

2. *The scientific abstract*

Among diabetics, peripheral neuropathy is common and ultimately accounts for significant morbidity. The consequence of such deficits involving the lower extremities may be foot ulceration initiated by trauma that is inapparent to the patient. Such ulcerations often lead to lower extremity amputation, a complication that is 15 times higher in diabetic versus non-diabetic patients. Pain, imbalance, and severe discomfort from numbness are additional features.

Preliminary clinical studies have demonstrated improvement in signs and symptoms of neuropathy in patients with lower extremity vascular occlusive disease following intramuscular injection of naked DNA encoding vascular endothelial growth factor (VEGF). To determine if such a strategy could be applied to diabetic patients, including those without evidence of large vessel occlusive disease, we investigated the hypothesis that experimental diabetic neuropathy results from destruction of the vasa nervorum and can be reversed by administration of an angiogenic growth factor. In two different animal models of diabetes, nerve blood flow and the number of vasa nervorum were found to be markedly attenuated, resulting in severe peripheral neuropathy. In contrast, following VEGF gene transfer, vascularity and blood flow in nerves of treated animals were similar to those of non-diabetic controls; constitutive overexpression of VEGF resulted in restoration of large and small fiber peripheral nerve function. These findings implicate microvascular disruption as the basis for diabetic neuropathy and suggest that angiogenic growth factors may constitute a novel treatment strategy for this pernicious disorder.

Accordingly, we now seek to address the following two objectives:

1. **Objective #1:** is to evaluate the safety and impact of VEGF2 gene transfer on diabetic neuropathy in patients with macrovascular disease involving the lower extremities.
2. **Objective #2:** is to evaluate the safety and impact of VEGF2 gene transfer on diabetic neuropathy in patients *without* macrovascular disease involving the lower extremities.

The protocol outlined in this Investigational New Drug Application has been designed as a Phase I/II, single-site, dose escalating, double-blind, placebo controlled study to evaluate the safety and impact of VEGF-2 gene transfer on sensory neuropathy in patients with diabetes with or without macrovascular disease involving the lower extremities. Diabetic Males or Females ≥ 21 years of age with sensory neuropathy with or without macrovascular disease will be eligible. A total of 192 patients (or up to 212) will be recruited into two arms of the study (each arm consisting of between 96 and 106 patients) over a period of 4 years (the fifth year will be limited to follow-up examinations). Questionnaires requesting information regarding the status of the patient will be sent to the patient's primary care physician annually for fourteen years following completion of the 12-month follow-up. The patients in each of the two arms of the study will comprise 3 cohorts, each consisting of 32 patients. Within each of these cohorts, patients will be randomized to receive VEGF2 transgene or placebo based upon a 3:1 randomization ratio. Thus, at the completion of the study, 24 patients will have each received a given dose (2, 4, or 8 mg VEGF2) and 24 patients will have received placebo. Doses will be employed in a serial dose-escalating fashion. The entire volume of the study drug will be divided and delivered in 8 intramuscular injections administered into the affected extremity, between the gluteal fold and the ankle. Two injections will be made adjacent to the sciatic nerve, in the posterior thigh; the injection sites will be at one-third and two-thirds the distance

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between the gluteal fold and the popliteal fossa. Three injections will be made adjacent to the tibial nerve in the calf; sites will be one-quarter, one-half, and three-quarter the distance between the popliteal fossa and the lateral malleolous. The three injection sites adjacent to the peroneal nerve will be at the same intervals as for the tibial nerve, but in the anterior-lateral foreleg. All injections will be above the ankle. Following the initial set of injections, repeat treatment with an identical dose will be provided 2 and 4 weeks after the initial treatment.