Biologic Therapy in the Treatment of Critical Limb Ischemia

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Presenter Disclosure Information
Richard J Powell, MD
FINANCIAL DISCLOSURE:
Consultant
AnGes Inc
Aestrom
Boston Scientific
DSMB
Aldagen
EV3
CLEVER –NIH NHLBI
Current Grants/Research Support:
NIH NHLBI

Angiogenesis

- Growth and proliferation of new blood vessels from existing vascular structures.
- **Therapeutic Angiogenesis**: promote the growth of new blood vessels for the treatment of disorders of inadequate tissue perfusion
- **Vasculogenesis**: De novo vascular development form stem cells
- **Arteriogenesis**: collateral blood vessel development

Angiogenic Therapy
Critical Limb Ischemia Trials

- Delivery of angiogenic factor to ischemic limb
  - Cell therapy
  - Growth factor or transcription factor
  - Gene therapy
    - Plasmid
    - Adenovirus
- **Potential Complications**
  - “Off Target” angiogenesis
  - Growth of occult tumor
  - Progression of diabetic retinopathy
**Gene Therapy by Intramuscular Injection of Angiogenic Growth Factor**

Pre-gene transfer
- DNA
- Muscle

Post-gene transfer
- Angiogenic growth factor
- Neovascularization

**TALISMAN 201 Trial**
*NV1FGF Plasmid vs Placebo in CLI*

- Primary end-point: rate of wound healing
- Secondary end-points, n=112

<table>
<thead>
<tr>
<th>12 month Endpoint</th>
<th>AFS</th>
<th>Amputation</th>
<th>Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>NV1FGF</td>
<td>73%</td>
<td>16%</td>
<td>12%</td>
</tr>
<tr>
<td>Placebo</td>
<td>52%</td>
<td>34%</td>
<td>23%</td>
</tr>
<tr>
<td>p</td>
<td>.009</td>
<td>.015</td>
<td>.10</td>
</tr>
</tbody>
</table>

*Nikol S et al, Molecular Therapy, 16, 972-978, 2008*

**ASO Gene Therapy using VEGF to Treat Peripheral Arterial Disease**

Before GT

4W

8W


**FGF Gene Transfer in CLI**
*Proof of Concept*

- 6 patients undergoing leg amputation treated with IM injection FGF -1 plasmid
- Amputation specimens positive for:
  - Plasmid expression
  - FGF-1 mRNA expression
  - FGF protein expression

Proves that CLI patients injected with FGF plasmid express the FGF protein

*Baumgartner I et al, Molecular Therapy; 17,914-21, 2009*
TAMARIS Trial
**NV1FGF Plasmid vs Placebo in CLI**
*Pivotal Trial*

- n=525

<table>
<thead>
<tr>
<th>12 month Endpoint</th>
<th>AFS</th>
<th>Amputation</th>
<th>Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>NV1FGF</td>
<td>63%</td>
<td>26%</td>
<td>18%</td>
</tr>
<tr>
<td>Placebo</td>
<td>67%</td>
<td>21%</td>
<td>15%</td>
</tr>
<tr>
<td>p</td>
<td>.48</td>
<td>.31</td>
<td>.53</td>
</tr>
</tbody>
</table>

Belch et al. Lancet 2011

A Phase II Double Blind Placebo-Controlled Study to Assess the Safety and Efficacy of AMG0001 to Improve Perfusion in Critical Limb Ischemia

**HGF-STAT Trial**

Richard Powell, M.D.
Principal Investigator
Professor of Surgery
Section of Vascular Surgery
Dartmouth Medical School

Powell et al, Circulation 118, 2008

Molecular Mechanisms of HGF Action

AG-CLI-0202 – Treatment Arms

Randomized, placebo-controlled, double-blind, multi-center

- Arm 1: Placebo (n=25)
- Arm 2: 0.4 mg AMG0001 on days 0, 14 and 28 (n=25)
- Arm 3: 4 mg AMG0001 on days 0 and 28 (n=25)
- Arm 4: 4 mg AMG0001 on days 0, 14 and 28 (n=25)

Each administration as 8 x 2.0ml injections at predefined anatomical locations
Inclusion Criteria

- Rest pain; or peripheral ischemic ulcer(s); or areas of gangrene
- TcPO\textsubscript{2} of \leq 40 mmHg
- Ankle systolic pressure of \leq 70 mmHg or toe systolic pressure \leq 50 mmHg
- Poor candidate for standard revascularization treatment options for peripheral arterial disease

Baseline Disease Status
(Efficacy population)

<table>
<thead>
<tr>
<th></th>
<th>Placebo (n=24)</th>
<th>AMG0001 3 x 0.4mg (n=25)</th>
<th>AMG0001 2 x 4.0mg (n=21)</th>
<th>AMG0001 3 x 4.0mg (n=23)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rutherford 4</td>
<td>25%</td>
<td>48%</td>
<td>43%</td>
<td>30%</td>
</tr>
<tr>
<td>Rutherford 5 + 6</td>
<td>75%</td>
<td>52%</td>
<td>57%</td>
<td>70%</td>
</tr>
<tr>
<td>TcPO\textsubscript{2} (dorsum of foot)</td>
<td>22.2</td>
<td>22.1</td>
<td>27.3</td>
<td>21.1</td>
</tr>
<tr>
<td>ABI</td>
<td>0.44</td>
<td>0.37</td>
<td>0.46</td>
<td>0.39</td>
</tr>
<tr>
<td>TBI</td>
<td>0.18</td>
<td>0.19</td>
<td>0.19</td>
<td>0.17</td>
</tr>
<tr>
<td>Ulcer (mean cm\textsuperscript{2})</td>
<td>3.63</td>
<td>3.73</td>
<td>5.83</td>
<td>3.20</td>
</tr>
</tbody>
</table>

Safety Assessment

- No safety concerns at 12 month follow-up
- 750 adverse events in 97 subjects equally distributed between groups
- 11 neoplasms (including benign) diagnosed and equally distributed between groups.
  - Two colon cancers: Placebo and middle dose (2 x 4.0mg) groups
- No progression of retinopathy

TcPO\textsubscript{2} at Baseline and at 6 Months

Baseline TcPO\textsubscript{2}  vs TcPO\textsubscript{2} at 6 months

\# = p.014 vs placebo at 6 months
Increase in TcPO₂ to ≥ 30 mmHg at 6 Months

<table>
<thead>
<tr>
<th>Dose</th>
<th>Placebo</th>
<th>Low Dose</th>
<th>Middle Dose</th>
<th>High Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Increase</td>
<td>39</td>
<td>57</td>
<td>67</td>
<td>80</td>
</tr>
</tbody>
</table>

\( p = 0.110 \)

HGF 0205

Change in Toe Brachial Index

<table>
<thead>
<tr>
<th>Time</th>
<th>HGF</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>0.10</td>
<td>0.27</td>
</tr>
<tr>
<td>3 months</td>
<td>0.22</td>
<td>0.14</td>
</tr>
<tr>
<td>6 months</td>
<td>0.24</td>
<td>0.11</td>
</tr>
</tbody>
</table>

\( P = 0.06 P = 0.05 \)

HGF 0205

Change in Pain Score by Visual Analogue Scale (VAS)

<table>
<thead>
<tr>
<th>Time</th>
<th>HGF</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>5.3</td>
<td>6.0</td>
</tr>
<tr>
<td>3 months</td>
<td>4.4</td>
<td>6.5</td>
</tr>
<tr>
<td>6 months</td>
<td>3.4</td>
<td>6.7</td>
</tr>
</tbody>
</table>

\( P = 0.21 P = 0.04 \)

*Powell et al., J Vasc Surg, Dec 2010*
Summary of Japanese Phase III Clinical Trial ASO Study

**Efficacy:**

<table>
<thead>
<tr>
<th>Dose Group</th>
<th>Subjects</th>
<th>Subjects Unimproved</th>
<th>Improvement Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>HGF</td>
<td>19</td>
<td>8</td>
<td>70.37%</td>
</tr>
<tr>
<td>Placebo</td>
<td>4</td>
<td>9</td>
<td>30.77%</td>
</tr>
</tbody>
</table>

Total 40 Subjects

MH Analysis

P=0.0140

Summary of HGF Trial Results

- Well tolerated, no safety concerns
- AMG0001 improved limb perfusion and wound healing in patients with CLI
- Phase III trial to start 2012

Bone Marrow Derived Stem Cells

- Cardiac Muscle
- Skeletal Muscle
- Bone Marrow Stromal Cells
- Bone Marrow Derived Stem Cells
- Hematopoietic and Endothelial Stem Cells
- Endothelial Precursor Cells
- Blood Vessels
- Macrophages, Lymphocytes & other Blood Cells
- Neurons & Glial Cells
- Epithelial Cells
- Fat Cells
Harvest Technologies Phase 2 Trial

**Bone Marrow Aspirate Concentrate in CLI**

- 48 patients

<table>
<thead>
<tr>
<th>End point at 3 months</th>
<th>Bone Marrow Concentrate (34)</th>
<th>Control (14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Major Amputation</td>
<td>17.6%</td>
<td>28.6% (p=.45)</td>
</tr>
<tr>
<td>Improved Pain</td>
<td>44%</td>
<td>25% (p=.54)</td>
</tr>
<tr>
<td>Improved ABI</td>
<td>32%</td>
<td>7% (p=.08)</td>
</tr>
<tr>
<td>Improved Rutherford Classification</td>
<td>35%</td>
<td>14% (p=.18)</td>
</tr>
</tbody>
</table>


Results from a Randomized, Placebo Controlled, Double-Blind Multi-Center Phase II Trial Comparing Expanded Autologous Bone Marrow Treatment in Patients with Critical Limb Ischemia

**RESTORE-CLI Trial**


Powell et al. Mol Therapy, March 2012

**Study Protocol**

- 2:1 randomization
  - Tissue repair cells
  - Placebo injection (acellular vehicle)
- One-time set of 20 intramuscular injections 0.5 ml each
  - Lower thigh
  - Calf
  - Foot
- 12 month follow-up

**Time to First Occurrence of Treatment Failure**

- Major amputation
- All-cause mortality
- Doubling in wound size
- De novo gangrene
- Revascularization
Bone Marrow Harvest (50 ml)– Cells Undergo Expansion in Bioreactor

Ixmyelocel-T Cell Expansion

Starting Bone Marrow
RBCs lymphocytes Granulocytes Monocytes Mesenchymal Stem Cells ~300 million cells

Expansion Process
RBC extraction ≤ 0.1% remaining
Cell Reduction
Cell Amplification
-5X
+200X +50X

ixmyelocel-T
~150 million cells

Therapeutic Effect
Remodelling of ischemic tissue Modulation of inflammation Promotion of angiogenesis

Safety Overview: All Aspirated Patients (N=77)

<table>
<thead>
<tr>
<th>Safety Parameter</th>
<th>Ixmyelocel-T  N = 53</th>
<th>Control N = 24</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>N (%) with Adverse Events</td>
<td>47 (89)</td>
<td>23 (96)</td>
<td>0.424</td>
</tr>
<tr>
<td>N (%) Serious Adverse Event</td>
<td>23 (43)</td>
<td>12 (50)</td>
<td>0.628</td>
</tr>
<tr>
<td>N (%) withdrawal due to AE</td>
<td>2 (4)</td>
<td>0 (0)</td>
<td>1.000</td>
</tr>
<tr>
<td>N (%) Deaths *</td>
<td>3 (6)</td>
<td>2 (8)</td>
<td>1.000</td>
</tr>
</tbody>
</table>

* An additional ixmyelocel-T patient died ~100 days after completing study.
** Based on Fisher’s Exact Test.

Time to First Occurrence of Treatment Failure – All Treated Patients (N=72)

62% risk reduction: HR 0.38, 95%CI = (0.20-0.74)
First Event Contributed to Treatment Failure – All Treated Patients (N=72)

<table>
<thead>
<tr>
<th>Endpoint (n)</th>
<th>Ixmyelocel-T (N = 48)</th>
<th>Control (N = 24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Major amputation</td>
<td>6 (12%)</td>
<td>4 (17%)</td>
</tr>
<tr>
<td>All-cause mortality</td>
<td>2 (4%)</td>
<td>1 (4%)</td>
</tr>
<tr>
<td>Doubling in total wound surface area*</td>
<td>5 (10%)</td>
<td>7 (29%)</td>
</tr>
<tr>
<td>De novo gangrene</td>
<td>6 (12%)</td>
<td>4 (17%)</td>
</tr>
<tr>
<td>Total n(%)</td>
<td>19 (39.6%)**</td>
<td>16 (66.7%)**</td>
</tr>
</tbody>
</table>

*For wound size doubling: patient must have come into the study with a wound to be eligible to contribute to this event. ** p = 0.0451, Fisher’s exact test.

Amputation-Free Survival – All Treated Patients (N=72)

<table>
<thead>
<tr>
<th>Days After Injection</th>
<th>Control</th>
<th>Ixmyelocel-T</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>24</td>
<td>48</td>
</tr>
<tr>
<td>100</td>
<td>23</td>
<td>47</td>
</tr>
<tr>
<td>200</td>
<td>22</td>
<td>46</td>
</tr>
<tr>
<td>300</td>
<td>21</td>
<td>45</td>
</tr>
<tr>
<td>400</td>
<td>20</td>
<td>44</td>
</tr>
</tbody>
</table>

32% risk reduction: HR 0.68, 95%CI = (0.28-1.65)

TTF and AFS – Baseline Wound Patients (N=45)

<table>
<thead>
<tr>
<th>Days After Injection</th>
<th>Control</th>
<th>Ixmyelocel-T</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>24</td>
<td>48</td>
</tr>
<tr>
<td>100</td>
<td>23</td>
<td>47</td>
</tr>
<tr>
<td>200</td>
<td>22</td>
<td>46</td>
</tr>
<tr>
<td>300</td>
<td>21</td>
<td>45</td>
</tr>
<tr>
<td>400</td>
<td>20</td>
<td>44</td>
</tr>
</tbody>
</table>

77% risk reduction: HR = 0.225
95% CI = (0.103, 0.490)
Cox PH p = 0.0002

61% risk reduction: HR = 0.391
95% CI = (0.131, 1.164)
Cox PH p= 0.0915

Conclusions from RESTORE-CLI

- Safety of TRC’s in CLI patients.
- Improved outcomes based on objective clinical endpoints.
  - Significant improvement in composite endpoint (major amputation, all-cause mortality, doubling in wound size, de novo gangrene)
  - Differences in outcome of patients with rest pain vs tissue loss
- Early results promising
  - Larger pivotal trial warranted
**Ongoing Cellular Therapy Trials**

- Autologous
  - Aastrom
    - Phase 3
  - Harvest Technology
    - Phase 3 - IDE
  - Biomet
    - Phase 3- IDE
- Allogeneic
  - Pluristem: placental derived
    - Phase 1 trial complete

**Conclusions**

- Potentially a disruptive technology
- No currently completed pivotal trial supports use of biologic therapy in CLI
- Large number of trials in progress with improved trial design

**PAD Pipeline**

- Interest from other Thought Leaders

**Patient Demographics—All Treated Patients (n=72)**

<table>
<thead>
<tr>
<th>Parameter* (Mean values)</th>
<th>Ixmyelocel-T ( N = 48 )</th>
<th>Control ( N = 24 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Male</td>
<td>71</td>
<td>58</td>
</tr>
<tr>
<td>Age</td>
<td>69</td>
<td>67</td>
</tr>
<tr>
<td>% Current, % Past smokers</td>
<td>17, 67</td>
<td>38, 46</td>
</tr>
<tr>
<td>% Current, % Past alcohol</td>
<td>44, 23</td>
<td>29, 33</td>
</tr>
<tr>
<td>BMI</td>
<td>27</td>
<td>28</td>
</tr>
<tr>
<td>Creatinine mg/dL</td>
<td>1.2</td>
<td>1.1</td>
</tr>
<tr>
<td>N (%) with known Diabetes</td>
<td>21 (44)</td>
<td>15 (63)</td>
</tr>
</tbody>
</table>

*No significant differences
Demographics
(Study population)

<table>
<thead>
<tr>
<th></th>
<th>Placebo (n=26)</th>
<th>AMG0001 3 x 0.4mg (n=26)</th>
<th>AMG0001 2 x 4.0mg (n=25)</th>
<th>AMG0001 3 x 4.0mg (n=27)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>68.2</td>
<td>70.5</td>
<td>73.0</td>
<td>67.6</td>
</tr>
<tr>
<td>Male (%)</td>
<td>62%</td>
<td>77%</td>
<td>56%</td>
<td>63%</td>
</tr>
<tr>
<td>Race (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>77%</td>
<td>92%</td>
<td>88%</td>
<td>85%</td>
</tr>
<tr>
<td>Black</td>
<td>15%</td>
<td>8%</td>
<td>8%</td>
<td>7%</td>
</tr>
<tr>
<td>Other</td>
<td>8%</td>
<td>0%</td>
<td>0%</td>
<td>7%</td>
</tr>
<tr>
<td>Diabetes (%) #</td>
<td>65%</td>
<td>54%</td>
<td>36%</td>
<td>41%</td>
</tr>
<tr>
<td>Creatinine &gt;1.5</td>
<td>35%</td>
<td>8%</td>
<td>12%</td>
<td>26%</td>
</tr>
<tr>
<td>Smoking (%)</td>
<td>85%</td>
<td>77%</td>
<td>72%</td>
<td>85%</td>
</tr>
</tbody>
</table>

#: p=0.141

Angiogenic Growth Factors

- Vascular Endothelial Growth Factor (VEGF)
- Fibroblast Growth Factor (FGF)
- Hypoxia Inducible Factor -1a (HIF-1a)
- Hepatocyte Growth Factor (HGF)