540. Sustained Correction of Phenylketonuria by a Single Dose of AAVHSC Packaging a Human Phenylalanine Hydroxylase Transgene

Seemin Ahmed, Jeff L. Ellsworth, Omar Francone, Deiby Faulkner, Arnold Sengooba, Serena Dollive, David Knowlton, Hillard Rubin, Diana Lamppu, Albert Seymour

Homology Medicines, Inc., Bedford, MA

A novel group of Clade F adeno-associated viruses has been isolated from normal human CD34+ hematopoietic stem cells (AAVHSCs) and has shown high-efficiency nuclease-free gene editing as well as gene transfer capabilities. In biodistribution studies in mice and non-human primates we have observed significant transduction of hepatocytes following intravenous delivery of AAVHSC demonstrating that AAVHSCs have tropism for mammalian liver. To evaluate whether a hepatotropic AAVHSC could be used to deliver a therapeutic gene and correct a disease-associated metabolic phenotype in the liver, AAVHSC15 was studied in PAHenu2 mice. These mice harbor a missense mutation (F263S) in the phenylalanine hydroxylase (PAH) gene resulting in less than one percent of wild-type levels of phenylalanine hydroxylase enzyme (PAH) activity, have 40- to 50-fold elevations in serum phenylalanine (Phe) on a normal chow diet, and are a model for the classic form of phenylketonuria (PKU) in humans. AAVHSC15 packaging a human PAH transgene driven by a ubiquitously expressing promoter (AAVHSC15-PAH) was prepared by triple transfection in HEK293 cells and purified through two rounds of CsCl density gradient ultracentrifugation. PAHenu2 mice were maintained on standard chow diet and had a constant serum level of Phe of ~2000 µM. Mice received a single intravenous (IV) injection of either vehicle alone or vehicle containing increasing amounts of AAVHSC15-PAH. Serum levels of Phe and of tyrosine (Tyr), the metabolic product of PAH activity, were analyzed weekly and tissues were harvested at various time-points for measurement of vector genomes and PAH mRNA by ddPCR, and PAH activity in liver. One week post-dose, the serum levels of Phe were normalized to less than 150 µM (p<0.0001) and the serum levels of Tyr were increased in animals treated with AAVHSC15-PAH (p<0.0001). These changes were associated with dose-dependent increases in human PAH vector genomes, human PAH mRNA, and PAH enzymatic activity in livers of treated animals. Durability of responses were dependent on dose of AAVHSC15-PAH, with sustained reductions in serum Phe out to >28 weeks post-dosing at the highest dose tested (p<0.0001). No changes in liver enzyme levels in sera were noted following treatment suggesting that AAVHSC15-PAH was well-tolerated at these doses. Neither changes in serum Phe nor hepatic PAH activity were observed in animals treated with vehicle alone. Vector sequences in AAVHSC15-PAH were optimized including the addition of a liver-specific promoter resulting in HMI-102. HMI-102 normalized serum Phe in PAHenu2 mice at ten-fold lower doses with durability in response seen out to >14 weeks in an ongoing study. Taken together, these data demonstrate that administration of AAVHSC-based gene transfer vectors packaging a human PAH transgene normalized serum Phe in PAHenu2 mice. The durable correction of serum Phe observed suggests that HMI-102 shows potential for further development as one-time, PAH gene replacement therapy for PKU in humans in the absence of dietary intervention.