Mesenchymal Stem Cells for Treatment of Acute Respiratory Distress Syndrome

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Stem Cell Transplant and Cellular Therapy
ARDS

- No current standard recommendation for treatment
- Berlin: *Acute* (<1 week) *diffuse inflammatory lung injury*, leading to
  - increased pulmonary vasculature permeability
  - increased lung weight
  - loss of aerated lung tissue
  with hypoxemia and bilateral radiographic opacities not explained by cardiac failure /anasarca.

- **PaO2/FiO2**
  - Mild 200-300
  - Moderate 100-200
  - Severe <100
Mesenchymal Stem Cells

- Mesenchymal stem cells
  - self-renewing multipotent stem cells
  - isolated from cord blood, adipose, bone marrow

- In vitro differentiated into multiple cell types

- Possess regenerative, immune-modulatory, and anti-microbial properties

- Proven to have protective effects in several preclinical models of ARDS including the ex vivo human lung perfusion model
MSCs in Pulmonary Disease; Completed

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<tr>
<th>Disease</th>
<th>Study/Phase</th>
<th>Dose/Schedule</th>
<th>Outcome</th>
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<tbody>
<tr>
<td>COPD</td>
<td>Mesoblast/Phase II</td>
<td>100X 10^6 X4 doses (prochymal)</td>
<td>Safe but no significant changes in PFTs, adverse events or QOL (↓ CRP only), (moderate 23, severe 39)</td>
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<td>BPD</td>
<td>PNEUMO-STEM</td>
<td>5 trials in South Korea, 1 published, IT in 9 infants, 10X10^6/kg, 20X10^6/kg</td>
<td>↓ IL-6, IL-8, MMP, TNFα, TGFβ1 in tracheal aspirates at day 7 BPD severity lower in the MSC recipients (rates of other adverse outcomes did not differ between the comparison group and MSC recipients)</td>
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<td>Bleo fibrosis</td>
<td>Phase 1</td>
<td>(placenta derived) First 4 1X10^6 /kg then 4 at 2X10^6/kg</td>
<td>Preclinical data highlighted importance of giving within 7 days after bleo administration At 6 months FVC, DLCO , 6MWD and CT fibrosis score were unchanged compared with baseline, no safety concerns (8 patients)</td>
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# MSCs in Pulmonary Disease; Ongoing

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<th>Outcome</th>
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<tr>
<td>IPF</td>
<td>Phase I</td>
<td>Autologous BM derived MSC IV</td>
<td>Actively recruiting in Spain (Universidad de Navarra) NCT01919827</td>
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<tr>
<td>Radiation induced PF</td>
<td>Phase I</td>
<td>1X10^6 /kg UCB MSC via bronchoscopy</td>
<td>Actively recruiting in China NCT02277145</td>
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<tr>
<td>Emphysema</td>
<td>Phase I</td>
<td>200X10^6 MSC conditioned in hypoxic media q2 months X 1 year</td>
<td>Actively recruiting in Russia NCT01849159</td>
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<tr>
<td>ILD</td>
<td>Phase I/II</td>
<td>2 IV weekly of 200X10^6 MSCs Repeated Q3 months for 1 year</td>
<td>Actively recruiting in Russia NCT02594839</td>
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*Others: MSC for BPD in preterm infants, for Non-Cystic Fibrosis Bronchiectasis, Paraquat Poisoning, Interstitial Lung Disease post SCT
ARDS Preclinical, In Vivo Models

- MSCs consistently demonstrate capacity to reduce inflammation and increase bacterial clearance

- MSCs attenuate inflammation (decrease TNF alpha, MIP-2, IFN gamma, beta, IL-6, IL-8, keratinocyte derived cytokine in plasma and BAL)

- Secretion of antibacterial protein lipocalcin-2
  

- Enhance activity of alveolar macrophages and host monocytes
  


- Rescue alveolar epithelial cells with mitochondrial dysfunction through mitochondrial transfer via direct cell contact and exosome formation

ARDS Preclinical, In Vivo Models

- Mitochondrial transfer from MSC to bronchial epithelial cells via tunneling nanotubes (TNT) was protective in in vivo asthma and COPD models
  
  *Ahmad T, et al. Miro1 regulates intercellular mitochondrial transport & enhances mesenchymal stem cell rescue efficacy. EMBO J 2014.*
  

- Mitochondrial donation from MSC to endothelial cells through TNT protective in in vitro reperfusion injury model
  
Mitochondrial Transfer via TNT is an Important Mechanism by which MSC Enhance Macrophage Phagocytosis in the in vitro and in vivo Models of ARDS

Stem Cells, April 5 2015, Jackson MV, et al

- Depletion of alveolar macrophages (AM) with IN liposomal clodronate resulted in complete abrogation of MSC anti-microbial effect in the in vivo model of E.coli pneumonia.

- AM depleted mice 2x higher bacterial CFU.
Cytokine Profiles

- Cytokine profile by membrane-based antibody array (validated by ELISA for IL-10, IL-16 and TNF-α)
- MSC demonstrated immunomodulatory effects with downregulation of levels of pro-inflammatory cytokines in normal mice:
  - MIP-1α, MIP-1β, IL-1α, IL-1β, IL-16, MIP-2, Eotaxin, TNF-α, IL-6, KC, IL-3, IL-27, I-309, IL-7, JE, IP-10, Trem1, MCP-5, MIG, IL-12p70, IL-17

- Upregulation of anti-inflammatory cytokines:
  - IL-4, IL-5, RANTES
Cytokine Profiles

• AM depletion decreased levels of pro-inflammatory cytokines (MIP-1α, MIP-1β, IL-1α, IL-1β, IL-16, MIP-2, Eotaxin, TNF-α, IL-6, KC, IL-27, I-309)

• MSC treatment of AM-depleted mice was not effective in restoring the levels of cytokines to those observed in the normal mice, suggesting AM are important mediators of immunomodulatory effect of MSC
MSC reduce ANC, WBC and Protein in Nondeplete

- BALF WBC & ANC decreased in the AM-depleted
- Adding MSC had no effect in AM-depleted but reduced absolute neutrophil counts in nondepleted mice
- BALF protein influx was significantly decreased in the AM-depleted group versus nondepleted mice treated with PBS
- MSC treatment significantly reduced BALF protein concentration in nondepleted mice and had no effect in AM-depleted animals
MSC administration was associated with enhanced AM phagocytosis in vitro

- *In vitro* human MDM were infected with *E. coli* with or without direct coculture with MSC
- MSC coculture significantly reduced extracellular *E. coli* CFU counts (80%) and significantly elevated levels of intracellular CFU
Fluorescent imaging demonstrated mitochondrial transfer from MSC to macrophages

- MSC were labeled with 200 nM MitoTracker Deep Red for mitochondrial staining
- Using immunofluorescent imaging, observed that post 4 hours in coculture with MSC all MDM acquire MSC mitochondria - occurred at least partially through TNT-like structures
Demonstration of mitochondrial transfer from MSC to macrophages

- Population of MDM cultured alone, stained with CD45-PE but negative for MitoRed-APC.
- Population of MSC cultured alone, stained with MitoRed-APC but negative for CD45-PE.
- After 4 hours in coculture, more than 90% of CD45⁺MDM demonstrate acquisition of MitoRed fluorescence (APC⁺), indicating extensive mitochondrial transfer from MSC.
Mitochondrial transfer is associated with enhanced phagocytic capacity

- In *E. coli* pneumonia MSC (MitoRed)-treated mouse BALF was harvested and phagocytic activity of alveolar macrophage was assessed using fluorescent *E. coli* bioparticles by flow cytometry.

- Macrophages that had internalized MSC mitochondria (Mito+) showed a significantly higher phagocytic index in comparison to those without (Mito-)
Additional Observations

• Route of MSC administration (IN vs IV) did not affect MSC capacity to decrease severity of lung injury, decrease inflammation and improve bacterial clearance

• Similar proportions of MSC were recovered from BALF post either route
Conclusions

• Inhibition of mitochondrial transfer through blockage of TNT formation MSC resulted in failure to improve macrophage bioenergetics and complete abrogation of the MSC effect on macrophage phagocytosis in vitro and the anti-microbial effect of MSC in vivo

• Demonstrates that mitochondrial transfer from MSC to innate immune cells leads to enhancement in phagocytic activity and reveals an important novel mechanism for the anti-microbial effect of MSC in ARDS
# MSCs and ARDS

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<td>Curley, 2012 and 2013</td>
<td>IV and IT</td>
<td>MSCs enhance repair following vent induced lung injury via KGF-dependent mechanism</td>
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<tr>
<td>China/Phase I</td>
<td>1X10^6/kg (one dose) UCB MSC</td>
<td>Ongoing NCT02444455</td>
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<tr>
<td>China/Phase I</td>
<td>1X10^6/kg (one dose) Adipose MSC</td>
<td>Ongoing NCT01902082</td>
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<tr>
<td>Korea/Phase I</td>
<td>Autologous BM der</td>
<td>Ongoing NCT02112500</td>
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| Compassionate use | 2×10(6)/kg | Successful recovery of 2 patients Resolution of respiratory, hemodynamic, and multiorgan failure, decrease in pulmonary and systemic markers of inflammation  
*Simonson OE, et al. In vivo effects of mesenchymal stromal cells in two patients with severe acute respiratory distress syndrome. Stem Cells Transl Med 2015* |
| UCSF/Phase I/II | 3 per cohort: 1X10^6/kg, 5X10^6/kg, 1X10^7/kg allo BM derived (one dose) | Ongoing multicenter START trial  
Completed phase I  
Completed interim analysis of Phase II (40/70) NCT02097641  
Hematologic Malignancy and ARDS

- ARDS patients with heme malignancy:
  - Retrospective analysis of 78 patients from the ARDS network trial showed a 28 day mortality of 55%.
  - 1004 pts with malignancies (78% with hematologic malignancies) showed a 63% hospital mortality for moderate ARDS and 69% mortality for severe ARDS using the Berlin Definition.

- Recent review at our institution of SCT patients admitted to ICU. Respiratory failure MCC for transfer to ICU
  - 377 patients.
  - Overall inpatient mortality 64%.
  - 1 year overall survival 15%.

- MSCs have been shown to decrease inflammation, protect alveoli against ALI via mitochondrial transfer and secrete antimicrobials that may reduce the severity of bacterial infections supporting their use in ARDS.
Clinical Trial: MSCs for ARDS in Cancer Patients

Two arm randomized comparative phase II trial of MSCs versus standard care for ARDS

Primary Objective
• To demonstrate the safety of allogeneic human mesenchymal stem cells administered by intravenous infusion in patients with ARDS. (10 x 10^6 cells/Kg once).

Secondary Objective
• To demonstrate the efficacy of allogeneic MSCs administered by intravenous infusion in patients with ARDS.
Study Goals

• **Primary Goal:** To demonstrate the safety of allogeneic MSCs administered by intravenous infusion in patients with ARDS as measured by the absence of acute infusion events including the following endpoints:

  • Increase in more than 10mcg/minute norepinephrine, 100 mcg/minute phenylephrine, 10 mcg/kg/minute dopamine, 0.1 mcg/kg/minute epinephrine or addition of 3rd vasopressor
  • New ventricular tachycardia
  • Ventricular fibrillation
  • Asystole
  • New arrhythmia requiring cardioversion
  • Hypoxemia requiring increased fraction of inspired oxygen 0.2 or more and an increase in PEEP pf 5cm H2O or more to maintain oxygen saturation in range of 88-95%

• SAEs within 24 hours of infusion include cardiac arrest and death
Study Goals

Secondary Goal: To demonstrate improvement in the following endpoints
- Delta SOFA (score reduction) at 72 hours and 7 days
- SAPS II (score reduction) at 72 hours and 7 days
- P/F ratio improvement (increase) at 72 hours and 7 days
- Murray score reduction at 72 hours and 7 days
- Oxygenation-index at 72 hours and 7 days
- Ventilator-free days at 30 days
- Vasopressor-free days at 30 days
- Organ failures-free days at 30 days
- Mortality at 30 and 60 days

Tertiary Goal: To determine inflammatory biomarkers at screening and at day 3:
- Markers of inflammation: IL-6, IL-8, IL-10, IL-1Ra, TNF alpha, Interferon gamma
- Markers of endothelial injury: von Willebrand factor, angiopoeitin-2
At 30 days post initial infusion: vent, vasopressor, and organ failure free days, mortality
At 60 days: mortality

At 7 days: pulmonary endpoints
At 3 days: biomarkers and pulmonary endpoints

IV infusion of MSCs (10X10^6/kg)

Eligibility for the infusion of MSCs
Measure biomarkers at screening

Patients with moderate to severe ARDS

Within 72 hours
Statistical Considerations

• 48 patients randomized between the two treatment arms

• Randomization balanced so that 8, 16, and 24 patients are treated on each arm at each of the interim analyses, if the trial is not stopped early

• Early termination if higher than historical death rate (63%) is seen at interim analyses
Conclusions

• MSCs show promise in acute lung disease in preclinical and early clinical use

• IV administration appears to be comparable to IT

• Several new clinical studies in cancer and non-cancer patients actively recruiting
## Acknowledgements

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