

## **PART 1: SCIENTIFIC ABSTRACT**

Angiogenesis, the formation of new blood vessels from existing blood vessels, is a complex physiologic process involving numerous mediators including the angiogenic growth factors Vascular Endothelial Growth Factor (VEGF), basic Fibroblast Growth Factor (bFGF), acidic FGF (aFGF), angiopoietins and others.<sup>1</sup> The term “therapeutic angiogenesis” has been used to describe a strategy employing gene transfer or protein formulations of angiogenic growth factors, which stimulate or augment new blood vessel development, to treat vascular insufficiency. Gene transfer, when administered into ischemic tissue permits targeted delivery of the therapeutic transgene and maintenance of a concentration of the angiogenic protein in that region for days to weeks following a single administration. Augmentation of collateral vessel formation has been achieved in animal models of peripheral and myocardial ischemia through administration of naked pDNA encoding VEGF<sup>2,3,4</sup>, and of recombinant adenovirus encoding VEGF.<sup>5,6</sup>

Whether any one growth factor is superior, or whether a combination of factors will provide better collateral vessel formation, is unknown. Animal studies have not demonstrated the superiority of one growth factor over another; however, studies propose that bFGF and VEGF are synergistic *in vivo*.<sup>7</sup> Studies also indicate that factors other than VEGF and FGF (e.g., angiopoietin-1 and PDGF- $\beta$ ) are likely required for full development of stable neovascularization.<sup>8,9</sup> This suggests that an angiogenesis strategy, which more effectively replicates physiologic angiogenesis, may provide improved collateral vessel formation and clinical benefit when compared to treatment with a single growth factor.

Genzyme has sought to exploit the natural adaptive response to hypoxia as an alternative approach for the treatment of ischemia associated with coronary artery disease. Specifically, the administration of HIF-1 $\alpha$  (Hypoxia-inducible factor-1  $\alpha$ ) via gene transfer might induce expression of a panel of potentially beneficial genes and ultimately lead to neovascularization of ischemic tissues. HIF-1  $\alpha$  plays a principal role in the regulation of gene expression in response to changes in oxygen tension. HIF-1  $\alpha$  potentiates adaptation to hypoxia at the systemic, tissue, and cellular levels through control of genes including inducible nitric oxide synthase (iNOS), VEGF, VEGF receptors (e.g., Flt-1 also known as VEGFR-1), and enzymes involved in glycolysis, 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 $\beta$

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  $\alpha$  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. In a pig model of chronic myocardial ischemia,<sup>4,10</sup> animals received 1.0 mL of  $1 \times 10^8$ ,  $1 \times 10^9$ ,  $1 \times 10^{10}$  Ad2/HIF-1 $\alpha$ /VP16 viral particles (or vehicle) administered as ten 100  $\mu$ L transepical intramyocardial (IMC) injections. In the Ad2/HIF-1 $\alpha$ /VP16 treated animals, at four weeks after administration, when compared to vehicle treated animals, there was significantly improved perfusion in the ischemic zone by colored microsphere blood flow analysis following pharmacologic stress. The improved perfusion was associated with an increase in ventricular function as left ventricular ejection fraction also significantly improved in Ad2/HIF-1 $\alpha$ /VP16-treated animals when compared to controls. Preclinical studies assessed safety and toxicity of a single 50  $\mu$ L IMC injection of  $1 \times 10^8$ ,  $1 \times 10^9$ , and  $1 \times 10^{10}$  Ad2/HIF-1 $\alpha$ /VP16 viral particles into the rat heart for as long as 90 days. The main toxicological finding to date is minimal to mild, apparently dose dependent, microscopic myocardial inflammation at the administration site with Ad2/HIF-1 $\alpha$ /VP16 related inflammation, seen at a high dose of  $1 \times 10^{10}$  viral particles. Systemic toxicity was not readily observed.

Specifically, Genzyme has conducted extensive efficacy, toxicity, and biodistribution studies in pigs and rats to support the proposed cardiac ischemia protocol and these data are presented in Appendix M-II-B-2-b-(3), Appendix M-II-B-2-d and in the clinical protocol. The following is a list of these studies:

- Efficacy:
  - Bioactivity of transepical intramyocardial injection in a pig model of chronic myocardial injection.
  - *In vitro* bioactivity studies.
  
- Safety:
  - 28 day toxicity study following intramyocardial injection in rats.
  - 90 day toxicity study following intramyocardial injection in rats.
  - 28, 60 and 120 day biodistribution studies following intramyocardial injection in rats.
  - 28 day toxicity study following intramyocardial injection in rats with pre-existing immunity.

- 28 day toxicity study following intra-arterial injection in rats.
- 28 day biodistribution study following intra-arterial injection in rats.

These studies are in addition to the intramuscular safety and toxicity studies conducted in support of our critical limb ischemia clinical protocols (NIH Protocols # 9907-327/328/329).

Viable but underperfused areas of the myocardium present a promising target for gene therapy designed to induce angiogenesis. Early gene therapy studies looking at the intramyocardial (transpicardial) injection of naked plasmid VEGF<sub>165</sub> (phVEGF<sub>165</sub>) as sole therapy<sup>11,12</sup> and adenoviral mediated gene transfer using the VEGF<sub>121</sub> transgene (ADGV VEGF<sub>121.10</sub>) as an adjunct to CABG surgery or as sole therapy<sup>13,14,15</sup> suggest that angiogenic gene therapy of VEGF is safe and may lead to reduced angina symptoms and improved myocardial perfusion in patients with intractable myocardial ischemia.

Genzyme proposes to conduct a Phase I, randomized, double-blind, placebo controlled, escalating dose, multi-center study investigating the safety, bioactivity, and potential clinical outcomes of direct intramyocardial injection of Ad2/HIF-1 $\alpha$ /VP16 into patients with multiple vessel coronary artery disease undergoing elective CABG who have areas of viable and underperfused myocardium which can not be effectively reached for revascularization by surgical or percutaneous methods. The study will include 5 separate dosing cohorts (each comprised of 4 patients). Within each dosing group, patients will be randomized to receive either Ad2/HIF-1 $\alpha$ /VP16 or placebo in the proportion of 3 to 1, respectively. Each patient will receive a single dose of study drug or placebo in one administration. The doses being evaluated start from 1 x 10<sup>8</sup> viral particles and increase in ½ log increments to 1 x 10<sup>10</sup> viral particles. An independent Data Safety Monitoring Board (DSMB) will assess the available safety data at the completion of the dosing cohort prior to moving on to the next higher dosing level in the study. This dose escalation schedule is comprised of the same five doses that are currently being studied in the Genzyme peripheral vascular disease (PVD) clinical program (NIH Protocols # 9907-327/328/329, BB-IND 8432).

CAD related safety variables include standard intra- and post-operative monitoring for elective CABG surgery performed with cardiopulmonary support, reported adverse events, physical examination and laboratory parameters. Following surgery, the patients will undergo routine monitoring and post-operative care for a CABG procedure performed with cardiopulmonary bypass support. The potential for myocardial inflammation and/or infection will be assessed by changes from pretreatment in cardiac specific laboratory parameters (e.g., CPK-MB and troponin I) and ECGs.

Echocardiography will be utilized to determine if there is myocardial edema, large pericardial effusions or new wall abnormalities. Non-routine measures specific to the study agent include adenoviral antibody titers, liver function tests, blood hemoglobin content, cancer screening, and fundoscopic examinations, the last being performed to assess newly occurring neovascularization. The outpatient study visit schedule includes follow-up on days 7 ( $\pm 2$ ), 14 ( $\pm 3$ ), 21 ( $\pm 3$ ), 30 ( $\pm 3$ ), 45 ( $\pm 3$ ), 60 ( $\pm 7$ ), 90 ( $\pm 14$ ), 180 ( $\pm 14$ ), and at one year, day 365 ( $\pm 21$ ) following surgery.

Bioactivity will be assessed by measuring plasma VEGF levels. The bioactivity of Ad2/HIF-1 $\alpha$ /VP16 will also be evaluated using imaging techniques in an attempt to detect and qualify increased perfusion and/or function resulting from capillary development and potential collateral artery remodeling in the treated area (i.e., SPECT nuclear imaging and MRI). In this study, changes in anginal class and exercise tolerance testing will also be collected. Although Ad2/HIF-1 $\alpha$ /VP16 will be administered to a myocardial area that is not amenable to conventional bypass, in this study it may not be possible to discern clinical benefit related to the gene transfer from clinical benefit resulting from the restoration of myocardial perfusion to other areas of the myocardium by the CABG surgery procedure.