Mesenchymal Stem Cells to Repair Vascular Damage after Chemotherapy: Past, Present and Future

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What are MSCs?

- Stem cell capacity
- Stromal property
- Non-hematopoietic cells
- Multipotent cells
- 0.001 - 0.01% of nucleated cells in BM
- Adhere to culture plate
- Express variable range of cell markers
Development of stem cells within the body. This is not a comprehensive diagram, for clarity only selected stem cells are shown. ESC = embryonic stem cell, NSC = neuronal stem cell, EpSC = epidermal stem cell, MSC = mesenchymal stem cell, HSC = haematopoietic stem cell. *Differentiation of MSCs along neuronal lineages has also been demonstrated, see text for information. Modified from R&D Systems website (http://www.rnksystems.com). Copyright BTR©

http://www.york.ac.uk/res/bonefromblood/background/osteogenesis.html
Self renewal

MSCs

Adipocytes (Fat)

Chondrocytes (Cartilage)

Osteoblast (Bone)

Muscle cells

Neurocytes
MSC Sources

Bone marrow

Peripheral blood

Cord blood

Placenta and fetal membrane

Adipose tissue

Hass et al 2011; Orbay et al 2012
### Common MSC Markers

<table>
<thead>
<tr>
<th>Negative Markers</th>
<th>Roles</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CD34</strong></td>
<td>Primitive hematopoietic cells and endothelial cells</td>
</tr>
<tr>
<td><strong>CD45</strong></td>
<td>Leukocytes</td>
</tr>
<tr>
<td><strong>CD11b and CD14</strong></td>
<td>Monocytes and macrophages</td>
</tr>
<tr>
<td><strong>CD79α &amp; CD19α</strong></td>
<td>B cells</td>
</tr>
<tr>
<td><strong>HLA Class II</strong></td>
<td>Antigen presenting cells and lymphocytes</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Positive Markers</th>
<th>Roles</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CD73/5’-Nucleotidase</strong></td>
<td>Catalyzes production of extracellular adenosine from AMP</td>
</tr>
<tr>
<td><strong>CD90/Thy1</strong></td>
<td>Wound repair, cell-cell and cell-matrix interactions</td>
</tr>
<tr>
<td><strong>CD105/Endoglin</strong></td>
<td>Vascular homeostasis; modulates TGF-β functions via interaction with TGF-β receptors (RI &amp; RII)</td>
</tr>
</tbody>
</table>

Dominici et al 2006
MSC Clinical Applications

- **Hematopoietic Stem Cell Transplantation**
  - Enhance engraftment
  - Reduce graft versus host disease

- **Solid Organ Transplantation**
  - Improved graft function
  - Reduced rejection

- **Repair Vascular Damage**
  - Chemotherapy
  - Radiotherapy
  - Others
## Chemotherapy and Vascular Toxicity

**TABLE 1. Cardiotoxicity Profiles of Chemotherapeutic Agents**

<table>
<thead>
<tr>
<th>Drug Class/Name, Generic (Brand)</th>
<th>Cardiac Adverse Events</th>
<th>Relative Frequency of Specific Adverse Effect[^1]</th>
<th>Relative Frequency of Therapeutic Use[^1]</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Anthracyclines/anthraquinolones</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Doxorubicin (Adriamycin)</td>
<td>CHF/LV dysfunction</td>
<td>+++</td>
<td>+++</td>
<td>Risk of CHF is cumulative dose and schedule dependent; LV dysfunction is secondary to free radical production; increased risk for young/elderly, after mediastinal XRT, female gender, history of cardiac disease; continuous infusion, liposomal delivery systems, or use of dextrazoxane can reduce toxicity; when appropriately administered, incidence of LV dysfunction is &lt;5%.</td>
</tr>
<tr>
<td>Daunorubicin (Cerubidine)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epirubicin (Ellence, Pharmorubicin)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Idarubicin (Idamycin)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mitoxantrone (Novantron)</td>
<td>CHF/LV dysfunction</td>
<td>++</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td><strong>Alkylating agents</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Busulfan (Myleran)</td>
<td>Endomyocardial fibrosis</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cardiac tamponade</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cisplatin (Platinol)</td>
<td>Ischemia</td>
<td>++</td>
<td>+++</td>
<td>CHF risk is increased in elderly, after chest XRT, or after prior anthracyclines.</td>
</tr>
<tr>
<td></td>
<td>Hypertension</td>
<td></td>
<td>+ + +</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CHF</td>
<td></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Cyclophosphamide (Cytoxan)</td>
<td>Pericarditis/myocarditis</td>
<td>+</td>
<td>++</td>
<td>Rare incidence of hemorrhagic myocarditis, more common with high dose.</td>
</tr>
<tr>
<td></td>
<td>CHF</td>
<td></td>
<td>+</td>
<td>CHF risk is increased with cumulative dose, in elderly, after chest XRT, or after prior anthracyclines.</td>
</tr>
<tr>
<td>Ifosfamide (Ifex)</td>
<td>CHF</td>
<td>++</td>
<td>++</td>
<td>CHF risk is increased with cumulative dose, prior anthracyclines.</td>
</tr>
<tr>
<td>Mitomycin (Mutamycin)</td>
<td>CHF</td>
<td>++</td>
<td>+</td>
<td>CHF risk is increased with cumulative dose, prior anthracyclines, chest XRT.</td>
</tr>
</tbody>
</table>

[^1]: Relative frequency scale: +, ++, +++ for adverse effect and Therapeutic Use.
Bu-Cy and Vascular Toxicity

Al-Hashmi et al, 2012
**Bu-Cy and Vascular Toxicity**

**Control**
- Unbroken endothelial cell-cell contact

**Bu-Cy**
- Detaching of endothelial cells
- Cell-cell contacts were disrupted

Al-Hashmi et al, 2012
Chemotherapy & Vascular Toxicity

- Cisplatin-based therapy
- Testicular cancer patients
- Before and after chemotherapy

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (years)</th>
<th>Event</th>
<th>Time</th>
<th>Platelets* ($\times 10^9$/L)</th>
<th>Fibrinogen* (g/L)</th>
<th>vWF* (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>31</td>
<td>MI</td>
<td>Day 15, course 2</td>
<td>393</td>
<td>5.0</td>
<td>170</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>673</td>
<td>6.4</td>
<td>339</td>
</tr>
<tr>
<td>2</td>
<td>37</td>
<td>MI</td>
<td>Day 9, course 1</td>
<td>378</td>
<td>4.8</td>
<td>185</td>
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<tr>
<td></td>
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<td></td>
<td></td>
<td>667</td>
<td>4.4</td>
<td>280</td>
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<tr>
<td>3</td>
<td>34</td>
<td>DVT (leg)</td>
<td>At diagnosis</td>
<td>348</td>
<td>3.9</td>
<td>104</td>
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<tr>
<td></td>
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<td></td>
<td></td>
<td>264</td>
<td>3.1</td>
<td>139</td>
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<tr>
<td>4</td>
<td>23</td>
<td>DVT (subclavian vein)</td>
<td>Day 22, course 4</td>
<td>239</td>
<td>ND</td>
<td>78</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td>117</td>
<td>ND</td>
<td>95</td>
</tr>
<tr>
<td>5</td>
<td>31</td>
<td>DVT (subclavian vein)</td>
<td>Day 9, course 2</td>
<td>217</td>
<td>ND</td>
<td>86</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>270</td>
<td>ND</td>
<td>140</td>
</tr>
<tr>
<td>6</td>
<td>28</td>
<td>PE</td>
<td>Day 11, course 4</td>
<td>285</td>
<td>4.6</td>
<td>88</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>196</td>
<td>3.4</td>
<td>173</td>
</tr>
<tr>
<td>7</td>
<td>22</td>
<td>PE</td>
<td>Day 15, course 2</td>
<td>755</td>
<td>7.3</td>
<td>133</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>245</td>
<td>4.4</td>
<td>145</td>
</tr>
</tbody>
</table>

MI, myocardial infarction; DVT, deep vein thrombosis; PE, pulmonary embolism; vWF, von Willebrand factor

Nuver et al, 2005
MSCs and Vascular Repair

- **MSC Source**
  - Rat BM

- **Injury induction**
  - Ligation of proximal left coronary artery
    - MI

- **MSC infusion**
  - Infarct area \((2 \times 10^6)\)

- **Outcomes**
  - Expressed muscle and endothelium markers
  - Improved LV function

Dai et al. 2005
MSCs and Vascular Repair

- **MSC Source**
  - Canine BM

- **Injury induction**
  - Ameroid constricctor placement
    - Chronic myocardial ischemia

- **MSC infusion**
  - Intramyocardial injections (10 x 10^7)

- **Outcomes**
  - Improved ejection fraction & vascular density
  - Increased vascularity
  - Improved cardiac function

- **MSC Differentiation**
  - SMCs & ECs

Silva et al 2005
MSCs and Vascular Repair

- MSC Source
  - Porcine BM

- Injury induction
  - Occlusion followed by reperfusion of the anterior coronary artery
    - MI

- MSC infusion
  - Intramyocardial injections ($20 \times 10^7$)

- Outcomes
  - Improved myocardial blood flow
  - Improved ventricular & late cardiac functions
  - Enhanced blood vessels maturation
  - Reduced endomyocardial apoptosis
  - Reduced infarct scarring

Schuleri et al. 2008
MSCs and Vascular Repair

- **MSC Source**
  - Porcine BM

- **Injury induction**
  - Carotid artery cannulation
    - MI

- **MSC infusion**
  - Transendocardial injections ($10 \times 10^7$)

- **Outcomes**
  - Differentiated into cardiomyocytes and vascular structures
  - Stimulated endogenous cardiac stem cells proliferation and differentiation
  - Stimulated cardiomyocyte replication
  - Reduced infarct size

Hatzistergos et al 2010
MSCs and Vascular Repair

- **MSC Source**
  - C57Bl/6 mice BM
    - Express GSK-3 β

- **Injury induction**
  - Carotid artery ligation
    - MI

- **MSC infusion**
  - Border zone of MI (1.5x 10⁵)

- **Outcomes**
  - GSK-3β increased MSCs survival
  - GSK-3 β-MSCs induced cardiomyocyte differentiation and angiogenesis
  - GSK-3 β–MSCs increased capillary density
  - GSK-3 β–MSCs upregulated paracrine factors (VEGF-A)

Cho et al 2011
Log-rank (Mantel-Cox) test: $P<0.05$
MSCs and Vascular Repair

- **MSC Source**
  - C57Bl/6 mice BM

- **Injury induction**
  - Removal of endothelium with a flexible wire
    - Carotid artery injury

- **MSC infusion**
  - IV injection (1 x 10⁵)

- **Outcomes**
  - Differentiated into neo-ECs in the injury
  - Contributed to vascular remodeling
  - Expressed different markers
  - Formed the tube-like structure

Li et al 2013
MSCs in clinical practice (Meta Analysis)

- Searched databases
  - PubMed, OVID, EMBASE, the Cochrane Library, and ClinicalTrials.gov

- Outcome
  - Improved LVEF in patients

- Efficacy of Cells in transplantation influenced by
  - Source of cells
  - Route of infusion
  - Type of injury

- BMCs & MSCs infusion is a safe and effective therapy to improve vascular repair

Xiao et al 2014; Xu et al 2014; Tian et al 2014
Precautions

- Intra-myocardial calcification
- Vascular calcification
- Increase aortic stiffness
- Systolic hypertension
- Transient improvement
- Unregulated differentiation

Liao et al 2012; Dai et al 2005
Prospective

- MSC therapy under intensive investigation
  - Establish clinical relevant vascular injury models
  - Sources & doses
  - Timing
  - Protocols

- Collaboration
  - Type of disease
  - Data sharing
Liao et al. 2012
## MSCs in clinical practice

<table>
<thead>
<tr>
<th>World</th>
<th>Condition</th>
<th>Intervention</th>
<th>Time frame</th>
<th>Phase/Status</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chronic ischemic LV dysfunction secondary to MI</td>
<td>10 and 20 intramyocardial injections of 2 million MSCs (low dose) or 20 million (high dose)/0.25–0.5 cm³ for a total of 20 million or 200 million cells, respectively</td>
<td>6–18 months</td>
<td>Phase I/II (unknown)</td>
</tr>
<tr>
<td></td>
<td>Chronic ischemic LV dysfunction and heart failure secondary to MI</td>
<td>Transendocardial injection of autologous human cells (bone marrow or mesenchymal); 40 million cells/mL delivered in either a dose of 0.25 mL/injection for a total of 100 million × 10 injections or a dose of 0.5 mL/injection for a total of 200 million × 10 injections</td>
<td>6–18 months</td>
<td>Phase I/II (unknown)</td>
</tr>
<tr>
<td>Florida (USA)</td>
<td></td>
<td>Transendocardial injection of autologous versus allogeneic MSCs; 40 million cells/mL delivered in either a dose of 0.5 mL/injection × 1 injection for a total of 20 million, a dose of 0.5 mL/injection × 5 injections for a total of 100 million, or a dose of 0.5 mL/injection × 10 injections for a total of 200 million MSCs</td>
<td>6–12 months</td>
<td>Phase I/II (active)</td>
</tr>
<tr>
<td></td>
<td>Nonischemic dilated cardiomyopathy</td>
<td>Transendocardial injection of autologous versus allogeneic MSCs; 20 million cells/mL delivered in a dose of 0.5 mL/injection × 1 injection for a total of 100 million of MSCs</td>
<td>6–12 months</td>
<td>Phase I/II (active)</td>
</tr>
<tr>
<td></td>
<td>Chronic ischemic LV dysfunction secondary to MI</td>
<td>10 and 20 intramyocardial injections of 2 million MSCs (low dose) or 20 million (high dose)/0.25–0.5 cm³ for a total of 20 million or 200 million of autologous human MSCs, respectively</td>
<td>6–18 months</td>
<td>Phase I/II (unknown)</td>
</tr>
<tr>
<td>Maryland (USA)</td>
<td></td>
<td>Transendocardial injection of autologous versus allogeneic MSCs; 40 million cells/mL delivered in either a dose of 0.5 mL/injection × 1 injection for a total of 20 million, a dose of 0.5 mL/injection × 5 injections for a total of 100 million, or a dose of 0.5 mL/injection × 10 injections for a total of 200 million MSCs</td>
<td>6–13 months</td>
<td>Phase I/II (active)</td>
</tr>
<tr>
<td>France (Europe)</td>
<td>Chronic myocardial ischemia: LV dysfunction</td>
<td>Transendocardial intramyocardial injections of 60 million autologous MSCs</td>
<td>30 days–2 years</td>
<td>Phase I/II (active)</td>
</tr>
<tr>
<td>China (East Asia)</td>
<td>ST-elevation MI</td>
<td>Intracoronary human umbilical WJ-MSC transfer</td>
<td>4 months–1 year</td>
<td>Phase II (active)</td>
</tr>
<tr>
<td>Korea (East Asia)</td>
<td>Acute MI</td>
<td>Intracoronary injection of single dose of autologous bone marrow-derived MSCs (1 million) cells/kg</td>
<td>6 months</td>
<td>Phase II (completed)</td>
</tr>
<tr>
<td>India (South Asia)</td>
<td>ST-elevation acute MI</td>
<td>A Single Dose of Intravenous infusion of Allogenic MSCs</td>
<td>6 months</td>
<td>Phase I/II (active)</td>
</tr>
<tr>
<td>Cultivation</td>
<td>Adherence to plastic in standard culture conditions</td>
<td></td>
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<tr>
<td>-------------</td>
<td>-----------------------------------------------------</td>
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<tr>
<td><strong>Phenotype</strong></td>
<td>Positive expression (≥ 95%)</td>
<td>Negative expression (≤ 2%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD 73</td>
<td>CD 14 or CD 11b</td>
<td></td>
<td></td>
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<tr>
<td>CD 90</td>
<td>CD 19 or CD 79α</td>
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<tr>
<td>CD 105</td>
<td>CD 34</td>
<td></td>
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<tr>
<td></td>
<td>CD 45</td>
<td></td>
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<tr>
<td></td>
<td>HLA-DR</td>
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</tbody>
</table>

**In vitro differentiation:** Under specific stimulus, cells will differentiate into osteoblasts, adipocytes, and chondroblasts.