Cardiac Gene Therapy: Beyond the Mouse

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Presenter Disclosure Information

FINANCIAL DISCLOSURE:

Equity: Osprey Medical
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UNLABELED/UNAPPROVED USES DISCLOSURE:
None
HF Epidemiology

Circ HF 2007
Heart Failure Treatment

Crude HF case-fatality rates after first diagnosis

Jhund et al; Circulation 2009;119:515-25

<55 yrs 55-64 yrs 65-74 yrs 75-84 yrs

30 days 1 year 5 years
Current state of play in advanced HF

**Survival**
- Baseline: 82%, 67%, 37%
- Post-intervention: 68%, 46%, 14%

**Mortality**
- Baseline: 18%, 33%, 63%
- Post-intervention: 32%, 54%, 86%

**Mean life expectancy**
- Baseline: 4.4 years
- Post-intervention: 2.5 years

**Baseline Characteristics**

**Clinical**
- Age: 74
- Gender: Male
- NYHA Class: 3B
- Weight (kg): 85
- EF: 20
- Syst BP: 108
- Ischemic

**Medications**
- ACE-I
- Beta-blocker
- ARB
- Statin
- Allopurinol
- Aldosterone blocker

**Diuretics**
- Furosemide: 160
- Bumetanide: 0
- Torsemide: 0
- Metolazone: 0
- HCTZ: 0

**Lab Data**
- Hgb: 13.6
- Lymphocyte%: 24
- Uric Acid: 9
- Total Chol: 190
- Sodium: 137

**Devices**
- None
- BiV Pacer
- ICD
- BiV ICD

**Interventions**
- ACE-I
- ARB
- Beta-blocker
- Statin
- Aldosterone Blocker

**Devices**
- Note: Some devices may be disabled if CMS clinical criteria are not met. See below.

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Options for the Patient with Advanced HF

Current Rx
Pharmacotherapy
BiV ICD

VAD
Co-morbidities
Support
Compl.\textsuperscript{ns}
Device durability
QOL
Cost effectiveness

Transplant
Recipient sel\textsuperscript{n}
Donors
Co-morbidities
Late compl.\textsuperscript{ns}

Palliative Care
Understanding HF Pathophysiology

Primary Myocardial Injury

- vasoconstriction
- NOS/ROS
- structural change
- cytokines

Endothelium
- vasoconstriction
- NOS/ROS
- structural change
- cytokines

CHF OUTCOMES: Sudden death, progressive pump failure, symptoms
## Mouse Models of Heart Failure

<table>
<thead>
<tr>
<th>Gene</th>
<th>Models</th>
<th>Cardiac defect</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Transcription factors</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NF-AT</td>
<td>Constitutively active</td>
<td>Hypertrophy, failure</td>
</tr>
<tr>
<td>CREB</td>
<td>Dominant negative</td>
<td>Cardiomyopathy</td>
</tr>
<tr>
<td>GATA4</td>
<td>Overexpression</td>
<td>Hypertrophy, failure</td>
</tr>
<tr>
<td>STAT3</td>
<td>Overexpression</td>
<td>Hypertrophy</td>
</tr>
<tr>
<td>MyoD</td>
<td>Overexpression</td>
<td>Embryonic lethal</td>
</tr>
<tr>
<td>NKX2.5</td>
<td>Mutant</td>
<td>Failure</td>
</tr>
<tr>
<td>RXR</td>
<td>Knockout</td>
<td>Embryonic lethal</td>
</tr>
<tr>
<td><strong>Growth factors, cytokines and other hormones</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNFα</td>
<td>Overexpression</td>
<td>Cardiomyopathy</td>
</tr>
<tr>
<td>TGFβ1</td>
<td>Constitutively active</td>
<td>Atrial fibrosis</td>
</tr>
<tr>
<td>ANP</td>
<td>Knockout</td>
<td>Hypertrophy, fibrosis</td>
</tr>
<tr>
<td>BNP</td>
<td>Knockout</td>
<td>Fibrosis</td>
</tr>
<tr>
<td>IGF1</td>
<td>Overexpression</td>
<td>Hypertrophy, fibrosis</td>
</tr>
<tr>
<td>NGF</td>
<td>Overexpression</td>
<td>Hypertrophy, fibrosis</td>
</tr>
<tr>
<td>VEGF</td>
<td>Knockout</td>
<td>Ischaemic cardiomyopathy</td>
</tr>
<tr>
<td>Mineralocorticoid R</td>
<td>Overexpression</td>
<td>Arrhythmias</td>
</tr>
<tr>
<td>Triadin-1</td>
<td>Overexpression</td>
<td>Hypertrophy, failure</td>
</tr>
<tr>
<td>FKBP12</td>
<td>Knockout</td>
<td>Failure</td>
</tr>
</tbody>
</table>
Regenerating the Failing Heart

• Improving (restoring) the function of myocytes
• Replacing myocytes
Multiple 'Targets' in HF
# Mouse Models of Cardiac 'Restoration' in HF

<table>
<thead>
<tr>
<th>Category</th>
<th>Heart Failure</th>
<th>Gene Therapy Restoration</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Contractile proteins</strong></td>
<td></td>
<td>√</td>
</tr>
<tr>
<td>alpha-MHC</td>
<td>Decreased</td>
<td></td>
</tr>
<tr>
<td><strong>Cytoskeletal proteins</strong></td>
<td></td>
<td>√</td>
</tr>
<tr>
<td>Dystrophin</td>
<td>Genetic DCM</td>
<td></td>
</tr>
<tr>
<td><strong>Ca(^{2+}) handling proteins</strong></td>
<td></td>
<td>√</td>
</tr>
<tr>
<td>PLB</td>
<td>Hypophosphorylation</td>
<td></td>
</tr>
<tr>
<td>SERCA2</td>
<td>Low</td>
<td></td>
</tr>
<tr>
<td>PP Inhibitor 1</td>
<td>Low</td>
<td></td>
</tr>
<tr>
<td>S100A</td>
<td>Low</td>
<td></td>
</tr>
<tr>
<td><strong>GPCRs and associated proteins</strong></td>
<td></td>
<td>√ (βARKct)</td>
</tr>
<tr>
<td>BARK1/GRK2</td>
<td>Increased</td>
<td></td>
</tr>
<tr>
<td>Beta2-AR</td>
<td>Low</td>
<td>√ (NB vs Marked XS)</td>
</tr>
<tr>
<td><strong>Others</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>miRNAs (various)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nerve growth factor</td>
<td></td>
<td></td>
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<tr>
<td>Insulin like growth factor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Relaxin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other (eg Pompe’s, Fabry’s Disease)</td>
<td></td>
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</tr>
</tbody>
</table>
From Mouse to Man?

What are the issues:

• Animal models (MI, TAC, transgenic) vs Human HF (often multi-factorial, duration)
  – Molecular signature may be different

• Route and mode of gene delivery
  • Tail vein, intra-myocardial, intra-ventricular

• Dose eg mouse- 10^{11} AAV particles

• Duration of follow-up and end-points used
  (vs clinical trials)
Gene Therapy Trials to Date

Indications Addressed by Gene Therapy Clinical Trials

- Cancer diseases 66.5% (n=871)
- Cardiovascular diseases 9.1% (n=119)
- Monogenic diseases 8.3% (n=109)
- Infectious diseases 6.5% (n=85)
- Neurological diseases 1.5% (n=20)
- Ocular diseases 0.9% (n=12)
- Other diseases 1.6% (n=21)
- Gene marking 3.8% (n=50)
- Healthy volunteers 1.7% (n=22)

The Journal of Gene Medicine, © 2007 John Wiley and Sons Ltd

www.wiley.co.uk/genmed/clinical
Viral Delivery

- Cardiomyocytes difficult to transfec

<table>
<thead>
<tr>
<th></th>
<th>Size of insert</th>
<th>Titre</th>
<th>Duration of expression</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenovirus</td>
<td>7–10 kb</td>
<td>High</td>
<td>7–14 days (E1-deleted) ~1 month (E1-E4 deleted)</td>
<td>High efficiency</td>
<td>Strongly immunogenic</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>High level of expression</td>
<td>Stimulates B &amp; T cells</td>
</tr>
<tr>
<td>Adeno-associated virus</td>
<td>~4.8 kb</td>
<td>Moderate</td>
<td>Onset of expression at 4 weeks; lifelong expression</td>
<td>Long term expression</td>
<td>Requires adenovirus to grow</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>No immune response</td>
<td>Limited insert size</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Integration at specific site</td>
<td>Complex to prepare</td>
</tr>
<tr>
<td>Lentivirus (pseudo-typed virus)</td>
<td>~8 kb</td>
<td>High</td>
<td>Lifelong</td>
<td>Long term expression</td>
<td>Complex preparation</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>High efficiency</td>
<td>Insertion site</td>
</tr>
<tr>
<td>Herpes virus/amplicon</td>
<td>&gt;35 kb</td>
<td>High</td>
<td>10–20 days</td>
<td>Large transgenes</td>
<td>Complex construction</td>
</tr>
<tr>
<td>Naked DNA</td>
<td>No size constraint</td>
<td>Very high</td>
<td>4–7 days</td>
<td>No viral proteins</td>
<td>Inefficient entry</td>
</tr>
<tr>
<td>DNA liposomes</td>
<td>No size constraint</td>
<td>Very high</td>
<td>4–10 days</td>
<td>More efficient entry</td>
<td>Lack of stability</td>
</tr>
<tr>
<td>Adenoviral polylysine DNA conjugates</td>
<td>No size constraint</td>
<td>High</td>
<td>7–14 days</td>
<td>More efficient entry</td>
<td>Limited persistence</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>Complex to construct</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Immunogenic</td>
</tr>
<tr>
<td>Desired Feature of Delivery System</td>
<td>Description of Feature</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>-----------------------------------</td>
<td>------------------------</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Safety</td>
<td>Achieving desired effect with minimal morbidity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Practicality (ease)</td>
<td>Readily adoptable by broad range of users, whilst also maintaining patient safety</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minimal Invasiveness</td>
<td>Limited procedural trauma may be more readily translated into patients with advanced disease eg. heart failure</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Achieves Delivery at a Critical Concentration</td>
<td>Allows delivery of biological at a threshold level to ensure effect</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Appropriate Regional Distribution</td>
<td>Dependent upon clinical need, provides either regional or global tissue/organ delivery.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Homogeneity of Expression and Biologic Effect</td>
<td>Ensures that all cells within an targeted area are impacted (rather than patchy distribution)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Limited Systemic Exposure and/or Toxicity</td>
<td>Minimizes induction of systemic responses (eg immunologic) or off-target consequences of accumulation in non-target tissue</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cost effectiveness</td>
<td>Determined by technical and equipment aspects</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Procedural Repeatability</td>
<td>Determined by technical aspects such as Invasiveness and cost in addition to biologic responses that limit the effect of repeat exposure</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
## Translatable Delivery Techniques

<table>
<thead>
<tr>
<th>Delivery Technique</th>
<th>Ease</th>
<th>Safety</th>
<th>Regionality</th>
<th>Critical Conc&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Systemic Exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intravenous (systemic)</td>
<td>+++</td>
<td>+++</td>
<td>-</td>
<td>-</td>
<td>+++</td>
</tr>
<tr>
<td>Intracoronary</td>
<td>++</td>
<td>++</td>
<td>+/-</td>
<td>+/-</td>
<td>+++</td>
</tr>
<tr>
<td>Intramyocardial (surgical inj&lt;sup&gt;n&lt;/sup&gt; via epicardial route)</td>
<td>++/+</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Intramyocardial (percutaneous route, endocardial injn)</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Intrapericardial</td>
<td>+/-</td>
<td>+/-</td>
<td>++</td>
<td>++</td>
<td>+/-</td>
</tr>
</tbody>
</table>
A ‘Clinical’ Percutaneous Approach to Whole Heart’ Delivery
The V-Focus Cardiac Delivery System

- Coronary perfusion catheters
- Coronary Sinus Catheter
- Occluding Balloon
- Nitinol Spreader
SERCA Gene Delivery
- *AAV mediated delivery*

16 sheep
High Rate Pacing

4 weeks (High Rate Pacing)

Heart Failure (n=21 met criteria)
Echocardiography & Hemodynamic Measurement

(n=5)
No Administration

(n=6)
Intra-coronary Infusion
*(2.5x10^{13} drp)*

(n=6)
V-Focus
*(1x10^{13} drp)*

6 weeks
High Rate Pacing

Study Termination
Echocardiography & Hemodynamic Measurement
**Ejection Fraction (%)**

**Fractional Shortening (%)**

**Positive dP/dt**

- * p<0.05 vs intra-coronary delivery
- # p<0.05 vs control

**Byrne et al Gene Therapy 2008**
SERCA Distribution after Gene Delivery

AAV-SERCA Treatment

SERCA Protein Abundance (au)

AAV2/1-SERCA 2a DNA (copies/100ng gDNA)

Endocardium Epicardium

Anterior Lateral Posterior

0.5
1.0
1.5

*
Conclusion

• Molecular pathogenesis of CVD well understood
• Optimization of vectors still required
• Variety of delivery tools available
• Future clinical advances will depend on optimal balance between safety, simplicity, efficiency of delivery and end-point effect relevance