Human Umbilical Cord-Derived Mesenchymal Stem Cells for Acute Respiratory Distress Syndrome

Hon-Kan Yip, MD[1–5]; Wen-Feng Fang, MD[6–8]; Yi-Chen Li, PhD[1]; Fan-Yen Lee, MD[9,10]; Chen-Hsiang Lee, MD[11]; Sung-Nan Pei, MD[12]; Ming-Chun Ma, MD[12]; Kuan-Hung Chen, MD, PhD[13]; Pei-Hsun Sung, MD[1]; Mel S. Lee, MD, PhD[14]

Objectives: To investigate the safety, feasibility, and possible adverse events of single-dose human umbilical cord-derived mesenchymal stem cells in patients with moderate-to-severe acute respiratory distress syndrome.

Design: Prospective phase I clinical trial.

Setting: Medical center in Kaohsiung, Taiwan.

Patients: Moderate-to-severe acute respiratory distress syndrome with a Pao_2/Fio_2 ratio less than 200.

Interventions: Scaling for doses was required by Taiwan Food and Drug Administration as follows: the first three patients received low-dose human umbilical cord-derived mesenchymal stem cells (1.0×10^6 cells/kg), the next three patients with intermediate dose (5.0×10^6 cells/kg), and the final three patients with high dose (1.0×10^7 cells/kg) between December 2017 and August 2019.

Measurements and Main Results: Nine consecutive patients were enrolled into the study. In-hospital mortality was 33.3% (3/9), including two with recurrent septic shock and one with ventilator-induced severe pneumomediastinum and subcutaneous emphysema. No serious prespecified cell infusion-associated or treatment-related adverse events was identified in any patient. Serial flow-cytometric analyses of circulating inflammatory biomarkers ($CD14^+CD33^+$/CD11b+CD16+/CD16+MPO+/CD11b+MPO+/$CD14^{dim}$CD33+) and mesenchymal stem cell markers (CD26+CD45−/CD29+CD45−/CD34+CD45−/CD44+CD45−/CD73+CD45−/CD90+CD45−/CD105+CD45−/CD26+CD45−) were notably progressively reduced (p for trend < 0.001), whereas the immune cell markers (Helper-T-$cell^{CD3+CD4+}$/Cytotoxity-T-$cell^{CD3+CD8+}$/Regulatory-T-$cell^{CD4+CD25+FOXp3+}$) were notably increased (p for trend < 0.001) after cell infusion.

Conclusions: The result of this phase I clinical trial showed that a single-dose IV infusion of human umbilical cord-derived mesenchymal stem cells was safe with favorable outcome in nine acute respiratory distress syndrome patients. (*Crit Care Med* 2020; XX:00–00)

Key Words: acute respiratory distress syndrome; allogenic mesenchymal stem cells; inflammation; in-hospital mortality; sepsis

[1]Division of Cardiology, Department of Internal Medicine, Kaohsiung Chang Gung Memorial Hospital and Chang Gung University College of Medicine, Kaohsiung, Taiwan.

[2]Institute for Translational Research in Biomedicine, Kaohsiung Chang Gung Memorial Hospital, Kaohsiung, Taiwan.

[3]Center for Shockwave Medicine and Tissue Engineering, Kaohsiung Chang Gung Memorial Hospital, Kaohsiung, Taiwan.

[4]Department of Medical Research, China Medical University Hospital, China Medical University, Taichung, Taiwan.

[5]Department of Nursing, Asia University, Taichung, Taiwan.

[6]Division of Pulmonary and Critical Care Medicine, Department of Internal Medicine, Kaohsiung Chang Gung Memorial Hospital and Chang Gung University College of Medicine, Kaohsiung, Taiwan.

[7]Department of Respiratory Therapy, Kaohsiung Chang Gung Memorial Hospital and Chang Gung University College of Medicine, Kaohsiung, Taiwan.

[8]Department of Respiratory Care, Chang Gung University of Science and Technology, Chiayi, Taiwan.

[9]Division of Thoracic and Cardiovascular Surgery, Department of Surgery, Kaohsiung Chang Gung Memorial Hospital and Chang Gung University College of Medicine, Kaohsiung, Taiwan.

[10]Division of Cardiovascular Surgery, Department of Surgery, Tri-Service General Hospital, National Defense Medical Center, Taipei, Taiwan.

[11]Division of Infectious Diseases, Department of Internal Medicine, Kaohsiung Chang Gung Memorial Hospital and Chang Gung University College of Medicine, Kaohsiung, Taiwan.

[12]Division of Hema-Oncology, Department of Internal Medicine, Kaohsiung Chang Gung Memorial Hospital and Chang Gung University College of Medicine, Kaohsiung, Taiwan.

[13]Department of Anesthesiology, Kaohsiung Chang Gung Memorial Hospital and Chang Gung University College of Medicine, Kaohsiung, Taiwan.

[14]Department of Orthopedics, Kaohsiung Chang Gung Memorial Hospital and Chang Gung University College of Medicine, Kaohsiung, Taiwan.

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Acute respiratory distress syndrome (ARDS), a globally growing disease frequently complicated with multiple organ failure (1–6), has been reported to lead to unacceptably high in-hospital mortality (1–7), especially in those of ARDS complicated by sepsis (7–11). Although the etiologies and underlying mechanisms of this disease have been extensively investigated (6, 7, 12), the effective therapeutic modality

is still limited (2, 6, 12–16), only leaving a primarily supportive care as the last resort. Therefore, a safe and effective management for ARDS is urgent and utmost important for clinicians and patients.

The mechanisms of ARDS causing extremely high mortality have been extensively investigated to be multifactorial, including enhancement of inflammatory reaction, overwhelming immune reaction, alveolar leukocytosis, protein leakage, mitochondrial oxidant production, generation of reactive oxygen species and an increase in lung oxidant stress, as well as apoptosis (17–20).

Abundant data from clinical trials and experimental studies have shown that mesenchymal stem cell (MSC) therapy has capacity of attenuating inflammation (21–25) and down-regulating innate and adaptive immunity (23–27) through suppressing immunogenicity (23–28). Our preclinical studies have shown that MSCs have strong capacity of immunomodulation that significantly reduced post-heart transplant acute rejection (29), effectively suppressed both overwhelming inflammatory and inflammatory-immune reaction (23, 24, 29–31), resulting in an improvement of prognostic outcomes. Additionally, our another study has further shown that xenogeneic human umbilical cord-derived mesenchymal stem cells (HUCDMSCs) significantly reduced the mortality in rats with ARDS complicated by sepsis (32). These findings (21–32) raise the hypothesis that MSC therapy might be beneficial on improving the clinical outcomes in the ARDS patients.

MATERIALS AND METHODS

Study Design

A total of nine ARDS patients were enrolled prospectively in this phase I clinical trial. The primary objectives were to test the safety, feasibility, and the occurrence rate of adverse events of single-dose HUCDMSCs. The secondary objective was to assess the potential efficacy of the therapy in patients with moderate to severe ARDS.

Inclusion and Exclusion Criteria

Inclusion criteria included patients with age of greater than 20 and less than 80 years who met the Berlin definition of moderate-severe ARDS (33) and already received conventional therapy, including ventilatory support, vasodilator and diuretic therapy for heart failure and fluid overload, inotropic agent for hypotension and shock, hemodialysis for end-stage renal disease, antibiotics for suspected infection or steroid/bronchodilator use for clinical presentation of acute asthma attack greater than or equal to 5 days after confirming the diagnosis of ARDS with no clinical improvement under optimal medications for any associated specific disease entities such as liver disease, chronic kidney disease (CKD), hypertension, diabetes mellitus, hypercholesterolemia, coronary artery disease, and left ventricular dysfunction. Additionally, patients and family were willing to participate in the clinical trial. On the other hand, patients with history of the following conditions were excluded from the study, including those less than or equal to 20 or greater than or equal to 80 years old, pregnancy, malignancy, immunologic disorders, adventitious agents, HIV carrier, or patient having already participated in another clinical trial.

Informed consent was obtained after discussion with the patient or an appropriate surrogate. After informed consent was acquired, the cell therapy laboratory was alerted of the enrollment. From December 2017 to August 2019, nine consecutive patients who fit the criteria were prospectively enrolled at the institute consecutively.

Moderate-Severe ARDS Under the Berlin ARDS Definition

In the present study, the ARDS was defined by the Berlin criteria (33) with minimal modification of previous studies (34, 35): 1) onset of ARDS (diagnosis) must be acute, defined as within 7 days of some prespecified events, which may be sepsis, pneumonia, or simply a patient's recognition of worsening respiratory symptoms; 2) positive pressure or positive-pressure ventilation applied by an endotracheal tube with a ratio of Pao_2 to Fio_2 less than 200 and with at least 8 cm H_2O positive end-expiratory airway pressure; 3) chest radiograph showing bilateral infiltrates, opacities, or lung edema that could not be attributed to heart failure; and 4) no clinical evidence of left atrial hypertension, or a measured pulmonary arterial occlusion pressure less than or equal to 18 mm Hg.

Requirement of Dose-Escalation for Safety in This Phase I Clinical Trial and Route of HUCDMSCs Administration

The Taiwan Food and Drug Administration (TFDA) recommended that no more than 10 patients to be enrolled in this phase I clinical trial. Additionally, patient dose-escalation protocol was finalized based on several discussions with and approved by the TFDA. For reasons concerning about patient's safety, the first three patients were assigned to receive low dose HUCDMSCs (1×10^6 cells/kg); the next three patients received intermediate dose HUCDMSCs (5×10^6 cells/kg); and the final three patients with high dose HUCDMSCs (1×10^7 cells/kg). The cell-based therapeutic regimen used for ARDS patients in this phase I clinical trial was based on the previous report (35), in which the protocol of regimen had been proved to be safe and to have a favorable clinical outcome. Requested by TFDA, data from every patient in the three dose cohorts should be reviewed for safety on a per-patient basis, before proceeding to the next enrolled patient. For the purpose of quick delivery into the pulmonary artery and lung parenchyma, the HUCDMSCs were administered from the central vein by being slowly dripped for at least 30 minutes. During infusion and 6 hours after complete infusion of the HUCDMSCs dose targeted, each patient was intensively observed to identify if there was any side effect or severe adverse event.

The Source and Preparation of the HUCDMSCs

The source of the HUCDMSCs was from the BIONET Corp. Company (Taipei City, Taiwan). These MSCs were derived from Wharton's jelly within the umbilical cord undergoing the specific

cell culturing in the good tissue practices (GTP) facility as the final product for this phase I clinical trial, that is, HUCDMSCs.

Primary and Secondary Objectives

This study was designed to test HUCDMSCs in patients with ARDS. The primary endpoints were to test the safety and tolerability of the HUCDMSCs transfusion. The secondary endpoints were to recognize and report the occurrence rate of all serious adverse events, including death, prespecified infusion-related events, and nonserious adverse events considered to be associated with the cell infusion. Additionally, we carefully measured the grade of respiratory and systemic organ dysfunctions, including Acute Physiology and Chronic Health Evaluation (APACHE) II score and Sequential Organ Failure Assessment (SOFA) score, duration of mechanical ventilatory support, length of stay in the ICU and total hospitalization days as well as biomarker values.

Scoring System for Mortality Prediction and Assessment of Severity of Organ Dysfunction

The APACHE II score was used to estimate in-hospital mortality. Additionally, SOFA score was used for daily assessing organ dysfunction with parameters of mechanical ventilation or not, mean atrial blood pressure, Glasgow Coma Scale (GCS) level, Pa_{O_2}/F_{IO_2}, coagulation status, and levels of serum creatinine, total bilirubin, and platelet count.

Flow cytometric quantifications of MSC, inflammatory and immune cell surface biomarkers (**Supplemental Digital Content—Methodology**, Supplemental Digital Content 1, http://links.lww.com/CCM/F364)

The HUCDMSCs Source

The HUCDMSCs were provided free of charge by BIONET Corp. Com for this phase I clinical trial. The cell sponsor of this trial did not play any role in the study design, data collection, data analysis, data interpretation, or writing of the report. The authors had fully examined all data and taken the final responsibility to submit the report for publication.

Statistics

Baseline condition, laboratory values including hemogram, biochemistry, and biomarker data, and clinical outcomes were described by using mean ± SD for continuous variables or number with percentage for categorical variables. Baseline and serial changes of lung consolidation number, SOFA score, APACHE score, and flow cytometric data among the treatment group were compared with repeated measures analysis of variance. Remaining descriptive analyses were presented and plotted with figures. Statistical analysis was performed using SPSS statistical software for Windows version 19 (Version 13; SPSS for Windows, SPSS, IL). A p value of less than 0.05 was considered statistically significant.

Study Approval

This clinical trial was approved by TFDA (number: 1066023736), Ministry of Health and Welfare, Taiwan, Republic of China, and the Institutional Review Committee on Human Research at Chang Gung Memorial Hospital (number: 201402853A0) in 2014, and conducted at Kaohsiung Chang Gung Memorial Hospital, a tertiary referral center. This study was supported by a program grant from Chang Gung Memorial Hospital and Chang Gung University (grant numbers: CMRPG8E1241 [1/2] and CMRPG8E1242 [2/2]). This study had been registered with International Standard Randomised Controlled Trial Number (ISRCTN) registry number of ISRCTN52319075.

RESULTS

Baseline Characteristics of Nine Patients and Laboratory Findings Upon Enrollment

Figure 1 shows the flow chart for elucidating the screening, exclusion, allocation, enrollment, follow-up, and outcome among the nine patients. On the other hand, **Table 1** lists the baseline characteristics and laboratory findings of nine patients. The mean age was 54 ± 18 years, more than 77% of study patients were male and mean body mass index was 25.9 ± 3.9. The prevalence of smoking, diabetes mellitus, and hyperlipidemia were less than 35%. On the other hand, there was no history of stroke or myocardial infarction. A patient with history of end-stage renal disease was on regular hemodialysis, while two patients with acute kidney injury received transient dialysis. The prevalence of CKD with estimated glomerular filtration rate less than 60 mL/min/1.73 m^2 was 55%. Additionally, two patients had history of alcoholic liver disease or cirrhosis. The mean GCS was 10.8 among the nine patients.

Serial changes of the clinical parameters, clinical conditions, and laboratory findings upon enrollment time among the nine patients (**Supplemental Fig. 1**, Supplemental Digital Content 2, http://links.lww.com/CCM/F365; **legend**, Supplemental Digital Content 1, http://links.lww.com/CCM/F364).

The expressions of Pa_{O_2} to F_{IO_2} ratio and daily urine output, two essential parameters for prediction of clinical outcome, were notably gradually improved during the clinical follow-up courses (Supplemental Fig. 1, Supplemental Digital Content 2, http://links.lww.com/CCM/F365; legend, Supplemental Digital Content 1, http://links.lww.com/CCM/F364). On the other hand, SOFA score, circulatory lactate, C-reactive protein, WBC count, number of lobular consolidations, and other crucial predictors of prognostic outcome were significantly reduced during the clinical follow-up (Supplemental Fig. 1, Supplemental Digital Content 2, http://links.lww.com/CCM/F365; legend, Supplemental Digital Content 1, http://links.lww.com/CCM/F364).

Six patients experienced severe sepsis or septic shock with multiple organ dysfunction syndrome (MODS), and all patients were found to have pneumonia during hospitalization necessitating empirical antibiotic therapy. The mean number of organ failure was identified up to 3.1 ± 0.6 and the mean Pa_{O_2}/F_{IO_2} ratio was 107.8 ± 59.2 on enrollment time. Five patients needed pharmacological circulatory support with inotrope or vasopressor for cardiogenic or septic shock. Three other patients required further mechanical circulatory support such as intra-aortic balloon pumping (IABP) or extracorporeal membrane oxygenation due to profound cardiogenic shock.

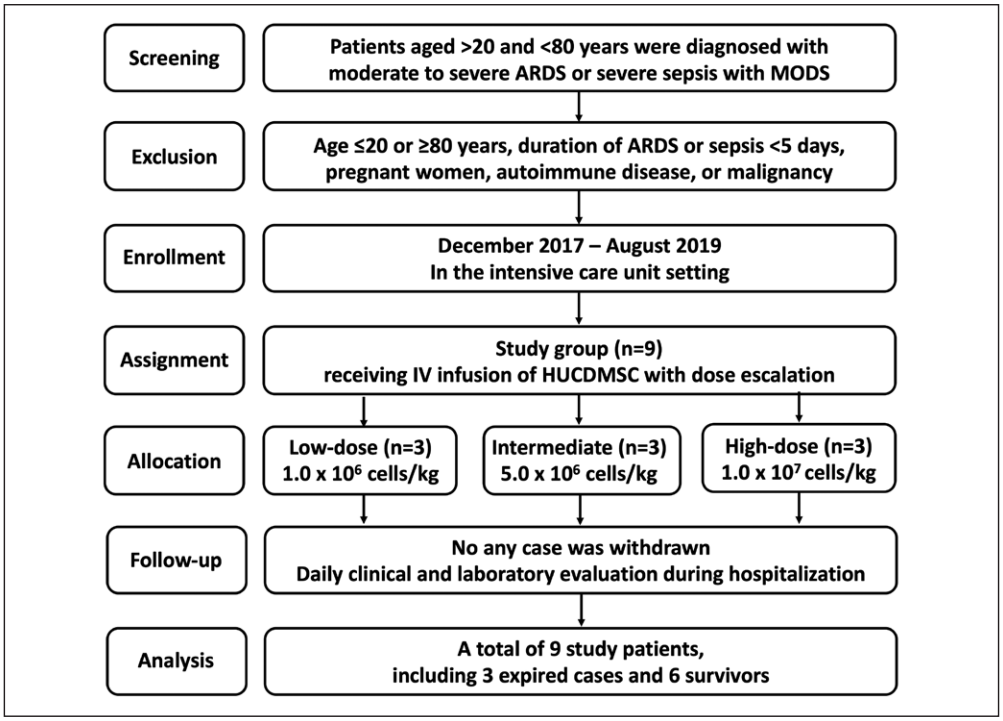

Figure 1. Flow chart of patient enrollment. Illustrating the flow chart of screening, inclusion, enrollment, allocation, follow-up, and analysis in this phase I clinical trial. ARDS = acute respiratory distress syndrome, HUCDMSC = human umbilical cord-derived mesenchymal stem cell, MODS = multiple organ dysfunction syndrome.

Plenty of laboratory findings which were usually used as useful parameters for prediction of prognostic outcome are listed in **Table 2**. Of these parameters, the serum lactate, C-reactive protein, procalcitonin, serum creatinine, liver biomarkers such as transferase and bilirubin, B-type natriuretic peptide, and cardiac troponin-I were markedly increased, whereas creatinine clearance rate was notably reduced upon presentation among nine patients.

Time courses of the flow cytometric results (**Supplemental Fig. 2**, Supplemental Digital Content 3, http://links.lww.com/CCM/F366; **Supplemental Fig. 3**, Supplemental Digital Content 4 http://links.lww.com/CCM/F367 [legend, Supplemental Digital Content 1, http://links.lww.com/CCM/F364])

We used the flow cytometric analyses to elucidate the time courses of inflammatory cell surface markers (i.e., CD11b+/CD16+, CD11b+/MPO+, CD16+/MPO+, and CD14+CD33+) in circulation (i.e., at baseline and after HUCDMSCs infusion). The results showed that these inflammatory parameters were substantially reduced at day 1 after cell infusion as compared with baseline, followed by notably stepwise increase from day 3 to day 7, then significantly reduced by day 30 after cell therapy as compared with baseline level (Supplemental Fig. 2, Supplemental Digital Content 3, http://links.lww.com/CCM/F366; legend, Supplemental Digital Content 1, http://links.lww.com/CCM/F364). These findings implied that the therapeutic effect of single-dose HUCDMSCs on suppressing the expression of inflammation in circulation might be only temporary. On the other hand, the number of $CD14^{dim}CD33^{+}$ cells, an indicator of dendritic cells (DCs) which are key mediators of the innate and adaptive immune responses, was substantially and persistently increased from baseline to day 7, followed by notable reduction at 1 month after HUCDMSCs therapy (Supplemental Fig. 2, Supplemental Digital Content 3, http://links.lww.com/CCM/F366; legend, Supplemental Digital Content 1, http://links.lww.com/CCM/F364). Intriguingly, the early apoptosis of mononuclear cells was found to be notably progressively increased after HUCDMSCs therapy, whereas the late apoptosis of mononuclear cells was first significantly decreased from baseline to day 3, and then significantly increased between day 4 to day 30 after HUCDMSCs infusion (Supplemental Fig. 2, Supplemental Digital Content 3, http://links.lww.com/CCM/F366; legend, Supplemental Digital Content 1, http://links.lww.com/CCM/F364).

Next, utilizing the same method of flow cytometric analyses, we assessed the time courses of immune cells and MSC surface markers in circulation. Interestingly, the surface markers of these immune cells were notably stepwise increased from day 7 to 30 after HUCDMSCs therapy, suggesting delayed intrinsic production of immune cells with response to stimulations of ARDS and HUCDMSCs therapy (Supplemental Fig. 3, Supplemental Digital Content 4 http://links.lww.com/CCM/F367; legend, Supplemental Digital Content 1, http://links.lww.com/CCM/F364). On the other hand, the majority of the MSC surface markers were notably stepwise decreased 1 day after HUCDMSCs infusion (Supplemental Fig. 3, Supplemental Digital Content 4, http://links.lww.com/CCM/F367; legend, Supplemental Digital Content 1, http://links.lww.com/CCM/F364).

Clinical Course and In-Hospital Mortality

Table 3 lists the initial clinical presentation of individual study patient. All the clinical parameters, such as need of IABP and EMCO supports, severity of ARDS, either septic or cardiogenic shock, or APACHE II score, indicated that the nine patients were at serious clinical situation.

Table 4 lists the clinical outcomes of each patient. The duration of hospitalization was 30 ± 31.8 days. Three of nine patients were dead during hospitalization, that is, the in-hospital mortality rate was 33.3%, and the remaining six patients were uneventfully discharged and followed up regularly at outpatient department.

TABLE 1. Baseline Characteristics of the Study Patients

Variables	Mean Value or *n* (%)
Background	Patients number ($n = 9$)
Age, yr	54 ± 18
Male sex	7 (77.8%)
Body height, cm	165.4 ± 10.8
Bodyweight, kg	72.6 ± 18.2
Body mass index, kg/m^2	25.9 ± 3.9
Smoking	3 (33.3%)
Hypertension	3 (33.3%)
Type 2 diabetes mellitus	3 (33.3%)
Dyslipidemia	2 (22.2%)
Chronic kidney disease ≥ stage II	5 (55.6%)
Regular or acute kidney injury for dialysis	3 (33.3%)
Chronic hepatitis or cirrhosis	2 (22.2%)
Old stroke	0 (0%)
Old myocardial infarction	0 (0%)
Coronary artery disease	0 (0%)
History of systolic heart failure	1 (11.1%)
Vital sign upon presentation	
Mean systolic blood pressure, mm Hg	108.1 ± 25.5
Mean diastolic blood pressure, mm Hg	67.3 ± 21.2
Mean arterial blood pressure, mm Hg	80.9 ± 22.1
Heart rate, beats/min	94.9 ± 28.5
Respiratory rate, breaths/min	23.8 ± 6.8
Body temperature, °C	36.9 ± 1.3
Glasgow Coma Scale	10.8 ± 4.0

Data were expressed as mean ± SD or *n* (%).

TABLE 2. Initial Clinical Presentation and Laboratory Findings

Variables	Mean Value or *n* (%)
Clinical condition and corresponding therapy	Total case number = 9
Moderate to severe acute respiratory distress syndrome	9 (100%)
Severe sepsis or septic shock with multiple organ dysfunction syndrome	6 (66.7%)
Number of organ dysfunction	3.1 ± 0.6
Pneumonia	9 (100%)
Fulminant myocarditis	2 (22.2%)
Mechanical ventilatory support	8 (88.9%)
Mechanical circulatory support	3 (33.3%)
Inotrope or vasopressor agent use	5 (55.6%)
Empirical antibiotic therapy	9 (100%)
Pa_{O_2}/F_{IO_2} ratio	107.8 ± 59.2
Laboratory data on enrollment time	
Arterial pH value	7.3 ± 0.1
Arterial lactate, mg/dL	32.4 ± 39.1
C-reactive protein, mg/L	166.4 ± 116.7
Procalcitonin, ng/mL	8.5 ± 15.9
WBC count, 1,000/μL	10.2 ± 6.6
Segment, %	81.3 ± 7.9
Lymphocyte, %	10.8 ± 4.8
RBC count, million/μL	4.2 ± 1.1
Hemoglobin, g/dL	12.2 ± 3.9
Platelet count, 1,000/μL	153.6 ± 56.2
Prothrombin time, s	16.3 ± 10.4
Activated partial thromboplastin time, s	44.2 ± 22.4
Serum creatinine, mg/dL	3.3 ± 4.3
Creatinine clearance rate, mL/min	44.7 ± 26.5
Aspartate aminotransferase, U/L	157.4 ± 125.5
Alanine aminotransferase, U/L	76.6 ± 74.0
Bilirubin (total), mg/dL	2.0 ± 1.8
B-type natriuretic peptide, pg/mL	1,774.4 ± 1,776.7
Cardiac troponin-I, ng/mL	4.0 ± 10.7
Transient hemodialysis	2 (22.2%)
Permanent hemodialysis	1 (11.1%)

Data were expressed as mean ± SD or *n* (%).

Detailed analysis demonstrated two patients (i.e., cases 2 and 6) (one with low dose and the other with intermediate dose) were expired due to recurrent sepsis with septic shock 3 days after cell infusion, implying that one-dose therapy could effectively maintain for 3 days. Another patient (i.e., case 4) recognized to have great improvement of clinical course and was already on ventilatory weaning process, also died from ventilator-associated mechanical complications, followed by severe septic shock.

Adverse Event During HUCDMSCs Infusion

Transient desaturation, dyspnea, and hypotension at 10–15 minutes after cell infusion were observed in cases 2 (i.e., arterial oxygen saturation [Sa_{O_2}] was 88% and systolic blood pressure was 86 mm Hg) and 4 (Sa_{O_2} was 86% and systolic blood pressure was 85 mm Hg). Additionally, case 3 presented with generalized skin rash that appeared a few

TABLE 3. Initial Clinical Presentation of Individual Patient

Case No.	Age/Sex	Severity of ARDS	Etiology of ARDS	Shock (Mechanical Circulatory Support)	No. of Organ Dysfunction	Acute Physiology and Chronic Health Evaluation II Score	Day and Dose of Cells/kg
1	66/male	Moderate	Pneumonia	No	5	16	$(D^{5})^{a}$ (1.0×10^{6})
2	54/female	Severe	Pneumonia	Yes	5	13	$(D^{9})^{a}$ (1.0×10^{6})
3	35/male	Severe	Pneumonia, SS, heart failure[b]	Yes, intra-aortic balloon pumping	2	22	$(D^{12})^{a}$ (1.0×10^{6})
4	69/male	Severe	Pneumonia	No	5	14	$(D^{5})^{a}$ (5.0×10^{6})
5	39/male	Severe	Pneumonia, SS, myocarditis[c]	Yes, ECMO	5	28	$(D^{7})^{a}$ (5.0×10^{6})
6	76/male	Severe	Pneumonia	Yes	5	15	$(D^{5})^{a}$ (5.0×10^{6})
7	31/male	Moderate	Pneumonia	No	5	14	$(D^{5})^{a}$ (1.0×10^{7})
8	75/female	Moderate	Pneumonia, SS, myocarditis[d]	Yes, ECMO	5	22	$(D^{5})^{a}$ (1.0×10^{7})
9	41/male	Moderate	Pneumonia, acute pericarditis[e]	Yes	4	15	$(D^{5})^{a}$ (1.0×10^{6})

ARDS = acute respiratory distress syndrome, ECMO = extracorporeal membrane oxygenation, SS = septic shock.
[a]The Arabic number indicated the human umbilical cord-derived mesenchymal stem (stromal) cells infusion at the day after ARDS was diagnosed.
[b]Indicated the left ventricular ejection fraction (LVEF) = 21% upon presentation.
[c]Indicated the LVEF < 20% upon presentation.
[d]Indicated the LVEF = 28% upon presentation.
[e]Indicated the LVEF = 45% upon presentation.

TABLE 4. Changes of Variables and Clinical Outcomes Before and After Cell Therapy

Case No.	Pao_2/Fio_2 Change	Sequential Organ Failure Assessment Score Change	No. of Lobar Consolidation	Ventilator Days	Left Ventricular Ejection Fraction Upon Presentation, %	ICU Days	Hospital Days	Clinical Outcome
1	198 → 549	6 → 3	5 → 0	6	> 70	8	13	Survival
2	72 → 125	12 → 17	5 → 5	22	54	22	22	Expired
3	31 → 442	14 → 6	2 → 0	15	21	19	29	Survival
4	62 → 208	9 → 15	5 → 4	21	57	21	21	Expired
5	63 → 194	16 → 8	5 → 2	44	< 20	50	69	Survival
6	93 → 101	7 → 16	5 → 5	15	76	15	33	Expired
7	139 → 440	8 → 4	5 → 2	8	> 70	11	56	Survival
8	122 → 275	6 → 3	5 → 2	8	28	12	21	Survival
9	178 → 204	5 → 1	4 → 2	0	45	12	15	Survival
Summary	106 → 282	9.2 → 8.1	4.6 → 2.4	15.4	49	18.9	31.0	Mortality 33.3%

→Indicated the variable change prior to and post cell therapy.
Pao_2/Fio_2 and Sequential Organ Failure Assessment were daily evaluated.

hours after cell therapy and was persistent for about 2 days. However, all these adverse events did not cause any life-threatening condition and these patients were recovered smoothly.

Serial imaging findings in each patient (**Supplemental Fig. 4**, Supplemental Digital Content 5, http://links.lww.com/CCM/F368; **Supplemental Fig. 5**, Supplemental Digital Content 6, http://links.lww.com/CCM/F369; and **Supplemental Fig. 6**,

Supplemental Digital Content 7, http://links.lww.com/CCM/F370 [legend, Supplemental Digital Content 1, http://links.lww.com/CCM/F364])

The detailed descriptions of serial changes of chest radiograph and chest CT imaging findings in nine cases were referred to figure legends.

DISCUSSION

This phase I clinical trial aiming to investigate the safety of cell-based therapy for ARDS with or without severe sepsis demonstrated that IV administration of a single-dose HUCDMSCs was quite well tolerated in these nine high-risk patients with moderate to severe ARDS complicated by sepsis, shock, or MODS. In fact, external review by the TFDA and Taiwan Centers for Disease Control and Prevention had thoroughly investigated and concluded that there was no evidence of prespecified infusion-associated adverse events, immediate clinical instability, or dose-limiting toxicity at any of the doses tested or any severe adverse events related to HUCDMSC infusion in our clinical trial, suggesting that the primary endpoints of these three different regimens of HUCDMSCs were safe in setting of moderate-to-severe ARDS patients.

The in-hospital mortality rate of moderate-severe ARDS has been previously identified to be more than 40% to 60% even undergoing the aggressive lung-protective management (1–7), highlighting an unacceptably high mortality and unmet needs in current therapeutic strategies for ARDS. An essential finding in the present study was that the in-hospital mortality was 33.3%. Of distinctive observation is that more than half patients (i.e., five of nine patients) fit the Berlin definition of severe ARDS upon enrollment time and six of them experienced shock in need of pharmacological or circulatory mechanical support. All of them suffered from multiple organ dysfunction during the treatment for ARDS. Accordingly, as compared with the results of previously clinical studies of ARDS with conventional therapy (1–7), our result was not inferior to that of conventional therapy (1–7) with an acceptable clinical outcome. Interestingly, recent three clinical trials (34–36), including two phase I and one phase II trials, have reported that allogenic MSCs therapy for acute moderate-severe ARDS was safe with in-hospital mortality of 35%, 30% in the phase I and 45% in the phase II, respectively. In this way, our result was not inferior to the findings from these three clinical trials (34–36).

Three patients were dead during hospitalization. Intriguingly, when this subgroup of patients was carefully analyzed, we found that those two dead patients (i.e., cases 2 and 6) expressed initial dramatic clinical improvement after HUCDMSCs infusion. However, recurrent sepsis, followed by septic shock occurred just after day 3's HUCDMSCs therapy. Notably, consistent finding (i.e., flow cytometric analysis) in the present study was that the cellular inflammatory surface markers were remarkably suppressed at day 1 after HUCDMSCs infusion. However, these parameters were notably upregulated at day 3 and even more upregulated at day 10, followed by notably downregulated or returned to baseline levels by day 30 after HUCDMSCs therapy. Additionally, the time course of circulating level of C-reactive protein and WBC count, two indicators of acute inflammation, also exhibited a similar pattern of inflammatory cell biomarkers in these patients. Concordantly, our recent preclinical studies (29, 32) have demonstrated that adipose-derived MSC and HUCDMSCs treatment substantially reduced inflammatory-immune reaction, resulting in reduction of rat post-transplanted acute heart rejection (29) and mortality in ARDS complicated with sepsis syndrome in rat (32). These clinical and laboratory distinctive features and the results of our preclinical studies (29, 32) call for the consideration of a second-round HUCDMSCs infusion to provide persistent and effective treatment to these patients, especially when recurrent sepsis-induced worsening clinical course are identified.

Circulating number of DCs (i.e., an innate immune cells) was significantly and progressively increased from days 1 to 7, followed by markedly reduced and lower than the baseline by day 30 after HUCDMSCs therapy. On the other hand, the adaptive immune T cells were identified to be slowly mildly increased from days 1 to 7, then markedly increased afterward and up to the peak level at day 30 after HUCDMSCs administration. These novel findings from time courses of flow cytometric analyses, which have not been investigated in a situation of ARDS with HUCDMSCs therapy, not only delineated the inherently mysterious correlation between the MSCs therapy and the host immune response, but also implied that HUCDMSCs could simultaneously regulate the intrinsic innate and adaptive immune responses for protecting the host against microorganism invasion or damage from overwhelming inflammatory-immune reactions.

As compared with those three reported clinical trials (34–36), advantages of relevant information gathered from the results of our clinical trial are as follows: 1) the flow cytometric analysis accurately displayed the correlation between downward, followed by upward of cellular inflammatory surface markers and relapsed severe sepsis after HUCDMSCs transfusion within 3 days; 2) the serial changes of DCs in circulatory system were also precisely clarified in the ARDS patients; and 3) the distinctive time courses of adaptive immune T cells in circulation were the first one to be delineated in ARDS patients by this phase I clinical trial.

Study limitation (**Supplemental Digital Content—Study limitation**, Supplemental Digital Content 1, http://links.lww.com/CCM/F364).

CONCLUSIONS

In conclusion, the results of this phase I clinical trial demonstrated that a single IV HUCDMSCs infusion of up to 1.0×10^7 cells/kg was excellently tolerated in nine moderate-to-severe ARDS patients without serious adverse events. Additionally, a good tolerability and favorable short-term outcome of this phase I clinical trial pave the way to initiate a phase II randomized placebo-controlled trial to test the long-term safety and the efficacy of HUCDMSCs for moderate to severe ARDS patients in the near future.

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This work was performed at Kaohsiung Chang Gung Memorial Hospital and Chang Gung University College of Medicine 83301, Kaohsiung, Taiwan.

Drs. Yip, Sung, and M. S. Lee conceived the experiments and designed the study. Drs. Fang, Li, and F. -Y. Lee carried out experiments. Drs. C. -H. Lee, Pei, Chen, and Ma analyzed data. Dr. Chen edited the article. Drs. Yip, Sung, and M. S. Lee drafted the article. Drs. Yip, Sung, and M. S. Lee had full access to all the data in the study and had final responsibility for the decision to submit for publication. All authors had final approval of the submitted and published versions.

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For information regarding this article, E-mail: e12281@cgmh.org.tw; mellee@cgmh.org.tw

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