Considerations for Cardiac Gene Therapy in Duchenne/Becker Muscular Dystrophy

H. Lee Sweeney, Ph.D.
Director, UF Myology Institute
Department of Pharmacology & Therapeutics
College of Medicine
University of Florida
Dilated Cardiomyopathy (DCM)
Associated with DMD

The human disease
DMD Cardiac Disease

Global LV Strain

http://diagnosoft.com/strain/clinical-value-of-strain
Inferior and Inferoseptal Region Showed the most Significant Decline in Strain

<table>
<thead>
<tr>
<th>Time Points</th>
<th>Anterior</th>
<th>Anteroseptal</th>
<th>Inferoseptal</th>
<th>Inferior</th>
<th>Inferolateral</th>
<th>Anterolateral</th>
</tr>
</thead>
<tbody>
<tr>
<td>B- Baseline</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>T- Two Years</td>
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</table>

**PEAK STRAIN %**

* indicates significant decline compared to Baseline.
Cardiac MRI showing late gadolinium enhancement in dystrophinopathy heart.
"Tonic Contraction" Su et al., Pediatric Cardiology, 2015

Blue = DMD boys who received ACEi prior to decreased EF
Red = DMD boys who received ACEi after EF decreased
The Dystrophin Complex transmits force across the muscle membrane, lowering the threshold for contraction-induced damage to muscle.

Contraction and even passive stretch of dystrophic (mdx) muscles causes membrane tears. (observed by dye entry)

A series of eccentric contractions results in a decrement in force generation in dystrophic muscle, indicating increased susceptibility to damage.

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Dystrophin Complex

complex connecting cytoskeleton to extracellular matrix
Duchenne Muscular Dystrophy:

Molecular Basis and Possible Treatment Strategies

**Functional Roles of Dystrophin:**

- **Mechanical** - transmits force from the contractile apparatus to connective tissue/tendon

- **Organizer** - positions a number of proteins at the muscle membrane (NOS, ion channels, etc.)

- **Signaling** - likely plays a number of signaling roles, including a key role in calcium homeostasis

Contraction causes **rupture of the muscle membrane**, which allows **calcium inflow**. There also may be **increased flux through ion (TRPC) channels** and **leakiness of the internal calcium storage compartment (SR)** via the ryanadine receptor (SR-calcium release channel).

Excessive calcium activates breakdown of muscle (via calpain and other proteases) and may trigger cell death program.

Cell death triggers an **inflammatory response**. Activation of fibroblasts can lead to **fibrosis**, which prevents **muscle regeneration** (modulated by IGF-I and myostatin).
Considerations for Gene Therapy for the Dilated Cardiomyopathy associated with DMD
Does dystrophin carry a cardiac-specific domain?

**Full-length**

| NT | H1 | 1 | 2 | 3 | H2 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | H3 | 20 | 21 | 22 | 23 | 24 | H4 | CR | CT |
|----|----|---|---|---|----|---|---|---|---|---|---|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|

**ΔH2-R19**

| NT | H1 | 1 | 2 | 3 |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|----|----|---|---|---|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|

**ΔH2-R15**

| NT | H1 | 1 | 2 | 3 |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|----|----|---|---|---|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|

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<th>ΔH2-R15</th>
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</tr>
<tr>
<td>QRS duration</td>
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</tr>
<tr>
<td>QT interval</td>
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<tr>
<td>Q amplitude</td>
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<tr>
<td>End-systolic volume</td>
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<td>normalized</td>
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<tr>
<td>dP/dt maximum</td>
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<tr>
<td>End-diastolic volume</td>
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</tr>
<tr>
<td>Ejection fraction</td>
<td>normalized</td>
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Wasala et al 2018 Hum Gene Ther.
Unlikely that AAV.μ-dystrophin will totally rescue the heart
Therapeutic Targets: For DMD skeletal muscle, there are at least six categories of therapies under development.

Current Duchenne muscular dystrophy therapeutic targets can be grouped into six categories. Only the first addresses the primary genetic defect (resulting in the loss of dystrophin protein). The rest address downstream aspects of the pathogenesis.

1) Replacement of dystrophin/utrophin (μdys gene therapy)
2) Increasing muscle mass and regeneration
3) Decreasing inflammation and fibrosis
4) Correcting blood flow regulation
5) Correcting perturbations in calcium handling (more utrophin?)
6) Mitochondria dysfunction and ROS generation
Duchenne Muscular Dystrophy:  

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Excessive calcium is taken up by mitochondria, ultimately contributing to mitochondrial uncoupling, free radical generation, and dysfunction. This can trigger apoptosis of the cardiomyocytes.
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Therapeutic Targets: For the DMD heart, are #5 and #6 the best targets if you cannot deliver full-length dystrophin?
Addressing the calcium leak with tadalafil
Potential Benefits of PDE5 Inhibition

Striated Muscle

TRPC

Ca²⁺

PKG

cGMP

PDE1

PDE5

sGC

NOX2

DMD/BMD

nNOS

ROSS

Injury

NO

NO

Blood flow deficit

Vascular Smooth Muscle

PDE5

cGMP

Sildenafil

Tadalafil

Sildenafil

PDE1

Unopposed vasoconstriction

Impaired dilation

Sympathetic nerve

NE

Regional Circumferential Strain Analysis

- Performed regional circumferential strain analysis (ECC) using Harmonic Phase method (HARP, Diagnostics Inc)
- Single slices were analyzed from mid-papillary region of left ventricle
- ECC measured at mid-wall of myocardium
- 16 frames acquired per cardiac cycle; In all cases, tags could be automatically followed beyond end-systole (~frame 4-7), but not always to the end of diastole
- Regional analysis were divided into 6 segments
Regional Circumferential Strain Analysis

Segment

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<th>Segment</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
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<tr>
<td>Ecc (%)</td>
<td>-16</td>
<td>-14</td>
<td>-12</td>
<td>-10</td>
<td>-8</td>
<td>-6</td>
<td>-4</td>
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- Treated
- Not treated

* indicates significant difference
Fractional Shortening (%)

Age (mo)

- Untreated
- Tadalafil

Treatment Start at 9 mo

p = 0.01
p = 0.04
p = 0.08

(Normal)
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<th>Protein</th>
<th>Tad</th>
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<td>μ-Calpain</td>
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<td></td>
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<tr>
<td>m-Calpain</td>
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<tr>
<td>Cleaved α-Actinin</td>
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PDE5 Inhibition (Tadalafil) Delays Progression of Cardiomyopathy

TRPC6 phosphorylation levels were increased >2-fold
Utrophin levels increased >1.5-fold
Progression of disease delayed by 15 months

Hammers et al., J Am Heart Assoc. 5: pii: e003911, 2016.
Conclusions from PDE5 Study

• PDE5 inhibition with tadalafil can partially silence TRPC6 channels (calcium leak) and decrease breakdown of utrophin (prevents calpain activation) thus slowing progression in dystrophin-deficient hearts.

• Tadalafil may slow cardiac disease progression in DMD/BMD.
Duchenne Muscular Dystrophy: Molecular Basis and Possible Treatment Strategies

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Excessive calcium is taken up by mitochondria, ultimately contributing to mitochondrial uncoupling, free radical generation, and dysfunction. This can trigger apoptosis of the cardiomyocytes.
Questions for Gene Therapy for DMD

• Can a micro-dystrophin totally rescue the heart?

• Would addressing calcium handling and/or mitochondrial function with gene therapy, either alone or in combination with micro-dystrophin, provide more benefit than micro-dystrophin delivery alone?

• It may be possible to deliver a cardiac-specific AAV to the heart at the same time a micro-dystrophin is delivered body-wide to the skeletal muscle. Unlike the case of skeletal muscle, the heart would likely never need to be re-dosed.
Preclinical Acknowledgments:

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Muscular Dystrophy
LEADING THE FIGHT TO END DUCHENNE

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