Regenerative medicine using Cardiac Stem Cells or Exosomes

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Disclosure: St. Jude Medical (research study).
Prevailing dogma before 2000

- Heart cells have little or no proliferative capacity
- Myocardial scar is irreversible
- Lost heart muscle cannot regrow
- The best hope for treating cardiac injury is to limit the injury or to block secondary maladaptive pathways
Stem Cell
Functional Definition

- Self-renewing.

- Able to form clones (clonogenicity).

- Potential for multi-lineage differentiation (multi-potentiality).

Types of stem cells

• Embryonic stem cells* or iPS (genetically induced pluripotent stem) cells#
  – Can evolve into all tissues
  – Tumors
  – Immune reactions
  – Ethically problematic*
  – Genetic modification#

• Adult stem cells
  – Skeletal myoblasts
  – Bone marrow stem cells
  – Cardiac-derived cells
Skeletal myoblasts

- First to be translated into human studies (Jan. 2001)
- Advantages: autologous, contractile
- Disadvantage: do not couple with surrounding myocardium, forming islands of conduction block
- VT, SCD observed in 10/22 phase 1 pts
- Clinically failed phase 2 trial as adjunct to surgery in patients with LV dysfunction (MAGIC)
- Experience with myoblasts raised generalized fears about stem cells and arrhythmias
Lessons learned from human bone marrow-derived cell trials

• >1000 subacute MI patients treated worldwide since Aug. 2001
• Modest functional benefit*, possibly transient#
• Sicker patients have greater functional improvement*
• Excellent safety profile with coronary catheter delivery, including arrhythmias and SCD
• MRI: small reductions in scar (~3 g), but no increase in viable myocardium
• Encouraging, but much room for improvement

#BOOST 2004, 2007  *REPAIR-MI 2006
Safety meta-analysis of five BMC trials

From: Clin Cardiol. 2009 Aug;32(8):458-66. Five trials (BOOST, REPAIR-AMI, ASTAMI, Janssens, and Yao) were included in the analysis.
Major landmarks 2000-present

• Cardiac stem cells (CSCs)
  – First recognized in 2000 (Deisher)
  – Antigenically-selected in rats and mice (Beltrami et al., Oh et al., 2003)

• Human cardiospheres (CSps)
  – Outgrowth of human surgical biopsies in primary culture (Messina et al., 2004)
  – Self-organize in suspension, increase post-ischemic function

• Cardiosphere-derived cells (CDCs)
  – Millions of cardiac stem cells from percutaneous endomyocardial biopsies (Smith et al., 2007)
  – Paradigm for autologous therapeutics
How we harvest and grow CDC’s

R. Smith et al., Circulation 115: 896 – 908, 2007

1. Biopsy specimen
   - Minced
   - Digested collagenase
   - Fibronectin

2. Explants (1)
   - Cardiac outgrowth flat & round cells (4)
   - Harvest (2,3) @ 1-2 wks
   - Trypsin

3. Cardiospheres (CSp’s, 5)
   - Cultured
   - Poly-D-lysine

4. Cardiosphere-derived cells (CDC’s, 6)
   - Seeded
   - Fibronectin

5. Cardiac outgrowth flat & round cells
   - Minced
   - Collagenase
   - Trypsin

6. Cardiosphere-derived cells
   - Collagenase
   - Trypsin

**Cell Surface Markers**

- c-Kit+
- CD133+
- CD105+
- CD90+

**Graph**

- % of cell total
  - c-Kit+
  - CD133+
  - CD105+
  - CD90+

**Images**

- Image 1: Explants (200 μm)
- Image 2: Cardiac outgrowth flat & round cells (200 μm)
- Image 3: Cardiospheres (100 μm)
- Image 4: Cardiosphere-derived cells (50 μm)
How we harvest and grow CDCs


1. Biopsy
2. Explants (1)
3. Cardiosphere-forming cells (4)
4. Cardiospheres (5)
5. Cardiosphere-derived cells (CDCs, 6)

Yield: 30M CDCs in 3-5 weeks
Biopsy

30 minutes digestion in 0.2 mg/ml collagenase

Cell harvest by 0.05% trypsin

Cardiospheres (CSps) Poly-D-lysine coated

Cardiosphere-derived cells (CDCs) Fibronectin coated

Infusion into the same patient
Vital Step Along The Translational Path

1. The idea
2. Proof of concept
3. Protocol optimization
4. Pivotal protocol
5. Clinical trial

Manufacturing protocols
Pharmacology
Toxicology
Efficacy
Safety

IND
Delivery, Dose
in vitro
Small animal models
CADUCEUS: 
CArdiosphere-Derived aUtologous stem 
CElls to reverse ventricUlar dySfunction

*Clinicaltrials.gov identifier: NCT00893360*

- Recent MI & ischemic LV dysfunction (EF 25-45%)
- NIH-funded, Phase I/II randomized, controlled, dose-escalation safety and preliminary efficacy study (MRI for scar size, volumes, & function)
- Two centers (Cedars-Sinai Heart Institute; Johns Hopkins)
- Endomyocardial biopsies; CDCs manufactured at Cedars-Sinai Heart Institute
- Intracoronary infusions of autologous CDCs
Representative CADUCEUS MR images

- **baseline**
  - 0005-CDC01-014
    - CDC-treated
  - 0005-CDC01-002
    - Control

- **6m**
Scar mass goes down in CDC-treated patients but not controls.

(g, 6 months minus baseline; independent samples t-test, pooled CDC group vs controls, means ± SD)
Meanwhile, viable myocardium increases (g, 12 months minus baseline; independent samples t-test, pooled CDC group vs controls, means ± SD)
Effects maintained at 12 months

Decrease in scar mass

Increase in viable mass

(g, 12 months minus baseline; independent samples t-test, pooled CDC group vs controls, means ± SD)
Intracoronary cardiosphere-derived cells for heart regeneration after myocardial infarction (CADUCEUS): a prospective, randomised phase 1 trial


ejection fraction (LVEF) was 39% (SD 12) and scar occupied 24% (10) of left ventricular mass. Biopsy samples yielded prescribed cell doses within 36 days (SD 6). No complications were reported within 24 h of CDC infusion. By 6 months, no patients had died, developed cardiac tumours, or MACE in either group. Four patients (24%) in the CDC group had serious adverse events compared with one control (13%; p=1·00). Compared with controls at 6 months, MRI analysis of patients treated with CDCs showed reductions in scar mass (p=0·001), increases in viable heart mass (p=0·01) and regional contractility (p=0·02), and regional systolic wall thickening (p=0·015). However, changes in end-diastolic volume, end-systolic volume, and LVEF did not differ between groups by 6 months.

**Interpretation** We show intracoronary infusion of autologous CDCs after myocardial infarction is safe, warranting the expansion of such therapy to phase 2 study. The unprecedented increases we noted in viable myocardium, which are consistent with therapeutic regeneration, merit further assessment of clinical outcomes.
Mechanism of benefit involves direct regeneration.

#Smith et al, Circulation, 2007;
Davis et al., PLoS One, 2009;
Davis et al., Stem Cells, 2010
Mechanism of benefit involves direct regeneration *as well as* paracrine effects*

*Chimenti et al., Circ. Res., 2010; Cheng et al., Circ. Res., 2010*
Presumptive mechanism (2004)

Transplanted stem cells

↓

Cell proliferation

↓

Differentiation

↓

New myocardium of donor origin
LV function in post-MI rats: syn = allo

K. Malliaras et al., Circ 2012

No surviving transplanted cells after 4 weeks
Intracoronary allogeneic CDCs regenerate the infarcted pig heart

K. Malliaras et al. Circulation 2013

All bars represent +/- 1 SEM.
Mechanistic rationale for allogeneic therapy

Transplanted CDCs

Cell proliferation

Differentiation

New healthy tissue of 

donor origin

Short-term engraftment

Secreted factors

New healthy tissue of 

host origin

CDCs anti-inflammatory, immunomodulatory and evanescent

1. I. Chimenti et al, Circ Res 2010
4. E. Tseliou et al., Basic Res Cardiol 2014
Therapeutic bioactivity of CDCs: beyond regeneration

- Regenerative √ 1,2
- Antifibrotic √ 1-4
- Anti-apoptotic √ 3-5
- Angiogenic √ 1,6
- Anti-inflammatory √ 9
- Immunomodulatory √ 9,10

Follow the data

2. Recognition of durable benefits despite cell transience (2010)
   ➔ Allogeneic paradigm (2012)
Summary of criteria for the perfect stem cell to treat heart disease

- Easily harvested using routine clinical methods
- Readily grown in large numbers
- Unmodified: no genes, limited processing
- “Off the shelf” availability for acute disease
- No ethical or moral quandaries
- Safe
  - No immune reaction or “rejection”
  - No tumors
  - No arrhythmias
- Regrows healthy heart after cardiac injury (heart attack or chronic heart failure)
# Cardiosphere-derived cells (CDCs)

First described by RR Smith et al., *Circulation* 2007

## Cell Type
- Human heart progenitor cell

## Characteristics
- CD105+, CD45-; secreted SDF-1

## Clinical Trials
- **CADUCEUS**-completed-autologous phase 1. Twenty-five patient study showed regeneration in CDC-treated post-MI subjects with mild HFrEF
- **ALLSTAR**-phase 1&2b study of allogeneic CDCs post-MI with mild HFrEF
- **DYNAMIC**- phase 2a study of allogeneic CDCs in patients with advanced HFrEF

## Mechanism of action
- Paracrine effects
  - Promote cardiomyomyogenesis
  - Prevent cardiomyocyte apoptosis
  - Anti-fibrotic
  - Anti-inflammatory

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![Cell progression diagram](diagram.png)
Additional allogeneic CDC trials in progress

- CDCs in HFpEF (Regress-HFpEF)
- CDCs in pulmonary hypertension (ALPHA)
- Duchenne muscular dystrophy (HOPE)
The graveyard of failed clinical trials
Study design

High salt diet (8%)

Low salt diet (0.3%)

Dahl salt sensitive rats

7 weeks of age

Baseline

6 w

Blood pressure

SBP

DBP

Wall thickness

E/A ratio

Diastolic function:

Systolic function:

Heart Weight

LVEF

Diastolic function:

Systolic function:

Inflammation: attenuated by CDCs

Histology for cardiac macrophages and leukocytes

Serum inflammatory cytokines

CD68+ cells

CD45+ cells
Fibrosis: attenuated by CDCs

Control Placebo CDC

Collagen 1A1

mRNA expression ratio to control

P<0.001

†

Control Placebo CDC

Collagen 3

mRNA expression ratio to control

P=0.001

†

Control Placebo CDC

Total fibrosis (% LV)

P<0.0001

†
“Clinical” impact

![Graphs showing survival, lung weight, lung/body weight, and body weight comparisons between control, placebo, and CDC groups.](image)

- Survival: Logrank $P=0.03$
- Lung weight: $P=0.02$
- Lung/body weight: $P=0.006$
- Body weight: $*P<0.05$ vs. placebo and CDC; $†P<0.05$ vs. control and CDC
Regress-HFpEF Trial

Regression of fibrosis & reversal of diastolic dysfunction in HFPEF patients treated with allogeneic CDCs

M. Zile, PI

NCT02941705 recruiting
Additional allogeneic CDC trials in progress

- CDCs in HFpEF (Regress-HFpEF)
- CDCs in pulmonary hypertension (ALPHA)
- Duchenne muscular dystrophy (HOPE)
CDCs reduce RV systolic pressure and hypertrophy in monocrotaline rat model of PAH

**Experimental Protocol**

- **MCT Treatment**
  - Day 0
- **CDC/Sham Treatment**
  - Day 14
- **Endpoint 1**
  - Day 28
- **Endpoint 2**
  - Day 35

**Tracking CDCs in the Lungs**

- **Total Radiant Efficiency** ($10^6$)
  - 0
  - 1 min
  - 15 min
  - 3 hrs
  - 24 hrs
  - 48 hrs
  - 72 hrs

**RV Systolic Pressure**

- **Day 28**
  - CTL
  - Sham
  - CDC
- **Day 35**
  - CTL
  - Sham
  - CDC

**RV Hypertrophy**

- **28 day**
  - CTL
  - Sham
  - CDC
- **35 day**
  - CTL
  - Sham
  - CDC

R. Middleton, M. Lewis et al., *PLoS One* 2018
CDCs reduce vessel wall thickness and macrophage migration in the lungs

**Arteriolar Wall Thickness**

- **Control**
- **Sham**
- **CDC**

**Macrophage Infiltration in the Lungs**

- **A.** 
- **B.**

**Wall Thickness Index (%)**

- **Vessel Diameter:** 20-50μm, 50-80μm, 80-110μm

- **Control**
- **Sham**
- **CDC**

**Ave. Macrophage Count/Animal**

- **Control**
- **Sham**
- **CDC**

**Macrophage Count/Field**

- **CTL 1, CTL 2, CTL 3, CTL 4, CTL 5**
- **Sham 1, Sham 2, Sham 3, Sham 4, Sham 5**
- **CDC 1, CDC 2, CDC 3, CDC 4, CDC 5**
ALpha Trial

ALlogeneic CDCs for Pulmonary arterial Hypertension therapy

M. Lewis, PI
NCT03145298

recruiting
Additional allogeneic CDC trials in progress

- CDCs in HFpEF (Regress-HFpEF)
- CDCs in pulmonary hypertension (ALPHA)
- Duchenne muscular dystrophy (HOPE)
HOPE-Duchenne trial

- Duchenne muscular dystrophy
- X-linked recessive disorder
- Skeletal myopathy
- Cardiomyopathy

Dystrophin deficiency

↑ Cellular [Ca^{2+}]

Cell membrane damage

Myocyte loss

Oxidative stress

Inflammation

Mitochondrial inefficiency/loss

Fibrosis
Duchenne cardiomyopathy:
Progressive increase in cardiac scar with patient age

Animesh Tandon et al. J Am Heart Assoc 2015;4:e001338
CDC treatment reduced cardiac collagen content and fibrosis in *mdx* mice

Aminzadeh et al., *Stem Cell Reports* 2018
Clinical trial of CDCs in Duchenne patients

Halt cardiomyopathy progression in Duchenne:

HOPE-Duchenne trial

• DMD patients age 12+ with ≥4 segments of scar by MRI
• N=25, 1:1 randomization to standard of care or multivessel infusion of alloCDCs
• Endpoints: safety, scar by MRI, function
HOPE 6-month data

Full data set at Capricor.com
Disadvantages of cells for therapeutics

- Cells work, but fragile living material
- QA/QC, release & identity criteria complex
- Suboptimal in closed compartments
- Immune memory?

Cell-free therapeutics

- Is there a single entity that can mimic all the salient benefits?
- Does the cell-free entity have features superior to those of the parent cells?
Exosomes are bioactive nanoparticles

- 30-150 nm particles
- Present in all body fluids
- Released by nearly all cell types
- Loaded with miRs and other bioactive contents
- Payload very cell-specific

**Blocking exosome biosynthesis abrogates CDC benefit**

**CDC exosomes ↑ EF**

Ibrahim et al., *Stem Cell Reports* 2014

[Graphs showing LVEF (%) over Days (after MI) for different treatments, including CDC, CDC-GW4869, Vehicle, CDC-exo, and Fibroblast-exo]
Exosome payloads mimic CDC effects on multiple biological processes

<table>
<thead>
<tr>
<th>CDCs</th>
<th>CDC-XO</th>
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<tbody>
<tr>
<td>• Regenerative</td>
<td>✓ 1,2</td>
</tr>
<tr>
<td>• Antifibrotic</td>
<td>✓ 1-4</td>
</tr>
<tr>
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<td>• Immunomodulatory</td>
<td>✓ 9,10</td>
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# Cardiosphere-derived cells (CDCs)

First described by RR Smith et al., *Circulation* 2007; methods and bioactivity reproduced by >40 labs worldwide.

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<td>Paracrine effects <strong>mediated by exosomes</strong></td>
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<td></td>
<td>• Promote cardiomyomyogenesis</td>
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<td></td>
<td>• Prevent cardiomyocyte apoptosis</td>
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Cardiosphere-derived cells (CDCs) are derived from cardiac tissue explants, which are then expanded into cardiospheres (CSps). The CSps are then differentiated into CDCs. These cells are believed to promote cardiomyomyogenesis, prevent cardiomyocyte apoptosis, and have anti-fibrotic and anti-inflammatory properties. They have been studied in clinical trials for the treatment of post-MI subjects with mild HFrEF.
When will therapeutic exosomes reach the clinic?
CDC-exosome clinical manufacturing

DONOR HEART → EDCs → EDC Expansion → MCB

MCB → CARDIOSPHERES → CDC Expansion → CDCs → CAP-1002

Confluent CDCs → Conditioned medium → Ultrafiltration → CAP-2003
Repeat xenogeneic dosing of human exosomes recapitulates effects of syngeneic CDCs in *mdx* mice

**Collagen content**

**Cardiomyogenesis**

**Functional benefit on heart**

Aminzadeh et al., *Stem Cell Reports* 2018
Follow the data

2. Recognition of durable benefits despite cell transience (2010)
   ➔ Allogeneic paradigm (2012)
3. Identification of exosomes as mediators (2014)
   ➔ Cell-free therapeutics (2018)
CDC-exosomes exhibit a distinctive miR profile

Ibrahim et al., Stem Cell Reports 2014
miRs are minority of exosomal RNA

L. Cambier et al., *EMBO Mol Med* 2017
Plentiful Y RNA fragment regulates IL-10 expression

**Predicted structure**

IL-10 transcript (left) and secreted protein (right) in macrophages

L. Cambier et al., *EMBO Mol Med* 2017
Exosomes: defined contents as next-gen TCs

- miR-181b
- miR-146a
- Y RNA fragment

Cardioprotection

↑ PKCδ

G. DeCouto et al., Circ 2017

↑ IL-10

L. Cambier et al., EMBO Mol Med 2017

↓ TRAF-6

A. Ibrahim et al., Stem Cell Reports 2014

Cardiomyocytes

Macrophage

Cedars Sinai
Heart Institute
Working hypotheses

• No single RNA species can account for all the benefits of exosomes
• Individual miRs or other RNA species may prevail in any given setting
• The totality of exosomal contents required for full manifestation of bioactivity
Deconstruction: follow the data

2. Recognition of durable benefits despite cell transience (2010)
   → Allogeneic paradigm (2012)
3. Identification of exosomes as mediators (2014)
   → Cell-free therapeutics (2018)
4. Mining of exosome contents identifies defined factors (2014-present)
   → Next-gen therapeutics (?)
Our initial goal with cell therapy (2004)

To repair the “permanently” injured heart

Our updated goals after 14 years of discovery work (2018)

To use cells, exosomes or defined factors as novel therapeutic candidates for a broad range of inflammatory/fibrotic diseases
Deconstruction: follow the data

Cardiosphere-derived cells (CDCs)

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<td>Canonical</td>
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Functional improvement in *mdx* mice with CDC treatment and repeat dosing

**Global LV function**

![Graph showing global LV function with different treatments and time points.](image)

**Exercise capacity**

![Graph showing exercise capacity with different treatments and time points.](image)
Candidate clinical indications for CDC exosomes

- Graft versus host disease
- Hypoplastic left heart syndrome
- Recurrent ventricular tachycardia
Exosomes and honey?

CDCs $\rightarrow$ conditioned media $\rightarrow$ exosomes

flowers $\rightarrow$ nectar $\rightarrow$ honey

E. Marbán, JACC 2018
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