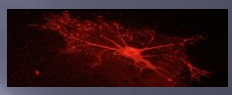
What's New in LE Wound Healing



Vickie R. Driver MS, DPM, FACFAS
Professor, Orthopedic Surgery, Brown University
President, Assoc. Advancement of Wound Care, AAWC
Chief, Podiatric Surgery VA Healthcare New England, RI
Director, Research, Wound Healing- RI Hospital

Limb Preservation Research and Education

BROWN

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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

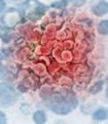
http://www.aeskinainternational.com/basic.php

History of Cellular Therapy

20th Century

Bone Marrow Transplants

Blood Transfusion



http://www.stjude.org/stjude/v/index.jsp?vgnextoid=0960fa2454e70110VgnVCM1000001e0215acRCRD&vgnextchannel=1b8bc86719171110VgnVCM1000001e0215acRCRD

http://www.redcrossblood.org/learn-about-blood/history-blood transfusion

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 –
  - Cell therapy – MSCs collected same day & injected

http://www.asgct.org/general-public/educational-resources/gene-therapy--and-cell-therapy-defined;
Freeman M, Fuerst M. Does the FDA have regulatory authority over adult autologous stem cell therapies? Journal of Translational Medicine 2012;10:60. doi10.1186/1479-5876-10-60http://c.ymcdn.com/sites/www.celltherapysociety.org

Today's Cellular Therapy

- **FDA and Autologous Cellular Therapy**
  - Stem Cell therapy - MSCs expanded then injected – days or weeks later
  - Cell therapy – MSCs collected same day & injected

http://www.fdalawblog.net/fda_law_blog_hyman_phelps/2014/02/dc-circuit-court-of-appeals-rules-that-fda-can-regulate-autologous-stem-cells.htmlhttp://c.ymcdn.com/sites/www.celltherapysociety.org
http://blogs.law.harvard.edu/billofhealth/2013/06/02/our-bodies-our-cells-fda-regulation-of-autologous-adult-stem-cell-therapies

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

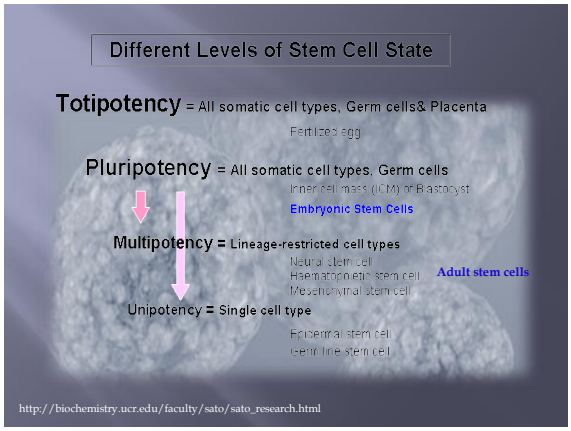
http://www.asgct.org/general-public/educational-resources/gene-therapy--and-cell-therapy-defined

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
http://www.stemcellnetwork.ca/index.php?page=stem-cell-timeline
http://www.cancer.gov/cancertopics/factsheet/Therapy/bone-marrow-transplant



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

Today's Cellular Therapy

Human Stem Cells

- Totipotent / Pluripotent
  - Embryonic stem cells/ stem cell lines
  - Induced pluripotent stem cell lines (iPSC)
- Multipotent
  - Adult stem cells / stem cell line
    - Hematopoietic stem cell transplantation
      Bone marrow, Umbilical cord blood/Placenta, Blood
    - Mesenchymal stem cell therapy (MSC)

http://biochemistry.ucr.edu/faculty/sato/sato_research.htmlhttp://www.stemcellnetwork.ca/index.php?page=stem-cell-timeline

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-definedActa Pharmacologica Sinica (2013) 34: 747–754; doi: 10.1038/aps.2013.50 Journal of Hematology & Oncology 2012, 5:19 doi:10.1186/1756-8722-5-19

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-definedActa Pharmacologica Sinica (2013) 34: 747–754; doi: 10.1038/aps.2013.50 Journal of Hematology & Oncology 2012, 5:19 doi:10.1186/1756-8722-5-19

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

International Consensus: Acellular matrices for the treatment of wounds, Wounds International
January 2011: http://www.woundsinternational.com/clinical-guidelines/acellular-matrices-for-the-treatment-of-wounds

Today's Cellular Therapy

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

- Permacol® (Covidien) porcine dermis
- MatriStem™ (Acell 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
PJ van Winterswijk, E. Nout. Wounds,2007;19(10):277-284.

Today's Cellular Therapy

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
PJ van Winterswijk, E. Nout. Wounds,2007;19(10):277-284.

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
PJ van Winterswijk, E. Nout. Wounds,2007;19(10):277-284.

Autologous *CELLULAR Therapy*
Tissue Engineered

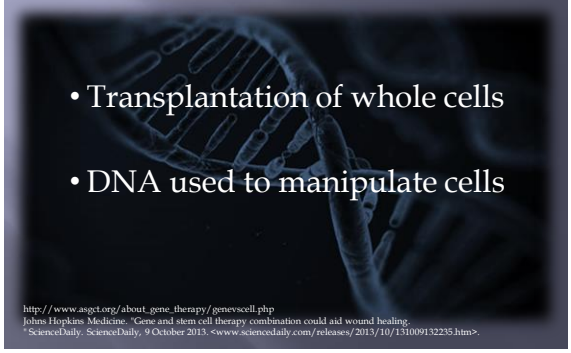
- Epicel® (Genzyme) – autologous lab produced skin graft

Tissue Engineering and Wound Healing: An Overview of the Past, Present, and Future
PJ van Winterswijk, E. Nout. Wounds,2007;19(10):277-284.

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

http://www.asgct.org/about_gene_therapy/genevscell.php
Johns Hopkins Medicine. "Gene and stem cell therapy combination could aid wound healing." ScienceDaily. ScienceDaily, 9 October 2013. <www.sciencedaily.com/releases/2013/10/131009132235.htm>.

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

What's New in LE Wound Healing II

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Section Chief, Podiatric Surgery, VA New England
Director Research HBO and Wound Care CTR RI Hospital
President, Association for the Advancement of Wound Care, AAWC

U.S. Department of Veterans Affairs

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Cell Therapy "Is it real"

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

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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

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Systematic Review and Meta-analysis of Gene Therapy in PAD

STUDY	DELTA (2007)	Kusumanto et al. (2006)	RAVE (2003)	TRAFFIC (2002)	Makinen et al. (2002)
GENE	Del-1	$VEGF_{165}$	$VEGF_{121}$	rFGF-2	VEGF
Sample size	105	54	105	190	54
Delivery method	i.m.	i.m.	i.m.	Intra-arterial	Intra-arterial

Ghosh et al. 2008

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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

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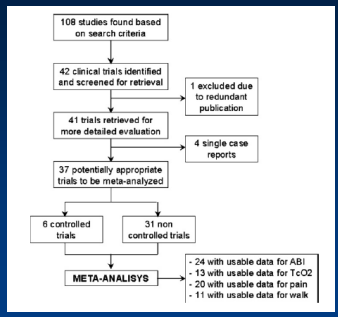
Systematic Review and Meta-analysis of Stem Cell Therapy in PAD



Fadini et al. 2009

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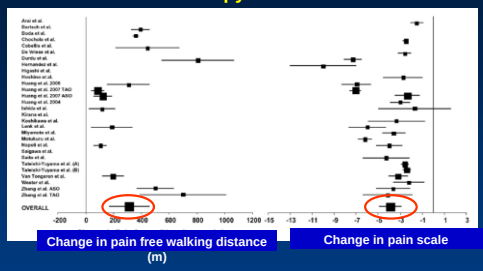
Systematic Review and Meta-analysis of Stem Cell Therapy in PAD



SIGNIFICANT IMPORVEMENT IN PAIN FREE WALKING DISTANCE

SIGNIFICANT REDUCTION IN PAIN

Fadini et al. 2009

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Review and Meta-analysis of Stem Cell Therapy in PAD

HARD ENDPOINTS

This met analysis only provides descriptive review of hard endpoints

ULCER HEALING: conflicting results

AMPUTATION: not reported in most studies

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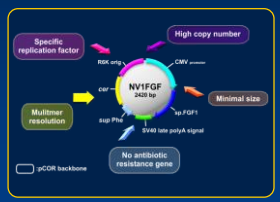
Therapeutic Angiogenesis NV1FGF

- 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



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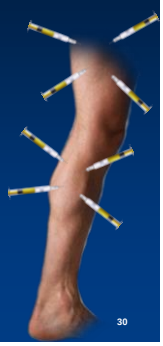
NV1FGF Phase 2 (TALISMAN study)

- 125 patients with CLI and ischemic ulcers (Fontaine IV) unsuitable for revascularization
- Randomized to 16 mg of NV1FGF or placebo **(4 mg administered as 8 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

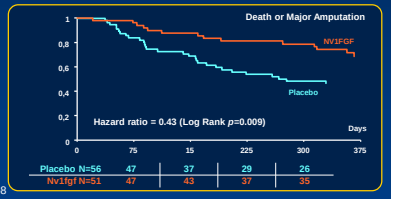


Nikol, et Al. Mol Ther 2008;16:972-8

30

NV1FGF Phase 2 Results

	Placebo (N=56)	NV1FGF (N=51)	Hazard ratio	*P* value
Primary end point at week 25				
Complete healing of at least one ulcer selected at baseline	8 (14.3)	10 (19.6)	-	0.514[a]
Secondary end points over 52 weeks				
Amputation rate				
All	31 (55.4)	19 (37.3)	0.498	0.015[b]
Major	19 (33.9)	8 (15.7)	0.371	0.015[b]
Death rate	13 (23.2)	6 (11.8)	0.460	0.105[b]
Combined major amputation and death rates	29 (51.8)	14 (27.4)	0.435	0.009[b]



Nikol. et Al. Mol Ther 2008;16:972-8

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

Lancet. 377(9781):1929-1937. June 2011

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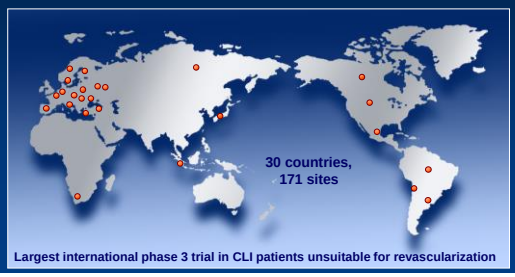
TAMARIS Study

525 CLI patients with skin lesions (Fontaine IV), unsuitable for standard revascularization

30 countries (US, Canada, South America, Europe, Asia, South Africa)

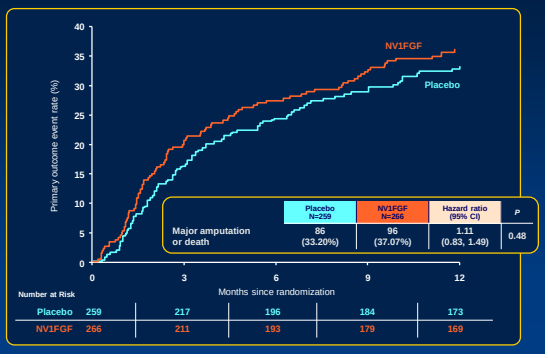
Randomized to **NV1FGF 4 mg every two weeks x 4** or placebo

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



CLI Phase I/IIa Study Design:
Two Dose Levels (n = 28);

CLI Patients (Rutherford Score IV or V)

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

↓

Baseline Assessment and Stabilization (2-4 weeks)

Cell Mobilization and Apheresis (5 days)

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

Follow-up Assessments:

Weeks 2, 4, 6, 8, & 12 and Months 6 & 12

DW Losordo, MR Kibbe, F Mendelsohn, W Marston, **VR Driver**, et al Circulation Cardiovascular Intervention. 5(6):821-830. December 2012

Autologous CD34+ Cell Therapy

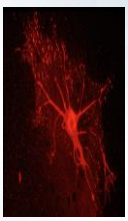
Multicenter Phase IIa Randomized

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)



DW Losordo, MR Kibbe, F Mendelsohn, W Marston, **VR Driver**, et al Circulation Cardiovascular Intervention. 5(6):821-830. December 2012

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

DW Losordo, MR Kibbe, F Mendelsohn, W Marston, **VR Driver**, et al; Circulation Cardiovascular Intervention. 5(6):821-830. December 2012

STEM CELL THERAPY IN CLI:

Autologous CD34+ cell therapy for critical limb ischemia

Multicenter Phase IIa RCT

n = 28	6-12 mo amputation rate (minor and major)	Median time to amputation (days)
Placebo	67% 75%	110
Low dose	43% 43%	183
High dose	22% 22%	

The control to high dose group resulted in a p-value of 0.011

DW Losordo, MR Kibbe, F Mendelsohn, W Marston, **VR Driver**, et al; Circulation Cardiovascular Intervention. 5(6):821-830. December 2012

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

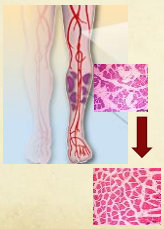
  } Liu et al., *Clin & Trans Immunol*. 2014; 3:e14
  - Angiogenic
  - Tissue-Reparative

  } Francki et al., Posters at Fall SAWC
- In phase II clinical trials for Peripheral Arterial Disease with Diabetic Foot Ulcer (NCT01859117) and (NCT02264288 (intramuscular injection)

PDA-002 Mechanisms of Action for DFU/PAD

Tissue Homeostasis
- Endothelial cell survival
- Epithelial cell proliferation/migration
- Myofibroblast differentiation

Vascular Growth Factors:
- *PDGF*
- *FGF*
- *VEGF, HGF*



Antioxidants & Others: Regulates
- *CAT*
- *SOD*
- *HMOX*
- *MMP's*
- *IDO*
- *PGE2*

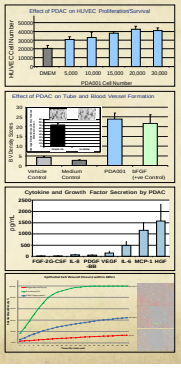
Cytokine Modulation
- *TNFa*
- *IL17*
- *IL23*
- *IL10*
- *IL6*
- *MCP-1*

Immune Cell Modulation
- *T-cells*
- *T-reg*
- *B-cells*
- *DC*
- *Monocytes*
- *Macrophages*

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

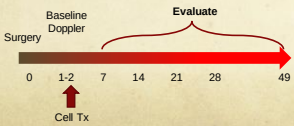
Characterization of PDA-002 in Vitro

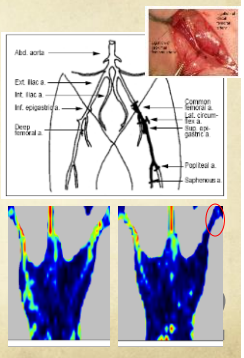
- PDA-002 induces proliferation/survival and migration of vascular endothelial cells- **quantity of tube formation**
- PDA-002 induces in vitro tube formation of endothelial cells and elevated blood vessel density in an ex vivo Chorioallantoic Membrane (CAM) assay-**quality**
- PDA-002 modulates epithelial 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



Animal Models of Limb Ischemia

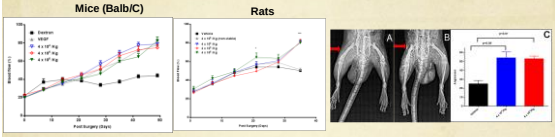
- Surgical resection of femoral artery to induce hind limb ischemia
- Mouse (BALB/c) and Rat
- Treatment with cells 1-2 days after surgery IM at 2 sites (above & below lesion)
- Endpoints evaluated up to 35-49 days after surgery
  - Blood flow (Laser Doppler)
  - Blood volume or angiography
  - Ischemic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





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

Mice (Balb/C) Rats



- A long term healing effect was observed with IM injection of PDA-002 (multiple models in rat, mice)
- Enhanced blood flow
- Increased blood volume
- Doses as low as 1000 cells were effective

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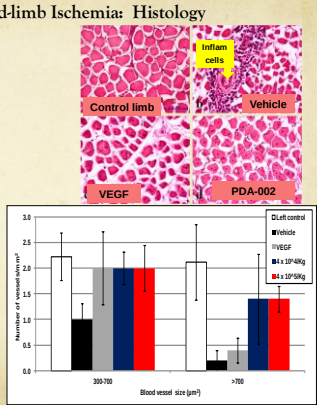
PDA-002 in Hind-limb Ischemia: Histology

- PDA-002 induces repair of damaged muscle in HLI model:
  - ➢ Reduction in inflammatory infiltrates
  - ➢ Myofibers containing multiple centrally located nuclei = new muscle tissue
- PDA-002 treatment results in increased number of larger blood vessels compared with Vehicle or VEGF



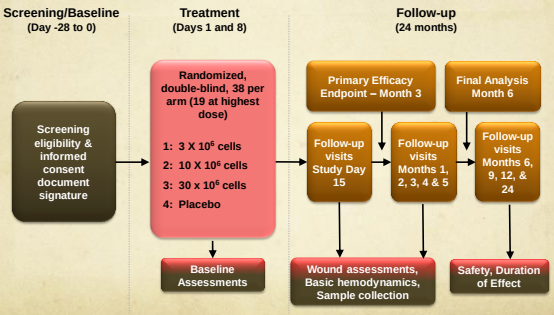
PDA-002 DFU-001: n24 Phase I

- Design: Phase 1 Multicenter, Open-Label, Dose-Escalation Safety and Efficacy of IM Injection of Human Placenta-Derived Cells (PDA-002) :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
  - 4 dose levels administered on Day 1 and 8
    - Dose-Level 1: 3 x 10^6 cells Dose-Level 2: 10 x 10^6 cells
    - Dose-Level 3: 30 x 10^6 cells Dose-Level 4: 100 x 10^6 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.
  - 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
  - Dose-Level 1: 3 x 10^6 cells (~38 subjects)
  - Dose-Level 2: 10 x 10^6 cells (~38 subjects)
  - Dose-Level 3: 30 x 10^6 cells (~19 subjects)
  - Placebo (~38 subjects)
- Sample size: N~133 Tcpo2 20-40 ABI .4-.8

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



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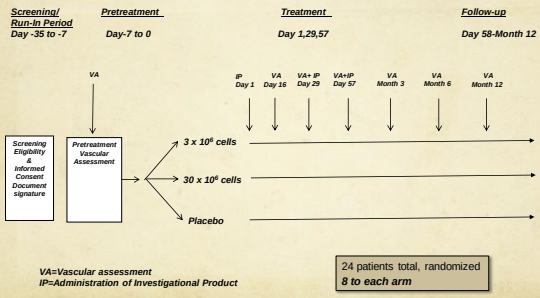
Proof of Mechanism Vascular Study in DFU: PDA-002 DFU-003

- Objective: Assess the safety, efficacy, and effect on vascular parameters caused by PDA-002 administered intramuscularly (IM) in subjects who have DFU with PAD.
  - Evaluate if increased peripheral vascularity can be detected in subjects who have DFU with PAD treated with PDA-002
- Design: A Phase 2 Double Blinded, 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

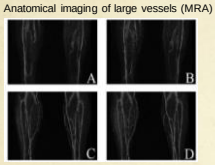
PI- Emile Mohler MD- UPenn

PDA-002 DFU-003 Proof of Mechanism

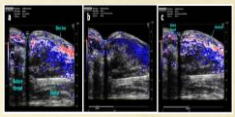


Techniques to Assess Tissue Oxygenation and Perfusion in PDA-002 DFU-003

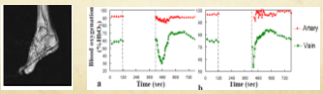
- Anatomical imaging of large vessels (MRA)



- Imaging of tissue perfusion and oxygenation (TcPO2, NIRS, Doppler US)



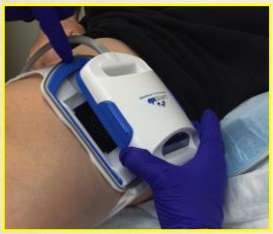
- Functional metabolic imaging and vascular measurements (FDG-PET CT, MR Oximetry)

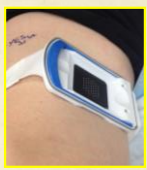


PDA-002 Summary

- 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

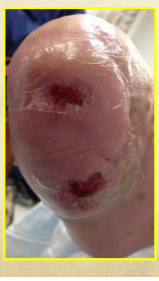




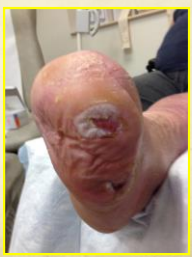
Tegaderm with Autogenous Graft

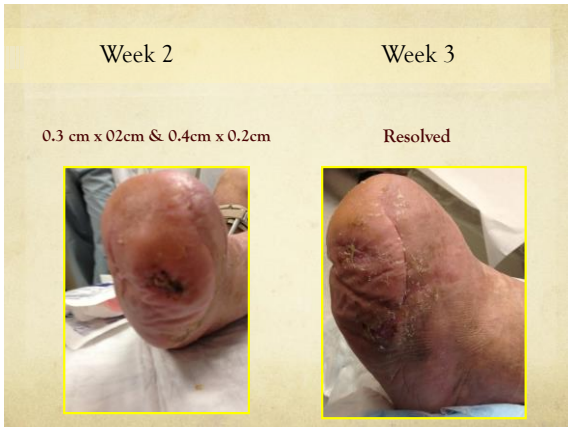
Week 1

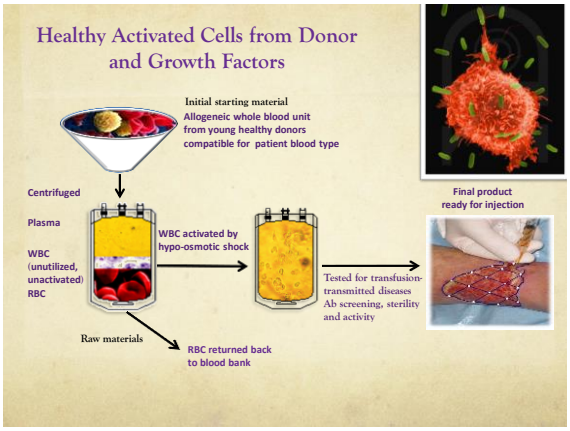
TMA day 1



1.4cm x 0.6 cm x 0.1cm







Wound Repair 2050

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

Thank you

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