TREATMENT of HUMAN DISEASES: CELL-BASED THERAPIES using ADULT MESENCHYMAL STEM CELLS.

October, 2008
NJ Symp Biomat & Regen Med
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Skeletal Research Center
Case Western Reserve University
The Impact of Regenerative Medicine

**Teeth:** Enamel matrix proteins are used to fill cavities. It works in dogs; human trials are a few years away.

**Saliva glands:** Proteins called *aquaporins* that allow cells to secrete water are used to recreate saliva glands damaged by disease or radiation. Glands are also being engineered to secrete healing drugs. The technique has proven successful in mice.

**Bone:** Bone-growth factors, or stem cells, are inserted into a porous material cut to a specific shape, creating new jaws or limbs. A product that creates shinbones is in clinical trials.

**Cartilage:** A product is already on the market that regrows knee cartilage. A chest has been grown for a boy and a human ear on a mouse.

**Heart valves, arteries, and veins:** A 10-year initiative to build a heart has just started. Genetically engineered proteins have been successfully used to regrow blood vessels.

**Breast:** In preclinical studies, several companies have been able to create a cosmetic nipple by inserting a ball of cartilage. Researchers are now trying to grow a whole cosmetic breast.

**Pancreas:** Insulin-manufacturing cells are harvested from pigs and injected into the abdomen. The method has been tested in animals and could be in human trials in 2 years.

**Liver:** A spongy membrane is built up and then seeded with liver cells. Organs the size of a dime have been grown, but a full-size liver could take 10 years because of its complexity.

**Spinal cord nerves:** Scientists are investigating nerve-growth factors, injecting them at the site of damage to encourage regeneration or seeding them along biodegradable filaments and implanting them. Rats have been made to walk again.

**Urinary tract:** Cartilage cells are taken from the patient, packed into a tiny matrix, and injected into the weakened ureter, where they bulk up the tissue walls to prevent urinary backup and incontinence. The method is in late-phase clinical trials.

**Bladder:** Doctors at Children’s Hospital in Boston have grown bladders from skin cells and implanted them in sheep. They are about to try the same process on a patient.

**Skin:** Organogenesis’ Apligraf, a human-skin equivalent, is the first engineered body part to win FDA approval, initially for leg ulcers. Other skins are in the works for foot ulcers and burns.
Regeneration (vs repair) of embryonic tissues is the central logic for the field of **REGENERATIVE MEDICINE**: The regenerative micro-environment in embryonic tissues is quite different from the regenerative micro-environment in adult tissues.
The Injury Response Cascade

- Acute Injury
- Inflammation
- “Regeneration”
- Fibrosis
- Scar Absent

Magnitude

Time

Acute Injury

EMBRYO
The Injury Response Cascade

**ADULT**

- **Inflammation**
- **"Regeneration"**
- **Fibrosis**

- **Magnitude**
- **Time**
- **Acute Injury**
- **Quick Fix**
- **Scar Complete**
The Injury Response Cascade

- **Acute Injury**
- **Inflammation**
- **"Regeneration"**
- **Scarless Regeneration**
- **Fibrosis**

**CELLS**

**Magnitude**

**Time**
The Injury Response Cascade

Acute Injury

IL-1α, IL-1β, TNF-α, IFN-γ, MCP-1, TGF-β...

IL-6, MMP-7, VEGF, BDNF, Endothelin, Angiogenin...

FGFs, TGF-β3, Type 1 Collagen, HA...

hMSC Rx

Scarless Regeneration
The Injury Response Cascade

**Acute Injury**

IL-1α, IL-1β, TNF-α, IFN-γ, MCP-1, TGF-β...

IL-6, MMP-7, VEGF, BDNF, Endothelin, Angiogenin...

FGFs, TGF-β3, Type I collagen, HA...

**Scarless Regeneration**

hMSC Rx
Adult Bone Marrow

HSC

MSC
THE MESENGENIC PROCESS

Mesenchymal Stem Cell (MSC)

Proliferation
- Osteogenesis
- Chondrogenesis
- Myogenesis
- Marrow Stroma
- Tendogenesis/Ligamentogenesis
- Other

Commitment
- Transitory Osteoblast
- Transitory Chondrocyte
- Myoblast
- Transitory Stromal Cell
- Transitory Fibroblast

Lineage Progression
- Osteoblast
- Chondrocyte
- Myoblast Fusion
- Unique Micro-niche

Differentiation
- Osteocyte
- Hypertrophic Chondrocyte
- Myotube
- Stromal Cells
- T/L Fibroblast

Maturation
- Bone
- Cartilage
- Muscle
- Marrow
- Tendon/Ligament
- Adipocytes, Dermal and Other Cells
- Connective Tissue
Bone Marrow Aspirate

Density Solution

Centrifugation

Plate cells at Interface

Adhere to Culture Dish

Colony Formation

Primary MSC Culture

CFU-F

Serum batch dependant

Passage Culture
Human MSCs Decline With Age:

Estimates obtained by CFU-F assay.
Bone Marrow Aspirate

Centrifugation

Density Solution

Plate cells at Interface

Adhere to Culture Dish

Colony Formation

Primary MSC Culture

Serum batch dependant

Passage Culture
MSCs

TISSUES

REGENERATE

TISSUE ENGINEERING

TURNOVER/MAINTENANCE

MICROENVIRONMENT/MILIEU
THE MESENGENIC PROCESS

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BONE CARTILAGE MUSCLE MARROW TENDON/LIGAMENT CONNECTIVE TISSUE
MSCs

TISSUES

REGENERATE

TISSUE ENGINEERING

TURNOVER/Maintenance

MICROENVIRONMENT/MILIEU
MSCs

REGENERATE

TISSUES

TURNOVER/MAINTAINANCE

MICROENVIRONMENT/MILIEU

BIOACTIVE FACTORS

IMMUNO-SUPPRESSIVE
hMSCs promote Th2 responses by inhibiting IFN-γ and TNF-α and increasing IL-10 (PGE₂-mediated). Also hMSCs alter antigen-presenting maturation and induce T-cell unresponsiveness (Beyth et al., Blood 2005;105:2214-19).
now: ALLOGENIC hMSCs.

Started Dec.1992: AIC FOUNDER.

BIO-ORTHOPEDICS
Osiris Pipeline in Cell Therapy:
Osiris Pipeline in Cell Therapy:
PROCHYMAL

GVHD PHASE II Trial:

(survival and the safety and tolerability of the drug): a randomized, prospective, open label trial at 16 cancer centers in the USA with 32 patients with grades II-IV GVHD. Now FDA OK PHASE III.

• 77% complete remission in 28 days.
• 61% at 6 months had a durable response requiring no additional immunosuppressive therapy or clinical intervention.
• 95% were alive at 6 months compared to patients receiving additional immunosuppression (25% survival). No adverse events.
**PROCHYMAL**

**GVHD** Compassionate use: 
Now (5/2008) APPROVED for PAYMENT & USE.

Prochymal infusions (3-21) in 12 pediatric patients (5 months to 15 years of age) suffering from treatment resistant, severe (grades III/IV), end-stage GVHD:

- All patients (12/12) showed a clinical response to therapy with 58% (7/12) achieving COMPLETE resolution of GVHD. Less than 15% who fail immunosuppression survive to 100 days.

- Clinical improvements in GI GVHD (75% COMPLETE REMISSION and 3 with regression to grade I).

No infusion toxicity or adverse events.

Dec. 10, 2007
Osiris Pipeline in Cell Therapy:
Osiris Therapeutics announced today that it has been awarded a $224.7 million contract, including purchase options, from the United States Department of Defense (DoD) to develop and stockpile Prochymal for the repair of gastrointestinal injury resulting from radiation exposure. Under the terms of the contract, the DoD will provide funding to Osiris for the development of Prochymal for acute radiation syndrome (ARS) in two stages, with an initial amount of $4.2 million in 2008. Upon Food and Drug Administration approval for ARS, the contract provides for the purchase of up to 20,000 doses, at $10,000 per dose, of Prochymal in four 5,000 dose increments. Prochymal was selected by the DoD as part of an open and competitive solicitation with pre-specified criteria that included safety and efficacy data, manufacturing capacity, soundness of the development plan, and time to final product delivery.
Osiris Pipeline in Cell Therapy:
Dose Response to Prochymal™ in Patients with Treatment-Resistant Crohn’s Disease

CDAI Response

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<tr>
<th></th>
<th>Low</th>
<th>Avg</th>
<th>High</th>
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<tbody>
<tr>
<td>7 days</td>
<td></td>
<td></td>
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<tr>
<td>28 days</td>
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Copyright 2007 Osiris Therapeutics, Inc.
**Phase III Clinical Evaluation of Prochymal in Treatment Resistant Crohn’s Disease:**

<table>
<thead>
<tr>
<th>Protocol 603</th>
<th>Phase III Trial Evaluating Prochymal for the Treatment of Moderate to Severe Crohn’s Disease Refractory to Biological Therapy</th>
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<tbody>
<tr>
<td>Design</td>
<td>270 patient, double-blind, placebo controlled, randomized 1:1:1 (high dose, low dose, placebo)</td>
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<tr>
<td>Population</td>
<td>Patients 18 to 70 years with CDAI &gt;250 that is not responsive to biological therapy</td>
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<tr>
<td>Dose</td>
<td><strong>Active High Dose:</strong> 2 infusions of $400 \times 10^6$ hMSC followed by 2 infusions of $200 \times 10^6$ hMSC over 2 weeks</td>
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<tr>
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<td><strong>Active Low Dose:</strong> 2 infusions of $200 \times 10^6$ hMSC followed by 2 infusions of $100 \times 10^6$ hMSC over 2 weeks</td>
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<td><strong>Placebo:</strong> Volume equivalent of excipient for each of 4 infusions</td>
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<td>End Points</td>
<td>Proportion of patients with CDAI &lt; 150 at day 28; 100* and 70 pt drop in CDAI at day 28</td>
</tr>
<tr>
<td>Centers</td>
<td>60 leading centers, U.S. and Canada</td>
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</tbody>
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*270 patients* Patients experiencing a 100 point drop in CDAI are eligible for participation in Protocol #610: a double-blind, placebo controlled evaluation of Prochymal for the maintenance of Crohn's Disease.
Osiris Pipeline in Cell Therapy:
12 million in USA have Chronic Obstructive Pulmonary Disease.
ASTHMA: MOUSE OVA MODEL

• Sensitize mouse to OVALBUMIN.
• After 2 wks, every other day, spray OVA into the lungs for 1, 4, 8, or 12 wks.
• Tail vein inject SALINE or $10^6$ hMSCs; continue OVA spray for 1 wk.
• Harvest / histology at the end of next wk.

AIC et al with Tracy L. Bonfield at CWRU.
4 Week OVA Challenge:

- Inject SALINE/1WK
- Inject hMSCs/1WK

Airway Hyperplasia Epithelium

Airway Epithelium
Anti-inflammatory effects of mesenchymal stem cells: novel concept for future therapies
TROPHIC

CAPLAN: CELL PRODUCED, BIOACTIVE FACTOR MEDIATED:

1. ANTI-APOPTOTIC/CYTOPROTECTIVE
   Limits field of ISCHEMIC injury

2. ANTI-FIBROTIC
   ANTI-SCARRING

3. ANGIOGENIC
   VEGF+ PERICYTE

4. MITOTIC/Regenerative Milieu
TROPHIC

CELL PRODUCED,
BIOACTIVE FACTOR MEDIATED:

1. STROMA: Bone marrow transplant.
2. HEART: Infarct (ischemia).
3. BRAIN: Stroke (ischemia).
4. SPINAL CORD: Axonal tracking.
5. TENDON: Tissue Regeneration.
6. MENISCUS: Tissue Regeneration.
7. KIDNEY: Acute Renal Failure (ischemia).
TROPHIC

CELL PRODUCED, BIOACTIVE FACTOR MEDIATED REGENERATIVE MILIEU.

MSCs as regulated multi-drug delivery vehicles.

MSCs as DRUG STORES.
MSCs

MICROENVIRONMENT/MILEAOU

IMMUNO-SUPPRESSIVE

BIOACTIVE FACTORS

TROPHIC

REGENERATE

TISSUE ENGINEERING

TiSSUES

TURNOVER/MAINTAINANCE

REGENERATIVE MEDICINE
Mesenchymal Stem Cells: The basis for new cell-based therapies.

- Regenerate Tissue
- Regulate Immune System
- Prevent Scar Formation
- Form New Blood Vessels
- Home To Injury Site
- Tissue Engineering
The Mesengenic Process

Mesenchymal Stem Cell (MSC)

Proliferation → Osteogenesis, Chondrogenesis, Myogenesis, Marrow Stroma, Tendogenesis/
Ligamentogenesis → Differentiation → Maturation → REGENERATIVE MEDICINE

Bone, Cartilage, Muscle, Marrow, Tendon
Pericytes: cells on capillaries and microvessels.

modified by BRUNO PEAULT
from http://www.geocities.co.jp/HeartLand-Suzuran/9389/kekkan
MSCs

**HYPOTHESIS**: ALL MSCs are PERICYTES and are, thus, found throughout the body.

Function to stabilize blood vessels, small & large.

Function to be at sites of local tissue damage:

1. Mute or suppress immuno-surveillance, thus, insuring that autoimmune reactions are not initiated (T-cell centric).
2. Limit the field and scope of injury (anti-apoptotic).
3. Stimulate angiogenesis.
4. Stimulate regeneration by mitotically expanding tissue-intrinsic progenitors at the injury site.
ALL blood vessels are not equal. Marrow MSCs are not fat MSCs. Marrow MSCs are not muscle MSCs. Marrow MSCs are not brain MSCs. Marrow MSCs are not X MSCs. Marrow MSCs are not Y MSCs. Marrow MSCs are not Z MSCs.
MSCs/ PERICYTES

REGENERATIVE:
- IMMUNO-MODULATORY
- ANITI-SCARRING
- CYTOPROTECTIVE / ANTI-APOPTOTIC
- ANGIOGENIC
- MITOTIC

*ie.* TROPHIC / REGENERATIVE
MSCs/ PERICYTES

TISSUES / SITES
SKIN
MUSCLE
SPINAL CORD
BONE MARROW
ORGANS

DELIVERY / DOSE
Regenerative Medicine and Adult Stem Cell Therapy.
Supported by

National Institutes of Health