Mesenchymal Stromal Cells
Large-scale Culture

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Hanley et al: Efficient manufacturing of therapeutic mesenchymal stromal cells with the use of the Quantum Cell Expansion System
Cytotherapy in Press
“Adult” Stem Cells

- Human Stem Cell
- Adult Stem Cell
- Adult Somatic Stem Cells

- Pancreatic
- Gut
- Mesenchymal
- Neuronal
- Epidermal
- Hepatic
- Hematopoietic

- Marrow Stromal Fat
- Cord Blood
- Limbal Stem cells
- Pancreas
- Islets
- Muscle
- Tendon
- Ligament
- Cartilage
- Fat
- Bone Marrow
- Peripheral Blood

- Eye
- Retina
Clinical Trials using MSC

www.ClinicalTrials.gov: 352 studies on Mesenchymal stem cells

- Osteoarthritis
- Severe Brain Injury (Adipose)
- Ischemic Stroke (BM)
- Ischemic cardiomyopathy (BM)
- Multiple sclerosis (Adipose, BM, Cord blood)
- Systemic sclerosis
- Liver cirrhosis
- Lateral epicondylitis (Adipose)
- Hereditary ataxia
- Rheumatoid arthritis (Cord Blood)
- Osteoarthritis (BM)
- Ulcerative colitis (Cord blood)
- Type 1 diabetes (Cord Blood)
- Type 2 diabetes
- Spinal Cord Injury (BM)
- Critical limb ischemia in diabetes (Adipose)
- Tibial & Femoral fractures
- Cerebral artery infarcts (BM)

- Crohn’s disease (BM, Adipose)
- Pulmonary fibrosis (BM)
- Parkinson’s disease (BM)
- Acute respiratory distress (Adipose)
- Myocardial infarction
- Liver failure
- Cleft lip and palate
- Cartilage defects (BM)
- Retinitis pigmentosa (BM)
- Degenerative disc disease
- Cerebellar ataxia (Adipose)
- Ulcerative colitis (Adipose)
- Lupus nephritis
- Mental retardation
- Muscular dystrophy (Cord Blood)
- Amyotrophic lateral sclerosis
- Chronic wound healing
- Emphysema
Other uses for MSCs

- Prevention of GvHD in hematological malignancies
- Prevention of subclinical rejection in organ transplant
- Treatment of chronic GvHD
- Promotion of engraftment in unrelated BMT
- Treatment of steroid refractory GvHD
- Poor graft function
- Co-infusion with cord blood stem cells
- Induction of renal transplant tolerance
- Co-infusion in mismatched mini-transplants
Aim

• Generation of allogeneic MSC for treatment of stroke
• FDA wanted us to use a method in clinical trials – Ed Horwitz, CHOP
• Used platelet lysate instead of serum
• Wanted to try to close up system
• Looked at methods that would do that
Traditional Culture Method

P Hanley et al. Manufacturing mesenchymal stromal cells for phase I clinical trials
Terumo Quantum Bioreactor

- Automated bioreactor
- Closed system
- Whole marrow as starting material
- Cells must meet all criteria for MSC
- Cleared for IND manufacturing
Terumo Quantum Bioreactor

- Stand-alone device
- Automated, hollow-fiber bioreactor system

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Quantum Manufacturing Procedure

Day 0: Bone Marrow

Flow rate starts @ 0.1ml/min. Monitor lactate & glucose. If >lactate 4mM then double feeding rate, until reaches 0.4ml/min and lactate is at 4mM.

Passage 1

10-14 days

Wash out non-adherent cells @ 24-48hr

Passage 2

7-10 days

Flow rate starts @ 0.1ml/min. Monitor lactate & glucose. If >lactate 4.5mM then double feeding rate, until reaches 1.6ml/min and lactate is at 5mM.

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Expansion of MSCs in the Quantum and Flasks

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Cell Doubling Times

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MSC Phenotype & Viability

CFU-F Recovery

P = 0.07

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MSC Differentiation Potential

Cells tested for ability to differentiate into:

- Adipogenic cells
- Osteogenic cells
- Chondrogenic cells

All three cell types seen

From: Research Center for Molecular Medicine, Debrecen, Hungary
http://rcmm.dote.hu/research-groups/oxidative-stress-and-adr-ribosylation/
T cells suppression by MSC

% CD4 T Cell Proliferation

Flasks
Quantum

1:0            1:1         1:05       1:0.1      1:0.05   Unstim

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# Open events using Flasks versus the Bioreactor

<table>
<thead>
<tr>
<th></th>
<th>T-175 Flask</th>
<th>Bioreactor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Per Flask/Expansion Set</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seeding Cells</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Exchanging Media</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Cell Dissociation</td>
<td>6</td>
<td>3</td>
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<tr>
<td>Total</td>
<td>10</td>
<td>7</td>
</tr>
<tr>
<td>Per Donor (340 Flasks)</td>
<td>340 Flasks</td>
<td>3,400</td>
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<td></td>
<td></td>
<td>7</td>
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<tr>
<td>Number of Donors / Expansion Sets</td>
<td>16 Donors*</td>
<td>19 Expansion Sets (1 donor)</td>
</tr>
<tr>
<td>Total</td>
<td>54,400</td>
<td>133</td>
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</tbody>
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Considerations

- Growth assessment is indirect – lactate production
- Disposable must be pre-coated with fibronectin
  - Can be accomplished in 4 hours
- Cost of disposable
  - Offset by cost of labor
- Cost of bioreactor
  - Offset by multiple applications (retrovirus manufacturing)
Regulatory Considerations

- Cleared by FDA for use in stroke Phase 1 trial
  - Selling point – closed system
  - Comparability of cells from flasks and bioreactor
  - Issues regarding allogeneic “cell bank”
    - Number of patients to be treated
    - Degree of testing required
Collaborators

- **Patrick Hanley** – Children’s National Medical Center, Washington D.C.
- **Zhuyong Mei** – CAGT
- **April Durett** – CAGT
- **Graca Cabreira-Harrison** – Texas Heart Institute
- **Mariola Klis, Wei Li, Yali Zhou** – CAGT
- **Peiman Hematti & Debra Bloom** – University of Wisconsin
- **Sean Savitz et al.** - University of Texas Health Science Center
- **Brent Rice** – Terumo BCT
Thank you