

## Scientific Abstract

The goal of this clinical study is to determine the safety, feasibility and potential efficacy of adenoviral vector-mediated gene transfer to the liver in adult patients with partial ornithine transcarbamylase deficiency (OTCD), the most common inborn error of urea synthesis. This study represents the first trial for the intravascular delivery of a recombinant adenovirus into the blood stream. OTCD is an X-linked disorder for which no effective treatment is yet available. Approximately 50% of hemizygous males have a null mutation and present in hyperammonemic coma in the first week of life with high morbidity and mortality. The other half of hemizygous males and females heterozygous have 10-20% normal activity, and manifest periodic episodes of life threatening hyperammonemic encephalopathy later in childhood or as an adult. Each episode of hyperammonemic coma carries a 5-10% risk of mortality and an even greater risk of brain damage. OTCD currently remains incompletely treated, and relies on severe nitrogen restriction combined with costly and unpleasant medications that attempt to provide an alternative pathway for waste nitrogen excretion. Adenoviral vectors are attractive gene delivery vehicles for gene therapy directed to liver. An extensive series of toxicology studies in mice and nonhuman primates support the safety of *in vivo* gene therapy with adenoviral vectors. Experiments in an authentic murine model of OTCD demonstrates the effectiveness of adenoviral vectors in correcting the underlying metabolic derangements in nitrogen metabolism and preventing hyperammonemic coma.

In this protocol we propose to study toxicity and efficacy of *in vivo* gene transfer by selective intraarterial infusion of a recombinant adenovirus containing human OTC cDNA into the right lobe of the liver of adults with partial OTCD. Intra-hepatic administration is chosen to minimize the risk of viral toxicity to other organs. This study will focus on toxicity with immune responses to the vector and genetically corrected cells and metabolic correction. The primary goal of the study is to establish a dose that will achieve effective gene transfer without toxicity. Toxicity will be assessed by biochemical parameters of liver, renal and hematologic function, as well as histologic analysis of liver biopsy material. Gene transfer will be measured by evidence of transduction of hepatocytes obtained by percutaneous liver biopsy. Metabolic efficacy will be measured by normalization of plasma ammonium, urea cycle intermediates, and urinary orotate excretion, and by rate of  $^{15}\text{N}$  flux through the urea cycle. The data analysis of this study will involve a repeated measures ANOVA. If any of the measures appear skewed, a logarithmic transformation will be done prior to analysis of variance. We intend to study both males and females with partial OTCD in a dose escalation toxicity study. Due to the paucity of male survivors, we predict an enrollment ratio of 1:2 males to females. The mutation responsible for OTCD has been seen in most races. Three patients will be treated at each dose starting with the lowest dose of  $1 \times 10^8$  plaque-forming units (pfu)/kg. with subsequent 1/2 log increases in the absence of toxicity. We anticipate a total of eighteen patients (6 dose increments, three in each group). Termination of the study will occur in the presence of either significant toxicity or efficacy. The study will take place in the outpatient and inpatient units of the General Clinical Research Center at the Hospital of the University of Pennsylvania. The adenoviral vector containing the human OTC cDNA is freshly prepared in the Human Applications Laboratory (HAL) of the Institute for Human Gene Therapy using previously described methodology. The specific virus used in the human trial must first be approved by the FDA. The potential risks are many and include direct or immune mediated liver injury which at high doses of vector could be severe or even fatal. Also, the patients may develop a brisk cell-mediated or humoral immune response, limiting duration of response or ability to readminister a subsequent dose. Occasional serious complications of angiography occur, including thrombosis of the femoral artery leading to poor circulation of the leg, or laceration of the artery leading to internal bleeding. Risks of the radiation dose administered may occur. A percutaneous liver biopsy will be performed, and bleeding from the puncture site may be severe enough to require surgery. Overall, we believe the benefits of the knowledge gained from this study far outweigh the potential risks to the patients.