Devices are So Old School: The New World of Myocardial Regeneration

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Disclosures

XyloCor Therapeutics
Regenerative Therapy for Cardiac Disease

Clinical Targets

**Ischemic Disease**

**Therapeutic angiogenesis:** The generation of new vasculature via delivery of angiogenic proteins or genes, specifically to “no option” patients not amenable to conventional interventions.

**Myocardial Infarction**

**Cellular Reprogramming:** Conversion of endogenous scar fibroblasts in areas of myocardial infarction into “induced cardiomyocytes”, thereby regenerating contractile myocardial tissue from infarcted myocardium.
Cardiac stem cell therapy
Introduction of *exogenous* (stem) cells into infarcted myocardium in order to improve cardiac function.

Cellular reprogramming
*In situ* conversion of *endogenous* infarct fibroblasts into functional cardiomyocytes.
Angiogenic Gene Therapy: Delivery of VEGF Induces Neovascularization

- Induce ischemia left circumflex
- Administer AdVEGF121

Evaluate:
- Safety
- Efficacy

3 wk → Control

4 wk → AdVEGF121

Adenovirus Delivery of VEGF 121 to Induce Cardiac Angiogenesis

- One-time, multi-site, direct myocardial injection
- Minimally invasive

Rosengart et al. *Circulation*. 1999;100:468-474

December 17, 1997, N.Y. Hospital: Phase I AdVEGF angiogenesis trial
Time to 1 min ST depression: change from baseline (min)

AdVEGF121 vs Control

- Week 12: 26 vs 27
- Week 26: 28 vs 27

*p = 0.024

REVASC Histology Results: Evidence of Angiogenesis in the Human Heart

Right ventricle – Untreated

Left ventricle – AdVEGF121 (19 days post-injection)

69 year old male died 19 days post-op from an MI in the RV (AdVEGF121 administered in the LV)

Long-term Outcomes after Cardiac Gene Therapy Exceeded Expectations

AdVEGF121 Trial 10 Year Follow Up

- Median survival 9.3 years
- 5-year survival greater with AdVEGF vs. natural history
- Apparent angiogenesis-induced collaterals

1Rosengart et al, Hum Gene Ther. 2013;24:203
Next-Gen Angiogenic Gene Therapy Trial

Subjects with diffuse moderate to severe coronary artery disease on optimal medical therapy with no other therapeutic options

Part A (Dose escalation)

Part B (randomized, double-blind, placebo controlled trial)

Direct myocardial injection via mini-thoracotomy

Assess safety and efficacy parameters up to 6 months (1 mm ST depression on ETT, PET)

Multi-isoform VEGF cassette
(100X greater potency)

Adenoviral vector

Cohort 1: AdVEGF-Minigene
Cohort 2: AdNull

3:1 Randomization

Treatment Group

Placebo Group

Subjects with diffuse moderate to severe coronary artery disease on optimal medical therapy with no other therapeutic options

Cohort 1
AdVEGF-Minigene

Cohort 2
AdNull

Randomization
Exogenous Stem Cell Delivery
• Inadequate implant phenotype
• Inadequate delivery, survival and integration
• Ventricular arrhythmias

Induced Pluripotent Stem Cells (iPSCs)
• Dedifferentiated stem cell ideal basis for CM phenotype
• BUT: still exogenous; immunogenicity, tumorigenicity risks

Exogenous Cell Therapy and iPSCs: A New Door Opens
Cardiac Stem Cell Implantation Therapy: Clinical Data

- Cochrane Review
- 100+ Phase I/II trials
- 41 randomized controlled trials
- 2732 patients
- Non-clinically relevant increase in LVEF of 2-5%


Cardiac Stem Cell Implantation Therapy: Angina Outcomes Data

- **IMPACT-CABG RCT** (JTCVS 2016): Autologous intramyocardial CD133+ (n=40) endothelial progenitor cells as adjunct to CABG:
  - No difference in improvement in angina

- **RENEW RCT** (JACC 2016): Autologous intramyocardial CD34+ cells (n=112)
  - Angina frequency was improved at 6 months (p = 0.05)
  - Exercise time difference 61 s at 3 months 36 s at 12 months (p = 0.43)
  - Study terminated

- **REGENT-VSEL RCT** (Circ Res 2016): Autologous intramyocardial CD133+ (n=31)
  - No change in ischemia by SPECT
  - No difference in improvement in angina
Obstacles to Exogenous Cell Therapy

...and If You had Any Doubt:
A New Era: Cardiac Cellular Reprogramming

Yamanaka (Oct4, Sox2, Kfl4, c-myc)

Srivastava (Gata4, Mef2c, Tbx5)

David et al. *Physiology* 2012
Induced Cardiomyocyte Generation

“Reprogramming” Genes
- Gata 4
- Mef2C
- Tbx5

Cardiac fibroblasts → Gene Transfer Vectors → Induced cardiomyocytes

DAPI  GFP  Cardiac Troponin T  Merge

GMT

GFP Control

Spontaneous iCM Contractility

Mathison et. al. J Am Heart Assoc. 2013
Myocardial Regeneration via In Situ Cellular Reprogramming

Fibroblasts

Cellular Reprogramming Factors

- Gata4, Mef2c, Tbx5
- Gata4, Mef2c, Tbx5, Hand2
- miR1, miR133, miR208, miR499, J11
- Mef2c, Myocardin, Tbx5
- Gata4, Mef2c, Tbx5, Myocardin, Srf
- Gata4, Mef2c, Tbx5, Hand2, Nkx2.5
- Gata4, Mef2c, Tbx5, Hand2, Nkx2.5, SB432542

Induced cardiomyocytes
Cellular Reprogramming *In Situ*: Experimental Studies

Create Infarct + Revascularization → Reprogramming → Assessment

- Fischer 344 rats
- LAD ligation
- AdVEGF or AdNull
- Gata 4, Mef2c, and Tbx5
- Lentivirus vectors
- Immunohistochemistry
- Echocardiography

3 wk Assessment
4 wk
Cellular Reprogramming *In Situ*: Evidence of Myocardial Regeneration

**Control**

**Treated (VEGF / GMT)**

- MYH7+ cardiac myocytes
- Fibrosis (trichrome)
- Echocardiography
Cellular Reprogramming: Improved Ventricular Function in Post-Infarct Model

Mathison et. al. J Am Heart Assoc. 2013
Triplet Vectors Enhance Reprogramming Efficacy Compared to Singlet Vectors

Lentivirus Singlet Vectors

Lentivirus GMT Triplet

Change in Ejection Fraction from Baseline

-40% -30% -20% -10% 0% 10% 20% 30% 40%

Pre-injection 2 weeks 4 weeks

VEGF/Triplet GMT*

VEGF/Singlet GMT

Double Control

*p < 0.01

Clinical Barriers to Reprogramming Therapy: Human Resistance to Cellular Plasticity

Human Cellular Reprogramming

• Cellular senescence
• Lack of stemness
• Cell cycle arrest
• Differentiation/anti-plasticity

Chromatin – based epigenetic modulation of genetic activation
Strategy to Overcome Human Resistance
More Factors in the Cocktail!

- Tbx5
- Gata4
- Mef2c
- Mesp1
- ESSRG
- miR-133
- Tbx5
- Gata4
- Mef2c
- Myocardin
- miR-1
- Hand 2
Overcoming Higher Order Species
Cellular Plasticity Barrier

GMT-treated porcine fibroblasts
(GFP/actinin)

GMT(HM)-treated porcine fibroblasts

GMT(HM)-treated porcine fibroblasts
Myocardial Regeneration: Lessons from the Tadpole
The p53 Family: “The Guardians of the Genome”
Key to Overcoming Barriers to Reprogramming?

**p53/p63 Regulation**
- Cellular senescence
- Stemness
- Cell cycle arrest
- Differentiation/plasticity

**Loss of p53/p63**
- Interferes with chromatin deacetylation
- “Unlocks” gene activation/promoter sites
- Enhanced iPS formation
- Confers increased cell plasticity

p63 Silencing Induces Cell Plasticity and Cardiac Cell Reprogramming

Murine Cardiac fibroblasts

Epigenetic Transdifferentiation
- p63 Knockdown
- Hand2
- Myocardin

Murine Induced cardiomyocytes

Ca Transient (Fura2 ratio)

Contractility

Cell length (µm)

Ca2+ Transient (Fura2 ratio)
Conclusions

• Gene therapy cardiac applications including angiogenesis trials have reappeared in the clinical arena

• Angiogenic pretreatment of infarcted myocardium improves outcomes of cell therapy

• Although formidable barriers remain, cardiac cellular reprogramming represents an exciting new potential treatment for individuals with end stage heart disease and heart failure
A New Paradigm for Treating Heart Disease
Thank you!

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