SCIENTIFIC ABSTRACT

Treatment is a critical problem in the management of severe Peripheral Artery Disease (PAD). Three therapeutic approaches are usually considered for patients suffering from PAD (Isner & Rosenfield, 1993): 1) risk factor modification such as tobacco, dietary changes, etc.; 2) when possible, percutaneous transluminal angioplasty (PTA) to revascularize the ischemic limb; 3) bypass surgery with the use of prosthetic material or vein graft. Both PTA and especially surgery carry a significant morbidity/mortality in these patients who often suffer concomitantly from an already existing heart or disseminated vascular disease.

The concept of therapeutic angiogenesis is based on the use of angiogenic factors such as Fibroblast Growth Factor (FGF) to promote neovascularization for the treatment of ischemic tissues. Both the administration of recombinant angiogenic factors, or gene transfer by viral and non viral DNA vectors that express these factors, were shown to induce angiogenesis in a number of preclinical animal models particularly those with surgically-induced limb and coronary ischemia, as well as more recently in humans.

We engineered an expression cassette containing a naturally occurring truncated form of FGF-1 fused to a heterologous secretion signal (sp.FGF 1) flanked by a strong viral promoter and polyadenylation sequences. This expression cassette was inserted in a proprietary plasmid backbone with a Conditional Origin of Replication (pCOR). The resulting plasmid was named NV1FGF.

Biological activity was demonstrated in vitro. Efficacy of IM administration of plasmids encoding sp.FGF1 was evaluated in rabbit and hamster models of hind limb ischemia. In the rabbit model, plasmid doses (0.1, 0.5 and 1 mg) were administered 10 days after induction of limb ischemia and efficacy was assessed 30 days after treatment by measuring different anatomical and hemodynamic parameters. sp.FGF-1 gene transfer led to the formation of angiographically visible collateral blood vessels. In the hypercholesterolemic hamster model, which exhibit sustained defects in collateral vessels formation after induction of ischemia, IM administration of NV1FGF (0.18 mg) significantly increased the angiographic score and the number of small arterioles in the injected ischemic limbs.

Expression of FGF-1 protein or/and FGF-1 mRNAs in the injected muscle was demonstrated after NV1FGF I.M. administration in various animal species (mice, rats and dogs) and in CLI patients. Expression of NV1FGF-derived FGF-1 protein was detected by immunohistochemistry in the myofibers of the injected muscle only. Expression of NV1FGF-derived mRNA was shown to be local and restricted to a few cm around the injection site as shown in animals and CLI patients.

In the rat, I.M. administration of NV I FGF was associated with limited and transient levels of NV1FGF sequences in blood and organs other than the injected muscle The NV1FGF sequences

were mostly product of plasmid degradation with no capability to express sp.FGF1. There was no evidence of NV1FGF-derived mRNA suggesting that the transient tissue distribution of NV1FGF sequences did not led to NV1FGF expression in organs other than the injected muscle.

IM NV1FGF toxicity studies did not show any treatment-related effect other than minimal to mild focal/multifocal inflammation at the injection site.

In an open label phase I trial involving 51 patients, single and repeated (two) IM doses of NV1FGF in patients with CLI were well tolerated and significantly improved symptoms (pain, ulcer size) and hemodynamic parameters in the treated limb. In a phase II double blind, randomized, placebo-controlled, multicentric study including 107 evaluable patients with CLI, with non-healing ulcer and no-option for revascularization, 4 IM administrations of NV1FGF 4mg at 2- week intervals have shown a similar improvement in ulcer healing as compared to placebo but significantly prolonged the time to first amputation and to first major amputations.

The data of this previous study lead us to perform a new single important study to confirm the benefit of NV1FGF in delaying the time to amputation or to death in CLI patients with skin lesions and no option for revascularization.

This trial will be randomized, parallel group, double blind, placebo-controlled since suitable treatments have not been established in the treatment of ischemic lesions. A 2 to 6-weeks screening period will be performed in order to provide some evidence of the disease chronicity and ulcers stability.

Only patients having CLI with skin lesions (either ulcers stable over the last 2 weeks interval or gangrene) and unsuitable for standard revascularization (surgical/interventional) should be included. Inclusion will be stratified both by diabetes status to insure equal distribution of patients with diabetes mellitus among the 2 and by study country.

In this study, usual medical care strategies in PAD are allowed and strongly recommended as per guidelines for the prevention of cardiovascular events in this high risk population.