American Society of Gene & Cell Therapy (ASGCT) Annual Meeting
Poster Session II
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5:15pm – 7:15pm
Stevens Salon C & D

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

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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 PAH^{enu2} 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. PAH^{enu2} 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 PAH^{enu2} 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 PAH^{enu2} 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.