

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

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Background—The Study to Assess the Safety of Intramuscular Injection of Hepatocyte Growth Factor Plasmid to Improve Limb Perfusion in Patients With Critical Limb Ischemia (HGF-STAT trial) determined the effect of hepatocyte growth factor (HGF) plasmid on safety and limb tissue perfusion as measured by transcutaneous oxygen tension (TcPo₂) in patients with critical limb ischemia (CLI).

Methods and Results—Randomized patients with rest pain or ischemic ulcers and TcPo₂ <40 mm Hg and/or toe pressure <50 mm Hg received placebo or HGF-plasmid intramuscular injection as follows: 0.4 mg at days 0, 14, and 28 (low dose); 4.0 mg at days 0 and 28 (middle dose); or 4.0 mg at days 0, 14, and 28 (high dose). Patients were evaluated for safety, changes in TcPo₂ and ankle and toe pressure, amputation, and wound healing. Ninety-three of 104 treated patients were evaluated for safety (mean age 70 years, 63% male, 53% diabetic, 64% with tissue loss, mean ankle-brachial index 0.41, and mean toe pressure 26 mm Hg). Adverse events occurred in 86% of the patients, most of which were related to CLI or comorbid conditions and were not different between groups. TcPo₂ (mean±SE) increased at 6 months in the high-dose group (24.0±4.2 mm Hg, 95% CI 15.5 to 32.4 mm Hg) compared with the placebo (9.4±4.2 mm Hg, 95% CI 0.9 to 17.8), low-dose (11.1±3.7 mm Hg, CI 3.7 to 18.7 mm Hg), and middle-dose (7.3±4.8 mm Hg, CI -2.2 to 17.0 mm Hg) groups (ANCOVA *P*=0.0015). There was no difference between groups in secondary end points, including ankle-brachial index, toe-brachial index, pain relief, wound healing, or major amputation.

Conclusions—Intramuscular injection of HGF plasmid was safe and well tolerated. Larger studies to determine whether HGF plasmid can improve wound healing and limb salvage in patients with CLI are warranted. (*Circulation*. 2008;118:58-65.)

Key Words: critical limb ischemia ■ angiogenesis ■ gene therapy ■ peripheral vascular disease ■ atherosclerosis

Critical limb ischemia (CLI), the most severe form of peripheral arterial disease, due to atherosclerosis is a common clinical problem that has no effective medical therapy. The incidence of CLI is 125 000 to 250 000 patients per year in the United States and is expected to grow as the population ages. Even with current medical therapy, up to 40% of patients with CLI will require major limb amputation within 12 months, and the yearly mortality rate for this condition exceeds 20%.^{1,2} The standard therapy for CLI has remained lower-extremity revascularization, either through open bypass surgery or by endovascular techniques, or

lower-extremity amputation when revascularization is not an option.³ Open bypass surgery in most instances results in a 5-year limb salvage rate of >80% but is associated with a perioperative mortality rate of 5% and a complication rate of 30% to 50%,^{3,4} and up to 40% of patients are not candidates for open surgery. Endovascular therapy in the treatment of patients with CLI is evolving; however, this therapy is frequently not an option and is associated with inferior mid- to long-term patency. At present, there is a need for less invasive therapies to improve limb perfusion in patients with CLI.

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Therapeutic angiogenesis is an investigational therapy that attempts to improve perfusion to ischemic vascular beds through the formation of new blood vessels from preexisting blood vessels.⁵ Therapeutic angiogenesis can be mediated through delivery of recombinant protein, cell therapy, or gene transfer.^{6–12} Gene transfer, with either naked plasmid or adenovirus, has been studied most extensively in both pre-clinical models and human trials. Open-label studies using vascular endothelial growth factor (VEGF)₁₆₅, VEGF₁₂₁, and acidic fibroblast growth factor (FGF) have shown some promise in improved ulcer healing or transcutaneous partial pressure of oxygen (TcPO₂) in patients with CLI.^{6,12,13} To date, there have been limited published randomized, placebo-controlled trials to determine whether angiogenic gene delivery can improve limb perfusion in patients with CLI.

Hepatocyte growth factor (HGF) is an angiogenic protein that activates the transcription factor Ets-1 through the c-Met receptor and that has been shown to regulate multiple genes involved in the angiogenic process.^{14,15} HGF expression, unlike expression of hypoxia-inducible factor (HIF)-1 α , is downregulated in the limbs of patients with CLI. HGF has been shown to induce robust collateral formation in preclinical models of peripheral arterial disease.^{14,15} Biodistribution studies have shown that transgene expression is limited to the site of injection. As such, this molecule has many attributes that suggest that it could induce clinically relevant increases in limb perfusion through angiogenesis. The purpose of the present study was to assess the safety of intramuscular injections of HGF plasmid into the limbs of patients with CLI and to determine whether this would result in increased limb perfusion as measured by changes in TcPO₂.

Methods

Patients

Patients were 40 years of age or older and had CLI, defined as severe, chronic symptoms and complications from inadequate limb perfusion that included ulceration, gangrene, and rest pain due to impaired peripheral blood flow. Patients with rest pain or ischemic ulcers were enrolled into the trial if their baseline TcPO₂ was <40 mm Hg and/or toe pressure was <50 mm Hg or ankle pressure was <70 mm Hg. In addition, patients were required to be poor candidates for standard revascularization treatment options for peripheral arterial disease on the basis of inadequate bypass conduit, unfavorable anatomy, or poor operative risk. Patients were required to be taking a statin and an antiplatelet agent (eg, clopidogrel, ticlopidine, or aspirin) for at least 4 weeks before the start of treatment unless contraindicated.

Exclusion criteria included patients who, in the opinion of the investigator, had a vascular disease prognosis that indicated they would require a major amputation (at or above the ankle) within 4 weeks of start of treatment, patients with a diagnosis of Buerger's disease (thromboangiitis obliterans), and those with hemodynamically significant aortoiliac occlusive disease, deep ulcerations with bone or tendon exposure, or clinical evidence of invasive infection (eg, cellulitis, osteomyelitis) uncontrollable by antibiotics. Patients receiving immunosuppressive medication, chemotherapy, or radiation therapy, with end-stage renal disease defined as significant renal dysfunction evidenced by a creatinine of ≥ 2.5 mg/dL, and those receiving chronic hemodialysis therapy were excluded from the study.

Exclusion criteria also included patients with evidence (clinical, laboratory, or imaging) of malignant neoplasm, except for fully resolved basal cell carcinoma of the skin. Patients who had successful tumor resection or radiochemotherapy of breast cancer more than 10 years before inclusion in the study, with no recurrence, were permitted in the study. Patients who had successful tumor resection or radiochemotherapy of all other tumor types more than 5 years before inclusion in the study, with no recurrence, were enrolled in the study. Patients who had proliferative diabetic retinopathy, severe nonproliferative retinopathy, recent (within 6 months) retinal vein occlusion, macular degeneration with choroidal neovascularization, macular edema on fundus evaluation by ophthalmologist, or intraocular surgery within 3 months were excluded from the study.

The investigation was approved by each institutional ethics committee and biosafety committee. All patients gave written informed consent to participate in the study.

Study Design

This was a double-blind, randomized, placebo-controlled trial conducted at 20 centers in United States. The eligible patients were randomized 1:1:1:1 to receive placebo or 1 of 3 dose regimens of HGF plasmid by intramuscular injection: 0.4 mg at days 0, 14, and 28 (low dose); 4.0 mg at days 0 and 28 (middle dose); and 4.0 mg at days 0, 14, and 28 (high dose). The study drug was administered via 8 intramuscular injections of 2 mL delivered to the leg, 4 above and 4 below the knee joint. The injections were delivered at 2 anterior and 2 posterior locations above the knee joint, and 4 injections were delivered posteriorly below the knee joint. Patients were followed up for 12 months with subsequent measurements at weeks 1, 2, 3, 4, 5, and 7 and months 3, 6, and 12 (month 12 for safety assessment only).

Safety Assessment

The primary objective for the present study was safety assessment. Patients were screened according to American Cancer Society guidelines for benign and malignant neoplasms and for proliferative diabetic retinopathy before the start of the study and during follow-up visits. Safety was assessed by evaluation of adverse events, concomitant medication use, ECG, blood chemistry, hematology, coagulation, urinalysis, vital signs, physical examination, cancer and retinopathy screening, and assays for HGF plasmid, HGF protein, and HGF antibodies.

TcPO₂ Assessment

Limb perfusion was assessed as change in TcPO₂ from baseline. TcPO₂ was assessed at screening and throughout the study. During screening, patients had TcPO₂ measured on 2 separate occasions at least 24 hours apart. Patients who met the inclusion criteria (TcPO₂ <40 mm Hg) were enrolled in the study and had TcPO₂ measured at baseline just before the first set of injections and again up to 6 months later. Patients with a >15-mm Hg increase in TcPO₂ from screening measurements to baseline (ie, before the first set of injections) were excluded from the primary efficacy analysis but were included in subsequent analysis on an intention-to-treat basis and in the safety analysis. The sensor locations were predefined as anterolateral, posteromedial calf, dorsum of the foot, and middle of the anterior chest wall. Measurements were recorded after 15 minutes by means of a sensor at a controlled temperature. For analyses, measurements observed at the dorsum of foot were used, because these are both the most clinically relevant measures and the most severe areas of ischemia in patients with CLI and as a result were uniformly the lowest values.

Hemodynamic Assessments

Ankle-brachial index (ABI) and toe-brachial index (TBI) were obtained bilaterally by measuring both the posterior tibial and dorsalis pedis arteries for the ABI and the first or second toe digital artery for the TBI. A vessel with no detectable Doppler signal (complete occlusion) was recorded as having a zero pressure.

Table 1. Subject Disposition: Study Population

	Placebo	Low Dose	Middle Dose	High Dose	Total
Subjects randomized	26	27	26	27	106
Subjects who received at least 1 dose of study drug	26	26	25	27	104
Subjects who completed the 6-month visit	24	23	22	22	91
Subjects discontinued early	2	3	3	5	13
Reason for discontinuation					
Adverse event	0	0	0	1	1
Death	1	2	1	2	6
Voluntary withdrawal	1	1	2	2	6

Ulcer Healing Assessment

Ulcer size was measured with EZ Graph. An independent central reader calculated ulcer size (in centimeters squared), and an additional central reviewer reviewed all photos to determine whether the ulcer was ischemic in nature and recorded correctly on EZ Graph. Complete ulcer healing was defined as skin closure without drainage or need for dressing of all ulcers per patient.

Additional Efficacy Assessment

The rate of major amputation, mortality rate, pain as measured by visual analogue scale, Rutherford classification (clinical classification of severity of chronic lower-extremity ischemia), and SF-36 (Short-Form 36-Item Health Survey, a quality-of-life measure; Medical Outcomes Trust, Waltham, Mass) were also assessed.

Statistical Analysis

Data were reviewed before unblinding to define the appropriate patient population for each end point; patients with markedly increased (>15 mm Hg) TcPO₂ values from screening to baseline and incomplete treatment were excluded from primary analysis. Mean values ±SE are given. Statistical significance was defined as $P < 0.05$. If data were missing, the last observation after day 28 (final dose of treatment) was carried forward and used for the variable of interest, unless another imputation technique was specified. Probability values were calculated with an ANCOVA with change from baseline as the response variable, treatment as the exploratory variable, and the baseline value as the covariate.

For TcPO₂ and hemodynamics analyses, multiple data convention rules were applied for data recorded after a major amputation. First, the missing data were set to 0 mm Hg, and second, the last available observation was used. In addition, a parametric covariate analysis was used for data with no imputation method. The residuals were calculated and tested with a Shapiro-Wilk test. To test for homogeneity of variances, a Levene test was also used. We have presented the analyses with the no-imputation method for TcPO₂ because there was no difference in the statistical interpretation of these analyses. Data analysis was performed with SAS (version 9.13 for Windows; SAS Institute, Inc, Cary, NC).

Determination of Sample Size

The planned sample size of 100 patients, which included 25 patients in each study arm, was considered appropriate for assessment of the safety of the drug and to provide statistical power sufficient for exploratory statistical data analysis. A sample size of 100 patients (25 per treatment arm) and an α -level of 5% would provide 95% power to detect a minimum mean difference of 15 mm Hg in the difference from baseline of TcPO₂ among the 4 treatment arms, if we assumed a common SD of 15 mm Hg.

The authors had full access to the data and take full responsibility for its integrity. All authors have read and agree to the manuscript as written.

Table 2. Demographics (Safety Population)

	Placebo (n=26)	Low Dose (n=26)	Middle Dose (n=25)	High Dose (n=27)	<i>P</i>
Age, y	70.2	70.1	73.0	68.1	0.36
Male, %	63	76	57	57	0.47
Race, %					0.38
White	75	96	86	87	
Black	17	4	10	9	
Other	8	0	5	4	
Diabetes, %	71	56	38	43	0.12
Smoking, %	83	76	71	83	0.73

Kruskal-Wallis test for continuous variables and χ^2 test for categorical variables.

Results

Baseline Characteristics

The subject disposition is shown in Table 1. There were 104 patients who received at least 1 dose of the study drug and were available for safety analysis. The treatment groups were well balanced for demographics and baseline characteristics (Table 2), although patients randomized to the placebo group tended to have a higher proportion of diabetes mellitus. The mean TcPO₂ for all groups ranged from 21 to 27 mm Hg, and TBI ranged from 0.17 to 0.19, consistent with the severe hemodynamic compromise anticipated in patients with CLI (Table 3).

Safety Analysis

The administration of HGF plasmid by intramuscular injections was well tolerated. Injection-related adverse events occurred infrequently and were similar in the placebo and the 3 HGF groups. There were no significant differences in the incidence of adverse events among the groups with the exception of pain in the limb, for which the percentage of patients reporting this adverse effect was significantly greater in the treatment groups (placebo, 0%; low dose, 15%; middle dose, 20%; high dose, 26%; $P < 0.05$). Most adverse events (68%) were considered to be unrelated to the treatment received. There were no significant differences between the treatment groups in any of the categories with regard to adverse event relationships (likely related or possibly related).

Adverse events that were severe in nature were reported by 50%, 46%, 44%, and 56% of patients in the placebo and low-, middle-, and high-dose groups, respectively. Most adverse events were due to CLI or to the comorbid conditions that are prevalent in this population. The differences were not significant. Serious adverse events by body system with an incidence >10% are shown in Table 4.

Retinopathy was reported as a serious adverse event in 1 patient in the placebo group. Five patients developed malignancy during follow-up, the distribution of which is shown in Table 5.

Seven patients died during follow-up (2 in the placebo group, 2 in the low-dose group, 1 in the middle-dose group, and 2 in the high-dose group). The cause of death was cancer in 1 patient (placebo group), cerebrovascular accident in 1

Table 3. Baseline Disease Status (Safety Population)

	Placebo (n=26)	Low Dose (n=26)	Middle Dose (n=25)	High Dose (n=27)	<i>P</i>
Rutherford classification, %					0.42
4	25	48	43	30	
5	67	48	43	65	
6	8	4	14	4	
TcPo ₂ (dorsum of foot), mm Hg	22.2±2.9	22.1±4.2	27.3±3.0	21.1±3.2	0.50
ABI	0.44±0.04	0.37±0.05	0.46±0.04	0.39±0.03	0.47
TBI	0.18±0.03	0.19±0.03	0.19±0.03	0.17±0.03	0.97
Mean total ulcer size, cm ²	3.63±1.24	3.73±1.28	5.83±2.88	3.20±0.65	0.89

Kruskal-Wallis test for continuous variables and χ^2 test for categorical variables.

(high-dose group), suicide in 1 (high-dose group), myocardial infarction in 1 (low-dose group), respiratory failure in 1 (middle-dose group), small-bowel obstruction in 1 (low-dose group), and worsening heart disease in 1 (placebo group). None of these deaths were considered to be related to treatment.

HGF plasmid was not detectable in the peripheral circulation at 2 weeks after injection in any patient. At no time was the HGF protein level found to be increased in the blood. No patients developed antibodies to the HGF protein during the study. No evidence of HGF related toxicity was observed in the study.

Transcutaneous Partial Pressure of Oxygen

Patients With Increase in TcPo₂>15 mm Hg Before Treatment Excluded

There were 73 patients available for efficacy analysis. High-dose-treated patients had a significant increase in TcPo₂ (23.9±4.2 mm Hg, 95% CI 15.5 to 32.4 mm Hg) at 6 months from baseline, which was significantly greater than in the placebo group (9.3±4.2 mm Hg, 95% CI 0.9 to 17.8 mm Hg; ANCOVA *P*=0.0015, high-dose versus placebo *P*=0.0018; Figures 1 and 2; n=73 patients). The TcPo₂ at 3 months was not different in the placebo, low-dose, middle-dose, and high-dose groups.

In clinical practice, a TcPo₂ ≥30 mm Hg is suggestive of wound healing.¹⁶ The percentage of patients with a TcPo₂

>30 mm Hg increased from 31% at baseline to 80% at 6 months in the high-dose group compared with an increase in the placebo group from 35% at baseline to 39% at 6 months. The corresponding values for the low- and middle-dose groups were from 38% to 57% and from 56% to 67%, respectively (Figure 3). These differences were not statistically significant (*P*=0.11, ANCOVA).

Patients With Increase in TcPo₂>15 mm Hg Before Treatment Included

In all evaluable patients (n=92), TcPo₂ at 6 months increased from baseline the most in the high-dose group (18.9±3.5 mm Hg) compared with the placebo (8.7±3.5 mm Hg), low-dose (9.1±3.6 mm Hg), and middle-dose (7.7±4.2 mm Hg) groups. This difference did not reach statistical significance (ANCOVA *P*=0.07; Figure 4).

Toe Pressure and ABI

In all evaluable patients (n=71), the change in TBI at 6 months was not different among the treatment groups (Figure 5). There was no difference in ABI among groups at any of the time points studied (data not shown).

Wound Healing, Major Amputation, and Survival

There were no differences in incidence of complete wound healing, major amputation, or death at 6 months among the groups. Patients with widely discrepant wounds were enrolled into the study, including patients with severe

Table 4. Incidence of Adverse Events by Body System

Serious Adverse Events, by Body System	Placebo (n=26)	Low Dose (n=26)	Middle Dose (n=25)	High Dose (n=27)	<i>P</i> *
At least 1 serious adverse event	15 (58)	16 (62)	12 (48)	19 (70)	0.44
Cardiac disorders	5 (19)	5 (19)	3 (12)	7 (26)	0.67
Gastrointestinal disorders	3 (12)	3 (12)	0	0	0.07
General disorders and administration-site conditions	1 (4)	1 (4)	1 (4)	3 (11)	0.72
Infections/infestations	5 (19)	5 (19)	5 (20)	6 (22)	1.00
Injury and procedural complications	0	0	1 (4)	3 (11)	0.16
Skin and subcutaneous tissue disorders	1 (4)	3 (12)	0	2 (7)	0.44
Vascular disorders	4 (15)	2 (8)	7 (28)	7 (26)	0.20

No. (%) of subjects with at least 1 serious adverse event within each body system are presented.

Only body systems with at least a 10% incidence rate are included in this Table.

*Fisher exact test.

Table 5. Incidence of New Malignancy

Cancers	Placebo	Low Dose	Middle Dose	High Dose
Adenocarcinoma	1	0	0	0
Basal cell carcinoma	0	2	0	0
Colon cancer	1	0	1	0
Total	2	2	1	0

gangrene (Rutherford class 6 wounds) and patients with combined CLI and venous stasis ulcers. Overall, there was no difference in wound healing among the groups. There was a modest trend toward stabilization or improvement in wound healing at 6 months in patients with ulcers <10 cm² at baseline in all treated groups compared with the placebo-treated group, which had a deterioration in wound size; however, these differences were not significant (Figure 6). Major amputations occurred in 11% of patients at 12 months; 3 of these occurred before completion of treatment. In those patients who received all 3 sets of injections, the 6- and 12-month major amputation rates were 8% and 9%, respectively. There was no difference in incidence of major amputation across treatment groups.

Discussion

At present, the standard therapy for CLI remains revascularization, either by endovascular techniques or open bypass surgery, with no effective medical management for this condition. Revascularization options are often limited and even when performed are associated with significant morbidity in this elderly patient population with a high prevalence of associated comorbidities.^{3,4,17} Major lower-extremity amputation frequently follows failed revascularization or is done when revascularization cannot be performed. Major amputation is associated with impairment of independence and a 12-month mortality rate as high as 40%. Less invasive alternatives to present revascularization strategies would have a positive impact on patients with CLI.

Early open-label gene transfer studies by Isner and coworkers laid the groundwork for the field of therapeutic angiogenesis. In those studies,^{6,13,18} intramuscular gene transfer of naked plasmid DNA encoding for the 165-amino acid isoform of vascular endothelial cell growth factor was shown to result in wound healing in a substantial number of patients with CLI or Buerger's disease. An additional open-label

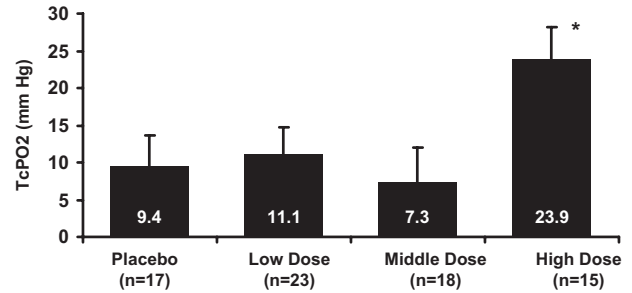


Figure 2. Mean change (\pm SE) in TcPO₂ from baseline at 6 months. Patients with ≥ 15 -mm Hg TcPO₂ increase before treatment were excluded. $P=0.0015$ (ANCOVA); * $P=0.018$ (placebo vs high dose).

study using VEGF₁₆₅ gene transfer has also been shown to improve chronic ischemic neuropathy from baseline in patients with CLI.¹⁹ In those studies, VEGF₁₆₅ gene transfer had an acceptable safety profile.

Comerota and coworkers¹² have reported improved lower-extremity perfusion as measured by improvement in TcPO₂ from baseline after intramuscular gene transfer of acidic FGF in patients with CLI. In their open-label, phase I dose-escalation trial, acidic FGF improved TcPO₂ from baseline and was associated with an acceptable safety profile. Data from the phase I trial were encouraging enough that a phase II randomized, placebo-controlled trial has been performed, the results of which have not yet been published.

A recent phase I clinical trial on the safety and efficacy of adenoviral delivery of HIF-1 α (adeno-HIF-1 α) in patients with CLI has been reported.²⁰ This open-label dose-escalation study demonstrated that the adeno-HIF-1 α was well tolerated and safe. Proof of efficacy was absent in this small study.

HGF is a disulfide-linked heterodimeric growth factor that signals through the transmembrane tyrosine kinase c-Met receptor. HGF has been shown to stimulate endothelial cell proliferation and migration, as well as smooth muscle cell migration but not proliferation.^{14,15,21} It has been established that HGF upregulates the expression of VEGF, matrix metalloproteinases, c-Met, and HGF. Preclinical hindlimb ischemia models in rabbits and rats have shown that intramuscular injection of HGF plasmid improved limb perfusion and blood vessel density compared with controls.

A phase I open-label clinical trial using intramuscular injection of HGF plasmid in patients with either CLI or

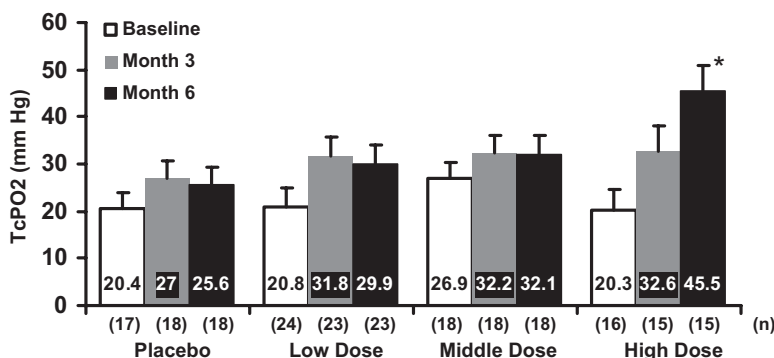


Figure 1. Mean TcPO₂ (\pm SE) at baseline and at 3 and 6 months. Patients with ≥ 15 -mm Hg TcPO₂ increase before treatment were excluded. * $P<0.05$ vs baseline.

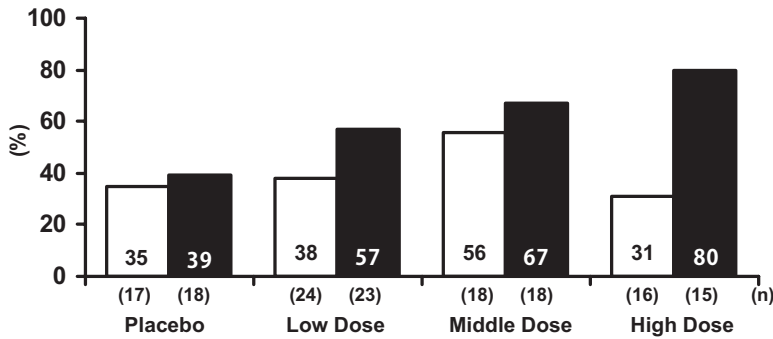


Figure 3. Percent of patients with TcPO₂ to ≥ 30 mm Hg at baseline and at 6 months. Patients with ≥ 15 -mm Hg TcPO₂ increase before treatment were excluded. $P=0.11$ (ANCOVA).

Buerger's disease has been completed in Japan.²² The study was conducted with 4 mg of HGF plasmid divided between 2 dosing sessions 4 weeks apart. The study found no safety concerns. Systemic HGF protein levels were unchanged during the study. Although the study population was small, there was a suggestion of efficacy. The ABI increased by 0.1 in all patients, and a decrease in ulcer size of $>25\%$ occurred in 8 of 11 ulcers.

In the present phase I/II study, patients with CLI who received the highest dose and frequency of HGF gene transfer had a suggestion of improved limb perfusion as measured by changes in TcPO₂ (Figures 1 and 2). The increase in TcPO₂ observed in the present study suggests that the high-dose HGF plasmid may promote a clinically relevant increase in limb perfusion. TcPO₂ measures of >30 mm Hg have been shown to be associated with wound healing.¹⁶ As shown in Figure 3, 80% of patients who received the high-dose HGF plasmid had a TcPO₂ >30 mm Hg at the 6-month time point compared with 39% in the placebo group. There was no difference between treatment groups observed in other secondary end points such as change in toe pressure index, wound healing, limb salvage, or survival; however, the present study was not powered to address differences in these secondary end points.

Lessons learned from the present trial design relate to variability in TcPO₂ measurement and variation in wound care. Because the putative mechanism of action of therapeutic angiogenesis involves the creation of blood vessels in the 300- μm range, TcPO₂ was thought to be a more sensitive measure of improved limb perfusion than toe pressure. For this reason, TcPO₂ was chosen in the present study as the surrogate marker for limb perfusion despite the increased variability known to occur with this measure.^{16,23} To attempt

to offset the increased variability of TcPO₂, 2 measures of TcPO₂ were required during the screening period before patient enrollment into the trial. Despite this, it was observed that 18% of patients who had low screening TcPO₂ at the time of baseline measurement had TcPO₂ levels that had increased without treatment by >15 mm Hg at the time of entry into the trial. Possible explanations for the apparent false-positive TcPO₂ values obtained during the screening period include leg edema, decreased limb perfusion from a reversible cause such as congestive heart failure, or operator error. Patients with an increase in TcPO₂ from screening to baseline of >15 mm Hg, although excluded in the primary analysis of TcPO₂ to improve the homogeneity of the patient population, were included in the safety analyses and the subsequent analysis of TcPO₂.

An unexpected weakness in the study included variability in wound care. Each center was allowed to continue preexisting wound care practice standards, which varied widely between centers. This variability in wound care likely affected the ability to determine whether HGF plasmid therapy had a beneficial effect on wound healing. Future studies in this area should consider rigorous wound assessment and care protocols to prevent such variability. Patients with CLI enrolled into the study also demonstrated a wide spectrum of lower-limb ischemia, from rest pain to extensive tissue loss. Attempts to apply more rigorous exclusion criteria may improve on the patient heterogeneity found in the present study; however, this may also severely limit patient recruitment.

Concerns about stimulation of systemic angiogenesis remote from the injection site include progression of proliferative retinopathy or growth of occult tumors. There were no safety concerns regarding intramuscular HGF plasmid injection identified in the present study. Five patients developed a

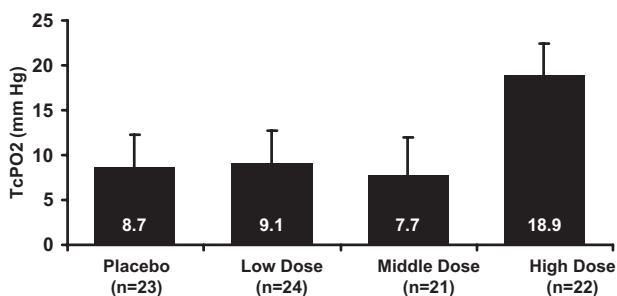


Figure 4. Mean TcPO₂ (\pm SE) at baseline and at 3 and 6 months. Data include all evaluable patients, with last observation carried forward. $P=0.07$ (ANCOVA).

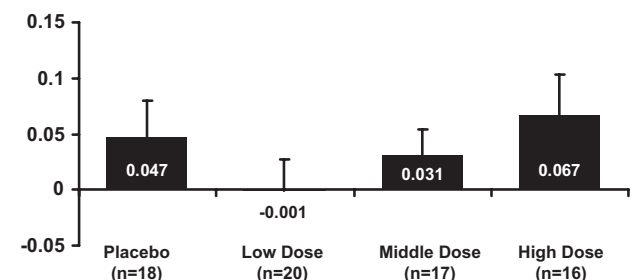


Figure 5. Mean change (\pm SE) in TBI from baseline at 6 months. Data include all evaluable patients. $P=0.41$ (ANCOVA).

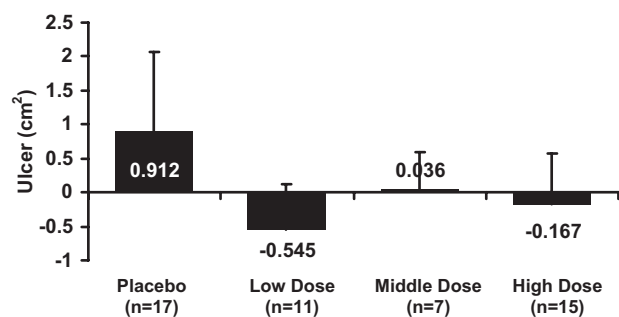


Figure 6. Mean change (\pm SE) in total ulcer size from baseline at 6 months. Patients with ulcer ≥ 10 cm² at baseline were excluded. $P=0.77$ (ANCOVA).

new malignancy during the trial. These were equally distributed between the placebo and treatment groups. There was no identifiable progression of proliferative retinopathy. Serious adverse events were equally distributed between groups. These data suggest that this therapy is reasonably well tolerated by this elderly patient population.

Gene therapy for the treatment of patients with lower-extremity vascular disease is in early development. The optimal gene and optimal delivery technique have not been determined. The strengths and weakness of plasmid, adenoviral, and adeno-associated viral cell transduction have been outlined in previous reports.⁵ Adenoviral and adeno-associated viral strategies have improved transfection efficiency compared with plasmid delivery. A major concern centers on the limited ability to re-dose patients with adenoviral vectors due to immune response development. Adeno-associated viral transfer vectors are in early clinical trials. In the present study, no patient developed antibodies to the HGF plasmid, and re-dosing was well tolerated.

Conclusions

The present study demonstrated that intramuscular injection of HGF plasmid has an acceptable safety profile for the treatment of patients with CLI. Intramuscular injection of HGF plasmid may have a favorable effect on limb perfusion as measured by TcPo₂ in patients with CLI. The results from this phase I/II trial are promising and warrant further study in a larger trial to determine whether HGF has a clinically meaningful effect on wound healing, limb salvage, and survival.

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Disclosures

Dr Powell, the primary investigator, is a paid consultant to AnGes Inc. Drs Simons and Annex are paid consultants to AnGes Inc. Dr Morishita is a board member and stockholder of AnGes MG, Inc. Minako Koga is a former employee of AnGes Inc. The other authors report no conflicts.

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CLINICAL PERSPECTIVE

Critical limb ischemia continues to be associated with a high incidence of limb loss and mortality. Patients frequently have multiple associated comorbidities that place them at high risk for open surgical bypass. Endovascular approaches are often not an option because of the extent of disease, and they have limited durability. There is no effective medical therapy for critical limb ischemia. Therapeutic angiogenesis through gene or stem cell delivery to the ischemic limb is a developing technology that attempts to improve perfusion to the ischemic limb. Concerns exist about the potential for gene therapy–mediated therapeutic angiogenesis to promote tumor growth or the progression of proliferative diabetic retinopathy. The present study demonstrates that hepatocyte growth factor plasmid gene therapy is safe and well tolerated. In addition, there is a potential indication of efficacy. Given the results of this phase I/II study, future studies are warranted to determine the efficacy of this agent on the more clinically relevant end points of wound healing, major amputation, and patient survival.

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