Evaluation of Myocardial Viability and Stem Cell Survival
- Cardiac Regeneration -

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CIRM Roundtable with FDA
October 16th, 2012
I. Myocardial viability
   - Tissue characterization
   - Clinical end-point

II. Stem cell survival and engraftment
   - Fundamental mechanism for myocardial restoration
   - Myocardial regeneration
   - Optimal cell population for clinical translation
I. Myocardial Viability

- Coronary artery disease: #1 killer in the US
  - 1.1 million new or recurrent MI and 500,000 deaths
  - Improved acute MI therapy shift in the landscape
- Heart failure (HF): #1 cause of hospital admission
  - Prevalence: 5 million patients
  - Incidence: 300,000 patients/year
  - Advanced therapy: 5-year survival \( \sim \)50%
  - Heart transplant: 1,000 pt/year
- Accurate diagnosis and optimal therapeutic intervention
Clinical Issues

• Challenge: dysfunctional myocardium
  – Dead, viable, or viable but injured myocardium
  – Sufficient viability to salvage the injured myocardium
  – Revascularization, device, and/or medicine

• Mandate: regenerate the myocardium
  – Permanent and sustained restoration of the myocardium
  – Increase myocardial viability survival
Delayed Gadolinium (Gd) Enhanced MRI (DEMRI)

- Relies on non-specific distribution of Gd into extracellular space
- Delayed Gd clearance from infarcted myocardium/scar produces T1 positive MRI signal
Recovery of Regional Contractility

Kim R, et al. NEJM ‘00
Hypothesis

Presence of peri-infarct ischemia predicts future CVE in patients with severe ischemic cardiomyopathy (CVE: death, MI, stroke, CHF, ventricular arrhythmia, syncope).
## Results: Peri-infarct Ischemia

<table>
<thead>
<tr>
<th></th>
<th>CVE (+) (n=6)</th>
<th>CVE (-) (n=17)</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Peri-infarct ischemia, n (%)</strong></td>
<td>4/6 (67)</td>
<td>2/17 (12)</td>
<td>0.03</td>
</tr>
<tr>
<td><strong>Scar volume, cm(^3)</strong></td>
<td>20 ± 15</td>
<td>21 ± 18</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Scar percentage of LV volume, %</strong></td>
<td>9 ± 7</td>
<td>15 ± 12</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Ejection fraction, %</strong></td>
<td>29 ± 10</td>
<td>23 ± 13</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Age, years</strong></td>
<td>51 ± 12</td>
<td>54 ± 11</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Coronary anatomy, n (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 vessel (include P-LAD or LMT)</td>
<td>2/6 (33)</td>
<td>8/17 (47)</td>
<td>NS</td>
</tr>
<tr>
<td>3 vessel disease</td>
<td>4/6 (67)</td>
<td>9/17 (53)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are expressed as a mean ± SD.
Hypothesis

Quantitative characterization of myocardial scar by CMRI can predict cardiovascular events in patients with severe ischemic cardiomyopathy.
# Results: Myocardial Scar

<table>
<thead>
<tr>
<th></th>
<th>CVE (+) n=33</th>
<th>CVE (-) n=53</th>
<th>p - value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scar volume (cm³)</td>
<td>16.8 ± 12.4</td>
<td>11.7 ± 12.6</td>
<td>0.023</td>
</tr>
<tr>
<td>Scar % of the myocardium</td>
<td>10.2 ± 6.9</td>
<td>7.2 ± 6.7</td>
<td>0.037</td>
</tr>
</tbody>
</table>
### Results: Transmural MI

<table>
<thead>
<tr>
<th>Description</th>
<th>CVE (+)</th>
<th>CVE (-)</th>
<th>(p) - value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Non-transmural MI</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(1-75% scar of myocardium)</td>
<td>18.4 ± 14.0%</td>
<td>13.8 ± 11.2%</td>
<td>0.049</td>
</tr>
<tr>
<td>1 – 25%</td>
<td>9.2 ±11.0%</td>
<td>6.7 ± 9.3%</td>
<td>0.12</td>
</tr>
<tr>
<td>26 – 50%</td>
<td>9.2 ± 10.6%</td>
<td>3.2 ± 3.6%</td>
<td>0.03</td>
</tr>
<tr>
<td>51 – 75%</td>
<td>3.5 ± 4.2%</td>
<td>4.0 ± 4.5%</td>
<td>0.30</td>
</tr>
<tr>
<td><strong>Transmural MI</strong> (76 – 100% scar of myocardium)</td>
<td>5.8 ± 10.2%</td>
<td>7.2 ± 11.4%</td>
<td>0.28</td>
</tr>
</tbody>
</table>
Quantitative Tissue Characterization of Infarct Core and Border Zone in Patients With Ischemic Cardiomyopathy by Magnetic Resonance Is Associated With Future Cardiovascular Events

Shahriar Heidary, MD,* Harendra Patel, MD,* Jaehoon Chung, MD,* Hajime Yokota, MD,* Sandeep N. Gupta, PhD,§ Mihoko V. Bennett, PhD,† Chandra Katikireddy, MD,* Patricia Nguyen, MD,* John M. Pauly, PhD,‡ Masahiro Terashima, MD, PhD,* Michael V. McConnell, MD,* Phillip C. Yang, MD*
Stanford, California; and Niskayuna, New York

Hypothesis

Evaluation of infarct heterogeneity in the peri-infarct region may be a stronger predictor of CVE than the traditional measurements.
## Results: Heterogeneity Analysis

### Significant predictors for CVE

<table>
<thead>
<tr>
<th></th>
<th>CVE (+) n=29</th>
<th>CVE (-) n=41</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Scar Mass (g)</td>
<td>36.7 ± 22.2</td>
<td>27.0 ± 21.0</td>
<td>0.03</td>
</tr>
<tr>
<td>Peri-Infarct Scar Mass (g)</td>
<td>17.0 ± 13.1</td>
<td>11.2 ± 11.0</td>
<td>0.02</td>
</tr>
<tr>
<td>Peri-Infarct Scar % of the Myocardium (%)</td>
<td>10.6 ± 7.9</td>
<td>7.3 ± 7.7</td>
<td>0.04</td>
</tr>
</tbody>
</table>

### Medicine vs. revascularization
- Medicine: Peri-infarct zone
- Revascularization: Total scar and core zone
# Results: LVEF and Volume

<table>
<thead>
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<th>CVE (+)</th>
<th>CVE (-)</th>
<th>( p ) - value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVEF (%)</td>
<td>25 ± 10</td>
<td>27 ± 13</td>
<td>0.26</td>
</tr>
<tr>
<td>LVEDV (ml)</td>
<td>234 ± 76</td>
<td>230 ± 88</td>
<td>0.41</td>
</tr>
<tr>
<td>LVESV (ml)</td>
<td>180 ± 73</td>
<td>175 ± 90</td>
<td>0.40</td>
</tr>
</tbody>
</table>
Manganese-Enhanced MRI (MEMRI)

- Manganese (Mn\(^{2+}\)) produces T1 shortening
- Enters **live** cells via L-type Ca\(^{2+}\) channels
- Uptake is specific for live cardiomyocytes

Dash et al., *Circulation: Cardiovasc Imaging*, 2011
MEMRI-DEMRI of Peri-Infarct Region

Dash et al, Circulation: Cardiovasc Imaging, 2011
II. In Vivo MEMRI of Stem Cell Survival

Manganese (Mn$^{2+}$)

Voltage gated calcium channel

Bright signal on MRI

In Vivo MEMRI in Murine Myocardium

Myocardial Regeneration: Human Amniotic Mesenchymal Stem Cells

Ge et al., Stem Cells Dev, 2012
iPSC Generation

- **Around day 10: colony formation**
  - At least ~20 hESC-like colonies were found from 20,000 cells (more than 0.1%)

- **At day 20, the characteristic iPSC colonies were observed**
  - high activity of alkaline phosphatases (ALP) was observed.
Immunomodulation *in vivo*

- 250K MiPSCs were injected into hind limbs of immunocompetent FVB mice.
- MiPSCs survived in the myocardium ~2 weeks post-injection.
- MiPSCs survived in FVB mice 7 days.
Cardiac Differentiation - Phenotype

- High levels of mature cardiac marker expression: cTNT, connexin 43 and sarcomeric actin
- Calcium transient through L-type calcium channel and contractile force
Functional restoration

- The EF improved by MiPSCs (control NS LVEF 15-20%)
- The myocardial viability was increased significantly (control NS 75%).

Kim PJ et al, Circulation, 2012, YIA
Porcine Myocardial Injury Model

8 Adult Farm Pigs

Day 0 Intramyocardial hAMSC
~80 million hAMSCs

Serial Cardiac MRI at d7, 14, 21 post-HAMSC

Cardiac PET-CT Imaging
Subpopulation of cells (10-15 million hAMSCs) transduced with HSV-tk PET reporter gene

Immunohistochemistry
Human Anti-Mitochondrial Ab
Porcine Ischemia-Reperfusion Model
- hAMSCs
- Increased LVEF

hAMSCs Improve LV Function

Control - 14 d

hAMSC - 14 d
Prolonged hAMSC Survival:
- Transduced w/ HSV-tk PET reporter gene
- PET-CT positive for live cell populations within apex and septum, corresponding to hAMSC injection sites
- IHC positive for human Anti-Mitochondrial antibody

d66 post-IR; d38 post-hAMSC
hAMSCs Increase Viable Myocardium

- hAMSCs generated higher MEMRI signal within the infarct zone: viable hAMSCs
- hAMSCs generated smaller infarct zones
- hAMSCs decreased remodeling
Cardiovascular Stem Cell Imaging

- Evaluation of myocardial viability: clinical end-point using MEMRI and DEMRI
  - IND approval for MEMRI

- Translational imaging technique will visualize: survival of cardiovascular stem cell

- Permanent and sustained restoration of the injured heart: myocardial regeneration by optimal cell population
Laboratory for Cellular and Molecular MRI of Cardiovascular Stem Cells

Yang Laboratory
Takayasu Arai
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Xiaohu Ge
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Luke Kwon
Yuka Matsuura
Garren Reichel
Yoriyasu Suzuki
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Ildiko Toma
Hunter Wang
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Joe Wu

CT Surgery
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Robert Robbins

CV Intervention
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Scott Metzler
Renee Reijo-Pera
Chelsey Simmons
Irv Weissman

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UM1-HL-12026
R01-HL39297
CIRM, CVI SEED, AHA
Direct cardiac transdifferentiation of hAMSCs

- VPA
- LIF
- CHIR
- Tra-1-60
- BMP4
- Activin A
- SU5402
- IWR
- Apelin
- RA

hAMSCs → Partial reprogramming → Tra-1-60+ hAMSCs → Mesoderm formation → Mesoderm → Cardiac specification → Cardiac differentiation → Cardiac Cells

Graph showing gene expression levels with bar charts for different conditions.
VI. Current and Future Clinical Studies

• Direct cardiac transdifferentiation
• Simulation of durable myocardial tissue
• Clinical trial
  – MEMRI FOR MYOCARDIAL VIABILITY: FDA IND
  – NIH Cardiovascular Cell Therapy Research Network
• Disease Modeling of Congenital Heart Disease
Durable Myocardial Tissue

- hCMs only
- hCMs + hAMSCs
- hCMs + hECs
- hCMs + hAMSCs + hECs

hCMs only

hCMs + hECs
hAMSC isolation

1. Removing chorion layer of the placenta body.
2. Peeling the amnion membrane underlying chorion layer.
3. Washing and digesting the membrane.
4. Culturing hAMSCs

Clinical Relevance of Hibernating Myocardium in Ischemic Left Ventricular Dysfunction
Arend F.L. Schinkel, MD, a,b Jeroen J. Bax, MD, c Victoria Delgado, MD, c Don Poldermans, MD, Shahbudin H. Rahimtoo, MD

Predicting Benefit From Revascularization in Patients With Ischemic Heart Failure: Imaging of Myocardial Ischemia and Viability
Orla Buckley and Marcelo Di Carli

Circulation
JOURNAL OF THE AMERICAN HEART ASSOCIATION

American Heart Association®
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MEMRI of Myocardial Viability

- Rapid assessment of myocardial viability
  - Tissue enhancement pattern established within 30 seconds
  - Persists for at least one hour and blood signal gone by 10 minutes
  - Viable cell specific
Mechanism of Delayed Enhancement

- Gd is injected & wait 10-20 min
- Gd accumulates in injured tissue
- 180° RF pulse inverts all the spins
- Tissues return to nl at different rates
- At time TI, image acquisition begins

In vivo MEMRI of viable hAMSCs

- hAMSCs survived over 5 weeks (and beyond?) in porcine heart with minimal cyclosporine immunosuppression
- hAMSCs improved cardiac function predictably and durably in subacute and chronic infarction model
- hAMSCs reduced infarct size and LV dilatation
- MEMRI tracked LIVE stem cells with no prior cell modification
Peri-infarct ischemia

Delayed-enhanced Stress Perfusion Image Peri-infarct Ischemia
Measurement of Myocardial Scar

Scar percentage of the myocardium (%) = Scar volume / myocardial volume
Analysis of Transmurality

1 - 50% (Non transmural)

76 - 100% (Transmural)
Tissue Heterogeneity Quantification

Tissue heterogeneity analysis performed with Cinetool version 7.1.2, GE Healthcare.
Evaluation Myocardial Viability

• Myocardial Function
  – Echo: safe vs. qualitative and acoustic window

• Cellular Metabolism
  – SPECT and PET: quantitative vs. ionizing radiation and low image quality

• Myocardial Scar and Infarct: gold standard
  – MRI: image quality and quantitative vs. Gd-contrast agent
II. *In Vivo* Evaluation of Stem Cell Survival

<table>
<thead>
<tr>
<th></th>
<th>MRI</th>
<th>RN</th>
<th>BLI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sensitivity</strong></td>
<td>$10^{-7} - 10^{-9}$ Mole/L</td>
<td>$10^{-11} - 10^{-12}$ Mole/L</td>
<td>$10^{-15} - 10^{-17}$ Mole/L</td>
</tr>
<tr>
<td><strong>Spatial resolution</strong></td>
<td>500 μm</td>
<td>3-5 mm</td>
<td>3-5mm</td>
</tr>
<tr>
<td><strong>Temporal resolution</strong></td>
<td>10 ms</td>
<td>seconds</td>
<td>seconds</td>
</tr>
</tbody>
</table>

In Vivo Molecular MRI - Teratoma
In Vivo Molecular MRI of Cell Survival

In Vivo Molecular MRI of Cell Survival

T2* effect

HA fluc

myc

EF-1α/Ubiquitin Igκ HA fluc myc PDGFR-TM
II. Stem Cell Survival in the Myocardium

Hendry et al, JTCS 2008
Mechanism of Myocardial Restoration