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Trial Design Paper

Design of the Del-1 for Therapeutic Angiogenesis Trial (DELTA-1), a Phase II Multicenter, Double-Blind, Placebo-Controlled Trial of VLTS-589 in Subjects with Intermittent Claudication Secondary to Peripheral Arterial Disease

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ABSTRACT

The objective of this phase II investigation is to assess the safety and efficacy of a plasmid mediated approach to induce angiogenesis/arteriogenesis with the angiomatrix protein Del-1 (developmentally regulated endothelial locus 1), in subjects with intermittent claudication (IC) secondary to peripheral arterial disease (PAD). VLTS-589 is an investigational nonviral therapeutic comprising a plasmid-expressing Del-1 formulated with poloxamer 188 (facilitating agent). One hundred subjects with bilateral PAD and IC will be randomized after careful screening to bilateral intramuscular delivery of VLTS-589 or placebo. A total of 84 mg of plasmid or placebo will be delivered as 42 intramuscular injections (2 ml per injection, 21 injections or 42 ml in each extremity of either plasmid or placebo) in both lower extremities. The subjects in the study will be followed at regular intervals for a year after study drug administration (days 30, 90, 180, and 365) with the primary endpoint being the safety and tolerability of VLTS-589 and change in peak walking time (PWT) at day 90. The secondary endpoints include percent and absolute change in resting ankle brachial Index, claudication onset time, and quality of life measured at various time points. DELTA-1 represents the largest plasmid-based gene transfer trial designed to test the efficacy of a Del-1 as a therapeutic approach in patients with IC caused by PAD. The novel aspects of the protocol include the usage of a Del-1 plasmid-polaxamer formulation to enhance gene transfer at doses that are an order of magnitude different than other comparable trials in a unique bilateral intramuscular dosing pattern to maximize transfection/clinical efficacy and general applicability to patients with PAD.

INTRODUCTION

INTERMITTENT CLAUDICATION (IC) caused by peripheral arterial disease (PAD) is increasingly prevalent in the United States (Criqui, 2001). While IC secondary to aortoiliac occlusive disease has effective and durable revascularization treatment options, the treatment of infrainguinal disease remains predominantly medical (Dormandy and Rutherford, 2000). Angiogenic approaches for the treatment of PAD with cell- or gene-based therapy relies on the induction of neovascularization with improvements in perfusion to the lower extremities (Muyayam and Ashara, 2000; Yla-Herttuala and Alitalo, 2003). Gene transfer approaches have the intrinsic advantage of sustained delivery (compared to protein approaches) and can be accomplished by viral and nonviral approaches (Nabel, 1995). Nonviral gene transfer is attractive, because it circumvents is-

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sues pertaining to preexisting immunity, cytotoxicity, and viral integration into the genome. However, transfection with naked DNA is intrinsically inefficient (Niidome and Huang, 2002; Herweijer and Wolff, 2003). Plasmid adjuncts such as polaxamers have been shown to enhance expression and may have additional properties that may prove beneficial in angiogenesis applications, including promoting macrophage activation and infiltration at the site of delivery (March *et al.*, 1995; Lemieux *et al.*, 2000; Moghini and Hunter, 2000).

This phase II trial will investigate the safety and efficacy of a plasmid expressing Del-1 (developmentally regulated endothelial locus 1) in conjunction with polaxamer 188, in patients with moderate to severe IC caused by PAD. Del-1 is a novel factor belonging to the angiomatrix family of proteins, transiently expressed during vasculogenesis (Hidai *et al.*, 1998) that induces a potent angiogenic response, through coordinate upregulation of the integrins $\alpha v\beta 5$ and $\alpha v\beta 3$ (Zhong *et al.*, 2003). As opposed to traditional angiogenic approaches that rely on overexpression of one or more growth factors, usage of such a strategy stimulates angiogenesis indirectly, through ligation of the integrin $\alpha v\beta 5$, which in turn increases expression of $\alpha v\beta 3$ and the transcription factor Hox D3, eventually resulting in activation of a host of cellular responses compatible with an angiogenic phenotype.

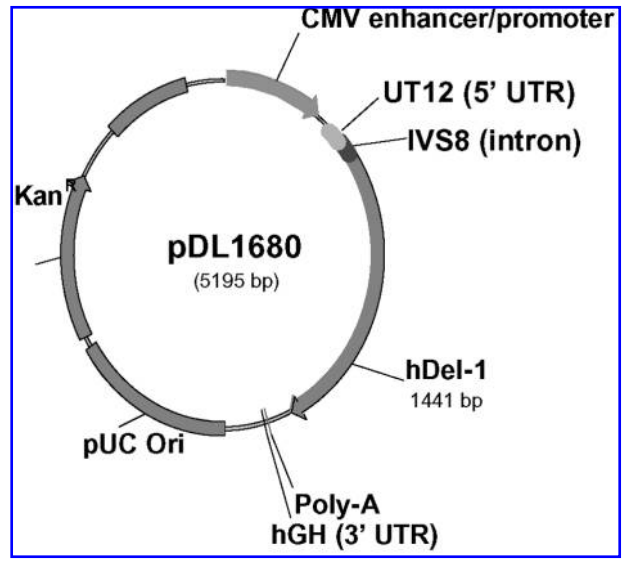

FIG. 1. Plasmid map of pDL1680 containing the 1441 bp human Del-1 gene. HGH, human growth hormone; 3′ UTR, 3′ untranslated region; 5′ UTR, 5′ untranslated region; CMV, cytomegalovirus; Kan^R, Kanamycin resistance.

MATERIALS AND METHODS

The protocol will be approved by the Institutional Review Board (IRB) in each participating institution and all patients will provide written informed consent. This is a phase II multicenter, double-blind, placebo-controlled trial in which subjects with IC secondary to bilateral infrainguinal PAD will receive a single treatment of VLTS-589 (Del-1 expressing plasmid plus polaxamer 188) or placebo administered as an intramuscular injection to the bilateral lower extremities during one procedure.

Study drug

VLTS-589 is an investigational nonviral, plasmid-based therapeutic comprising a plasmid expression system formulated with poloxamer 188 (facilitating agent). The plasmid preparation is identical to that used in preclinical studies (Zhong *et al.*, 2003). With the exception of the addition of the plasmid, the manufacture and content of the placebo in this trial is identical to that of the drug product, VLTS-589.

The plasmid (pDL1680) is 5 kilo-base pairs in length and contains a eukaryotic expression cassette encoding the full-length human developmentally regulated endothelial locus-1 (Del-1) protein under the control of a cytomegalovirus promoter (Fig. 1). Del-1 expression is enhanced approximately 10 times by a 5′ untranslated region (UTR; UT12) sequence containing a proprietary synthetic IVS8.

Endpoints

The primary endpoints of the investigation are to evaluate the safety and tolerability of VLTS-589, with peak walking time (PWT) at day 90 as the primary efficacy endpoint, compared to subjects receiving placebo. The secondary endpoints include (1) change in PWT at days 30, 180, and 365; (2) percent and absolute change in resting ankle brachial index (ABI) at days 30, 90, and 180; (3) percent and absolute change in the claudication onset time (COT) from baseline to days 30, 90, and 180; and (4) changes from baseline to days 90, 180, and 365 in quality-of-life questionnaires (Walking Impairment Questionnaire [WIQ] and Medical Outcome Scales Health Survey Short Form [SF-36v2™; McHorney *et al.*, 1993; Hiatt *et al.*, 1995]). The subjects will be treated as outpatients during the course of the trial.

Trial phases

The trial is comprised of three phases: screening, dosing, and follow-up as illustrated in Figure 2.

Screening phase. The inclusion exclusion criteria for the trial are listed in Table 1. At the time of screening, subjects will be required to be in compliance with the American Cancer Society (ACS) current recommended guidelines for screening tests for malignancies of the colon, breast (females only), cervix/uterus (females only), prostate (males only), and lung. Any subject found to have cancer by these screening tests will be excluded from the trial. The patients will undergo a drug washout of contraindicated medications. These include drugs that may potentially interfere with or potentiate the angiogenic response, cyclooxygenase-2 (COX-2) inhibitors, angiotensin-converting enzyme (ACE) inhibitors, and agents such as cilostazol that have been shown to be beneficial in improving walking performance (Simons *et al.*, 2000). These drugs may not be initiated during the 4-week screening phase or at any time during 90 days of the protocol; however if the subject has been on a stable dosage of any of these for at least 3 months, it may be allowed in the study provided no dosing adjustments are allowed for 6 months following randomization, except because of medical necessity.

The patient will undergo a history and physical examination,

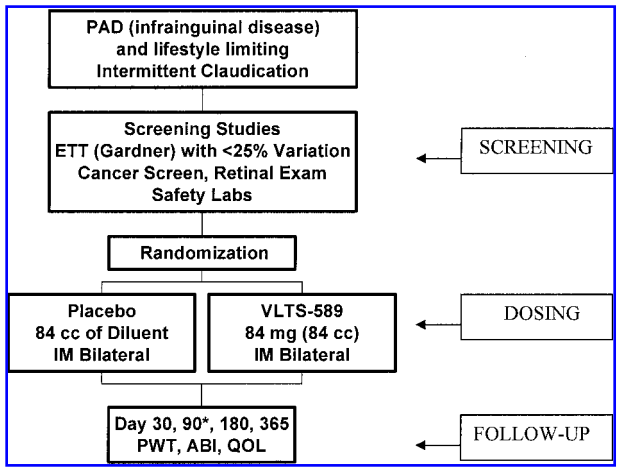

FIG. 2. Study stages and endpoints. *Corresponds to primary end-point time point.

ABI, and PWT measurements to establish eligibility and will return to repeat these tests in screening visit 2. The variability in PWT recorded at screening visit 1 (PWT1) and 2 (PWT2) will have to be less than 25% of the difference between PWT1 and PWT2 using a standardized Gardner protocol ETT (Exercise Tolerance Test; Gardner *et al.*, 1991). If variability is more than 25%, a third ETT may be performed at the discretion of the medical monitor between 3 and 14 days of visit 2. The subjects will also undergo administration of quality-of-life questionnaires (i.e., WIQ, SF-36v2™) during screening qualification visit 2.

Dosing phase. The patients will undergo administration of the study drug as an outpatient after ensuring that they meet all the safety criteria including a pregnancy test, if female of reproductive age. Under local anesthesia, patients will undergo bilateral drug administration using the schema illustrated in Figure 3. The bilateral administration scheme is designed to provide coverage of the largest area possible. The paired injection pattern is designed to deposit the investigational agent in a manner that bridges the region of adequate to inadequate perfusion. Subjects will be monitored during administration of VLTS-589 for signs of systemic or local treatment-related toxicity.

Scheduled physical examinations, eye examinations, laboratory tests, and disease assessments will be performed during the course of the trial. Serum samples from all subjects will be collected and stored for potential assay of hDel-1 protein and antibodies.

Follow-up phase. Subjects in the study will be followed at regular intervals for a year after study drug administration (day 3, 7, 30, 90, 180, and 365). At each of these time points study-specific procedures will be conducted. For any subject who requires bypass surgery, lower extremity percutaneous intervention or amputation, an exit ABI or TBI and a Gardner protocol ETT will be obtained (provided subject is still ambulatory). For any subject who undergoes posttreatment amputation, attempts will be made to obtain tissue samples and these will be analyzed for the presence of hDel-1 DNA, and vascularity (vessel count and CD31 positivity).

Statistical considerations

Sample size. The study was designed to detect a difference of 1.5 min in PWT from baseline to day 90 between the placebo and the VLTS-589 groups. This translates into roughly a treadmill stage difference in walking distance, and was felt to be clinically meaningful (the observed difference between drug and placebo arms in a prior study with fibroblast growth factor was 1.17 min and was 2.0 min in a prior cilostazol study) (Dawson *et al.*, 1998; Lederman *et al.*, 2002). The average of these two studies is approximately 1.5 min. Assuming a standard deviation of 2.5 min (based on previous studies), a two-sided *t*-test for independent samples with a significance level of 0.05 would require 45 completed subjects per treatment group in order to have 80% statistical power to detect a difference of at least 1.5 min between VLTS-589 arm and placebo. Assuming a 10% attrition rate between dosing assignment and

TABLE 1. INCLUSION AND EXCLUSION CRITERIA

Inclusion criteria
Male or female, ≥40 and ≤80 years old
Exercise-limiting IC in lower extremities, of >2 months duration
Diagnosis of PAD at screening:
ABI of ≤0.80 in both lower extremities after 10 minutes of rest at screening or a toe-brachial index (TBI) of <0.70
Mean peak walking time (PWT) of 1–10 minutes (inclusive) on a standardized Gardner protocol
Exclusion
Inflow disease (>50% stenosis in the distal aorta, common/external iliac or common femoral artery)
Critical limb ischemia
Termination of treadmill for other than claudication reasons
Percutaneous intervention within 2 months or lower limb surgical vascularization within 6 months prior to entry
Participation in a structured excercise treatment protocol within 30 days
Presence of the following medical conditions (unstable angina, myocardial infarction, CABG, PTCA, stroke, CHF or deep vein thrombosis)
Malignant neoplasm within the previous 5 years (except curable nonmelanoma skin malignancies) or the presence of proliferative retinopathy.

IC, intermittent claudication; PAD, peripheral artery disease; ABI, ankle brachial index; CABG, coronary artery bypass graft; PTCA, percutaneous transluminal coronary angioplasty; CHF, congestive heart failure.

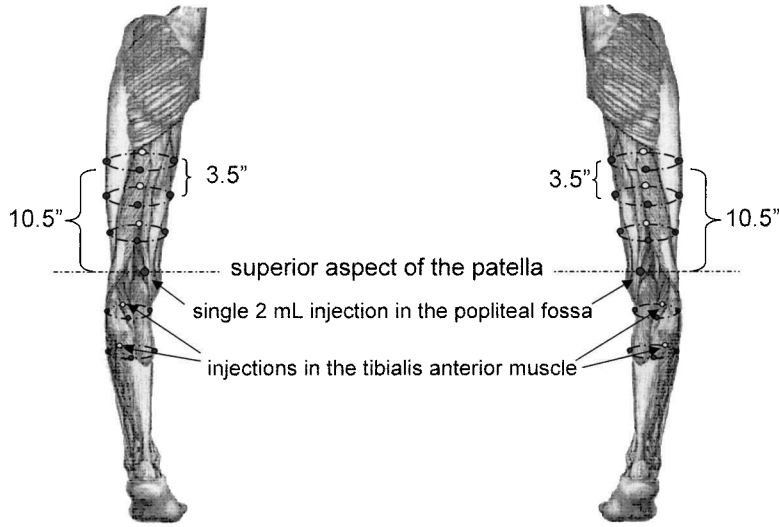

FIG. 3. Injection strategy employed in the trial and anatomic areas injected. A total of 21 intramuscular (IM) injections of 2 ml each (42 mg of plasmid or placebo) will be delivered into each lower extremity in the dosing pattern illustrated. The dots indicate areas of delivery.

study completion, at least 50 subjects per treatment group (100 total subjects) will be randomized.

Efficacy analysis. The primary and secondary analysis will be based on an analysis of covariance (ANCOVA) to compare the effects of VLTS-589 and placebo on variables (change in PWT and ABI from baseline to day 90). Other efficacy endpoints such as the change in ABI and PWT at day 180 and 360 will also be evaluated. Exploratory analysis will be conducted on the primary endpoint to investigate the effects of risk factors such as smoking status, diabetes, and age on PWT.

Safety analysis. Adverse events and serious adverse events (defined using Food and Drug Administration [FDA] criteria) will be summarized (frequency and percentage) by body system and preferred term by treatment group. All chemistry, hematology, and vital sign measurements will be presented using summary statistics including change from baseline and shift tables by visit for each treatment group.

DISCUSSION

DELTA-1 represents the largest plasmid based gene transfer trial designed to test the efficacy of a Del-1–based therapeutic approach in patients with IC caused by PAD. There are several novel aspects of the design of this trial that are noteworthy: (1) the usage of a novel angiomatrix protein Del-1 that may represent a growth factor independent strategy to induce therapeutic angiogenesis; (2) the usage of plasmid-polaxamer combination to enhance gene transfer; (3) the usage of plasmid doses that are an order magnitude different than other comparable trials; and (4) a unique dosing pattern and bilateral intramuscular dosing to maximize both transfection/clinical efficacy and general applicability, respectively.

The use of Del-1 as the transgene in this trial for claudication is well grounded by strong preclinical observations that support Del-1 as a potent angiogenic agent. Del-1 is an $\alpha v\beta 3$ integrin that exerts potent arteriogenic and angiogenic effects both *in vitro* and *in vivo* (Penta *et al*., 1999; Rezaee *et al*., 2002). Preclinical and early phase I data in animal models of hind limb ischemia and humans respectively, have demonstrated efficacy that is comparable to other angiogenic growth factors (Zhong *et al*., 2003). Traditional approaches to therapeutic angiogenesis in ischemic vascular disease have centered on the usage of soluble growth factors such as vascular endothelial growth factor (VEGF) to initiate angiogenesis, although there is emerging literature to support the contention that VEGF levels in ischemic tissues may not be limiting (Blann *et al*., 2001, 2002; Heeschen *et al*., 2003). An alternate approach to angiogenesis that has recently been described involves a class of proteins referred to as angiomatrix proteins because these are typically found in the matrix and promote $\alpha v\beta 3$ integrin-dependent angiogenesis in the absence of exogenously added growth factors (Penta *et al*., 1999). Among the members of this family are proteins such as Del-1, Cyr 61, Nov, and connective tissue growth factor/Fisp12 (Babic *et al*., 1998, 1999; Kireeva *et al*., 1998; Hidai *et al*., 1999). Del-1 is a 52-kd protein that contains multiple epithelial growth factor (EGF) domains and an arginine-glycine-aspartic acid (RGD) motif that stimulates angiogenesis through ligation of $\alpha v\beta 5$, which is turn results in the expression of $\alpha v\beta 3$ and increased expression of the angiogenic transcription factor Hox D3. This results in transformation of resting endothelial cells to an angiogenic/invasive state (Penta *et al*., 1999; Aoka *et al*., 2002; Rezaee *et al*., 2002; Zhong *et al*., 2003). Expression of Hox D3 even in the absence of growth factors such as fibroblast growth factor (FGF) results in angiogenesis and may represent an alternate mechanism for angiogenesis that centers on activation of pathways involved in remodeling that may be important in the maintenance of the angiogenic phenotype (Boudreau *et al*., 1997).

The delivery of intramuscular naked DNA in the absence of

a carrier moiety is inefficient because of inactivation of the DNA by nucleases and clearance by the phagocyte system (Herweijer and Wolff, 2003). While techniques such as electroporation and other bioballistic approaches are efficient in ramping up transfection rates, these methods are associated with side effects including skeletal muscle injury that may limit widespread applicability (Niidome and Huang, 2002; Herweijer and Wolff, 2003). The usage of nanoparticles such as polaxamers have been shown to enhance delivery of drugs and genes (March *et al.*, 1995; Lemieux *et al.*, 2000; Moghimi and Hunter, 2000). Polaxamer 188, used in this study, consists of a central polyoxypropylene (POP) molecule that is surrounded by two hydrophilic chains of polyoxyethylene (POE) in the configuration $(POE)_a(POP)_b(POE)_a$ where a = 52 molecules and b = 30 (Moghimi and Hunter, 2000).

The dosage of Del-1 used in this study derives from earlier phase I experience that has demonstrated safety at doses up to 84 mg delivered unilaterally (Rajagopalan *et al.*, 2004). The dosage used in this trial will be 42 mg per extremity or 84 mg bilaterally, delivered via an intramuscular route. Although not comparable, it is of interest that the total dose used in this trial will exceed the maximal doses used in other plasmid-based trials by an order of magnitude (Baumgartner *et al.*, 1998; Simovic *et al.*, 2001; Comerota *et al.*, 2002). Finally another unique feature of this trial is its general applicability to the IC population. PAD is generally a bilateral disease and treatment of both extremities is important in order to avoid limitation due to the nontreated limb (Rajagopalan *et al.*, 2003). DELTA-1 represents an important trial and as such assimilates lessons learned from prior therapies and trials in this area and promises renewed hopes for patients with PAD.

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