Stem cell therapy for myocardial repair

Birgit Assmus
The heart is regenerating!

Undisputable Evidence from DNA Integration of C-14
– generated during nuclear bomb testing during cold war -

The heart muscle is younger than the body!

Bergmann O et al., Science 2009; 324:98-102
Cells for functional cardiac repair

Embyronic-like stem cells (iPS)

4 genes: Oct4, Klf4, Sox2, myc

somatic cells (skin fibroblasts)

Modified from Dimmeler et al, JCI 2005
Clinical evolution of BMC therapy for cardiovascular diseases

Phase I/II clinical trials

Bone marrow-derived cells
- Bone marrow mononuclear cells (BMC)
  - CD133+
- CD34+CXCR4+
  - CD34+
  - Mesenchymal Stromal Cells

Adipose tissue-derived cells

2001/2002

Cardiac stem cells
- c-kit+ cells
  - Cardiospheres

Cell enhancement, target region preconditioning
- Shock waves for enhancing cell engraftment
- Factors to enhance cardiac differentiation
- Repeated administration

2006

Bone marrow mononuclear cells (BMC)
- CD34+CXCR4+

2008

CD133+

2009

2013

Phase III trials

Bone marrow-derived cells stopped end 2013

Bone marrow mononuclear cells (BMC)
Cell therapy in cardiovascular diseases

- Acute Myocardial Infarction
- Refractory Angina
- Peripheral arterial occlusive disease
- Chronic post-infarction heart failure
Cell Therapy in Acute Myocardial Infarction: therapeutic targets

- Acute Myocardial Infarction
- Chronic Heart Failure

Vascularization ↓
Apoptosis ↑
Paracrine factors
Cardiac Regeneration

Adverse LV Remodeling
- Infarct expansion
- Chronic LV- dilatation

BMC/CPC

Ischemia
Cytokines, e.g. VEGF, SDF-1
Cell Therapy for STEMI

- The patient population at risk post-AMI
- Effects of cell therapy in patients at risk
- Derivation of the clinical benefit
LV contractile recovery within 1 week after successful reperfusion determines clinical outcome in STEMI

There is **no linear** correlation between mortality and ejection fraction after AMI!
Enhanced contractile recovery by BMC is confined to patients with failed initial recovery.

**4 months data LV angiography**

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>BMC</th>
</tr>
</thead>
<tbody>
<tr>
<td>EF below median (≤ 48.9 %)</td>
<td>2.5 ± 1.1</td>
<td>7.5 ± 1.1</td>
</tr>
<tr>
<td>p = 0.002</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EF above median (&gt; 48.9 %)</td>
<td>3.7 ± 0.7</td>
<td>4.0 ± 0.6</td>
</tr>
<tr>
<td>p = 0.81</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**12 months data MRI**

Change of endsystolic volumes over time (MRI)

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>BMC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>80 ± 10</td>
<td>120 ± 14</td>
</tr>
<tr>
<td>p = 0.065</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 months</td>
<td>70 ± 10</td>
<td>110 ± 14</td>
</tr>
<tr>
<td>p = 0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12 months</td>
<td>60 ± 10</td>
<td>100 ± 14</td>
</tr>
<tr>
<td>p = 0.7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Repair-AMI: n= 204 patients; 1:1 multicentre double-blind randomized intracoronary placebo or BMC infusion 3 -7 days after successful acute reperfusion therapy.
Do beneficial effects of BMC therapy on adverse remodeling translate into clinical benefit?

Therapies preventing adverse remodeling...

ACEI, ARB, ß-Blocker, Aldosteron-Ant., CRT
**BMC therapy post-AMI**

**Improved clinical outcome in meta-analysis**

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Peto OR</th>
<th>95% Confidence Interval</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>All-cause mortality</td>
<td>0.39</td>
<td>0.27 to 0.55</td>
<td>&lt;0.00001</td>
</tr>
<tr>
<td>Cardiac deaths</td>
<td>0.41</td>
<td>0.22 to 0.79</td>
<td>0.005</td>
</tr>
<tr>
<td>Recurrent MI</td>
<td>0.25</td>
<td>0.11 to 0.57</td>
<td>0.001</td>
</tr>
<tr>
<td>Heart failure</td>
<td>0.52</td>
<td>0.27 to 1.00</td>
<td>0.05</td>
</tr>
<tr>
<td>Stent thrombosis</td>
<td>0.34</td>
<td>0.12 to 0.94</td>
<td>0.04</td>
</tr>
<tr>
<td>In-stent restenosis</td>
<td>0.87</td>
<td>0.47 to 1.62</td>
<td>0.66</td>
</tr>
<tr>
<td>TVR</td>
<td>0.83</td>
<td>0.55 to 1.23</td>
<td>0.35</td>
</tr>
<tr>
<td>CVA</td>
<td>0.28</td>
<td>0.08 to 1.07</td>
<td>0.06</td>
</tr>
<tr>
<td>VT / VF</td>
<td>1.14</td>
<td>0.52 to 2.53</td>
<td>0.74</td>
</tr>
</tbody>
</table>

Abbreviations: BMC, bone marrow cell; CVA, cerebrovascular accident; MI, myocardial infarction; OR, odds ratio; TVR, target vessel revascularization; VF, ventricular fibrillation; VT, ventricular tachycardia.

*N = 2625 patients from 50 studies*
Baseline LVEF determines 5-year survival in Repair-AMI

5-year survival

Baseline LVEF > 45%

Baseline LVEF ≤ 45%

Assmus et al; Eur Heart J 2014
Factors influencing function of autologous BMC

Cell intrinsic factors

- Patient characteristics:
  - Age
  - Diabetes
  - Heart failure
  - Acute MI

- Cell functionality:
  - Survival
  - Migration/Homing
  - Paracrine activity
  - Differentiation capacity
  - Colony formation

Extrinsic factors

- Cell preparation:
  - Purity
  - Contaminations (e.g. Red blood cells, granulocytes?)

- Cell storage:
  - pH (NaCl)
  - Temperature
  - Serum vs Plasma
  - Heparin
  - Nutrients/Metabolism
## Selection of clinical BMC Trials in AMI

### EU- Ficoll isolated BMC trials

<table>
<thead>
<tr>
<th>Study</th>
<th>Number of pts.</th>
<th>Cells</th>
<th>Heparin in Final Cell Product</th>
<th>Primary Endpoint</th>
<th>Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ASTAMI</strong></td>
<td>100</td>
<td>i.c.; BM-MNC vs. standard therapy</td>
<td>5 U/ml</td>
<td>LVEF (SPECT)</td>
<td>(-) after 6 / 12 months</td>
</tr>
<tr>
<td><strong>BONAMI</strong></td>
<td>101</td>
<td>i.c.; BM-MNC vs. standard therapy</td>
<td>No heparin</td>
<td>Vitality (SPECT)</td>
<td>(+) vitality after 3 months (-) LVEF</td>
</tr>
<tr>
<td><strong>FINCELL</strong></td>
<td>80</td>
<td>i.c.; BM-MNC vs. medium</td>
<td>heparinized serum</td>
<td>LVEF (QLVA, echo)</td>
<td>(+) LVEF after months</td>
</tr>
<tr>
<td><strong>HEBE</strong></td>
<td>200</td>
<td>i.c.; BM-MNC vs. peripheral MNC vs. standard therapy</td>
<td>20 U/ml</td>
<td>reg. LV-function (MRI)</td>
<td>(-) after 4 months</td>
</tr>
<tr>
<td>Janssens-Trial</td>
<td>67</td>
<td>i.c.; BM-MNC vs. NaCl + serum</td>
<td>No heparin</td>
<td>LVEF (MRI)</td>
<td>(+) reduction infarct size (+) regional LV function</td>
</tr>
<tr>
<td>Plewka et al</td>
<td>60</td>
<td>i.c.; BM-MNC vs. standard therapy</td>
<td>?</td>
<td>LVEF (echo)</td>
<td>(+) LVEF after 6 months</td>
</tr>
<tr>
<td><strong>REGENT</strong></td>
<td>200</td>
<td>i.c.; BM-MNC vs. CXCR4+ BM-MNC vs. standard therapy</td>
<td>No heparin</td>
<td>LVEF (MRI)</td>
<td>((+)) LVEF after 6 months in cell treated groups</td>
</tr>
<tr>
<td><strong>REPAIR-AMI</strong></td>
<td>204</td>
<td>i.c.; BM-MNC vs. medium</td>
<td>No heparin</td>
<td>LVEF (QLVA)</td>
<td>(+) LVEF after 4 months (+) after 12 &amp; 24 months</td>
</tr>
</tbody>
</table>
Migratory / Invasion Capacity of BMC: Effects of Heparin

BMC/CPC

Ischemia

Cytokines, e.g. VEGF, SDF-1

in-vitro migration assay

Migration

Homing of BMC in ear wound model

![Graph showing migration results](image)

- Control
- 0.05 U/ml Heparin
- 0.5 U/ml Heparin
- 1 U/ml Heparin
- 2 U/ml Heparin
- 20 U/ml Heparin
- 7.5 µg/ml Bivalirudin
- 15 µg/ml Bivalirudin
- 30 µg/ml Bivalirudin

Homed BMC / high power field

- Untreated BMC
- Heparin-treated BMC
- Bivalirudin-treated BMC

* p<0.05 vs Control; n=3

p= n.s.

p=0.02

p=0.04

Circ Res 2012
Functional capacity of the applied BMC predicts clinical outcome at 5 years

![Graph showing event-free survival rates for different migration levels.](Eur_Heart_J_2014)

- **SDF-1-ind. migration > mean**
- **SDF-1-ind. migration ≤ mean**

**Event-free survival [%]**
- (cardiac, cardiovascular and unknown death, rehospitalization for heart failure)

**Numbers at risk**
- **Migration ≤ 170**: 53, 51, 49, 48, 45, 44
- **Migration > 170**: 48, 48, 48, 46, 45, 42

**p = 0.01 (log rank)**

**Years follow-up**
- 0, 1, 2, 3, 4, 5
BAMI Clinical Trial Design
(ESC Cell Therapy Trial Consortium)
'The effect of intracoronary reinfusion of bone marrow-derived mononuclear cells on all-cause mortality in STEMI'

- 1:1 randomized, controlled, no placebo group
- intracoronary BMC administration vs. standard care
- approx. 3000 patients, event-driven trial design
- primary endpoint: all-cause mortality
- Inclusion criterion: LVEF < 45% 3-6 days after successful reperfusion by quantitative echo core lab analysis
- Aim: to reduce 2-year mortality by 25%
- anticipated mortality in control group: 11.5% at 2 years
- 11 participating European countries
- 6 core cell processing facilities across Europe
- first patient in: Q3 / 2013
Patients with acute myocardial infarction post primary PCI

N = 3000 eligible AMI patients 3-6 days post primary PCI
Central reading of echocardiography (EF ≤ 45%)

Randomisation 1:1

Group 1: Control, n= 1500
No intervention

Day 30 ± 7 days: Site visit follow-up
Month 3: Telephone follow-up
Every 3 months: Telephone follow-up
Following observation of full no. of events:
End of study visit (on site)

Group 2: BM-MNC, n= 1500
Bone marrow aspiration

Intracoronary infusion of BMCs
4-8 days post PCI

Day 30 ± 7 days: Site visit follow-up
Month 3: Telephone follow-up
Every 3 months: Telephone follow-up
Following observation of full no. of events:
End of study visit (on site)
Cell processing centers and patient distribution

- The Royal London Hospital, London, UK
- Hospital Gregorio Maranon, Madrid, Spain
- Rigshospitalet University Hospital, Copenhagen, Denmark
- Universitaire Ziekenhuizen, Leuven, Belgium
- BSD, Institute for Transfusion Medicine, Frankfurt, Germany

potentially eligible patients @ day 4-5 post AMI (EF≤45)

Start Sept 2013, 8 Sept 2014: n = 51 patients
Cell therapy in cardiovascular diseases

- Acute Myocardial Infarction
- Refractory Angina
- Peripheral arterial occlusive disease
- Chronic post-infarction heart failure
Challenges in Cell Therapy of Chronic Heart Failure

Acute Infarction

- 'healed' infarction
- established scar
- lack of inflammation
- remodeled LV
- chronically ill patient

Reverse LV Remodeling

Impaired homing of progenitor cells in chronic heart failure

- Mean Indium activity (%; AUC/time)

Acute MI
MI > 1 year

Effects of i.c. administration of progenitor cells in CHF

- Abs. delta LVEF (baseline – FU; %)

Pooled analysis (N=70)

Schächinger et al, Circ 2008
Assmus et al, NEJM 2006
Kang et al, Circ 2006
Need for novel cell sources or enhancement strategies for cell therapy in chronic heart failure

Pretreatment of progenitor cells

- Bone marrow
- Blood
- Skeletal muscle
- Adipose tissue
- Cardiac stem cells

Pretreatment of the target region

- Shock wave pretreatment
- Nanofiber-based delivery

Repetitive applications

Recruitment in target tissue

Early Cardiac Retention of Administered Stem Cells Determines Clinical Efficacy of Cell Therapy in Patients With Dilated Cardiomyopathy

Birgit Assmus, Andreas M. Zeiher
Shock Wave Application may Improve Cell Homing to Target Area

1. Shock Wave Pretreatment

2. Release of chemoattractant factors in target tissue after 24 h
   - VEGF & SDF-1 (attraction & retention of BMC)

3. Injection of cells

- Homing

- Number of Cells (% of control)

- Untreated limb
- 500
- 1000
- 2000

- Number of pulses
- 0.05 mJ/mm²

- P < 0.05

(Aicher et al, Circulation 2006)
2-D-Echo-guided cardiac shockwave application

- Coil High Voltage pulse
- Membrane (Magnetic field)
- Rapid membrane movement
- Shock wave produced in water bellow
- Shockwave focused by acoustic lens

Power generator with Energy level control unit and ECG synchronization

ECG Trigger

Live 2D integrated Ultrasound Imaging

Shockwave Source
- Flat coil
- Membrane
- Acoustic lens
- Bellow
- Shockwave path
- SW target area

Custom build by Dornier Med Tech Systems Wessling, Germany

Assmus et al., JAMA 2013
Primary endpoint: absolute change in LVEF at 4 months

- pooled groups -

Assmus et al., JAMA 2013
**Mechanistic Insights by MRI substudy**

**Increased infarct wall thickening**

- SW & Placebo: N=11
- Placebo-SW & BMC: N=7
- SW & BMC: N=15

P for trend = 0.01

**Decreased infarct size**

- SW & Placebo: N=12
- Placebo-SW & BMC: N=6
- SW & BMC: N=13

P for trend = 0.002

*Assmus et al., JAMA 2013*
Hazard ratios for MACE at 3-year follow-up

All-cause death
Cardiac Death
Re-MI
Rehospitalization for CHF
Ventricular Tachycardia
Cardiac death and rehospitalisation for heart failure
Cardiac death, rehospitalisation for heart failure, and re-AMI
Cardiac death, rehospitalisation for heart failure, re-AMI and VT

Assmus et al., JAMA 2013
Cell therapy with autologous BMC is a realistic option in patients with large acute myocardial infarction.

EU-sponsored mortality trial

Cell therapy with autologous BMC in patients with chronic post-infarction heart failure: application and single therapy are safe, but have minor efficacy. Enhancement strategies are under way:
- different cell types (cardiomyocyte progenitor cells)
- pretreatment of target tissue (shockwave)
- repeated applications (Repeat trial)
Andreas Zeiher & Stefanie Dimmeler
Florian Seeger, Stephan Fichtlscherer, Jörg Honold, Brigitte Luu

Institute of Cardiovascular Regeneration Centre for molecular medicine

Former contributors:
Volker Schächinger, Salvatore de Rosa,
David Leistner, Ulrich Fischer-Rasokat
Ralf Lehmann

Cardiology Studies
Coordinating Centre (CSCC):
Stephanie Estel, Daniela Höhl,
Carmelo Smorta, Anne Krämer,
Marga Müller-Ardogan

Support:
DFG (SFB 834, TR-SFB23)
Excellence Cluster ECCPS
LOEWE
Leducq Foundation: Transatlantik Network of Excellence
European Union: ERC Advanced Grant, Endostem
Need for novel cell sources or enhancement strategies for cell therapy in chronic heart failure

Pretreatment of progenitor cells

Recruitment in target tissue

Bone marrow
Blood
Skeletal muscle
Adipose tissue
Other sources

Cell therapy

shock wave pretreatment
nanofiber-based delivery

Comparison of observed and model-predicted * mortality in 297 consecutive patients treated with intracoronary BMC infusion.

Seattle Heart Failure Model (SHFM):
- multivariable risk model that predicts all-cause and cause-specific mortality in CHF
- age, gender, etiology of cardiomyopathy, hemodynamics, LVEF, treatment incl. devices, lab values
- validated in 9942 patients from large clinical trials: ELITE2, Val-HeFT, UW, RENAISSANCE, IN-CHF)

Small but significant effects on LV function

- initiation of an ongoing registry in 2005
- open to all patients presenting with CHF at our centre
- patients were offered repeated treatment at 4 months at time of first treatment (not to long-distance travellers)
Only repeated intracoronary BMC treatment is associated with lower mortality than SHFM-model predicted mortality.

N = 297 patients; repeated treatment offered at 4 months FU.

De Rosa, ... Zeiher, Assmus under revision.
Only repeated intracoronary BMC treatment is associated with lower mortality than SHFM-model predicted mortality.

**Estimated cumulative survival**

- **Total cohort analysis**
  - Repeated BMC administration
  - Single BMC administration
  - P = 0.02

- **Landmark analysis**
  - Repeated BMC administration
  - Single BMC administration
  - P = 0.07

**Estimated event-free survival**

- **Total cohort analysis**
  - Death and rehospitalization
  - Repeated BMC administration
  - Single BMC administration
  - P = 0.02

- **Landmark analysis**
  - Death and rehospitalization
  - Repeated BMC administration
  - Single BMC administration
  - P = 0.06
Design REPEAT trial

N = 668 patients with post-infarction heart failure
Open infarct vessel / bypass

1. intracoronary infusion of BMC
N=334

2. intracoronary infusion of BMC
4 months

Randomisation 1:1

8 months

Group 1

24 months; primary endpoint

60 months; end of study

Group 2

1. intracoronary infusion of BMC
N=334

2. intracoronary infusion of BMC

4 months

Started Dec. 2013

Primary endpoint: Mortality at 2 years
Secondary endpoint: Rehospitalization for CHF, cardiac death, HTX/LVAD
Need for novel cell sources or enhancement strategies for cell therapy in chronic heart failure

Pretreatment of progenitor cells

- Bone marrow
- Blood
- Skeletal muscle
- Adipose tissue
- Other sources

Recruitment in target tissue

- shock wave pretreatment
- nanofiber-based delivery

Cardiac stem cells: the heart’s little helper

- **c-kit (mouse, dog, human)**
  (Beltrami, Cell 2003)

- **Sca-1 (mouse)**
  **Sca-1-like (dog, human)**
  (Oh et al, PNAS 2003)

- **Cardiospheres (murine, human)**
  (Messina et al, Circ Res 2004)

- **Islet (postnatal mouse, human)**
  (Laugwitz et al, Nature 2005)

Cell therapy with cardiac stem cells

**c-kit^+ CSC (Scipio trial)**
(Bolli et al Lancet 2011)

- **Ejection Fraction at 4 Months After CSCs**

<table>
<thead>
<tr>
<th></th>
<th>Control patients (n=7)</th>
<th>CSC-treated patients (n=14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>50%</td>
<td>50%</td>
</tr>
<tr>
<td>4 months</td>
<td>40%</td>
<td>55%</td>
</tr>
</tbody>
</table>

- Cells expanded from atrial samples obtained during CABG

**Cardiospheres (Caduceus trial)**
(Makkar et al Lancet 2012)

- **Infarct size reduction at 6 and 12 months after Cardiospheres**

- No difference in EF or volumes

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>CSCs</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 months</td>
<td>N=17</td>
<td>N=8</td>
</tr>
<tr>
<td>12 months</td>
<td>N=17</td>
<td>N=17</td>
</tr>
</tbody>
</table>

- Cells expanded from endomyocardial biopsies

2009
First patient treated

2011
Phase I data: presented AHA 2011