

A phase I safety study in patients with severe hemophilia B (factor IX deficiency) using adeno-associated viral (AAV) vector to deliver the gene for human factor IX to skeletal muscle.

SCIENTIFIC ABSTRACT

This clinical protocol is a phase I trial to determine the safety of an adenoviral associated viral (AAV) vector in delivery of the gene for human coagulation factor IX to muscle. Hemophilia B is an X-linked bleeding disorder resulting from the deficiency of coagulation factor IX. Current therapy using factor IX protein concentrates is suboptimal because of the inevitable delay between the onset of bleeding and hemostasis following infusion of factor IX. Such delays result in tissue damage. A newer strategy of prophylactic infusion of factor IX is effective in decreasing central nervous bleeding and the development of chronic arthropathy. This approach suffers from the high cost of recombinant or plasma-derived factor IX concentrates and in some patients with limited venous access, the requirement for placement of a central venous catheter with the attendant risks of infection. Although plasma-derived factor IX concentrates undergo a series of viral inactivation steps and are safe with respect to hepatitis B, C and HIV, there are still real and theoretical risks of contamination of these products with blood-borne agents such as hepatitis A, parvovirus B-19 and new agents that may escape inactivation by heat or solvent/detergent treatment. By resulting in a steady plasma level of factor IX, a gene therapy approach to the treatment of hemophilia may provide the benefits observed with prophylactic infusion therapy without the expense of factor IX concentrate or the inconvenience of obtaining intravenous access. Hemophilia B is a good model for gene therapy because tissue specific (*i.e.* hepatic) expression of the transgene is not required and precise regulation of expression is not necessary. Even low levels of plasma factor IX (2-3%) resulting from transgene expression are adequate to change the phenotype of an individual with severe hemophilia B, such that spontaneous hemarthroses and soft tissue hemorrhages (including central nervous system bleeds) will be prevented. The vector contains the human factor IX mini-gene consisting of all sequences coding for the mature protein, the signal sequence which directs the protein for secretion, the pro-peptide which is required for proper γ -carboxylation, a portion of intron 1, and the first 227 nucleotides of the 3' untranslated region. The transgene is under the control of a cytomegalovirus immediate early promoter and the cassette contains an SV40 polyadenylation signal. Pre-clinical toxicity studies in rodents and dogs with hemophilia B have shown no local or systemic toxicity related to vector administration. Efficacy studies Rag-1 mice treated at a dose of 1×10^{13} vector genomes/kg demonstrated expression of factor IX at levels of 250-350 ng/ml (5-7% of normal human plasma levels), and studies in hemophilia B dogs treated at a dose of 8.5×10^{12} vector genomes/kg have demonstrated levels of canine factor IX between 1-2% of normal human factor IX. A low-titer transient inhibitory antibody to canine factor IX developed in one of five hemophilic dogs. The inhibitory antibody lasted for a period of ~8 weeks and disappeared without any specific therapy. Given our data on safety and promising data on efficacy of AAV-mediated muscle directed gene transfer of the factor IX gene, a phase I/II trial is proposed. Three patients will be enrolled in three dose escalation groups beginning with a low dose of 2.0×10^{11} vector genomes/kg and a high dose of 1.0×10^{13} vector genomes/kg (a dose expected to result in factor IX levels of >1% based on dose response observed in mice and dogs). All patients will be observed closely for 12 months for signs of local toxicity (including biopsy of the injection site) and systemic toxicity (including evidence for the development of inhibitory antibodies to factor IX). Factor IX antigen and activity levels will be determined, and transgene presence and expression will be assessed on muscle biopsy.