Scientific Abstract

This randomized, double-blind, placebo-controlled, dose-escalating gene therapy clinical study will evaluate the effects of several dose levels of intramuscular pVGI.1(VEGF2) plasmid deoxyribonucleic acid (DNA) versus placebo with respect to safety and efficacy in patients with moderate-risk critical limb ischemia (CLI). The pVGI.1(VEGF2) plasmid contains the complementary DNA sequence for the vascular endothelial growth factor-2 (VEGF-2) protein, a member of a class of natural growth factors that promote angiogenesis. This study will obtain information regarding the safety, duration of activity, and optimal dose of this gene for the treatment of critical limb ischemia.

The primary objectives of this study are as follows:

- To evaluate, through dose escalation in defined increments, the safety of intramuscular administration of pVGI.1(VEGF2) versus placebo in adult patients with moderate-risk CLI (Rutherford score 4) by assessing the frequency, duration, and severity of adverse events

- To assess, in adult patients with moderate-risk CLI, the effect and duration of activity on resting leg pain (as assessed by frequency of rest pain, pain medication use history, sleeping history, and intensity of rest pain) of single, defined, increasing doses of pVGI.1(VEGF2) given by direct intramuscular injection into the affected leg when compared to placebo

A secondary objective of this study is to assess, in adult patients with moderate-risk CLI, the effect and duration of activity on Rutherford Clinical Severity Score, exercise test, ankle-brachial index (ABI), and great-toe index (GTI) of single, defined, increasing doses of pVGI.1(VEGF2) given by direct intramuscular injection into the affected leg when compared to placebo. Key inclusion criteria for patients in this study are critical limb ischemia as defined by a Rutherford score of 4, no reproductive potential, an ABI of less than 0.6 or a great-toe index GTI of less than 0.3, and angiographic evidence of total occlusion in an artery of the affected leg. Key exclusion criteria are the following: concomitant disease resulting in a life expectancy of less than 1 year; nonhealing ulcers or other evidence of tissue loss in the ischemic leg; a history of neoplasm or active retinopathy; a history of recent, successful aortic or lower extremity surgery or angioplasty; and presentation as an optimal candidate for revascularization of the affected limb.

This study will include 12 patients who will be enrolled sequentially into 3 dosing cohorts. Each cohort will consist of 4 patients. Within each dosing cohort, patients will be randomized to receive either pVGI.1(VEGF2) or placebo in a 3:1 ratio. Three pVGI.1(VEGF2) dose levels will be used: 2, 4, or 8 mg. The patient will receive the total dose by 8 intramuscular injections into the affected limb given in a single treatment session.

All patients in a dosing cohort will be evaluated for safety prior to progressing to the next dosing cohort; therefore, dosing in successive cohorts will occur not less than 4 weeks apart. The overall study duration will be approximately 3 months, consisting of a Screening/Baseline Phase (up to 2 weeks), a Treatment Phase (1 day), and a Post-treatment Phase (12 weeks following the final treatment).

An efficacy analysis will be conducted on all patients randomized into the study (*i.e.*, the intent-to-treat population). A secondary analysis for those patients that have completed the Weeks 4 and 12 post-treatment assessments will also be performed.

During this dose-escalating study, safety will be evaluated based on the adverse events experienced by the patients. Efficacy will be evaluated primarily based on resolution of rest pain. Efficacy will also be examined using the Rutherford clinical severity score, hemodynamic measurements (ankle-brachial index and great-toe index), and results of an exercise test.