Mesenchymal stem cells & Clinical applications

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WHAT ARE MESENCHYMAL STEM CELLS (MSC) ?

• First isolated by Friedenstein et al in 1974
• Fibroblastoid cells - spindle-shaped
• Adherent to tissue culture glass or plastic
• High growth potential
Mesenchymal Stem Cells

**SOURCE**
- Bone Marrow
- Adipose Tissue
- Umbilical Cord
- Amniotic Fluid
- Skeletal Muscle
- & Others

**ESC/IPSC**

**CHARACTERIZATION**
- CD Surface Antigens
- Chemokine Receptors
- Cytokines
- Mitogens
- Immunological Features

**APPLICATIONS**
- Basic Biology
- Cancer Biology
- Genomics
- Drug Discovery
- Cell Therapy

**ISOLATION**

**EXPANSION**

**MESODERM DIFFERENTIATION**
- Chondrocyte (cartilage)
- Osteocyte (bone)
- Adipocyte (fat)
- Myoblasts (muscle)

**TRANSDIFFERENTIATION**
- Neural
- Hepatic
- Endothelial

**MULTI-LINEAGE ADULT STEM CELLS** (i.e. MAPC, MPLC, MIAMI, etc.)
Centrifugation
Passage Culture
Bone Marrow Aspirate
Adhere to Culture Dish
Primary MSC Culture
Colony Formation
Plate cells at Interface
Density Solution
Serum batch dependant
CFU-F
HOW TO ISOLATE MSC?

• Sources: BM, periosteum, synovium, fat, cord blood, peripheral blood, foetal liver and lung
• Adherent to culture bottle; haemopoietic stem cells do not
• Easily subcultured
In vitro MSC growth pattern-

doubling time 33hr

(Conget et al 1999)
HOW TO IDENTIFY MSC?

• Morphology
• Adherent to glass or plastic
• Surface markers: SH2, SH3, SH4, STRO-1, ICAM-2, NCAM, integrins, PDGF.
• CD105, CD73, and CD90 while lacking CD45, CD34, CD14, CD11B, CD79, CD19, and human leucocyte antigen (HLA)-DR.
• They adhere to plastic and are capable of massive *in vitro* expansion in culture. Capable to be induced in vitro to differentiate into osteoblasts, chondrocytes, and adipocytes
TRANSDIFFERENTIATION

• MSC – mesodermal origin
• Can differentiate into cells of endodermal and ectodermal origins
• Neurons, hepatocytes, islet cells, skin
MSC-> Neurons (Martin et al 2002; Choong & Cheong 2003)

160 kDa neurofilament M

the nerve growth factor receptor

βIII tubulin
REPORTED USE OF MSC
ADENOSINE DEAMINASE DEFICIENCY

• Gerson et al 1997 implanted subcut MSC graft transduced with a functional adenosine deaminase gene
FABRY’S DISEASE

- X-linked genetic disorder - deficiency of lysosomal enzyme alpha-galactosidase
- Using patient’s own MSC
- Transduced with a functional galactosidase gene
- Return MSC to the patient
- Correction of deficiency (Osiris, 2000)
OSTEOGENESIS IMPERFECTA

- Horwitz et al 1999 reported 3 children transplanted with allogeneic MSC from HLA-compatible siblings
- New lamellar bone formation, improved osteogenesis with fewer fractures
- Engrafted MSC were shown to differentiate into osteoblasts
ANAEMIA CORRECTION

• Erythropoietin shown to be useful in chronic renal failure and anemia of cancers
• MSC transduced with erythropoietin gene (Lim & Cheong 2003)
• May be as good as external injection
INSULIN FOR TYPE I DIABETES MELLITUS

• Insulin deficiency
• Can be corrected with islet cell transplant
• MSC -> brand new autologous islet cells
• MSC -> transduced with gene responsible for insulin production
• Allogeneic transduced MSC also feasible
REPARATIVE MEDICINE

- Since MSC are pluripotent and capable of being induced to differentiate into different types of cells
- Exploited to replace diseased cells or tissues
- ? Heart cells for infarcted myocardium
- ? Neurons for Parkinson’s disease
- ? Meniscus regeneration and replacement
Figure 1 Number of registered clinical trials of mesenchymal stem cell-based therapy on ClinicalTrials.gov.

Figure 3 Percentages of the common diseases now treated with mesenchymal stem cells

- Myocardial infarction (22.9%)
- Diabetes (10.3%)
- Spinal cord injury (9.2%)
- Crohn’s disease (3.8%)
- Aplastic anemia (1.5%)
- Rheumatoid arthritis (1.1%)
- Brain injury (0.4%)
- Graft versus host disease (16.0%)
- Liver cirrhosis (10.3%)
- Osteoarthritis (8.0%)
- Multiple sclerosis (3.4%)
- Systemic lupus (1.1%)
- Parkinson’s disease (0.8%)
- Others (11.0%)

A bone marrow transplant is a procedure that replaces abnormal or missing cells in the bone marrow with healthy stem cells derived from either the marrow of one self- Autologous or from a donor- Allogeneic.
Transplant prehistory
Ulster, Ireland circa 500 BC
Indication: major trauma
Stem cell source: bos taurus xenograft Outcome: died d+ 7 (major trauma)
Reference: Tain Bo Cuialnghe (The cattle raid of Ulster)
Clinical Stem Cell Transplantation and the beginnings of HLA typing

- **1957-Thomas**
  - Safe infusion of marrow into humans

- **1959-Mathé**
  - First bone marrow transplants for radiation accident victims.

- **1958-Dausset**
  - First HLA antigen described (A2)

- **1963-Mathé**
  - First successful complete engraftment and survival of over 1 year, description of acute and chronic GVHD in men

- **1968-van Rood/Terasaki**
  - Modern HLA serologic typing available
  - Secondary disease-running syndrome-GVHD

- **1968-Good (Minneapolis) De Vries (Leiden)**
  - First successful HLA-matched sibling transplant for SCID
Transplanters hall of fame

Georges Mathé  E Donnall Thomas  JJ van Rood
Jean Dausset  Robert Good  Paul Terasaki
Bone Marrow Transplant – Autologous

1. Collection
   Stem cells are collected from the patient's bone marrow or blood.

2. Processing
   Blood or bone marrow is processed in the laboratory to purify and concentrate the stem cells.

3. Cryopreservation
   Blood or bone marrow is frozen to preserve it.

4. Chemotherapy
   High dose chemotherapy and/or radiation therapy is given to the patient.

5. Reinfusion
   Thawed stem cells are infused into the patient.

Bone Marrow Transplant – Allogeneic

1. Collection
   Stem cells are collected from the patient's bone marrow or blood.

2. Processing
   Bone marrow or peripheral blood is taken to the processing laboratory where the stem cells are concentrated and prepared for the freezing process.

3. Cryopreservation
   Bone marrow or blood is preserved by freezing (cryopreservation) to keep stem cells alive until they are infused into the patient's bloodstream.

4. Chemotherapy
   High dose chemotherapy and/or radiation therapy is given to the patient.

5. Infusion
   Thawed stem cells are infused into the patient.
Types of Transplant

Autologous and Allogenic Donors:
  Syngeneic twin
  Matched sibling donor
  Mis-matched family donor (Haplo)
  Matched unrelated donor
  Cord blood donation
  “Saviour” sibling
  “Matched” unrelated cord-blood
A fatal syndrome of skin abnormalities and diarrhea (or wasting, in newborns) given allogeneic spleen cells after irradiation.

BILLINGHAM AND BRENT -1953
• The graft must contain immunologically competent cells.
• The host must possess alloantigens that are lacking in the graft (must appear foreign).
• The host must be incapable of mounting an effective anti-graft reaction, at least for a period of time.
• alloreactivity  
  • degree of mismatch  
  • strength of mismatch  
• sex mismatch  
• donor parity  
• age  
  • recipient age  
    • 20%, 30%, and 80%  
    • at <20, 45-50, and >50  
  • donor age  
• stem cell source  
  cord: ?less 
  marrow 
  PBSC: more chronic 
  whether T cell depleted 
microbes 
  particularly herpes family 
conditioning  
  immune suppression  
  organ damage  
prophylaxis
<table>
<thead>
<tr>
<th>Classification- Filipovich et al-2005</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time of Symptoms</td>
</tr>
<tr>
<td>------------------</td>
</tr>
<tr>
<td>ACUTE GVHD</td>
</tr>
<tr>
<td>Classic acute</td>
</tr>
<tr>
<td>Persistent, recurrent or late onset acute</td>
</tr>
<tr>
<td>CHRONIC GVHD</td>
</tr>
<tr>
<td>Classic Chronic</td>
</tr>
<tr>
<td>Overlap syndrome</td>
</tr>
<tr>
<td>Organ</td>
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<td>-------</td>
</tr>
<tr>
<td>Skin</td>
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<td>Liver</td>
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</table>
Diagnosis

- Clinical evaluation
- Histology
- Biomarkers (IL-2Rα, TNFR1, IL-8, and HGF)
- Imaging
Rectal biopsy in a patient with acute graft-versus-host disease (GVHD) shows crypt cell necrosis with the accumulation of degenerative material in the dead crypts.
### Efficacy of drug prophylaxis for acute graft-versus-host disease

<table>
<thead>
<tr>
<th>Drug(s)</th>
<th>GVHD, percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>52-100</td>
</tr>
<tr>
<td>Methotrexate</td>
<td>56-70</td>
</tr>
<tr>
<td>Cyclosporine</td>
<td>33-54</td>
</tr>
<tr>
<td>ATG-methotrexate-prednisone</td>
<td>21</td>
</tr>
<tr>
<td>Cyclosporine-methotrexate</td>
<td>15-33</td>
</tr>
<tr>
<td>Cyclosporine-prednisone</td>
<td>12-21</td>
</tr>
<tr>
<td>Cyclosporine-methotrexate-prednisone</td>
<td>9-32</td>
</tr>
</tbody>
</table>
Treatment- Gr 1 Ac GVH

- Therapeutic CSA
- Topical and oral steroids
- Cholestyramine
- ? octreotide
Treatment- GR2-4

• Steroids- usually MET Pred @ 2mg/kg bw
  - Infections- Prophylaxis and monitoring
  - PTLD risk
Steroid refractory GVHD has been reported to have a survival rate of only 17% at 2 years.

Steroid Refractory GVHD

- MMF
- TNF α inhibitors
- IL2 receptor antagonists
- Camptah
- ATG
- Thalidomide
- Pentostatin
- Ontak- Denileukin diftitox
- ECP
- MSC’s
Leukocytes undergo apoptosis after ECP treatment and the apoptotic leukocytes phagocytosed by APCs, including macrophages and dendritic cells, lead to downregulation of costimulatory molecules and proinflammatory cytokines and upregulation of anti-inflammatory cytokines by APCs to create a tolerogenic environment that facilitates the generation of regulatory T cells.
MSC’s
(1) MSCs support the growth and differentiation of HSCs

(2) Infused MSCs home to sites of tissue injury in mice and non-human primates.

(3) MSCs are immunomodulatory and anti-inflammatory.

(4) MSCs are tolerogenic and avoid immune destruction.

(5) MSCs promote tissue repair

(6) Human MSCs can be isolated from BM, culture ex vivo, and expanded many fold.
**Characteristics**

- Differentiation:
  - osteoblasts
  - adipocytes
  - chondroblasts

- Proliferation

- Plastic adherence

- Negative markers:
  - CD45, CD34, CD14, CD11B, CD79, CD19, HLA-DR

**Immune function**

- Promotion of proliferation and function

- Inhibition of proliferation and function

- Treg
- DC2
- IL10
- CTL
- B cell
- NK
- IL-12
- IFNγ
- TNFα
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).
<table>
<thead>
<tr>
<th>STUDY</th>
<th>N</th>
<th>SUBJECTS</th>
<th>INDICATION</th>
<th>SOURCE</th>
<th>DOSE</th>
<th>HOW MANY</th>
<th>TIMING</th>
<th>FINDINGS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Le Blanc- Lancet</td>
<td></td>
<td></td>
<td>Steroid refractory</td>
<td>20,</td>
<td>10x10^5/k</td>
<td></td>
<td>day 73,</td>
<td>prompt response</td>
</tr>
<tr>
<td>2004</td>
<td>1</td>
<td>AML Ac GVHD</td>
<td></td>
<td>Haplo g</td>
<td>twice 120</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ringden Transplant-2006</td>
<td>8</td>
<td>Hame and non haem malignancy</td>
<td>7-90-</td>
<td>10x10^5/k</td>
<td>once</td>
<td>96 hours</td>
<td></td>
<td>Better survival than controls</td>
</tr>
<tr>
<td>2008</td>
<td></td>
<td>Leukaemia</td>
<td>Steroid refractory 2x sib, Haplo 6, 3rd</td>
<td><strong>MSC=10, No</strong></td>
<td>15x10^5/k</td>
<td></td>
<td>4 hours</td>
<td>Reduced GVHD in MSC arm, but more relapse.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ac GVHD party-4</td>
<td><strong>MSC=15</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Le Blanc Lancet</td>
<td></td>
<td>HM=43, NHM=2, Non</td>
<td>Steroid refractory</td>
<td>Sib=5, Haplo=18, 3rd</td>
<td>90x10^5/k</td>
<td></td>
<td>27 days Complete response in 30 pts. No diff according to source</td>
<td></td>
</tr>
<tr>
<td>2008</td>
<td>55</td>
<td>malign=10</td>
<td>Ac GVHD</td>
<td>69</td>
<td>twice</td>
<td></td>
<td>29-498</td>
<td></td>
</tr>
<tr>
<td>Von Bonin BMT</td>
<td>13</td>
<td>HM=12, Non malign=1</td>
<td>Steroid refractory</td>
<td>g</td>
<td>2 doses</td>
<td></td>
<td>7 showed response</td>
<td></td>
</tr>
<tr>
<td>2009</td>
<td></td>
<td>Ac GVHD 3rd party</td>
<td></td>
<td></td>
<td>days</td>
<td></td>
<td></td>
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</tbody>
</table>
In a major setback, Osiris Therapeutics announced this morning that preliminary results indicate that its stem cell therapy Prochymal failed both of its late-stage clinical trials. One analyst swiftly dubbed the preliminary results "horrible," and shares of the company swiftly plunged 52 percent.
Reasons for failure

• **Early Passage MSCs Have Greater Potency** - In EU trials for GVHD, 1-year survival was 75% in patients who received early-passage MSCs (from passages 1–2) in contrast to 21% using later passage MSCs (from passages 3–4)–( p < 0.01). von Bahr, H et al. Biol Blood Marrow Transplant 18, 557, 2012.

• **Donor heterogeneity** - IFNg responsiveness is not uniform among human subjects and that MSCs derived from low indoleamine 2,3-dioxygenase (IDO) inducers may be substantially less potent than cells derived from high inducers.- So do we need to screen for the best donor.
IDO-mediated tryptophan degradation by DCs results in multiple effects, including inhibition of T-cell proliferation, increased T-cell apoptosis, and de novo formation of Tregs.


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Allogeneic Human Mesenchymal Stem Cell Therapy (Remestemcel-L, Prochymal) as a Rescue Agent for Severe Refractory Acute Graft-versus-Host Disease in Pediatric Patients

Joanne Kurtzberg, Susan Prockop, Pierre Teira, Henrique Bittencourt, Victor Lewis, Ka Wah Chan, Biljana Horn, Lolie Yu, Julie-An Talano, Eneida Nemecek, Charles R. Mills, Sonali Chaudhury

Biology of Blood and Marrow Transplant
Volume 20, Issue 2, Pages 229-235 (February 2014)
DOI: 10.1016/j.bbmt.2013.11.001
MSCs to enhance engraftment after HSCT

• The incidence of graft failure is <5% in fully ablative HLA-matched transplants.

• Can be a significant issue in subjects who receive less intense conditioning, HLA-mismatched grafts (including cord blood transplants) or in recipients who are HLA-alloimmunized.

### Major studies regarding engraftment

<table>
<thead>
<tr>
<th>STUDY</th>
<th>N</th>
<th>SUBJECTS</th>
<th>BMT TYPE</th>
<th>SOURCE</th>
<th>DOSE</th>
<th>TIMING</th>
<th>NENGRT</th>
<th>PLTENGRT</th>
<th>MAJOR FINDINGS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Koc et al, JCO 2000</td>
<td>28</td>
<td>Breast cancer</td>
<td>Auto</td>
<td>Auto</td>
<td>10-22x10^5/kg</td>
<td>1-24 hrs post</td>
<td>8 days</td>
<td>8.5 days</td>
<td>2yr DFS 53%, Safe</td>
</tr>
<tr>
<td>Lazarus et al, 2005</td>
<td>46</td>
<td>HM</td>
<td>MRD</td>
<td>Donor</td>
<td>10-30x10^5/kg</td>
<td>4 hrs prior</td>
<td>14 days</td>
<td>20.5 days</td>
<td>Safe and feasible</td>
</tr>
<tr>
<td>Le Blanc et al, Leukaemia 2007</td>
<td>7</td>
<td>AML-3, SCID-2, SAA-2</td>
<td>MRD+VUD</td>
<td>MRD+Haplo</td>
<td>10-10x10^5/kg</td>
<td>4 hours post</td>
<td>12 days</td>
<td>&lt;12 days</td>
<td>Rapid engraftment in pts with previous graft failure</td>
</tr>
<tr>
<td>Ball et al, Blood 2008</td>
<td>14</td>
<td>HM-11, NM-3</td>
<td>Haplo</td>
<td>Haplo donors</td>
<td>10-20x10^5/kg</td>
<td>4 hrs prior</td>
<td>12 days</td>
<td>10 days</td>
<td>0% graft failure, 15% historic</td>
</tr>
<tr>
<td>Ning et al, Leukaemia 2008</td>
<td>Yes-10, No-15</td>
<td>Leukaemia</td>
<td>MRD</td>
<td>MRD donor</td>
<td>0.3-15x10^15/kg</td>
<td>4 hours prior</td>
<td>16 days</td>
<td>NA</td>
<td>Less GVHD in MSC arm but more relapse.</td>
</tr>
<tr>
<td>MacMillan BMT 2009</td>
<td>8</td>
<td>Paeds ac Leuk</td>
<td>Cord</td>
<td>Haplo</td>
<td>9-50x10^%/kg</td>
<td>day 0</td>
<td>19 days</td>
<td>NA</td>
<td>5 alive and disease free after 5 years</td>
</tr>
</tbody>
</table>
Mesenchymal Stem Cells versus Mesenchymal Stem Cells Combined with Cord Blood for Engraftment Failure after Autologous Hematopoietic Stem Cell Transplantation: A Pilot Prospective, Open-Label, Randomized Trial

Yi-Ying Xiong, Qian Fan, Fen Huang, Yu Zhang, Yu Wang, Xiao-Yong Chen, Zhi-Ping Fan, Hong-Sheng Zhou, Yang Xiao, Xiao-Jun Xu, Min Dai, Na Xu, Jing Sun, Peng Xiang, Xiao-Jun Huang, Qi-Fa Liu

Biology of Blood and Marrow Transplant
Volume 20, Issue 2, Pages 236-242 (February 2014)
DOI: 10.1016/j.bbmt.2013.11.002
MSC and tissue repair

• Leukemia. 2007 Nov;21(11):2271-6. Tissue repair using allogeneic mesenchymal stem cells for hemorrhagic cystitis, pneumomediastinum and perforated colon: Ringdén O et al

• The Effect of Bone Marrow-Derived Mesenchymal Stem Cell Transplantation on Allodynia and Hyperalgesia in Neuropathic Animals: A Systematic Review with Meta-Analysis; Biol Blood Marrow Transplant. 2015 Sep;21(9), Hosseini M et al
Challenges to commercialization

- Funding,
- Intellectual property issues,
- Lack of equivalence between different sources of MSCs,
- Possible batch to batch variability,
- Absence of a universally acceptable potency assay,
- And the lack of regulatory guidance of a cellular product.
NCT01633229 Study Plan – 10 subjects

MSC infusions 2x 10^6/kg

Day: Pre 0 1 4 7 14 30 42
Blood Samples

HLA-alloimmunization, cytokines, biomarkers, and lymphocyte phenotype

Safety Clinical response
Results

• MSCs induce rapid clinical responses and biomarker (Reg 3a, CK18, Elafin) normalization in patients with steroid-refractory GVHD and tissue injury.
• MSCs appeared ineffective in patients with more aggressive GVHD with lower lymphocyte counts.
• A relatively intact immune system with higher absolute lymphocyte counts and favorable cytokine and T-cell phenotype patterns may be required for effective GVHD control by MSCs.
• So early detection and treatment is required.
Thank you very much