P1-171

mRNA Therapy Reduces Phenylalanine in a Mouse Model of Phenylketonuria

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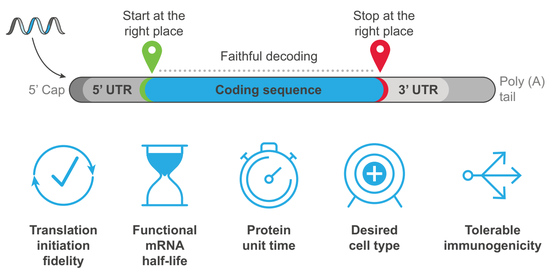
BACKGROUND

- mRNA therapeutics are composed of lipid nanoparticles (LNPs) designed with specific ratios of ionizable lipids, cholesterol, phospholipids, and polyethylene glycol–conjugated lipids, which encapsulate specific mRNA construct sequences
- The LNP and mRNA constructs are designed with chemical and physical stability intended to increase biodistribution, cellular uptake, and endosomal escape, resulting in protein expression to provide the desired therapeutic effect
- The features of the mRNA sequence significantly contribute to therapeutic success (**Figure 1**)

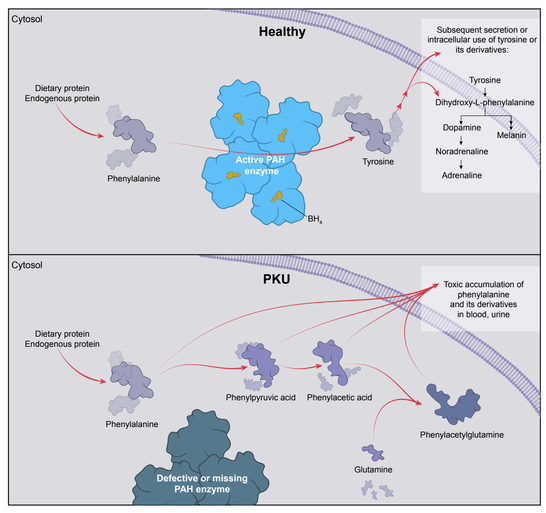
Figure 1. Desired mRNA Features



Poly (A), polyadenylated; UTR, untranslated region.

- Phenylketonuria (PKU) is a rare, inherited metabolic disorder that results from defects in phenylalanine hydroxylase (PAH; **Figure 2**)[1]
  - PKU is typically identified by newborn screening and occurs in approximately 1 of 24,000 live births, affecting approximately 450,000 patients worldwide[2]
- Clinically, PKU is characterized by neurologic conditions such as attention-deficit/hyperactivity disorder (ADHD), poor executive function, worsening IQ, anxiety, depression, seizures, and eczema[1]
- Biochemically, PKU is characterized by the toxic accumulation of phenylalanine (Phe) and its derivatives[1]
- The current standard of care includes a restricted diet with tyrosine (Tyr) supplementation, pegvaliase-pqpz, and/or tetrahydrobiopterin supplementation[1]

Figure 2. PKU Results From Defects in PAH

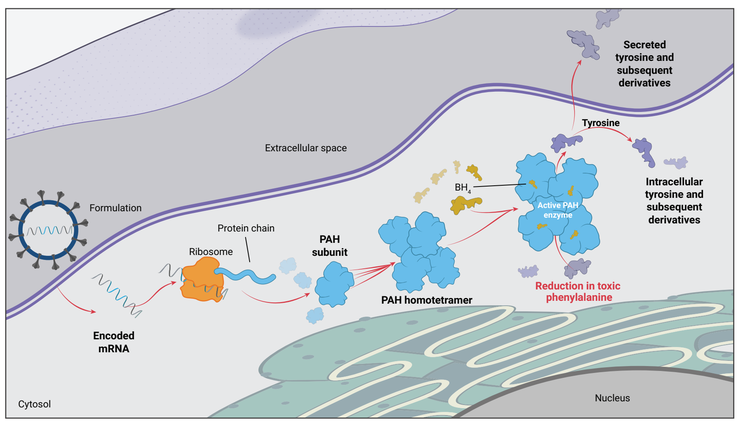


BH_4, tetrahydrobiopterin; PAH, phenylalanine hydroxylase; PKU, phenylketonuria.

OBJECTIVE

- To characterize the pharmacology and pharmacokinetics of an investigational mRNA-based intravenous therapy for PKU that encodes human (h) PAH-mRNA in a PAH-deficient mouse model (**Figure 3**)

Figure 3. Approach to Phenylketonuria Treatment



BH_4, tetrahydrobiopterin; PAH, phenylalanine hydroxylase.

METHODS

- The PKU mouse model PAH^{enu2} was used for preclinical evaluation of the candidate hPAH-mRNA construct and LNP development following administration of a single intravenous 0.5-mg/kg dose of the therapeutic candidates
  - Whole blood was collected via the submandibular or submental vein and analyzed for Phe concentrations using liquid chromatography and a triple quad mass spectrometer system
  - Blood samples were soaked directly onto duplicate Mitra® microsamplers that carried 10 µL VAMS® tips (Neoteryx, Microsampling Solutions by TRAJAN). Samples were placed onto the 96-Autorack storage plate and dried for a minimum of 4 hours at room temperature prior to Phe analysis
  - Samples were analyzed for Phe using an optimized Agilent Liquid Chromatography and 6460 Triple Quad Mass Spectrometer system
- The lead therapeutic candidate was selected for subsequent preclinical pharmacokinetic and pharmacodynamic evaluation
  - PAH^{enu2} mice were administered single intravenous 0.25-, 0.5-, and 1.0-mg/kg doses
  - Quantitation of serum and liver mRNA was performed using a real-time reverse transcription-quantitative polymerase chain reaction assay using QuantStudio™ Real-Time PCR System (Thermo Fisher Scientific)
  - Liver tissue samples were analyzed for hPAH concentration of each peptide using liquid chromatography and tandem mass spectroscopy (LC/MS-MS) in positive ionization mode
  - Blood Phe and Tyr were analyzed using an Aglient 1200 Liquid Chromatography system and a Waters XBridge BEH HILIC column. The analytes were detected using a SCIEX API 5000 Triple Quad LC-MS/MS system equipped with an ESI (TurboIonSpray®) ionization source operated in the positive ionization mode
- Data were analyzed using Agilent Quantitative Analysis, Excel, and GraphPad Prism

CONCLUSIONS

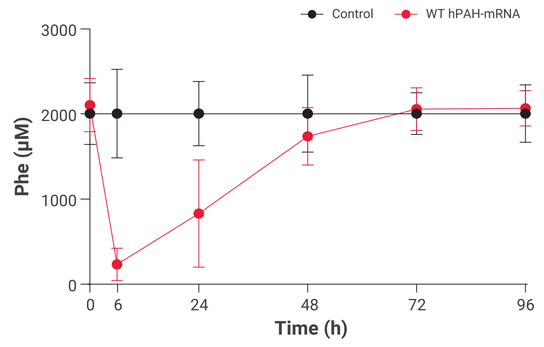
- Our mRNA-based therapy is designed to produce the hPAH protein, the enzyme that is defective in PKU
- hPAH-mRNA administration significantly lowered circulating Phe in PAH^{enu2} mice
- Preclinical studies have been completed and results are consistent with other mRNA-based therapeutics in development
- hPAH mRNA therapy with LNPs could have a significant therapeutic effect for patients with PKU who have limited treatment options

RESULTS

Evaluation of a Single Intravenous Dose of hPAH-mRNA Therapeutic Candidates in PAH^{enu2} Mice

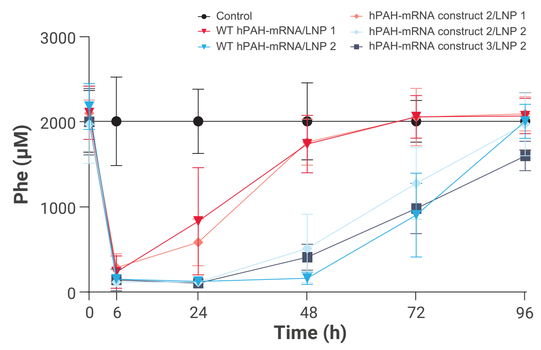
- After administration of a single dose of the full-length wild-type (WT) hPAH-mRNA candidate, blood Phe levels in PAH^{enu2} mice were reduced to therapeutic levels (120-360 µM) before returning to predose levels by approximately 72 hours postdose (**Figure 4**)
- To further optimize pharmacologic effect, several hPAH-mRNA constructs and lipids for LNP composition were screened to identify the lead therapeutic candidate for further preclinical evaluation (**Figure 5**)

Figure 4. Reduced Blood Phe Levels in PAH^{enu2} Mouse Models Treated With WT hPAH-mRNA



enu2, N-ethyl-N-nitrosourea; hPAH, human phenylalanine hydroxylase; PAH, phenylalanine hydroxylase; Phe, phenylalanine; PKU, phenylketonuria; WT, wild-type.
PAH^{enu2} mice (n = 8 per treatment group) were treated with a single intravenous 0.5-mg/kg dose. Blood Phe levels were assessed at baseline and 6, 24, 48, 72, and 96 hours postdose.

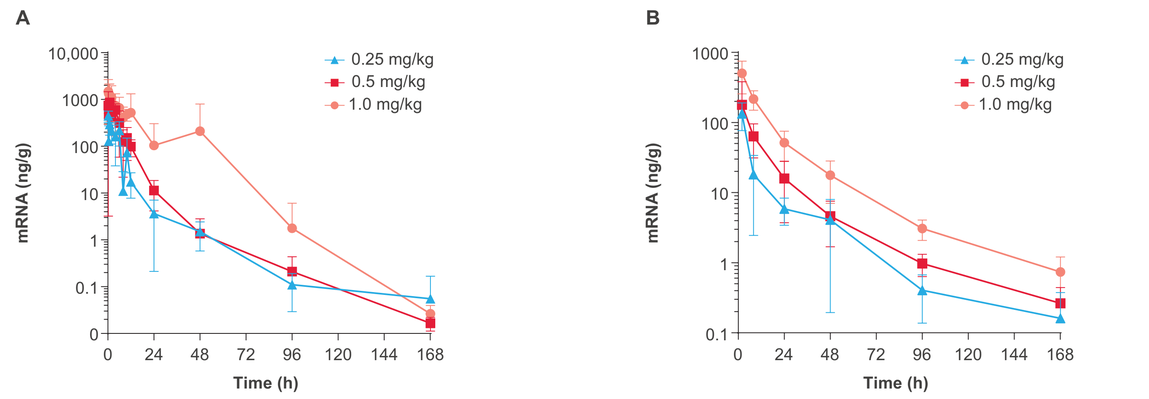
Figure 5. hPAH-mRNA Construct and Lipid Selection Evaluation Identified a Candidate for Further Preclinical Evaluation



enu2, N-ethyl-N-nitrosourea; hPAH, human phenylalanine hydroxylase; LNP, lipid nanoparticle; Phe, phenylalanine; PKU, phenylketonuria; WT, wild-type.
PAH^{enu2} mice (n = 8 per treatment group) were treated with a single intravenous 0.5-mg/kg dose. Blood Phe levels were assessed at baseline and 6, 24, 48, 72, and 96 hours postdose.

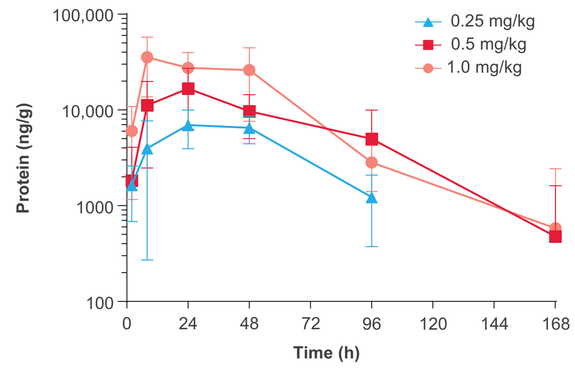
- Preclinical pharmacology and pharmacokinetics analyses were conducted in the PAH^{enu2} mouse model to evaluate the lead therapeutic candidate (**Figures 6-8**)
- Biodistribution and toxicology studies have been completed

Figure 6. Dose Response and Time Course Studies of (A) Serum hPAH-mRNA and (B) Liver hPAH-mRNA Concentrations in PAH^{enu2} Mice



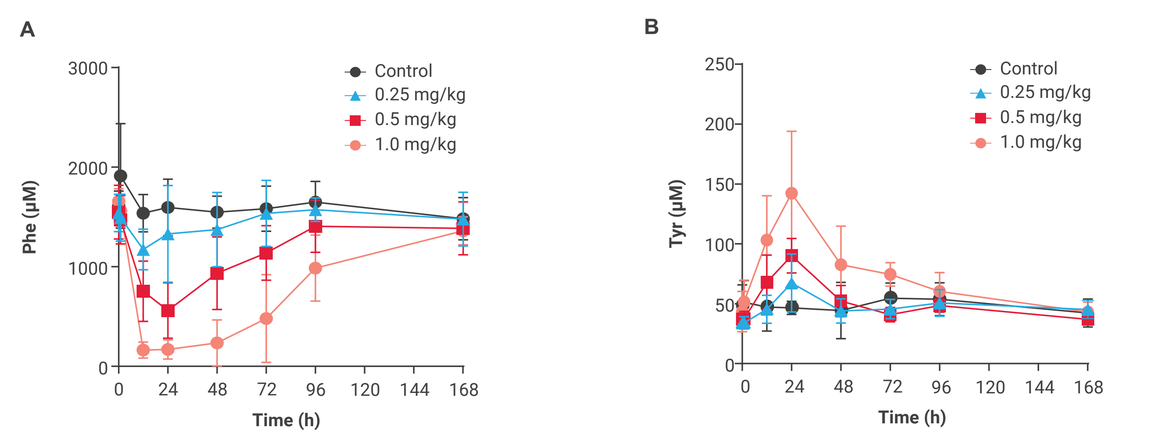
enu2, N-ethyl-N-nitrosourea; hPAH, human phenylalanine hydroxylase; PAH, phenylalanine hydroxylase; PKU, phenylketonuria.
PAH^{enu2} mice (n = 6-16 per treatment group) were treated with a single intravenous dose and mRNA levels were assessed at baseline and 6, 24, 48, 72, 96, and 168 hours postdose.

Figure 7. Dose Response and Time Course Studies of Liver hPAH-Protein Concentrations in PAH^{enu2} Mice



enu2, N-ethyl-N-nitrosourea; hPAH, human phenylalanine hydroxylase; PAH, phenylalanine hydroxylase; PKU, phenylketonuria.
PAH^{enu2} mice (n = 6-16 per treatment group) were treated with a single intravenous dose and protein levels were assessed at baseline and 6, 24, 48, 72, 96, and 168 hours postdose.

Figure 8. Dose Response and Time Course Studies of (A) Blood Phe and (B) Blood Tyr Concentrations in PAH^{enu2} Mice



enu2, N-ethyl-N-nitrosourea; PAH, phenylalanine hydroxylase; Phe, phenylalanine; Tyr, tyrosine.
PAH^{enu2} mice (n = 8 per treatment group) were treated with a single intravenous dose. Blood Phe and Tyr levels were assessed at baseline and 6, 24, 48, 72, 96, and 168 hours postdose.

References

1. van Spronsen FJ, et al. *Nat Rev Dis Primers*. 2021;7(1):36.
2. Hillert A, et al. *Am J Hum Genet*. 2020;107(2):234-250.

Acknowledgments

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Disclosures

RB, LC, SZ, AC, LR, RD, AF, ME, PGVM, and PF are employees of Moderna, Inc., and hold stock/stock options in the company.

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