

1. Scientific Abstract

Progressive atherosclerosis in the peripheral arterial circulation leads to multi-segmental stenosis and occlusions causing peripheral arterial occlusive disease (PAOD). This leads to ischemia in the affected extremities causing rest pain. Any therapeutic strategy that improves peripheral arterial blood flow may ameliorate rest pain as well as prevent the progressive nature of the disease. Pharmacotherapy is widely used, but generally ineffective for symptomatic treatment except possibly in patients with intermittent claudication. No available pharmacotherapy has been shown to alter disease progression. Revascularization with peripheral arterial bypass surgery or catheter-based percutaneous intervention is the only effective standard treatment for symptomatic disease with appropriate anatomy for such interventions. Despite modern surgical and endovascular techniques, effective results are often short-lived. Also, for technical and anatomic reasons there are always a significant number of patients who are not candidates for revascularization. Ultimately, failed or ineffective revascularization may lead to amputation. Both revascularization surgery and amputation are costly and associated with significant morbidity and mortality.

Recognizing the morbidity, mortality and amputation rates associated with advanced-stage PAOD (Fontaine stages III and IV), there is clearly an urgent need for improved therapy for patients with severe leg ischemia, which can be life-threatening. The incidence of major amputations due to PAOD is estimated to be between 300 to 500 cases per million patients per year. Thus, new treatment options, such as therapy with an angiogenic growth factor, which may improve peripheral arterial blood supply, may have important therapeutic potential for this progressive, debilitating disease.

Angiogenic gene therapy using an adenovirus 5.1 vector encoding a fibroblast growth factor-4 construct (Ad5.1FGF-4) administered intramuscularly significantly increased peripheral blood flow in a rat model of limb ischemia. An efficacy study was performed in a rabbit hind-limb ischemia model that compared neovascularization by adenoviral gene transfer of human fibroblast growth factor-4 (FGF-4) and murine vascular endothelial growth factor (VEGF-164). This study demonstrated that adenoviral gene transfer of FGF-4 induces vascular permeability, angiogenesis and arteriogenesis comparable to that of VEGF-164 gene transfer, consistent with improved peripheral blood flow. This implies that adenoviral gene transfer of FGF-4 could be useful for the treatment of peripheral arterial occlusive disease.

The proposed clinical study will evaluate the safety and potentially beneficial effects of the FGF-4 angiogenic gene therapy after intramuscular injection in PAOD patients with chronic leg ischemia who are moderately to severely incapacitated by the disease, have a high risk of progression, and face permanent disability due to amputation. This new therapeutic technique, with its simple mode of application, compared with reconstructive surgery or endovascular procedures, is expected to relieve ischemic pain and/or lower the rate of deterioration due to the disease. Furthermore, this type of therapy could provide a

treatment option for patients wishing to avoid the risks associated with revascularization, or could serve as an adjunct treatment to revascularization or other invasive procedures.

The study will enroll patients with PAOD Fontaine stage III or IV in a double-blind, randomized, placebo-controlled, multicenter, ascending dose trial. A minimum of 8 evaluable patients (6 active, 2 placebo) will be evaluated per dose group with 4 ascending doses (increasing by 1 log steps) unless safety concerns prevent dose increases.

The following dose groups will be evaluated:

1. 2.87×10^8 viral particles
2. 2.87×10^9 viral particles
3. 2.87×10^{10} viral particles
4. 2.87×10^{11} viral particles

At each dose group, patients will be randomly assigned to one of the two treatment groups:

1. Ad5.1FGF-4 given as 6 to 10 intramuscular injections into the respective leg on a single day
2. Placebo (Vehicle) given as 6 to 10 intramuscular injections into the respective leg on a single day

A Safety Review Committee will review 2-week safety data from all patients in the last dose group before enrolling patients in the next higher dose group. The decision to progress to the next dose group will be made by the sponsor based upon a satisfactory review of the information. The study will be conducted in up to 15 different medical centers and will recruit a total of up to 52 suitable patients, aged 40 and above. The study ends 12 weeks after the study treatment, but additional safety data will be obtained outside of the study at 6, 12, 24 and 36 months after the study treatment.

The objectives of this study are to evaluate the safety of ascending doses of Ad5.1FGF-4; to find suitable doses for subsequent study, and to evaluate potentially beneficial effects of Ad5.1FGF-4. Potential beneficial effects will be assessed as follows:

- Assessment of rest pain by the use of analgesics
- Assessment of ulcers
- Ankle and toe blood pressure for collateral development
- Tissue analyses of the amputated leg (if applicable).

- Assessment of the need for and extent of amputation, examination of deaths (if applicable)
- Digital subtraction angiography for prevention of disease progression and angiogenesis