PART 1: SCIENTIFIC ABSTRACT

Angiogenesis, the formation of new blood vessels from existing blood vessels, is a complex coordinated physiologic process involving numerous mediators including the angiogenic growth factors Vascular Endothelial Growth factor (VEGF), basic Fibroblast Growth Factor, acidic FGF, angiopoietin and others.[i] The term "therapeutic angiogenesis" has been used to describe a novel strategy employing angiogenic growth factors to stimulate or augment collateral blood vessel development, to treat vascular insufficiency. Gene therapy affords the potential for localized delivery of a therapeutic transgene and transient expression of the angiogenic protein in that region following a single administration. Augmentation of collateral vessel formation has been achieved in animal models of peripheral ischemia through administration of naked pDNA encoding VEGF[ii,iii,iv], and of recombinant adenovirus encoding VEGF.[v,vi] Early studies looking at the intramuscular injection of "naked" plasmid VEGF165 (phVEGF165) suggest that it may be sufficient to induce therapeutic angiogenesis in selected patients with critical limb ischemia.[vii,viii,ix]

Genzyme has sought to exploit the natural adaptive response to hypoxia as an alternative approach for the treatment of ischemia associated with vascular disease. Specifically, the administration of HIF-1-alpha via gene therapy might induce expression of a panel of potentially beneficial genes and ultimately lead to neovascularization of ischemic tissues. HIF-1 (Hypoxia-inducible factor-1) plays a principal role in the regulation of gene expression in response to changes in oxygen tension. HIF-1 potentiates adaptation to hypoxia at the systemic, tissue, and cellular levels through control of genes encoding inducible nitric oxide synthase (iNOS), VEGF, VEGF receptors (e.g., Flt-1 also known asVEGFR-1), and glycolytic enzymes among others. HIF-1 is a heterodimer which requires the convergence of both the alpha and beta subunits to induce the expression of these genes. While the HIF-1-alpha subunit is present and stable during periods of normoxia, HIF-1-alpha is stable and available for dimerization only in a hypoxic environment.

Consequently, Genzyme has modified the transactivation domain of HIF-1 to enhance its stability, and developed the Ad2/HIF-1-alpha/VP16 recombinant adenoviral vector in an attempt to maximize the expression of this modified HIF-1-alpha transcription factor within targeted ischemic tissues. The bioactivity of Ad2/HIF-1-alpha /VP16 vector has been documented in vivo in the rabbit ischemic hindlimb model. In a study of two escalating doses of Ad2/HIF-1-alpha/VP16 (10^9 and 10^{10} viral particles), both stimulated therapeutic angiogenesis that was at least as effective as phVEGF165 in the same model. Safety studies of Ad2/HIF-1-alpha/VP16 in rats found peripheral intramuscular injection of adenovirus to be associated with a small area of inflammation at the injection site that appears to be dose-dependent. Preliminary data suggest that the level of therapeutic bioactivity of Ad2/HIF-1-alpha/VP16 may be sufficiently potent to allow a dose that will minimize potential adenovirus associated inflammation.

Genzyme proposes to conduct clinical trials to evaluate the safety and bioactivity of intramuscular injection of a replication deficient adenovirus, Ad2/HIF-1-alpha/VP16, to evaluate the extent of new collateral artery development for the purpose of improving perfusion within the ischemic limb in patients with critical limb ischemia who are not suitable for surgical or percutaneous revascularization. Safety variables will include reported adverse experiences, and change from baseline in physical examinations, laboratory parameters, vital signs and adenoviral antibody titers. Eye examinations will be performed to assess newly occurring or worsened abnormalities relative to baseline. Bioactivity will be assessed by measuring serum VEGF levels. In addition, the development of collateral vessels will be assessed in patients by magnetic resonance angiography. Preliminary clinical efficacy assessments will include ischemic rest pain, ankle-brachial index, ischemic ulcers improved or healed and clinical improvement in limb status.