MSCs for Acute Lung Injury: The Manufacturing Center's Perspective

ISCT Paris
Bench to Bedside: MSCs for ARDS
Thursday, April 24, 2014

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University of Minnesota
Disclosures

• No conflicts of interest
• Cell manufacturing support through Production Assistance for Cellular Therapies (PACT)/NIH
Objectives

• Describe clinical laboratory support of pre-clinical studies of MSCs in acute lung injury (ALI).
• Discuss approach to manufacturing of MSCs for the clinical trial in ALI.
• Review testing of MSCs, including an early phase potency assay.
Roadmap for Translation of Human Mesenchymal Stem Cells to a Clinical Trial for Acute Lung Injury

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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

From PACT website (MOP): http://www.PACTgroup.net
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 (several thousand patients)
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)
Our Experience

• Visited Tulane several years ago (D. Prockop)

• Based clinical manufacturing on their approach
  – Low seeding density
  – Low passage
  – FBS
## Flask Seeding Calculator

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### CELL FACTORIES

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MSC Manufacturing

- 3rd party MSCs
- Limit donors (2)
- 3-5 billion cells/lot
- Ph II:
  - 1 billion cells/pt
  - 40 patients (cells)
  - 8-13 lots needed
  - Packaging

- Trilineage assays
- KGF assay

Human KGF/FGF-7 Immunoassay

Catalog Number DKG00

For the quantitative determination of human keratinocyte growth factor (KGF) concentrations in cell culture supernates, serum, and plasma.
Potency Testing

When?

Please be advised that you will be required to establish a potency assay before initiating Phase 3 trials and validate this assay prior to submission of a license application. Your potency assay should be a measure of “the specific ability or capacity of the product…to effect a given result”.

Summary from Pre-IND Call (FDA/CBER/OCTGT) for an MSC-based IND
Potency Testing

When? and Why?

ASAP because…

There are a number of advantages, such as allowing you to:

• Demonstrate product activity, quality and consistency throughout product development
• Generate data to support specifications for lot release
• Provide a basis for assessing manufacturing changes
• Evaluate product stability
• Recognize technical problems or reasons a different assay might be preferable
• Evaluate multiple assays
• Collect sufficient data to support correlation studies, if necessary
Potency

How?

- **Biological assay** – potency in a living biological system (in vivo animal, in vitro organ, tissue, cell culture)
- **Non-biological analytical assay** – surrogate of biological activity; immunochemical, biochemical, molecular attribute with correlation to biological activity
- **Multiple assays** (assay matrix) – should have at least one quantitative component

Using progressive implementation...

- Early product development
- Later phase product development
- Biological license

*Much more detail in the Draft Guidance*

KGF Mediates Much of the Protective Effect of MSC-Conditioned Media Treated in the Perfused Human Lung

* P<0.001 vs. Control
\( \sqrt{ } \) P<0.03 vs. LPS (0.1 mg/kg)
# P<0.01 vs. LPS + CM MSC (KGF siRNA)

Lee et al, PNAS, 2009
KGF as potency assay

• KGF has been suggested to be a paracrine effector for a number of different epithelial cell types

• Synthesized by several cell types, including fibroblasts and MSCs, it is proposed to act locally on the overlying epithelial sheet

• In addition to its ability to induce cell proliferation, it may also promote epithelial differentiation
KGF as potency assay

• Employs a quantitative sandwich enzyme immunoassay technique (R&D Systems, Mpls, MN)
• Monoclonal antibody specific for KGF pre-coated onto microplate
• Standards and samples are pipetted into the wells and any KGF present is bound by the immobilized antibody
• After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for KGF is added to the wells
• Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of KGF bound in the initial step
• The color development is stopped and the intensity of the color is measured
KGF as potency assay

• Batching testing
• Performing on each lot
• All phase I patients received same lot
• Analysis to follow
The path to the clinic...
Roadmap for Translation of Human Mesenchymal Stem Cells to a Clinical Trial for Acute Lung Injury

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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

-Baylor College of Medicine, Center for Cell and Gene Therapy Contract#HHSN268201000007C
-Center for Human Cell Therapy Boston Contract#HHSN26820100009C
-City of Hope, Center for Applied Technology Development Contract#HHSN26820100011C
-University of Minnesota, Molecular and Cellular Therapeutics Contract#HHSN26820100008C
-University of Wisconsin - Madison, Waisman Clinical BioManufacturing Facility Contract# HHSN26820100010C

The EMMES Corporation serves as the Coordinating Center Contract#HHSN26820100006C

PACT website www.pactgroup.net

PACT is federally funded by National Heart, Lung, and Blood Institute, National Institutes of Health, Department of Health and Human Services
Research/pre-clinical: NIH MSC Repository (Tulane/Texas Tech, Prockop)
Pre-clinical/pre-IND: NIH PACT Group (U of MN)
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 preclinical 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.

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.
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.
Lessons learned as university-based programs...

**Engage regulators (FDA):**

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.

**Creatively secure funds:**

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

2. 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.

**Gather the team and focus on realistic goals:**

1. 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.

2. Team approach and collaboration among centers/labs is critical.
Challenges for Phase II

• Large dose (10M MSC/kg = 1B MSC/pt)
  – Time
  – Cost

• Maintaining blinding of investigators
  – Inventory
  – On site/infusion
Long-term Challenges
Standardization of CT Manufacturing

• Possible?
  – *Donor variability, batch to batch variability and variability induced by culture conditions*
  – *Many clinical trials, little detail in methods*

• At early stages of therapeutic development…
  balance standardization against learning and innovation

• At minimum effort should be made to standardize for given trial (and development to trial)
Long-term Challenges

• Move toward optimizing MSC manufacturing
  • Culture (starting material, media, additives, length/passages, etc.)
  • Cryopreservation/thaw (Hubel et al)


• Centralized QC assay sites for voluntary participation
Acknowledgements

MCT

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Kristen Reyna
Julie LaTour
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Maria Opitz

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