880. Towards a Gene Therapy Treatment for Peripheral Vascular Disease – High Level of Expression of Angiogenic Genes in Rat Limb Muscle Following Intravenous Delivery of Plasmid DNAs

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Despite a lack of success in clinical trials, gene therapy remains a highly promising therapeutic approach for the treatment of peripheral vascular disease. The precise reasons for the poor success rates in the clinical studies remain unclear but it has been postulated that low levels of angiogenic gene transfer and expression may be a primary contributing factor.

To address this problem, we have recently developed an intravenous injection procedure that allows for the efficient and widespread delivery of genes (encoded within plasmid DNAs) to skeletal muscle groups of isolated limbs. Using this injection procedure, we have undertaken a systematic study to determine if various angiogenic genes can be expressed at high levels in rat limb muscle in normal and ischemic animals. Angiogenic genes being tested include; vascular endothelial growth factor (VEGF165), fibroblast growth factor 2 (FGF2), hypoxia inducible factor 1 alpha (HIF1a), insulin-like growth factor 1 (IGF-1), platelet derived growth factor B (PDGF-B) and Angiopoietin 1 (ANG1). The primary goal of this study is to determine the relative levels and duration of transgene expression that can be obtained in muscle groups of the lower limb following a single intravenous injection. In addition the physiological response to high level angiogenic gene expression is being analyzed for each of the genes individually and in select combinations.

Following the injection of 500 micrograms of pDNA encoding human VEGF165 under the control of the CMV promoter/enhancer, extremely high levels of VEGF protein were detected in limb muscle tissue by 1 day post-injection (>1600 pg/mg total protein) while serum levels of VEGF165 remained very low. Histology (H&E staining) and immunohistochemistry analysis indicated that these high levels of VEGF165 expression stimulated a massive proliferation of endothelial cells (cells stained positive for RECA1 marker) within the muscle tissue. As expected however, VEGF165 expression levels were dose dependent with regards to the amount of injected pDNA and when lower levels of pDNA were injected the endothelial cell proliferation could be restricted or prevented. Expression and histology results for all the indicated angiogenic genes will be presented.

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NEUROLOGIC: APPLICATIONS TO BRAIN AND EYE; VECTOR DEVELOPMENT; IMMUNE RESPONSES

881. Safety, Efficacy and Biodistribution of Recombinant AAV2-RPE65 Vector Delivered by Ocular Subretinal Injection

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Introduction: AAV2 delivery of the RPE65 cDNA to the retina of blind RPE65-deficient dogs or rd12 mice restores vision as determined electrophysiologically and behaviorally. This strategy is being considered for human trials in RPE65-associated Leber congenital amaurosis, but safety and dose-efficacy within a non-toxic range with this vector have not been defined. Here we report the results of toxicology and biodistribution studies in two mammalian species, dogs and rats. Methods: Vector related pathology and spread after subretinal delivery of AAV2-CBA-RPE65 was studied in RPE65-mutant dogs and normal Spague-Dawley rats. Doses of vector delivered bracketed those intended for the clinical study. Results: There was no systemic toxicity at any vector dose in either species. In dogs ocular examinations showed mild or moderate inflammation that resolved over an initial 3-month period. Retinal histopathology indicated that traumatic lesions from the injection were common, but retinal thinning within the injection region only occurred at the highest vector doses. Biodistribution studies were also performed in RPE65-mutant dogs at various times after vector injection and in normal rats at about 2 weeks and 2 months post-injection. Vector DNA was not widespread outside the injected eye with blood and gonadal tissue consistently negative. Concomitant dose-response results in the RPE65-mutant dogs indicated that the highest 1.5 log unit range of vector doses was efficacious. Conclusions: Given the efficacy and toxicity limits defined in this study, a range for safe vector dose escalation of subretinal AAV2-CBA-RPE65 is suggested for initial human trials.

W.W.H. and the University of Florida, G.M.A. and Cornell University and G.D.A. and University of Pennsylvania could be entitled to patent royalties for inventions related to this work, W.W.H. and the University of Florida both own equity in a company that may commercialize some of the technology described herein.