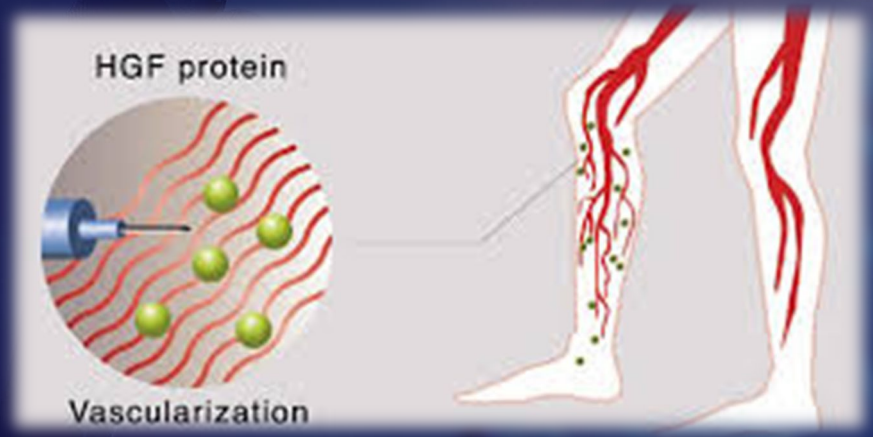
Peripheral Arterial Diseases
and Plasmid DNA Therapy:

# From Current Standards of Care to Emerging Clinical Innovation




Supported by an educational grant from AnGes

# Faculty

- David G. Armstrong, DPM, MD, PhD
  Distinguished Professor of Surgery and Neurological Surgery; Director, USC Limb Preservation Program; Keck School of Medicine of University of Southern California (Los Angeles, CA)
- John Lantis, MD
  Site-Chief of Surgery; Mount Sinai Hospital; Professor of Surgery, Icahn School of Medicine at Mount Sinai (New York, NY)
- Dennis Orgill, MD, PhD
  Division of Plastic Surgery; Medical Director, Wound Care Center; Brigham and Women's Hospital; Professor of Surgery, Harvard Medical School (Boston, MA)
- Richard Powell, MD
  Director of the Dartmouth Health Heart and Vascular Center; Professor of Surgery and Radiology at Geisel School of Medicine at Dartmouth; Board of Trustees; Dartmouth Health (Lebanon, NH)
- Charles Zelen, DPM
  Medical Director, President; Professional Education and Research Institute; Foot and Ankle Section Chief; Department of Orthopedic HCA Lewis Gale Hospital; ACFAS Recognized Fellowship Director (Roanoke, VA)

# Faculty Disclosures

- David G. Armstrong, DPM, MD, PhD
  Principal Investigator: AnGes 0211 Pivotal Trial
- John Lantis, MD
  Advisory Board and Author: AnGes 0211 Pivotal Trial
- Dennis Orgill, MD, PhD
  Senior Wound Validator and Author: AnGes 0211 Pivotal Trial
- Richard Powell, MD
  Principal Investigator and Senior Author: AnGes Clinical Program
- Charles Zelen, DPM
  Medical Director and President; PERI; CRO for AnGes 0211 Pivotal Trial

# Disclosures

The faculty have been informed of their responsibility to disclose to the audience if they will be discussing off-label or investigational use(s) of drugs, products, and/or devices (any use not approved by the US Food and Drug Administration)

- Applicable CME staff have no relationships to disclose relating to the subject matter of this activity
- This activity has been independently reviewed for balance
- All AI-assisted content has been reviewed, edited, and validated by the presenter for accuracy, balance, and alignment with current evidence. Author(s) assume full responsibility for the content presented
- This CME activity may include brand names for participant clarity purposes only. No product promotion or recommendation should be inferred.

# Learning Objectives

- Review the current standard of care and treatment approaches for patients with arterial ulcers associated with peripheral arterial disease (PAD)
- Examine the scientific rationale and clinical development of plasmid DNA therapy, including key findings from two decades of clinical investigation
- Analyze the design, methodology, and clinical outcomes of the LEGEND-1 registrational trial evaluating investigational gene therapy in patients with moderate PAD and ulcers
- Evaluate wound validation methodologies and review clinical case data from the pivotal trial to better understand patient selection, wound assessment, and treatment outcomes

PAD = peripheral arterial disease.

# Introduction



Charles Zelen, DPM

Medical Director and President; Professional Education and Research Institute
Foot and Ankle Section Chief; Department of Orthopedic HCA Lewis Gale Hospital
ACFAS Recognized Fellowship Director
Roanoke, VA

# PAD and CLTI: A Major Unmet Need

A growing PAD population and constant amputation risk represents a significant unmet need

**31.1M**

**Projected North America Cases by 2050**

Prevalence is scaling rapidly, with global projections climbing to between 113-363M patients

**10-12M**

**Americans Affected by PAD**

Roughly 200 million people are impacted worldwide by PAD

**10%-20%**

**Progress to Severe CLTI**

A significant portion of PAD patients progress to chronic limb-threatening ischemia (CLTI) associated with clinical amputation

**≈1.6M**

**Diabetic Foot Ulcers (DFUs) Annually**

Occur annually in the U.S.; approximately half of DFU patients also suffer from PAD

**1.3%**

**U.S. Adult Prevalence**

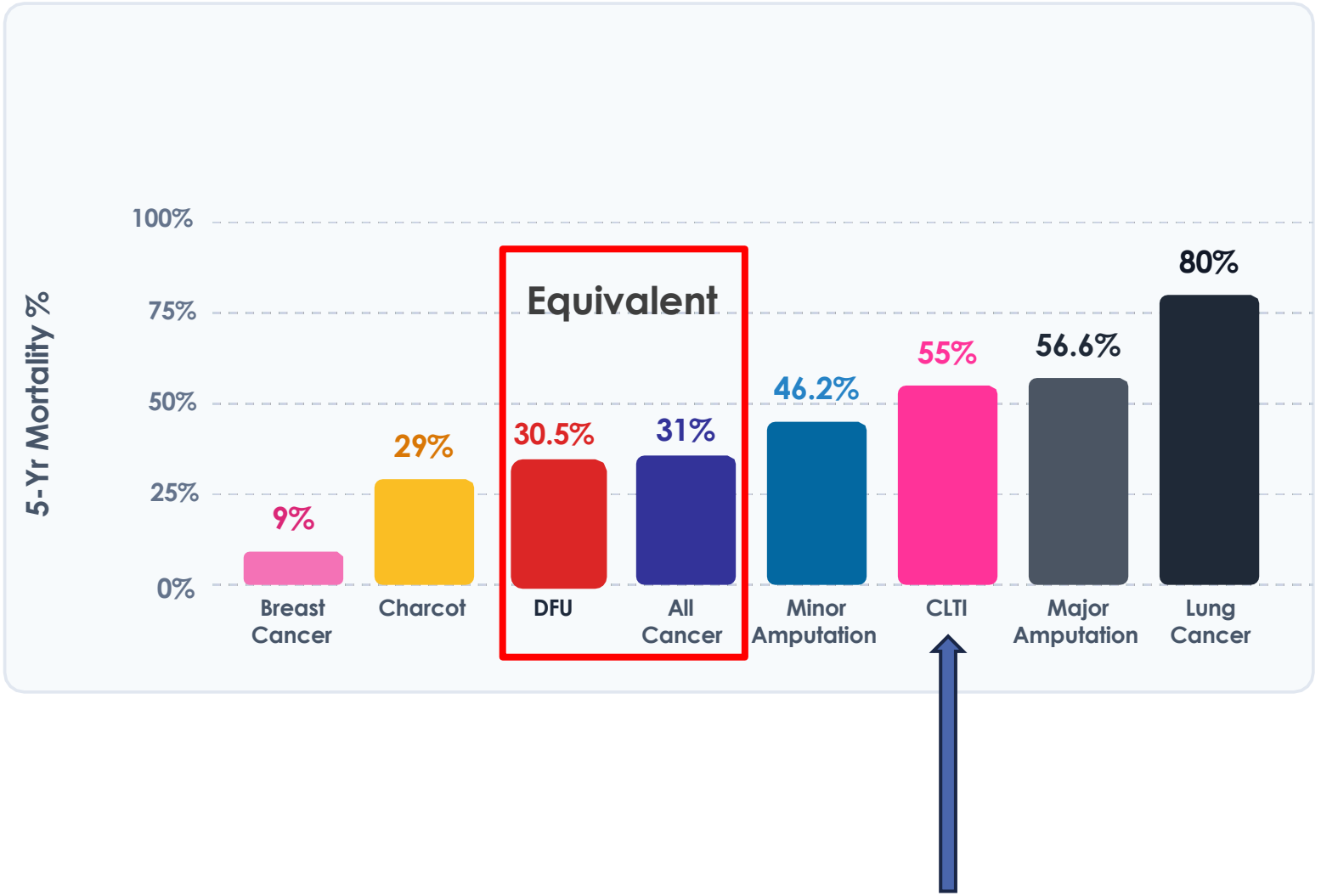
Severe PAD with CLTI affects a massive segment, placing actual prevalence between 2-3.4 million patients in the U.S. alone



DFU = diabetic foot ulcer; CLTI = chronic limb-threatening ischemia.

Allison MA, et al. *Circulation*. 2023;148:286-296. Writing Committee Members; Gornik HL, et al. *J Am Coll Cardiol.* 2024;83(24):2497-2604. Deng L, et al. *Research*. 2025;8:Article 0702. Armstrong DG, et al. *NEJM.* 2017;376:2367-2375.

# 5-Year Mortality Burden Matches Many Cancers




## Why This Matters

### Survival Imperative

- **Early, effective treatment** of PAD/CLTI is critical for both life and limb
- DFU 5-yr mortality: **30.5%**
- All-cancer 5-yr mortality: **31%**
- **Mortality following CLTI or major amputation is 2nd/3rd only to the mortality rate of lung cancer(80%)**

Armstrong DG, et al. *J Foot Ankle Res.* 2020;13(1):16.

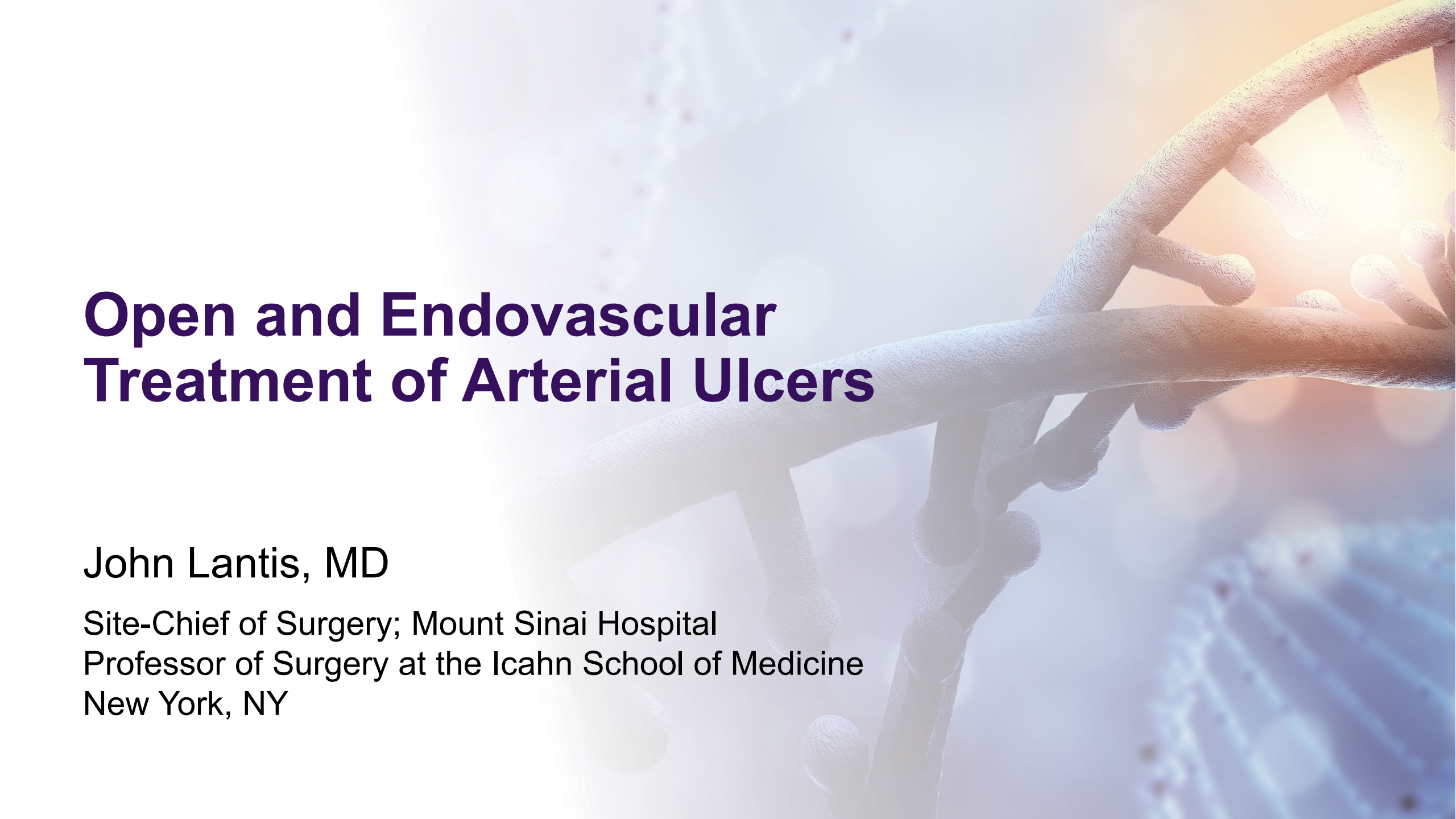
# Beperminogene Perplasmid



| | |
|---|---|
| **1984** | Human hepatocyte growth factor (HGF) is initially discovered in Japan as a potent regenerative growth factor in the liver |
| **1995** | Osaka University discovered that HGF has the capacity to generate new vessels and began the development of an HGF gene therapy product |
| **1999**<br>**2019** | AnGes was founded with the aim of developing drugs based on the action of the HGF gene to regenerate blood vessels. 20 yrs of pre-clinical and clinical studies conducted |
| **2019** | Conditional approval of hepatocyte growth factor (HGF) in Japan |
| **2024** | Completion of USA – Registrational Pivotal Trial AnGes 0211<br>FDA (CBER) grant breakthrough designation |
| **2026** | Clinical and CMC Agreement with FDA<br>FDA grants approval for Rolling – BLA submission for beperminogene perplasmid |

HGF = hepatocyte growth factor.

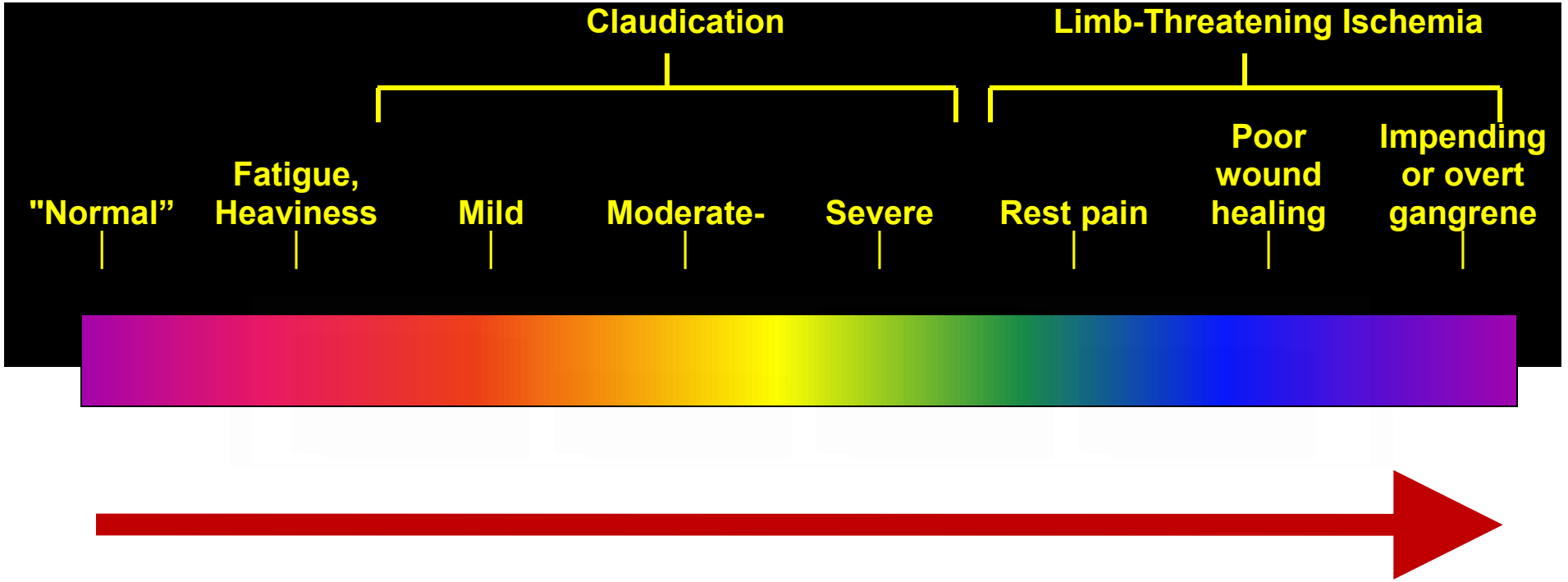
# Open and Endovascular Treatment of Arterial Ulcers



John Lantis, MD

Site-Chief of Surgery; Mount Sinai Hospital
Professor of Surgery at the Icahn School of Medicine
New York, NY

# Spectrum of Peripheral Artery Disease (PAD)



# Critical Limb Ischemia with Rest Pain

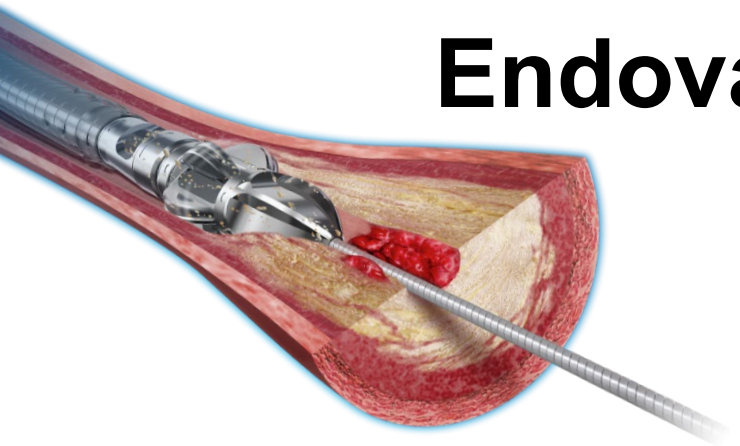
Dependent Rubor



“Classic CLTI Patient”

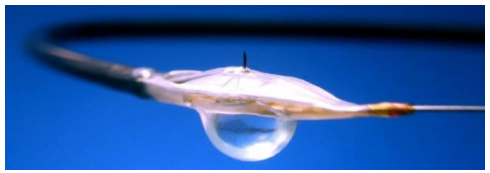


# Endovascular in the Treatment of CLI

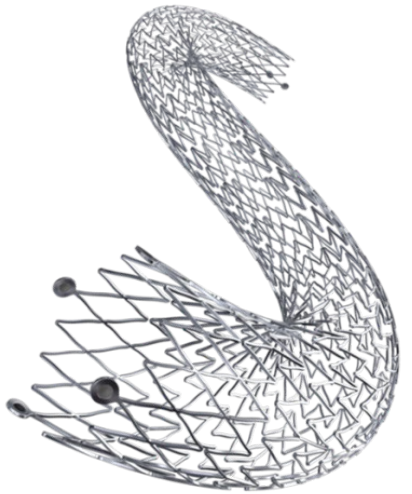


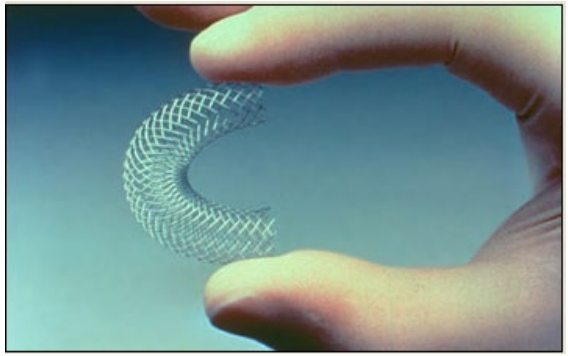


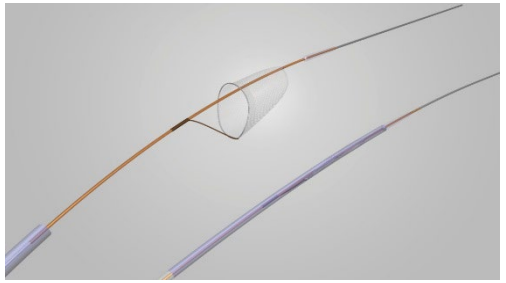




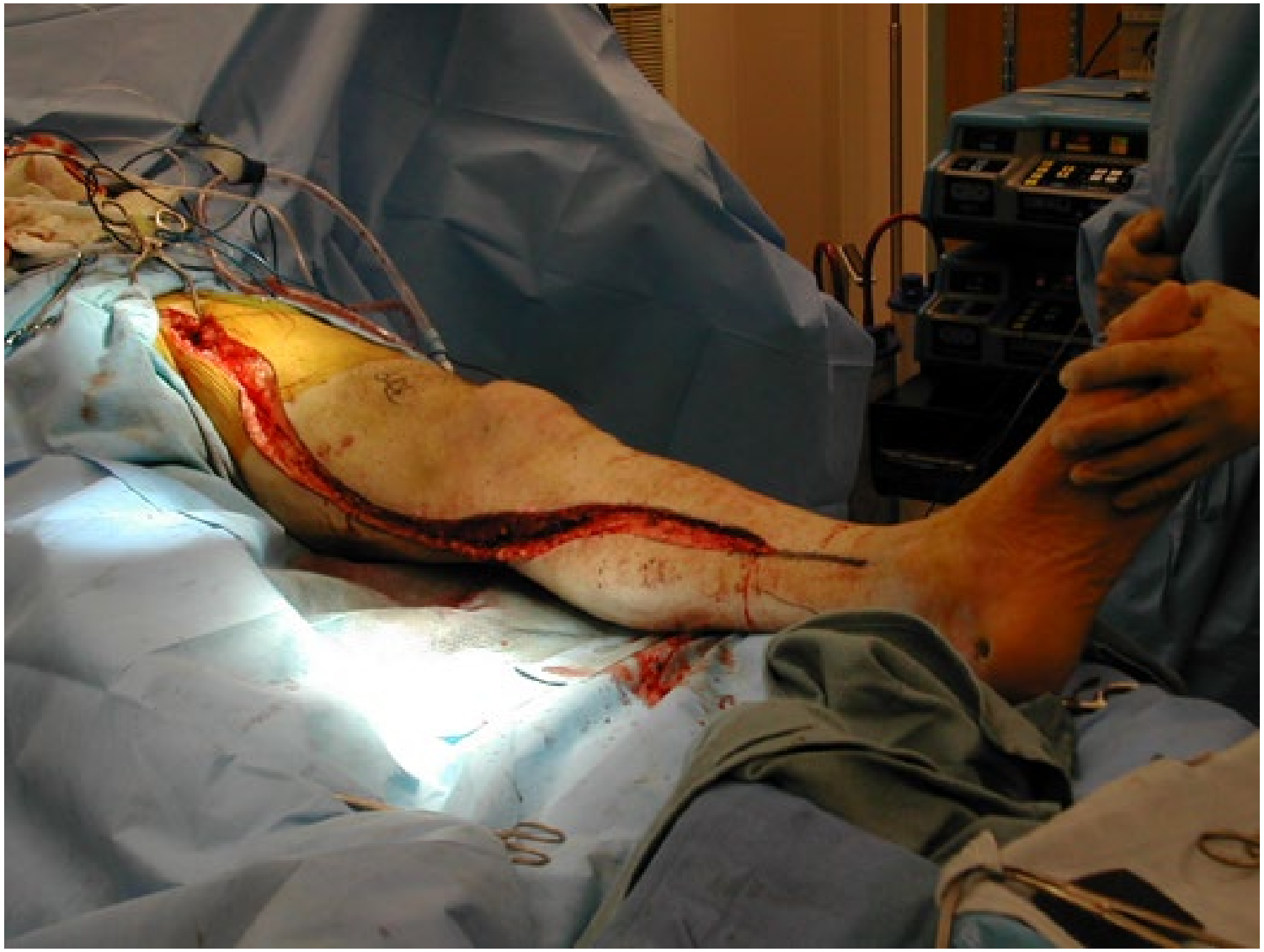








# Open Bypass in the Treatment of CLTI





# BEST-CLI

**B**est **E**ndovascular vs. Best **S**urgical **T**herapy in Patients with **C**ritical **L**imb **I**schemia

Sponsored by the National Heart, Lung, and Blood Institute (NHLBI)

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New England Research Institutes

The NEW ENGLAND JOURNAL of MEDICINE

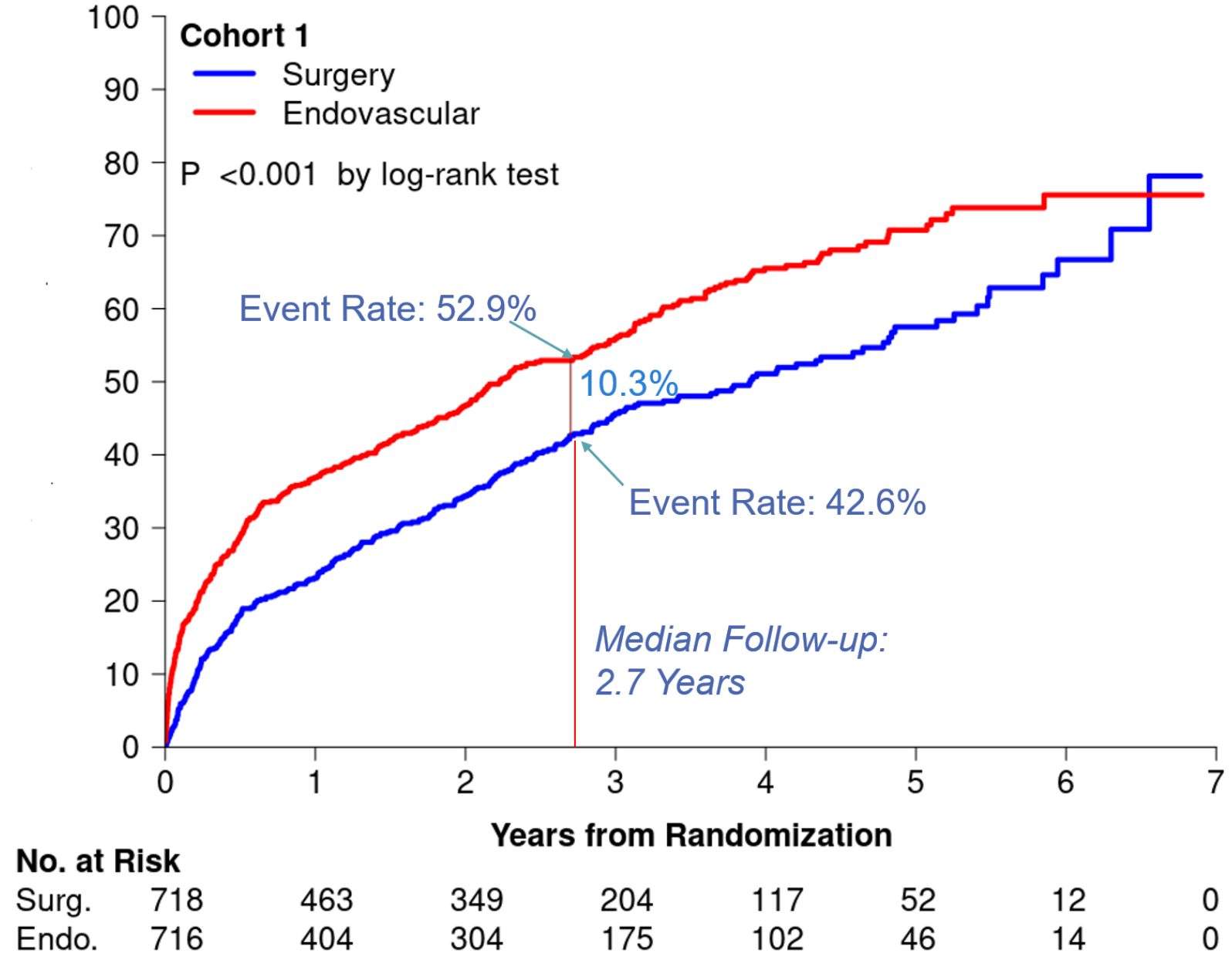
ESTABLISHED IN 1812 DECEMBER 22, 2022 VOL. 387 NO. 25

# Surgery or Endovascular Therapy for Chronic Limb-Threatening Ischemia

A. Farber, M.T. Menard, M.S. Conte, J.A. Kaufman, R.J. Powell, N.K. Choudhry, T.H. Hamza, S.F. Assmann,* M.A. Creager, M.J. Cziraky, M.D. Dake, M.R. Jaff, D. Reid, F.S. Siami, G. Sopko, C.J. White, M. van Over, M.B. Strong, M.F. Villarreal, M. McKean, E. Azene, A. Azarbal, A. Barleben, D.K. Chew, L.C. Clavijo, Y. Douville, L. Findeiss, N. Garg, W. Gasper, K.A. Giles, P.P. Goodney, B.M. Hawkins, C.R. Herman, J.A. Kalish, M.C. Koopmann, I.A. Laskowski, C. Mena-Hurtado, R. Motaganahalli, V.L. Rowe, A. Schanzer, P.A. Schneider, J.J. Siracuse, M. Venermo, and K. Rosenfield, for the BEST-CLI Investigators†

# Primary Endpoint

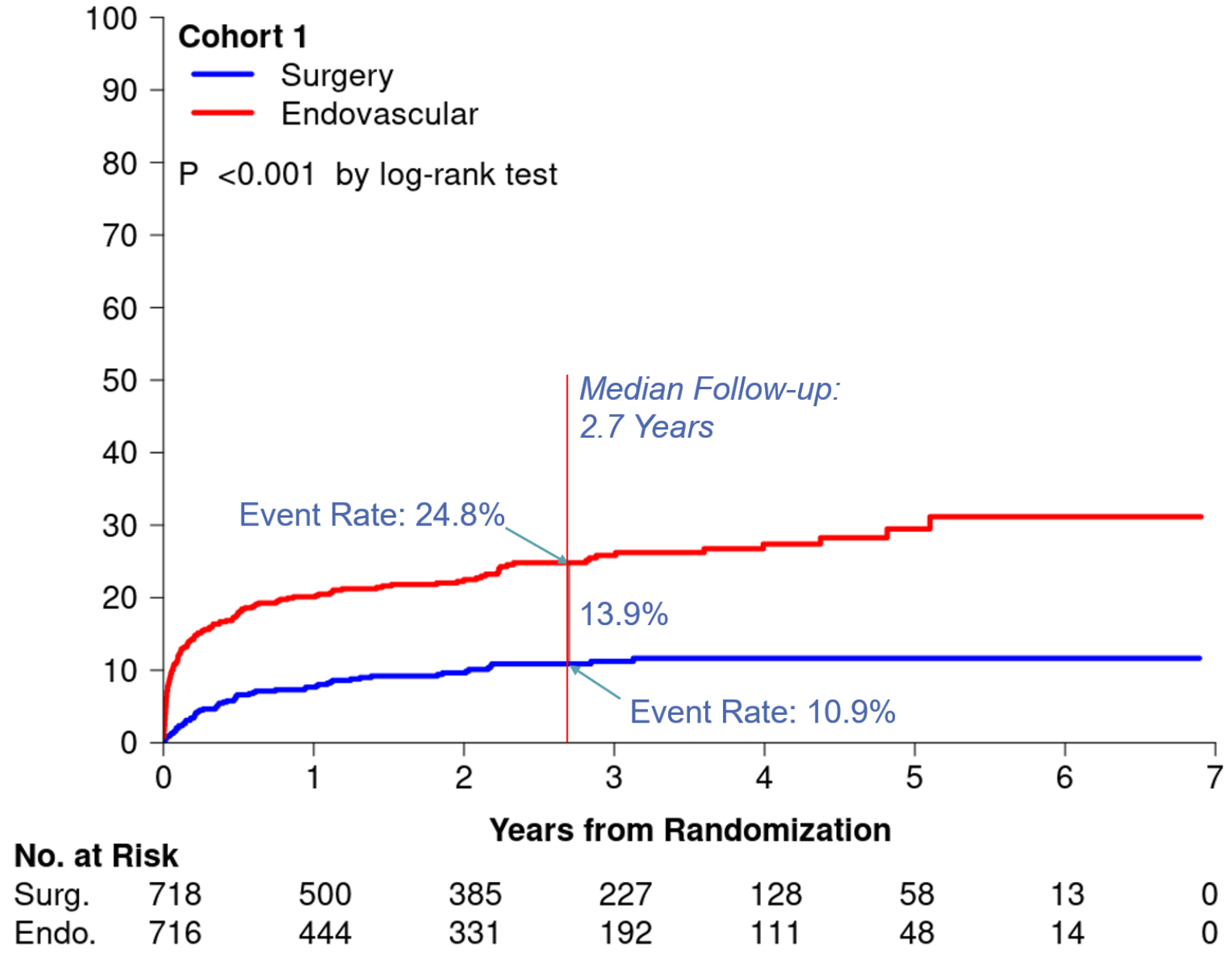
MALE (Major reintervention, or above-ankle amputation) or all-cause death (%)




BEST-CLI

# Primary Endpoint Component

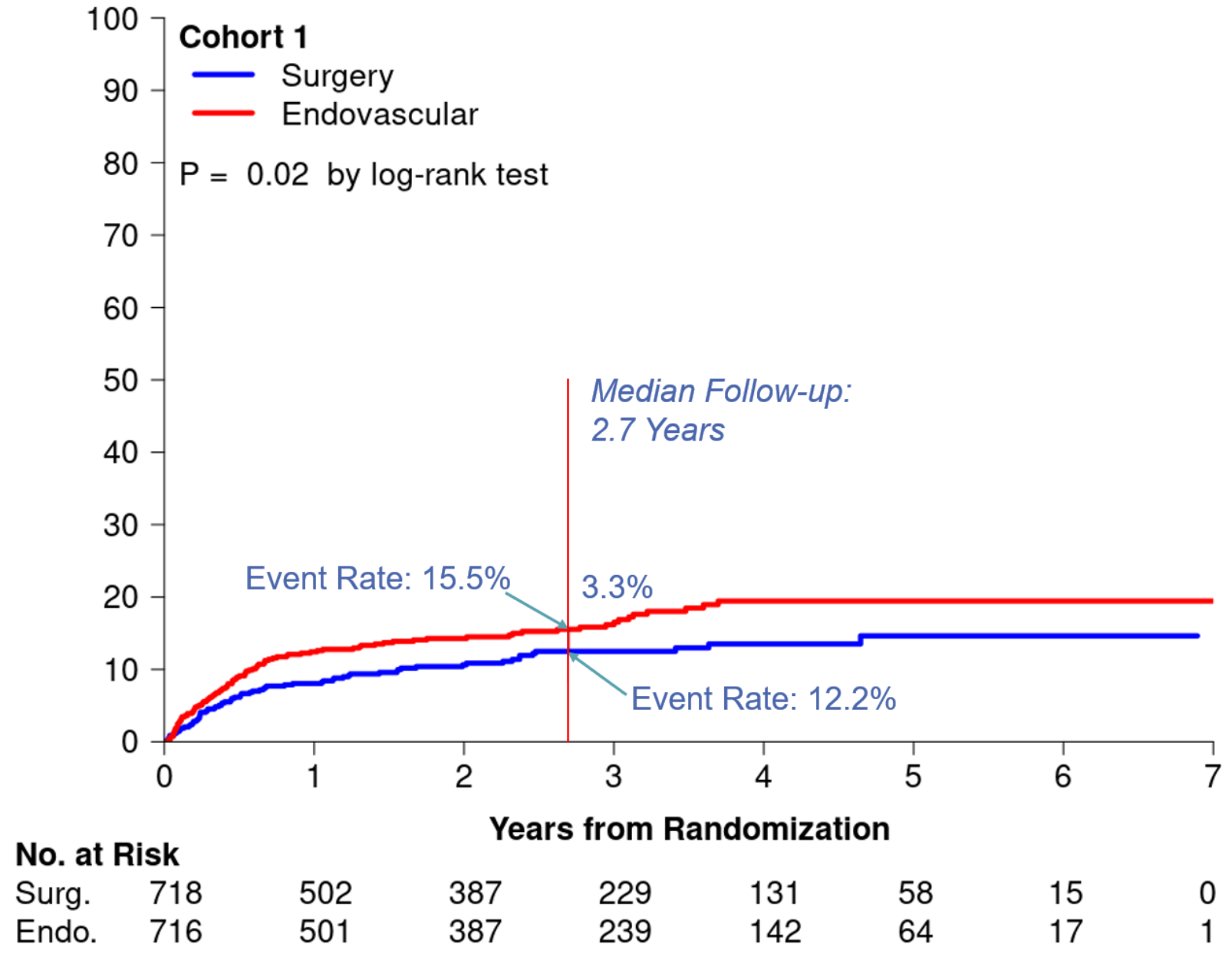
Major reintervention (%)




BEST-CLI

# Primary Endpoint Component

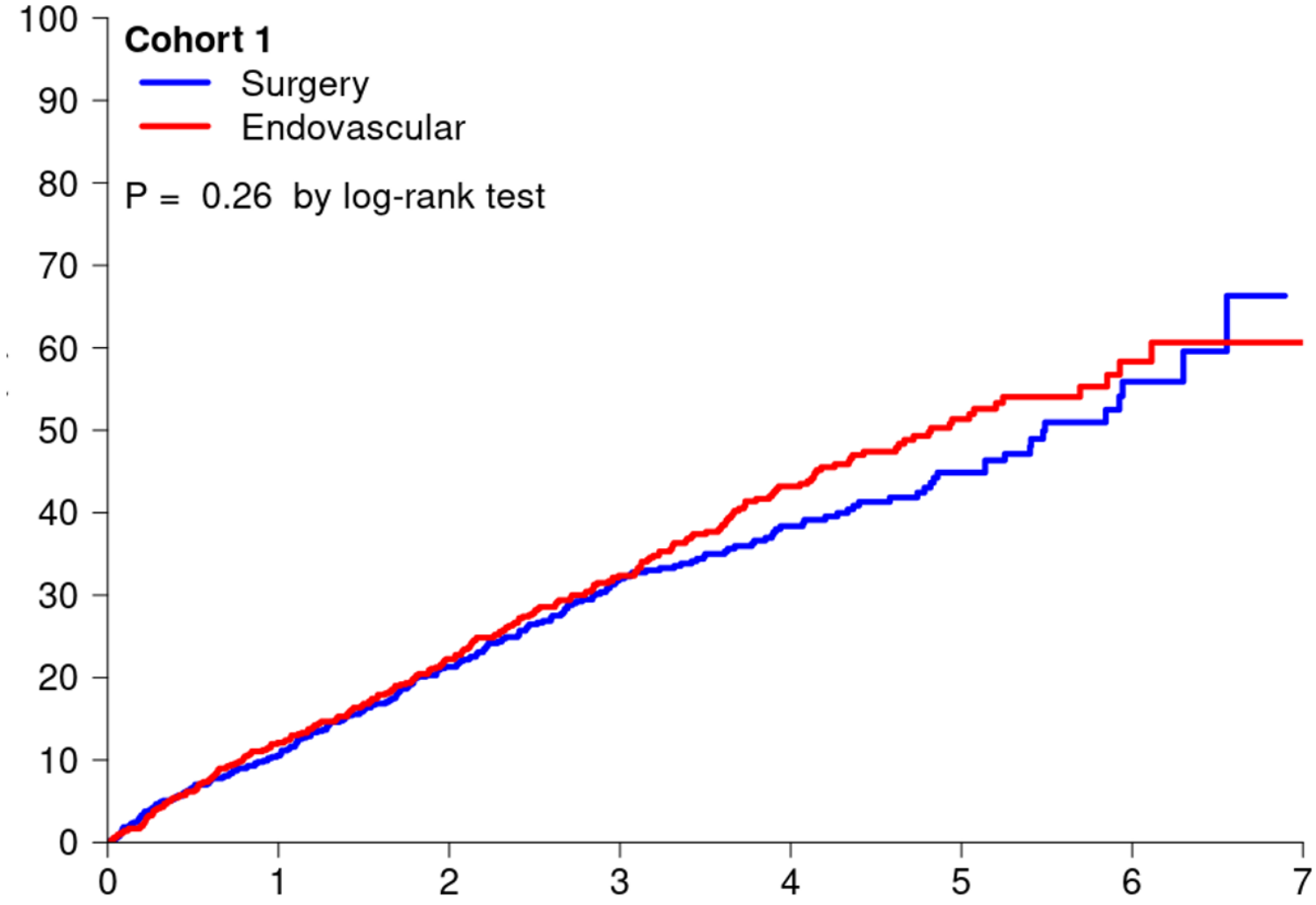
Above-ankle amputation (%)




BEST-CLI

# Primary Endpoint Component

Death (%)




**No. at Risk**

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Surg. | 718 | 577 | 457 | 282 | 168 | 80 | 20 | 0 |
| Endo. | 716 | 586 | 462 | 298 | 182 | 85 | 23 | 1 |

BEST-CLI

# PAD and CLTI: A Major Unmet Need

## Treatment Limitations

Despite advances in revascularization and medical therapy, CLTI remains associated with **severe outcomes** in patients with advanced or non-reconstructable disease

- High amputation rates
- High mortality
- Poor wound healing
- Treatment failure

**We Need More Options**

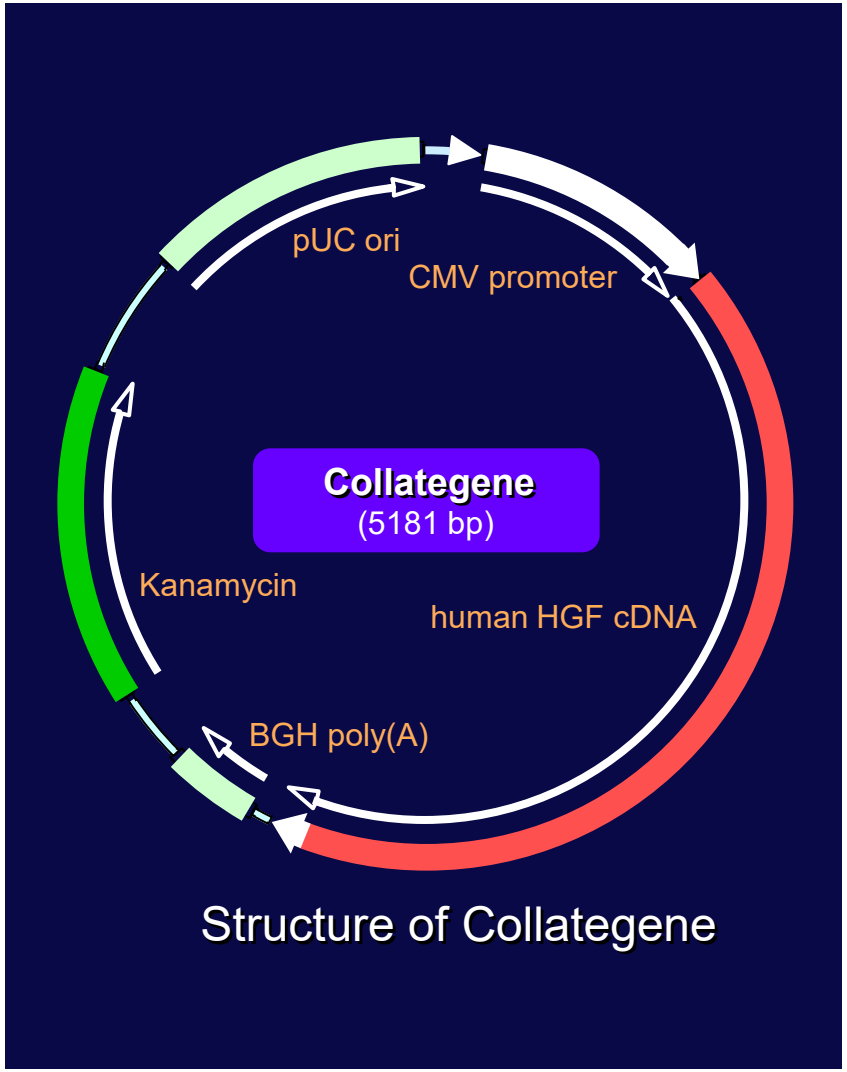


# Beperminogene Perplasmid
# HGF Plasmid
# Pre-Clinical and Early Phase Studies

Richard Powell, MD

Director of the Dartmouth Health Heart and Vascular Center
Professor of Surgery and Radiology at Geisel School of Medicine at Dartmouth
Board of Trustees; Dartmouth Health
Lebanon, NH

# Structure of HGF Plasmid (Collategene®)




**Plasmid:** A DNA molecule that is separate from the nuclear DNA (chromosome), mainly parasitic in bacteria. Although circular and double-stranded DNA molecules are stably transmitted to the descendants, it is capable of autonomous replication separately from chromosomal (host) DNA.

**CMV Promoter:** A promoter derived from cytomegalovirus that increases HGF protein production.

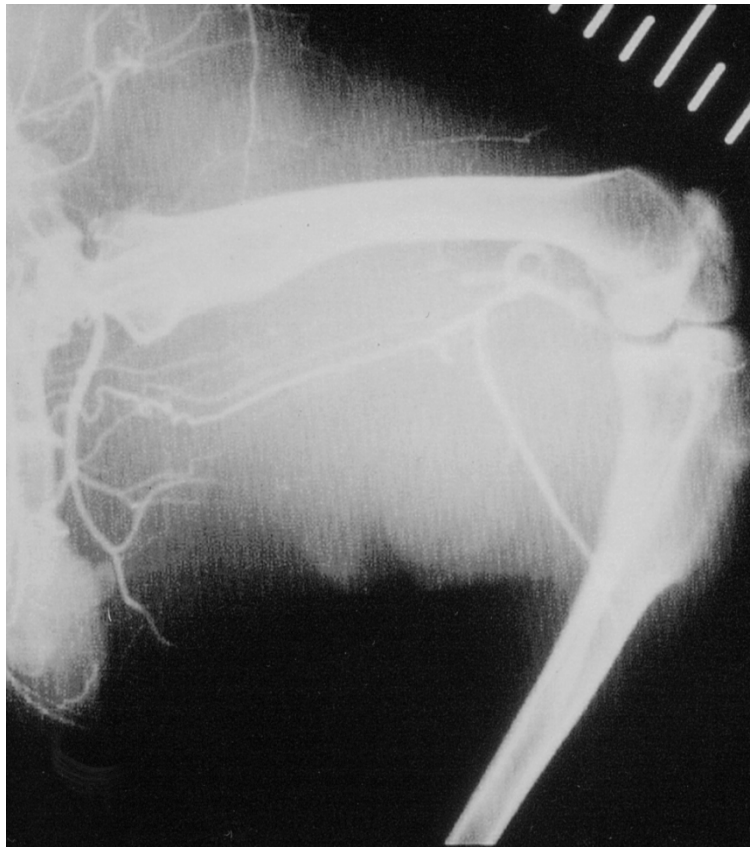
**Human HGF cDNA:** Human HGF (hepatocyte growth factor) gene. Located on chromosome 7q21.

**BGH poly(A):** Poly-A signal from the bovine growth hormone gene that improves the stability of HGF mRNA and HGF protein production.

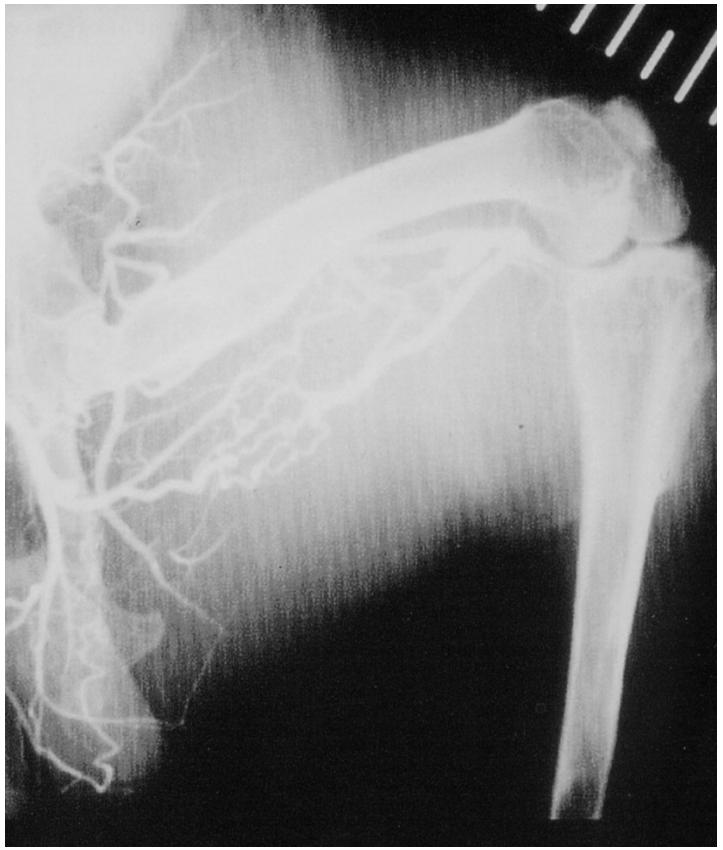
**Kanamycin-Resistance Gene:** Marker to specify the plasmid in host *E. coli*.

**pUC ori:** pUC derived region. Necessary for plasmid growth in *E.coli*.

# Neovascularization by HGF Plasmid Rabbit ASO Model – 1 intramuscular dose of 2mg



Control Plasmid pVAX1



HGF Plasmid pVAX1HGF/MGBI

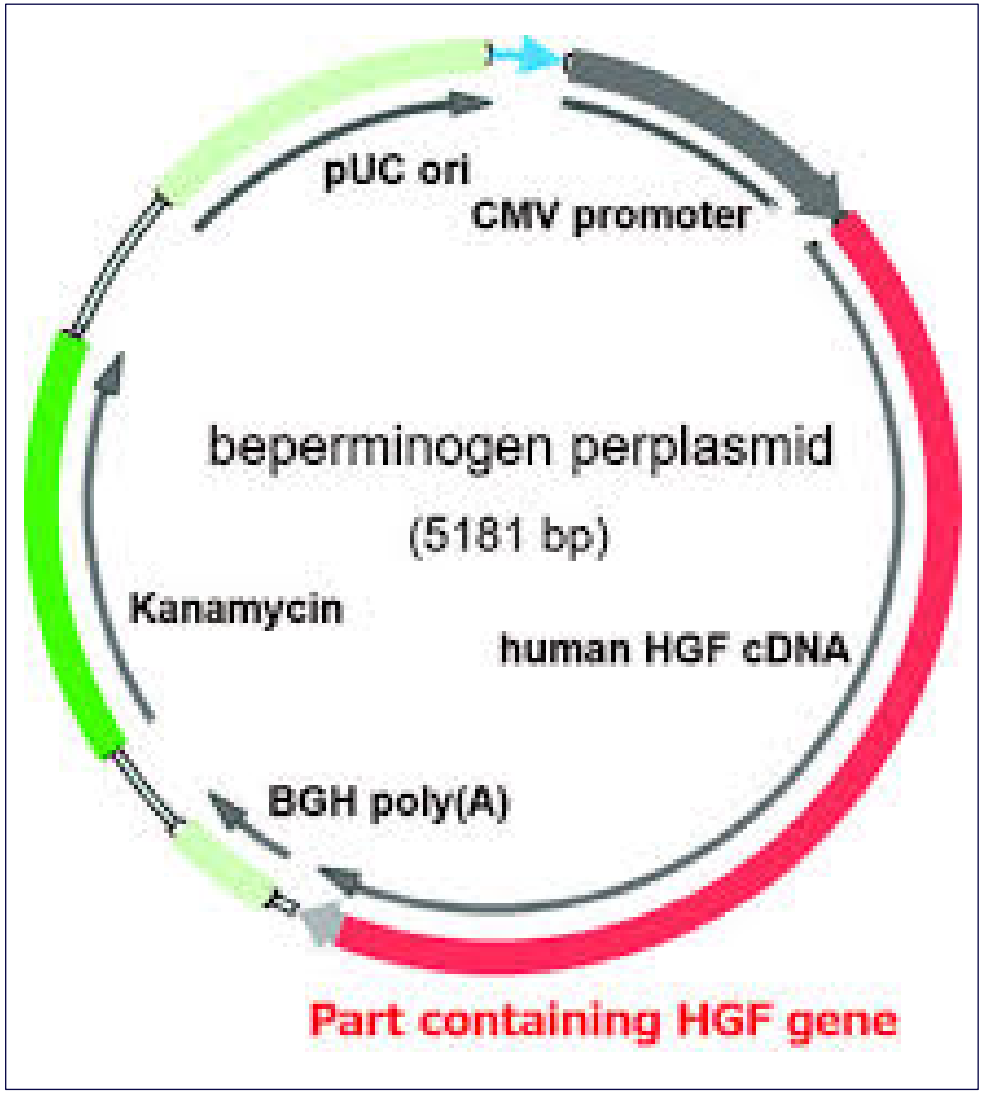
Taniyama Y, et al. *Gene Ther*. 2001;8(3):181-189.

# Preclinical Safety Data

- Biodistribution in rats by single- and multiple-dose administrations
- Different routes: Intramuscular and intravenous
- Single-dose toxicity studies in rats and monkeys
- Multiple-dose toxicology (4 monthly injections) in rats and monkeys
- 5 weekly repeated dosing toxicology in rats
  - Current trial dose 1/80$^{th}$ of animal preclinical
- Tumor promotion study in tumor-bearing mice

- No safety issues. No tumor promotion.

# Early Phase Studies with Plasmid HGF in Critical Limb Ischemia (CLI)



# Clinical Studies Undertaken in PAD

| Study | Disease | Study Design | Safety Follow-Up (Months) |
|---|---|---|---|
| AMG0001-JN-100 (Osaka University, Japan) | IC/CLI & TAO | Open label | 27 |
| AMG0001-JN-101 (Japan) | CLI | DB-placebo-controlled (DBPC) | 24 for HGF; 3 for Placebo |
| AMG0001-JN-102 (Japan) | TAO | Open label | 24 |
| AG-CLI-0202 (USA) | CLI | (DBPC) (dose-response) | 12 |
| AG-CLI-0205 (USA) | CLI | (DBPC) | 12 |
| AG-CLI-0209 (USA) | CLI | Open label | 6 |
| AG-CLI-0206 International | CLI | DBPC | 56 |

# JN-101: Double-Blind Placebo-Controlled Study in CLI

*Gene Therapy.* 2010;17:1152-1161

## *Randomized, double-blind, placebo-controlled clinical trial of hepatocyte growth factor plasmid for critical limb ischemia*

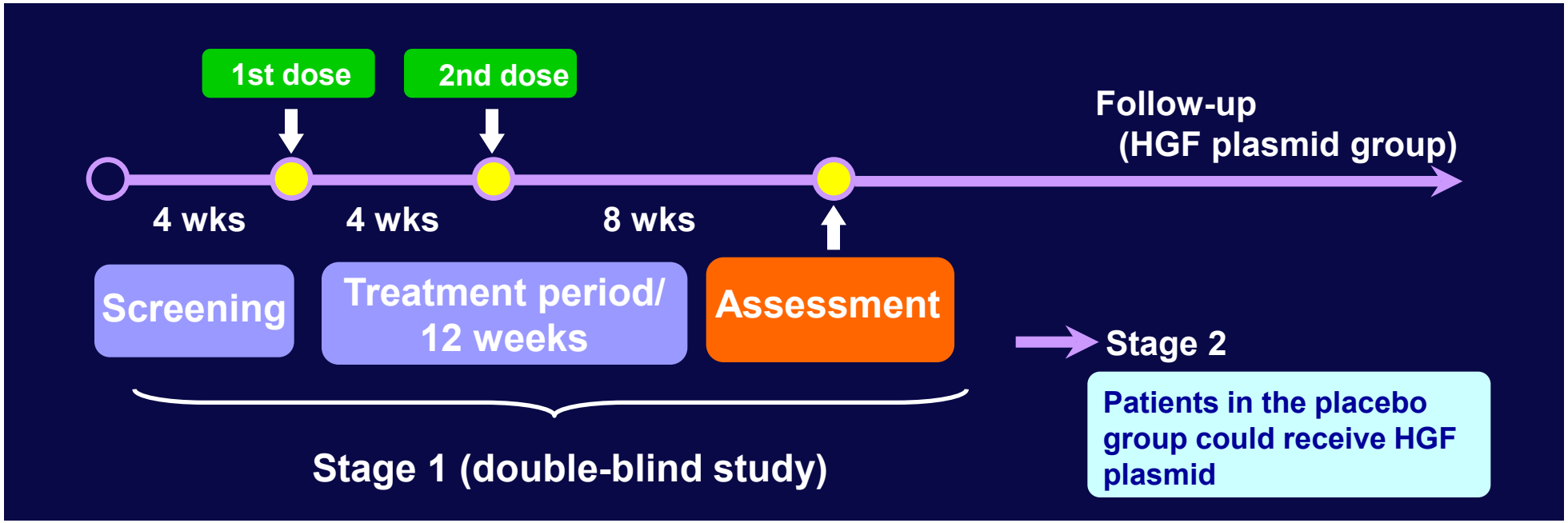
H Shigematsu[1], K Yasuda[2], T Iwai[3], T Sasajima[4], S Ishimaru[5], Y Ohashi[6], T Yamaguchi[7], T Ogihara[8] and R Morishita[9]

*[1]Department of Vascular Surgery, Tokyo Medical University, Tokyo, Japan; [2]Hokkaido Chuo Rosai Hospital Spinal Cord Injury Center, Japan Labour Health and Welfare Organization, Hokkaido, Japan; [3]Tsukuba Vascular Center and Buerger's Disease Research Institute (NPO), Moriya Keiyu Hospital, Ibaraki, Japan; [4]First Department of Surgery, Asahikawa Medical University, Hokkaido, Japan; [5]Center for Endovascular Therapy, Toda Chuo General Hospital, Saitama, Japan; [6]Department of Biostatistics, School of Public Health, University of Tokyo, Tokyo, Japan; [7]Department of Clinical Trial Data Management, Graduate School of Medicine, University of Tokyo, Tokyo, Japan; [8]Osaka General Medical Center, Osaka, Japan and [9]Department of Clinical Gene Therapy, Osaka University Graduate School of Medicine, Osaka, Japan*

Confidential

# JN-101: DB, Phase 3, Placebo-Controlled Study in CLI

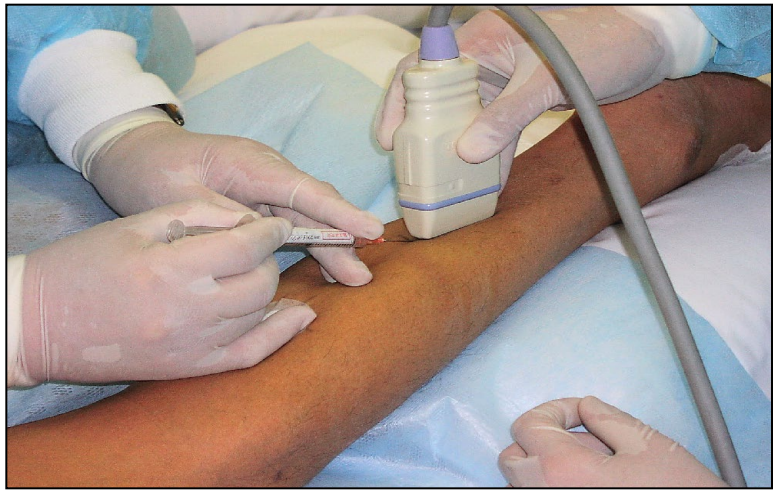
| | |
|---|---|
| Subjects: | CLI (Fontaine III, IV) |
| Treatment groups: | HGF plasmid group and placebo group |
| Stage progression: | Patients in the placebo group could receive HGF plasmid in Stage 2 |



# Gene Transfer (Injection Method)

- The study drug is diluted in saline and injected intramuscularly at 8 sites in the ischemic limb (0.5mg in 3mL per site; total dose: 4mg)
- Study drug injection was performed twice at a 4-wk interval
- The injection sites are determined for each participant depending on the location of the diseased vessels





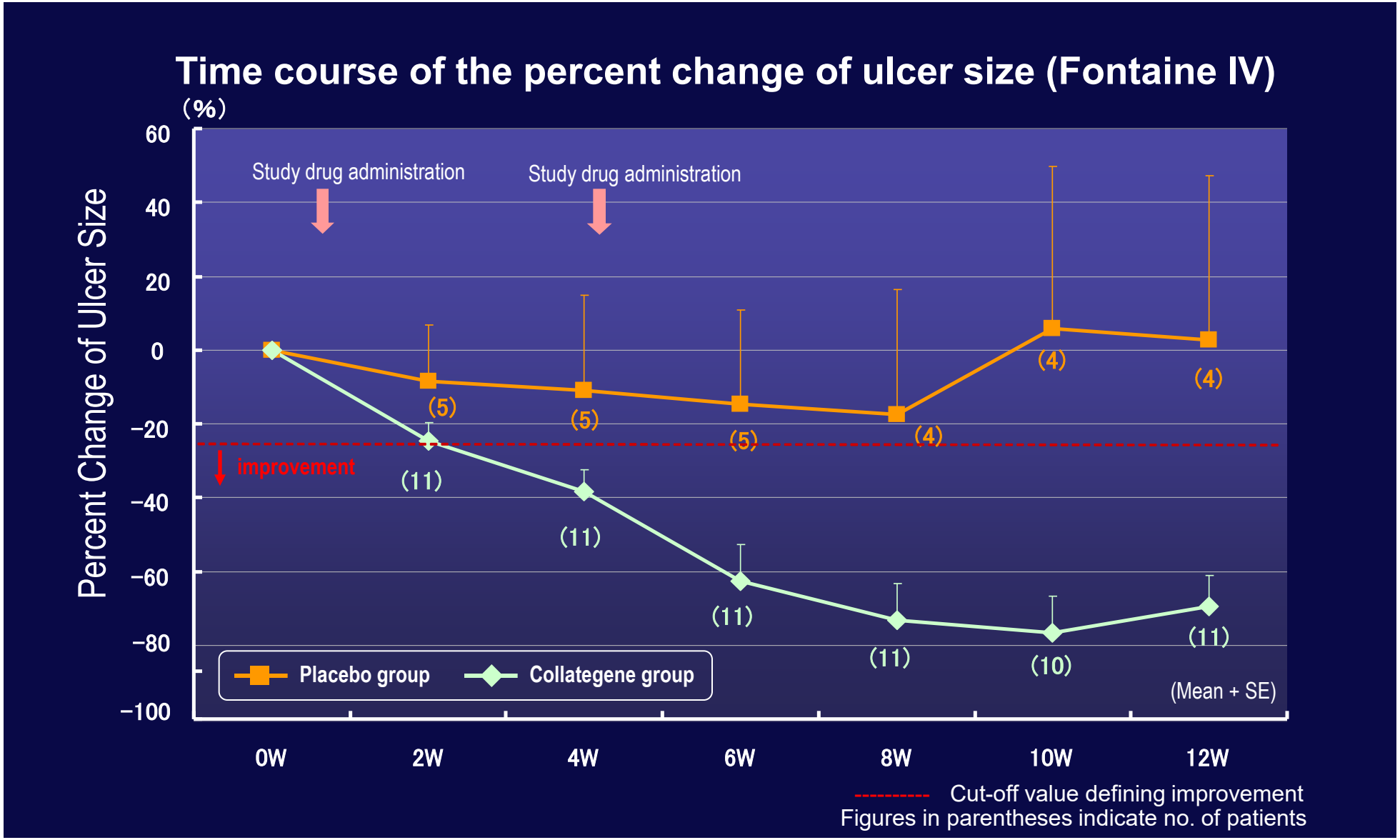
Confidential

# JN-101 Study: Primary Efficacy Endpoint Responder Rate

| Dose Group | Responders | Non-Responders | Percent Responder |
|---|---|---|---|
| HGF | 19 | 8 | 70% |
| Placebo | 4 | 9 | 31% |

***P*=.014**

# JN-101: Primary Endpoint – Ulcer Size



# U.S. Study 0202

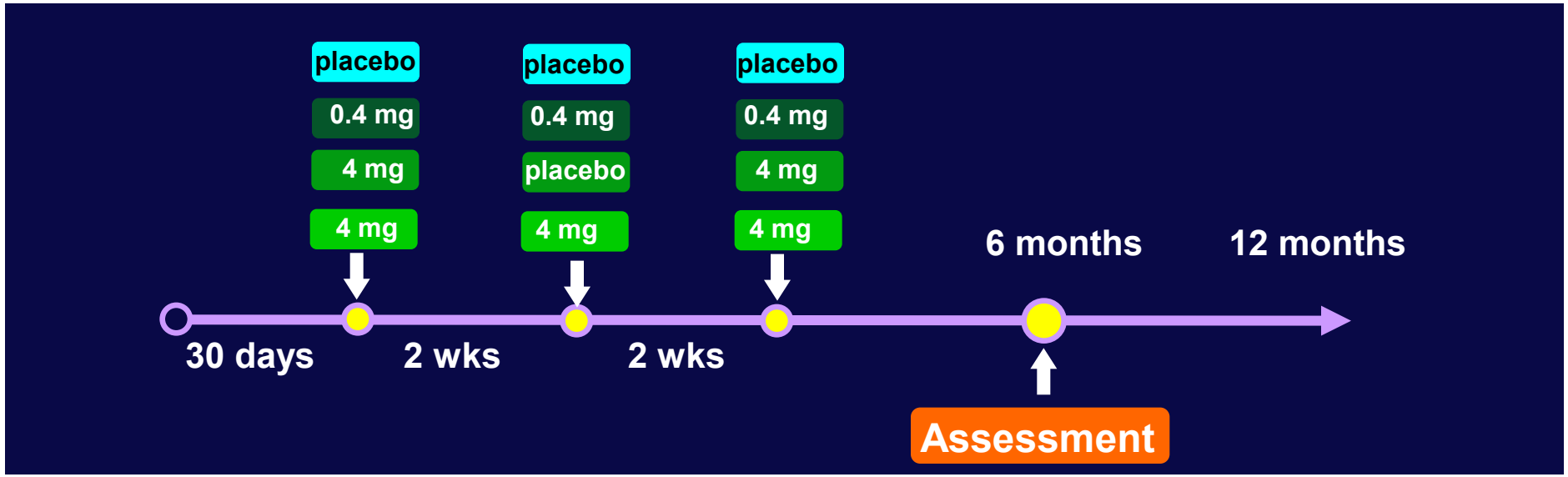
*Circulation*. 2008;118: 58-65

## Results of a Double-Blind, Placebo-Controlled Study to Assess the Safety of Intramuscular Injection of Hepatocyte Growth Factor Plasmid to Improve Limb Perfusion in Patients With Critical Limb Ischemia

Richard J. Powell, MD; Michael Simons, MD; Farrel O. Mendelsohn, MD; George Daniel, MD; Timothy D. Henry, MD; Minako Koga, BS; Ryuichi Morishita, MD; Brian H. Annex, MD

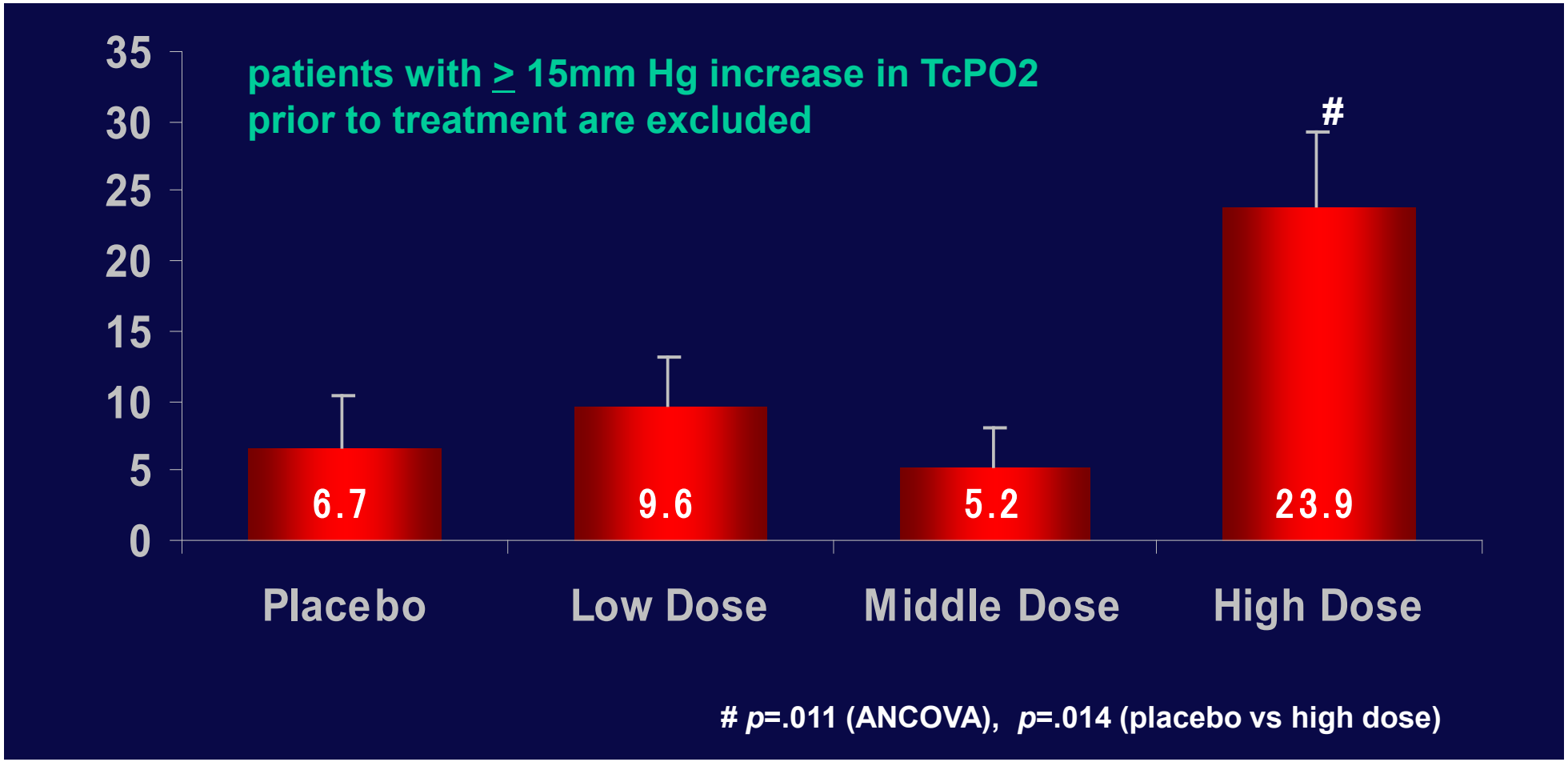
# U.S. Study 0202

- Randomized, DB placebo-controlled, dose-response study in CLI (Rutherford 4-6)
- Placebo, AMG0001 0.4mg x 3, AMG0001 4.0mg x 2, AMG0001 4.0mg x 3
- IM injections (8 sites per dose) in the affected leg (4 above and 4 below the knee)
- Duration of follow-up: 12 months
- Primary endpoint: Change in TcPO2 at 6 months (surrogate for improved perfusion)

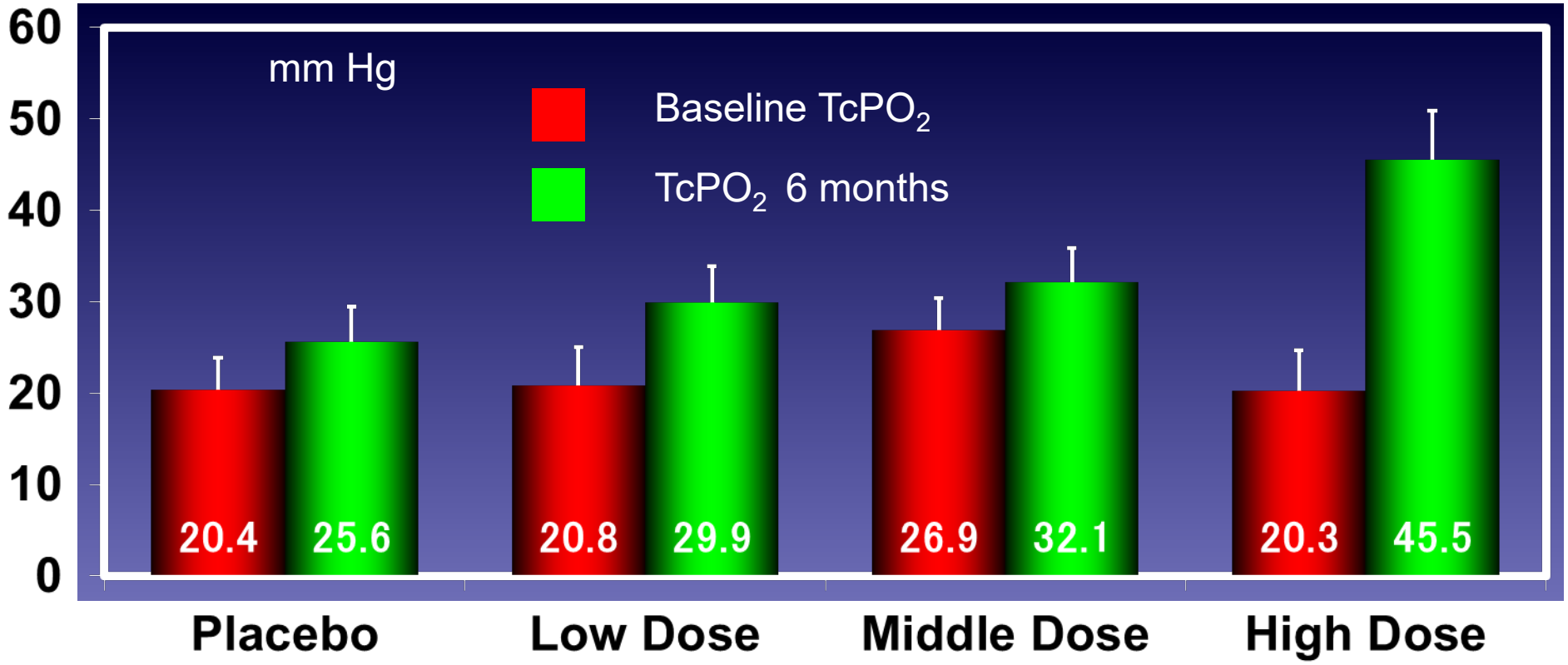


# US Study 0202: Primary Endpoint

Mean change (± SE) in TcPO2 from baseline, at 6 months



# $TcPO_2$ at Baseline and at 6 Months




#=*p*.014 vs placebo at 6 months

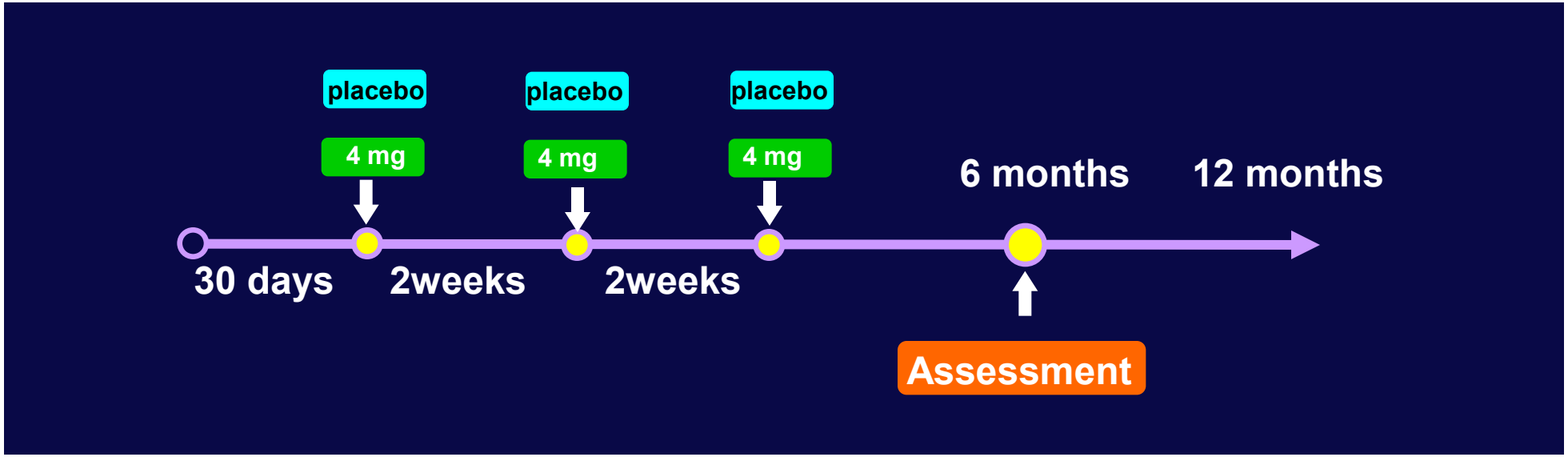
# Safety Assessment

- No excess cancers
- No progression of retinopathy
- No angiomas
- Most adverse events due to complications of CLI or comorbidities; SAEs not different in the 2 groups
- No safety concerns; doses well tolerated

# U.S. Study 0205

*J Vasc Surg.* 2010;52:1525-1530.

- A DBPC study to assess the safety and efficacy of plasmid HGF IM 4mg x 3, 2 wks apart in patients with CLI with ulcers
- Clinic follow-up up to 12 months
- Randomization 3:1 in favor of plasmid HGF
- Total patients enrolled 27; placebo 6, HGF 21



# U.S. Study 0205: Evaluations

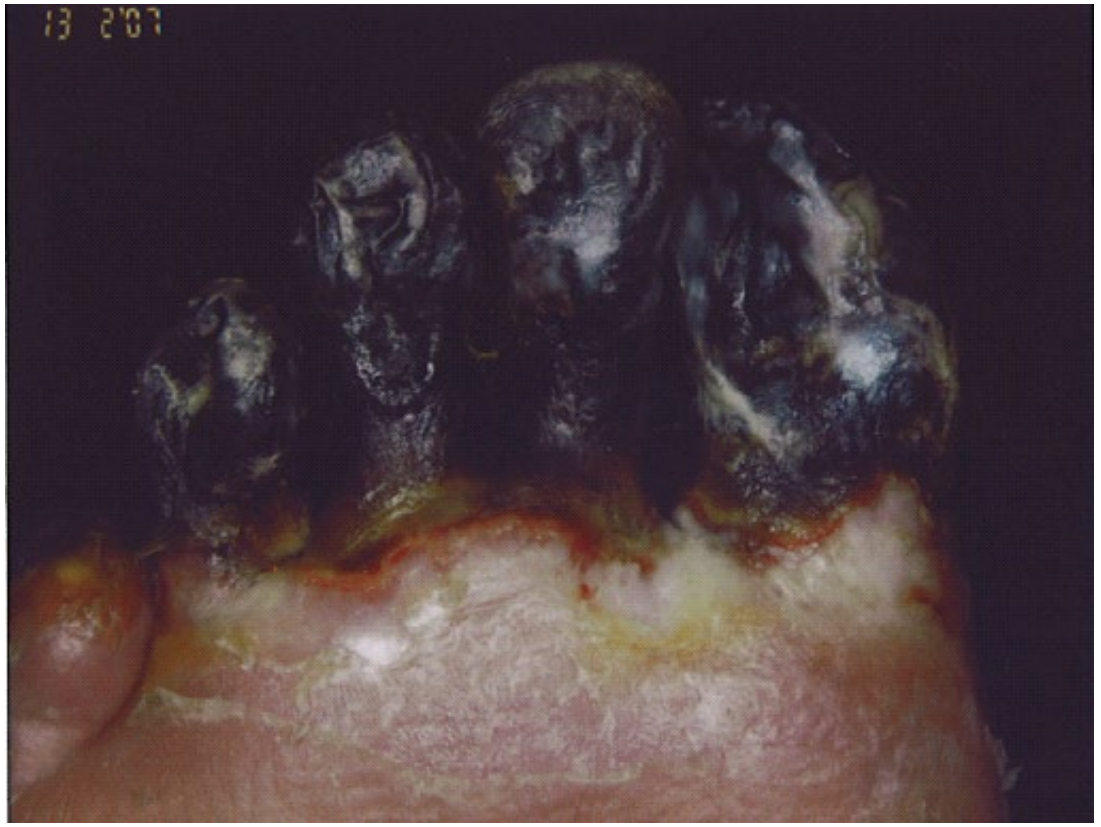
- Safety evaluation up to 12 months
- Efficacy evaluation at 3, 6, and 12 months
- Efficacy parameters included ulcer healing, rest pain relief (VAS score), and hemodynamic measurement changes

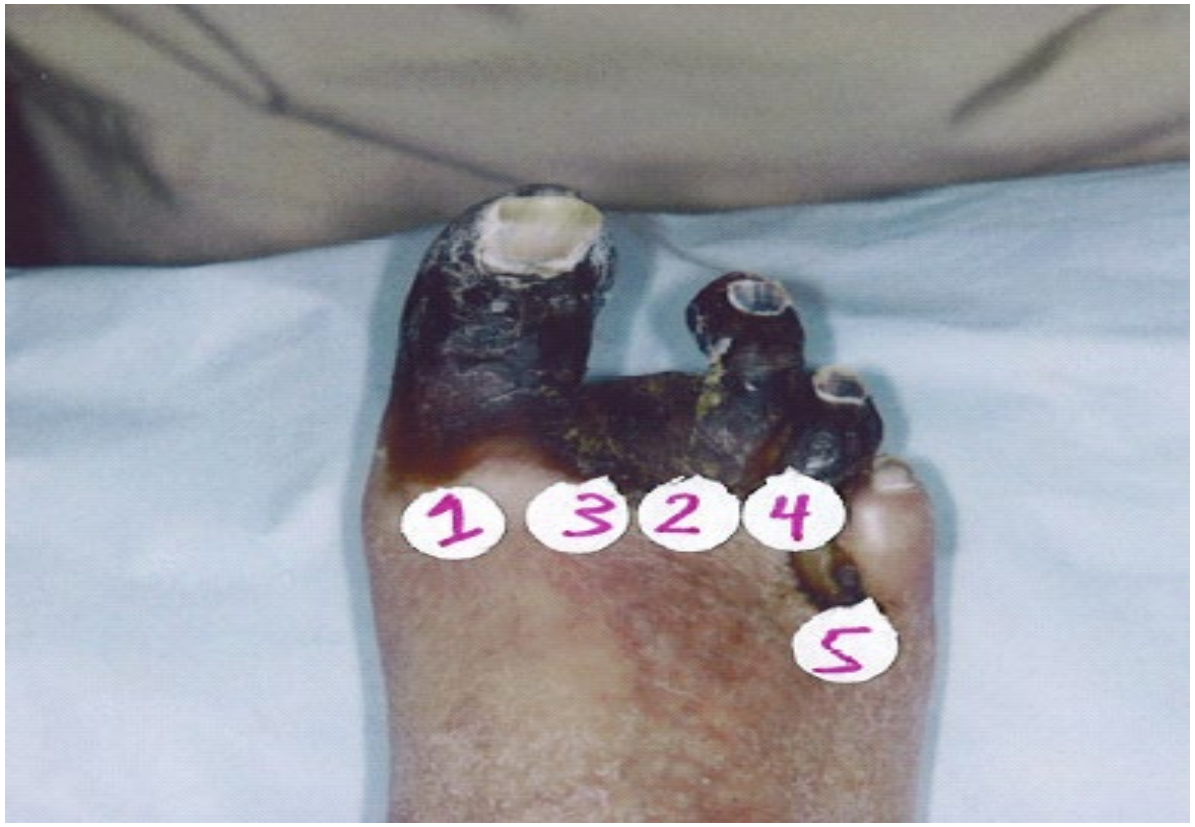
**Efficacy Results**

- Significant improvement in toe pressure, TBI, and rest pain in the HGF plasmid group
- Thirty-one percent (31%) of all (and largest) ulcers healed in the HGF plasmid group, but none in the placebo group (*p*=.28)

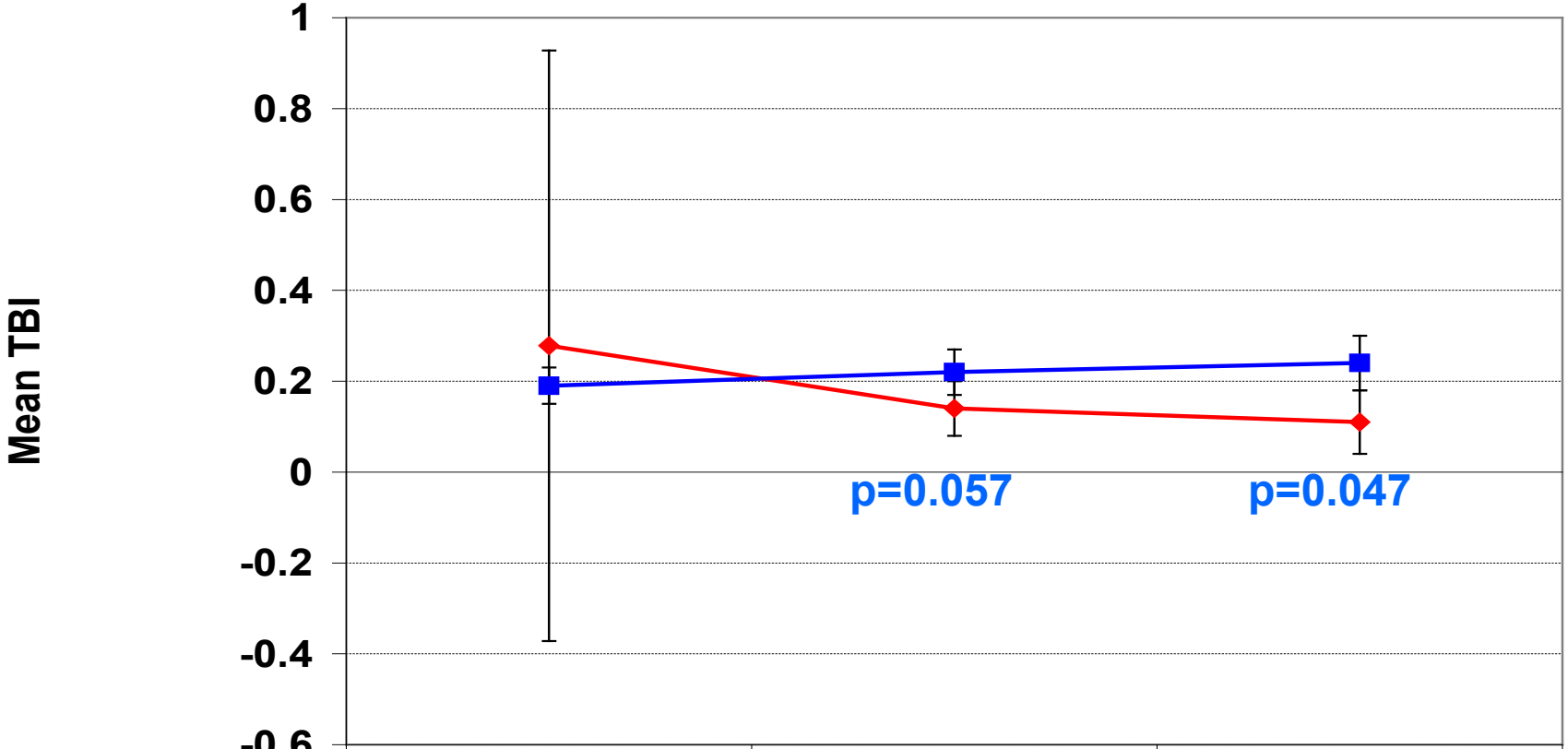
VAS = visual analogue scale; TBI = toe-brachial index.

# AG-CLI-0205 – Baseline Ulcer Severity



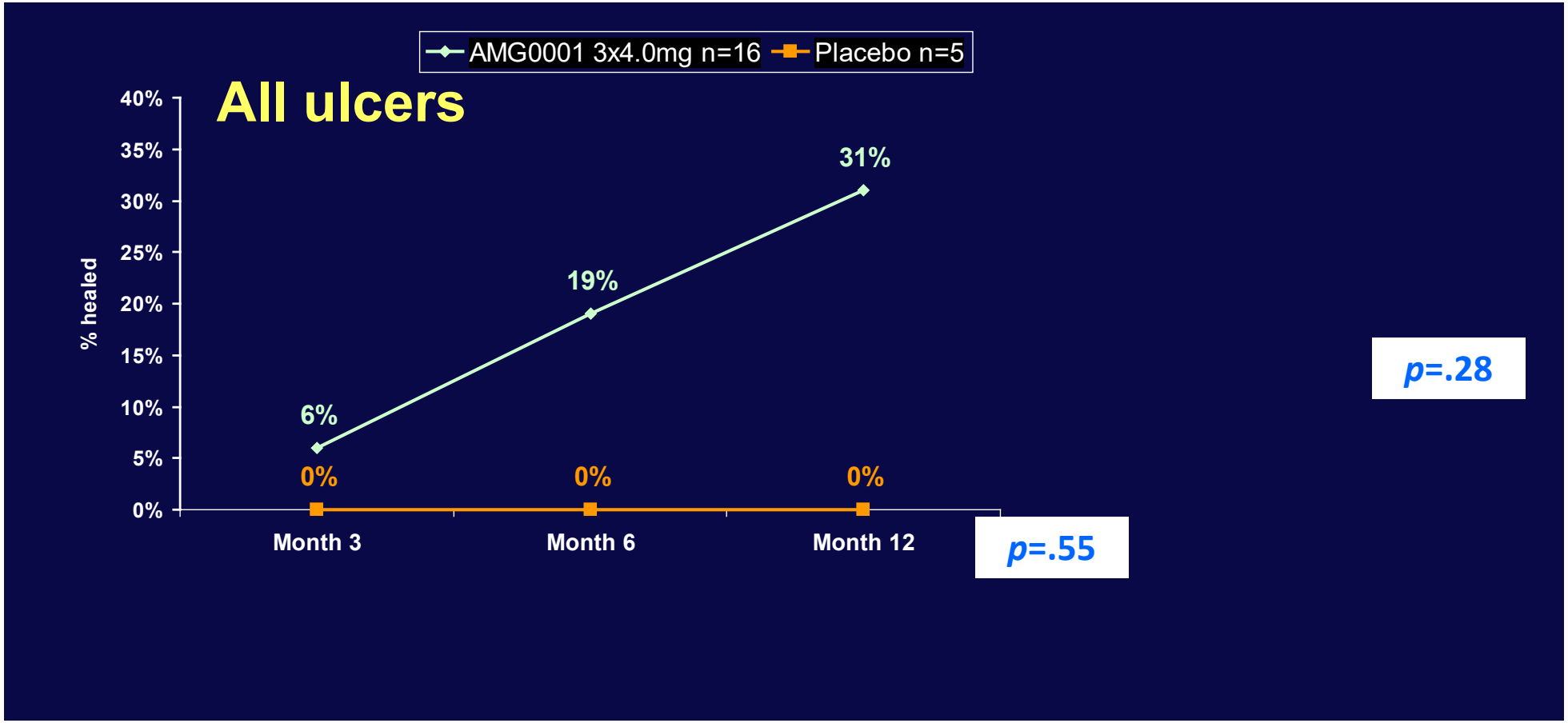


# U.S. Study 0205:
## Hemodynamic Measurements – TBI




| | Baseline | Month 3 | Month 6 |
|---|---|---|---|
| Placebo | 0.278 | 0.14 | 0.11 |
| AMG0001 | 0.19 | 0.22 | 0.24 |

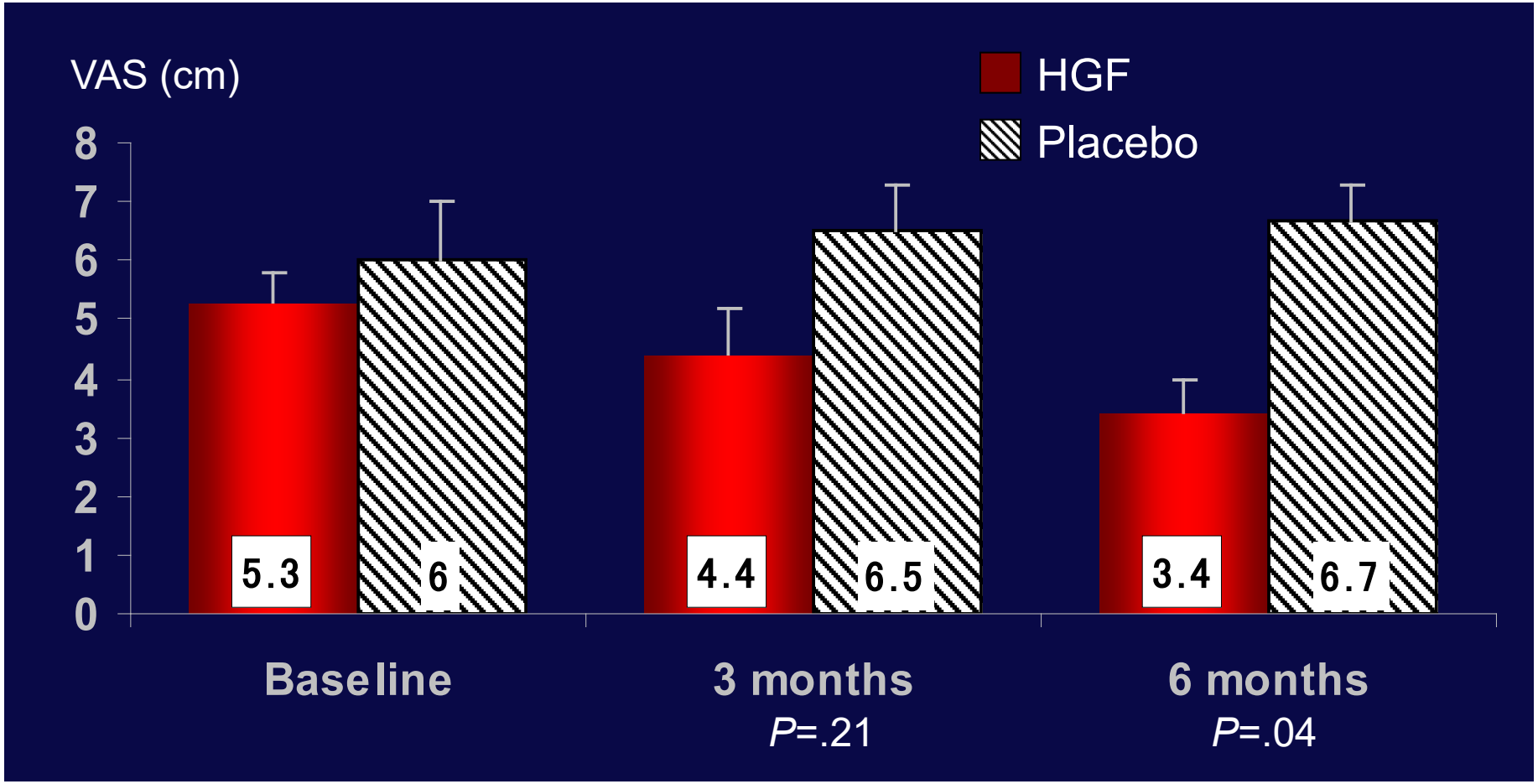
# U.S. Study 0205: Complete Wound Healing




*p-value*: vs placebo

# U.S. Study 0205: Pain Score

Change in Pain Score by Visual Analogue Scale (VAS)



# U.S. Study 0205: Safety

- AMG0001 4.0mg x3 was well tolerated
- AEs mostly due to study disease, intercurrent illness, or comorbid conditions (eg, MI, CVA, renal failure, diabetic complications)
- No cancers reported up to the 12-month follow-up
- No angioma, worsening retinopathy

MI = myocardial infarction; CVA = cerebrovascular accident;

# Protocol Highlights

## AG-CLI-0206 Protocol Title

A phase 3 multinational, double-blind, randomized, placebo-controlled study to evaluate the safety and efficacy of AMG0001(HGF Plasmid) in participants with critical limb ischemia (CLI)

# AG-CLI-0206 Study Population (continued)

**In addition, participants will have**

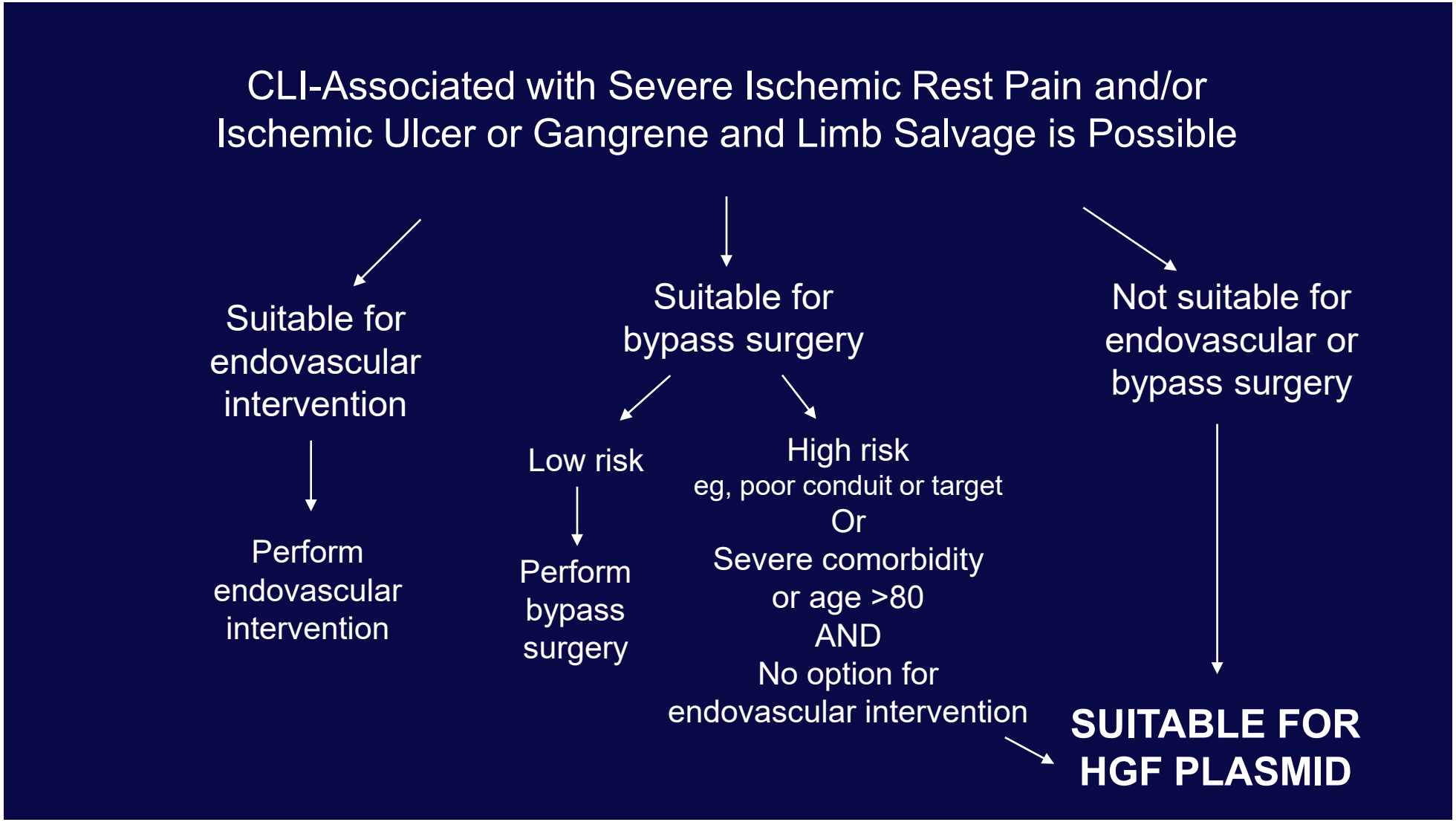
- No option for revascularization by endovascular intervention or bypass surgery (see definitions below)

**OR**

- Poor option (high-risk) for revascularization by surgery (see definition next slide), but no option for revascularization by an endovascular procedure (Type D lesions using the TASC II classification)[1]

1 Norgren L, et al. *Eur J Vasc Endovasc Surg.* 2007;33(Suppl 1):S1-S75.

# HGF Plasmid: Suitable Patients for Study 0206



# AG-CLI-0206 Study Population

Participants (40-90 yrs old) with chronic CLI who have

- Severe ischemic pedal rest pain (nocturnal metatarsalgia, Rutherford 4) in the index lower limb with
  - Ankle and/or toe systolic pressures of less than 50 and 30 mm Hg, respectively
  - Classical features of rest pain and on narcotics for pain relief

# AG-CLI-0206 Endpoints

## Primary Endpoint

Time to major amputation (of the treated leg)
or all-cause death up to 18 months

# AG-CLI-0206 Exclusion Criteria

Evidence or history of malignant neoplasm (clinical, laboratory, or imaging) except for successfully excised basal cell or squamous cell carcinoma or successfully excised early melanoma of the skin.

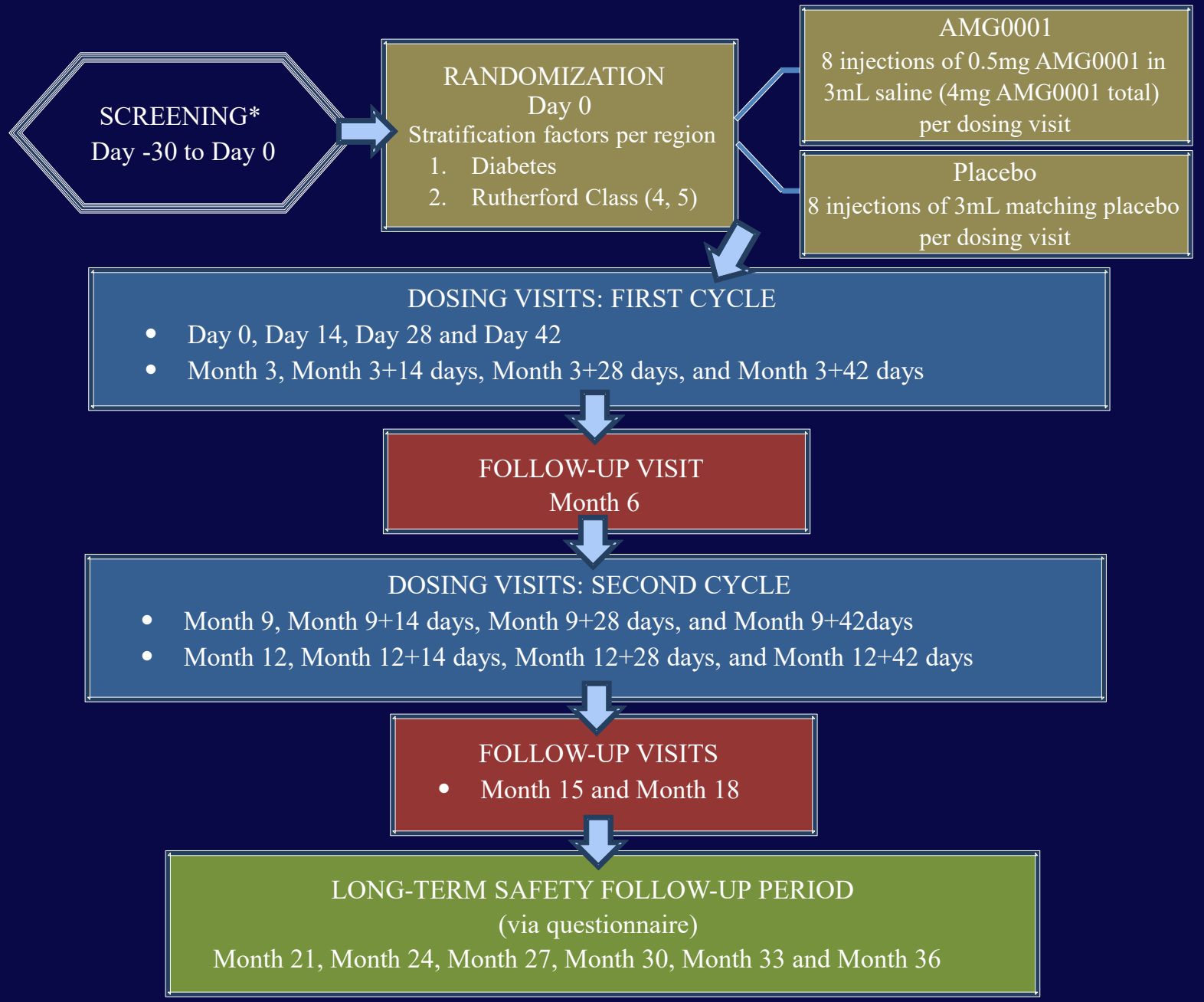
- Successful tumor resection or radio-chemotherapy of breast cancer more than 10 yrs prior to inclusion in the study, and with no recurrence, may be enrolled in the study
- Successful tumor resection or radio-chemotherapy of all other tumor types and have been in remission for more than 5 yrs prior to inclusion in the study, and with no recurrence, may be enrolled in the study
- A dermatological exam will have ruled out any skin cancer

Participants who have proliferative retinopathy, or moderate or severe non-proliferative retinopathy, from any cause (ETDRS Score >35), clinically significant macular oedema or previous panretinal photocoagulation therapy. Results from the Early Treatment Diabetic Retinopathy Study (ETDRS). *Ophthalmology*. 1991;98(5 suppl):823-833.

# AG-CLI-0206 Dosage Regimen

| | |
|---|---|
| **First Cycle** | **Day 0, Day 14, Day 28, and Day 42**<br>(4 sets of injections, 2 wks apart; each set is 4mg subdivided into 8 injections of 0.5mg of HGF plasmid in 3mL saline or matching placebo)<br><br>**Month 3, Month 3+14 days, Month 3+28 days, and Month 3+42 days**<br>(4 sets of injections, 2 wks apart; each set is 4mg subdivided into 8 injections of 0.5mg of HGF plasmid in 3mL saline or matching placebo) |
| **Second Cycle** | **Month 9, Month 9+14 days, Month 9+28 days, and Month 9+42 days**<br>(4 sets of injections, 2 wks apart; each set is 4mg subdivided into 8 injections of 0.5mg of HGF plasmid in 3mL saline or matching placebo)<br><br>**Month 12, Month 12+14 days, Month 12+28 days, and Month 12+42 days**<br>(4 sets of injections, 2 wks apart; each set is 4mg subdivided into 8 injections of 0.5mg of HGF plasmid in 3mL saline or matching placebo) |

# AG-CLI-0206 Study Flow Chart



# AG-CLI-0206: HGF Plasmid in CLTI Patients

## Safety and Efficacy of Prolonged AMG0001 Administration

**Change from baseline in hemodynamic parameters**

| | AMG0001 | | | Placebo | | | |
|---|---|---|---|---|---|---|---|
| | n | mean | SD | n | mean | SD | P-value |
| TBI | 17 | 0.168 | 0.2692 | 14 | -0.044 | 0.1846 | 0.033 |
| AP (dorsalis pedis) | 14 | 32.9 | 41.55 | 12 | 16.7 | 34.25 | 0.324 |
| AP (posterior tibial) | 12 | 35.1 | 33.97 | 11 | 13.2 | 24.98 | 0.051 |
| Pain (VAS) | 16 | -1.61 (34%) | 2.86 | 11 | -0.20 (3%) | 3.09 | 0.002 |

- No subject tested positive for anti-HGF antibody
- No long-term safety concerns or delayed AEs

# AG-CLI-0206: HGF Plasmid in CLTI Patients

## Safety and Efficacy of Prolonged AMG0001 Administration

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# QoL（SF-36): Comparison of Change Between Pre-, Post-Treatment

| Health Concept | Amount of Change | | Difference in Average | 95%CI |
|---|---|---|---|---|
| | Placebo | AMG0001 | | |
| Physical Functioning | 11.25 | 13.33 | 2.08 | -11.93-16.10 |
| Role physical | 15.91 | 9.26 | -6.65 | -38.47-25.17 |
| **Bodily pain** | 6.58 | 20.63 | 14.05* | 1.00-27.10 |
| General Health Perceptions | 1.25 | 6.78 | 5.53 | -6.93-17.99 |
| Mental Health | -12.00 | 11.69 | 23.69* | 3.44-43.94 |
| Role Emotional | 0.00 | 18.52 | 18.52 | -3.39-40.42 |
| Social Functioning | 8.33 | 15.28 | 6.94 | -25.91-39.80 |
| Vitality | -3.33 | 12.31 | 15.64 | -1.64-32.92 |
| Summary Score : | | | | |
| Physical Component Summary | 8.68 | 6.79 | -1.89 | -9.85-6.08 |
| Mental Component Summary | -4.49 | 5.70 | 10.18 | -0.54-20.91 |

*$p<.05$

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|---|---|---|---|---|
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| Mental Component Summary | -4.49 | 5.70 | 10.18 | -0.54-20.91 |

*$p<.05$

# Summary: Overall Efficacy from All Studies

- Evidence of increase in tissue perfusion and hemodynamic parameters (0202, 0205, 0206)
- More ulcer heal (0205) or reduce in size (JN-101)
  - Rest pain severity is reduced (0205, 0206)
  - Small number of patients in each study
  - Too few events to evaluate major amputation or death

# Summary: Overall Safety from All Studies

- HGF injections are well-tolerated
- Short- and medium-term safety is reassuring
- Majority of adverse events are due to worsening of CLI or due to comorbid conditions or intercurrent illnesses, and so far, no evidence of unwanted angiogenesis

# Registrational Trial
# LEGEND 1




David G. Armstrong, DPM, MD, PhD

Distinguished Professor of Surgery and Neurological Surgery
Director, USC Limb Preservation Program
Keck School of Medicine of University of Southern California (USC)
Los Angeles, CA

#AHA24

# ANATOMICALLY DIRECTED LOWER EXTREMITY GENE THERAPY FOR ULCER HEALING: A DOUBLE BLIND, RANDOMIZED, PLACEBO CONTROLLED STUDY – LEGEND-1

David G. Armstrong, DPM, MD, PhD*^, Michael S. Conte, MD*#, Joseph L. Mills, MD, Matthew T. Menard, MD, Dennis P. Orgill, MD, PhD, Robert D. Galiano, MD, Robert S. Kirsner, MD, PhD, Alik Farber, MD, MBA, John C. Lantis, MD, Charles M. Zelen, DPM, Marissa J. Carter, PhD, MA, Caitlin W. Hicks, MD, MS, Richard J. Powell, MD

Division of Surgery, Keck School of Medicine of USC, Division of Surgery; University of California; San Francisco, Brigham and Women's Hospital, Harvard Medical School, Boston Medical Center, Feinberg School of Medicine, Northwestern University, Icahn School of Medicine, University of Miami, Johns Hopkins University School of Medicine, Dartmouth Geisel School of Medicine

American Heart Association.

American Heart Association. 100 YEARS Bold Hearts

# INTRODUCTION

## Peripheral Artery Disease (PAD) and its sequelae

- 200 million people, worldwide
- Half of people with PAD have concomitant diabetes
- Approximately 18.6 million people worldwide develop DFUs annually
- 50–60% of patients with DFUs have PAD



Conte, et al, J Vasc Surg, 2019
Alison, Armstrong, Goodney, Hamburg et al, Circulation, 2023
Armstrong, Tan, Boulton, and Bus, JAMA, 2023
Armstrong, Boulton, Bus, N Engl J Med, 2017

# INTRODUCTION

Previous studies enrolled end-stage patients, often with **gangrene** using **amputation free survival as the endpoint in "no option" CLTI**

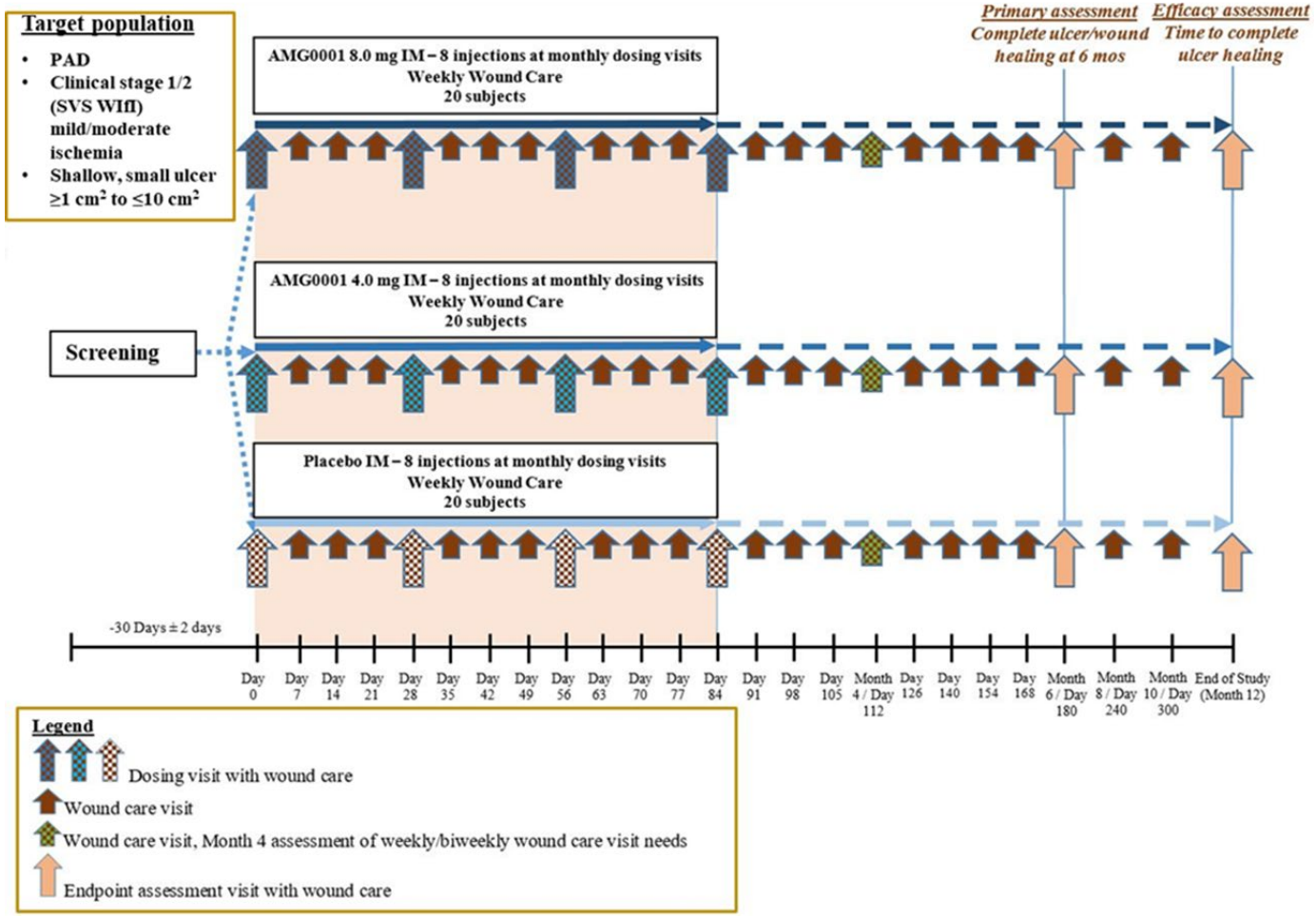
Purpose of the current study: Safety and effectiveness of gene therapy in **lower extremity wound healing in mild-moderate CLTI**

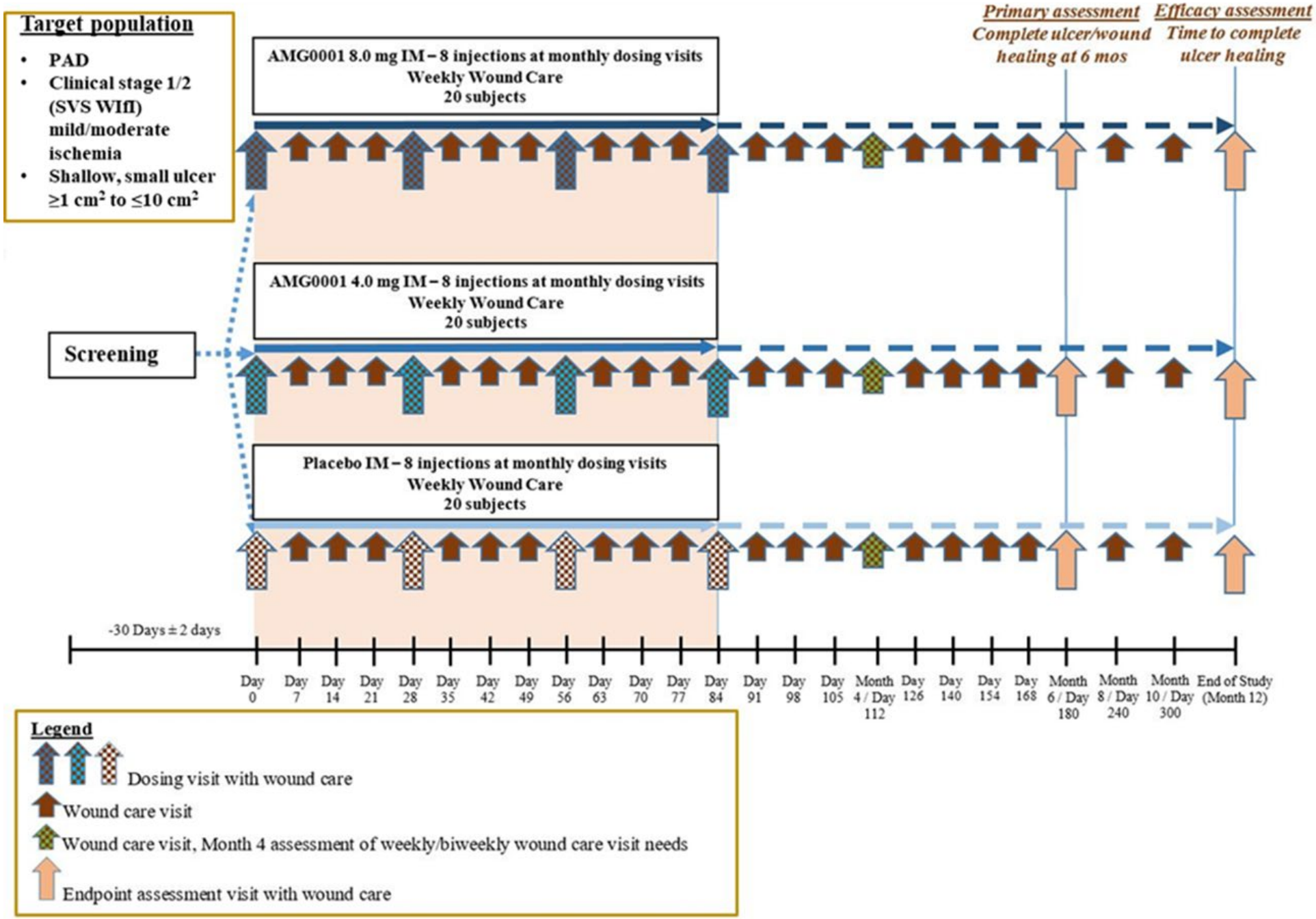


Norgren, et al, BJS, 2024
Belch, et al, Lancet, 2011
Powell, et al, J Vasc Surg, 2010

# STUDY DESIGN

- The Phase II **LEGenD 1** trial was a double-blind, randomized, placebo-controlled study
- 75 subjects with neuro-ischemic ulcers and transcutaneous oxygen pressure ($TcPO_2$) or Toe Pressure (TP) levels of 30–59 mmHg
- Participants were randomized to:
  - AMG0001 4 mg
  - AMG0001 8 mg
  - Placebo
- Subjects received four treatments at one-month intervals **anatomically-directed** along a **target artery path**
- Co-primary endpoints **(pooled across doses)** were **time to healing** and proportion of ulcers **healed by 6 months using blinded assessors**
- Additional endpoints included ulcers **healed by 12 months, hemodynamic changes, and recurrence**



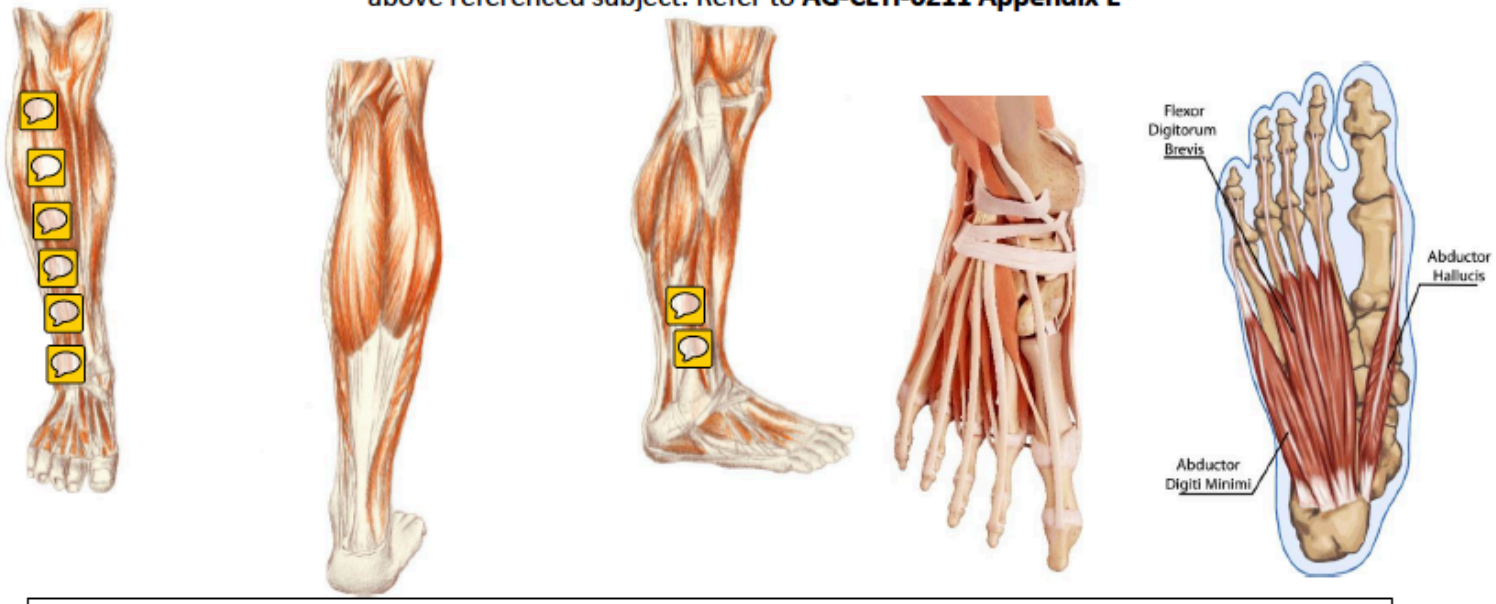

Target population
PAD
Clinical stage 1/2 (SVS WIfI) mild/moderate ischemia
Shallow, small ulcer ≥1 cm² to ≤10 cm²
Primary assessment
Complete ulcer/wound healing at 6 mos
Efficacy assessment
Time to complete ulcer healing
AMG0001 8.0 mg IM – 8 injections at monthly dosing visits
Weekly Wound Care
20 subjects
AMG0001 4.0 mg IM – 8 injections at monthly dosing visits
Weekly Wound Care
20 subjects
Placebo IM – 8 injections at monthly dosing visits
Weekly Wound Care
20 subjects
Screening
-30 Days ± 2 days
Day 0
Day 7
Day 14
Day 21
Day 28
Day 35
Day 42
Day 49
Day 56
Day 63
Day 70
Day 77
Day 84
Day 91
Day 98
Day 105
Month 4 / Day 112
Day 126
Day 140
Day 154
Day 168
Month 6 / Day 180
Month 8 / Day 240
Month 10 / Day 300
End of Study (Month 12)
Legend
Dosing visit with wound care
Wound care visit
Wound care visit, Month 4 assessment of weekly/biweekly wound care visit needs
Endpoint assessment visit with wound care

# ANATOMICALLY DIRECTED GENE THERAPY

**- Individualized treatment plan based on objective independent assessment**

- Ulcer location
- CTA/MRA of limb
- Primary and secondary target artery pathways selected **based on angiosomes**
- Total of 8 injection sites mapped per subject

The "**X**"s on the diagram below indicates where the 8 Investigational Product injections should occur for the above referenced subject. Refer to **AG-CLTI-0211 Appendix E**

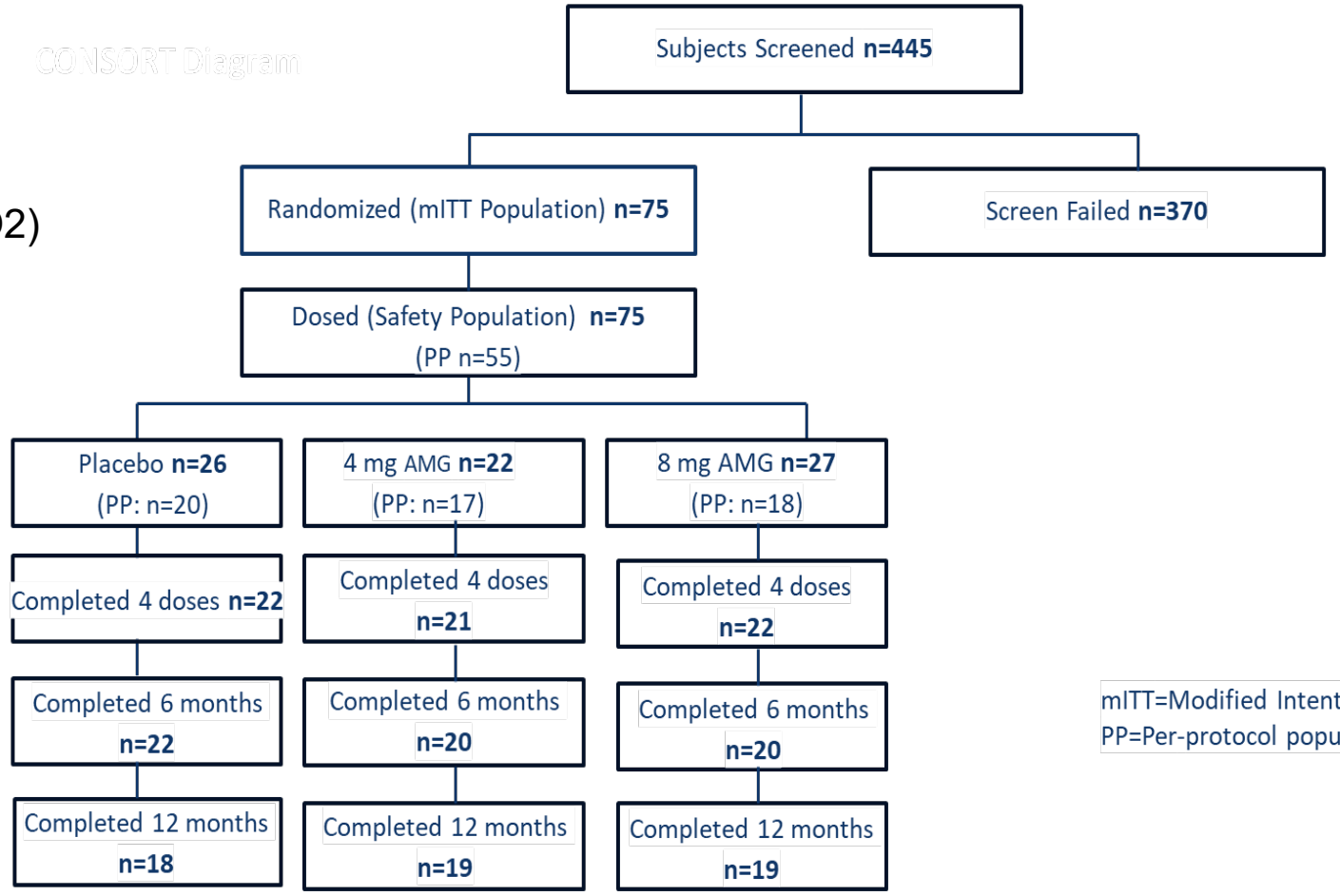



Specific injection instructions: 6 injections equally spaced longitudinally through the anterior tibial artery distribution; 2 injections in the distal peroneal distribution

# STUDY DESIGN

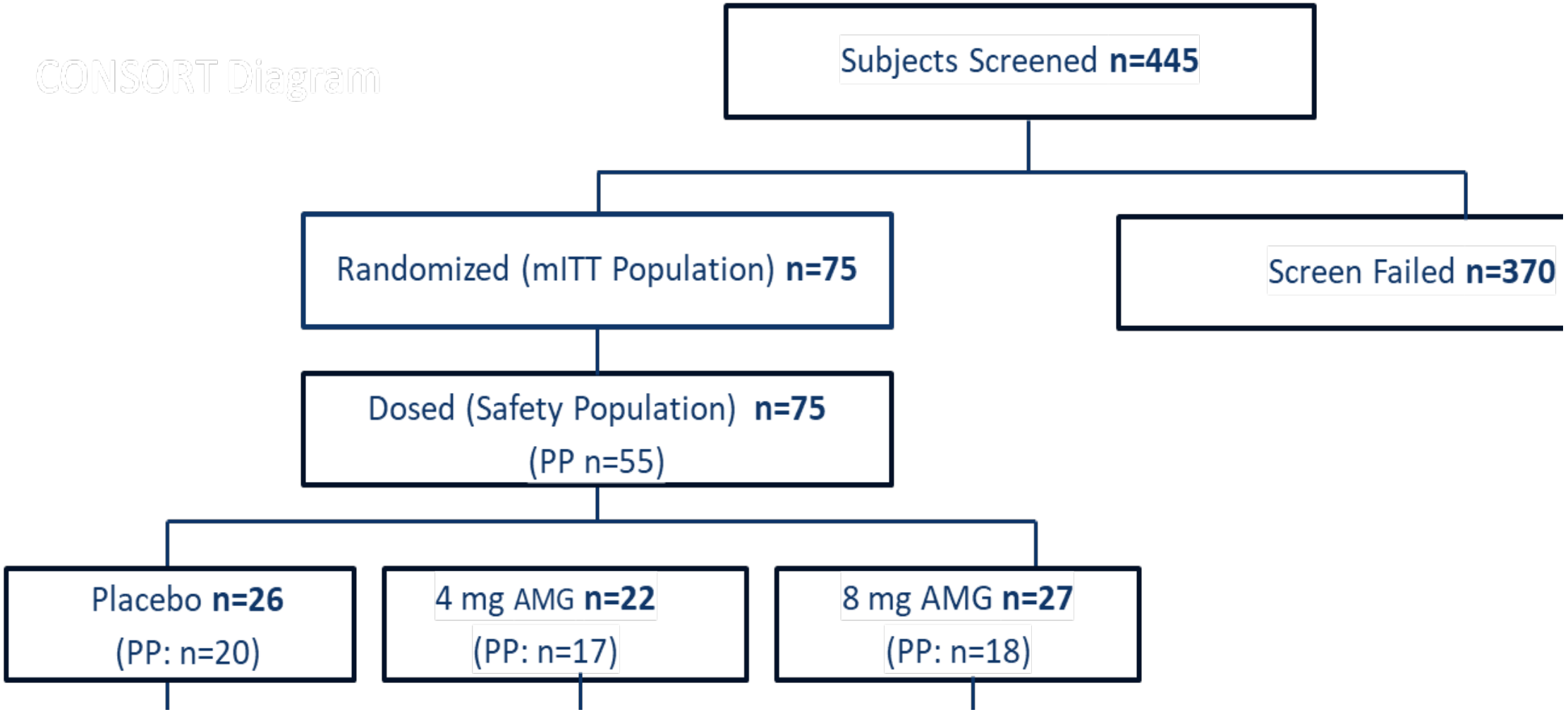
**Key exclusion criteria**

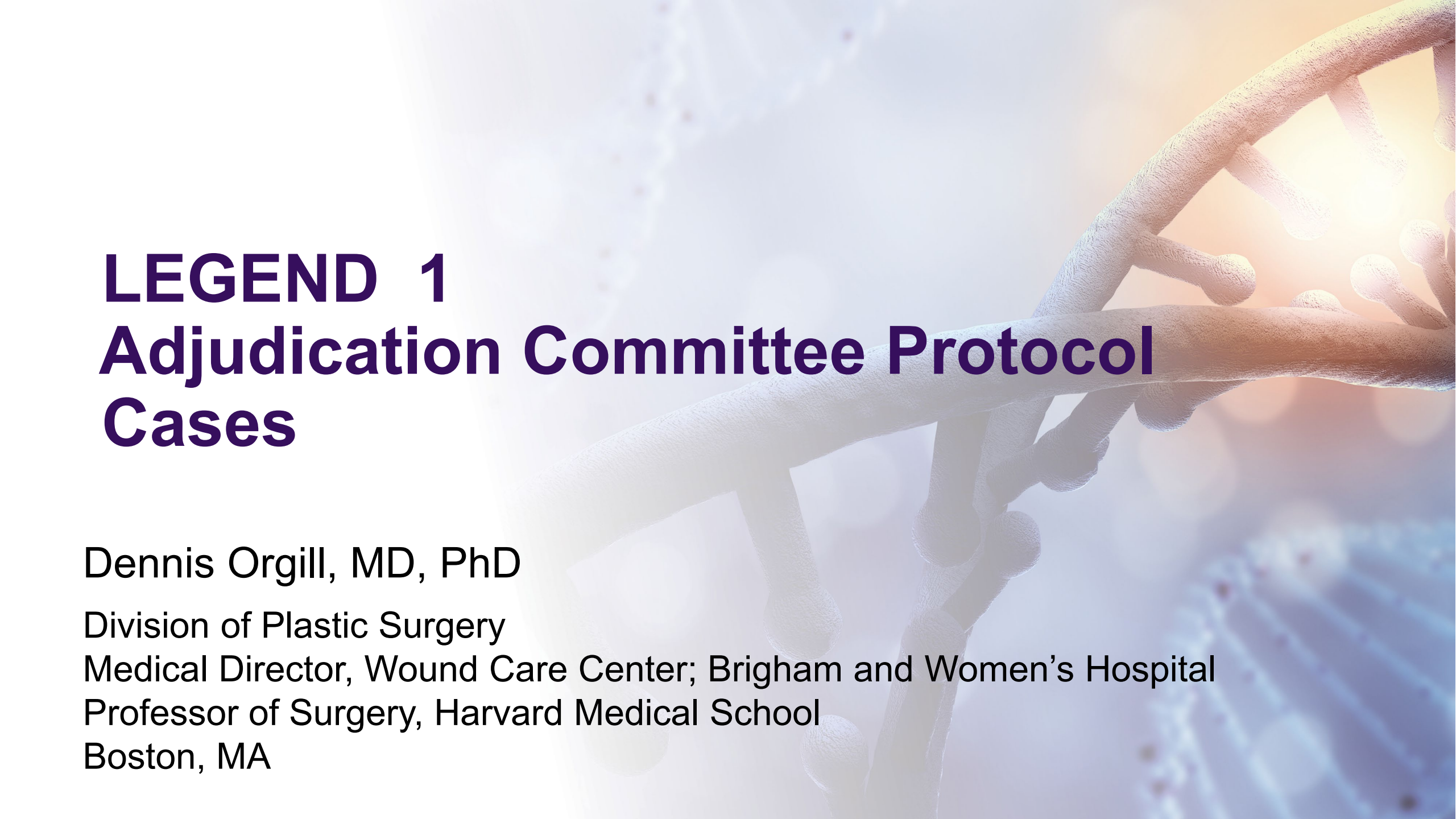
- Severe ischemia (<30mmHg TP or TCPO2)
- Gangrene or tissue loss >10cm2
- Significant aortoiliac occlusive disease
- <2m hx of technically successful revasc
- History of malignant neoplasm <5y
- (eGFR) <30 mL/min
- Proliferative retinopathy




mITT=Modified Intention to Treat
PP=Per-protocol population

# CONSORT Diagram





# LEGEND 1
# Adjudication Committee Protocol Cases

Dennis Orgill, MD, PhD

Division of Plastic Surgery
Medical Director, Wound Care Center; Brigham and Women's Hospital
Professor of Surgery, Harvard Medical School
Boston, MA

# Adjudication Process

After the study center has determined the subject is healed (investigator + physician not associated with study), CRO forwards images of the subject's wound to 3 independent adjudicators who determine if the subject is "healed."

"Healing" is defined as: 100% re-epithelialization of the wound surface, with no discernible exudate and without drainage or dressing requirements.

# Adjudication Process

# Adjudication Protocol Criteria

## 6. ADJUDICATION PROCESS

Confirmation evaluations of wound closure will be subject to oversight to reduce bias. This will be completed by the adjudication committee. Per protocol:

- The photographs will be forwarded to the group of blinded Adjudicators within one week
- If two-thirds of the Adjudicators agree that the wound is healed, the subject proceeds to an additional consecutive study visit 2 weeks apart to confirm wound has remained healed.
- If two-thirds of the Adjudicators do not agree or have questions, more information regarding the wound will be provided by the PERI and forwarded to the group of Adjudicators. If this does not provide resolution or if the Adjudicators deem the wound not healed, the subject will continue study participation at the next scheduled visit. Wound closure will be reassessed at the next visit.
- In the event of one adjudicators absence, two of the three adjudicators can confirm the wound heal, if they both agree. In the event those two adjudicators do not agree, then the final adjudication decision will be determined once the third adjudication can be completed.

# Adjudication Case Report Form

☐☐☐-☐☐☐

**Subject ID**

**Date of Photo used for Adjudication:** ☐☐ ☐☐☐ ☐☐☐☐
DD MMM YYYY

**Adjudicator Instructions**

Adjudicators are blinded to the study treatment the subject received and cannot discuss any subject data or images with other Adjudicators.

Please answer the following questions as either "yes" or "no."

**If additional images are needed to answer these questions please contact PERI Adjudication team via email at adjudication@periedu.com, or the Clinical Trail Manager via phone 513-319-8169**

**Adjudicator Questions**

1. Is the wound 100% epithelialized and there is no pink, red, yellow or black slough or dried exudate concealing the surface of the skin?

   ☐ YES ☐ NO

2. The entire skin surrounding the former wound skin is either normal in color, pink or red, callused or macerated but without signs of an ulcer in the tissue that surrounds the former wound. (i.e. no excoriations, no marginal recurrence).

   ☐ YES ☐ NO

3. Is there a complete absence of exudate, meaning there cannot be any clear, serous, serosanguinous or purulent drainage?

   ☐ YES ☐ NO

4. There are no clinical signs of infection in and around the former wound site?

   ☐ YES ☐ NO

**Adjudicator Name / Signature**

**Adjudicator Name:**

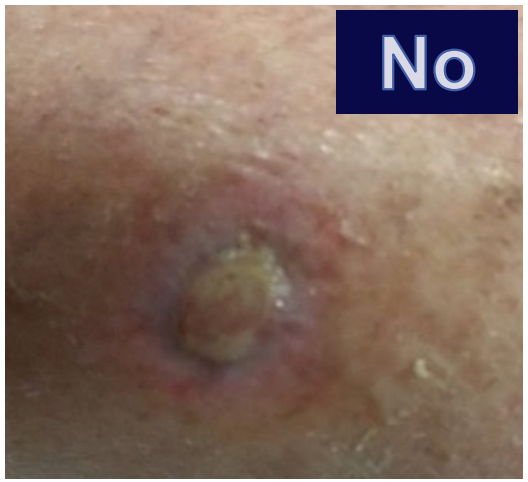
**Adjudicator Signature:** **Date:**

# Adjudication Case Examples

*Confidential*

# Adjudication Examples

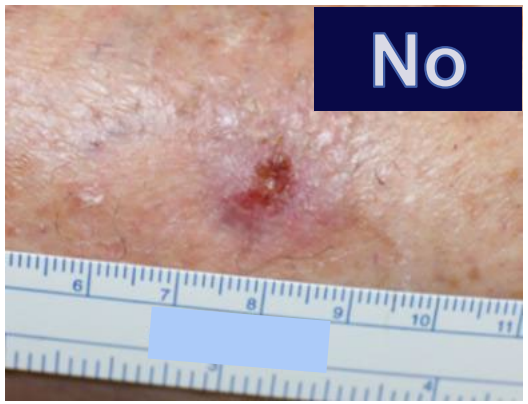


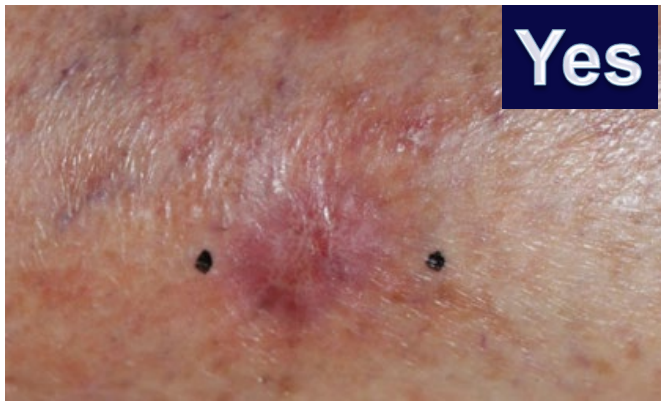









# Clinical Trial Cases

*Confidential*

# Case 1: Baseline



Initial wound size at baseline: 3.6cm$^2$

# Case 1: Week 9



50% improvement in wound area

# Case 1: Week 15



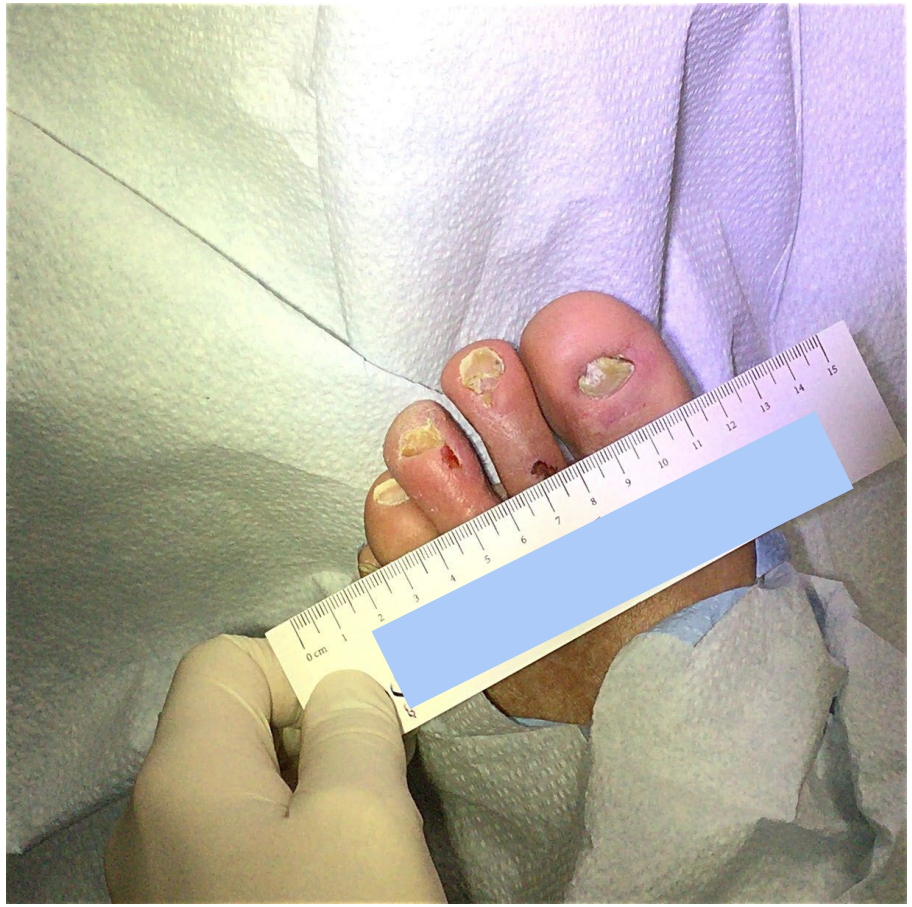
Healed after 4 injections

# Case 2: Baseline




Initial wound size at baseline: 1.9cm$^2$

# Case 2: Week 4



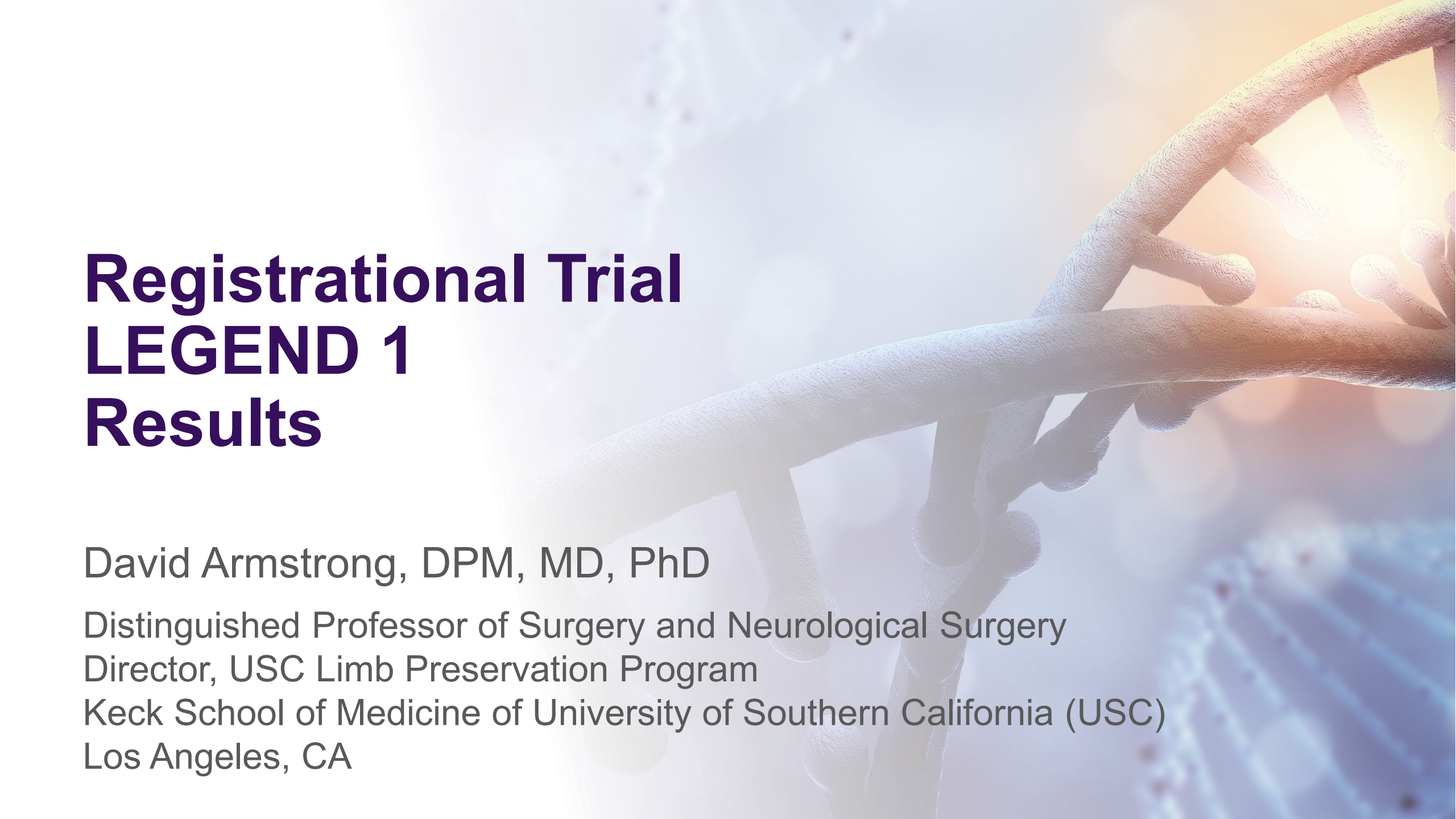
85% wound area reduction

# Case 2: Week 7



Healed after 2 injections

# Registrational Trial LEGEND 1 Results



David Armstrong, DPM, MD, PhD

Distinguished Professor of Surgery and Neurological Surgery
Director, USC Limb Preservation Program
Keck School of Medicine of University of Southern California (USC)
Los Angeles, CA

# BASELINE DEMOGRAPHICS AND SUBJECT CHARACTERISTICS

| | Placebo (N=26) | 4.0 mg AMG0001 (N=22) | 8.0 mg AMG0001 (N=27) | Overall (N=75) |
|---|---|---|---|---|
| Age* [years] | | | | |
| Mean (SD) | 63.0(11.6) | 62.9(12.5) | 62.1(13.6) | 62.7(12.6) |
| Sex | | | | |
| Male | 23 (88.5%) | 18 (81.8%) | 19 (70.4%) | 60 (80%) |
| Female | 3 (11.5%) | 4 (18.2%) | 8 (29.6%) | 15 (20%) |
| Ethnicity | | | | |
| Hispanic or Latino | 8 (30.8%) | 9 (40.9%) | 6 (22.2%) | 23 (30.7%) |
| Not Hispanic or Latino | 18 (69.2%) | 13 (59.1%) | 21 (77.8%) | 52 (69.3%) |
| Race | | | | |
| Asian | 0 | 0 | 1 (3.7%) | 1 (1.3%) |
| Black or African American | 1 (3.8%) | 5 (22.7%) | 5 (18.5%) | 11 (14.7%) |
| White | 25 (96.2%) | 17 (77.3%) | 21 (77.8%) | 63 (84%) |
| BMI Mean (SD) | 32.9(7.7) | 32.3(6.1) | 31.1(6.6) | 32.1(6.8) |
| Diabetes | 19 (73.1%) | 14 (63.6%) | 20 (74.1%) | 53 (70.7%) |

p>0.05 for all

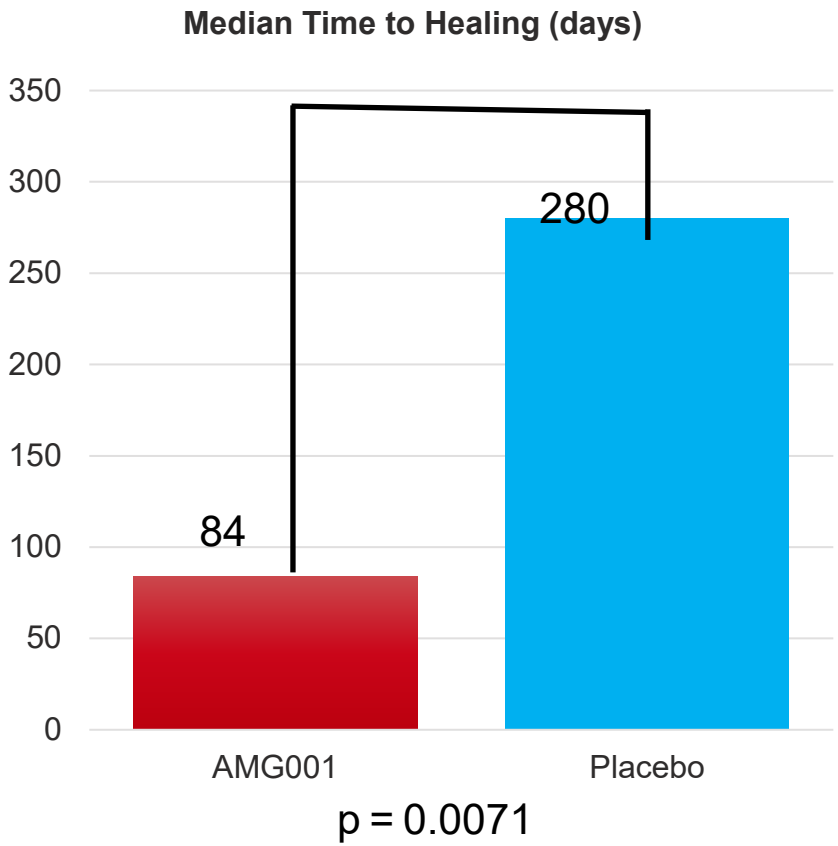
# BASELINE WOUND CHARACTERISTICS

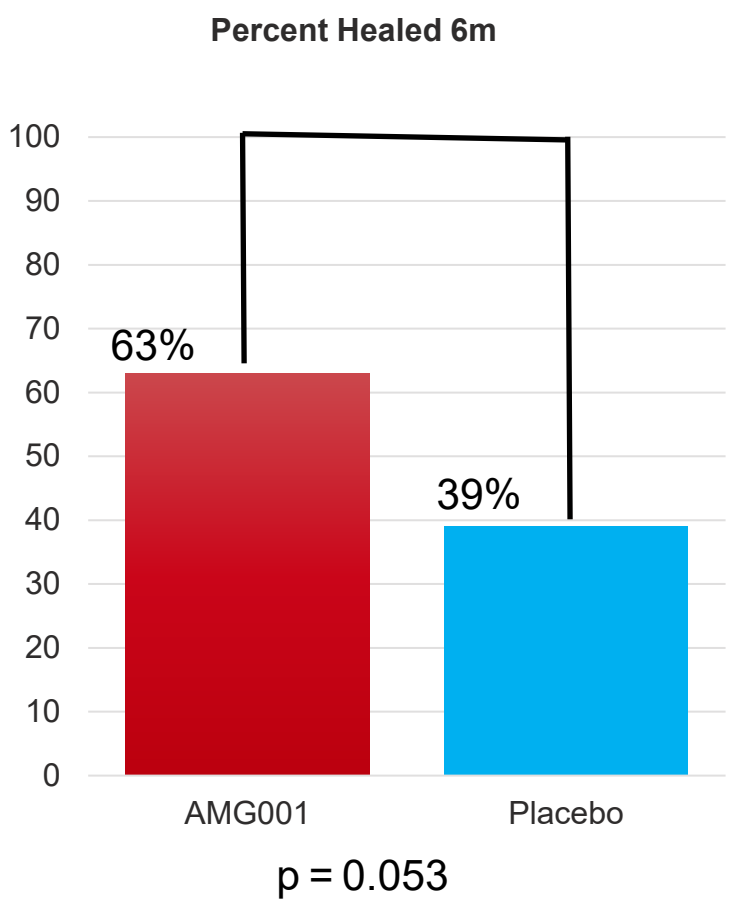
| Characteristic | Placebo (N=26) | 4.0 mg AMG0001 (N=22) | 8.0 mg AMG0001 (N=27) | Overall (N=75) |
|---|---|---|---|---|
| Ulcer Size ($cm^2$) | | | | |
| Mean/Median/SD (Range) | 3.6/2.7/2.5 (1.0-10.0) | 3.5/2.8/2.5 (1.0-9.6) | 3.2/1.8/2.4 (1.1-8.6) | 3.4/2.3/2.5 (1.0-10.0) |
| Ulcer ≤5 $cm^2$ | 20 (76.9%) | 18 (81.8%) | 21 (77.8%) | 59 (78.7%) |
| Ulcer >5 $cm^2$ | 6 (23.1%) | 4 (18.2%) | 6 (22.2%) | 16 (21.3%) |
| Wound age (months) | | | | |
| Median (median range) | 5.2 | 6.3 | 4.6 | (4.6-6.3) |
| ≤9 months | 20 (76.9%) | 17 (77.3%) | 20 (74.1%) | 57 (76.0%) |
| > 9 months | 6 (23.1%) | 5 (22.7%) | 7 (25.9%) | 18 (24.0%) |
| Hemodynamics (mm Hg)* | | | | |
| Index Leg Toe pressure mean (SD) | 47.0 (8.1) | 45.3 (6.9) | 45.3 (7.0) | 46.1 (7.0) |
| Index Leg Ankle pressure mean (SD) | 67.1 (NA) | 90.0 (NA) | 63.5 (9.2) | 71.0 (13.8) |
| $TcPO_2$ mean (SD) | 52.2 (5.8) | 49.6 (8.9) | 47.9 (8.3) | 49.8 (7.8) |
| WIfI Ischemia Grade | | | | |
| Grade 1 | 23 (88.5%) | 18 (81.8%) | 18 (66.7%) | 59 (78.7%) |
| Grade 2 | 3 (11.5%) | 4 (18.2%) | 9 (33.3%) | 16 (21.3%) |

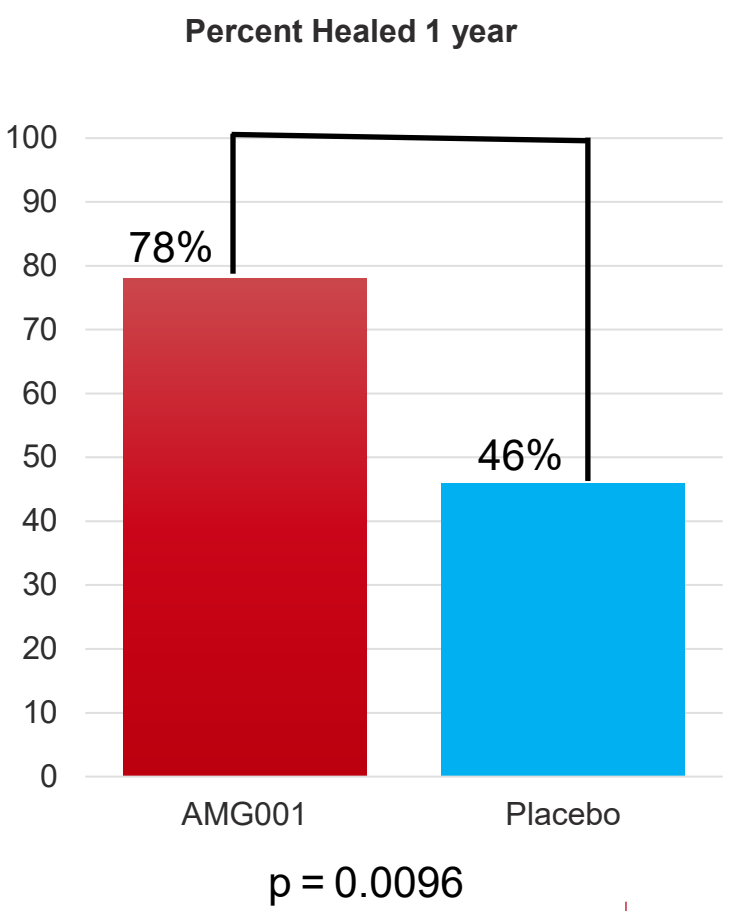
p>0.05 for all

# RESULTS

## Time to Healing and Proportion of Healing (pooled 4 and 8mg)

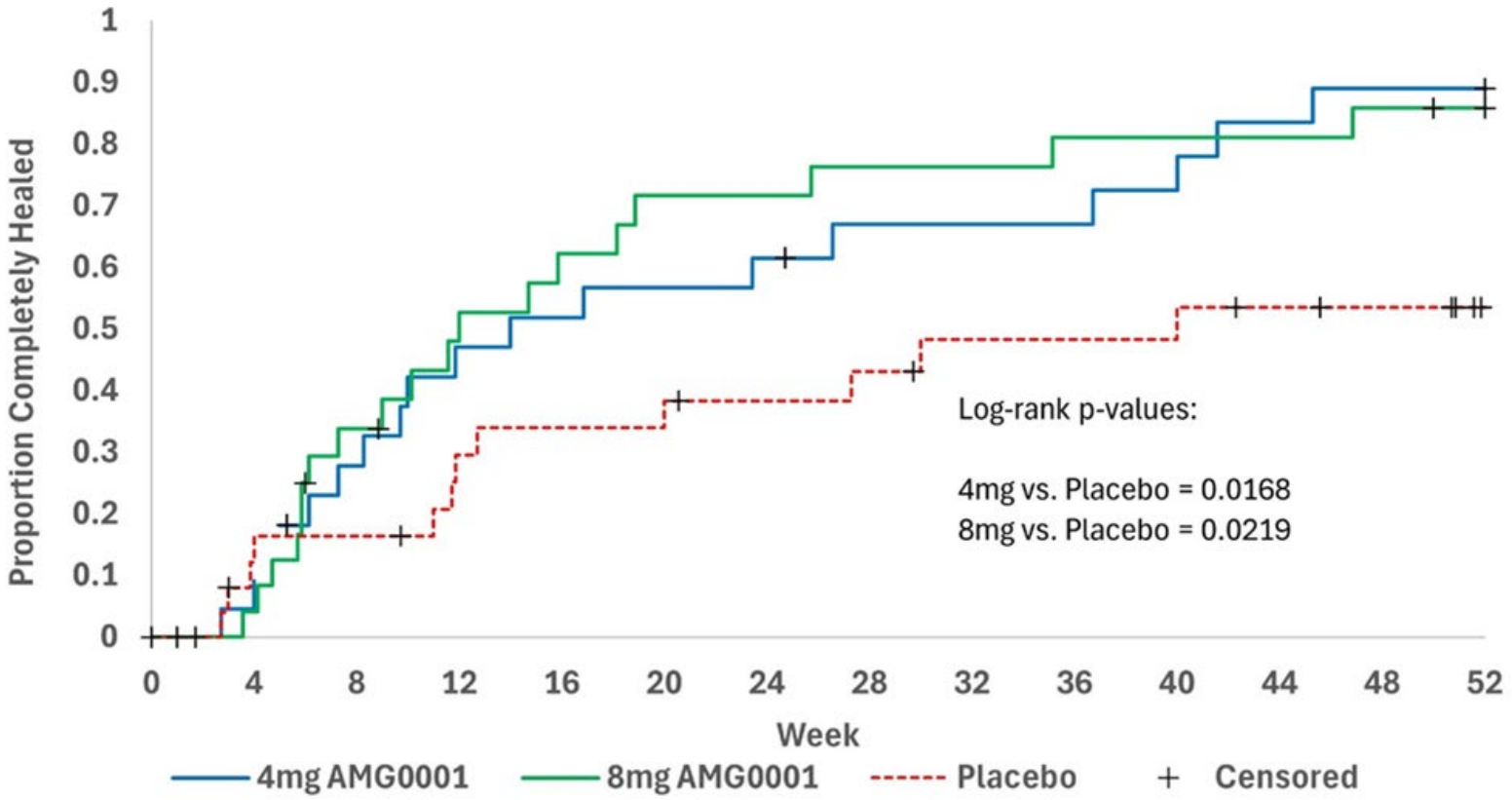


# RESULTS

**Kaplan-Meier Plot for Time to Complete Ulcer Healing by AMG0001 Doses and Placebo – mITT Population**



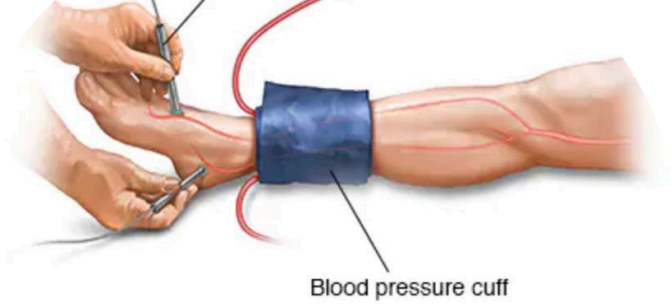
# SUBGROUP AND SECONDARY OUTCOMES

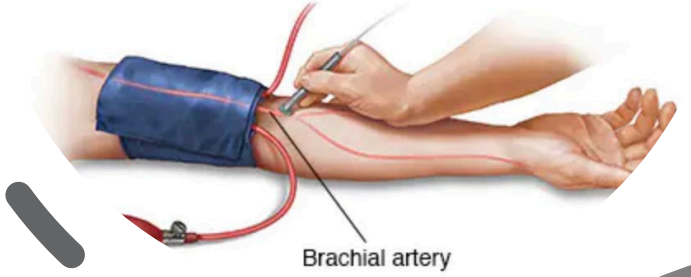
- AMG0001 doses effective regardless of ulcer size (dichotomized at 5 cm2) (p=0.03)
- Ulcer recurrence
  - AMG0001-treated patients (31.6%) compared to placebo (50%) (p>0.05)



# EXPLORATORY OUTCOMES

- Hemodynamics
  - Increase in ABI at 6 months for the AMG0001 4.0 mg group compared to placebo (+0.13; p=0.03)
- Tissue Perfusion
  - Increase from baseline in the dorsal foot TcPO2 at 12 months in the AMG0001 4.0 mg group compared to placebo (+18.5mmHg; p=0.02)






# SAFETY PROFILE

- Overall, there was a low incidence of treatment-emergent adverse events (TEAEs) without evidence of a dose-related trend
  - Most common: bruising, muscle spasm, pain
  - No injection-site infections requiring abx
- **Three deaths** were reported: two in the placebo group and one in the 8.0 mg AMG0001 group
- TEAEs were **evenly distributed** across treatments with no dose-related trend, including serious AEs
- Two TEAEs in the 8.0 mg group led to withdrawal from the study drug; however, these were not considered related to the treatment by the investigator.
  - MVA and DVT

# DISCUSSION AND CONCLUSION

- Among people with CLTI (WIfI Stage 1 and 2), first anatomically-directed gene therapy to demonstrate improved wound healing
- Appears to be well-tolerated
- HGF gene transfer may offer a promising non-surgical option for treating people with CLTI and lower extremity tissue loss
- Further studies are warranted to confirm these encouraging findings



# DISCUSSION AND CONCLUSION

**Next Step:**
**Successful**
**BLA License Application**

