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Nuclease-free genome editing by AAVHSC vectors leads to *in vivo* genome correction and amelioration of disease phenotype in a mouse model of phenylketonuria (PKU)

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The correction of pathogenic mutations has great potential for the treatment of genetic disorders. A panel of adeno-associated viruses isolated from normal human hematopoietic stem cells (AAVHSCs) have shown nuclease-free gene editing through the homologous recombination pathway. Here we explore application of AAVHSCs for *in vivo* nuclease-free correction of phenylketonuria (PKU), a recessive disorder caused by loss of function mutations in the gene phenylalanine hydroxylase (*PAH*) which results in elevated levels of phenylalanine and decreased tyrosine production. To correct PKU, an AAVHSC vector containing a human *PAH* cDNA flanked by targeting sequences homologous to the murine *Pah* gene is administered by a single IV injection into Pah^{enu2} mice, a disease model. Correction was assessed by monitoring serum Phe levels. Treatment with AAVHSC vectors led to long-term correction of phenylalanine levels while on a normal diet. Molecular characterization of treated mouse livers shows efficient and precise gene editing and expression of human PAH concordant with phenotypic correction. Human-specific editing of human liver was explored using AAVHSC editing vectors constructed with human-specific targeting sequences and administered to mice whose liver is populated with implanted human hepatocytes. Characterization of humanized livers display human-specific gene editing and expression comparable to those sufficient to reverse PKU phenotypes. Further, no "off-target" editing of the orthologous mouse loci is detected supporting sequence specificity. Together these results support AAVHSC vectors as a clinically feasible platform to address the genetic liver disorder PKU via targeted genome correction and led to development candidate, HMI-103, currently in IND-enabling studies.