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A Randomized, Controlled Pilot Study of Autologous CD34+ Cell Therapy for Critical Limb Ischemia

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Abstract

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Background—Critical limb ischemia (CLI) portends a risk of major amputation of 25-35% within 1 year of diagnosis. Pre-clinical studies provide evidence that intramuscular injection of autologous CD34+ cells improve limb perfusion and reduce amputation risk. In this randomized, double-blind, placebo-controlled pilot study, we evaluated the safety and efficacy of intramuscular injections of autologous CD34+ cells in subjects with moderate or high-risk CLI who were poor or non-candidates for surgical or percutaneous revascularization (ACT34-CLI).

Methods and Results—Twenty-eight CLI subjects were randomized and treated: 7 to 1×10^5 (low-dose) and 9 to 1×10^6 (high-dose) autologous CD34+ cells/kg; 12 to placebo (control). Intramuscular injections were distributed into 8 sites within the ischemic lower extremity. At 6 months post-injection 67% of control subjects experienced a major or minor amputation versus 43% of low-dose and 22% of high-dose cell-treated subjects (P=0.137). This trend continued at 12 months with 75% of control subjects experiencing any amputation versus 43% of low-dose and 22% of high-dose cell-treated subjects (P=0.058). Amputation incidence was lower in the combined cell-treated groups compared with control group (6 months: P=0.125; 12 months: P=0.054), with the low-dose and high-dose groups individually showing trends towards improved amputation free survival at 6 and 12 months. No adverse safety signal was associated with cell administration.

Conclusions—This study provides evidence that intramuscular administration of autologous CD34+ cells was safe in this patient population. Favorable trends toward reduced amputation rates in cell-treated versus control subjects were observed. These findings warrant further exploration in later phase clinical trials.

Keywords

peripheral vascular disease; revascularization; reperfusion; randomized trial; stem cells

The age-adjusted prevalence of peripheral arterial disease (PAD) in the U.S. population has been estimated to approach 12%.[1] The clinical consequences of symptomatic occlusive peripheral arterial disease (PAD) include intermittent claudication (IC), i.e., pain with walking, and critical limb ischemia (CLI), which includes pain at rest and loss of tissue integrity in the distal limbs, i.e., non-healing ulcers or gangrene.[2,3] The worldwide incidence of CLI is estimated to be 500 to 1000 cases per million people per year.[2]

The first objective in treating CLI is to increase perfusion to the affected limb. Surgical bypass techniques and percutaneous catheter-based interventions have both been used to successfully revascularize the limbs of patients with CLI. In many patients, however, heanatomic extent and distribution of arterial occlusive disease is too severe or advanced to permit relief of pain and/or facilitate healing of ischemic ulcers. It is estimated that up to 50% of CLI patients are not suitable candidates for surgical options.[4] No effective medical therapy is available for the treatment of such patients.

The rationale for this clinical study is based upon preclinical studies of CD34+ cell transplantation using in vivo models of hind limb ischemia which demonstrated that intramuscular administration of human CD34+ cells could augment perfusion and reduce the incidence of amputation.[5,6] We evaluated the safety and potential efficacy of intramuscular injection of autologous CD34+ cells in subjects with moderate or high-risk CLI who were poor or non-candidates for surgical or percutaneous revascularization (ACT34-CLI).

Methods

Study Design

The ACT34-CLI study was a prospective, double-blind, randomized, placebo-controlled clinical pilot study conducted at 14 centers in the United States. A total of 28 subjects were randomized 1:1:1 to 3 treatment groups: low-dose (1×10^5 Auto-CD34+ cells/kg, N=7), high-dose (1×10^6 Auto-CD34+ cells/kg, N=9), and control (placebo, N=12). The objectives of this Phase I/IIa clinical trial were to evaluate the safety and bioactivity of intramuscular injection of Auto-CD34+ cells in subjects with Rutherford Categories 4 and 5 who were not amenable to percutaneous or surgical revascularization. The institutional review board at each center approved the protocol, and all subjects provided written informed consent. The principal investigator (D. Losordo) was the IND holder and had responsibility for the conduct of the study, and Baxter Healthcare funded the study. Safety data were monitored by an independent Data Safety Monitoring Board.

Study Population

Male or female patients aged 21 years or older with Rutherford categories 4 or 5 CLI and no suitable revascularization options (determined by independent vascular surgeons and vascular interventionists) were eligible for this study. In addition, demonstrated infra-inguinal atherosclerosis with a stenosis (>70%) or occlusion (100%) of a major vessel and an absolute ankle pressure in the affected limb of <60 mmHg or a reduced toe pressure of <40 mmHg or abnormal PPG, diagnostic of microvascular insufficiency (flat wave forms) were required.

Candidates were excluded* if arterial insufficiency in the lower extremity was the result of a non-atherosclerotic disorder, including but not limited to, advanced scleroderma (CREST syndrome). Additional exclusion criteria included patients with advanced CLI (Rutherford Category 6), expected amputation within 4 weeks of screening, clinical evidence of sepsis, advanced AV block or NYHA Class III or Class IV heart failure, myocardial infarction within 3 months, or clinically successful aortic or lower extremity arterial surgery, percutaneous revascularization, or lumbar sympathectomy within 3 months preceding screening.

Auto-CD34+ Cell Mobilization, Collection, and Preparation

To maintain the double-blind design, all subjects underwent cell mobilization with 5 μg/kg per day doses of granulocyte colony stimulating factor (G-CSF) (Filgrastim/Neupogen®, Amgen, Thousand Oaks, CA) administered subcutaneously for 4 or 5 days followed by leukapheresis on the fifth day. The following day, the leukapheresis product was enriched for CD34+ cells using the ISOLEX 300*i* Magnetic Cell Selection System (Baxter Healthcare, Deerfield, IL). Lot release testing was performed on the final cell preparation to document sterility (gram stain and subsequent culture), viability (7-AAD apoptosis staining)[7] and purity (fluorescence activated cell sorting for CD34+ cells). Auto-CD34+ cells were suspended in 4 mLs of 0.9% NaCl (saline) plus 5% autologous plasma and provided to the investigator in 8 syringes.

Randomization and Blinding

Once the cell product passed all lot release criteria, the subject was randomized to 1 of the 3 treatment arms. Subjects were prospectively stratified centrally for Rutherford category 4 or 5, presence or absence of diabetes mellitus, and smoker or non-smoker. The investigator,

*Thromboangiitis obliterans (Buerger's Disease) was allowed subject, study site personnel, core laboratory(ies), blinded study statistician, and all Sponsor and CRO personnel remained blinded to all subject treatment.

Cell Injection Procedure

On the day of randomization, the total cell dose was delivered via intramuscular injection into 8 distinct sites (0.5 mL/site) in the ischemic lower extremity using a 1 mL syringe fitted with a 27 gauge needle. In the majority of subjects in which ischemia was most prominently manifested in the distal lower extremity (below knee), the 8 injections were distributed in the proximal, mid and distal calf, according to the subject's clinical status and vascular anatomy, targeting ischemic muscle supplied by occluded or stenotic arteries.

Endpoints

Safety—The primary endpoint of this exploratory study was the safety of intramuscular injection of Auto-CD34+ cells. Adverse events (AEs), vital signs, and laboratory assessments (clinical chemistry, hematology, cardiac biomarkers and urinalysis) were assessed during the treatment period (G-CSF cell mobilization, apheresis and intramuscular injection) and during the follow-up period at weeks 2, 4, 6, 8 and 12 weeks and 6 and 12 months.

Efficacy—To assess limb salvage, the occurrence of amputation, nature of amputation (toe or transmetatarsal, below or above knee, preserving or not preserving function), and time to amputation were recorded during the 12 month follow-up period.

A subject diary was used to record rest pain. Subjects began recording rest pain in their diaries 7 days prior to each follow-up visit. Changes from baseline in the duration, frequency and intensity (numerical rating pain scale from 1 [least pain] to 10 [greatest pain]) of rest pain, analgesic use, and sleep history were assessed.

The Six Minute Walk test was performed at baseline, week 12, and months 6 and 12 to assess functional improvement in subjects. The Modified Borg Scale was used to measure fatigue and a baseline score was determined before beginning the test. All symptoms, walking distance, time to onset of leg cramping/pain were recorded.

A core lab (Canfield) was utilized for wound assessment. Assessment included ulcer tracing and photography of the wound. Acetate tracings of the wound and digital planimetry were used to assess changes from baseline in ulcer size (area). Time to complete healing or change to a state of potentially successful surgical closure or skin grafting was recorded.

Quality of life (QoL) was assessed using the SF-36 QoL questionnaire (version 1).

Disease severity was assessed by changes from baseline in the Rutherford Clinical Severity score, absolute ankle and toe pressure, and ankle and toe brachial index (ABI and TBI, respectively).

Statistical Analysis

This study was designed to help determine the selection of endpoints, time points and the appropriate sample sizes for subsequent clinical studies of Auto-CD34+ cells for subjects with CLI. All analyses performed were intent to treat. Efficacy analyses were exploratory in nature and no corrections for multiple comparisons or formal sample size calculations were performed. Baseline characteristics were summarized. One-way ANOVA was used to test for differences in the treatment groups for continuous variables and Fisher's exact test was used for categorical variables. AEs were summarized. Fisher's exact test was used to test for differences between treatment groups in percent of subjects with amputations. Log-Rank tests were used to test for differences in the distributions of time to first amputation. The adjusted amputation rates were calculated assuming that amputations have a negative binomial distribution. Changes in function and disease severity over time are presented descriptively; no statistical analysis was performed.

Results

Subject Disposition and Baseline Characteristics

Between November 2007 and April 2010, 14 centers across the United States screened 43 subjects; 28 subjects met the entry criteria for this study and underwent G-CSF cell mobilization, apheresis to collect total mononuclear cells, randomization and intramuscular injections of Auto-CD34+ cells or placebo (Figure 1). In total 20 subjects completed the 1 year study follow-up period.

There were no statistically significant differences in subject baseline demographics, medical history and disease characteristics between treatment groups (Table 1). The study population included 9 females and 19 males with a mean age of 67 years. Previous lower extremity bypass surgery or PCI had been performed in all subjects.

Safety of Auto-CD34+ Cell Therapy during Treatment and Follow-up Period

A total of 60 serious AEs (SAEs) in 22 (79%) subjects occurred during the study of which 59 occurred after intramuscular injection and 1 occurred during mobilization. The majority of SAEs were considered unrelated to study treatment by the investigator with the exception of 2 SAEs which were considered possibly study-related: 1 subject experienced moderate hypotension during mobilization which required prolonged hospitalization, and 1 subject experienced severe worsening of CLI in the target leg after injection which required prolonged hospitalization. Only 1 SAE was cardiac-related: 1 subject in the control group experienced an acute non-ST segment elevation MI approximately 4.5 months post-injection. There were 2 deaths during the study which were not considered study-related and these were the only subjects to discontinue due to an AE.

Predominantly modest and exclusively asymptomatic elevations in cardiac enzyme levels were observed during the mobilization (G-CSF) and injection period (Table 2). Of the subjects with cardiac enzyme measurements, elevated troponin, CK-MB and CK levels ($> 1 \times$ ULN) were observed in 9 (56.3%), 13 (54.2%) and 4 (15.4%) of subjects, respectively, during the mobilization and injection period. During the follow-up period, elevated troponin, CK-MB and CK levels ($> 1 \times$ ULN) were observed in 5 (27.8%), 8 (30.8%) and 5 (17.9%) of subjects, respectively.

Amputation

All efficacy analyses were exploratory in nature as the study was not powered to detect differences among treatment groups in efficacy parameters. At 6 months post-injection, 8 (66.7%) subjects in the control group, 3 (42.9%) subjects in the low-dose group and 2 (22.2%) subjects in the high-dose group experienced an amputation ($P = 0.137$, Table 3). Major amputations occurred in 4 subjects in the control group, in 3 subjects in the low dose group and 2 in the high dose group ($P=0.780$). At 12 months post-injection there was no increase in the incidence of amputations in the cell-treated groups from the 6-month post-injection time point, but the incidence increased slightly in the control group (9 [75.0%] subjects; $P=0.058$). The incidence of major amputations was slightly higher in the control group (N=6 [50%]) compared with the cell-treated groups, but this difference was not statistically significant ($P = 0.488$). Trends toward lower amputation rates in the cell-treated groups versus the control group were observed at 6 months (P=0.187) and 12 months post-injection (P=0.121). Statistically significant differences in major amputation rates among the control and cell-treated groups at 6 months (P = 0.303) and 12 months (P = 0.430) post-injection were not detected.

When subjects in the cell-treated groups are combined, the incidence of total amputations at 6 months and 12 months post-injection was 66.7% in control versus 31.3% in cell-treated subjects (P=0.125) and 75.0% in control versus 31.3% in cell-treated subjects (P=0.054), respectively. The incidence of major amputations at 6 months and 12 months post-injection was 33.3% in control versus 31.3% in cell-treated subjects (P=1.000) and 50.0% in control versus 31.3% in cell-treated subjects (P=0.441), respectively.

There were trends toward an increased probability of amputation free survival in the low-dose and high-dose groups compared with the control group during 12 month post-injection follow-up period (P=0.35, Log Rank Test, Figure 2a). When the cell-treated groups are combined, the probability of amputation free survival was significantly increased in the cell-treated group compared with the control group (P=0.013; Figure 2b). A trend toward improved major amputation free survival was observed in the individual cell-treated groups (Figure 2c) and combined cell-treated group (Figure 2d) compared with the control group (P=0.414 and P=0.294, respectively).

Functional Improvement, Wound Healing, and Rest Pain

Of the 28 subjects enrolled in the study, 22 completed the 6 minute walk test at baseline (Table 4). A total of 11 subjects completed the 6 minute walk test at the 6 and/or 12 month post-injection timepoints (Figure 3). In the control group (N=4), the distance walked increased for 2 subjects and decreased for 2 subjects. In the cell-treated groups (N=7), the distance walked increased for 6 subjects and decreased slightly for 1 subject. Eleven subjects did not complete the test at 6 and 12 months post-injection for the following reasons: amputation (N=7), withdrawal from study (N=3) and unknown (N=1).

A total of 14 subjects had leg ulcers at baseline (Table 4). At 6 months post-injection, wound area measurements were not reported in 5 of the 14 subjects with ulcers at baseline due to amputation (Figure 3). At 12 months post-injection, wound area measurements were not reported in an additional 4 subjects due to subject withdrawal (N=2) or assessment not performed (N=2). There were no treatment-related trends in terms of wound healing observed at 6 months or 12 months post-injection.

A total of 27 subjects completed the pain diary at baseline. Overall, decreases in the median number of pain episodes per week and the average pain intensity scores were observed in all groups at 6 months and 12 months post-injection (Table 4).

Disease Severity

There were minor fluctuations in the ABI and TBI among subjects in all treatment groups with preserved limbs and measurements at 6 and 12 months (Table 5). Of the subjects with Rutherford score data at 6 months and 12 months post-injection (N=13 and N=11, respectively), the mean Rutherford score decreased from baseline in all treatment groups at 6 months post-injection and in the control and high-dose groups at 12 month post-injection. The mean Rutherford score in the low-dose group remained unchanged from baseline at 12 months post-injection.

Quality of Life

At 6 months post-injection, improvements from baseline in the majority of health domain scores were observed in all treatment groups (Figure 4). At 12 months post-injection, improvements in the mean scores were observed in 8, 5 and 3 of the health domains in the low-dose, high-dose and control groups, respectively.

Discussion

The results from this Phase I/IIa pilot study provide initial evidence that intramuscular injection of Auto-CD34+ cells is safe and well-tolerated in patients with moderate or high-risk CLI who are poor or non-candidates for surgical or percutaneous revascularization.

Trends toward decreased amputation in Auto-CD34+ cell-treated subjects compared with control subjects demonstrate the potential efficacy of Auto-CD34+ cell therapy in this population. One must use caution in interpreting these results, however, since a higher percentage of subjects experienced amputations during this study relative to other recent clinical studies of CLI.[8,9,10,11,4]

Several surrogate markers (ABI, TBI, leg pain, walking distance, wound healing) of limb perfusion were explored and no differences were detected between the cell-treated and control groups; however, this study was not powered to detect differences in efficacy endpoints. In addition, the high rate of amputation observed in this study resulted in missing data for several of these endpoints, making it difficult to draw any conclusions. No differences in QoL were detected among the cell-treated and control groups.

In theory, increased blood flow could be achieved by increasing the number of vessels that supply the ischemic tissue with blood. The use of pharmacological or biological therapies to induce new blood vessel growth for the treatment or prevention of pathological clinical conditions has been termed therapeutic angiogenesis.[12,13] The mechanism of action for the majority of pharmacological therapies tested for CLI is vasodilation and promotion of angiogenesis with agents such as prostaglandins. Two randomized, double-blind, phase 3 studies of lipo-ecraprost as a parenteral therapy or as an adjunctive parenteral therapy after distal revascularization in subjects with CLI did not, however, improve major amputation or survival outcomes.[9,10]

Biological therapies, including gene therapy and stem cell therapy, have been evaluated in patients with CLI for improving perfusion in ischemic tissues.[14,15,16,17,11,18,19] Stem cell therapy for the treatment of CLI is an emerging therapy in which unselected bone marrow mononuclear cells (BM-MNC) or BM-MNC selected to express particular cell surface markers are delivered via intramuscular or intraarterial injection. While a limited number of blinded, randomized controlled trial (RCTs) evaluating cell therapy for no-option CLI patients have been performed, results from several early phase studies show no safety signal and demonstrate favorable trends in efficacy parameters for cell-treatment versus control. In the TACT study a significant increase in ABI and TcPO2 was observed in subjects treated with BM-MNC compared with those treated with peripheral blood MNC.[20] Interim results from RESTORE-CLI, a blinded RCT in which bone marrow aspirate was processed to generate the tissue-repair cell (TRC) population of stem and progenitor cells, demonstrated that TRC-treated subjects had increased amputation free survival and time to treatment failure compared with placebo subjects.[21] Treatment with BM-MNCs in the PROVASA trial was associated with improved ulcer healing and reduced rest pain compared with placebo.[22] A recent report of 12-week data from a RCT of bone marrow aspirate concentrate (BMAC) demonstrated favorable trends for BMAC versus control in major amputations and improved pain, ABI, Rutherford classification and QoL.[23] The results of these studies are encouraging; however, the variability between studies in the efficacy endpoints that detected differences between the cell-treated and control groups highlight the challenges of choosing clinically meaningful measures of efficacy in this population.

In our study we chose to isolate and administer CD34+ cells for 2 principal reasons: 1 - because of their demonstrated pro-angiogenic potential in vivo, [5,6] and 2- because an available, approved technology permitted the "manufacturing" of CD34+ cell preparations by standardized methods. The advantage of this approach is that selection of CD34+ cells results in a higher concentration of endothelial progenitor cells in each dose compared with unselected MNCs resulting in greater therapeutic potency in preclinical models. There are no known disadvantages of this approach other than the added step in cell processing. A theoretical disadvantage of this approach is the possibility that other cell types, which may exert pro-angiogenic or reparative functions, are removed; however the evidence from pre-clinical models does not support this concept. Similar to the studies described above, we observed favorable trends in efficacy including reduced amputation rates and improved amputation free survival in the cell-treated groups compared with the control group. The high rate of amputations observed in our study, however, limited the interpretation of other efficacy endpoints.

Taken together, the results from our pilot study and other early stage studies provide evidence for the safety and potential bioactivity of stem cell therapy for CLI. Multiple early phase studies of additional stem cell therapies are currently underway.[24] Large randomized, placebo-controlled, double-blind studies are necessary; however the high cost of conducting trials in this patient population remains a significant challenge, particularly for earlier stage companies attempting to develop novel therapeutics. In addition, the large variability observed in amputation rates in the phase 2 and phase 3 studies of FGF1 gene therapy[8,17] suggests that a better understanding of the no-option CLI population is necessary such that patient demographics, physiological characteristics, biomarkers or yet to be defined genetic markers can be used to better predict event rates in this population.

In conclusion, the overall positive safety profile of collecting and administering autologous CD34+ cells in this patient population as well as the potential efficacy of preventing amputations warrant larger scale studies to verify these findings, and to further refine the methods for collecting and administering Auto-CD34+ cells to patients with disabling CLI.

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What is known

Human CD34+ cells are well known as hematopoietic stem cells used for stem cell transplants in patients who have bone marrow ablation by chemotherapy or radiation therapy.

Preclinical studies in models of myocardial or limb ischemia show that local delivery of human CD34+ cells improves perfusion and function in ischemic tissue.

What this article adds

In a double-blind, randomized, placebo controlled pilot clinical trial in patients with Rutherford Class 4 and 5 critical limb ischemia, direct intramuscular injection of autologous CD34+ cells was associated with reductions in the frequency of amputation.

The strategy of mobilizing and collecting autologous CD34+ cells in CLI patients was shown to be feasible and was not associated with an adverse safety signal. Further study is warrented.

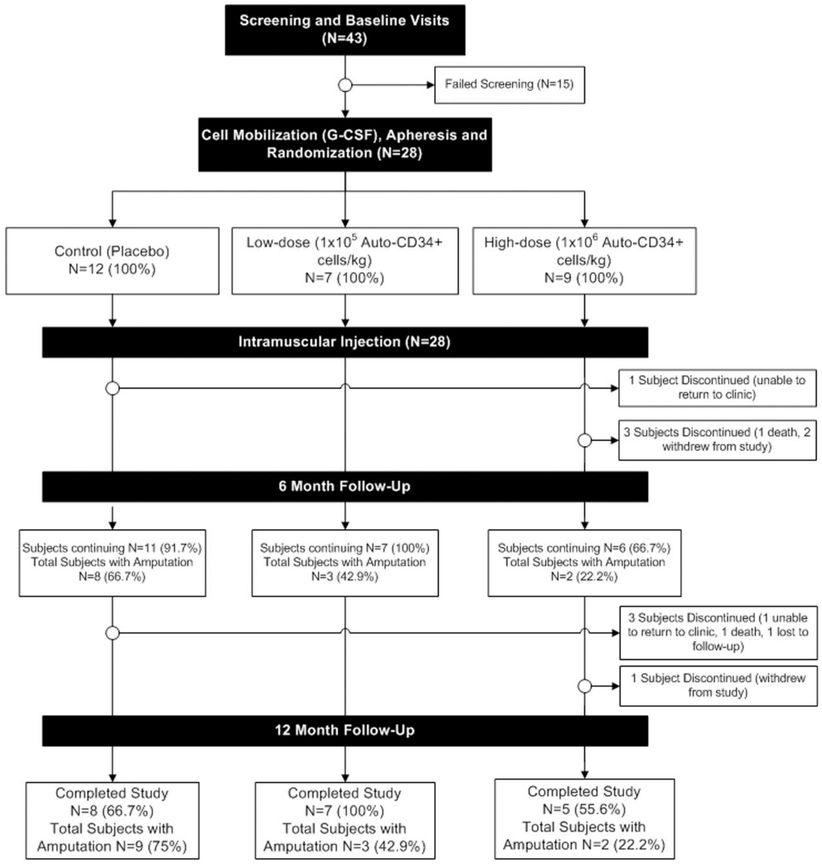

Figure 1. Study Design and Subject Disposition

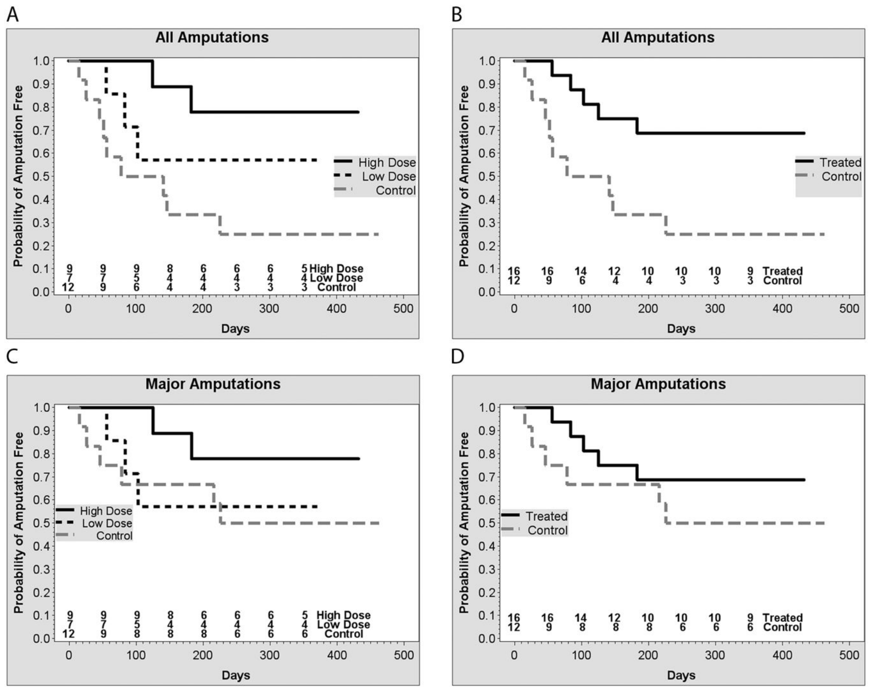

Figure 2. Probability of Amputation Free Survival

Probability of amputation free survival in low-dose, high-dose and control groups (A) and in combined cell-treated and control groups (B). Probability of major amputation free survival in low-dose, high-dose and control groups (C) and in combined cell-treated and control groups (D).

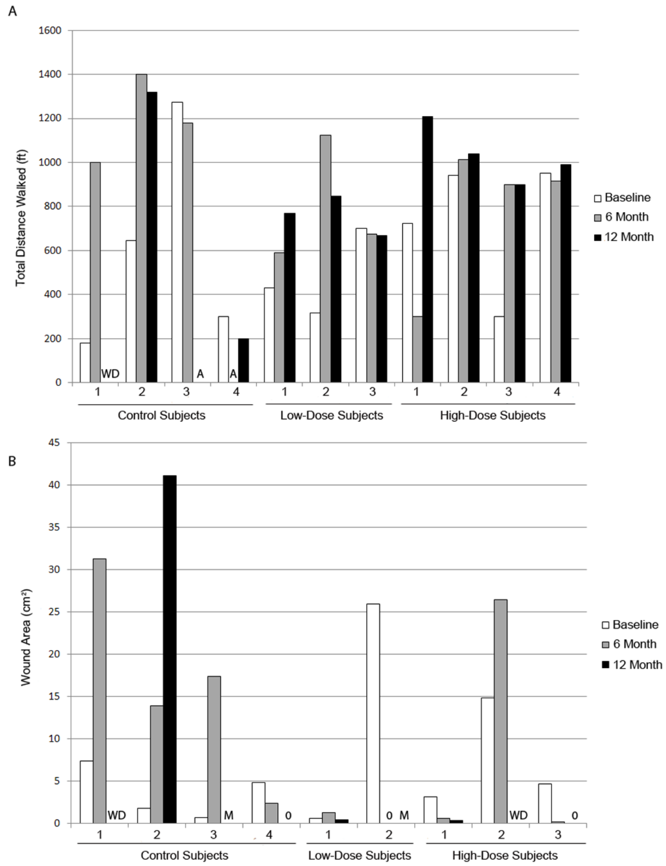

Figure 3. Functional Improvement and Wound Healing at 6 and 12 Months

3A: Total distance walked on 6 minute walk test at baseline and 6 and 12 months post-injection in subjects that completed the test at 6 and/or 12 months post-injection. **3B**: Total wound area in the treated leg in subjects with ulcers reported at 6 and/or 12 months post-injection. Zero (0) indicates no leg ulcer present. C=control, L=low-dose, H=high-dose, WD=withdrawal, A=amputation, M=missing.

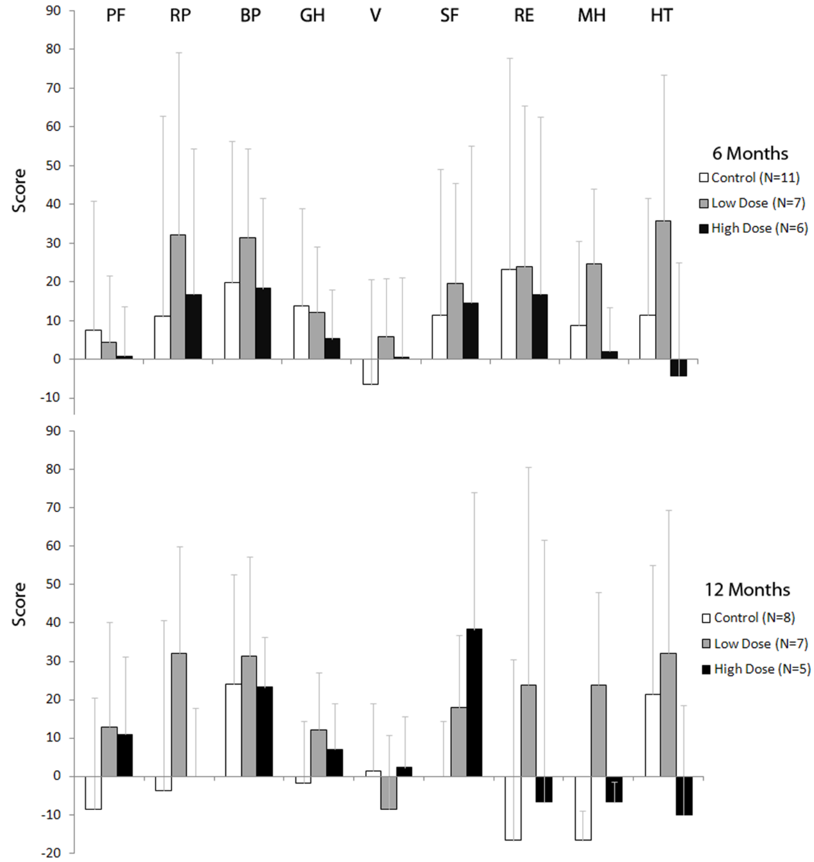

Figure 4. Summary of Mean Change from Baseline in SF-36 Health Domains

Mean (±SD) change from baseline in SF-36 health domains at 6 months and 12 months post-injection. PF=physical functioning, RP=role physical, BP=bodily pain, GH=general health, V=vitality, SF=social functioning, RE=role emotional, MH=mental health, HT=health transition.

Table 1
Baseline Characteristics of Study Population

	Control (N=12)	1×0^{5} c/kg (N=7)	1×10^{6} c/kg (N=9)	P-value
Demographics				
Age (mean ± SD)	67.1 ± 14.2	61.8 ± 13.9	69.7 ± 10.9	0.497
Female (%)	50.0	28.6	11.1	0.180
BMI (mean ± SD)	27.0 ± 4.7	29.3 ± 6.0	31.0 ± 7.9	0.348
PAD Risk Factors				
Hypertension (%)	83.3	85.7	77.8	1.000
Current, Former Smoker (%)	8.3, 58.3	57.1, 42.9	33.3, 44.4	0.179
Diabetes (%)	41.7	71.4	55.6	0.446
Hyperlipidemia (%)	58.3	57.1	66.7	1.000
Buerger's Disease (%)	0.0	14.3	0.0	0.250
Previous Vascular Interventions				
Prior Bypass Graft (%)	58.3	71.4	44.4	0.495
Prior Angioplasty (%)	58.3	57.1	55.6	1.000
Prior Endarterectomy (%)	33.3	14.3	11.1	0.507
Prior Stent Placement (%)	58.3	42.9	66.7	0.716
Concomitant Cardiovascular Disease				
Angina Pectoris (%)	16.7	0.0	0.0	0.492
Myocardial Infarction (%)	25.0	14.3	22.2	1.000
Congestive Heart Failure (%)	8.3	14.3	33.3	0.390
Coronary intervention (%)	41.7	42.9	44.4	1.000
Stroke (%)	8.3	0.0	0.0	1.000
Pulmonary embolism (%)	0.0	14.3	0.0	0.250
Medications				
Beta Blocker (%)	33.3	42.9	66.7	0.390
Ca^{2+} Blocker (%)	16.7	71.4	33.3	0.076
ASA (%)	83.3	71.4	66.7	0.645
Clopidogrel (%)	58.3	57.1	33.3	0.623
Statin (%)	83.3	71.4	88.9	0.690
ACE-inhibitor/ARB (%)	58.3	71.4	55.6	0.790
Vascular Measures*				
Absolute Ankle Pressure (mean ± SD)	0.37 ± 0.20	0.50 ± 0.32	0.48 ± 0.23	0.487
Toe Pressure (mean ± SD)	0.07 ± 0.10	0.30 ± 0.48	0.28 ± 0.39	0.326
Ischemic Rest Pain (Rutherford 4, [%])	41.7	57.1	55.6	0.746

	Control (N=12)	1×0^{5} c/kg (N=7)	1×10^{6} c/kg (N=9)	P-value
Minor Tissue Loss (Rutherford 5, [%])	58.3	42.9	44.4	

*In affected limb

Table 2

Summary of Subjects with Cardiac Enzyme Elevations

		Treatment Period % (N)						Follow-Up Period % (N)						
	Baseline % (N)	G-CSF D1	G-CSF D2	G-CSF D3	G-CSF D4	G-CSF D5	IM Injection	Week 2	Week 4	Week 6	Week 8	Week 12	Month 6	Month 12
≥1 × ULN														
CK (IU/L)	3.8% (1)	8.0% (2)	8.0% (2)	0	3.8% (1)	4.2% (1)	11.5% (3)	3.7% (1)	4.3% (1)	4.5% (1)	22.7% (5)	4.0% (1)	4.2% (1)	0
CK MB (μg/L)	28.6% (6)	17.4% (4)	26.1% (6)	26.1% (6)	37.5% (9)	39.1% (9)	37.5% (9)	12.5% (3)	14.3% (3)	25.0% (5)	28.6% (6)	18.2% (4)	20.0% (4)	6.3% (1)
Troponin I (ng/mL)	13.3% (2)	6.7% (1)	26.7% (4)	40.0% (6)	25.0% (4)	25.0% (4)	50.0% (8)	12.5% (2)	6.7% (1)	0	0	12.5% (2)	6.7% (1)	0
≥3 × ULN														
CK (IU/L)	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CK MB (μg/L)	0	4.3% (1)	4.3% (1)	4.3% (1)	4.2% (1)	8.7% (2)	12.5% (3)	4.2% (1)	0	5.0% (1)	9.5% (2)	0	0	0
Troponin I (ng/mL)	0	0	6.7% (1)	6.7% (1)	18.8% (3)	18.8% (3)	37.5% (6)	6.3% (1)	0	0	0	6.3% (1)	0	0

Percentages based on total number of subjects with enzyme measurements at the particular timepoint.

Table 3
Summary and Analysis of All Amputations by Treatment Group

	Control (N=12)	1×10^5 c/kg (N=7)	1×10^6 c/kg (N=9)	P-Value
6 Months				
% with Amputation (N)	66.7% (8)	42.9% (3)	22.2% (2)	0.137
% with Major Amputation (N)	33.3% (4)	42.9% (3)	22.2% (2)	0.780
Total Number of Amputations	9	5	2	
Adjusted Amputation Rate * (Lower, Upper 95% CI)	1.50 (0.78, 2.87)	1.42 (0.59, 3.42)	0.44 (0.11, 1.77)	0.187
Adjusted Major Amputation Rate * (Lower, Upper 95% CI)	0.66 (0.25, 1.77)	1.42 (0.59, 3.42)	0.44 (0.11, 1.77)	0.303
12 Months				
% with Amputation (N)	75.0% (9)	42.9% (3)	22.2% (2)	0.058
% with Major Amputation (N)	50.0% (6)	42.9% (3)	22.2% (2)	0.488
Total Number of Amputations	12	5	2	
Adjusted Amputation Rate * (Lower, Upper 95% CI)	1.00 (0.57, 1.76)	0.72 (0.30, 1.73)	0.26 (0.06, 1.03)	0.121
Adjusted Major Amputation Rate * (Lower, Upper 95% CI)	0.50 (0.22, 1.11)	0.72 (0.30, 1.73)	0.26 (0.06, 1.03)	0.430

* Amputations per year. Adjusted for different rates for each subject using Negative Binomial model.

Table 4

Summary of Change from Baseline in Function, Wound Healing and Pain

Parameter		Treatment Group	Baseline (N)	Change from Baseline (N)	
				Month 6	Month 12
6 Minute Walk		Control	175.4±88.7 (7)	66.5±263.8 (2)	-258.0 (1)
	Time to Leg Pain (s) *Mean ± SD*	Low Dose	212.2±89.4 (5)	−158.0±264.5 (2)	−92.0±108.9 (2)
		High Dose	142.4±34.7 (5)	122.7±78.0 (2)	−26.5±4.9 (2)
		Control	307.1±234.6 (7)	355.0±629.3 (2)	−300.0 (1)
	Distance to Leg Pain (ft) *Mean ± SD*	Low Dose	356.8±172.6 (5)	−55.0±304.1 (2)	64.0±161.2 (2)
		High Dose	306.7±135.9 (4)	321.5±454.7 (2)	−33.5 (1)
		Control	717.2±402.0 (9)	493.3±510.5 (3)	287.5±548.0 (2)
	Total Distance Walked (ft) *Mean ± SD*	Low Dose	527.3±371.9 (6)	315.0±438.5 (3)	279.3±286.9 (3)
		High Dose	704.0±334.3 (7)	53.5±421.5 (4)	307.0±278.5 (4)
Leg Ulcer		Control	5.7±6.2 (7)	2.8 ±11.2 (4)	17.3±31.2 (2)
	Area (cm^2) *Mean ± SD*	Low Dose	1.7±2.0 (3)	12.7±0.7 (2)	−0.2 (1)
		High Dose	8.7±5.8 (4)	0.1±10.4 (3)	−3.7±1.3 (2)
Rest Pain		Control	33.0 [3,123] (12)	-19.5 [-112, 18](10)	--20.0 [−37, −3](6)
	Number of Pain Episodes per Week *Median [min, max]*	Low Dose	10.0 [4,27] (7)	-8.5[-13,0] (4)	−8.5 [-10, -7](2)
		High Dose	17.5 [7,125] (8)	-11.0 [-15,0] (5)	-5.5 [-12,0](4)
		Control	5.4±1.3 (12)	−1.1±2.2 (10)	−0.9±3.3 (6)
	Pain Intensity Score *Mean ± SD*	Low Dose	6.7±3.0 (7)	−2.8±0.8 (4)	−3.3±1.6 (2)
		High Dose	6.0±2.0 (8)	−0.6±1.4 (5)	−0.5±1.8 (4)

Table 5

Summary of Change from Baseline in Disease Severity

Parameter		Treatment Group	Baseline (N)	Change from Baseline (N)	
				Month 6	Month 12
Ankle and Toe Pressure	***ABI (treated leg)*** *Median*[*min, max*]	Control	0.3 [0,0.8] (11)	0.1[−0.1,0.4] (6)	0.1 [−0.1,0.6] (5)
		Low Dose	0.5 [0,0.9] (7)	0.2 [−0.5,1.1] (4)	0.2 [−0.3,0.6] (4)
		High Dose	0.5 [0,0.7] (9)	0 [−0.2,0.4] (6)	0.1 [−0.1,0.1] (5)
		Control	0 [0,0.3] (9)	0 [0,0] (2)	−0.1 (1)
	TBI (treated leg) *Median*[*min, max*]	Low Dose	0.1 [0,1.3] (7)	0 [−0.5,0.2] (4)	0 [−0.4,0.3] (4)
		High Dose	0.1 [0,1.1] (8)	0 [0,0.1] (5)	0.1 [0.1,0.2] (4)
Rutherford Score	***Treated Leg*** *Mean ± SD*	Control	4.6±0.5 (12)	−1.7±2.0 (6)	−1.5±1.9 (4)
		Low Dose	4.4±0.5 (7)	−0.7±1.2 (3)	0.0±2.0 (3)
		High Dose	4.4±0.5 (9)	−1.0±2.4 (4)	−0.8±2.2 (4)