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Single Intravenous Administration of AAVHSC15 Packaging a Human Phenylalanine Hydroxylase Transgene Sustainably Corrects Phenylketonuria in Mouse Model

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Human CD34+ hematopoietic stem cell-derived adeno-associated viruses (AAVHSCs) that map to Clade F show potential for hepatic gene therapy because of their high liver tropism. Phenylketonuria (PKU) is a rare autosomal recessive metabolic disease resulting from loss of function mutations in the phenylalanine hydroxylase (*PAH*) gene that abolish activity of phenylalanine hydroxylase (PAH) in the liver. PKU is a suitable candidate for AAV-based gene therapy as there are no current treatments that address the underlying genetic defect, and a recent five-year retrospective chart review demonstrates that even closely monitored classic PKU patients' serum phenylalanine (Phe) levels are well above target therapeutic levels. PAH^{enu2} mice are a murine model for severe PKU. There is a missense mutation (F263S) in the *Pah* gene that greatly reduces enzyme activity in PAH^{enu2} mice, causing a 40-fold elevation in Phe on a normal chow diet (containing 1% Phe). Preclinical studies were conducted with these mice and an AAV vector encoding the human *PAH* gene.

The human *PAH* transgene, packaged in AAVHSC15 and driven by a ubiquitous promoter (AAVHSC15-CBA-PAH), was administered as a single intravenous injection in PAH^{enu2} mice. Mice were fed normal chow throughout the study ensuring a constant intake of Phe in their diet. Serum levels of Phe and tyrosine (Tyr) [byproduct of Phe metabolism required for production of neurotransmitters] were measured weekly. Livers were harvested and processed to measure vector genomes, mRNA, and PAH enzyme activity. An optimized vector (AAVHSC15-PAH; HMI-102) was tested at ten-fold lower doses compared to the initial vectors and examined for durability in response.

One week post-dosing with AAVHSC15-CBA-PAH, Phe levels decreased from 1500μM to <150μM ($p<0.0001$) even as mice had a dietary intake of 1% Phe. Dose-dependent increases in PAH vector genomes, mRNA, and enzymatic activity were observed. Correction in phenotype was sustained out to 28 weeks post-dosing ($p<0.0001$). The optimized vector (AAVHSC15-PAH) decreased serum Phe and increased serum Tyr in PAH^{enu2} mice on normal chow within a week at ten-fold lower doses compared to the initial research vector and sustained correction out to 48 weeks post-injection, consistent with the lifespan of the model. Coat color change was also observed, indicating an effect on melanin (another byproduct of Phe metabolism).

These data demonstrated that a single dose of AAVHSC15-based gene therapy resulted in long-term correction of PKU in PAH^{enu2} mice on a normal chow diet. HMI-102 is expected to enter the clinic in 2019.