What's New in LE Wound Healing

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Director, Research, Wound Healing, RI Hospital

What's New in LE Wound Healing

Cellular Therapy

Also called Cell Therapy
Also called Cytotherapy
Also called Cell-based Therapy

History of Cellular Therapy

Early 19th Century into 20th Century
Injection of extracts from animal organs and plants
Implantation of animal organs in humans
Swiss physician Paul Niehans
1930-1960
over 50,000 cellular therapy treatments

http://www.aeskininternational.com/basic.php

20th Century
Bone Marrow Transplants
Blood Transfusion

http://www.asgct.org/general-public/educational-resources/gene-therapy--and-cell-therapy-defined

Today's Cellular Therapy

• Allogeneic Cell Therapy
  • Cell therapy – HEMACORD
  • Tissue engineered wound care products
  • Stem Cell therapy
• Autologous Cell Therapy
  • Stem Cell therapy - MSCs expanded then injected – days or weeks later
  • Cell therapy – MSCs collected same day & injected

Today’s Cellular Therapy
FDA Approved Stem Cell Product

November 10, 2011
FDA announces first stem cell product approval

HEMACORD

- ALLOGENIC
- Cell Therapy – transplantation targeting stem cells

http://www.asgct.org/general-public/educational-resources/gene-therapy--and-cell-therapy-defined

Today’s Cellular Therapy
Human Stem Cells

- Totipotent
- Pluripotent
- Multipotent and
- Unipotent

http://www.asgct.org/general-public/educational-resources/gene-therapy--and-cell-therapy-defined
http://biochemistry.ucr.edu/faculty/sato/sato_research.html

Different Levels of Stem Cell State

**Totipotency**
A somatic cell can become any cell in the body.

**Pluripotency**
A pluripotent cell can become any cell in the body.

**Multipotency**
A multipotent cell can become several cell types in the body.

**Unipotency**
A unipotent cell can become one cell type in the body.

http://biochemistry.ucr.edu/faculty/sato/sato_research.html

Today’s Cellular Therapy
Human Stem Cells

- Multipotent
- Adult stem cells
  - Mesenchymal stem cell therapy (MSC therapy)

http://www.asgct.org/general-public/educational-resources/gene-therapy--and-cell-therapy-defined
http://biochemistry.ucr.edu/faculty/sato/sato_research.html

Today’s Cellular Therapy
Human Stem Cells

- Multipotent
  - Adult stem cells – Other than Mesenchymal
    - Hematopoietic – Mammary – Intestinal
    - Endothelial – Neural – Olfactory
    - Neural crest – Testicular

http://www.asgct.org/general-public/educational-resources/gene-therapy--and-cell-therapy-defined
http://biochemistry.ucr.edu/faculty/sato/sato_research.html
Today's Cellular Therapy

Cellular versus Acellular

Tissue Engineered Products - Acellular - Scaffold/Matrix

INTEGRA™ Dermal Regeneration Template
Bi-layered: bovine tendon collagen and glycosaminoglycan with a polysiloxane (silicone) Membrane


Today's Cellular Therapy

Examples of Non-human tissue-engineered Acellular wound products:

- Permacol® (Covidien) porcine dermis
- MatriStem™ (Acel Inc) porcine urinary bladder
- Oasis Wound Matrix® (S&N) porcine intestine
- PriMatrix™ (TEI Biosciences) fetal bovine dermis

Tissue Engineering and Wound Healing: An Overview of the Past, Present, and Future

Acellular Human Therapies

Examples of Human tissue-engineered Acellular wound products:

- Graft Jacket (KCI)
- AlloDerm (LifeCell)
- AmnioFix (Mimedix)

Tissue Engineering and Wound Healing: An Overview of the Past, Present, and Future

Allogenic Tissue-engineered CELLULAR Therapy

- DermaGraft (Organogenesis)
- Apligraf (Organogenesis)
- TransCyte® (Smith&Nephew)
- OrCel® (FortiCell)
- EpiFix® (Mimedix)

Tissue Engineering and Wound Healing: An Overview of the Past, Present, and Future

Autologous CELLULAR Therapy

Tissue Engineered

- Epicel® (Genzyme) – autologous lab produced skin graft

Tissue Engineering and Wound Healing: An Overview of the Past, Present, and Future

APPROVED Products- Cellular Therapies

- Provenge® – Autologous - Expanded Cells in Lab –
  - TRANSPLANTATION
  - Immunotherpay for advanced prostate cancer
- Carticel® – Autologous - Expanded Cells in Lab
  - TRANSPLANTATION
- LaVIV® – Autologous - Expanded Cells in Lab
  - TRANSPLANTATION
Cell Therapy versus Gene Therapy

- Transplantation of whole cells
- DNA used to manipulate cells

The most glorious moments in your life are not the so-called days of success, but rather those days when out of dejection and despair you feel rise in you a challenge to life, and the promise of future accomplishments.

Gustave Flaubert

Cell Therapy “Is it real”

- Failures: NV1FGF
- Successes: Autologous CD34
- Upcoming therapies: Placenta

Current Treatment Options for CLI

- Limited
  - Revascularization options predominantly above knee
  - No approved drugs that reverse / arrest condition

- 21-26 studies currently recruiting CLI patients*
  - 4 Drug studies
  - 13 Biologic studies
  - 9 Other (devices, behavior, etc.)

* www.clinicaltrials.gov - as of September 1, 2013

Systematic Review and Meta-analysis of Gene Therapy in PAD

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<tr>
<td>GENE</td>
<td>Del-1</td>
<td>VEGF-125</td>
<td>VEGF-121</td>
<td>rFGF-2</td>
<td>VEGF</td>
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<tr>
<td>Sample</td>
<td>105</td>
<td>54</td>
<td>105</td>
<td>190</td>
<td>54</td>
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<tr>
<td>size</td>
<td></td>
<td></td>
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<tr>
<td>Delivery method</td>
<td>i.m.</td>
<td>i.m.</td>
<td>i.m.</td>
<td>Intra-arterial</td>
<td>Intra-arterial</td>
</tr>
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</table>

Ghosh et al. 2008
Systematic Review and Meta-analysis of Gene Therapy in PAD

HARD ENDPOINTS

Met analysis shows that gene therapy doesn’t modify ABI values, peak walking distance or claudicate onset time

ULCER HEALING: not reported
AMPUTATION: not reported

Systematic Review and Meta-analysis of Stem Cell Therapy in PAD

Fadini et al. 2009

HARD ENDPOINTS

This met analysis only provides descriptive review of hard endpoints

ULCER HEALING: conflicting results
AMPUTATION: not reported in most studies

Therapeutic Angiogenesis NV1FGF

• Via gene transfer of angiogenic growth factors may improve perfusion the development of new blood vessels
• NV1FGF is a non-viral recombinant DNA plasmid, containing a gene encoding FGF1
• Human acidic growth factor (FGF1) protein promotes activation, migration, proliferation and differentiation of relevant vascular cells

NV1FGF Phase 2 (TALISMAN study)

• 125 patients with CLI and ischemic ulcers (Fontaine IV) unsuitable for revascularization
• Randomized to 16 mg of NV1FGF or placebo (4 mg administered as 4 IM injections every two weeks for a total of 4 doses)
• Primary endpoint = Complete ulcer healing at 25 weeks
• Secondary endpoints = major amputations, death, amputation free survival at 12 months

Nikol, et Al. Mol Ther 2008;16:972-8
NV1FGF Phase 2 Results

Primary end points at week 25

Complete healing of at least one ulcer selected at baseline

<table>
<thead>
<tr>
<th></th>
<th>NV1FGF (N=51)</th>
<th>Placebo (N=56)</th>
<th>Hazard ratio</th>
<th>P value</th>
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<td>Complete healing of at least one ulcer</td>
<td>24 (47.1%)</td>
<td>22 (39.3%)</td>
<td>0.93</td>
<td>0.59</td>
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Secondary end points over 52 weeks

Amputation rate

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<td>8 (15.7%)</td>
<td>13 (23.2%)</td>
<td>0.49</td>
<td>0.015</td>
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<tr>
<td>Minor</td>
<td>16 (31.4%)</td>
<td>19 (34.1%)</td>
<td>0.85</td>
<td>0.49</td>
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Death rate

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Combining major amputation and death rates

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<td>0.85</td>
<td>0.49</td>
</tr>
<tr>
<td>Death</td>
<td>14 (27.4%)</td>
<td>26 (46.4%)</td>
<td>0.43</td>
<td>0.009</td>
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</tbody>
</table>

NV1FGF Gene Therapy on Amputation-Free Survival in Critical Limb Ischemia - Phase 3 Randomized Double-Blind Placebo-Controlled Trial (TAMARIS)

Iris Baumgartner, Jill Belch, Eric Van Belle, Vickie R Driver, William R. Hiatt, Sigrid Nikol, Lars Norgren


TAMARIS Study

525 CLI patients with skin lesions (Fontaine IV), unsuitable for standard revascularization.
30 countries (US, Canada, South America, Europe, Asia, South Africa)

Randomized to NV1FGF 4 mg every two weeks x 4 or placebo.
Double blind assessment, stratification by country and diabetes.

Largest international phase 3 trial in CLI patients unsuitable for revascularization.

Primary Endpoint

Time to Major Amputation or Death

Autologous CD34+ Cell Therapy

Multicenter Phase Ila Randomized Double-blind Placebo-Controlled Pilot

1) Placebo
2) Low dose (1x10^5 CD34/Kg body wt)
3) High dose (1x10^6 CD34/Kg body wt)


CLI Phase I/IIa Study Design:

Two Dose Levels (n = 28);

- Non-optimal candidate for surgical or percutaneous revascularization or have refused revascularization

Baseline Assessment and Stabilization (2-4 weeks)

Cell Mobilization and Apheresis (3 days)

6 Intramuscular Injections or Placebo Rx (in lower leg and distal thigh)

Follow-up Assessments: Weeks 2, 4, 6, 8, & 12 and Months 6 & 12

Randomized Controlled Pilot Study
Autologous CD34+ Cell Therapy for CLI

- A total of 28 pts were randomized and completed the injection procedure and 24 completed the 6 and 12 mo evaluation.
- The mean age of subjects was 66.6±13.0. there were 9 female and 19 male subjects

Placenta-Derived Adherent Cells (PDA-002)
- A novel cell therapy
- Mesenchymal stromal cells isolated-like population from full term human placenta
- Properties
  - Immunomodulatory
  - Anti-inflammatory
  - Angiogenic
  - Tissue-Regenerative
- In phase II clinical trials for Peripheral Arterial Disease with Diabetic Foot Ulcer (NCT01859117) and (NCT02264288) intramuscular injection

Characterization of PDA-002 in Vitro
- PDA-002 induces proliferation/survival and migration of vascular endothelial cell
- PDA-002 induces in vitro tube formation of endothelial cells and elevated blood vessel density in an ex vivo Chorioallantoic Membrane (CAM)
- PDA-002 modulates epithelial and endothelial to mesenchymal transition (EMT) and endothelial cell activity via secretion and regulation of growth factors and cytokines
- PDA-002 induces closure of epithelial cell wounds in vitro

PDA-002 Mechanisms of Action for DFU/PAD

Immune Cell
- T cells
- B cells
- DC
- Macrophages

Multi-task cells, HGF proliferation of endothelial cell, maintenance of vessels,

Animal Models of Limb Ischemia
- Surgical incision of femoral artery to induce hind limb ischemia
- Mouse (BALB/c) and Rat
- Treatment with cells 1-2 days after surgery IM at 2-sites (above & below lesion)
- Endpoints evaluated up to 35-49 days after surgery
  - Blood flow (Doppler)
  - Blood volume or angiography
  - Ischemic severity (score)
  - Histology

STEM CELL THERAPY IN CLI:
Autologous CD34+ cell therapy for critical limb ischemia
Multicenter Phase IIa RCT

<table>
<thead>
<tr>
<th>n = 28</th>
<th>6-12 mo amputation rate (minor and major)</th>
<th>Median time to amputation (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>67% 75%</td>
<td>110</td>
</tr>
<tr>
<td>Low dose</td>
<td>43% 43%</td>
<td>183</td>
</tr>
<tr>
<td>High dose</td>
<td>22% 22%</td>
<td>The control to high dose group resulted in a p-value of 0.011</td>
</tr>
</tbody>
</table>
A long term healing effect was observed with IM injection of PDA-
Reduction in inflammatory
Preliminary results suggest that PDA-
cells (~19 subjects)
Objective: Assess the safety, dose and efficacy
Objective: Assess the safety, efficacy, and effect on vascular parameters caused
Objective: Assess the safety and efficacy of PDA-
Enhanced blood flow
Month 3
cells (~38 subjects)
Myofibers containing multiple
cells Dose
Dose
>700
Increased blood volume
Emile
Doses as low as 1000 cells were effective
UPenn
cells
(28 to 0)
- Ischemic or neuro-ischemic diabetic foot ulcer
- PAD with ABI > 0.6 and ≤ 0.9 or TBI > 0.35
and ≤ 0.7
- Open label, Dose-escalation
  (1) 4 dose levels administered on Day 1 and 8
  (2) Dose-Level 1: 3 x 10^5 cells
  (3) Dose-Level 2: 3 x 10^5 cells
  (4) Dose-Level 3: 3 x 10^5 cells
  (5) Dose-Level 4: 3 x 10^4 cells
- Preliminary results suggest that PDA-002 has a therapeutic effect in wound
healing and increasing peripheral vascular flow

Efficacy of PDA-002 in Hind-Limb Ischemia: Improved Blood Flow and Angioscore

PDA-002 DFU-001: n=24  Phase 1

- Design: Phase 1 Multicenter, Open-Label, Dose-escalation Safety and efficacy of IM Injection of Human Placenta-Derived Cells (PDA-002) in PAD and DFU
- Objective: Assess the safety, dose and efficacy
  - Ischemic or neuro-ischemic diabetic foot ulcer
  - PAD with ABI > 0.6 and ≤ 0.9 or TBI > 0.35 and ≤ 0.7
- Open-label, Dose-escalation
  (1) 4 dose levels administered on Day 1 and 8
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  (4) Dose-Level 3: 3 x 10^5 cells
  (5) Dose-Level 4: 3 x 10^4 cells
- Preliminary results suggest that PDA-002 has a therapeutic effect in wound
healing and increasing peripheral vascular flow

Phase 2 Clinical Study: PDA-002 DFU-002

- Objective: Assess the safety and efficacy of PDA-002 administered
  intramuscularly (IM) in subjects with DFU with PAD.
  (1) The primary endpoint is complete wound closure DFU and PAD at
  3 months.
- Design: A Phase 2 Multicenter, Randomized, Double-Blind, Placebo-
  Controlled, Dose Range Finding Study to Evaluate the Safety and
  Efficacy of IM Injection of Human Placenta-Derived Cells (PDA-002)
  - Double-Blind, Placebo-Controlled 3 dose levels and placebo administered on Day 1 and 8
    (1) Dose-Level 1: 3 x 10^5 cells (38 subjects)
    (2) Dose-Level 2: 3 x 10^4 cells (38 subjects)
    (3) Dose-Level 3: 3 x 10^3 cells (39 subjects)
  (a) Placenta (38 subjects)
- Sample size: N=133  Top2040 ABI: 4.8

PDA-002 Phase 2 Study:
Diabetic Foot Ulcer with Peripheral Artery Disease

Screening/Baseline (Day -30 to 0)  Treatment (Days 1 and 8)  Follow-up (24 months)

Randomized, double-blind, 38 per
Arm (10 at highest
dose)
1: 3 x 10^5 cells
2: 3 x 10^4 cells
3: 30 x 10^3 cells
4: Placebo

Baseline
Assessments
Wound assessments, Basic hemodynamics, Sample collection
Safety, Duration of Effect

Primary Efficacy Endpoint - Month 2
Follow-up visits: Study, Day 15
Follow-up visits: Months 1, 2, 4 & 5
Follow-up visits: Months 6, 12, 18 & 24

Proof of Mechanism Vascular Study in DFU:
PDA-002 DFU-003

- Objective: Assess the safety, efficacy, and effect on vascular parameters caused
  by PDA-002 administered intramuscularly (IM) in subjects who have DFU with
  PAD.
  (1) Evaluate if increased peripheral vascularity can be detected in subjects
  who have DFU with PAD treated with PDA-002
- Design: A Phase 2 Double-Blind, Dose Range Finding Study to Evaluate the
  Safety, Vascular Effect and Efficacy of Intramuscular Injection of Human
  Placenta-Derived Cells (PDA-002) in subjects who have DFU with PAD
- Vascular Assessments
- Sample size: N=24

PI: Emilie Mohler MD, UPenn
PDA-002 DFU-003 Proof of Mechanism

Screening/ Pretreatment
Day 1
Day 7 to 0

Treatment
Day 1, 2, 16

Follow-up
Day 30 to Month 12

Techniques to Assess Tissue Oxygenation and Perfusion in PDA-002 DFU-003

• Anatomical imaging of large vessels (MRA)
• Imaging of tissue perfusion and oxygenation (TcPO2, NIRS, Doppler US)
• Functional metabolic imaging and vascular measurements (FDG-PET CT, MR Oximetry)

PDA-002 Summary

• PDA-002 (IM) is well tolerated in immune suppressed and immune competent animal models
• Efficacy demonstrated in multiple models of hind-limb ischemia (rats and mice)
• Specific efficacy for angiogenesis was demonstrated
  ➢ increased number of capillaries
  ➢ increased number of large blood vessels
  ➢ enhanced maturation of collateral blood vessels
• A reparative effect on muscle pathology was observed
• Working hypothesis for potential MoA: PDA-002 modulates the local innate and possibly adaptive immune response, which enhances endogenous repair
• Clinical Studies
  ➢ Phase 1 (Safety and Efficacy of PDA-002 in Subjects with PAD / DFU)
    ➢ Preliminary results suggest that PDA-002 has a therapeutic effect in wound healing and increasing peripheral vascular flow
  ➢ Phase 2 ongoing

Harvester

Tegaderm with Autogenous Graft

Week 1

TMA day 1
1.4 cm x 0.6 cm x 0.1 cm
Week 2

0.3 cm x 0.2 cm & 0.4 cm x 0.2 cm

Week 3

Resolved

Wound Repair 2050

- Surgeon/Clinician dispensing genes/cells
- Cell lab group
- Biologics center
- Diagnostics lab
- Wound telemetry
- Personalized therapy
  - Micro-bio lab

Healthy Activated Cells from Donor and Growth Factors

Initial starting material centrifuged whole blood unit from young healthy donors compatible for patient blood type

Centrifuged

Plasma

WBC

RBC

Activated by hypo-osmotic shock

WBC returned back to blood bank

Final product ready for injection

Ab screening, sterility and activity

Initial starting material

Tested for transfusion transmitted diseases

Thank you
drvdriver@aol.com