediated immunosuppression occurs via concerted action of chemokines and nitric oxide. *Cell Stem Cell* 2008;2:141–150.
- 7 Kode JA, Mukherjee S, Joglekar MV et al. Mesenchymal stem cells: Immunobiology and

role in immunomodulation and tissue regeneration. *Cytotherapy* 2009;11:377–391.

8 Weiss DJ, Kolls JK, Ortiz LA et al. Stem cells and cell therapies in lung biology and lung diseases. *Proc Am Thorac Soc* 2008;5:637–667.

9 Ortiz LA, Gambelli F, McBride C et al. Mesenchymal stem cell engraftment in lung is enhanced in response to bleomycin exposure and ameliorates its fibrotic effects. *Proc Natl Acad Sci U S A* 2003;100:8407–8411.

10 Weng JY, Du X, Geng SX et al. Mesenchymal stem cell as salvage treatment for refractory chronic GVHD. *Bone Marrow Transplant* 2010;45:1732–1740.

11 Weiss DJ, Casaburi R, Flannery R et al. A placebo-controlled, randomized trial of mesenchymal stem cells in COPD. *Chest* 2013;143:1590–1598.

12 Fischer UM, Harting MT, Jimenez F et al. Pulmonary passage is a major obstacle for intravenous stem cell delivery: The pulmonary first-pass effect. *Stem Cells Dev* 2009;18:683–692.

13 Braun RK, Koch JM, Hacker TA et al. Cardiopulmonary and histological characterization of an acute rat lung injury model demonstrating safety of mesenchymal stromal cell infusion. *Cytotherapy* 2016;18:536–545.

14 Franquesa M, Hoogduijn MJ, Reinders ME et al. Mesenchymal Stem Cells in Solid

Organ Transplantation (MiSOT) Fourth Meeting: Lessons learned from first clinical trials. *Transplantation* 2013;96:234–238.

15 Reinders ME, de Fijter JW, Roelofs H et al. Autologous bone marrow-derived mesenchymal stromal cells for the treatment of allograft rejection after renal transplantation: Results of a phase I study. *STEM CELLS TRANSLATIONAL MEDICINE* 2013;2:107–111.

16 Tan J, Wu W, Xu X et al. Induction therapy with autologous mesenchymal stem cells in living-related kidney transplants: A randomized controlled trial. *JAMA* 2012;307:1169–1177.

17 McAuley DF, Curley GF, Hamid UI et al. Clinical grade allogeneic human mesenchymal stem cells restore alveolar fluid clearance in human lungs rejected for transplantation. *Am J Physiol Lung Cell Mol Physiol* 2014;306:L809–L815.

18 Chambers DC, Enever D, Lawrence S et al. Mesenchymal stromal cell therapy for chronic lung allograft dysfunction: Results of a first-in-man study. *STEM CELLS TRANSLATIONAL MEDICINE* 2017;6:1152–1157.

19 Keller CA, Erasmus DE, Alvarez FG et al. Preliminary report on the effect of mesenchymal stem cell (MSC) infusion in lung function on patients with chronic allograft dysfunction (CLAD). *J Heart Lung Transplant* 2016;35:S43.



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