Mechanisms of Bone Marrow Derived Cell Therapy in Ischemic Cardiomyopathy with Left Ventricular Assist Device (LVAD) Bridge to Transplant

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In animal models, bone marrow cells improve vascularity and function.

In human studies:

- In vitro: bone marrow cells differentiate into blood vessel components and provide paracrine factors to promote angiogenesis and cell survival.

- In some Clinical Trials injection of bone marrow cells improves function and perfusion.

In vivo mechanisms remain uncertain in humans.
Potential Mechanism(s) for Bone Marrow Stem Cells to Improve Ischemic Cardiomyopathy

Cardiomyocyte

\[ \uparrow \text{Survival/Proliferation} \]

\[ \text{CD34}^+ \]

\[ \text{CD34}^{-} \]

\[ \downarrow \text{FIBROSIS/INFLAMMATION} \]
HYPOTHESIS

CD34+ stem cells are necessary to improve vascularity and decrease fibrosis in the ischemic human heart.
Patient needs LVAD as bridge to cardiac transplantation

Patient undergoes cardiac transplantation

- BMMC
- CD34+
- CD34neg
- saline
Study Population

• **Major Inclusion Criteria**
  – >18 yrs of age
  – Ischemic cardiomyopathy
  – LVAD as bridge to transplant

• **Major Exclusion Criteria**
  – Emergent LVAD
  – Inability to consent
  – Patient on IABP
Marrow Aspiration (15-20cc)

Ficoll Separation

25%

Feridex Labelling (25%)

Pooled BMMC

Resuspend each fraction in saline

CD34+/CD34neg fractions

75%

CliniMACS Selection

Prepare vials:
- 0.5 x 10^6 CD34+
- 1.0 x 10^6 CD34neg
- 1.0 x 10^6 BMMC
- Saline control

Release Testing
- Viability
- Gram stain
- FACS
Experimental Design: Injection Details

Each subject receives all 4 injections

- Product delivered in sterile vials labeled #1-4
  - Content blinded to all investigators
  - Content rotated from subject to subject

Injections placed in areas of moderate ischemia

- SPECT definition of areas of 50-75% perfusion
- Sample/injection area
Endpoints: Safety and Histologic

**SAFETY:**
- First 7 post-operative days
  - Ventricular Arrhythmias
  - Bleeding/Re-Operation

**HISTOLOGIC:**
- Primary Histologic Endpoints
  - Endothelial Density (CD31)
  - Fibrosis (picrosirius red)
- Secondary Histologic Endpoints
  - Inflammation/Macrophage content (CD68 density)
  - Fibroblast Content (αSMA density, vessels excluded)
  - Proliferation Index (Ki67 density)
Statistical Analysis

• Each Subject serves as his/her own Control:
  – Paired t-test between each cell treatment and saline for primary and secondary end-points

• Correlation Coefficient between specific treatment groups
Study Enrollment

26 patients w/ IsCM scheduled for BTT LVAD screened

18 Excluded
• 5 refused
• 6 required IABP
• 5 converted to DT LVAD
• 2 logistical issues

8 patients consented to study

7 patients bone marrow was collected (1 with low cell #)

6 patients had cells injected intra-operatively

1 patient withdrew

1 patient too unstable in OR for injection
<table>
<thead>
<tr>
<th>Subject</th>
<th>Age</th>
<th>Gender</th>
<th>Time to Transplant (days)</th>
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<tbody>
<tr>
<td>A1</td>
<td>70</td>
<td>M</td>
<td>177</td>
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<tr>
<td>A6</td>
<td>60</td>
<td>M</td>
<td>53</td>
</tr>
</tbody>
</table>
Results: Safety Data

- **Ventricular Arrhythmias:**
  - first 7 days post-operatively
  - 8/13 controls
  - 4/6 cases

\[ P = \text{NS by t test and Mann Whitney} \quad \quad P = \text{NS by Chisquare and Fishers} \]
Results: Primary Endpoints

Endothelial Density

Fibrillar Collagen Content

* $P = 0.02$ by paired t-test versus saline; dots represent mean values +/- SEM
CD31 for Endothelial Cells Subject A5

Saline

BMMMC

CD34neg

CD34+
Results: Secondary Endpoints

**INFLAMMATION**

![Bar graph showing CD68 positive cells/mm² for different treatments.](image)

**PROLIFERATION**

![Bar graph showing Ki67 positive cells/mm² for different treatments.](image)

**Activated Fibroblasts**

![Bar graph showing αSMA percent positive area for different treatments.](image)

Change from saline injected sample

*P = 0.02 by paired t-test versus saline

dots represent mean values +/- SEM
Histologic Effects of Treatment with CD34+ Compared to CD34neg Cells

Fibroblasts (% αSMA+ area)

Fibrillar Collagen (% picred area)

Macrophages (CD68+ cells/mm²)

correlation 0.904
p-value 0.035

correlation 0.926
p-value 0.023

correlation 0.977
p-value 0.004
Iron-labeled Cells Correlate with CD68+ Cells

Prussian Blue (iron)  CD68 (macrophages)
Conclusions

• Injection of bone marrow derived cells at the time of LVAD placement is safe and feasible
• Injection of CD34+ stem cells does not increase vascularity in unloaded ischemic tissue.
• Injection of cells may induce cellular changes via paracrine effects that are independent of the origin of the cell therapeutic delivered.
Potential Mechanism(s) for Bone Marrow Stem Cells to Improve Ischemic Cardiomyopathy

Activated Fibroblasts

Survival, Proliferation

Cardiomyocyte

CD34+

= = =

CD34 neg

FIBROSIS/INFLAMMATION
Limitations

• Left ventricle is unloaded during LVAD and remodeling and effect of cell injection may not be the same as in a non-unloaded heart.

• Small numbers increase risk of both Type I and Type II statistical errors.
Acknowledgements

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