Cardiosphere-derived cells for heart failure in Duchenne muscular dystrophy

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Concepts underlying my talk

• Cell therapy with CDCs can regenerate “irreversibly” scarred heart (CADUCEUS)
• Safety has been demonstrated with allogeneic CDCs (ALLSTAR phase 1)
• Triple-vessel delivery safe preclinically and achieves broad coverage
• Disease-modifying activity demonstrated in mdx mice
Properties of CDCs

• CD105+/CD45- cells\textsuperscript{1,2} of intrinsic cardiac origin\textsuperscript{3}
• Not MSCs, fibroblasts, myofibroblasts, or cardiomyocytes\textsuperscript{1}
• Shrink scar and increase viable myocardium\textsuperscript{1,2,4-8}
• Functionally superior to other clinically-applied cells\textsuperscript{5}
• Multipotent & clonogenic,\textsuperscript{4} but long-term engraftment & differentiation not necessary for benefit\textsuperscript{6-9}

CADUCEUS
Final 12 mo data (Malliaras et al., JACC 2014)

Δ scar mass
6 mos 12 mos

Δ viable mass
6 mos 12 mos

Final 12 mo data; all bars represent +/- 1 SEM
Limitations of autologous therapy

• Timing constraints (3-5 weeks for CDCs)
• Expense
• Risk
  – Harvesting procedure
  – Manufacturing failure
• Suboptimal QA/QC
• Interpatient variability in cell potency
• Is allogeneic therapy safe and effective?
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Safety of triple vessel infusion

**TIMI flow LAD**

**TIMI flow LCX**

**TIMI flow RCA**

**Tnl levels Pre Infusion**

**Tnl levels 24hrs Post Infusion**

- Placebo
- CDCs Stop Flow
- CDCs Continuous Flow
The DYNAMIC trial

Dilated cardiomyopathy intervention with allogeneic myocardially-regenerative cells

• Ischemic or nonischemic DCM with LVEF ≤ 35%
• Allogeneic CDCs - non-occlusive intracoronary infusion in 2-3 vessels
• Phase 1a open-label (N=14, single center): safety funded by NIH grant to Capricor
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Duchenne Muscular Dystrophy (DMD)

• **X-linked recessive disorder** (Male)

• **Myopathy** (Cell membrane damage in muscle fibers):

  **Skeletal muscle weakness:**
  - Starts from 3-5 y/o
  - Progressive weakness
  - Wheelchair dependency ~ 13 y/o

  **Cardiomyopathy:**
  - 1/3 < 13 y/o; 1/2 < 18 y/o; all > 18 y/o
  - DCM:
    - LV posterobasal fibrosis
  - Conduction abnormalities:
    - mainly intra-atrial: SVT
    - AV nodal conduction abn.

  **Smooth muscle myopathy:** Vascular dysfunction
  GI and urinary tract systems involvement

• **Prognosis:** Most die of heart failure or respiratory insufficiency
Duchenne Muscular Dystrophy (DMD)

\[ \text{Dystrophin gene mutation (Deletion)} \]

\[ \text{Loss of Dystrophin} \]

\[ \text{Cell membrane damage} \]

\[ \text{Leakage of extracellular Ca}^{2+} \text{ into cell} \]

\[ \uparrow \text{Intracellular [Ca}^{2+}] \]

\[ \text{Activation of Calpain (Proteolytic pathway)} \]

\[ \text{Muscle proteolysis & Apoptosis & FIBROSIS} \]

Vascular dysfunction & GI/ GU system Abn.

Ambulation

Heart failure

Heart

Skeletal muscle

Smooth muscle

Oxidative/Nitrosative stress & Inflammation
Rationale for use in DMD

<table>
<thead>
<tr>
<th>CDCs</th>
<th>DMD pathophysiology</th>
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<tbody>
<tr>
<td>- Anti-oxidative</td>
<td>- Oxidative/Nitrosative stress</td>
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<tr>
<td>- Anti-inflammatory</td>
<td>- Inflammation</td>
</tr>
<tr>
<td>- Anti-apoptotic</td>
<td>- Apoptosis</td>
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<tr>
<td>- Anti-remodeling</td>
<td>- Remodeling</td>
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<tr>
<td>- Regenerative</td>
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Hypothesis: CDC administration is beneficial in retarding/reversing DMD

Tested in *mdx* mouse model
Cardiac global function is improved with CDC Rx

*** p<0.001
* p<0.05

n=12 Mdx+CDC, Mdx+Vehicle
n=5 CTL(WT)
CDC treatment reduced cardiac collagen content and fibrosis

A

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<thead>
<tr>
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<th>CTL(WT)</th>
<th>Mdx+Vehicle</th>
<th>Mdx+CDC</th>
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B

Collagen I A1/GAPDH

Collagen III A1/GAPDH

†

CDC treatment reduced cardiac collagen content and fibrosis.
CDCs increased maximal exercise capacity

n = 6-11
Nrf2 antioxidant pathway
CDC treatment heightened activity of Nrf2 antioxidative pathway

**B**

### Cytoplasmic Nrf2-p^{S40}

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<tbody>
<tr>
<td>Nrf2-p^{S40}/Nrf2</td>
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### Nuclear Nrf2

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<tr>
<td>Nrf2/Histone H1</td>
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CDC treatment heightened activity of Nrf2 antioxidative pathway and increased expression of Nrf2 downstream gene products.
CDC treatment enhanced mitochondrial respiratory protein expression and increased mitochondrial content

\[ \text{Nrf2} \rightarrow \text{Nrf1} \rightarrow \text{mtTFA} \rightarrow \text{Mitochondrial biogenesis} \]
\[ \text{&} \]
\[ \text{ATP Synthesis} \]

Mitochondrial respiratory complex proteins

\[ \text{CDC} \] treatment enhanced mitochondrial respiratory protein expression and increased mitochondrial content.
Mitochondrial ultrastructure corrected by CDCs

A

B

MITOCHONDRIAL LENGTH
n=350 per group

ROUNDED CRISTAE
n=100 per group

CRISTAE/MITO
n=100 per group
Respiratory function of isolated mitochondria restored by CDCs

n=6 CTL
n=8 Mdx+Vehicle & Mdx+CDC
* p<0.05 vs Mdx+CDC
Normalized to CS activity
Cardiomyogenesis augmented by CDCs

A

CTL(WT)  Mdx+Vehicle  Mdx+CDC

WGA  Ki67  DAPI

B

WGA  Aurora  DAPI

C

C-kit  Nkx2.5  DAPI

23
mdx cardiomyopathy and CDCs

- Injection of CDCs into mdx hearts
  - improves global function
  - decreases fibrosis
  - improves exercise capacity
  - exerts potent anti-oxidant effects
  - reverses abnormalities in mitochondrial abundance, structure and function
  - increases cardiomyocyte proliferation and activation/recruitment of endogenous repair
Totality of the data motivate clinical trial of CDCs in Duchenne patients

Halt cardiomyopathy progression in Duchenne:

HOPE-Duchenne trial