Vascular Endothelial Growth Factor Gene Transfer for Diabetic Polyneuropathy: A Randomized, Double-Blinded Trial

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Objective: Randomized, blinded trial of intramuscular gene transfer using plasmid vascular endothelial growth factor (VEGF) to treat diabetic polyneuropathy.

Methods: Diabetic patients with polyneuropathy were randomized to receive a VEGF-to-placebo ratio of 3:1. Three sets of injections were given at eight standardized sites adjacent to the sciatic, peroneal, and tibial nerves of one leg. Primary outcomes were change in symptom score at 6 months and a prespecified overall clinical and electrophysiological improvement score. Secondary outcomes were differences in symptoms, examination scores, visual analog pain scale, nerve conduction, and quantitative sensory testing.

Results: Thirty-nine patients received plasmid VEGF and 11 received placebo. Mean symptom score improved in both legs at 6 months, favoring VEGF over placebo (-1.2 ± 0.5 vs -0.9 ± 0.5; $p < 0.01$ after adjustment for change in the untreated leg) and compared with the untreated leg (-0.7 ± 0.5; $p = 0.02$). The region of sensory loss and visual analog pain scale improved in the treated group (-1.5 vs -0.5; $p = 0.01$). Twelve of 39 VEGF versus 2 of 11 placebo patients met criterion for overall improvement. Other measures including nerve conduction potentials did not improve. There were 84 adverse events in VEGF patients, and 22 were serious; there were 51 events in placebo patients, and 2 were serious.

Interpretation: Intramuscular plasmid VEGF gene transfer improved diabetic neuropathic symptoms, meeting primary endpoint criteria for efficacy but not affecting most secondary measures. Treatment was associated with more serious adverse events that did not reach statistical significance. These results are not conclusive but may justify further clinical study.

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A predominantly sensory polyneuropathy is present in 7% of diabetic patients at the time of diagnosis and affects more than 50% after 25 years of disease.[1] The consequences of diabetic polyneuropathy include pain, numbness, imbalance, and a predisposition to foot ulceration, the last of which is facilitated by autonomic and vascular changes as a result of neuropathy. Painless ulcerations are often unrecognized by the patient for long periods, and lead to infection and toe or foot amputation at a rate 15 times greater in diabetic patients compared with individuals without diabetes.

Preclinical studies from our and other laboratories have demonstrated improved sensory behavioral features (tail flick and paw withdrawal), nerve vascularization, sciatic nerve blood flow by laser–Doppler measurement, and nerve conduction studies in diabetic animals treated with intramuscular injections of plasmid DNA encoding the vascular endothelial growth factor (VEGF) gene.[2] Two other models have given similar results.[3,4] In addition, clinical studies have indicated that the signs and symptoms of local neuropathy in patients with lower extremity critical limb ischemia improve with intramuscular injection of VEGF gene.[5] These findings implicate microvascular ischemia as an important and possibly principal cause of diabetic polyneuropathy, and suggest

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that angiogenic growth factors are a potential treatment.

Gene transfer consists of the introduction of genetic material into somatic cells to achieve gene expression. Of the three methods of introducing this material; viral vector, liposome DNA, and naked plasmid DNA; the last avoids the risks for viral exposure and persistent uncontrolled expression. Although transfection efficiency is low with plasmid DNA (fewer than 1% of cells), it is highly site specific and leads to local levels of protein that are biologically active and have therapeutic effects both in vitro[6] and in vivo[7] without inciting a host response. Furthermore, ischemic tissues show transfection efficiencies that are fivefold greater than in normal tissue.

We conducted a randomized phase 2 trial of VEGF gene transfer for the treatment of symptomatic diabetic polyneuropathy. One leg was injected with active agent or placebo; the contralateral leg was not injected and served as an additional control.

Subjects and Methods

Patient Recruitment and Selection

Diabetic patients with pain or numbness in the feet and legs were solicited by print and radio advertising in the Boston and New York metropolitan areas, and from the diabetes clinics of our hospitals. Respondents were screened through phone calls and by review of medical records by a specially trained nurse (AP). Those with established diabetes who were taking oral or insulin therapy, had symptoms of polyneuropathy, and reported being free of cancer and active diabetic retinopathy were invited for screening. Those who fulfilled the study's entry criteria and had no contraindications for gene therapy (see Supplemental Appendix A) signed informed consent for further testing and for gene therapy administration. They underwent a general physical, neurological, funduscopic, and standard laboratory examinations, and had screening for cancer (see Supplemental Appendix B), alternative causes of polyneuropathy (see Supplemental Appendix C), and nerve conduction studies of the peroneal, tibial, and sural nerves in both legs.

The study was initiated at St. Elizabeth's Medical Center, Boston, and performed at this institution and at Columbia University Medical Center, New York. The conduct of the study and informed consent methods were approved by the institutional review boards of the two institutions, as well as the RAC (Recombinant DNA Advisory Committee) and data safety monitoring board established by the sponsor, National Heart, Lung, and Blood Institute.

Study Measures

Patients were assessed by neurologists who were blinded to treatment assignment and to the patient's laboratory data. For all scores, greater values indicate more severe involvement. Therefore, a greater decline in comparison with baseline or with the opposite leg indicates a better outcome. The symptom score (SS) encompassed five neuropathy-related features in each leg: (1) distal leg weakness, (2) proximal leg weakness, (3) numbness, (4) paresthesias, and (5) pain (excluding at the site of skin ulceration, pain from vascular claudication, or ischemic rest pain). Each symptom was graded from 0 to 3 (0 = none; 1 = mild; 2 = moderate; 3 = severe). The maximum possible SS (indicating most severe symptoms) was thus 15 (a score more than 4 was required for study entry).

Lower extremity sensory testing was graded by a sensory examination score, which evaluated the following: (1) sensory deficit for pinprick and light touch when compared with a proximal, normal region, graded 0 to 4 (0 = normal; 1 $\geq$ 75% of normal; 2 = 50–74%; 3 = 25–49%; 4 $\leq$ 25%); (2) distribution of sensory symptoms for light touch and pinprick: graded 0 to 4 (0 = normal; 1 = abnormal to toes; 2 = to ankle; 3 = to midcalf; 4 = above midcalf); (3) vibration sense at the toes and ankle, graded 0 to 4 (0 = normal; 1 = mild loss; 2 = moderate; 3 = severe; 4 = absent); and (4) proprioception at the great toe (6 trials), graded 0 to 4 (0 = 6/6 correct; 1 = 4–5/6; 2 = 3/6; 3 = 1–2/6; 4 = 0/6). The maximum possible sensory examination score was 28.

The motor examination score evaluated strength in both proximal and distal leg muscles using a 0 to 4 scale (reverse of the Medical Research Council scale of 1 to 5, as higher scores indicate more weakness). Proximal muscles tested were the iliopsoas, quadriceps, and hamstrings; distal were tibialis anterior, gastrocnemius, and extensor hallucis longus/brevis. Reflex score assessed deep tendon reflexes at the knees and ankles, graded 0 to 4 (0 = normal; 2 = reduced; 4 = absent); a score of 2 or more at the ankles was required as an entry criterion.

A total examination score (TES) was calculated as the sum of sensory examination score, motor examination score, and reflex score. The maximum possible TES, indicative of the most severe impairment, was 64. Examination scores and nerve conduction studies were obtained within 4 weeks before the first injection and at 12, 24, and 52 weeks; quantitative sensory testing was performed in both legs before treatment and at 6 months. Ankle–brachial index, Rutherford vascular scores, and funduscopy with retinal photographs to screen for active proliferative diabetic retinopathy were obtained before treatment and at 12, 24, and 52 weeks.

This scoring system and the electrophysiological measures described later were used in our previous study of ischemic limb neuropathy and are adopted from scales that are incorporated into the NIS-LL (Neuropathy Impairment Score in the Lower Limbs), which was devised for quantifying the symptoms and deficits of diabetic neuropathy,[8] INCAT (Inflammatory Neuropathy Cause and Treatment) criteria sensory sum score,[9] Average Muscle Score,[10] and NTSS-6 (Neuropathy Total Symptom Score-6)[11] that have been used in other therapeutic trials for peripheral neuropathy.[12]

Standardized nerve conduction studies of the tibial and peroneal motor nerves and the sural sensory nerve were performed in both legs. Quantitative sensory testing was performed using a CASE IV (Computer Aided Sensory Evaluator; WR Medical Electronics, Stillwater, MN) to determine thresholds for vibration and cold sensation in the legs using the "4-2-1" algorithm. Vibration testing used calibrated 125Hz mechanical oscillations in 25 graded steps from 0.1 to 576μm. One trial each for vibration and cold threshold

was performed using the 4-2-1 testing algorithm.[13] The results are given as units of "just noticeable difference."

Randomization

Patients were randomized within 4 weeks of the screening assessments to receive VEGF or placebo based on a 3:1 randomization ratio, initially planned to be stratified by the presence or absence of symptomatic macrovascular disease of the legs (see protocol in supplementary material), but so few patients fell into the former group that the protocol was amended after the first six patients to omit stratification.

Study Agent

Two VEGF agents were used in this study. Preclinical data indicated bioequivalency of therapeutic effect using either VEGF-1/VEGF-A or VEGF-2/VEGF-C. The initial cohort of 16 patients was treated with VEGF-1, which was manufactured at St. Elizabeth's Medical Center, thereby limiting the application to a single center. Because of interest in moving forward into larger-scale studies, a change was made to VEGF-2 that was developed as a therapeutic agent by a commercial entity that manufactures clinical-grade material suitable for distribution to multiple sites. At the completion of the first dose cohort (1mg), evidence of toxicity and all patient data relevant to adverse events were reviewed by the data safety monitoring board, and an endorsement was made to continue and escalate the dose to 4mg (Fig). The preparation of the plasmid is given in Supplemental Appendix D. Placebo injections were 0.9% sterile saline in 2.5ml, the same volume as used for the active agent.

The active agent or placebo was divided and delivered to the patient in eight intramuscular injections into the hamstring (two injections spaced one-third and two-thirds of the distance from the buttock fold to the midpopliteal space), the meridian of the gastrocnemius (three injections spaced one-, two-, and three-quarters of the distance between the malleoli and the popliteal fossa), and the meridian of the tibialis anterior (three injections spaced one, two-, and three-quarters of the distance between the malleoli and the bottom of the patella). A second and a third series of eight injections was administered at 2 and 4 weeks after the first treatment. The doses were developed based on animal data showing that reasonable levels of gene expression are limited to 2 weeks, whereas gene expression at 3 weeks is diminished and by 4 weeks is altogether absent. Three doses at intervals of 2 weeks were thus designed to sustain gene expression for approximately 6 weeks.

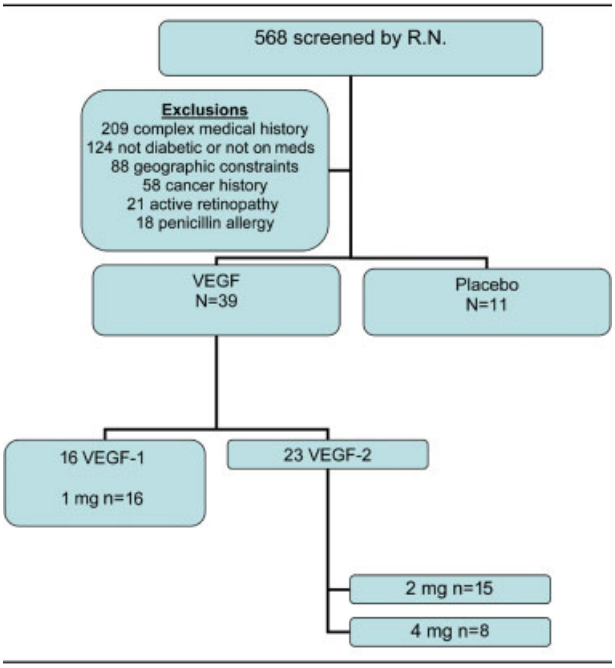

Fig. Patient recruitment CONSORT (Consolidated Standards of Reporting Trials) diagram.

Outcomes and Statistical Analysis

The prespecified primary outcome was change in SS at 6 months in the treated leg. The protocol listed as a coprimary outcome the proportion of patients achieving two of the following: (1) $\geq$2 point decrease in SS; (2) $\geq$4 point decrease in TES; (3) $\geq$30% improvement in peroneal or (4) tibial nerve amplitude or (5) summed motor amplitude; or (6) more than 30% improvement in sural nerve amplitude, if present at baseline. The secondary outcomes were the changes at 6 months in the treated and untreated legs in the symptoms, examination, and nerve conduction values.

Because of the small cohort sizes, VEGF-1 and VEGF-2 results, and those with and without macrovascular disease were analyzed as one group.

Treatment group means of clinical and just noticeable difference variables at baseline and 6 months were compared by the two-sample *t* test. For these variables, the prespecified method for analyzing changes from baseline to 6 months between treatment groups was an analysis of covariance with change in the treated leg as the outcome and change in the untreated leg as a continuous covariate. This method was chosen before opening the data set and performing statistical analysis to improve the precision of estimation of treatment effects. Because values of 0 were common in the electrophysiological variables, Wilcoxon rank-sum tests were used to compare the distributions of the baseline, 6-month, and change measurements for these variables.

Results

A total of 1,262 patients inquired about the study, and 568 were screened by interview and review of records by 1 study nurse. Patients were excluded for the following reasons: complex medical history, blood chemistry abnormalities in record or prior amputations (209); did not have diabetes (115) or not taking diabetic medications (9); geographic constraints (88); history of cancer (58); active proliferative retinopathy (21); and penicillin allergy (18) (see Fig). Thirty-nine patients were randomized to the active agent (20 to VEGF-1 and 19 to VEGF-2), and 11 were randomized to placebo. All protocol clinical and nerve conduction studies were completed with the exception of three patients who did not have full data from quantitative sensory studies.

Baseline clinical characteristics of the active and pla-

cebo groups are shown in Table 1. There were more male and type 1 diabetics but fewer with insulin requirement in the VEGF group. Hemoglobin A1c values were similar. The initial symptom and examination scores and nerve conduction values are shown in Table 2. There were no significant differences in the clinical or neurological features, but SSs and TESs tended to be greater (worse) in the treated legs of VEGF patients relative to placebo-treated patients. There was a difference in the mean just noticeable difference sensitivity to cold in the treated leg. Two placebo patients received a score of 0 in the treated leg at baseline and two had missing data.

Six-Month Absolute Measures

At 6 months, mean absolute SSs (see Supplementary Table 1A) were lower in the placebo group for both the treated and untreated legs, as they were at baseline, with the difference achieving statistical significance in the untreated leg at 6 months (4.64 vs 6.61; $p = 0.04$). TES was also lower (better) in the placebo group, but none of the absolute differences between treatment groups achieved statistical significance. The median peroneal nerve amplitude was greater in the injected leg of placebo group subjects than in the treated leg (2.6 vs 0.6mV; $p = 0.1$). However, the peroneal amplitude was greater in the treated leg of VEGF patients than in the untreated leg (0.6 vs 0.3; see Supplementary Table 1B). None of these amplitudes was significantly different from baseline. Hemoglobin A1c increased slightly in both groups, with a greater elevation in the placebo group (0.15 for VEGF vs 0.76 for placebo).

Six-Month Outcomes

The primary and secondary outcomes of changes between baseline and 6 months are shown in Tables 3 (clinical) and 4 (nerve conduction and quantitative sensory testing). A greater decline in clinical measures

Table 1. **Baseline Clinical Features**

Clinical Features	VEGF (n = 39)	Placebo (n = 11)
Mean age ± SD, yr	61.4 ± 9.9	65.6 ± 8.4
Male sex, n	18 (46%)	1 (9%)
Diabetes type I, n	7 (18%)	0
Diabetes type II, n	32 (82%)	11 (100%)
Diabetes duration, yr	12.7 ± 10.6	13.2 ± 7.4
Insulin use, n	17 (44%)	7 (64%)
HgbA1c baseline	7.6 ± 1.4	7.6 ± 2

VEGF = vascular endothelial growth factor; HgbA1c = hemoglobin A1c.

Table 2. **Baseline Scores and Electrophysiological Values by Leg and Treatment Group**

	VEGF (n = 39)	Placebo (n = 11)	*p*
Mean Baseline Scores (SD)			
Symptom Score			
Untreated leg	7.26 (2.46)	6.55 (2.50)	0.40
Treated leg	7.62 (2.57)	6.18 (1.54)	0.09
Sensory examination score			
Untreated leg	20.10 (5.28)	17.55 (6.59)	0.19
Treated leg	20.33 (5.32)	17.55 (5.47)	0.13
Motor score			
Untreated leg	2.82 (3.89)	2.55 (3.27)	0.83
Treated leg	2.92 (4.04)	2.09 (3.24)	0.53
Reflex score			
Untreated leg	5.64 (2.19)	4.36 (1.75)	0.08
Treated leg	5.44 (2.10)	4.18 (1.40)	0.07
Total examination score			
Untreated leg	28.56 (8.43)	24.45 (8.68)	0.16
Treated leg	28.69 (8.52)	23.82 (8.41)	0.10
VAS score			
Untreated leg	5.15 (2.94)	4.45 (2.11)	0.47
Treated leg	5.44 (3.08)	4.45 (2.54)	0.34
Median Electrophysiological Variables (IQR)			
Tibial amplitude, mV			
Untreated leg	0.3 (0.0–3.6)	0.9 (0.1–9.2)	0.32
Treated leg	0.4 (0.0–2.5)	3.7 (0.1–8.3)	0.15
Peroneal amplitude, mV			
Untreated leg	0.4 (0.0–1.9)	2.8 (0.1–4.3)	0.10
Treated leg	0.6 (0.0–2.4)	1.8 (0.2–3.5)	0.21
Amplitude sum, mV			
Untreated leg	0.6 (0.2–4.8)	3.1 (0.2–15.1)	0.26
Treated leg	1.1 (0.1–6.4)	4.8 (0.3–13.3)	0.19
Sural amplitude, μV			
Untreated leg	0.0 (0.0–0.0)	0.0 (0.0–2.7)	0.51
Treated leg	0.0 (0.0–1.7)	1.5 (0.0–4.4)	0.22
Mean JND Variables (SD)			
Cold			
Untreated leg	19.31 (6.64)	17.74 (5.04)	0.51
Treated leg	20.97 (5.40)	15.19 (9.19)	**0.02**
Vibration			
Untreated leg	22.80 (5.01)	23.48 (1.97)	0.69
Treated leg	22.81 (4.60)	23.13 (1.63)	0.84

VEGF = vascular endothelial growth factor; SD = standard deviation; VAS = visual analog pain scale; IQR = interquartile range; JND = "just noticeable difference."

Table 3. **Changes in Clinical Measures between Baseline and Six Months by Leg and Treatment Group**

Treatment Group	VEGF (n = 39)	Placebo (n = 11)	p[a]
Mean Clinical Variable (SE)			
Symptom score[b]			
Untreated leg	−0.74 (0.50)	−1.91 (0.59)	0.24
Treated leg	−1.21 (0.53)	−0.91 (0.65)	**0.01**
Sensory examination score			
Untreated leg	−0.42 (0.64)	−1.18 (1.55)	0.60
Treated leg	−0.89 (0.57)	−1.18 (1.43)	0.71
Motor score			
Untreated leg	−0.18 (0.36)	−0.09 (0.49)	0.90
Treated leg	−0.39 (0.34)	0.09 (0.41)	0.36
Reflex score			
Untreated leg	−0.05 (0.30)	0.73 (0.49)	0.21
Treated leg	0.16 (0.30)	0.91 (0.41)	0.72
Total examination score			
Untreated leg	−0.66 (0.83)	−0.55 (1.61)	0.95
Treated leg	−1.13 (0.75)	−0.18 (1.66)	0.37
VAS score			
Untreated leg	−0.92 (0.42)	−1.45 (0.82)	0.56
Treated leg	−1.47 (0.48)	−0.45 (1.11)	**0.01**

[a]p values for treated leg obtained from analysis of covariance adjusting for change in the untreated leg. [b]Primary outcome. VEGF = vascular endothelial growth factor; SE = standard error; VAS = visual analog pain scale.

in Tables 3 and 4 indicates a better outcome in relation to the comparator group. No significant differences between treatment groups were observed for the untreated leg. For comparisons in the treated leg, change in the untreated leg was used as a covariate. This resulted in substantially better precision. The 6-month change in SS was significantly different between treatment groups. Though the unadjusted changes in the treated leg were similar in the two treatment groups, the decline in mean SS in the untreated leg was larger for placebo-treated patients (−1.91) than for VEGF-treated patients (−0.74). Because change in the untreated leg was a strong predictor of change in the treated leg, the adjusted estimate of the difference between groups in change in the treated leg was −1.40 (standard error, 0.52; $p = 0.01$).

Twelve of 39 patients in the treated group versus 2 of 11 in the placebo group met the prespecified coprimary outcome of change in at least 2 of the 5 primary categories. No patient in either group showed greater than 30% increase in sural nerve action potential.

Among the secondary outcomes, comparing the active treatment leg with placebo, changes in the visual analog pain scale scores and distribution of pinprick loss favored the treatment group, but changes in the other examination subscores (Table 5), nerve conduction studies, and quantitative sensory measures (see Table 4) were unchanged when adjusted for changes in the untreated leg.

Adverse Events

Adverse events over 52 weeks are tabulated in Table 6. Ten patients in the active treatment group had 22 serious adverse events (Table 7); the placebo group had two serious adverse events in unique patients (χ^2 for patients affected, one-tailed $p = 0.47$). No instances of worsening of active proliferative retinopathy were found. Increased claudication, diabetic foot infections, or amputations occurred in the contralateral (untreated) limb. Overall, there were 84 adverse events in the 39 treated patients, and 51 events in the 11 placebo patients. There were no deaths in the 52 weeks

Table 4. **Changes in Electrophysiological and Just Noticeable Difference Variables between Baseline and Six Months by Leg and Treatment Group**

Treatment Group	VEGF	Placebo	p
Median Changes in Electrophysiologic Variables (IQR)			
Tibial amplitude			
Untreated leg	0.0 (−0.3, 0.1)	−0.1 (−1.3, 0.0)	0.33
Treated leg	0.0 (−0.2, 0.1)	−0.4 (−2.3, 0.0)	0.27
Peroneal amplitude			
Untreated leg	0.0 (−0.2, 0.1)	0.0 (−0.3, 0.6)	0.88
Treated leg	0.0 (−0.3, 0.0)	0.0 (−0.1, 0.9)	0.12
Amplitude sum			
Untreated leg	0.0 (−0.3, 0.1)	0.0 (−3.0, 0.4)	0.80
Treated leg	−0.1 (−0.4, 0.0)	0.0 (−1.0, 0.1)	0.64
Sural amplitude			
Untreated leg	0.0 (0.0, 0.0)	0.0 (−0.5, 0.0)	0.44
Treated leg	0.0 (0.0, 0.0)	0.0 (−0.8, 0.0)	0.36
Mean Changes in JND Measurements (SE)			
Cold			
Untreated leg	−0.15 (1.11)	−3.14 (3.98)	0.31
Treated leg	−0.68 (1.21)	2.01 (4.65)	0.30
Vibration			
Untreated leg	0.84 (0.81)	−1.66 (1.31)	0.15
Treated leg	0.07 (0.88)	−0.43 (0.51)	0.69

VEGF = vascular endothelial growth factor; IQR = interquartile range; JND = "just noticeable difference"; SE = standard error.

Table 5. **Changes in Sensory Examination Score Subscores between Baseline and Six Months by Leg and Treatment Group**

Treatment Group	VEGF	Placebo	p[a]
Mean SES Subscores (SE)			
Sensation: Pin			
Untreated leg	−0.11 (0.15)	−1.09 (0.48)	**0.01**[b]
Treated leg	−0.21 (0.18)	−0.64 (0.45)	0.15
Sensation: Touch			
Untreated leg	0.24 (0.20)	0.27 (0.75)	0.95
Treated leg	0.11 (0.22)	0.36 (0.69)	0.42
Distribution: Pin			
Untreated leg	−0.18 (0.14)	−0.27 (0.24)	0.76
Treated leg	−0.26 (0.12)	0.18 (0.18)	**0.017**
Distribution: Touch			
Untreated leg	−0.13 (0.14)	0.00 (0.40)	0.70
Treated leg	−0.16 (0.15)	−0.09 (0.34)	0.92
Vibration: Toe			
Untreated leg	−0.16 (0.15)	−0.73 (0.33)	0.10
Treated leg	−0.32 (0.15)	−0.73 (0.36)	0.70
Vibration: Ankle			
Untreated leg	−0.16 (0.19)	−0.09 (0.25)	0.23
Treated leg	−0.11 (0.19)	−0.09 (0.25)	0.47
Proprioception: Toe			
Untreated leg	0.08 (0.25)	−0.18 (0.46)	0.62
Treated leg	0.05 (0.20)	−0.18 (0.46)	0.60

[a]p values for treated leg obtained from analysis of covariance adjusting for change in the untreated leg. [b]The p value assuming unequal variances is 0.07. VEGF = vascular endothelial growth factor; SES = sensory examination score; SE = standard error.

after enrollment, and no hospitalizations related to the trial or to the study agent or the injections.

Discussion

The primary outcome of SS and secondary outcomes of visual pain scores and distribution of sensory loss improved in the VEGF-treated leg in comparison with the placebo-treated leg and compared with the opposite (untreated) leg. Parallel improvement in these measures is plausible and is distribution of pinprick loss in the treated patients. The coprimary outcome of change in at least two of five categorical measures that included clinical and electrophysiological change favored the treated group (12 of 39 vs 2 of 11). Other measures, including nerve conduction studies, showed no statistically significant change. The improvement in the treated leg of 1 or 2 points on a summed symptom scale of 15 has clinical significance. Because most of the motor and sensory nerve electrophysiological measures were moderately to severely abnormal or absent on study entry, it is unlikely that a regenerative effect could have restored enough function to achieve measurable change in electrical function over a 6-month period. The lack of change in nerve conduction measures may have been related to the extent of electrophysiological abnormality at baseline.

It is unlikely that the levels of circulating VEGF were adequate to explain these findings.[14] Hemoglobin A1c concentrations did not differ significantly between groups at any point, and it is not reasonable that any improvement in glycemic control in an individual patient affected one limb preferentially. An alternative interpretation of the improved sensory and pain scores was that VEGF worsened nerve function and thereby reduced symptoms. This is unlikely in view of the parallel improvement in the total area of distribution of sensory loss.

The adjustment for covariance was prespecified and chosen to improve precision of estimated treatment effect in a small study. Presumably by chance, placebo-treated legs showed a larger decline in SS. As a result, analysis of covariance both increased the estimated treatment effect and reduced its standard error, resulting in a significant treatment effect. This method and the improvement in the untreated leg, although valid and specified before analysis of the data set, temper any conclusions.

We used VEGF for its potential to promote neovascularization of the microcirculation of peripheral nerves, although it may also have neurotrophic activity. Both isoforms of VEGF have been shown to be bioequivalent. Because VEGF plays an important role in the pathogenesis of diabetic retinopathy and poses a theoretical risk for promoting neovascularization within occult tumors, we chose a delivery method designed to deliver the gene to the target organ without generating high circulating blood levels of the factor. This was accomplished by injecting naked plasmid DNA adjacent to the main nerve trunks of the leg with the aim of promoting VEGF expression in nerve. As in a previous study,[5] we established that circulating VEGF above baseline levels could not be detected after these injections, and that diabetic retinopathy did not worsen through the period of the study. There were also no instances of neoplastic disease in the year after injections. Mild leg swelling was observed in the treated leg of a few patients, and it is not possible to determine whether this led to unblinding.

The weaknesses of this study are its small sample size, the two isoforms and dose escalation of VEGF, and the use of a nonstandard scale for grading diabetic neuropathy. We have used this scale in a previous

Table 6. **All Adverse Events over 52 Weeks**

Adverse Events	Events/Subject (% of Subjects)		
	Placebo Group (n = 11)	Treatment Group (n = 39)	Total (n = 50)
Chest pain	2/1 (9.1)	2/2 (5.1)	4/3 (6.0)
Diabetic retinopathy	2/2 (18.2)	4/3 (7.7)	6/5 (10.0)
Ecchymosis	2/1 (9.1)	0/0 (0.0)	2/1 (2.0)
Epistaxis	0/0 (0.0)	6/1 (2.6)	6/1 (2.0)
Excoriation	0/0 (0.0)	7/5 (12.8)	7/5 ((10.0)
Eye arterial narrowing	2/1 (9.1)	0/0 (0.0)	2/1 (2.0)
Eye hemorrhage	3/2 (18.2)	0/0 (0.0)	3/2 (4.0)
Eye vascular periphery	2/1 (9.1)	0/0 (0.0)	2/1 (2.0)
Narrowing			
Hemoglobin decreased	2/2 (18.2)	1/1 (2.6)	3/3 (6.0)
Hematocrit decreased	2/2 (18.2)	0/0 (0.0)	2/2 (4.0)
Macular degeneration	2/1 (9.1)	0/0 (0.0)	2/1 (2.0)
Muscle cramp	0/0 (0.0)	6/3 (7.7)	6/3 (6.0)
Pain	5/2 (18.2)	4/2 (5.1)	9/4 (8.0)
Pain in extremity	5/3 (27.3)	11/7 (17.9)	16/10 (20.0)
Peripheral edema	13/5 (45.4)	35/15 (38.5)	48/20 (40.0)
Rectal hemorrhage	0/0 (0.0)	6/3 (7.7)	6/3 (6.0)
Red blood cell count decreased	2/2 (18.2)	0/0 (0.0)	2/2 (4.0)
Retinal exudates	3/1 (9.1)	0/0 (0.0)	3/1 (2.0)
Urinary tract infection	2/2 (18.2)	2/2 (5.1)	4/4 (8.0)
Vertigo	2/1 (9.1)	0/0 (0.0)	2/1 (2.0)

Subjects could have more than one adverse event.

Table 7. **Serious Adverse Events over 52 Weeks**

Subject No.	SAEs Reported
Treatment Group (10 subjects)	
01–1102	2 episodes of myocardial ischemia
001–1103	3 episodes of congestive heart failure; 1 episode of worsening of vascular disease with gangrene
01–1108	2 episodes of calf claudication; 2 episodes of carotid artery disease; 1 episode of coronary artery disease
01–1113	2 episodes of severe asthma
001–2102	1 episode of colorectal bleeding
001–2106	1 episode of colitis
001–2109	2 episodes of diabetic foot infection
001–2110	1 episode of worsening depression
001–2115	1 episode of CHF; 1 episode of myocardial infarction
001–2116	1 episode of stroke
Placebo Group (2 subjects)	
01–1121	1 episode of left foot trauma: cellulitis
001–2114	1 episode of hospitalization for presyncopal episode

SAE = serious adverse event; CHF = congestive heart failure.

study[5] and chose it in preference to other scales because of its simplicity and ease of administration, focus on symptoms and signs in the legs, and its ability to show therapeutic effect in a prior study. During our prior studies, several other scales and subscores failed to show changes even with gross alterations in foot and leg symptoms. The scale that Notermans and colleagues[12] derived, from which our measures were derived, is discussed in Subjects and Methods. However, the scale used in our study has not been validated against other scales.

Growth factor therapy for diabetic neuropathy had been attempted before in the form of nerve growth factor protein injected subcutaneously, three times per week for 48 weeks by Apfel and colleagues.[15,16] Their goal was to create a sustained circulating level of the protein that would act as a trophic factor for damaged nerves. Their initial trial with this approach yielded positive results, or at least a signal for benefit, but a larger randomized trial by the same investigators showed no effect. An analysis of the promising results of the two phase II trials and failure of the single phase III trial concluded that a "robust placebo effect, inadequate dosage, different study populations, and changes to the formulation of rhNGF for the phase III trial" may have explained the lack of benefit of growth factor therapy.[17] A similar trial with subcutaneous injections of recombinant brain-derived neurotrophic factor also failed to show an effect.[18] All of these issues also apply to our study. In addition, we studied a severely affected group of patients with marked sensory symptoms and signs, and absence or marked reduction of motor and sensory nerve conduction potentials, thus limiting the chances for regeneration or improved nerve function. However, our preclinical studies suggested that the delivery of a trophic factor in physical proximity to nerve trunks had a likelihood of success, and we designed our clinical trial based on these models.

Our study provides evidence that intramuscular VEGF gene therapy may improve symptoms of diabetic polyneuropathy and support a larger study to determine the therapeutic effects and safety of VEGF gene transfer in diabetic neuropathy.

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