Cell and Gene Therapies for Non-Reconstructable Critical Limb Ischemia
Critical Limb Ischemia

- Defined as chronic limb ischemia with either rest pain or tissue loss (non-healing ulcers or gangrene)

- Prognosis
  - 25% major limb amputation within 1 year
  - 25% die of cardiovascular complications within 1 year
  - 50% mortality at 5 years

- Treatment
  - Medical
    - ASA, statin
    - Wound care
  - Intervention
    - Amputation
    - Revascularization
      - Surgical bypass
      - Endovascular intervention
Non-reconstructable Critical Limb Ischemia

- No option for intervention
  - No suitable target vessel for bypass
  - Small vessel disease in the foot
  - Extensive co-morbidities

- Dismal prognosis
  - Almost 40% amputation rate at 6 months

- Quality of Life comparable to patients with advanced cancer

- Treatment Options
  - Intensive wound care (NPWT, debridement, abx) at a dedicated wound center
    - Some reports with up to 55% healing rates
    - Slow, laborious, unpredictable outcomes
  - Pharmacotherapy (antiplt, vasodilators, hyperbaric O2) of unproven benefit
  - Primary amputation
    - Non-reconstructable disease accounts for ~ 60% of secondary amputations
      - Failed revasc 2/2 disease progression, recurrent ischemia, persistent infection/necrosis despite patent revascularization
Primary Amputation as a Viable Option in a Subset of Patients with Non-reconstructable Critical Limb Ischemia

- Maintenance of ambulation has been shown to be an important factor in preserving independence and quality of life

- Amputation and prosthetic rehabilitation may be an excellent option to achieve independence and preserve quality of life
  - Good-risk patients after BKA (SM Taylor et al, JVS 2005)
    - Maintenance of ambulation may approach 70%
    - Maintenance of independence may approach 90%
  - Use of iPop may lead to
    - Faster return to ambulation (EM Burgess et al 1969)

- Palliative AKA appropriate for patients too ill to realize the benefit of revascularization
  - Nonambulatory, elderly, knee contractures
  - Preop functional status is most important predictor of postop outcome (SM Taylor et al JVS 2006)
Novel Therapeutic Approaches for Non-reconstructable CLI

- **Regenerative Therapies**
  Enhance intrinsic cellular/tissue physiologic mechanisms to provide increased blood flow to ischemic limbs
  - Targeting growth factors and gene products involved in angiogenesis and arteriogenesis
  - Stem and progenitor cells participating in vascular repair and proliferation

- **Advanced delivery methods**
  - Gene therapy
  - Molecular therapy
  - Cellular delivery
- All 3 mechanisms come into play in PAD

- Significant heterogeneity between patients in vascular response to chronic ischemia
  - Same degree of occlusive disease may yield very different functional impairments in different patients
  - Same degree of occlusive disease can yield variable amounts of collaterals
Therapeutic Angiogenesis for Critical Limb Ischemia

- Concept:
  Use of angiogenic growth factors or stem cells in ischemic limbs to
  - Grow blood vessels
  - Improve blood flow
  - Increase tissue perfusion

- VEGF, FGF, HGF studied in animal models
  - Collateral vessel formation
  - Increased blood flow
  - Increased capillary density

- VEGF, FGF, HGF have been studied in the setting of RCT, with mixed results
  - All have confirmed feasibility and safety
  - No “off-target” angiogenesis
  - No occult tumor growth
  - No progression of diabetic retinopathy
Effect of fibroblast growth factor NV1FGF on amputation and death: a randomised placebo-controlled trial of gene therapy in critical limb ischaemia

Jill Belch, William R Hiatt, Iris Baumgartner, I Vickie Driver, Sigrid Nikol, Lars Norgren, Eric Van Belle, on behalf of the TAMARIS Committees and Investigators

Lancet 2011; 377: 1929-37

- Phase III trial
- 525 patients with non-reconstructable disease, 30 countries
- Randomized to treatment vs. placebo
- 8 IM injections on day 1, 15, 29, 43

Endpoints
  - Primary:
    - Major amputation or death within 1 yr

Results:
  - No difference in 12-month amputation-free survival when compared to placebo (63% vs. 67%, P=0.48)
  - Major amputation/death in 20-25% of patients
Multicenter, randomized, double blind, placebo controlled
- 44 pts with non-reconstructable CLI
- Evaluate for efficacy and safety
- End points:
  - Primary
    - Reduction of ulcer size
    - Decrease in rest pain
  - Secondary
    - QoL
- Decrease in ischemic ulcer size
- Other studies with similar results
- Large phase III trial started but terminated in 2016 due to low enrollment
<table>
<thead>
<tr>
<th>Study design</th>
<th>FGF type 1 (NV1FGF) [14]</th>
<th>FGF plasmid, NV1FGF TAMARIS trial, Phase III [16]</th>
<th>HGF plasmid, AMG0001, AnGes trial [18]</th>
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</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>51</td>
<td>525</td>
<td>Drug, 156; placebo, 50</td>
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<tr>
<td>Study design</td>
<td>Phase I, randomized, placebo-controlled</td>
<td>Phase III, randomized, placebo-controlled</td>
<td>Phase II and III, randomized, placebo-controlled</td>
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<td>Rutherford class CLI severity</td>
<td>4 and 5</td>
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<td>Method of drug delivery</td>
<td>IM injection</td>
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<tr>
<td>No. of treatments</td>
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<td>2 or 3</td>
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<td>Study length (months)</td>
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<td>12</td>
<td>3–36</td>
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<tr>
<td>Study endpoints</td>
<td>Pain reduction; $P &lt; .001$</td>
<td>Amputation free-survival: drug, 65%; placebo, 67%; $P = .48$</td>
<td>Improved ulcer healing; $P &lt; .05$</td>
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<tr>
<td>Ulcer healing; $P &lt; .01$</td>
<td>Amputation: drug, 26%; placebo, 21%; $P = .31$</td>
<td>Increase in tissue TcPO$_2$; $P &lt; .01$</td>
<td></td>
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<tr>
<td>Increased TcPO$_2$; $P &lt; .01$</td>
<td>Death: drug, 18%; placebo, 15%; $P = .53$</td>
<td>Reduction in rest pain; $P &lt; .05$</td>
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<tr>
<td>Efficacy demonstrated</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
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<tr>
<td>Safety issues</td>
<td>Yes</td>
<td>None</td>
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</table>

CLI = critical limb ischemia; FGF = fibroblast growth factor; HGF = hepatocyte growth factor; IM = intramuscular.
Stem Cell Therapies
Mesenchymal Stem Cells (MSCs)

- Multipotent non-hematopoietic stem cells
  - Found in myriad tissues
    - 1st isolated from bone marrow
    - Optimal source for therapeutic use yet TBD
  - Capacity for self-renewal
  - Differentiation into many different cell types

- Home to and survive in ischemic environments
  - Transdifferentiation -> become vascular cell types
  - Paracrine effects -> stimulate angiogenesis/arteriogenesis via growth factor release

- Currently most actively studied at preclinical and clinical levels
  - Ease of isolation
  - Capacity for ex vivo expansion

Yan, J et al, Stem Cell Rev and Rep, 2013
Once in ischemic tissue, stem cells have capacity to perform all functions required during angio/arteriogenesis.
Mesenchymal Stem Cells

- Preclinical studies showed promising results in animal models
  - MSCs transplanted into ischemic areas express endothelial markers and promote angiogenesis, arteriogenesis
    - Leading to significantly increased limb blood flow recovery

- Intramuscularly injected MSCs localize to ischemic hind limb
  - No significant migration to other tissues

- Multiple active clinical trials to study the effect of stem cell therapy in CLI
Interim analysis results from the RESTORE-CLI, a randomized, double-blind multicenter phase II trial comparing expanded autologous bone marrow-derived tissue repair cells and placebo in patients with critical limb ischemia

Richard J. Powell, MD, Anthony J. Comerota, MD, Scott A. Berceli, MD, Raul Guzman, MD, Timothy D. Henry, MD, Edith Tzeng, MD, Omaida Velazquez, MD, William A. Marston, MD, Roudna L. Bartel, PhD, Amy Longcore, MS, Theresa Stern, PhD, and Sharon Watling, PhD, Lebanon, NH; Toledo, Ohio; Gainesville, Fla; Nashville, Tenn; Minneapolis, Minn; Pittsburgh, Pa; Miami, Fla; Chapel Hill, NC; and Ann Arbor, Mich

- Prospective, randomized, double-blinded, placebo controlled multicenter trial (18 US centers)
- 86 unreconstructable CLI patients
- BM aspirate expanded ex vivo, then injected into 20 sites in ischemic LE

Endpoints
- Primary:
  - Safety
- Secondary
  - Major amputation-free survival
  - Time to 1st occurrence of treatment failure (amp, death, new gangrene, doubling of wound size)
  - Major amputation rate
  - Wound healing

Results
- No difference in adverse events
- Increased time to treatment failure and amputation-free survival in treated group
- Decreased major amp rate (19% vs 43%)
- Improved wound healing
REVIVE-CLI

- Phase III RCT based on findings of RESTORE-CLI
- Started in 2013
- Enrolled 40 pts with non-reconstructable disease (594 planned)
- Halted due to slow enrollment and company shift of focus to dilated cardiomyopathy

FDA approved phase II pilot study

- BMAC allows for immediate IM injection of stem cells prepared in the OR
- 48 pts randomized 2:1 BMAC:placebo
- 3 months f/u in pts with CLI (tissue loss)
  - Major amputation in 39% treated vs. 71% in placebo patients
  - Duration to amputation was increased
- Phase III trial now under way
MOBILE
- Phase III trial, currently in progress
- Double blind RCT
- BMAC vs placebo
- 152 participants

Outcomes assessed
- Time to major amputation or death
- Several secondary outcome measures incl perfusion and QoL
- 5 year amputation free survival

Estimated study completion May 2020
- Treatment and 1yr f/u has been completed
- Long-term follow up is ongoing
- No initial data analysis published to date
Conclusions

- There is no FDA-approved biological therapy for CLI
- Biologic therapies have shown promise in the treatment of patients with CLI several studies
- Work by promoting tissue angiogenesis in the skeletal musculature
  - tissue regeneration and promotion of distal wound repair
- Recent clinical trials have shown that these biologic therapies are safe
- On-going phase III trials are focusing on stem cell therapy (BMAC)
  - Powered to determine if amp-free survival can be increased
  - Help elucidate frequency of therapy, dose optimization
- Currently no large phase III gene therapy trials underway
  - Phase I/II trials involving genetically engineered stem cells overexpressing vascular growth factors – recruiting
- The effect of concomitant comorbidities, such as DM, on these treatment modalities remains to be elucidated
- Future applications may include biologic therapies in CLI patients before or as an adjunct to endovascular and/or open repair
Thank you for your attention