pheEDIT: A Phase 1, Open-Label, Dose-Escalation Safety and Efficacy Gene Editing Study Evaluating HMI-103 in Adults with Classical PKU

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Introduction

Phenylketonuria (PKU) is a rare autosomal recessive inborn error of metabolism. If left untreated, phenylalanine hydroxylase (PAH) deficiency may result in progressive, irreversible neurological impairment. Neither a phenylalanine (Phe)-restricted diet nor currently available therapeutic treatments address the core underlying defect of the disease - the presence of biallelic pathogenic variants in the *PAH* gene.

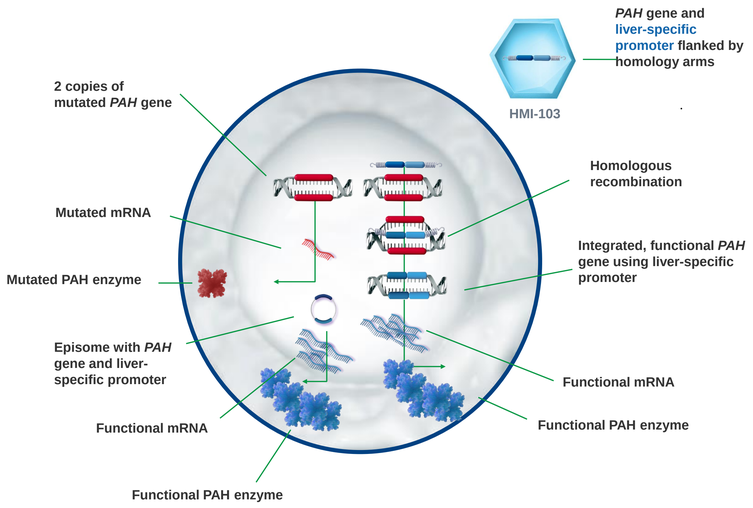
This leaves a significant unmet medical need for patients with PKU due to PAH deficiency.

- Standard of care → onerous low Phe diet has poor compliance
- Phe-controlled diet not sufficient to reduce Phe levels to within American College of Medical Genetics and Genomics (ACMG) targets (120-360 µmol/L)[1] or EU targets (120-600 µmol/L)[2]
- Approved therapeutics do not reconstitute normal biochemical pathways for ~95% of patients; all require chronic dosing vs. a potential one-time treatment
- Physicians and patients seek new treatment options

HMI-103 Mechanism of Action

HMI-103 is an investigational gene editing vector designed to:

- Address underlying genetic cause of PKU
- Deliver functional copies of the *PAH* gene to hepatocytes
- Integrate into target *PAH* locus in the genome via non-nuclease-based AAV-mediated homologous recombination (HR), which is a high-fidelity natural DNA repair process for gene editing
- Treat adult and pediatric PKU with dual mechanism of action in transduced liver cells
- Maximize PAH expression through *PAH* and liver-specific promoter integration and episomal expression in transduced cells
- Restore Phe metabolism following a single intravenous (I.V.) infusion



Preclinical Efficacy and Safety

Objectives:

- Evaluate pre-clinical safety and efficacy of HMI-103, a nuclease-free gene editing candidate for humans, and its mouse surrogate vector in appropriate models
- Assess on-target and potential off-target integration

Materials:

- Vectors packaged in AAVHSC15 (adeno-associated virus [AAV] serotype HSC15) capsid using Homology's platform process
- Mouse surrogate vector evaluated in *Pah*^{enu2} mouse model of PKU and Wild Type (WT) mice
- HMI-103 tested in humanized-liver xenograft mouse model

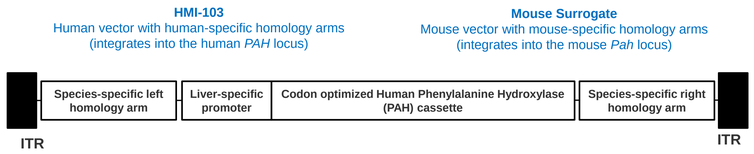


Figure 1: HMI-103 and mouse surrogate vectors share the same liver-specific promoter and human PAH cassette but have species-specific homology arms

Long-term efficacy in *Pah*^{enu2} mice using the mouse surrogate vector; sustained normalization of blood Phe (41 weeks post-dose) in mice on a normal chow diet

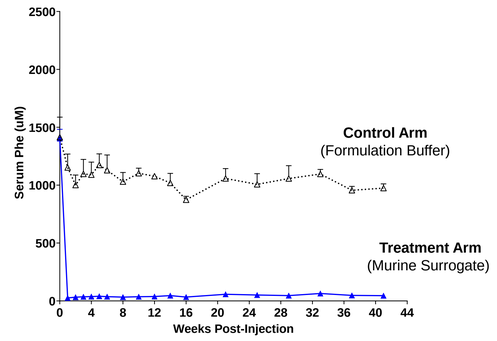


Figure 2: Phe values were determined by RFMS (RapidFire mass spectrometry); individual mouse data values (an average of 8 technical replicates per mouse) group by study arm. Data (mean values) for the arm at each time point are represented. Error bars denote standard deviation between adult male mice (N=4 per group).

HMI-103 transduced human hepatocytes in the humanized-liver xenograft model, integrating into human *PAH* locus (on-target) at rates shown to result in sustained reduction of serum Phe in the PKU mouse model (*Pah*^{enu2}) with the mouse surrogate vector

✓ Hepatocyte Transduction* ✓ ON-target Integration ✓ Liver Expression**

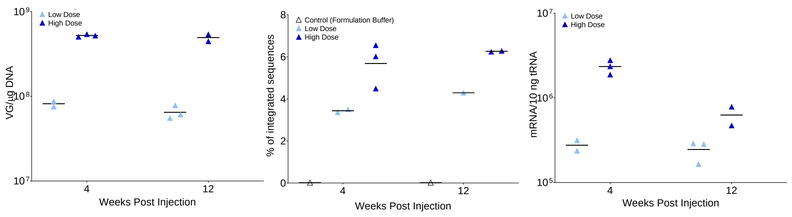


Figure 3: Vector genomes (VGs) measured by qPCR as copies per total µg of genomic DNA; mRNA measured by RT-qPCR as copies per 10 ng of total RNA. On-target integration was measured by next generation sequencing (NGS). Integration rates are calculated based on number of sequences with integration divided by total number of WT, integrated and concatemer sequences (with and without the integration event) multiplied by the PCR efficiency coefficient. Individual mice are represented by symbols and the mean represented by the line. (N = 1 to 3 per group/timepoint evaluated). *VG levels for control animals were below lower level of detection (LOD); ** mRNA levels for control animals were below LOD.

Molecular methods demonstrated no unwanted on-target mutations following *in vivo* editing with HMI-103 in a humanized liver murine model

Method	Outcome
Sequencing across homology arm of integrated alleles Sequence coverage >10,000 reads per base	**No *de novo* mutations** introduced
Long-read sequencing to capture entire sequence from inserted DNA ➔ homology arm ➔ native genome	**No inverted terminal repeats (ITRs)** detected

No adverse findings or evidence of germline transmission were observed in GLP studies using the mouse surrogate vector

GLP toxicology study in *Pah*^{enu2} Mouse Model:

- All doses normalized Phe levels
- Phe levels never dropped below normal even at the highest dose tested
- Dose-dependent levels of on-target integration
- No adverse findings observed

Germline transmission study in WT mice:

- No test article-related effects in P generation or F1 pups; no test article-related effects in reproductive parameters
- No evidence of germline transmission

HMI-103 was ten times more potent than non-integrating gene therapy construct HMI-102 in *Pah*^{enu2} mice at reducing blood Phe levels

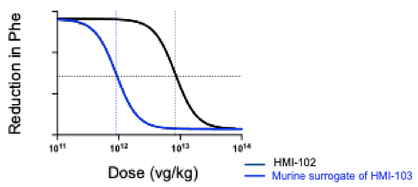
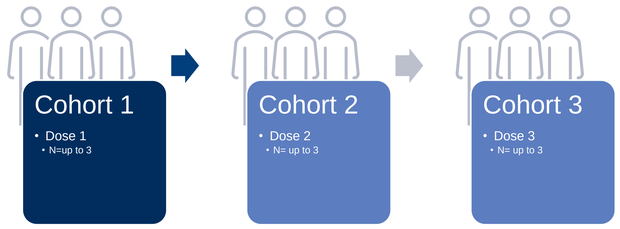


Figure 4: The analysis compared the dose at which fifty percent Phe reduction was achieved in the model

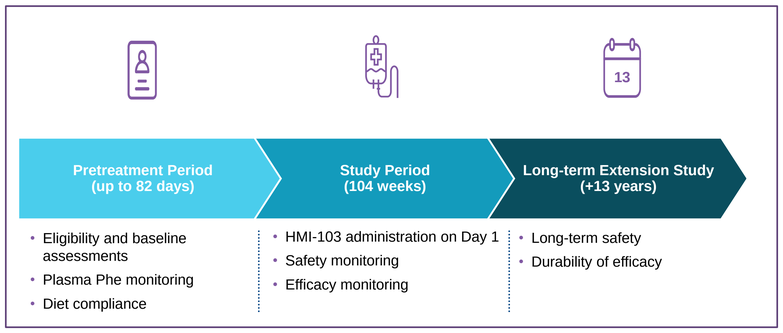
pheEDIT Clinical Trial Design

pheEDIT is a Phase 1, open label, dose-escalation trial evaluating the safety and efficacy of a one-time single I.V. dose of HMI-103 in participants with Classical PKU due to PAH deficiency



To decrease the potential for an immune response to HMI-103, pheEDIT uses a prophylactic immunosuppressive regimen with a corticosteroid and the T-cell inhibitor, tacrolimus.

pheEDIT and Long-term Extension Study Overview



pheEDIT primary study endpoints include:

- Incidence and severity of treatment-emergent adverse events (TEAEs) and adverse events (AEs) of special interest (Hepatic assessments and low Phe)
- Mean percent change from Baseline in plasma Phe concentrations within each dose cohort post-administration of HMI-103

pheEDIT Key Eligibility Criteria

Inclusion

- Adults 18-55 years of age
- Diagnosis of classical PKU due to PAH deficiency
- Uncontrolled disease despite Phe-restricted dietary management
- Four baseline plasma Phe values ≥ 600 µmol/L (10 mg/dL) during pretreatment period and at least one Phe level ≥ 600 µmol/L in the preceding 24 months
- Ability and willingness to maintain baseline diet, unless otherwise directed

Exclusion

- Participants with PKU that is not due to PAH deficiency
- Presence of anti-AAVHSC15 neutralizing antibodies during screening
- Elevated Hemoglobin A1c or fasting glucose
- Elevated liver enzyme levels or INR
- Abnormal hematology values
- Previously received gene therapy for the treatment of any condition

Based on IND-enabling studies demonstrating efficacy of HMI-103 studies in the mouse, HMI-103 received Fast Track designation by the United States (U.S) Food and Drug Administration (FDA). The pheEDIT Phase 1 gene editing clinical trial (NCT05222178) has been initiated, and recruitment is ongoing.

Demonstration of positive safety and efficacy results in an adult population may allow for enrollment of younger participants in future studies.

References

[1]Vockley J et al. Genetics in Medicine 2014
[2]van Spronsen FJ et al. Lancet Diabetes Endocrinol 2017.

ClinicalTrials.gov https://clinicaltrials.gov/ct2/show/NCT05222178