Stem Cell -- “Fountain of Youth?”
Heart - self-renewing organ

- Myocyte regeneration occurs throughout organism lifespan

- Cardiac niches contain stem cells ➔ after activation ➔ give rise to myocytes and vascular structures

Piero Anversa, Jan Kajstura, Annarosa Leri. Circulation. 2006;113:1451-1463
Regeneration of Human Heart

- Rapid, Large Myocardial Injury,
- Continuous Myocyte loss through Apoptosis
- Stem cells inadequate in number & quality
  - Hypertension, Diabetes, hypercholesterolemia, aging and IHD
Stem Cell Therapy for Heart Failure
Clinical Questions

• What cell source do you use?
• How should cells be delivered?
• What cells within that pool are beneficial?
• How many cells do you need?
• When should you deliver the cells?
• What is the Experience so far?
• What’s the role of Tissue Engineering in heart disease?

These answers all depend on each other
Classification of Stem Cells

• The classification of stem cells is still in evolution based on a large number of cell markers

  Primary distinction is between:

  1) Embryonic stem cells
  2) Non-embryonic stem cells
     - adult stem cell
     - cord blood
  3) Induced Pluripotent Stem cells
  4) Somatic Cell Nuclear Transfer
Patient-Specific Somatic Cell Nuclear Transfer

Perry ACF, NEJM 2005
Induced Pluripotent Stem Cell Lines (iPS)

Four factors (Oct 4, NANOG, Sox 2, LIN28) sufficient to reprogram a human somatic stem cell (fibroblast) to a pluripotent cell with all the characteristics of hES (Yu J, et al., Thomson J, Science 2007)

Adult Stem Cell Therapy and the Heart

Bone Marrow
- Mesenchymal stem cells (CD 34-)
- Hematopoietic stem cells (CD 34+)
- Multipotent stem cells

Skeletal Muscle
- Satellite cells (myoblast)

Blood Vessel
- Endothelial Progenitor Cells (Hemangioblasts)

Other (Adipose)

Heart
- Side Population cells/
  Cardiac specific progenitor

Adapted from M. Schneider MD
Possible routes for cell therapy to the heart

Uptake only 3%

Uptake 33%

Transendocardial Targeted Injection Technique

NOGA Myostar injection catheter

Injection catheter Advanced into LV

Perin EC et al Circulation 2003;107: 2294-2302
Mechanisms of action

- Progenitor cells
  - Differentiation to an endothelial phenotype
  - Production of paracrine factors
  - Differentiation to a cardiac phenotype
  - Fusion

- Mechanisms
  - Perivascular incorporation

- Functions
  - Improvement of neovascularization
  - Cardiac regeneration
Adult Stem Cell Trials in Cardiac Patients

- Acute Myocardial Infarction
- Myocardial ischemia and no revascularization options
- Ischemic and non-ischemic cardiomyopathy

Majority of these trials have delivered bone marrow cells to the heart by intra-coronary infusion
Randomized control trials of intracoronary BMSC therapy following acute myocardial infarction

<table>
<thead>
<tr>
<th>Study name (ref)</th>
<th>Date published</th>
<th>n</th>
<th>Days after AMI</th>
<th>Primary outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOPCARE-AMI⁴¹</td>
<td>2002</td>
<td>59</td>
<td>4.3 ± 1.5</td>
<td>Improvement in global LVEF from 51.6 ± 9.6% to 60.1 ± 8.6% (P = 0.003) at 4 months</td>
</tr>
<tr>
<td>BOOST⁴²</td>
<td>2004</td>
<td>60</td>
<td>5.1 ± 1.3</td>
<td>Improvement in global LVEF at 6 months but effect was only maintained in large infarcts at long-term follow-up</td>
</tr>
<tr>
<td>REPAIR-AMI⁴³</td>
<td>2006</td>
<td>187</td>
<td>3–6</td>
<td>Improvement in the LVEF at 4 months by 2.5% above baseline</td>
</tr>
<tr>
<td>ASTAMI⁴⁶</td>
<td>2006</td>
<td>97</td>
<td>6 ± 1</td>
<td>No change in the LVEF at 6 months</td>
</tr>
<tr>
<td>LEUVEN-AMI⁴⁵</td>
<td>2006</td>
<td>66</td>
<td>1</td>
<td>No change in global LVEF at 4 months but there was improvement in regional contractility and infarct size in patients with the largest infarcts</td>
</tr>
<tr>
<td>FINCELL⁴⁴</td>
<td>2008</td>
<td>77</td>
<td>3</td>
<td>Improvement in the LVEF at 6 months by 5% above baseline</td>
</tr>
<tr>
<td>HEBE⁴⁷</td>
<td>2010</td>
<td>200</td>
<td>3–8</td>
<td>No change in global LVEF at 4-month follow-up</td>
</tr>
</tbody>
</table>

AMI, acute myocardial infarction; BMSC, bone marrow stem cells; n, number of patients; LVEF, left ventricular ejection fraction.
# Meta-analysis of intra-coronary cell therapy clinical trials

## Table 1: Main Features of Included Studies

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>Design</th>
<th>Patients Enrolled (Patients at Follow-Up)</th>
<th>Cell Type</th>
<th>Follow-Up (Months)</th>
<th>Primary End Point</th>
<th>Imaging Modality for LVEF Assessment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strauer et al. (10)</td>
<td>2002</td>
<td>Non-RCT</td>
<td>20 (20)</td>
<td>BMC</td>
<td>3</td>
<td>LVEF</td>
<td>LV angiography</td>
</tr>
<tr>
<td>Bartunek et al. (11)</td>
<td>2005</td>
<td>Non-RCT</td>
<td>35 (35)</td>
<td>BMC</td>
<td>4</td>
<td>Safety, LVEF</td>
<td>LV angiography, SPECT</td>
</tr>
<tr>
<td>Jannsens et al. (8)</td>
<td>2006</td>
<td>RCT</td>
<td>67 (66)</td>
<td>BMC</td>
<td>4</td>
<td>LVEF</td>
<td>Cardiac MRI</td>
</tr>
<tr>
<td>BOOST (7)</td>
<td>2006</td>
<td>RCT</td>
<td>60 (60)</td>
<td>BMC</td>
<td>18</td>
<td>LVEF, safety</td>
<td>Cardiac MRI</td>
</tr>
<tr>
<td>Zhan-Quan et al. (13)</td>
<td>2006</td>
<td>Non-RCT</td>
<td>70 (58)</td>
<td>PMC</td>
<td>6</td>
<td>LVEF, LV volumes, WMSI</td>
<td>Echocardiography</td>
</tr>
<tr>
<td>MAGIC CELL-3-DES (12)</td>
<td>2006</td>
<td>RCT</td>
<td>56 (50)</td>
<td>PMC</td>
<td>6</td>
<td>LVEF</td>
<td>Cardiac MRI</td>
</tr>
<tr>
<td>TCT-STAMI (15)</td>
<td>2006</td>
<td>RCT</td>
<td>20 (20)</td>
<td>BMC</td>
<td>6</td>
<td>LVEF</td>
<td>Echocardiography, SPECT</td>
</tr>
<tr>
<td>ASTAMI (2,4)</td>
<td>2006</td>
<td>RCT</td>
<td>100 (97)</td>
<td>BMC</td>
<td>6</td>
<td>LVEF, EDV, infarct size</td>
<td>SPECT, MRI, echo</td>
</tr>
<tr>
<td>REPAIR-AMI (5)</td>
<td>2006</td>
<td>RCT</td>
<td>204 (187)</td>
<td>BMC</td>
<td>12</td>
<td>LVEF</td>
<td>LV angiography</td>
</tr>
<tr>
<td>Meluzin et al. (16)</td>
<td>2006</td>
<td>RCT</td>
<td>66 (66)</td>
<td>BMC</td>
<td>3</td>
<td>Infarct zone systolic function</td>
<td>SPECT</td>
</tr>
</tbody>
</table>

BMC = bone marrow cells; EDV = end-diastolic volume; LV = left ventricular; LVEF = left ventricular ejection fraction; MRI = magnetic resonance imaging; PMC = peripheral mononuclear cells; RCT = randomized controlled trial; SPECT = single-photon emission computed tomography; WMSI = wall motion score index.

Lipinski MJ et al, JACC 2007;50: 1761-7
3% ↑EF with cell therapy (p<0.001)

5.6% reduction in infarct size (p<0.001)

7.4 ml reduction in LV ESV (p=0.002)

Non-significant trend towards LV EDV reduction (4.6 ml) (p=0.11)
Clinical “Stem Cell” Studies to Date

Table 1  Heart cell implantation after myocardial infarction: clinical trials

<table>
<thead>
<tr>
<th>Cell source</th>
<th>Method of delivery</th>
<th>Patients (n)</th>
<th>Inclusion criteria</th>
<th>Follow-up (duration)</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autologous mononuclear BMCs</td>
<td>IM injection at the time of CABG</td>
<td>5</td>
<td>Old MI; no graftable coronary arteries</td>
<td>1 year</td>
<td>Increased myocardial perfusion (in 3 patients)</td>
</tr>
<tr>
<td>Autologous mononuclear BMCs</td>
<td>IC infusion at the time of PTCA</td>
<td>10</td>
<td>5–9 days post-MI</td>
<td>3 months</td>
<td>Decreased infarct region, improved wall motion, increased myocardial perfusion</td>
</tr>
<tr>
<td>Autologous mononuclear BMCs and cultured blood-derived EPCs</td>
<td>IC infusion at the time of PTCA</td>
<td>20</td>
<td>&lt;3 days post-MI</td>
<td>4 months</td>
<td>Improved LVEF, EDV, myocardial perfusion, improved contractile function</td>
</tr>
<tr>
<td>AC133 + bone marrow cells</td>
<td>IM injection at the time of CABG</td>
<td>12</td>
<td>&gt;10 days but &lt;3 months post-MI</td>
<td>3–9 months</td>
<td>4/6 patients showed increase in EF; 5/6 showed increased myocardial perfusion</td>
</tr>
<tr>
<td>Autologous mononuclear BMCs</td>
<td>Percutaneous IM injection guided by electromechanical mapping.</td>
<td>8</td>
<td>Severe ischemic heart disease</td>
<td>3 months</td>
<td>Improved anginal symptoms, myocardial perfusion and contractile function</td>
</tr>
<tr>
<td>Autologous mononuclear BMCs</td>
<td>Percutaneous IM injection guided by electromechanical mapping.</td>
<td>14</td>
<td>CHF</td>
<td>2 months</td>
<td>Increased LVEF, myocardial perfusion and contractile function</td>
</tr>
<tr>
<td>Skeletal muscle myoblasts</td>
<td>IM injection at the time of CABG</td>
<td>10</td>
<td>CHF</td>
<td>10.9 months</td>
<td>Improved LVEF, improved contractile function</td>
</tr>
<tr>
<td>Autologous skeletal myoblasts</td>
<td>IM injection at the time of CABG</td>
<td>12</td>
<td>Old MI and ischemic CAD</td>
<td>Improved LVEF, improved regional contractility</td>
<td>Hemeros et al. (2003) [12]</td>
</tr>
<tr>
<td>Autologous skeletal myoblasts</td>
<td>IM injection at the time of LVAD implantation</td>
<td>5</td>
<td>Ischemic cardiomyopathy and refractory heart failure</td>
<td>68–191 days</td>
<td>Development of myotubes in scarred myocardium</td>
</tr>
<tr>
<td>Autologous skeletal myoblasts</td>
<td>Percutaneous IM injection guided by electromechanical mapping.</td>
<td>5</td>
<td>Ischemic heart failure</td>
<td>6 months</td>
<td>Increased LVEF and wall thickening at areas of injection</td>
</tr>
<tr>
<td>Autologous BMCs</td>
<td>IIC infusion at the time of PTCA</td>
<td>30 (in BMC group)</td>
<td>&lt;5 days post-MI</td>
<td>6 months</td>
<td>Improved LVEF and contractile function</td>
</tr>
<tr>
<td>Autologous BMSCs</td>
<td>IC infusion 18 days after PTCA</td>
<td>34 (in BMSC group)</td>
<td>10 days post-MI</td>
<td>3–6 months</td>
<td>Decreased wall movement dysfunction, perfusion defects and ventricular EDV and ESV; increased wall movement velocity and LVEF</td>
</tr>
<tr>
<td>Autologous peripheral blood stem cells + IV infusion of G-CSF</td>
<td>IC infusion after PTCA</td>
<td>10 (in cell infusion group)</td>
<td>Acute MI (&gt;48 hours) or old MI</td>
<td>6 months</td>
<td>Increased in-sent restenosis in the G-CSF group.</td>
</tr>
</tbody>
</table>
Adjunctive TMR or CABG plus TMR

- Standard of care is CABG but the patient would be incompletely revascularized by CABG alone

- Incomplete revascularization is estimated to occur in 15-25% of CABG patients

BMLR Procedure

1 mm Holmium Yag Laser Fibre

3 Needles deploy along with Fibre to deliver biologic throughout myocardium
HARVEST BMAC System: Facilitates Autologous Bone Marrow Therapy
Result: Safety Profile

- 7/8 patients (87.5%) were off pump procedures
- Harvest system concentrated CD34+ cell count 7.12 times

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean +/- SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration of Surgery (min.)</td>
<td>295.6 +/- 91.7</td>
</tr>
<tr>
<td>Duration of BMLR (min.)</td>
<td>16.9 +/- 8</td>
</tr>
<tr>
<td>Duration of Ventilation (hours)</td>
<td>22.07 +/- 5.1</td>
</tr>
<tr>
<td>CCU Stay (hours)</td>
<td>81.78 +/- 19.1</td>
</tr>
<tr>
<td>Intraoperative Blood transfusion (ml)</td>
<td>500 +/- 144</td>
</tr>
</tbody>
</table>
## Results: Outcome

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>6 months</th>
<th>1 year</th>
</tr>
</thead>
<tbody>
<tr>
<td>NYHA Class</td>
<td>3.25+/-.46</td>
<td>1.38+/-.51</td>
<td>1.5+/-.75**</td>
</tr>
<tr>
<td>6 Min Walk (m)</td>
<td>241.6+/-.44</td>
<td>360.8+/-.65</td>
<td>347.4+/-.78**</td>
</tr>
<tr>
<td>QOL score (1-5)</td>
<td>1</td>
<td>3</td>
<td>3.8</td>
</tr>
</tbody>
</table>

** p<0.001
Case 5: Improvement in Anterolateral wall Perfusion Defect on MRI

- Grafts to PLv, PDA.
- BMLR in anterior and Anterolateral wall.
Feasibility Study of Autologous Concentrated Bone Marrow Nucleated Cell Therapy for Congestive Heart Failure Patients Undergoing Treatment with Coronary Artery Bypass Grafting (CABG) Surgery

**INCLUSION CRITERIA:**
Patients with Ischemic CHF & Ejection Fraction of ≤ 40% Undergoing Treatment with Coronary Artery Bypass Grafting (CABG) Surgery

**STUDY COHORTS:** Randomization 3:1

- **Treatment group: CABG / BMAC (15 subjects):**
  - 180 ml bone marrow aspirated
  - Increasing volume of injectate (BMAC)
    - 10 mL
    - 15 mL
    - 20 mL

- **Control Group: CABG (5 subjects):**
  - 21 Subjects Enrolled
  - Effect of Injectate volume on Cardiac Events
  - complete 1Yr FUP
Tables for the final study Report

Figure 3: Change in EF from Baseline measured by Cardiac MRI

Change in EF from Baseline measured by Cardiac MRI

- Control
- BMAC 10 ml
- BMAC 15 ml
- BMAC 20 ml

Baseline
Month 6
Month 12
Visits

EJECTION FRACTION (%) Change from Baseline

8
6
4
2
0
-2
-4
6.93
5.03
1.54
-1.35
-3.38
Feasibility Study of Retrograde Delivery of Autologous Concentrated Bone Marrow Nucleated Cell Therapy for Patients Diagnosed with Congestive Heart Failure (CHF)

• Randomized, multicentre Study to evaluate efficacy and safety of bone marrow derived nucleated cell Therapy by retrograde method in subjects of Ischemic and Non-ischemic heart failure
Study Design

Congestive Heart Failure (51 patients)

Ischemic Patients (27 patients)
- Ischemic Treatment (21 patients)
- Ischemic Control (6 patients)

Non-Ischemic Patients (24 patients)
- Non-Ischemic Treatment (20 patients)
- Non-Ischemic Control (4 patients)
Change in NYHA Class

Treatment Ischemic

Control Ischemic

Treatment Non-Ischemic

Control Non-Ischemic
Cardiovascular Tissue Engineering

Cell Source
- Embryonic stem cells
- Mesenchymal stem cells
- Endothelial progenitor cells
- Resident Cardiac SCs

Signals
- VEGF
- TGF-β
- FGF
- BMP
- PDGF
- Shear stress
- Axial strain

ECM
- Matrigel
- Collagen
- Alginate
- Fibrin
- Decellularized Tissue
- PLA
- PGA
The Scaffold

Biodegradable polymer On Nitenol Stent
The Bioreactor

(A) respirator, peristaltic pump, (B, C) three chambers: (i) air chamber, (ii) ventricular chamber, and (iii) recirculation chamber. During systole, the silicone membrane separating the air and ventricular chambers is periodically displaced by a respirator (small arrow in B shows direction of displacement), with culture medium propelled through the lumen of the valve.
The Valve ready in a month
The Stent Deployment

- Trans Catheter deployment
- Degradable stent expected in 5 yrs
The Bypass Surgery
The Scaffold

The Regrown Vessel

Image courtesy of Science/AAAS
Problems with tissue engineering for Heart

Two of these critical aspects, namely

• Vascularization
• Inerrvation

have not received all the attention they deserve.
Induction of Myocyte Differentiation By Electrical Stimulation

- Conductivity: Carbon Nanoparticles
- Cells: Cardiac Myocyte Precursors
Stem cell therapy: More questions to be answered.

Routes of delivery
Surgical vs percutaneous

Timing of delivery
Acute vs chronic

Cell type and marker
Myoblast vs BM
CD34/AC133/SP

Target patient population
Best criteria

Dose
Dose response

Cell origin
Embryonic vs adult
BM vs peripheral
Culture expanded vs fresh

Courtesy of Timothy Henry, MD.
THANK YOU
Tissue heterogeneity and stem-cell functionality for homeostasis and repair.

Rando Nature 441, 1080-1086 (29 June 2006)
Human Embryonic Stem Cells

Advantages

- Highly Expandable
- Pluripotent

Disadvantages

- Ethical objections
- Difficult to isolate
- Risk of rejection
- Immune-suppressive Rx required
- Arrhythmogenic potential
- Risk of teratocarcinomas
- Lack of specific markers

Strauer BE and Kornowski R 2003;107: 929-934
Somatic Cell Nuclear Transfer

**Advantages**
- Highly Expandable
- Pluripotent

**Disadvantages**
- ? Ethical objections
- **Difficulty in obtaining oocytes**
- ? Risk of rejection
- ? Immune-suppressive Rx required
- ? Arrhythmogenic potential
- ? Risk of teratocarcinomas
- ? Lack of specific markers
Adult Stem Cells

**Advantages**
- Likely more easily obtainable
- No ethical objections
- Highly compatible
- Autologous transplantation
- No need for immunosuppressive Rx
- Clinical application already realized

**Disadvantages**
- Lack of specific markers
- ? arrhythmogenic

Strauer BE and Kornowski R 2003;107: 929-934
Stem Cell Application in Heart disease

Angiogenesis

Myogenesis