STEM CELLS FOR CARDIAC REGENERATION

REPLICABILITY OF SCIENTIFIC DATA

Lucian Beer 2016
1. Stem cells for Cardiac regeneration (Orlic Paper)

2. Prof. Anversa´s group

3. Lessons to be learned
“Orlic paper” published 2001 in Nature
Showed that stem cells derived from bone marrow can transdifferentiate into cardiomyocytes and “generate de nova myocardium”
(6032 citations on google scholar 05/2016)
STEM CELLS FOR CARDIAC REGENERATION

Myocardial infarct after injection on bone marrow derived stem cells. Arrowheads indicate regenerating myocardium; VM, viable myocardium.

red, cardiac myosin
green, propidium iodide labelling of nuclei

Orlic et al. Nature 410, 701-705 (5 April 2001)
Stem cells regenerate the myocard from the endocardium (EN) to the epicardium (EP)

EGFP (green); b, cardiac myosin (red); c, combination of EGFP and myosin (red–green), and propidium-iodide-stained nuclei (blue). Infarcted tissue (IT)
Subendocardial myocytes (SM)

Newly built myocardium forms regular cell-cell contacts

Stem cells improve cardiac function.

**Proposed scheme**

- Infarcted myocardium
- Unknown molecular signal(s)
  - Cell migration to damaged area
  - Proliferation and differentiation
    - Cytoplasmic proteins
      - Cardiac myosin
      - α-Sarcomeric actin
      - Connexin 43
    - Nuclear proteins
      - Csx/Nkx2.5
      - MEF2
      - GATA-4
  - Functional competence

Orlic et al. *Nature* 410, 701-705 (5 April 2001)
Pubmed search with tags: „stem cell“ AND heart
STEM CELLS HOME TO THE TRANSPLANTED HEART

Cardiac myocytes

Smooth-muscle cells

Arrowhead = Y-chromosome in a female donor heart

C-kit cells expressing the transcription factor Nkx2.5 (white dots)

In vivo data: 20 day after AMI; Cardiac myosin, red; PI, green. (D) Connexin 43 (yellow; arrows). (E) N-cadherin (yellow; arrows). (D and E) BrdU-PI labeled nuclei, white-green

Immediate translation from basic science to clinical trials!

**Study characteristics**

<table>
<thead>
<tr>
<th>Name of Study</th>
<th>Sample size (Cell therapy/Controls)</th>
<th>Mean follow-up duration (month)</th>
<th>Cell type</th>
<th>Location of AMI</th>
<th>Time from AMI to cell delivery (days)</th>
<th>Imaging modality</th>
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</thead>
<tbody>
<tr>
<td>CADUCEUS</td>
<td>17/8</td>
<td>12</td>
<td>Cardiosphere-derived cells</td>
<td>anterior (except 1)</td>
<td>62±11</td>
<td>MRI</td>
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<tr>
<td>BONAMI</td>
<td>52/49</td>
<td>3</td>
<td>BM-MNC</td>
<td>anterior</td>
<td>9±2</td>
<td>SPECT, RNV</td>
</tr>
<tr>
<td>Aalst Study</td>
<td>19/16</td>
<td>4</td>
<td>BM-MNC</td>
<td>multiple</td>
<td>12±1</td>
<td>LV Angiography</td>
</tr>
<tr>
<td>REPAIR-AMI</td>
<td>101/103</td>
<td>4</td>
<td>BM-MNC</td>
<td>multiple</td>
<td>4±1</td>
<td>LV Angiography</td>
</tr>
<tr>
<td>BOOST</td>
<td>30/30</td>
<td>6</td>
<td>BM-MNC</td>
<td>multiple</td>
<td>5±1</td>
<td>MRI</td>
</tr>
<tr>
<td>LATE-TIME</td>
<td>58/29</td>
<td>6</td>
<td>BM-MNC</td>
<td>multiple</td>
<td>17±5</td>
<td>MRI</td>
</tr>
<tr>
<td>ASTAMI</td>
<td>50/50</td>
<td>6</td>
<td>BM-MNC</td>
<td>anterior</td>
<td>6±1</td>
<td>SPECT, Echocard.</td>
</tr>
<tr>
<td>REGENT</td>
<td>160/40</td>
<td>6</td>
<td>BM-MNC, or selected CD34+CXCR</td>
<td>anterior</td>
<td>7±2</td>
<td>MRI</td>
</tr>
<tr>
<td>SWISS-AMI</td>
<td>133/67</td>
<td>4</td>
<td>BM-MNC</td>
<td>multiple</td>
<td>13±10</td>
<td>MRI</td>
</tr>
<tr>
<td>TIME</td>
<td>79/41</td>
<td>6</td>
<td>BM-MNC</td>
<td>multiple</td>
<td>5±2</td>
<td>MRI</td>
</tr>
<tr>
<td>SCAMI</td>
<td>29/13</td>
<td>12</td>
<td>BM-MNC</td>
<td>multiple</td>
<td>6±1</td>
<td>MRI</td>
</tr>
<tr>
<td>FINCELL</td>
<td>39/39</td>
<td>6</td>
<td>BM-MNC</td>
<td>multiple</td>
<td>3±1</td>
<td>Echocard.</td>
</tr>
</tbody>
</table>

SPECT: single photon emission computed tomography, RNV: radionuclide ventriculography, Echocard.: Echocardiography

CLINICAL IMPACT

Haematopoietic stem cells do not transdifferentiate into cardiac myocytes in myocardial infarcts

Charles E. Murry¹, Mark H. Soonpaa², Hans Reinecke¹, Hidehiro Nakajima², Hisako O. Nakajima², Michael Rubart², Kishore B. S. Pasumarthi²*, Jitka Ismail Virag¹, Stephen H. Bartelmez³, Veronica Poppa¹, Gillian Bradford², Joshua D. Dowell², David A. Williams²* & Loren J. Field²

several clinical trials¹⁶,¹⁷. Here, we used both cardiomyocyte-restricted and ubiquitously expressed reporter transgenes to track the fate of haematopoietic stem cells after 145 transplants into normal and injured adult mouse hearts. No transdifferentiation into cardiomyocytes was detectable when using these genetic techniques to follow cell fate, and stem-cell-engrafted hearts showed no overt increase in cardiomyocytes compared to sham-engrafted hearts. These results indicate that haematopoietic stem cells do not readily acquire a cardiac phenotype, and raise a cautionary note for clinical studies of infarct repair.
OUTLINE

1. Stem cells for Cardiac regeneration (Orlic Paper)

2. Prof. Anversa’s group

3. Lessons to be learned
LESSONS TO BE LEARNED

1. Reproducibility of scientific data is <50%  
   • No correlation between IF and reproducibility

Prinz et al. Nature Reviews Drug Discovery 2011 ;10
### Lessons to be Learned

**Table 1. Examples of Some Reported Reproducibility Concerns in Preclinical Studies**

<table>
<thead>
<tr>
<th>Author</th>
<th>Field</th>
<th>Reported Concerns</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ioannidis et al (2009)²²</td>
<td>Microarray data</td>
<td>16/18 studies unable to be reproduced in principle from raw data</td>
</tr>
<tr>
<td>Baggerly et al (2009)²³</td>
<td>Microarray data</td>
<td>Multiple; insufficient data/poor documentation</td>
</tr>
<tr>
<td>Sena et al (2010)²⁴</td>
<td>Stroke animal studies</td>
<td>Overt publication bias: only 2% of the studies were negative</td>
</tr>
<tr>
<td>Prinz (2011)¹</td>
<td>General biology</td>
<td>75% to 80% of 67 studies were not reproduced</td>
</tr>
<tr>
<td>Begley &amp; Ellis (2012)²</td>
<td>Oncology</td>
<td>90% of 53 studies were not reproduced</td>
</tr>
<tr>
<td>Nekrutenko &amp; Taylor (2012)²⁵</td>
<td>NGS data access</td>
<td>26/50 no access to primary data sets/software</td>
</tr>
<tr>
<td>Perrin (2014)²⁶</td>
<td>Mouse, in-vivo</td>
<td>0/100 reported treatments repeated positive in studies of ALS</td>
</tr>
<tr>
<td>Tsilidis et al (2013)²⁷</td>
<td>Neurological studies</td>
<td>Too many significant results, overt selective reporting bias</td>
</tr>
<tr>
<td>Lazic &amp; Essioux (2013)²⁸</td>
<td>Mouse VPA model</td>
<td>Only 3/34 used correct experimental measure</td>
</tr>
<tr>
<td>Haibe-Kains et al (2013)²⁹</td>
<td>Genomics/cell line analysis</td>
<td>Direct comparison of 15 drugs and 471 cell lines from 2 groups revealed little/no concordant data</td>
</tr>
<tr>
<td>Witwer (2013)³⁰</td>
<td>Microarray data</td>
<td>93/127 articles were not MIAME compliant</td>
</tr>
<tr>
<td>Prassas et al (2013)³²</td>
<td>Commercial ELISA</td>
<td>ELISA Kit identified wrong antigen</td>
</tr>
<tr>
<td>Baker et al (2014)³⁴</td>
<td>Journals</td>
<td>Top tier fail to comply with agreed standards for animal studies</td>
</tr>
<tr>
<td>Vaux (2012)³⁵</td>
<td>Journals</td>
<td>Failure to comply with their own statistical guidelines</td>
</tr>
</tbody>
</table>

ALS indicates amyotrophic lateral sclerosis; MIAME, minimum information about a microarray experiment; NGS, next generation sequencing; and VPA, valproic acid (model of autism).
LESSONS TO BE LEARNED

1. Reproducibility of scientific data is <50%
   • No correlation between IF and reproducibility
2. Publishing policies (e.g., Publication pressure, splitting of data analysis – PI does all final data analysis…)
3. Academic culture:
   • Journal clubs
   • Cooperation
   • Publishing of raw data….
CONCLUSION

“I think you should be more explicit here in step two.”

http://www.huffingtonpost.com/david-h-bailey/set-the-default-to-open-r_b_2635850.html
reproducibility
a principle of the scientific method

separates scientists from other researchers and normal people

http://xkcd.com/242/