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**Poster Session**  
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Sustained Correction of a Murine Model of Phenylketonuria and Integration Into the Genome Following a Single Administration of an AAVHSC15 Phenylalanine Hydroxylase Gene Editing Vector

Benard L, Lamppu D, Von Stetina JR, Thompson J, Wright T, Avila N, Bailey M, Francone O, Seymour A and Wright J.

Homology Medicines, Inc.

Phenylketonuria (PKU) is a rare autosomal recessive inborn error of metabolism. If left untreated, severe forms of PKU due to phenylalanine hydroxylase (PAH) deficiency result in progressive, irreversible neurological impairment during infancy and early childhood. Neither phenylalanine (Phe)-restricted diet nor the currently available therapeutic treatments address the core biological defect of the disease, which is, the presence of biallelic pathogenic variants in the PAH gene.

HMI-103 is an investigational gene editing vector designed to deliver functional copies of PAH to hepatocytes, integrate into the genome, and has the potential to restore PAH activity and normalize Phe metabolism. HMI-103 contains locus- and species-specific homology arms (HA) flanking the human PAH sequence (cDNA) that are designed to guide the cDNA to the target PAH locus in the genome and integrate through non-nuclease-based, AAV-mediated homologous recombination.

Vectors expressing PAH were packaged in AAVHSC15. Both HMI-103 and a murine surrogate vector were used in preclinical studies; the murine surrogate is different from HMI-103 in that it contains HAs specific to the murine Pah locus to enable integration into the murine genome. The mouse-specific vector was tested in a murine model of PKU (Pahenu2), while HMI-103 was tested in a humanized-liver murine xenograft model in which the liver is repopulated with human hepatocytes. The vectors were administered as a single injection. Blood Phe was measured by mass spectrometry. Livers were processed to measure vector genome (vg) copy number and mRNA by ddPCR and integration in genome by next generation sequencing (NGS).

Following a single administration, the murine surrogate vector resulted in long-term normalization of blood Phe, dose-responsive vg copy number, mRNA expression, and on-target genome integration in Pahenu2 mice. Administration of HMI-103 in the humanized-liver murine model resulted in stable, dose-responsive vg copy number, mRNA expression, and on-target integration into the PAH locus with no unwanted changes to the target site in the genome. The mRNA and integration levels achieved were consistent with levels in Pahenu2 mice that corresponded with correction of the PKU phenotype. Additionally, a long-read, genome-wide assay capable of detecting vector integrations at 0.5% or higher showed no evidence of off-target integration into the human genome. The murine surrogate was also evaluated in a GLP toxicology study in Pahenu2 mice and a germline transmission study in C57BL/6J mice. In the PKU mouse model, blood Phe was normalized at all doses tested and there were no test-article related clinical pathology or necropsy findings. There was no evidence of germline transmission.

These data in mice demonstrate efficacy, integration, fidelity and specificity for the target locus and preclinical safety of HMI-103 and supported clinical trial initiation.