MSCs for Acute Lung Injury

2013 ISCT Regional Meeting
Workshop 6, Sunday, September 8th
Optimizing Bench to Bedside Translation of Research – An Update from PACT

David McKenna, M.D.
University of Minnesota
Acute Lung Injury

- Most common cause of ALI/ARDS is severe bacterial pneumonia
- Nosocomial pneumonia in 25% of ICU patients
- Occurs in 200,000 ventilated patients annually in USA
- Leading cause of death among hospital acquired infections
  - Mortality of 20-35%

NHLBI ARDS Network Trials, NEJM 2000 & NEJM 2006
Acute Lung Injury

- Expected to increase with aging population
- Concerns for antibiotic resistance
- Large public health impact
- Current treatment primarily supportive
  – Lung-protective ventilation and fluid conservative strategy
- Pharmacologic therapies to reduce lung injury not yet translated to effective clinical treatments
Pulmonary Edema in ALI

- Underlying inflammatory response (e.g., pneumonia)
- Integrity of alveoli compromised
- Leads to leaky alveoli (junctions)
- Blood-air barrier fails
- Fluid accumulates
- Gas exchange down
The acute respiratory distress syndrome (ARDS) is an important cause of acute respiratory failure that is often associated with multiple organ failure. Several clinical disorders can precipitate ARDS, including pneumonia, sepsis, aspiration of gastric contents, and major trauma. Physiologically, ARDS is characterized by increased permeability pulmonary edema, severe arterial hypoxemia, and impaired carbon dioxide excretion. Based on both experimental and clinical studies, progress has been made in understanding the mechanisms responsible for the pathogenesis and the resolution of lung injury, including the contribution of environmental and genetic factors. Improved survival has been achieved with the use of lung-protective ventilation. Future progress will depend on developing novel therapeutics that can facilitate and enhance lung repair.
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MSC & ALI - Rationale

- Anti-inflammatory properties
- Restore endothelial & epithelial barrier integrity
- Enhance alveolar & lung edema fluid clearance
- Anti-microbial properties
- Anti-apoptotic effects
- Cell contact dependent & independent effects

Ware & Matthay, NEJM, 2000
Proof-of-Principle/Pre-Clinical Studies
In Vitro

- Measurement of fluid transport and protein permeability from apical to basolateral membrane of alveolar type II cells
- Transwell plate - 2 compartments
- AT II cells separated from MSCs by membrane (0.4 micron)
- AT II cells exposed to cytomix (IL-1β, TNF-α, IFN-γ; 50ng/mL) with ¹³¹I-albumin
- MSCs plated (250,000/well) prior to insult exposure
- Measurement reveals improvement of protein permeability
In Vivo

- C57BL/6 (non-immunosuppressed) male mice (8-10 wks old)
- Intra-trach administration endotoxin (5 mg/kg)
- 4 hr later MSCs (750K) given (intra-trach)
- PBS (30 uL), fibroblasts, apoptotic MSCs – controls
- Measured a variety of parameters/read-outs
Gupta N, et al. continued

Survival:

80% vs. 42% at 48 hr
64% vs. 18% at 72 hr
Gupta N, et al. continued

Lung injury and endothelial/epithelial permeability:
Gupta N, et al. continued

**MSC**

**PBS**


MSCs from NIH Repository – Tulane Prockop

Lung injury score

- Hemorrhage
  - PBS
  - MSC

- Edema
  - PBS
  - MSC

Gupta N, et al. continued

Decrease in pro-inflammatory cytokines:

- **A**: 24 h MIP-2 BAL (pg/ml) - PBS: ~6500, MSC: ~1100
  - Statistical significance: ****

- **B**: 24 h TNF-α BAL (pg/ml) - PBS: ~3000, MSC: ~1000

- **C**: 48 h MIP-2 BAL (pg/ml) - PBS: ~1200, MSC: ~300
  - Statistical significance: *

- **D**: 48 h TNF-α BAL (pg/ml) - PBS: ~1500, MSC: ~300
  - Statistical significance: *
Gupta N, et al. continued

Increase in anti-inflammatory cytokines:

![Bar charts showing increase in IL-10 levels]
MSCs Improved Survival in Live *E. coli* Pneumonia-Lung Injury in Mice with Marked Anti-Inflammatory and Anti-Bacterial Effects

*MSC vs PBS p < 0.01
#MSC vs 3T3 p = 0.03
3T3 vs PBS p = 0.26

Gupta et al, Thorax, 2012 (live bacteria)

Human MSCs Give IV Just As Effective as IT MSCs
Stem Cell, 2011
AJP:Lung, 2012
Summary of Results of MSC Studies in Mice

1. IT or IV MSCs improved survival and reduced lung injury in both endotoxin and live bacterial models of ALI, both associated with a reduction in the pro-inflammatory responses to endotoxin, increase in anti-inflammatory cytokines and reprogramming of M1 to M2 monocytes.

2. The antibacterial effect of MSC in mice was mediated by antimicrobial peptides and enhanced monocyte phagocytosis.
Ex Vivo
Ex Vivo Perfused Human Lung Preparation

- Peristaltic pump
  - Rate: 0.32 liters/min

- Lung
  - CPAP 10 cm H₂O
  - 95% O₂, 5% CO₂
  - Pulmonary Artery Pressure = 10 - 12 mm Hg
  - Left Atrial Pressure = 0 mm Hg

- Perfusate Reservoir
  - 37°C water bath

References:
- Frank J et al. AJP:Lung, 2007
- Lee JW et al, PNAS, 2009
After Severe E. coli Endotoxin injury in the Human Lung, MSC or MSC Conditioned Medium Restored Barrier Properties and Reduced Pulmonary Edema

Lee et al, PNAS, 2009
KGF Mediates Much of the Protective Effect of MSC-Conditioned Media Treated in the Perfused Human Lung

* P<0.001 vs. Control
\( \sqrt{\text{P}<0.03 \text{ vs. LPS (0.1 mg/kg)}} \)

# P<0.01 vs. LPS + CM MSC (KGF siRNA)

Lee et al, PNAS, 2009
MSCs
MSCs

• 1\textsuperscript{st} described in 1968 (Friedenstein)
  – Adherent, clonogenic, fibroblastic marrow cells
• Multiple sources
• ISCT definition (2006)
  – Plastic adherence
  – CD73, CD90, CD105 (+); lineage markers (-)
  – In vitro differentiation to bone, fat, cartilage
MSCs

• Immunologically well-tolerated
  – Low expression of MHC
  – Lack of T cell co-stimulatory molecules (CD80, CD86)
• Safe profile (>2,000 patients)
MSCs...
MSCs for ALI – Why?

• Ability to modulate immune system
  – DCs, T and B cells, other
  – Anti-inflammatory cytokines
  – Angiopoietin-1 (improves endothelial barrier)
  – Growth factors with cytoprotective and repair properties (e.g., VEGF, KGF, HGF)
  – Lipid mediators (e.g., prostaglandin-E2)
MSCs

- Marrow (small volume)
- MNC (density gradient)
- Plate on tissue culture flasks
- Carry adherent cells through (low seeding density)
- Limit passages (1)
- QC testing
  - Phenotype
  - Viability
  - Sterility
  - Endotoxin
  - Mycoplasma
  - In vitro differentiation
  - KGF (ELISA)
The path to the clinic...
Barriers to Translating hMSC Therapy to Testing in Patients with Acute Lung Injury/ARDS in 2010

1. No reliable source of high quality clinical grade human MSCs.

2. Funding not available for a clinical trial.

3. Although UCSF had been a major center for NHLBI sponsored phase 2 & 3 clinical trials of ARDS since 1995, they did not have the complete expertise at UCSF and in their group for how to proceed with an IND and the steps to testing hMSCs in the clinical setting.
This project is funded in part by PACT

PACT provides assistance for cellular therapy translational research and the manufacture of cellular therapy products

**PACT Cell Processing Facilities**

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- Center for Human Cell Therapy Boston  Contract#HHSN26820100009C
- City of Hope, Center for Applied Technology Development  Contract#HHSN268201000011C
- University of Minnesota, Molecular and Cellular Therapeutics  Contract#HHSN268201000008C
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PACT website
www.pactgroup.net

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Molecular & Cellular Therapeutics
Product Development Process

Proposal
- Product Information
- Process Information
- Regulatory and Clinical Information
- Funding Information
- Contractual Considerations
- Intellectual Property

Assessment
- Opportunity and Strategy
- Clinical Requirements
- Regulatory Pathway
- Compliance Requirements
- Technology Requirements
- Space Requirements
- Contractual Issues
- Cost Estimates
- Biosafety Issues
- Facility Master File

Development
- Process
- Test Methods
- Supplies
- Equipment
- Clinical Strategy
- Documentation
- Budget
- Timeline
- Contract and Billing
- CMC
- Suppliers

Validation
- Timeline
- Validation Plan
- Documentation
- Training
- Test Methods
- Equipment Qualification
- Validation Report
- Validation Approval
- Risk Management
- Budget Review
- Facility Master Plan

Clinical Use
- Product Review
- CAPA
- Clinical Review (SAEs, outcomes)
- Regulatory Compliance
- Quality Monitors
- Financial Review
- Project Review
- Customer Satisfaction
The Team

- PI/PI Team
- Cell Manufacturing Team
  - Lab/Med Dir
  - Operations/Facility Dir
  - QA Dir
  - R&D Lead
- Regulatory
MSCs for ALI

*In preparation for Ph I Trial*

Clinical-Grade (UM-made) MSC in the Ex Vivo Perfused Human Lung Model of ALI

Michael Matthay, M.D.
1. Clinical target of ALI/ARDS for MSC reasonable.

2. Proposed hMSC from PACT Program at the University Minnesota acceptable with some minor modifications.

3. But preclinical data **not** sufficient to support safety in the proposed clinical trial – would be preferable to test in a large animal model.

4. Single versus multiple dosing requires appropriate pre-clinical support in animal models.
Follow Up Pre-clinical Studies in Response to the Pre-IND FDA Review

1. Rat studies over 6 hours with acid induced lung injury with three doses of MSCs under GLP-like conditions.

2. Sheep studies over 24 hours in a model of severe lung injury in 2011 with Dr. Traber’s group at U. Texas.

3. Further discussions with the FDA in Jan, 2012 to plan a second round of sheep studies with two doses of MSCs and administration with DMSO 3%.

4. Further discussions with the FDA during the course of these 2012 experiments
Testing of Allogeneic Human MSC in Severe Acute Lung Injury in Sheep

1. Severe acute lung injury in anesthetized sheep injured by inhalation of cotton wood smoke plus direct instillation of live Pseudomonas aeruginosa in three lobes followed by mechanical ventilation, as in patients with ALI.

2. Sheep prepared to measure pulmonary and systemic hemodynamics, respiratory function, urine output and hourly samples of key biochemical indices.

3. Experiments carried out over 24 hours with the primary focus on the safety of administering intravenous hMSCs one hour after the injury (5 or 10 x 10^6 cells/kg) plus post mortem end points.
Systemic Arterial Blood Pressure Not Different Among Control versus hMSC Treated Sheep (2011)
Pulmonary Arterial Pressure (mmHg)

Plasmalyte  (n = 7)
MSC-Low  (n = 7)
MSC-High  (n = 4)

Pulmonary Arterial Blood Was Not Different Among the Controls or hMSC Treated Sheep (2011)
Oxygenation (\( \text{PaO}_2/\text{FiO}_2 \))
Was Improved with hMSC (2011)

* \( P = 0.02 \) by ANOVA test with Bonferroni adjustments.
Pulmonary Edema Was Reduced in Sheep Treated with the Higher Dose of hMSC (2011)

Wet/Dry Ratio (g H\(_2\)O/g dry weight)

- PlasmaLyte (n=7)
- MSC-Low (n=7)
- MSC-High (n=4)

* P = 0.01 compared to PlasmaLyte A alone group by Wilcoxon rank-sum test.
# P = 0.02 compared to PlasmaLyte A alone group by Wilcoxon rank-sum test.
Research/pre-clinical: NIH MSC Repository (Tulane/Texas Tech, Prockop)
Pre-clinical/pre-IND: NIH PACT Group (U of MN)
Michael Matthay, et al. submitted the 1200 page IND on December 19, 2012 and received the IND without any questions or changes on January 18, 2013.
Plans for the Clinical Trials of hMSCs in Severe Clinical Acute Lung Injury (ARDS)

1. Use washed hMSCs from University of Minnesota PACT program.

2. Phase 1 trial - dose escalation at 3 doses (1, 5, & $10 \times 10^6$ cells/kg) in patients with ARDS ($\text{PaO}_2/\text{FiO}_2 < 200$) to test safety, especially for hemodynamic and respiratory end points.

3. Phase 2 trial of 60 patients with ARDS with 2:1 randomization.

4. Safety end points will be primary but also include clinical respiratory and biological end points from plasma and mini-bronchoalveolar lavage.
Lessons learned as university-based programs...

1. High value of the pre-IND meeting with the FDA for design of appropriate pre-clinical studies to support clinical trial design.

2. Large animal model to support the design of the clinical trial very important, including ongoing consultation with the FDA.

3. Support from the NHLBI PACT program and the U01 funding mechanism to support the clinical trial.

4. Support from the UCSF CTSI (NIH T1 Catalyst) to obtain expert consultation to assist with preparation of the IND plus partial support for the Phase 1 trial was critical.

5. DMSO may interfere with efficacy of hMSCs.

6. Design of the initial clinical trials in ARDS need to be primarily focused on safety although some clinical and biologic efficacy end points can be tested but underpowered for most of these.
# Roadmap for Translation of Human Mesenchymal Stem Cells to a Clinical Trial for Acute Lung Injury

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Trial Update

- First 3 patients treated (1 million MSC/kg)
- DSMB review and approval to move forward
- Next 3 patients at 5 million MSC/kg...
- Stanford and MGH nearly ready for patient enrollment
- Pittsburgh, VT to follow
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MEDICAL SCHOOL
University of Minnesota