Gene Transfer Therapy

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Director, Advanced Heart Failure Treatment Program
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Disclosures

Dr. Greenberg is a consultant for and has received honoraria from Celladon
Evolution of Heart Failure Therapy

*From Withering to the Present*

1700's - 1960's
- Dig and diuretics

1970's
- Vasodilators

*The Age of Empiricism*  
1700’s - 1960’s

*Myocardial Mechanics*  
1970’s
Vasodilator Therapy of Chronic Aortic Insufficiency

Figure 8-4. Effects of long-term hydralazine therapy in a patient with severe aortic insufficiency. Perimeter drawings of the left ventricle from the contrast angiogram are shown. The end-diastolic volume is shown by the unbroken line and the end-systolic volume by the dashed line. A more complete description of the results is included in the text.
ACEIs Inhibit LV Remodeling: SOLVD Echo Sub-study

Mortality in Studies of Left Ventricular Dysfunction (SOLVD) Treatment

Risk Reduction = 16% (95% CI: 5% - 26%)  
\( p = .0036 \)

Effects of Carvedilol on LV Remodeling in Heart Failure

Effects of Beta-Blockers on Mortality in CHF

US CARVEDILOL (n=1094)  CIBIS II (n=2647)  MERIT-HF (n=3991)  COPERNICUS (n=2289)

<table>
<thead>
<tr>
<th>Time (years)</th>
<th>ß-blocker</th>
<th>Placebo</th>
<th>Risk ↓</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.0</td>
<td>1.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.6</td>
<td>0.8</td>
<td>34 %</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>2</td>
<td>0.8</td>
<td>0.8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Risk ↓ 65 %, P = 0.0001

Mortality

Evolution of Heart Failure Therapy
From Withering to the Present

1700’s - 1960’s
Dig and diuretics

1970’s
Vasodilators

1980’s - 2013
Neurohormonal Antagonists

The Age of Empiricism

Myocardial Mechanics

Device Therapy

The Remodeling Paradigm

1980’s - 2013
## Current Heart Failure Therapies That Reduce Morbidity and Mortality

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Devices</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>• ACEIs/ARBs</td>
<td>• BiV pacemakers</td>
<td>• Cardiac Transplant</td>
</tr>
<tr>
<td>• Beta blockers</td>
<td>• ICDs</td>
<td></td>
</tr>
<tr>
<td>• Aldosterone receptor antagonists</td>
<td>• LVAD’s</td>
<td></td>
</tr>
<tr>
<td>• Hydralazine/nitrates (in AAs)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Diuretics…probably</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Has Heart Failure Been Cured?
Survival Trends in HF Patients With and Without Preserved EF

A. Patient with Reduced Ejection Fraction

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>No. at Risk</td>
<td>819</td>
<td>525</td>
<td>424</td>
</tr>
<tr>
<td></td>
<td>424</td>
<td>336</td>
<td>274</td>
</tr>
<tr>
<td></td>
<td>220</td>
<td>274</td>
<td>336</td>
</tr>
</tbody>
</table>

P = 0.005

B. Patient with Preserved Ejection Fraction

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>No. at Risk</td>
<td>510</td>
<td>377</td>
<td>313</td>
</tr>
<tr>
<td></td>
<td>537</td>
<td>447</td>
<td>375</td>
</tr>
<tr>
<td></td>
<td>263</td>
<td>314</td>
<td>262</td>
</tr>
</tbody>
</table>

P = 0.36

Owan et al. NEJM. 2006; 355:251-259.
Why Are Outcomes in Heart Failure Less Favorable Than Expected?

• Problems with patient access to healthcare and inadequate use of proven therapies.
• Little or no progress in improving outcomes in HFpEF or acute decompensated HF.
• Not all patients respond to treatment.
• Current treatments are palliative, not curative.
Even With Optimal Therapy the Heart May Run Out of Steam

• The emergence of a cohort of patients with ‘advanced chronic HF’ is a consequence of recent therapeutic advances.

• Implications: This “emerging cohort of patients with advanced chronic heart failure (ACHF) represents a population for which additional treatments are required”.

COPERNICUS: Carvedilol vs. Placebo in Patients Receiving ACEIs

All-cause mortality rates:
Placebo 18.5%; Carvedilol 11.4%

Risk Reduction ↓35% (19%, 48%) $P=0.0014$

Gene Transfer Therapy
Targets for New Treatments of Heart Failure

Gene Transfer Therapy for Treating Heart Failure

- Introduction of recombinant human genetic material to a patient in order to alter levels of a protein that will directly or indirectly (e.g. paracrine or systemic effects) alter cardiac structure and function.
Gene Therapy of CV Disease

Requirements depend on pathology to be treated:

1. Proportion of cells that need to be gene-modified (e.g. restoration of pacemaker activity vs. recovery of contractility).
2. Temporal expression (e.g. angiogenic factors versus recovery of contractility).
3. Direct effect of transgene on cell compared to paracrine or systemic effects.
# Gene Delivery Systems

<table>
<thead>
<tr>
<th><strong>Non-viral</strong></th>
<th><strong>Recombinant viral</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Strengths:</strong></td>
<td><strong>Strengths:</strong></td>
</tr>
<tr>
<td>1. easy to produce</td>
<td>1. high efficiency</td>
</tr>
<tr>
<td>2. larger cassette size</td>
<td>2. capacity for long-term transgene expression</td>
</tr>
<tr>
<td>3. minimal biosafety risk</td>
<td></td>
</tr>
<tr>
<td><strong>Weaknesses:</strong></td>
<td><strong>Weaknesses:</strong></td>
</tr>
<tr>
<td>1. low transfection efficiency</td>
<td>1. reduced packaging capacity</td>
</tr>
<tr>
<td>2. transient due to intracellular degradation</td>
<td>2. inconsistent bioavailability and purity</td>
</tr>
<tr>
<td></td>
<td>3. biosafety</td>
</tr>
</tbody>
</table>
## Viral Vectors

<table>
<thead>
<tr>
<th></th>
<th>Adenovirus</th>
<th>AAV</th>
<th>Lentivirus</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Functional titre/ml</strong></td>
<td>Up to $10^{12}$</td>
<td>Up to $10^{10}$</td>
<td>Up to $10^{10}$</td>
</tr>
<tr>
<td><strong>Genome</strong></td>
<td>ds DNA</td>
<td>ss DNA</td>
<td>ssRNA</td>
</tr>
<tr>
<td><strong>Insert Capacity</strong></td>
<td>7-30 kb</td>
<td>4.8 kb</td>
<td>7-10 kb</td>
</tr>
<tr>
<td><strong>Integration</strong></td>
<td>No</td>
<td>No (recomb vectors)</td>
<td>Pseudo-random</td>
</tr>
<tr>
<td><strong>Pattern of TG expression</strong></td>
<td>Transient</td>
<td>Long term</td>
<td>Long term</td>
</tr>
<tr>
<td><strong>Cell cycle-dependence</strong></td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td><strong>Host Vector interactions</strong></td>
<td>Cytotoxic and immunogenic</td>
<td>Minimally Immunogenic</td>
<td>Minimally Immunogenic</td>
</tr>
</tbody>
</table>
How Does Gene Therapy Work?

AVV entry. 1 indicates receptor binding and endocytosis; 2, escape into cytoplasm; 3, nuclear import; 4, capsid disassembly; 5, double-strand synthesis; and 6, transcription.

Antegrade Coronary Artery Infusion

V-Focus System and Retrograde Coronary Venous Infusion

Direct Myocardial Injection and Pericardial Injection

Key Role of SERCA2a in Regulating Ca^{2+} in Cardiomyocytes

SERCA2a Declines in Cardiomyocytes in the Explanted Failing Human Heart

Non-Failing  Failing

SERCA2a

Phospho-
lamban

ATPase Activity (mmol/mg.min)

Restoring SERCA2a Activity in Cardiomyocytes Using Gene Transfer
MYDICAR: AAV1/SERCA2a

- **MYDICAR® (AAV1/SERCA2a):**
  - **DNA:** Inverted Terminal Repeats (ITR) derived from AAV2, CMV promoter, human SERCA2a cDNA, PolyA
Kinetics of MYDICAR Driven SERCA2 Expression in the Heart Demonstrates Durability of Expression for 12 Months

The data are expressed as the means ± standard error of the mean.
Survival in Rats with Post-MI HF: SERCA2a vs. Current Inotropes

Gene Transfer

Sham

HF+ Ad.SERCA2a

HF+ Ad.GFP

HF

+ Dobutamine

Gene Transfer

Intracoronary Delivery of SERCA2a in CUPID
CUPID 1 Phase 2 Study Schema

Double-blind, randomized, placebo controlled trial

Screening: N=8
- MYDICAR® Low 6 X 10^{11} DRP (~8.6 X 10^9 DRP/kg)
- MYDICAR® Mid 3 X 10^{12} DRP (~4.3 X 10^{10} DRP/kg)
- MYDICAR® High 1 X 10^{13} DRP (~1.4 X 10^{11} DRP/kg)
- Placebo

Randomization: N=9

Observe 12 Months
- Weeks 1, 2, 3, 4, 5 & 6
- Months 2, 3, 6, 9 & 12

Long-Term Follow-Up
- Semi-Annual Phone Questionnaire for 2 years

CUPID 1 – Entry Criteria

### Main Inclusion Criteria

- Age 18-75 years old
- NYHA Class III/IV
- Ischemic or non-ischemic cardiomyopathy
- Maximal oxygen consumption ($\text{VO}_2\text{max}$) of $\leq 20 \text{ mL/kg/min}$
- Left ventricular ejection fraction $\leq 35\%$
- ICD implanted
- If indicated, biventricular pacemaker implanted for $>6$ months
- Stable, optimized HF regimen for 30 days, except for diuretics

### Main Exclusion Criteria

- Anti-AAV1 neutralizing antibody titer (NAb) $\geq 1:2$
- Clinically significant MI within 6 months
- Likely need for HF-related surgery within next 6 months
- Expected survival $<1$ years
  - Based on investigator's clinical judgment of HF and co-morbid conditions

---

## Baseline Patient Characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All Subjects N=39</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age, years, mean (SD)</strong></td>
<td>60.5 (11.5)</td>
</tr>
<tr>
<td><strong>Sex, n</strong></td>
<td>34 Male</td>
</tr>
<tr>
<td><strong>Race, n</strong></td>
<td>34 White</td>
</tr>
<tr>
<td><strong>HF Etiology, n (%)</strong></td>
<td></td>
</tr>
<tr>
<td>Ischemic cardiomyopathy</td>
<td>19 (48.7)</td>
</tr>
<tr>
<td>Idiopathic cardiomyopathy</td>
<td>14 (35.9)</td>
</tr>
<tr>
<td>Hypertensive cardiomyopathy</td>
<td>4 (10.3)</td>
</tr>
<tr>
<td>Other</td>
<td>3 (7.7)</td>
</tr>
</tbody>
</table>

# Baseline Patient Characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All Subjects N=39</th>
</tr>
</thead>
<tbody>
<tr>
<td>6MWT, m, mean (SD)</td>
<td>343 (124)</td>
</tr>
<tr>
<td>VO$_{2}\text{max}$, mL/kg/min, mean (SD)</td>
<td>13.9 (3.9)</td>
</tr>
<tr>
<td>LVEF, %, mean (SD)</td>
<td>25 (7)</td>
</tr>
<tr>
<td>LVESV, mL, mean (SD)</td>
<td>202 (91)</td>
</tr>
<tr>
<td>NYHA Class III, n (%)</td>
<td>39 (100)</td>
</tr>
<tr>
<td>MLWHFQ, mean (SD)</td>
<td>46 (22)</td>
</tr>
<tr>
<td>NT-proBNP, pg/mL, mean (SD)</td>
<td>2932 (3028)</td>
</tr>
</tbody>
</table>

CUPID Pre-Screen Anti-AAV1 Antibody Results
~50% Heart Failure Patients Qualify

Percent by NAb Titer
N=506

- <1:2: 49% (n=244)
- 1:2: 31% (n=160)
- ≥1:16: 7% (n=3)
- 1:4: 6% (n=30)
- 1:8: 6% (n=36)
- 1:16: 7% (n=36)
Efficacy Measures: Prospectively Defined Thresholds and Primary Endpoint Analysis

- Primary endpoint
  - Concordant changes across multiple efficacy domains
    - Group Level Analysis
    - Individual Level Analysis
    - Outcome Analysis

<table>
<thead>
<tr>
<th>EFFICACY DOMAIN</th>
<th>MEANINGFUL CHANGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>SYMPTOMATIC</td>
<td></td>
</tr>
<tr>
<td>NYHA Class</td>
<td>1 Class</td>
</tr>
<tr>
<td>MLWHFQ</td>
<td>10 Points</td>
</tr>
<tr>
<td>FUNCTIONAL</td>
<td></td>
</tr>
<tr>
<td>6MWT</td>
<td>50 meters</td>
</tr>
<tr>
<td>Peak VO₂</td>
<td>1.5 mL/kg/min</td>
</tr>
<tr>
<td>BIOMARKER</td>
<td></td>
</tr>
<tr>
<td>NT-proBNP</td>
<td>35% or 300 pg/mL *</td>
</tr>
<tr>
<td>REMODELING</td>
<td></td>
</tr>
<tr>
<td>Ejection Fraction</td>
<td>3 or 5% Absolute **</td>
</tr>
<tr>
<td>End Systolic Volume</td>
<td>20 mL or 10% *</td>
</tr>
</tbody>
</table>

* Whichever is Greater
** 3% for Group, 5% for Individual

Serum Biomarker: NT-ProBNP

Left Ventricular Ejection Fraction

**Mean (SE) Change From Baseline (%)**

- **Low**: -10, -8, -6, -4, -2, 0, 2, 4, 6, 8
- **Mid**: -9, -7, -5, -3, -1, 1, 3, 5, 7
- **High**: 1, 3, 5, 7, 9, 11, 13, 15, 17
- **Placebo**: 1, 3, 5, 7, 9, 11, 13, 15, 17

**Improvement**

### Summary of 12-Month Analysis of CUPID 1*

<table>
<thead>
<tr>
<th>Efficacy Domain</th>
<th>MYDICAR (HIGH)</th>
<th>Placebo / Optimized</th>
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</thead>
<tbody>
<tr>
<td><strong>Symptomatic</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quality Of Life questionnaires</td>
<td>↑↑</td>
<td>↓</td>
</tr>
<tr>
<td><strong>Functional</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 Minute Walk Test</td>
<td>←→</td>
<td>↓↓↓↓</td>
</tr>
<tr>
<td>VO\textsubscript{2}max</td>
<td>←→</td>
<td>↓↓↓↓</td>
</tr>
<tr>
<td><strong>Biomarker</strong></td>
<td>←→</td>
<td>↓↓↓↓</td>
</tr>
<tr>
<td>NT-proBNP</td>
<td>←→</td>
<td>↓↓↓↓</td>
</tr>
<tr>
<td><strong>Remodeling</strong></td>
<td>←→</td>
<td>↓</td>
</tr>
<tr>
<td>Ejection Fraction</td>
<td></td>
<td></td>
</tr>
<tr>
<td>End Systolic Volume</td>
<td>↑</td>
<td>↓↓↓</td>
</tr>
</tbody>
</table>

* Circulation. 2011 Jul 19;124(3):304-313. Double arrows indicate that change from baseline at 6 months (primary endpoint) reached prespecified criteria for a clinically meaningful change.
CUPID 1 Met Primary Efficacy Endpoint for High dose vs. Placebo

1. **Group-level analysis success**: Improvement in 6MWT (p=0.14), ESV (p=0.057) with no clinically significant worsening in any endpoint and numerical superiority in all other endpoints

   AND

2. **Individual-level analysis success**: The mean individual efficacy “score” for MYDICAR is greater than that for Placebo, p=0.052

   AND

3. **Time-to-event (death, LVAD implantation or heart transplant)**: MYDICAR numerically better than Placebo

   AND

4. **Duration of CV hospitalizations**: duration for MYDICAR less than that for Placebo, 2.1 ± 3 in placebo versus high dose 0.2 ± 0.7 days p=0.08.

The probability of achieving above outcomes by chance alone is <0.1% based on permutation test
MYDICAR Reduced CV Clinical Events: 3-Year Follow-up

Clinical Events
- Worsening Heart Failure
- Myocardial Infarction
- Heart Transplant
- Chronic Use of Inotrope
- Insertion of LVAD
- All-Cause Death

CUPID 1: MYDICAR High Dose
Reduced Recurrent Hospitalization

Cumulative Rate of Non-Terminal Events

<table>
<thead>
<tr>
<th></th>
<th>Through 1 Year</th>
<th>Through 3 Years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High Dose MYDICAR</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Hazard Ratio (CI)*</th>
<th>0.12 (0.03, 0.49)</th>
<th>0.18 (0.03, 0.99)</th>
</tr>
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<tbody>
<tr>
<td>Risk Reduction</td>
<td>88%</td>
<td>82%</td>
</tr>
<tr>
<td>p-Value</td>
<td>p = 0.003</td>
<td>p = 0.048</td>
</tr>
</tbody>
</table>

*CI= Confidence Interval

CUPID 1: MYDICAR Effects on 3 Year Survival

## Persistence of SERCA2a Transgene in the Myocardium

<table>
<thead>
<tr>
<th>STUDY PHASE</th>
<th>Treatment Group</th>
<th>Time Point</th>
<th>AAV1/SERCA2a Copies DNA/µg Total DNA*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase 2</td>
<td>Placebo</td>
<td>Month 2</td>
<td>Negative</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Month 10</td>
<td>Negative (all)</td>
</tr>
<tr>
<td>Phase 2</td>
<td>Placebo</td>
<td>Month 7</td>
<td>Negative</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Month 10</td>
<td>Negative (all)</td>
</tr>
<tr>
<td>Phase 1</td>
<td>MYDICAR Very Low-dose</td>
<td>Month 8</td>
<td>Negative (all tissues)</td>
</tr>
<tr>
<td>Phase 1</td>
<td>MYDICAR Mid-dose</td>
<td>Month 1</td>
<td>Negative</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Month 21</td>
<td>Negative (all)</td>
</tr>
<tr>
<td>Phase 2</td>
<td>MYDICAR Mid-dose</td>
<td>Month 5</td>
<td>Negative (all)</td>
</tr>
<tr>
<td>Phase 2</td>
<td>MYDICAR Mid-dose</td>
<td>Month 10</td>
<td>Negative (all)</td>
</tr>
<tr>
<td>Phase 2</td>
<td>MYDICAR Mid-dose</td>
<td>Month 11</td>
<td>Negative</td>
</tr>
<tr>
<td>Phase 1</td>
<td>MYDICAR High-dose</td>
<td>Month 18</td>
<td>Negative</td>
</tr>
<tr>
<td>Phase 2</td>
<td>MYDICAR High-dose</td>
<td>Month 11</td>
<td>&gt;20 to &lt;200 Copies</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Month 23</td>
<td>561 Copies (AS)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>365 Copies (PS)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>&gt;20 to &lt;200 Copies (AW and PLW)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>230 Copies (LVAC)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>250 Copies (RVAC)</td>
</tr>
<tr>
<td>Phase 1</td>
<td>MYDICAR High-dose</td>
<td>Month 31</td>
<td>&gt;20 to &lt;200 Copies</td>
</tr>
<tr>
<td>Phase 2</td>
<td>MYDICAR High-dose</td>
<td>Month 22</td>
<td>223 Copies (PW)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>&gt;20 to &lt;200 Copies (AS, PS and AW)</td>
</tr>
</tbody>
</table>

CUPID 2: A Phase 2b Confirmatory Ongoing International Study

Study Population
- 18-80 years of age
- Systolic HF
- Ischemic or non-ischemic
- EF ≤35%
- NYHA Class II to IV
- Maximal, optimized HF regimen & high risk for HF-related hospitalizations
- AAV NAb titer negative

MYDICAR 1x10^{13} DRP, N=125
Placebo, N=125

Sample Size/Power:
N=125 per treatment group with 186 recurrent events provides:
83% power, 0.05 two-sided significance level, to detect at least a 45% risk reduction (HR=0.55)

All Subjects Followed Quarterly for Clinical Events Until:
Last enrolled subject completes 12 months of observation AND
186 adjudicated HF-related hospitalizations have occurred

Primary Endpoint
Time-to-recurrent HF-related hospitalizations in presence of terminal events
(all-cause death, heart transplant, and LVAD implantation)

Secondary Endpoint
Time-to-first terminal event (all-cause death, heart transplant, LVAD implantation)

Additional Endpoints
Symptoms, Exercise Capacity and Quality of Life

Greenberg B et al. JACC:HF (2013)
CUPID 2 – 36 U.S. Centers

LabCorp (Los Angeles, AAV NAb)
Celladon (San Diego)
Integrium (Tustin, US CRO, WW Safety DB, Endpts)
Optum Insight (Auburndale, MA, Health Economics)
CTL (Shaker Heights, ELISPOT)
CEC (Boston, Clinical Endpoints)
LabCorp (Cranford, NJ Central Lab)
B Braun (Allentown, PA Perfusor Space Pump)
Almac (Durham, P&L, Drug Depot, IVRS)

★ = Investigative Center
## CUPID 2: 20 European Centers

<table>
<thead>
<tr>
<th>Country</th>
<th>NCI</th>
</tr>
</thead>
</table>
| Belgium              | Dr. Jozef Bartunek  
O.L.V Ziekenhuis, Aalst                                           |
| Denmark              | Prof Jens Kastrup  
Rigshospitalet University Hospital, Copenhagen                      |
| Germany              | Prof Dr med Veselin Mitrovic  
Kerckhoff-Klinik, Bad Nauheim                                        |
| Poland               | Prof Piotr Ponikowski  
4 Wojskowy Szpital Kliniczny, Wroclaw                               |
| Sweden               | Thomas Kahan  
Danderyds Sjukhus, Stockholm                                        |
| The Netherlands      | Prof Dr Adriaan Voors  
University Medical Center, Groningen                                 |
| United Kingdom       | Dr Alex Lyon  
Royal Brompton Hospital, London                                     |
Future Directions for Gene Therapy of Heart Failure

• New targets to improve cardiac structure and function.
• Improved vectors that increase specificity of uptake in the heart.
• Novel promoters that regulate gene expression according to the pathologic milieu.
• Using GT to transform fibroblasts to pluripotent stem cells or cardiomyocytes.
• Strategies to reduce the level of neutralizing antibodies to AAV vector.
A Novel Pathway of the RAS

Angiotensinogen

Renin

Ang I

Ang II

ACE

ACE2

Chymase

ACE2

ACE

Ang-(1-9)

Ang-(1-7)

AT\textsubscript{1}

AT\textsubscript{2}

AT\textsubscript{1-7} (Mas)

Cell Growth

Vasoconstriction

Uncertain

Anti-growth

Vasodilation
ACE2 Inhibition Reduces Systolic Function and Increases Infarct Size

C16 given from days 2 through 28 post-MI

ACE2 Inhibition Reduces Systolic Function and Increases Infarct Size

ΔFS between days 2 and 28

ΔMI size between Days 2 and 28

Kim M et al. J Card Failure 2010
Ang-(1-7) Effects in the Post-MI Heart

- Direct and indirect anti-growth effects on cardiomyocytes
- Anti-fibrotic effects
- Reduces MI size
- Attenuates post-MI remodeling and improves cardiac function

Hypothesis: Does GTT to deliver Ang-(1-7) to the post-MI mouse heart attenuate remodeling?
Ang Peptide Effects on ET-1 and LIF mRNA Expression in Cardiac Fibroblasts

Ang Peptide Effects on Collagen Production in ARCFs

AAV9.45 Bicistronic Vector

- Recombinant adeno-associated viral vectors (AAV):
  - Non-pathogenic, non-replicating & non-integrating
  - Serotypes (e.g., AAV9.45) have high myocyte selectivity in vivo
- Ang-(1-7) Fusion Protein
  - Generated in the lab of Dr. Tim Reudelhuber
  - Immunoglobulin/furin cleavage site/Ang-(1-7)
- TdTomato
  - Fluorescent reporter for tracking (can also use luciferase as bioluminescent reporter)
Fluorescence Imaging of the Heart Using AAV9 Vector

Bioluminescence imaging 28 days after AAV-6 injection

Fluorescence imaging of heart after AAV-9 cTnT-eGFP injection

Prasad et al. (2011) Gene Therapy. 18, 43-52.
GTT Collaborators

**UCSD Greenberg Lab**
- Randy Cowling, PhD
- Seung Hee Kim, MD
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**Other**
- David Hall, PhD

**Heart Failure Program**
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- Eric Adler, MD
- Jorge Silva, MD
- Melanee Schimmel, RN

**Celladon**
- Kris Zsebo, PhD
- Jeff Rudy
- Janice Pagoda, PhD
- Roger Hajjar, MD

**The CUPID Investigators**
Evolution of Heart Failure Therapy

1700’s - 1960’s
Dig and diuretics

1970’s
Vasodilators

1980’s - 2010
The Remodeling Paradigm
Neurohormonal Antagonists

2010 - Future
Gene Transfer, Stem Cells, Novel Pharmacologic Agents

The Age of Empiricism
1700’s - 1960’s
Myocardial Mechanics

The Molecular Age
2010 - Future
Gene Transfer, Stem Cells, Novel Pharmacologic Agents