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# Investigational Genetic Medicine Approaches for Phenylketonuria (PKU)

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PKU is an autosomal recessive monogenic disorder. >98% of cases are due to mutations in the phenylalanine hydroxylase (*PAH)* gene, resulting in deficient PAH activity, a hepatic enzyme that catalyzes the formation of tyrosine from phenylalanine (Phe). Untreated PKU results in progressive, irreversible neurological impairment during infancy and early childhood (Lichter-Konecki, 2019). Restricting protein and Phe intake is standard of care for most PKU patients. Relaxing of dietary restrictions results in loss of metabolic control and wide fluctuations in Phe that are associated with progressive neurological damage (Christ, 2010; Enns, 2010; Janzen, 2010). Current U.S. treatment guidelines (Vockley, 2014) indicate that blood Phe ≤360 µM does not require therapy or dietary modification.

Gene therapy has the potential to deliver a functional copy of human *PAH* (hPAH) to the liver, restoring PAH enzyme activity and normal Phe levels thus potentially eliminating dietary restrictions. A gene transfer (GT) approach, with episomal gene expression, is feasible in a fully developed liver. Because the human liver undergoes rapid growth during the first 10-15 years, (Pryce, 2014; Shankle, 1983; Chouker, 2004), a gene editing (GE) approach with integration of the *PAH* gene into the target locus would allow maintained expression throughout the period of growth as the cells divide.

GT and GE vectors expressing hPAH were packaged in AAVHSC15. The GE vector transgene has locus- and species-specific homology arms flanking the *hPAH* sequence that are designed to guide the DNA to the target *PAH* locus and integrate through non-nuclease-based, AAV-mediated homologous recombination. Mouse surrogate vectors were used in a murine model of PKU ($Pah^{enu2}$), while a human-targeted GE vector was used in a humanized-liver murine model. The vectors were administered as a single intravenous (IV) injection.

The $Pah^{enu2}$ mouse has baseline blood Phe >1000µM. These mice were fed normal chow throughout the studies for consistent Phe intake. The xenograft mouse has a liver comprised of >90% human hepatocytes, following gradual repopulation of the liver compartment with human hepatocytes (Azuma, 2007). The xenograft model does not have *Pah/PAH* gene mutations.

Phe was measured by mass spectrometry. Livers were processed to measure vector genomes and mRNA by ddPCR, and integration of the GE vector transgene in the target locus by next generation sequencing (NGS).

Data from $Pah^{enu2}$ mice were used to establish a dose-response relationship between hPAH mRNA expression and Phe levels. Results from the humanized-liver murine model were compared to results from $Pah^{enu2}$ mice, providing the basis for dose extrapolation from the mouse surrogate to the human-specific construct.

Blood Phe normalization (<120 μM) in Pah$^{enu2}$ mice was achieved within 1-2 weeks post-dose and maintained for the duration of the studies with both GT and GE vectors. Similar hPAH mRNA levels were observed across species with the mouse- and human-specific constructs and were consistent with levels needed for Phe reduction in Pah$^{enu2}$ mice. Gene insertion at the target locus was confirmed by NGS analysis. Integration rates were similar in Pah$^{enu2}$ mice and human-liver xenograft mice.

These data demonstrated that a single IV dose of AAVHSC15-based GT or GE gene therapy resulted in a sustained reduction of Phe in Pah$^{enu2}$ mice on a normal chow diet. The human-targeted GE vector demonstrated human-specific editing. The option of a GT or GE approach has the potential to deliver the hPAH gene to hepatocytes resulting in sustained expression in a fully developed liver or a growing liver, respectively.