



Mesenchymal stem (stromal) cells for treatment of ARDS: a phase 1 clinical trial

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Summary

Background No effective pharmacotherapy for acute respiratory distress syndrome (ARDS) exists, and mortality remains high. Preclinical studies support the efficacy of mesenchymal stem (stromal) cells (MSCs) in the treatment of lung injury. We aimed to test the safety of a single dose of allogeneic bone marrow-derived MSCs in patients with moderate-to-severe ARDS.

Methods The STem cells for ARDS Treatment (START) trial was a multicentre, open-label, dose-escalation, phase 1 clinical trial. Patients were enrolled in the intensive care units at University of California, San Francisco, CA, USA, Stanford University, Stanford, CA, USA, and Massachusetts General Hospital, Boston, MA, USA, between July 8, 2013, and Jan 13, 2014. Patients were included if they had moderate-to-severe ARDS as defined by the acute onset of the need for positive pressure ventilation by an endotracheal or tracheal tube, a PaO₂:FiO₂ less than 200 mm Hg with at least 8 cm H₂O positive end-expiratory airway pressure (PEEP), and bilateral infiltrates consistent with pulmonary oedema on frontal chest radiograph. The first three patients were treated with low dose MSCs (1 million cells/kg predicted bodyweight [PBW]), the next three patients received intermediate dose MSCs (5 million cells/kg PBW), and the final three patients received high dose MSCs (10 million cells/kg PBW). Primary outcomes included the incidence of prespecified infusion-associated events and serious adverse events. The trial is registered with ClinicalTrials.gov, number NCT01775774.

Findings No prespecified infusion-associated events or treatment-related adverse events were reported in any of the nine patients. Serious adverse events were subsequently noted in three patients during the weeks after the infusion: one patient died on study day 9, one patient died on study day 31, and one patient was discovered to have multiple embolic infarcts of the spleen, kidneys, and brain that were age-indeterminate, but thought to have occurred before the MSC infusion based on MRI results. None of these severe adverse events were thought to be MSC-related.

Interpretation A single intravenous infusion of allogeneic, bone marrow-derived human MSCs was well tolerated in nine patients with moderate to severe ARDS. Based on this phase 1 experience, we have proceeded to phase 2 testing of MSCs for moderate to severe ARDS with a primary focus on safety and secondary outcomes including respiratory, systemic, and biological endpoints.

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Introduction

Despite advances in our understanding of the pathogenesis of acute respiratory distress syndrome (ARDS), no drug has reduced mortality in ARDS.¹ Treatment remains primarily supportive, with lung-protective ventilation and a fluid conservative strategy, as well as early neuromuscular blockade and prone positioning in more severe cases.²⁻⁷ Mortality of ARDS has declined modestly with improved ventilator and fluid management, but remains high (between 20% and 40% in clinical studies).^{1,8}

Treatment with allogeneic bone marrow-derived human mesenchymal stem (stromal) cells (MSCs) is attractive as a potential new treatment for ARDS for several reasons. MSCs are multipotent cells with low immunogenicity that secrete multiple paracrine factors including endothelial and epithelial growth factors, anti-inflammatory cytokines, and antimicrobial peptides.⁹⁻²⁰ They are also capable of transferring mitochondria to

injured epithelial cells.²¹ These characteristics are directly relevant to the main abnormalities that underlie lung injury in patients with ARDS.¹

Preclinical studies in small animal (mouse and rat) and large animal (sheep) experiments, as well as in an ex-vivo perfused human lung model, showed potential efficacy and safety of MSC administration for the treatment of ARDS.^{9,10,12,13,15,22-24} In April, 2014, Zheng and colleagues²⁵ published the results of a single-centre trial testing a single dose of 1 million cells/kg adipose-derived human MSCs in 12 patients with moderate-to-severe ARDS and reported no infusion-related adverse events. Additionally, MSCs have been tested in more than 2000 human patients for a variety of conditions, with no apparent major adverse effects.¹⁹ Based on these studies, we aimed to assess the safety of bone marrow-derived human MSCs for the treatment of moderate-to-severe ARDS and to determine the

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maximum tolerated MSC dose up to a dose of 10 million cells/kg predicted bodyweight (PBW).

Methods

Study design and participants

The STem cells for ARDS Treatment (START) trial was a multicentre, open-label, phase 1 clinical trial. Patients were enrolled in the intensive care units at University of California, San Francisco, CA, USA, Stanford University, Stanford, CA, USA, and Massachusetts General Hospital, Boston, MA, USA, between July 8, 2013, and Jan 13, 2014. Patients were included if they had moderate-to-severe ARDS as defined by (1) the acute onset of the need for positive pressure ventilation by an endotracheal or tracheal tube, (2) a PaO₂:FiO₂ ratio less than 200 mm Hg with at least 8 cm H₂O positive end-expiratory airway pressure (PEEP), and (3) bilateral infiltrates consistent with pulmonary oedema on frontal chest radiograph. The PEEP threshold was set at 8 cm H₂O instead of 5 cm H₂O to decrease the chance that a patient's hypoxaemia was due in measure to atelectasis, and to narrow the patient population to those with moderate-to-severe ARDS who would be most likely to benefit from the therapy.

To avoid enrolling patients with late ARDS, the study design excluded patients in whom more than 96 h had passed since meeting the Berlin definition for ARDS.⁸ Additionally, the MSC infusion had to be initiated within 120 h of meeting the Berlin definition for ARDS. If the PaO₂:FiO₂ improved to more than 300 mm Hg after enrolment but before infusion, the patient was considered no longer eligible to receive MSCs. Patients were also excluded if they had an active malignancy requiring treatment within the past 2 years, major trauma in the preceding 5 days, severe chronic respiratory disease requiring home oxygen or with a baseline PaCO₂ greater than 50 mm Hg, moderate-to-severe liver failure (Childs-Pugh score >12), recent deep vein thrombosis or pulmonary embolism, WHO class III or IV pulmonary hypertension, or if they were moribund or there was not a commitment to full supportive measures other than cardiopulmonary resuscitation. Panel 1 shows the full inclusion and exclusion criteria.

Informed consent was obtained after discussion with the patient or an appropriate surrogate. After informed consent was gained, the cell therapy laboratory was alerted to the enrolment, and a 2 h period of bedside observation of haemodynamic and respiratory parameters was initiated to ensure that the patient was stable before the MSC infusion. In terms of baseline stability criteria, in the supine position, patients must have sustained the following for 2 h before MSC infusion: transcutaneous oxygen saturation in the target range of 88–95% without any increase in ventilator settings and stable use of vasopressor if the patient required vasopressors for blood pressure support. The dose of vasopressor could be increased no more than 5 µg per

min for norepinephrine, 50 µg per min for phenylephrine, 5 µg/kg per min for dopamine, and 0·5 µg/kg per min for epinephrine.

The nine patient dose-escalation protocol was selected based on several discussions with and approval by the US Food and Drug Administration (FDA). The protocol included a provision that the Data Safety Monitoring Board (DSMB), the FDA, or the study sponsor could decide to enrol more patients at any dose level if there were any prespecified infusion-associated adverse events or serious adverse events related to the MSCs.

Procedures

The first three patients were assigned to receive low dose MSCs (1 million cells/kg PBW); the next three patients were assigned to receive intermediate dose MSCs (5 million cells/kg PBW); and the final three patients were assigned to receive high dose MSCs (10 million cells/kg PBW). The dose of 10 million cells/kg PBW was selected as the final target dose of MSCs based on preclinical experiments in a large animal model of

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Panel 1: START inclusion and exclusion criteria

Inclusion criteria

- 1 Positive pressure ventilation by an endotracheal or tracheal tube with a PaO₂:FiO₂ <200 mm Hg with at least 8 cm H₂O positive end-expiratory airway pressure
- 2 Bilateral infiltrates consistent with pulmonary oedema on frontal chest radiograph
- 3 No clinical evidence of left atrial hypertension, or if measured, a pulmonary arterial occlusion pressure ≤18 mm Hg
- 4 Criteria 1–3 must all be present within a 24 h time period and at the time of enrolment

Exclusion criteria

- 1 Age younger than 18 years
- 2 >96 h since first meeting acute respiratory distress syndrome criteria per the Berlin definition
- 3 Pregnant or breastfeeding
- 4 Prisoner
- 5 Presence of any active malignancy (other than non-melanoma skin cancer) that required treatment within the past 2 years
- 6 Any other irreversible disease or condition for which 6-month mortality is estimated to be >50%
- 7 Moderate to severe liver failure (Child-Pugh score >12)
- 8 Severe chronic respiratory disease with a PaCO₂ >50 mm Hg or the use of home oxygen
- 9 Patient, surrogate, or physician not committed to full support (exception: a patient will not be excluded if he or she would receive all supportive care except for attempts at resuscitation from cardiac arrest)
- 10 Major trauma in the previous 5 days
- 11 Lung transplant patient
- 12 No consent or inability to obtain consent
- 13 Moribund patient not expected to survive 24 h
- 14 WHO class III or IV pulmonary hypertension
- 15 Documented deep venous thrombosis or pulmonary embolism within past 3 months
- 16 No arterial line or no intent to place an arterial line
- 17 No intent or unwillingness to follow lung protective ventilation strategy or fluid management protocol
- 18 Currently receiving extracorporeal life support or high-frequency oscillatory ventilation

Panel 2: Prespecified infusion-associated events

Any of the following occurring within 6 h of mesenchymal stem-cell infusion:

- Addition of a third vasopressor or an increase in vasopressor dose greater than or equal to the following:
 - Norepinephrine: 10 µg per min
 - Phenylephrine: 100 µg per min
 - Dopamine: 10 µg/kg per min
 - Epinephrine: 0.1 µg/kg per min
- Hypoxaemia requiring an increase in the fraction of inspired oxygen of ≥ 0.2 and increase in positive end-expiratory airway pressure level of 5 cm H₂O or more to maintain transcutaneous oxygen saturations in the target range of 88–95%
- New cardiac arrhythmia requiring cardioversion
- New ventricular tachycardia, ventricular fibrillation, or asystole
- A clinical scenario consistent with transfusion incompatibility or transfusion-related infection
- Cardiac arrest or death within 24 h of mesenchymal stem-cell infusion

	Age (years)	Sex	APACHE III	Primary cause of ARDS	Tidal volume (mL/kg PBW)	Plateau pressure (cm H ₂ O)	PEEP (cm H ₂ O)	PaO ₂ :FiO ₂ (mm Hg)	Lung injury score
1 million cells/kg PBW									
Patient 1	29	Female	81	Pre-eclampsia	7.0	28	10	173	3.25
Patient 2	86	Female	121	Pneumonia	6.6	31	10	101	3.25
Patient 3	59	Female	130	Aspiration	6.0	25	10	168	2.50
5 million cells/kg PBW									
Patient 4	67	Female	133	Aspiration	6.3	21	10	105	2.75
Patient 5	62	Female	109	Pneumonia	5.6	20	14	111	2.50
Patient 6	46	Female	83	Aspiration	6.0	19	10	153	3.00
10 million cells/kg PBW									
Patient 7	52	Male	121	Pneumonia	7.1	23	10	154	2.75
Patient 8	55	Female	127	Sepsis (biliary)	5.9	34	10	194	3.50
Patient 9	38	Male	68	Pneumonia	6.0	Not measured	8	118	2.33

APACHE=Acute Physiology and Chronic Health Evaluation. ARDS=acute respiratory distress syndrome. PBW=predicted bodyweight. PEEP=positive end-expiratory pressure.

Table 1: Baseline characteristics

ARDS, which showed maximum efficacy and favourable safety with this dose.²³ Data from the first patient of each cohort and each complete cohort were reviewed for safety before proceeding with enrolment of the next patient or escalation of the dose.

The allogeneic, bone marrow-derived human MSCs were prepared from bone marrow obtained from a healthy male donor (age 18–45 years), with support from the National Heart, Lung, and Blood Institute Production Assistance for Cellular Therapies (PACT) programme. The mononuclear cell fraction of the bone marrow was enriched and tested for nucleated cells, differential, viability, flow cytometry, and sterility before seeding for culture. At 70% confluence, MSCs were lifted and

passed at a low density into a cell factory. At 70–80% confluence of the MSCs, the product was washed, harvested, resuspended, and cryopreserved. Karyotyping and G-banding were normal.

The cryopreserved MSCs were shipped frozen to the clinical sites in a validated liquid nitrogen dry shipper with continuous temperature monitoring device. Upon receipt, the cellular product was inspected and stored in a controlled, continuously monitored liquid nitrogen storage tank. Before administration, the MSCs were thawed, washed to remove dimethyl sulphoxide, and resuspended in Plasmalyte-A by the local cell therapy laboratory. The total volume of the MSC infusion was 100 mL regardless of dose. The percent viability of the infused MSCs was determined by trypan blue exclusion after the MSCs had been thawed and prepared for infusion. The viability ranged from 50–63% (mean 56%).

After 2 h of clinical stability, the infusion was initiated using a standard blood filter tubing set. The cells were infused via gravity over roughly 60–80 min, with the infusion rate controlled by the investigator based on droplet count. The physician investigator remained at the bedside for duration of the infusion and for 6 h after the infusion was initiated, to observe for any signs of an adverse reaction. All patients were ventilated according to the modified ARDS Network lower tidal volume protocol.³ Data collection and on-study measurements are described in a previous publication.²⁶

Because of the concern that infusion of MSCs could lead to transient obstruction of the pulmonary microcirculation with subsequent haemodynamic or respiratory compromise, all patients were monitored closely for any changes in respiratory or cardiovascular parameters by at least one study physician at the bedside during the 1 h infusion and for 6 full hours after the start of the infusion. Prespecified infusion-associated events are shown in panel 2. The incidence and nature of all serious adverse events were reviewed and independently evaluated by the DSMB to determine whether they were believed to be related to MSC administration, with special focus on events that would be unexpected in a critically ill patient with ARDS. Additionally, serum creatinine, total bilirubin, and alanine aminotransferase (ALT) were measured on days 3, 7, and 14 (after administration of the MSCs) for safety monitoring if patients were still in hospital.

The lung injury score (LIS), a widely used measure of severity of lung injury, is composed of four components: (1) chest radiograph; (2) PaO₂:FiO₂; (3) PEEP; and (4) static compliance of respiratory system.^{27,28} At timepoints when the patient was not ventilated with positive pressure ventilation, the PEEP was treated as 5 cm H₂O or less, the PaO₂:FiO₂ was treated as 300 mm Hg or greater, the compliance was not calculated, and the total of points was divided by three instead of four.

The Sequential Organ Failure Assessment (SOFA) score incorporates the severity of organ dysfunction and

predicts outcomes in critically ill patients.^{29,30} We calculated the SOFA score daily for study days 1–14, using the worst values for each parameter in the 24 h period. When a single value required for calculation of the SOFA score was missing, we carried forward the value from the previous measurement.

We also measured biological markers in plasma collected at baseline, 6 h post-infusion, and days 1, 2, and 3. These included markers of inflammation, epithelial injury, and endothelial injury, selected based on the proposed mechanism of action of MSCs in ARDS and on the results of previous preclinical studies. Specifically, we measured inflammatory markers, interleukin 6 and interleukin 8, a marker of lung epithelial injury (receptor for advanced glycation endproducts [AGER]), and a marker of endothelial injury (angiopoietin-2 [ANGPT2]). Biomarkers were measured by enzyme-linked immunoassays (ELISAs) at baseline, 6 h, day 1, and day 3 (all ELISA kits from R&D Systems, Minneapolis, MN, USA). The remaining biomarkers listed in our clinical protocol were not measured in the phase 1 portion of this trial.

The DSMB was comprised of critical care physicians and a biostatistician with phase 1 trial experience, and was responsible for reviewing data for each cohort of three patients at each dosing level and making recommendations regarding continuing, stopping, or altering the trial. Additionally, a designated external medical monitor and scientific review committee (SRC) comprised of study investigators evaluated the first patient in each dosing cohort after 7 full days of observation before enrolment proceeded. At the conclusion of the trial, the DSMB and SRC determined whether or not phase 2 testing was recommended, and if so, the dose of MSCs that should be administered.

Outcomes

Because this study was one of the first trials to test MSCs in patients with ARDS, the primary objectives were to test the safety and tolerability of the MSC infusion and determine a safe dose of MSCs for our planned phase 2 study. We report the incidence of all serious adverse events, including death, and the incidence of prespecified infusion-associated events and non-serious adverse events thought to be related to the MSC infusion. The secondary objectives were to measure standard respiratory and systemic organ endpoints, including LIS, SOFA score, and Acute Physiology and Chronic Health Evaluation (APACHE) score, and duration of mechanical ventilation, ventilator-free days, duration of vasopressor, and intensive care unit [ICU]-free days, and biomarker values.

Statistical analysis

Baseline and on-study LIS, SOFA score, and APACHE scores among the treatment groups were compared with analysis of variance. Systemic clinical outcomes and biomarker values were compared using Kruskal-Wallis

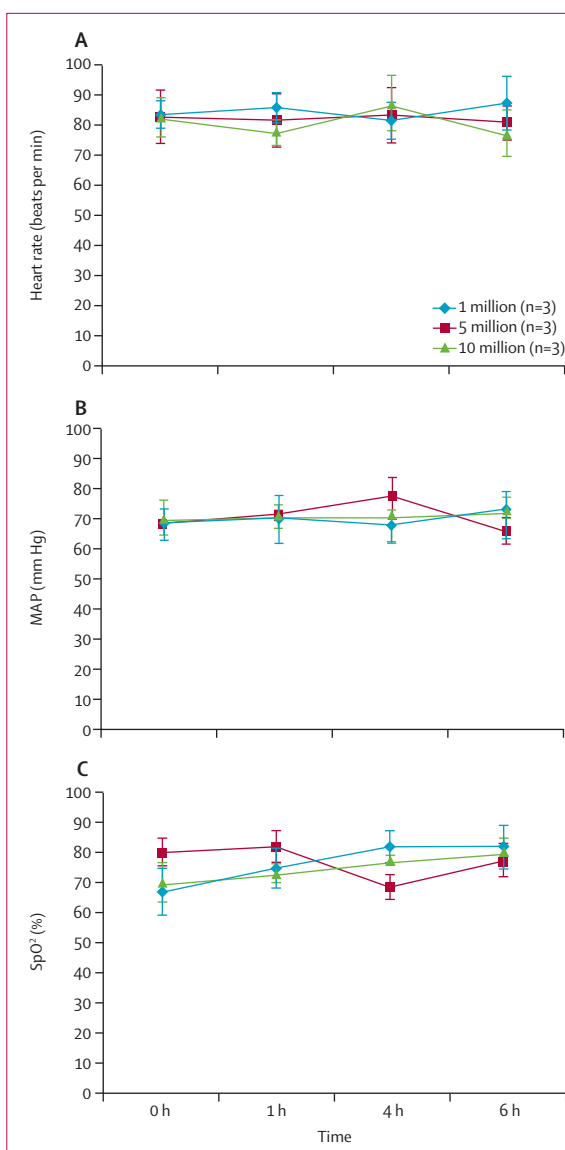


Figure 1: Respiratory and haemodynamic parameters during and after mesenchymal stem-cell infusion

Mean (SD) values for each dosing group for (A) heart rate (beats per minute), (B) mean arterial pressure (mm Hg), and (C) arterial oxygen saturation as measured by pulse oximeter (SpO₂; %) at baseline, 1, 4, and 6 h from start of mesenchymal stem-cell infusion.

one-way analysis of variance. Data were analysed with STATA version 12.1 (College Station, TX, USA). Remaining analyses are descriptive.

This study is registered with ClinicalTrials.gov, number NCT01775774.

Role of the funding source

The sponsors of the trial had no role in the study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author (MAM) had full access to all of the data and the final responsibility to submit the report for publication.

	Prespecified infusion-associated events	Other adverse events	Description
Patient 2	None	Death on day 9	Patient never recovered from ARDS or sepsis, developed worsening multiorgan failure and shock on study day 6, died on study day 9
Patient 3	None	Infarcts of kidneys, spleen, and brain	Multiple, probably embolic infarcts of spleen, kidneys, and brain discovered incidentally by CT scan on study day 0 and study day 1. Believed to have occurred before MSC infusion based on MRI results. Extensive work-up for embolic source was negative except for small abnormality of the mitral valve. Culture-negative endocarditis was cited as a possible cause of emboli. Patient recovered and was discharged to an acute rehabilitation facility
Patient 5	None	Respiratory arrest on day 24, death on day 31	Patient recovered from ARDS and was discharged from intensive care unit. On study day 24 had a respiratory arrest believed to be related to aspiration and was transferred back to intensive care. Developed worsening multiorgan failure and died on study day 31. No resuscitation was attempted in keeping with family wishes

ARDS=acute respiratory distress syndrome. MSC=mesenchymal stem cells.

Table 2: Serious adverse events by patient

	Duration of mechanical ventilation (days)	Ventilator-free days (up to day 28)	Oxygenation index (day 3)	Duration of vasopressor use (days)	Intensive care unit-free days (up to day 28)	Vital status and day of discharge
Low dose						
1	5	24	2.33	0	24	Alive, day 8
2	10	0	13.91	10	0	Dead, day 9
3	11	18	5.78	4	14	Alive, day 22
Intermediate dose						
4	7	22	4.63	0	21	Alive, day 34
5	31	0	5.36	2	0	Dead, day 31
6	3	27	*	3	26	Alive, day 5
High dose						
7	3	26	10	0	22	Alive, day 7
8	17	12	*	0	9	Alive, day 25
9	19	20	6.39	0	18	Alive, day 14

Oxygenation index=FiO₂ × mean airway pressure/PaO₂. *Extubated.

Table 3: Secondary respiratory and systemic results by dosing cohort

Results

As planned, nine patients were enrolled: three patients received the low dose (1 million cells/kg PBW), three patients received the intermediate dose (5 million cells/kg PBW), and three patients received the high dose (10 million cells/kg PBW) MSCs. Baseline characteristics of each patient are shown in table 1. Most patients (seven of nine) had pneumonia or aspiration as the primary cause of ARDS. Although several patients met criteria for severe ARDS when first identified by the study team, all nine had moderate ARDS by PaO₂:FiO₂ at the official time of enrolment. Clinical variables, including mean age, APACHE III score, PaO₂:FiO₂, and LIS were similar across the three dosing groups at baseline.

All patients tolerated the MSC infusion well and no prespecified infusion-associated adverse events were observed. No patient suffered any immediate complication or respiratory or cardiovascular compromise in the 6 h after the MSC infusion, and there were no cardiac arrests or deaths within 24 h of the MSC infusion. Specifically, no significant changes in heart rate, mean arterial pressure, or oxygen saturation were reported in any of the three dosing groups during the infusion or in the immediate post-infusion period (figure 1). Additionally, safety laboratory values (mean serum creatinine, total bilirubin, and alanine aminotransferase) were not significantly changed for any of the three dosing groups (data not shown).

Three patients subsequently developed serious adverse events in the weeks after the infusion: two patients died more than 7 days after the MSC infusion, and one patient was discovered to have multiple embolic infarcts of the spleen, kidneys, and brain that were age-indeterminate, but thought to have occurred before the MSC infusion based on MRI results. This third serious adverse event was determined by investigators to be unexpected in ARDS. All three serious adverse events were independently reviewed by the SRC and DSMB, and in all cases both the SRC and DSMB concurred with the investigators' assessments that none were related to MSC administration. Details of the serious adverse events are shown in table 2.

Two patients died within 60 days of study infusion, for a mortality rate of 22% (two of nine patients). One death occurred in the low dose group on study day 9, and one death occurred in the intermediate dose group on study day 31. Each death was reviewed in detail and neither was believed to be related to study participation. Vital status and study day at discharge for each patient are listed in table 3.

The mean LIS declined (improved) between baseline and day 3 in all three dosing groups (figure 2). Numerically, the greatest decrease in LIS was noted in the high dose cohort and the smallest decrease was observed in the low dose cohort (high dose 2.9 to 1.6 [-45%], intermediate dose 2.8 to 1.8 [-36%], low dose 3 to 2.1 [-30%]), but these differences between groups were not statistically significant (p=0.872). None of the patients received rescue therapies for refractory hypoxaemia (no extracorporeal membrane oxygenation and no inhaled nitric oxide or vasodilators). Two of nine patients were extubated before study day 3. One patient was extubated on two different occasions, however, this patient required reintubation within 48 h both times (mainly because of hepatic encephalopathy) and was never successfully liberated from the ventilator before death. Finally, one of the nine patients was never extubated and remained on mechanical ventilation with non-resolving ARDS and worsening multiorgan failure until death on study day 9. The duration of mechanical ventilation, number of ventilator-free days (as of day 28), and oxygenation index for each patient are shown in table 3.

Mean SOFA score declined in all three dosing groups over the first 3 days (figure 3). As with LIS, the greatest numerical decline was noted in the high dose cohort and the smallest decrease was noted in the low dose cohort (high dose 7 to 3.7 [−48%], intermediate dose 8.7 to 6.7 [−23%], low dose 8 to 7.7 [−4%]). The differences between groups were not statistically significant ($p=0.765$). Duration of vasopressor administration and number of ICU-free days are listed in table 3.

Table 4 shows the concentrations of biomarkers at baseline, 6 h, day 1, and day 3. Median concentrations of all four biomarkers declined between baseline and day 3. No significant differences in the magnitude of decline among groups for any of the biomarkers were noted ($p=0.3679$ for interleukin 6, $p=0.3189$ for interleukin 8, $p=0.3189$ for ANGPT2, and $p=0.8669$ for AGER).

Discussion

Intravenous administration of a single dose of bone marrow-derived human MSCs was well tolerated in this phase 1 trial in nine patients with moderate to severe ARDS, with no evidence of prespecified infusion-associated adverse events, immediate clinical instability, or dose-limiting toxicity at any of the doses tested (panel 3). Based on external review by the SRC and DSMB, none of the severe adverse events reported in our trial were related to MSC infusion. Thus, the primary outcomes suggest that all three doses of MSCs are safe in patients with moderate-to-severe ARDS.

The mortality in this cohort was 22%, which is lower than the expected mortality in patients with moderate ARDS according to the Berlin severity stages (32%), and similar to the mortality reported by Kangelaris and colleagues in patients with ARDS with a similar baseline LIS (23%).^{8,28} Thus, the mortality in our trial is in keeping with (or lower than) the expected mortality in patients with moderate ARDS and critically ill patients more generally.

The favourable changes observed in LIS and SOFA score with the high dose of MSCs (10 million cells/kg PBW) compared with both reduced doses are consistent with the hypothesis that increased doses of MSCs might provide increased clinical benefit. However, none of these differences were statistically significant, and in view of the absence of a control group in this phase 1 trial, we cannot conclude that these differences reflect a true dose response.

Although median levels of interleukin 6, interleukin 8, AGER, and ANGPT2 concentrations all decreased between baseline and day 3, no apparent dose effect was noted. Additionally, these markers of inflammation and epithelial or endothelial injury are known to decline with time in patients with ARDS treated with low tidal volumes.^{31–33} Thus, without a matched control group, we cannot conclude that the recorded changes in biomarkers were related to MSC therapy. The phase 2 iteration of this trial will include a control group to compare the same biomarkers in the patients given MSCs and patients

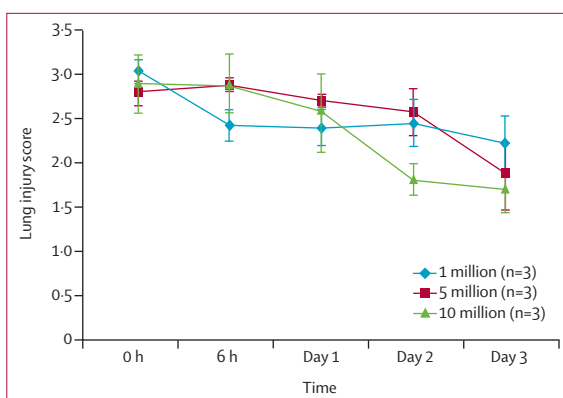


Figure 2: Lung injury score (LIS)

Mean (SD) LIS for each dosing group at baseline, 6 h from start of mesenchymal stem-cell infusion, and study days 1, 2, and 3. LIS is calculated from four variables: (1) number of affected quadrants on chest radiograph; (2) severity of hypoxia as measured by $\text{PaO}_2:\text{FiO}_2$; (3) level of positive end-expiratory pressure; and (4) the static compliance of respiratory system.²⁷

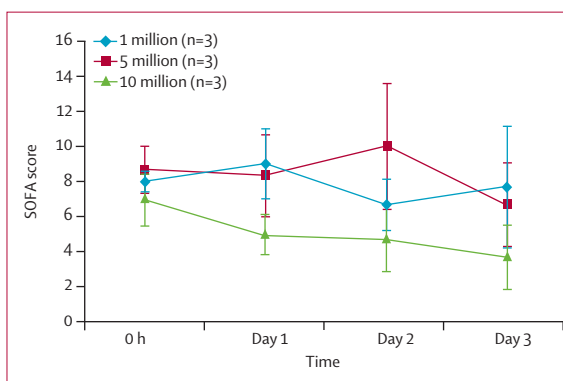


Figure 3: Sequential Organ Failure Assessment (SOFA) score

Mean (SD) SOFA score for each dosing group at baseline, 6 h from start of mesenchymal stem cell infusion, and study days 1, 2, and 3. The SOFA score quantifies the severity of organ dysfunction in six systems (respiratory, coagulation, hepatic, cardiovascular, renal, and neurological), and predicts outcomes in critically ill patients.^{28,29}

	Day 0	6 h	Day 1	Day 3
Interleukin 6 (pg/mL)	762 (419–1198)	557 (91–734)	317 (150–736)	62 (20–140)
Interleukin 8 (pg/mL)	35 (18–49)	26 (16–39)	29 (19–55)	16 (8–47)
ANGPT2 (pg/mL)	7507 (3977–14 950)	8168 (4415–13 000)	10 900 (4593–18 200)	6922 (4783–18 700)
AGER (pg/mL)	2749 (894–5060)	2841 (1055–4333)	1790 (882–5080)	1308 (1268–2437)

Data are median (IQR). ANGPT2=angiopoietin-2. AGER=receptor for advanced glycation endproducts.

Table 4: Biomarker concentrations at baseline, 6 h, day 1, and day 3

given placebo. Additionally, the phase 2 protocol includes mini-bronchoalveolar lavage at 48 h to sample the distal airspaces and permit measurement and comparison of the same biomarkers sampled from the control patients given placebo versus the patients given MSC.

Panel 3: Research in context**Systematic review**

We planned our trial based on extensive preclinical testing of mesenchymal stem (stromal) cells (MSCs) for acute lung injury, and based on a review of articles published between January, 1968, and June, 2013, identified by searches of Medline, Current Contents, PubMed, and references from relevant articles using the search terms "MSC", "mesenchymal stem cells", "mesenchymal stromal cells", "marrow stromal cells", "acute respiratory distress syndrome", "acute lung injury", and "sepsis". We also reviewed studies of MSCs in humans for other indications, such as acute myocardial infarction and chronic obstructive pulmonary disease. The many preclinical studies reviewed suggest that MSC therapy holds substantial therapeutic promise for acute respiratory distress syndrome (ARDS), and the human trials suggest that MSCs are well tolerated in various disease states, but no human trial of bone-marrow-derived human MSCs for patients with ARDS had been reported when we embarked on this trial.

Interpretation

Our trial shows that a single intravenous dose of MSCs of up to 10 million cells/kg predicted bodyweight was well tolerated in nine patients with moderate-to-severe ARDS. This safety profile is in keeping with the favourable safety record of MSCs in previous trials for other indications, and also the small number of trials that have tested MSCs for respiratory problems. These findings indicate that it is safe to proceed to phase 2 testing of MSCs for ARDS in a larger cohort of patients, at the highest dose tested. At this time, it is premature to make any conclusions about the long-term safety or efficacy of MSCs for the treatment of ARDS.

Interestingly, in April, 2014, Zheng and colleagues²⁵ published the results of a single-centre, randomised, double-blind, placebo-controlled trial in which 12 patients with moderate-to-severe ARDS were randomly assigned (1:1) to receive either a single dose of 1 million cells/kg allogeneic adipose-derived human MSCs or saline placebo. In this trial, as in ours, no infusion toxicities or MSC-related serious adverse events were noted. Secondary outcomes, including ventilator-free days and ICU-free days, were similar in both groups. Although no changes in biomarkers (including surfactant protein D, interleukin 6, and interleukin 8) were noted in the placebo group, day 5 serum concentrations of surfactant protein D were significantly lower in the MSC group, and interleukin 6 concentrations were non-significantly reduced as well. The study by Zheng and colleagues had several important limitations: (1) the only dose tested in the six patients who received MSCs was the lowest dose tested in our trial (1 million cells/kg), which is 1/10th of the dose that showed maximum efficacy, and no increased toxicity, in the large animal model we previously described;²⁴ and (2) the MSCs were adipose-derived and recultured in the patient's own serum after enrolment, a technique that diverges from the standard within the field. These important differences restrict the generalisability of their findings, and further comparison to the phase 1 trial that we are reporting.

Another relevant recent trial was a phase 1 dose-escalation trial of intratracheal human umbilical cord blood-derived MSCs in nine preterm infants at high risk for bronchopulmonary dysplasia (BPD).³⁴ Again, similar

to the results of our trial, the therapy was well tolerated, although in this case there was also a suggestion of benefit in terms of respiratory outcomes. For biomarker response, the authors noted a decrease in inflammatory cytokines after MSC therapy, although it is unclear whether this was due to the immunomodulatory effects of the MSCs or merely reflected the natural course of inflammation in the development of BPD.³⁴ Therefore, although the source and dose of MSCs differed among these trials, and conclusions about efficacy and biomarker response are unwarranted, the consistency in the results in terms of tolerability and short-term safety is encouraging.

Finally, Weiss and colleagues³⁵ did a multicentre, double-blind, placebo-controlled randomised trial of four monthly intravenous infusions of 100 million MSCs in 62 patients with moderate-to-severe chronic obstructive pulmonary disease. No toxicities during infusion, deaths, or serious adverse events were deemed related to MSC administration. Together with our trial, these findings suggest, but do not prove, that MSC infusions are well tolerated in patients with either acute or chronic respiratory compromise.

Our small phase 1 trial has some limitations. First and foremost, with only nine patients, we can neither generalise our phase 1 experience, nor draw conclusions about either the efficacy or long-term safety of MSCs for ARDS. Indeed, the absence of any statistically significant differences in secondary outcomes should be interpreted as a reflection of the absence of statistical power in this small study, rather than as confirmation of absence of effect.

The limitations of a small sample size are further amplified by the inherent challenges of undertaking clinical trials in critically ill patients, in whom it is often difficult to discern whether medical events are related to underlying critical illness or the experimental therapy being tested. In this trial, the requirement of baseline stability before infusion was intended to decrease the noise of critical illness and make it more feasible to identify potential harmful effects of the MSC infusion.

Finally, although no significant differences in baseline LIS, SOFA score, or APACHE III score were noted among the different dosing cohorts, it remains possible that differences in baseline severity of illness confounded the secondary outcomes we recorded in terms of change in LIS and SOFA score. For example, no patients in the high dose cohort were treated with vasopressors, which could mean the improvement observed in that cohort was due to the absence of shock, rather than to the increased dose of MSCs. Indeed, the optimum dose of MSCs remains unclear; although the high dose of 10 million MSCs/kg showed greater efficacy in the preclinical study of severe lung injury in sheep,²⁴ and the highest dose in this trial was well tolerated, it remains uncertain if that dose was more effective than lower doses in this trial, or if an even higher dose or repeated doses would be tolerated or provide additional benefit.

In conclusion, a single intravenous MSC infusion of up to 10 million cells/kg PBW was well tolerated in patients with moderate-to-severe ARDS in this phase 1 trial. No serious adverse events were related to MSC administration after 6 months of follow-up. This favourable tolerability and short-term safety profile is in keeping with previous research on MSCs for other clinical indications. Based on the recommendations of the DSMB, we are undertaking a randomised, double-blind, placebo-controlled phase 2 clinical trial of 10 million MSCs/kg PBW in 60 patients with moderate-to-severe ARDS, with a primary focus on safety and secondary outcomes including respiratory, systemic, and biological endpoints.

Contributors

KDL, HZ, LC, CSC, J-WL, AL, DM, BTT, and MAM designed the trial. JGW, LC, MM, XF, KC, RV, AJR, JL, JW-K, EKB, AL, DM, BTT, and MAM collected data. JGW, KDL, HZ, XF, AJR, JL, JW-K, EKB, DM, BTT, and MAM analysed data. JGW, KDL, HZ, CSC, J-WL, AJR, JL, JW-K, EKB, AL, DM, BTT, and MAM interpreted data. JGW, KDL, and MAM provided the plan and wrote the report, while CSC, AJR, JL, AL, DM, and BTT provided editorial overview and modified the report. HZ prepared the figures.

Declaration of interests

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