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1.0 SCIENTIFIC ABSTRACT

Lower extremity peripheral arterial disease (PAD) is increasingly prevalent in the United States. It is currently estimated to affect 8 to 12 million individuals. One-third to one-half of these patients suffer from intermittent claudication (IC). Individuals with PAD have a reduced functional capacity, lower quality of life, and an extremely high risk of cardiovascular morbidity and mortality. IC is classically defined as pain in one or both legs that occurs with walking or exertion, does not resolve with continued activity, and abates (within 10 minutes) upon rest or a reduction in walking pace. Symptoms vary depending on the extent and levels of disease involvement, but are commonly described as a cramping pain with or without muscle weakness. Regardless of the location and distribution of PAD within the lower extremity vasculature, claudication symptoms are most frequently localized to the muscles of the calf and are manifested as alteration in resting hemodynamic measurements in the lower extremity. Patients with IC generally have an ankle-brachial index (ABI) between 0.4 and 0.9, with lower values being associated with increasing disease severity and cardiovascular risk.

Most patients with IC and/or PAD are treated primarily to relieve lower extremity symptoms, increase functional walking capacity and quality of life, prevent the progression of disease and ulcer formation, and preserve limb tissue. Management of risk factors, lifestyle interventions, and pharmacologic treatment with agents to provide symptomatic relief have a central role in improving function, quality of life and retarding the progression to advanced endpoints such as rest pain, non-healing ulcers, gangrene and cardiac death. Surgical or percutaneous revascularization for aorto-iliac disease provides durable treatment for individuals with disabling symptoms. Infra-inguinal disease, even if extensive, very rarely justifies surgical intervention for claudication. Although select patients with superficial femoral artery disease and claudication may be considered for surgical treatment or percutaneous recanalization, these techniques are not successful in the vast majority. Similarly, in the patient with distal disease afflicting the tibio-peroneal circulation, there is a limited role for primary infrapopliteal angioplasty or surgery unless the patient is

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experiencing critical limb ischemia. Thus the treatment of infra-inguinal disease is predominantly medical in patients with IC.

The current therapeutic options available to patients with symptomatic IC are primarily exercise, pentoxifylline, and cilostazol. The latter agent is the only approved agent for the treatment of IC. However, this vasodilator drug does not result in biologic modification of the underlying disease and characteristically the symptoms return on cessation of the drug. In addition, in clinical trials evaluating this agent, there is high incidence of side effects such as headaches, palpitations and GI disturbances. In the patient population with IC, a substantial proportion has concomitant coronary disease and depression of ejection fraction owing to antecedent myocardial infarction, thus precluding the use of this agent. Another agent called pentoxifylline (Trental) provides only marginal benefits on walking ability and symptom relief, with a meta-analysis of eight well-controlled studies concluding that pentoxifylline yields an increase of 44 meters in total walking distance.

The establishment and maintenance of a vascular system is a fundamental requirement for the growth of both normal and neoplastic tissue. The last decade has seen tremendous advances in our understanding of the process of vessel wall development and has led to the identification of novel angiogenesis inducers which may have application in a wide range of disorders including the manifestations of atherosclerosis. The cellular and molecular mechanisms in angiogenesis are complex and involve a series of steps including cell proliferation, migration, cell-cell and cell-matrix interactions, extra cellular matrix turn-over and eventual formation of a primitive capillary tube that then evolves through a process of myocyte and pericyte recruitment into a blood vessel. Thus, this entire process requires careful orchestration in a temporal and spatially specific fashion, of a multitude of events likely through a host of growth factors. These developments have spurred the field of therapeutic angiogenesis or the deliberate induction of a network of blood vessels with the ostensible reason of improving tissue perfusion. Angiogenesis can be achieved through either delivery of recombinant protein or gene therapy approaches. The former approach, using delivery of recombinantly manufactured growth factors, has been shown to be effective

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in inducing an angiogenic response in a variety of animal models of acute limb and coronary ischemia, sometimes with the use of a single dose of an agent. Although studies in animals with systemic growth factors have been encouraging, recent results from Phase II trials involving systemic administration of recombinant basic fibroblast growth factor (bFGF or FGF-2) and vascular endothelial growth factor isoform 165 (VEGF₁₆₅) protein in subjects with myocardial ischemia have not demonstrated clinical benefit. Studies in PAD with bFGF suggest a benefit in patients with advanced IC. These equivocal findings with protein therapy approaches, at least in coronary artery disease, have been attributed to the short tissue residence of recombinant proteins owing to their rapid degradation or alternately to the concentration of a recombinant protein at a target site being insufficient to obtain the desired therapeutic effect. This has resulted in the hypothesis that localized approaches that result in sustained expression of a protein such as that resulting from gene transfer approaches may translate into phenotypic effects that are physiologically meaningful. In many ways, angiogenesis offers some of the greatest opportunities in the field of gene transfer. The advantages of using PAD as the initial testing field for angiogenic treatments include: (a) easy access to afflicted tissue; (b) simple intramuscular delivery strategies that do not require sophisticated catheter based technology; (c) amplification of the angiogenic response secondary to the ischemic up regulation of the receptor for vascular endothelial growth factor- flk-1 in hypoxic areas; and (d) a non-requirement of persistence of transgene expression, owing to the concerns of sustained and distant angiogenic responses.

The Developmentally regulated Endothelial Locus-1 (Del-1) is an endothelial cell-specific matrix protein expressed during embryological development of the vascular tree. Postnatally, it is also expressed at sites of angiogenesis. Del-1 supports the adherence and migration of endothelial cells, mediated via binding to the $\alpha v \beta 3$ integrin receptor. Del-1 also increases vessel formation in the chorioallantoic membrane and disk assays. Results from in-house preclinical studies with recombinant murine Del-1 protein and with formulated Del-1 plasmid compare favorably to results obtained with bFGF and VEGF165. The reasoning behind using a plasmid-DNA approach is that sustained local delivery offers the prospect of enhancing local concentrations with a consequent reduction in systemic exposure to the angiogenic

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growth factor in conjunction with the avoidance of issues that may arise with the use of a viral platform. Formulated Del-1 plasmid has been shown to elicit angiogenic and therapeutic effects in multiple animal models. In mice, injection of Del-1 plasmid was shown to increase capillary density in hindlimb muscle and to increase treadmill run time in a mouse model of hindlimb ischemia. In a rabbit model of hindlimb ischemia, injection of Del-1 plasmid was found to increase formation of collateral arteries and expression of the capillary endothelial marker CD-31. These results with Del-1 plasmid compare favorably to results obtained with VEGF165 plasmid, which was used as a comparator in these experiments. To date, no toxicity has been directly attributable to VLTS-589 or other formulated hDel-1 plasmids in preclinical animal studies, even at elevated levels of Del-1 expression (achieved by using electroporation to increase expression approximately 100-fold). These studies demonstrated that VLTS-589 injected in the muscle has the same effect on re-vascularization as the Del-1 recombinant protein.

A Phase I clinical trial with VLTS-589 in human subjects with PAD is currently ongoing to evaluate the safety and tolerability of the investigational drug (RAC Protocol #0010-424). To date, a total of 18 subjects have received the investigational drug, in which 3 – 84 mg has been administered per individual in escalating dose. To date, there have been no adverse events (other than two cases of mild ecchymosis) considered to be by the investigators and the sponsor to be related to administration of the investigational drug. Subjects have been treated as outpatients and have not had any drug-related signs or symptoms requiring treatment or hospitalization following administration of the investigational drug. The proposed Phase II trial with VLTS-589 will commence following conclusion of enrollment, investigational drug administration, and the primary observation period of subjects in the Phase I trial

The proposed Phase II trial is a multi-center, double-blind, placebo-controlled trial designed to evaluate the safety and tolerability of VLTS-589 in subjects with intermittent claudication secondary to peripheral arterial disease and to evaluate the effect on the subject's peak walking time (treadmill) at the end of day 90 compared to placebo. Following the completion of the currently ongoing Phase I safety trial, the proposed Phase II trial will enroll

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approximately 100 subjects; half of which will receive a placebo in place of the investigational drug.