OMTM, Volume *23*

Supplemental information

Engineered mesenchymal stromal cell therapy during human lung *ex vivo* lung perfusion is compromised by acidic lung microenvironment

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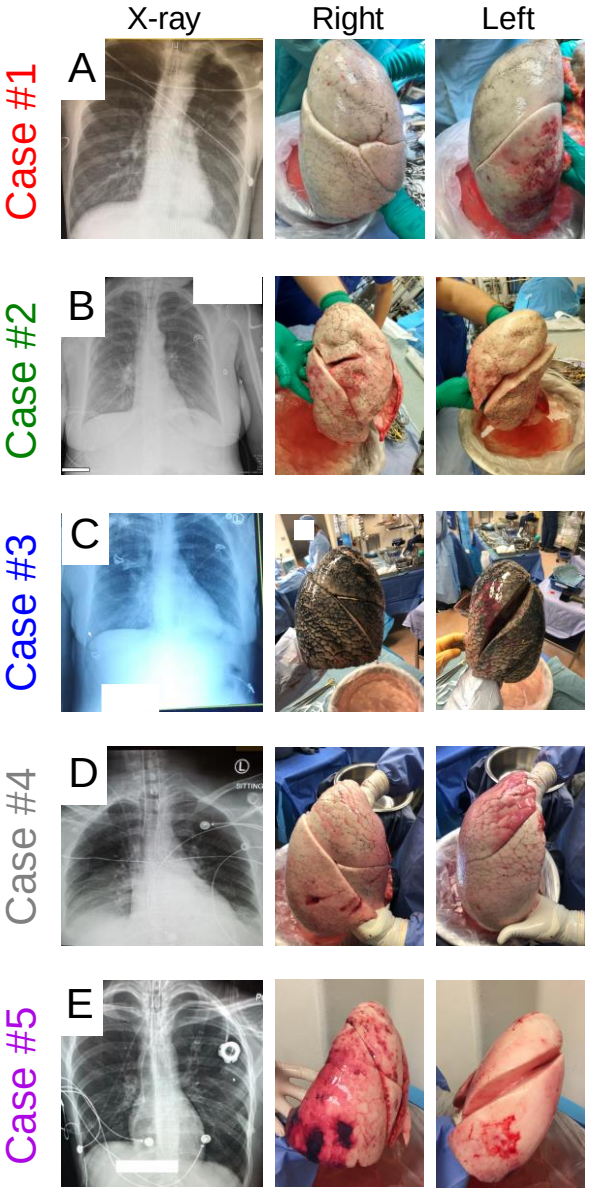
Table S1. EVLP and MSC-treatment baseline parameters

	Control	MSC^{IL-10}	p-value
n=	4	5	
Cold ischemia time, h	9.0 (7.6-12.3)	8.9 (7.9-11.6)	0.90
EVLP 1-hour (pre-MSC) parameters			
PAP, mmHg	8.0 (7.3-10.3)	8.0 (6.5-11.5)	0.97
PVR, dynes/sec/cm^{-5}	400 (263-599)	444 (282-607)	0.99
Peak airway pressure, cmH2O	13.5 (12.3-18.5)	13 (11.0-17.0)	0.63
Mean airway pressure, cmH2O	7.0 (6.3-7.8)	7.0 (6.0-7.5)	0.98
Dynamic compliance, ml/cmH2O	36 (26-63)	38 (27-45)	0.99
Static compliance, ml/cmH2O	56 (50-97)	72 (43-78)	0.96
Delta PaO2, mmHg	401 (216-503)	369 (334-444)	0.90
MSC treatment			
MSC administration after thawing, min		74 (66-91)	NA
MSC administration after EVLP start, min		85 (82-92)	NA
MSC viability at administration, %		97 (91-97)	NA

EVLP, Ex Vivo Lung Perfusion; IL-10, interleukin-10; MSC, mesenchymal stromal cell; PAP, pulmonary artery pressure; POD, postoperative day; PVR, pulmonary vascular resistance
Data median (interquartile range), analyzed by Mann-Whitney test

Supplemental Figure 1



Donor: 46 male, DCD
Cause of death: choking
Last P/F-ratio: 458 mmHg
Lung injury: LLL aspiration pneumonia
Right: control
Left: $40x10^6$ MSC^{IL-10}

Donor 54 female, DBD
Cause of death: over-dose
Last P/F-ratio: 260 mmHg
Lung injury: RLL aspiration pneumonia
Right: $40x10^6$ MSC^{IL-10}
Left: control

Donor 31 female, DCD
Cause of death: over-dose
Last P/F-ratio: 180 mmHg
Lung injury: bilateral aspiration pneumonia
Right: control
Left: $40x10^6$ MSC^{IL-10}

Donor 49 male, DBD
Cause of death: choking
Last P/F-ratio: 97 mmHg
Lung injury: RLL aspiration pneumonia
Right: Not used
Left: $40x10^6$ MSC^{IL-10}

Donor 23 female, DBD
Cause of death: drowning
Last P/F-ratio: 472 mmHg
Lung injury: Concerns of LL necrotic areas
Right: control
Left: $40x10^6$ MSC^{IL-10}

Figure S1. Human donor lungs used in the study. Human lungs rejected from clinical transplantation for various reasons were used in the study (n=5). (A-E) Chest x-ray and lung retrieval photos, and donor and treatment group information are given for each case. The double lung was split, each lung was connected to separate single-lung EVLP circuits, and one lung was randomized to $MSC^{IL\text{-}10}$ treatment while the contralateral lung served as a control (n=4). (D) In one case, the right lung with aspiration pneumonia had extensive right lower lobe atelectasis requiring recruitment with high airway pressures up to 35mmHg during the donor lung retrieval. Due to lung quality issues and a clinical concern that it was too damaged to last the planned 12-hour EVLP experiment, the right lung was not used for the experiment, and only the left lung was connected to EVLP and treated with $MSC^{IL\text{-}10}$ cells. DBD, donation after brain death; DCD, donation after circulatory death; IL-10, interleukin-10; LL, lower lobe; LLL, left lower lobe; MSC, mesenchymal stromal cell; P/F-ratio, ratio of arterial oxygen partial pressure to fractional inspired oxygen; RLL, right lower lobe.

Supplemental Figure 2

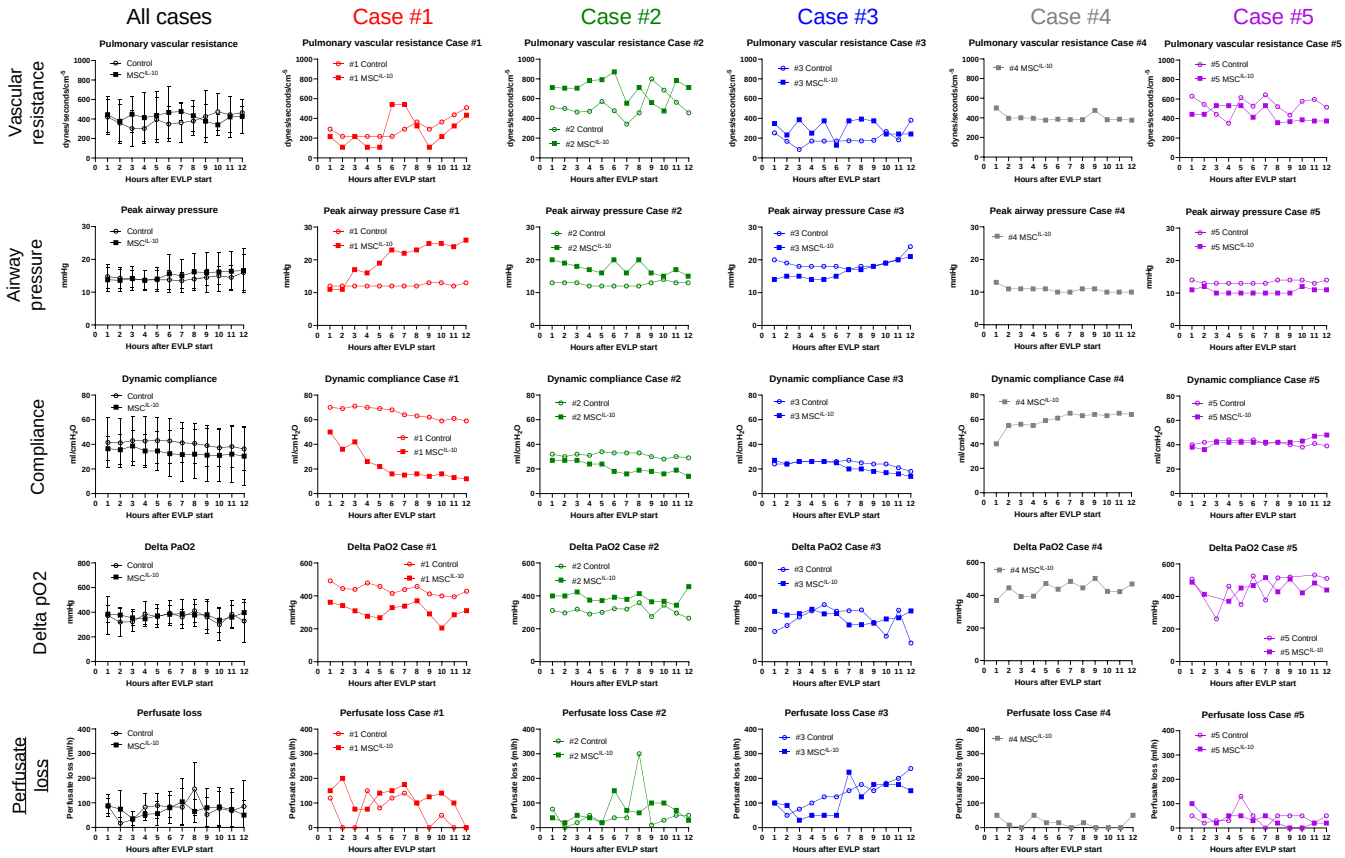


Figure S2. Lung function during EVLP with median values of all cases and individual cases. Pulmonary artery resistance, peak airway pressure, dynamic compliance, delta PaO2 and perfusate loss were recorded hourly. Median values for control (n=4) and $MSC^{IL\text{-}10}$ (n=5) groups are given in the left column and individual values of cases 1-5 in the remaining columns. Data on the left column expressed as median±interquartile range and analyzed by 2-way ANOVA. EVLP, ex vivo lung perfusion; IL-10, interleukin-10; MSC, mesenchymal stromal cell.

Supplemental Figure 3

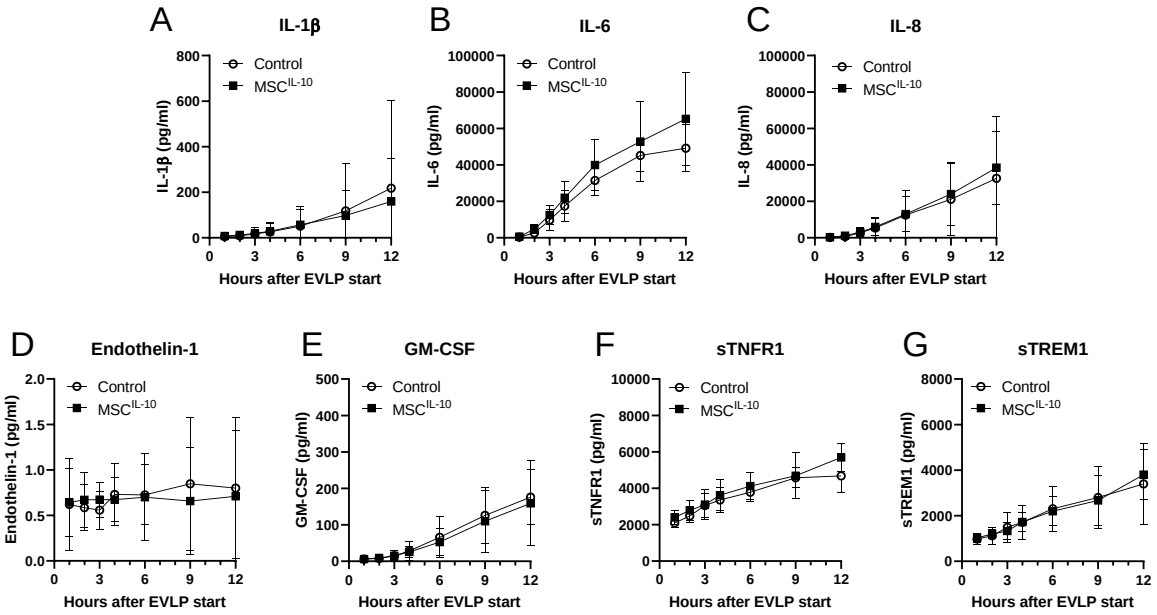


Figure S3. Perfusate cytokines during EVLP. Perfusate samples were taken every hour and assessed for (A) IL-1β, (B) IL-6, (C) IL-8, (D) endothelin-1, (E) GM-CSF, (F) sTNFR1 and (G) sTREM1 using multianalyte immunoassays. Control (n=4) and MSCIL-10 (n=5). Data expressed as median±interquartile range and analyzed by 2-way ANOVA. EVLP, ex vivo lung perfusion; GM-CSM, granulocyte-macrophage colony-stimulating factor; IL, interleukin; sTNFR1, soluble tumor necrosis factor receptor 1; sTREM1, soluble triggering receptor expressed by myeloid cells 1.

Supplemental Figure 4

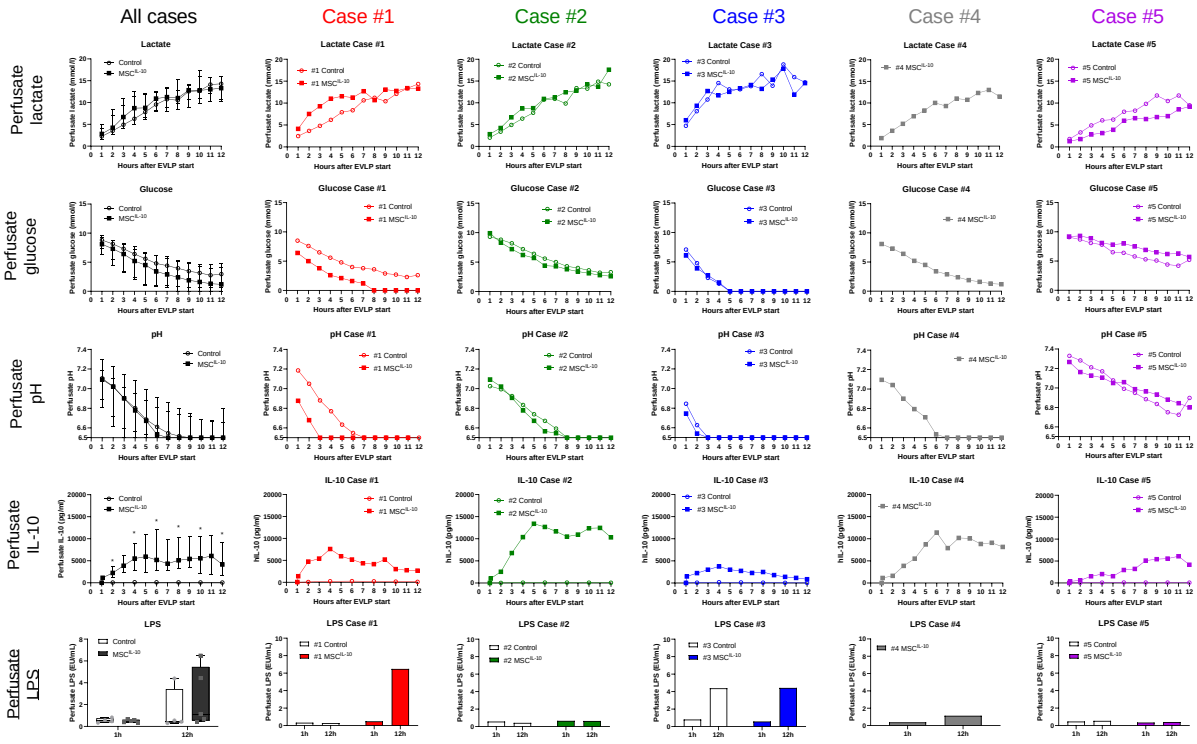


Figure S4. Perfusate metabolic markers, IL-10 and LPS during EVLP with median values of all cases and individual cases. Perfusate samples were taken every hour and assessed for glucose, lactate, pH, IL-10 and LPS levels. Median values (±interquartile range) for control (n=4) and $MSC^{IL\text{-}10}$ groups (left column) and individual values for cases 1-5. Data on the left column analyzed by 2-way ANOVA. EU, endotoxin unit; EVLP, ex vivo lung perfusion; IL-10, interleukin-10; LPS, lipopolysaccharide; MSC, mesenchymal stromal cell.

Supplemental Figure 5

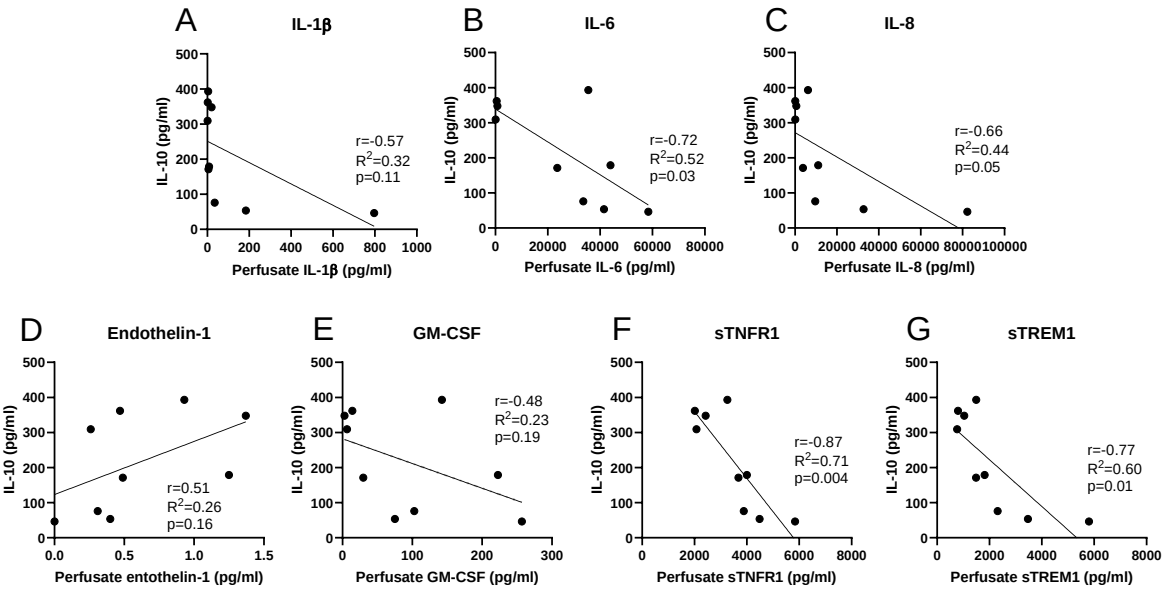


Figure S5. Perfusate proinflammatory and damage-related conditions are associated with decreased MSC^{IL-10} IL-10 production *in vitro*. MSC^{IL-10} were plated in vitro, cultured in 9 different human EVLP perfusate samples, and IL-10 secretion was measured at 4 hours. The 9 samples used consisted of 1-, 6- and 12-hour perfusate samples of control group cases 1-3 resulting in a spectrum of different baseline cytokine concentrations that were measured by ELISA. Correlation of 4-hour IL-10 concentration with respective baseline perfusate (A) IL-1β, (B) IL-6, (C) IL-8, (D) endothelin-1, (E) GM-CSF, (F) sTNFR1 and (G) sTREM1 concentrations. Data analyzed by linear regression and Pearson coefficient test. EVLP, ex vivo lung perfusion; GM-CSF, granulocyte-macrophage colony-stimulating factor; IL, interleukin; MSC, mesenchymal stromal cell; sTNFR1, soluble tumor necrosis factor receptor 1; sTREM1, soluble triggering receptor expressed by myeloid cells 1.