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Children's Hospital of Philadelphia (CHOP)
Center for Cellular and Molecular Therapeutics

Type B Meeting, Pre-IND
Meeting Request

Product Name: CHOP- LNP1.UCD.ABE2
Meeting ID#: 21721 and Submission PTS# PS010343

Sponsor Information:
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Director, Gene Therapy for Inherited Metabolic Disorders Frontier Program
The Children's Hospital of Philadelphia
[redacted]

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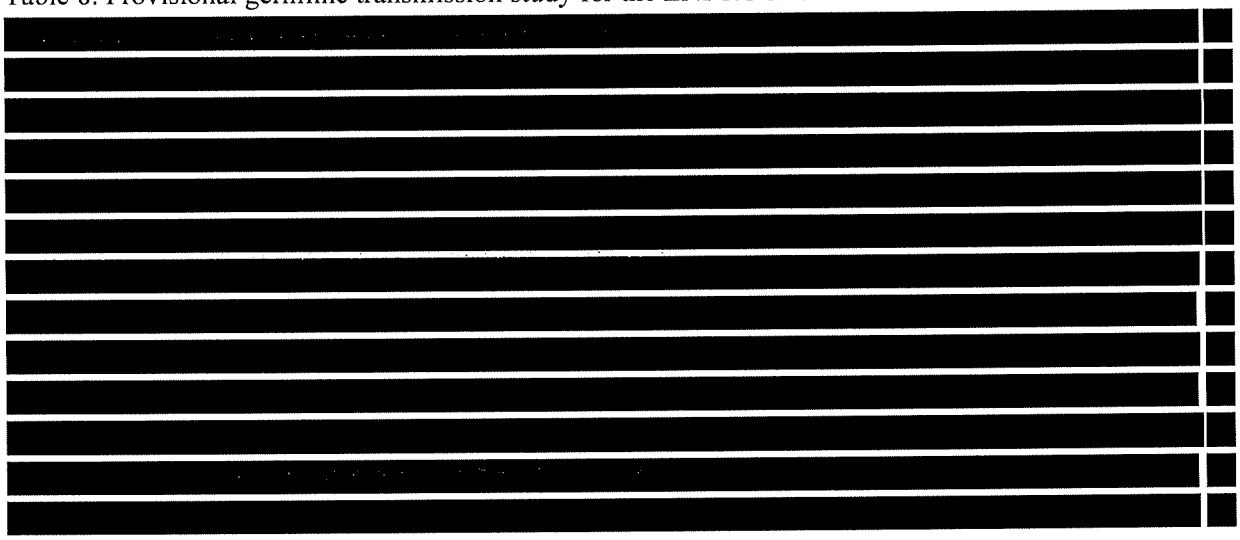


1. PRODUCT NAME

CHOP- LNP1.UCD.ABE2

2. CHEMICAL NAME AND STRUCTURE

The leader drug product (DP), designated **LNP1.UCD.ABE2**, is a lipid nanoparticle (LNP)-based editing therapeutic comprising lipid excipients, a messenger RNA (mRNA) drug substance (DS) encoding an adenine base editor (ABE), and a single guide RNA (gRNA) DS. The mRNA encodes an ABE that contains a common ABE8e TadA deaminase domain with a V106W variant and otherwise is >99% identical among all versions of the DP, with the ABE varying in its protospacer-adjacent motif (PAM) specificity. The gRNA DS is ≥80% identical among all versions of the DP.

The follower drug product (DP), designated **LNP1.UCD.ABE1** and **anticipated to be the focus of a separate Investigational New Drug (IND) application from the one focused on LNP1.UCD.ABE2**, is an LNP-based editing therapeutic comprising lipid excipients, an mRNA DS encoding an ABE, and a single gRNA DS. The mRNA encodes an ABE that contains a common ABE8.8 TadA deaminase domain and otherwise is >99% identical among all versions of the DP, with the ABE varying in its PAM specificity. The gRNA DS is ≥80% identical among all versions of the DP. The Sponsor proposes that efficacy of the leader DP, **LNP1.UCD.ABE2**, and the follower DP, **LNP.UCD.ABE1**, will ultimately be evaluated together in **a Phase III extension in a single clinical trial conducted under a master protocol IND**.

gRNA Drug Substance

Each version of the leader **LNP1.UCD.ABE2** DP comprises a gRNA DS, with each gRNA DS comprising (1) a distinct 20-nucleotide spacer sequence that corresponds to a protospacer DNA sequence matching the region of a urea cycle disorder (UCD) gene—*CPS1*, *OTC*, *ASS1*, *ASL*, *ARG*, *NAGS*, or *SLC25A15*—spanning the target variant, which includes the target adenosine nucleotide to be corrected by the DP, and (2) a common 80-nucleotide tracrRNA domain that complexes with a Cas9 nickase domain in an ABE.

The gRNA DS for the version of LNP1.UCD.ABE2 that corrects the *CPS1* c.1003C>T variant (p.Gln335Ter, or Q335X), designated **CPS1-001**, is a 100-mer oligonucleotide [redacted] as shown in **Table 1**. [This gRNA DS has previously been used in an LNP DP administered to a patient under a single patient expanded access IND (Musunuru et al., 2025).]

The gRNA DS for the version of LNP1.UCD.ABE2 that corrects the *CPS1* [redacted] variant [redacted], designated **CPS1-002**, is a 100-mer oligonucleotide [redacted] as shown in **Table 1**.

The gRNA DS for the version of LNP1.UCD.ABE2 that corrects the *ASS1* [redacted] variant [redacted], designated **ASS1-001**, is a 100-mer oligonucleotide [redacted] as shown in **Table 1**.

It is anticipated that, after the initial clearance of the leader IND for the **LNP1.UCD.ABE2** DP, **additional gRNA DSs targeting other variants in *CPS1*, *OTC*, *ASS1*, *ASL*, *ARG*, *NAGS*, or *SLC25A15* will be added by amendment to the IND.** These gRNA DSs cannot be specified beforehand, as each new gRNA DS will be selected **in real time** based on a variant that is: (1) identified upon genetic testing of a patient diagnosed with an infantile-onset UCD; and (2) demonstrated to be amenable to corrective editing by an ABE with an **ABE8e TadA deaminase domain with a V106W variant**, encoded by an mRNA DS specified below in **Table 3**.

Table 1. Exemplary gRNA DS sequences for LNP1.UCD.ABE2 DP.

CPS1-001	[redacted]
CPS1-002	[redacted]
ASS1-001	[redacted]

[redacted]

Each version of the follower **LNP1.UCD.ABE1** DP also comprises a gRNA DS, with each gRNA DS comprising (1) a distinct 20-nucleotide spacer sequence that corresponds to a protospacer DNA sequence matching the region of a UCD gene—*CPS1*, *OTC*, *ASS1*, *ASL*, *ARG*, *NAGS*, or *SLC25A15*—spanning the target variant, which includes the target adenosine nucleotide to be corrected by the DP, and (2) a common 80-nucleotide tracrRNA domain that complexes with a Cas9 nickase domain in an ABE.

The gRNA DS for the version of LNP1.UCD.ABE1 that corrects the *CPS1* [redacted] variant, [redacted], designated **CPS1-003**, is a 100-mer oligonucleotide [redacted] as shown in **Table 2**.

The gRNA DS for the version of LNP1.UCD.ABE1 that corrects the *ASL* [redacted] variant, [redacted], designated **ASL-001**, is a 100-mer oligonucleotide [redacted] as shown in **Table 2**.

The gRNA DS for the version of LNP1.UCD.ABE1 that corrects the *ASL1* [redacted] variant [redacted], designated **ASL-002**, is a 100-mer oligonucleotide [redacted] as shown in **Table 2**.

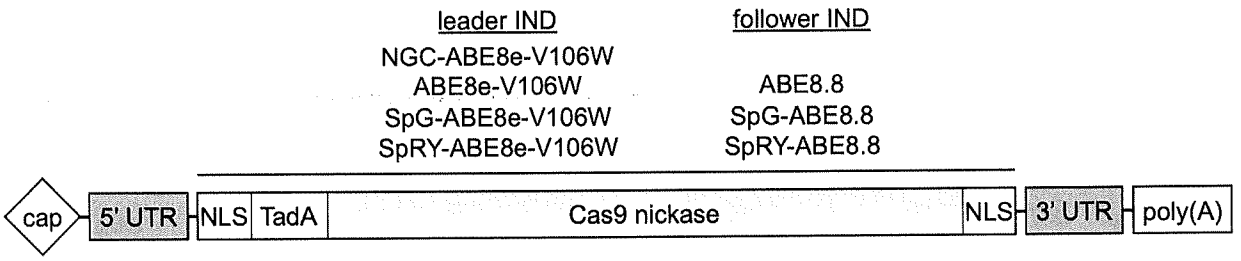
It is anticipated that, after the initial clearance of the follower IND for the **LNP1.UCD.ABE1** DP, **additional gRNA DSs targeting other variants in *CPS1*, *OTC*, *ASS1*, *ASL*, *ARG*, *NAGS*, or *SLC25A15* will be added by amendment to the IND.** These gRNA DSs cannot be specified beforehand, as each new gRNA DS will be selected **in real time** based on a variant that is: (1) identified upon genetic testing of a patient diagnosed with an infantile-onset UCD; and (2) demonstrated to be amenable to corrective editing by an ABE with an **ABE8.8 TadA deaminase domain**, encoded by an mRNA DS specified below in **Table 4**.

Table 2. Exemplary gRNA DS sequences for LNP1.UCD.ABE1 DP.	
CPS1-003	[redacted]
ASL-001	[redacted]
ASL-002	[redacted]
[redacted]	

mRNA Drug Substance

Each version of the leader **LNP1.UCD.ABE2** DP has one of several highly similar mRNA DSs. The first mRNA DS encodes the adenine base editor 8e protein with a V106W variant in the TadA deaminase domain (Richter et al., 2020) and with a version of the *Streptococcus pyogenes* Cas9 (SpCas9) D10A nickase that prefers NGC PAM sequences (hereafter referred to as **NGC-ABE8e-V106W**) (Musunuru et al., 2025; Silverstein et al., 2025). [This mRNA DS has previously been used in an LNP DP that was administered to a patient under an expanded access IND (Musunuru et al., 2025).] The other mRNA DSs encode essentially the same ABE, with the identical TadA deaminase domain and with a limited number of amino acid substitutions in the nickase domain that modify its PAM preference. One mRNA DS encodes **ABE8e-V106W**, which has the standard NGG PAM preference of SpCas9; another mRNA DS encodes **SpG-ABE8e-V106W**, which has a more relaxed NGN PAM preference (Walton et al., 2020); yet another mRNA DS encodes **SpRY-ABE8e-V106W**, which is near-PAMless, i.e., accommodates almost all PAM sequences (Walton et al., 2020). Each mRNA comprises the same 5′ cap, 5′ untranslated region (UTR), 3′ UTR, and 3′ polyadenylate tail (**Figure 1**). In each mRNA, the ABE coding sequence is codon-optimized with uridine minimization and has substitution of all uridines with the modified nucleotide N1-methylpseudouridine. The sequences of the mRNA DSs are ≥99.6% identical, with no more than 23 nucleotides differing between any two of the sequences; the encoded amino acid sequences differ by no more than 11 amino acids, exclusively in the Cas9 nickase domain. The mRNA and encoded protein sequences and the limited number of positions that differ among the sequences (indicated in bold underline) are shown in **Table 3**.

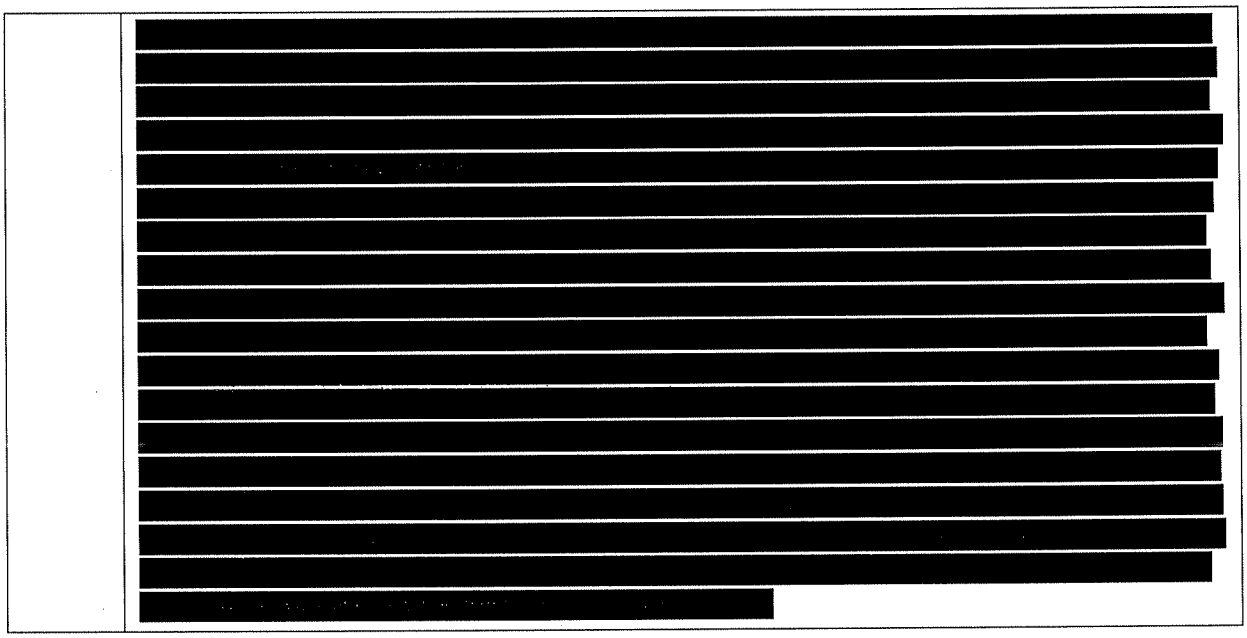
Figure 1. Structure of mRNA DS.



Abbreviations: Cap = methylated 5′-Cap-1; UTR = untranslated region; NLS = nuclear localization signal; TadA = evolved tRNA-specific adenosine deaminase; Cas9 = clustered regularly interspaced short palindromic repeats (CRISPR)-associated protein 9; poly(A) = polyadenylic acid. Not to scale.

Table 3. ABE mRNA sequences, not including 5′ cap or 3′ polyadenylate tail, and protein sequences for LNP1.UCD.ABE2 DP.

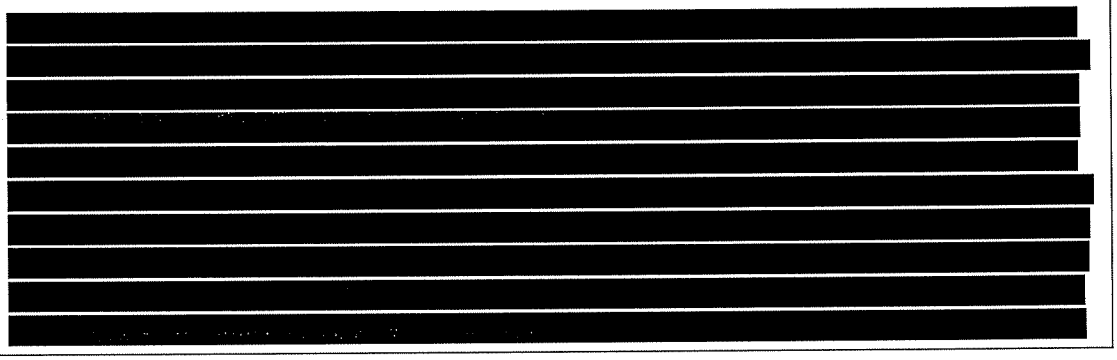
NGC-ABE8e-V106W mRNA	[redacted]



Underline = TadA domain; bold underline = differences among all mRNAs/proteins.

Each version of the follower **LNP1.UCD.ABE1** DP also has one of several highly similar mRNA DSs. The first mRNA DS encodes the adenine base editor 8.8 protein which has the standard NGG PAM preference of SpCas9 (**ABE8.8**) (Gaudelli et al., 2020). The other mRNA DSs encode essentially the same ABE, with the identical TadA deaminase domain and with a limited number of amino acid substitutions in the nickase domain that modify its PAM preference. One mRNA DS encodes **SpG-ABE8.8**, which has an NGN PAM preference; another mRNA DS encodes **SpRY-ABE8.8**, which is near-PAMless. Each mRNA comprises the same 5′ cap, 5′ UTR, 3′ UTR, and 3′ polyadenylate tail (**Figure 1**). In each mRNA, the ABE coding sequence is codon-optimized with uridine minimization and has substitution of all uridines with the modified nucleotide N1-methylpseudouridine. The sequences of the mRNA DSs are ≥99.5% identical, with no more than 28 nucleotides differing between any two of the sequences; the encoded amino acid sequences differ by no more than 11 amino acids, exclusively in the Cas9 nickase domain. The mRNA and encoded protein sequences and the limited number of positions that differ among the sequences (indicated in bold underline) are shown in **Table 4**.

Table 4. ABE mRNA sequences, not including 5′ cap or 3′ polyadenylate tail, and protein sequences for LNP1.UCD.ABE1 DP.

ABE8.8 mRNA	

	[redacted]
SpRY-ABE8.8 protein	[redacted]

Underline = TadA domain; bold underline = differences between mRNAs/proteins.

Lipid Nanoparticle Drug Product

The leader **LNP1.UCD.ABE2** DP (as well as the follower **LNP1.UCD.ABE1** DP) comprises the mRNA and gRNA DSs and four lipid excipients: [redacted]. The structures of these components and the target composition of lipid excipients in the DP are provided in **Table 5** below. [redacted]

Table 5. Lipid excipients of the LNP formulation.

[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]

[redacted]

3. PROPOSED INDICATION FOR USE

The leader DP, **LNP1.UCD.ABE2**, is proposed for the treatment of hyperammonemia in patients [redacted] with deficiencies in enzymes or a related transporter of the urea cycle who are homozygous or compound heterozygous for a pathogenic variant in any UCD gene, including

CPS1, *OTC*, *ASS1*, *ASL*, *ARG*, *NAGS*, and *SLC25A15*, that can be efficiently corrected by an adenine base editor (ABE) with a *Streptococcus pyogenes* Cas9 (SpCas9) nickase and an ABE8e TadA deaminase domain with a V106W variant.

The follower DP, **LNP1.UCD.ABE1**, is proposed for the treatment of hyperammonemia in patients [redacted] with deficiencies in enzymes or a related transporter of the urea cycle who are homozygous or compound heterozygous for a pathogenic variant in any UCD gene, including *CPS1*, *OTC*, *ASS1*, *ASL*, *ARG*, *NAGS*, and *SLC25A15*, that can be efficiently corrected by an ABE with an SpCas9 nickase and an ABE8.8 TadA deaminase domain.

4. DOSAGE FORM, ROUTE OF ADMINISTRATION, AND DOSING REGIMEN

LNP1.UCD.ABE2 (or **LNP1.UCD.ABE1**) will be administered via an intravenous (IV) infusion at a dose based on body weight. There is the potential for repeat dosing to achieve the desired therapeutic effect.

5. LIST OF SPONSOR ATTENDEES

Attendee	Affiliation	Title
[redacted]		

6. HISTORY OF THE PROJECT/BACKGROUND

Introduction

Hepatic inborn errors of metabolism (IEMs) are individually rare but collectively affect 1:1000–1:2500 births (Applegarth et al., 2000; Sanderson et al., 2006). Most arise from recessive loss-of-function variants in genes encoding key enzymes in hepatic biochemical pathways. Loss of enzyme activity results in accumulation of upstream toxic metabolites and/or insufficient production of downstream products. In many cases, abnormal liver biochemistry induces secondary organ dysfunction, especially in the brain. Each disorder has a distinct molecular etiology, with more than 140 hepatic IEMs cataloged to date. However, many hepatic IEMs share cardinal features that make them ideal candidates for a platform-based gene editing approach including: (1) the molecular etiology (i.e., editing target) is unambiguous; (2) accumulated metabolites are well-established disease and therapeutic biomarkers; (3) studies demonstrate the clinical benefit of liver correction (an organ that is accessible with current delivery technologies); (4) restoring 10-20% of hepatic enzyme activity often corrects disease phenotypes; and (5) many patients in the U.S. are identified as neonates through universal newborn screening.

Humans ingest protein to support growth and the synthesis of a number of key macromolecules. Nitrogen waste generated from protein catabolism is converted to ammonia, which under normal physiologic conditions is converted to urea via the urea cycle (**Figure 2**). Urea is then excreted in urine to maintain whole-body nitrogen homeostasis. Loss of function of any of the six enzymes of the urea cycle—encoded by *CPS1* (carbamoyl phosphate synthetase 1), *OTC* (ornithine transcarbamylase), *ASS1* (argininosuccinate synthetase), *ASL* (argininosuccinate lyase), *ARG* (arginase), and *NAGS* (*N*-acetylglutamate synthetase)—results in a urea cycle disorder (UCD). In

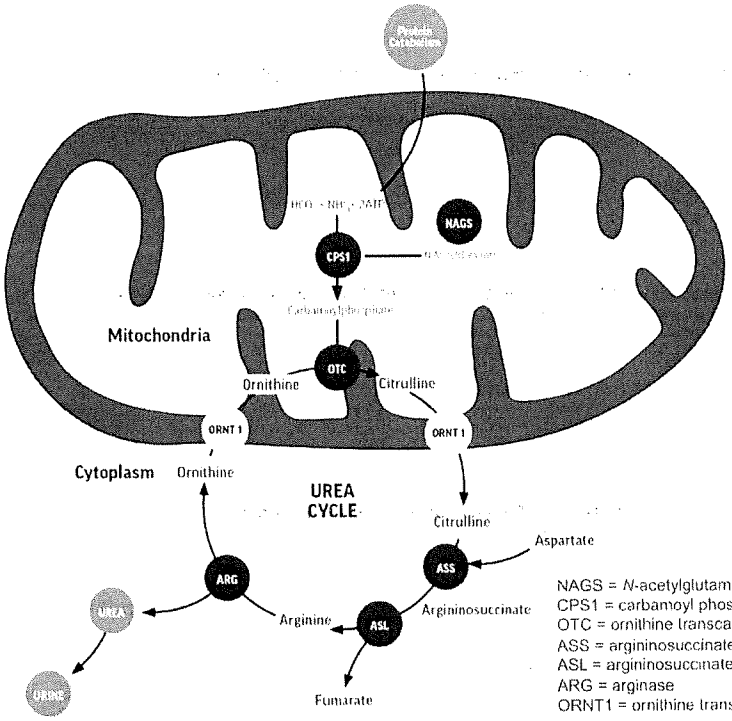


Figure 2. The urea cycle. In the liver, six enzymes either in the mitochondria or cytoplasm enable the conversion of ammonia into urea, starting with the condensation of ammonia (NH_4^+) and bicarbonate to form carbamoyl phosphate, a reaction catalyzed by CPS1 with its cofactor *N*-acetylglutamate, generated by NAGS. Carbamoyl phosphate enters the urea cycle, with the excess nitrogen passed through a serious of intermediates via the actions of OTC, ASS, ASL, and ARG and then excreted as urea in urine. Ornithine is shuttled into the mitochondria via the action of the ORNT1 transporter. A complete or near-complete deficiency of any of the six enzymes or the transporter can result in hyperammonemia and its clinical sequelae. Adapted from https://ravicti.eu/this-is-a-ucd/.

addition, a UCD can arise from defects in the mitochondrial ornithine transporter (ORNT1), encoded by *SLC25A15*.

Unmet clinical need

Severe UCD patients typically present as neonates and have a profound decrease in enzymatic function in any one of the six enzymes of the urea cycle, or a lack of function of a transporter that carries urea cycle intermediates. This results in toxic accumulation of ammonia in the blood and accumulation of specific urea cycle amino acids that aid in diagnoses and therapeutic monitoring. Patients are at risk of developing extreme hyperammonemia that can lead acutely to coma and death (Blair et al., 2015) and chronically to profound neurologic dysfunction. Current UCD medical management includes lifelong dietary protein restriction with administration of medical formulas and nitrogen scavenger medications (**Table 6**). While these standard-of-care medical interventions reduce the risk of dangerous hyperammonemic crises, they are insufficient to protect most UCD patients, who experience recurrent episodes with any minor physiologic stressors (Choi et al., 2022). Each hyperammonemic episode is associated with a high risk of permanent neurologic damage and death. It is estimated that 50% of infants with infantile-onset UCDs die in early infancy (Nettesheim et al., 2017).

Table 6. Current supportive therapy for UCDs.

Therapeutic aim	Chronic management	Emergent management during decompensations
Reduce toxic ammonia accumulation through scavenger therapy or direct removal	• Oral glycerol phenylbutyrate (Ravicti) • Liver transplantation after patient is large enough	• Intravenous (IV) sodium phenylacetate / sodium benzoate (Ammonul) • Dialysis (if needed)
Prevent fasting or inadequate caloric intake, which triggers catabolism of endogenous protein	• Frequent feeds, avoid fasting • Closely monitor caloric need and provide fats and carbohydrates as extra calories as needed	• High-dextrose IV fluids • IV lipids
Limit protein intake while avoiding any essential amino acid deficiencies, which trigger catabolism of endogenous protein	• Carefully provide non-essential amino-acid-free medical formula (i.e., "incomplete protein") to maintain normal essential amino acid levels • Strictly limit intake of natural (i.e., "complete") protein • Supplement specific urea cycle amino acids that are lacking in certain UCDs, such as citrulline in OTC deficiency	• Stop all protein intake for a limited period of time • Provide IV arginine (for all UCDs except arginase deficiency), an essential amino acid generated in the urea cycle

Given that medical measures are not very effective, liver transplantation has become the standard of care for severely affected patients at many institutions (Leonard & McKiernan, 2004; Pritchard et al., 2022; García Vega et al., 2023). As the urea cycle occurs entirely within the liver, transplantation can normalize the metabolic defect. However, transplantation is often delayed by donor availability and the need for neonates to grow to an appropriate size for

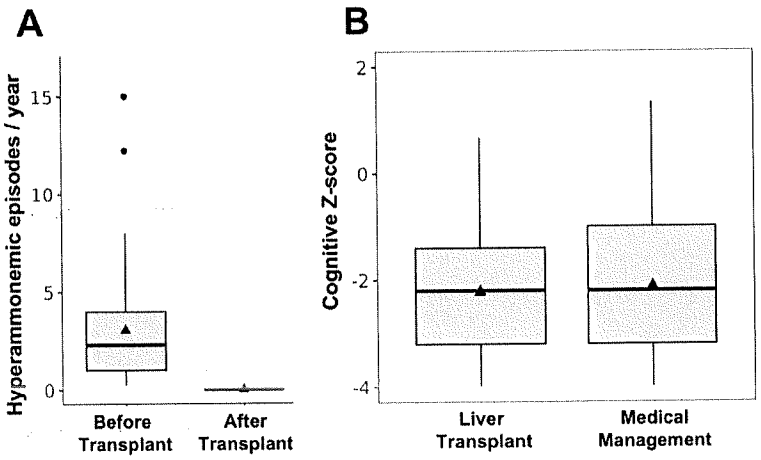


Figure 3. Severe urea cycle disorder patients have a high risk of repeat hyperammonemia and neurologic injury despite the standard of care. **(A)** Hyperammonemic episodes occurred frequently in severe UCD patients before liver transplantation but resolved after transplantation. **(B)** Transplantation did not improve cognitive outcomes as compared to medical management, likely because of neurologic injury that occurred prior to transplantation. Data are shown as medians (thick line) and means (triangle). The length of the box represents the interquartile range. Each point represents an outlier. Adapted from Posset et al., 2024.

transplantation (Yamamoto et al., 2019). Patients often are not large enough to be transplant candidates until after their first birthday. During this first year of life, most patients experience irreversible neurologic damage, even with close medical management (Posset et al., 2024) (**Figure 3**). Liver transplantation is also associated with acute and chronic risks and challenges—perioperative and postoperative complications including mortality, risk of rejection of the transplanted organ, and increased risk of infections and cancer due to the lifelong need for immunosuppressive therapy.

Treatment with the **LNP1.UCD.ABE2** DP (or the follower **LNP1.UCD.ABE1** DP) may effect the same metabolic correction as liver transplantation, but without the risks of transplantation. It may also be given earlier in the disease course, resulting in much better long-term outcomes.

Population with potential for benefit

While all UCDs can present as late-onset, attenuated forms, the most severe infantile-onset patients have the greatest potential for benefit from the leader **LNP1.UCD.ABE2** DP (or the follower **LNP1.UCD.ABE1** DP), given their high risk of neurologic injury and death. Hundreds of variants in UCD genes annotated as pathogenic or likely pathogenic have been cataloged in UCD patients, with a large subset being transition mutations, specifically G>A or C>T variants on the sense strand. As such, each of these variants is potentially amenable to correction by adenine base editing, which can drive site-specific A>G changes on either DNA strand (Gaudelli et al., 2017; Gaudelli et al., 2020; Richter et al., 2020). In the vast majority of UCD patients, the disease-causing variants are relatively unique, i.e., *N*-of-1 or *N*-of-few, which requires a high degree of **personalization** to individual patients. Notwithstanding the challenge of deploying bespoke versions of the leader **LNP1.UCD.ABE2** DP (or the follower **LNP1.UCD.ABE1** DP) in a short enough timeframe to be of maximal benefit for patients with infantile-onset UCDs—ideally, within several months after birth or the initial diagnosis—many UCD patients stand to benefit from **personalized** corrective editing treatments. The seven diseases that are the focus of the leader **LNP1.UCD.ABE2** IND, as well the follower **LNP1.UCD.ABE1** IND, are:

- Carbamoyl phosphate synthetase 1 (CPS1) deficiency, which has an estimated incidence of 1:1,300,000 (Summar et al., 2013). In the mitochondria, CPS1 catalyzes the first rate-

limiting step of the urea cycle, the condensation of ammonia and bicarbonate to form carbamoyl phosphate. There are no highly recurrent pathogenic variants in *CPS1*. For the leader IND application, the Sponsor proposes versions of the **LNP1.UCD.ABE2** DP that can correct the *CPS1* Q335X and [redacted] variants. For the follower IND application, the Sponsor proposes a version of the **LNP1.UCD.ABE1** DP that can correct the *CPS1* [redacted] variant. [redacted]

- Ornithine transcarbamylase (OTC) deficiency, which has an estimated incidence of 1:56,500 (Summar et al., 2013). In the mitochondria, OTC catalyzes the second step of the urea cycle, conversion of carbamoyl phosphate and ornithine to citrulline, which is then transported out of the mitochondria. There are no highly recurrent pathogenic variants in *OTC*. Unlike the other UCD genes—which cause disease in an autosomal recessive fashion—*OTC* resides on the X chromosome, and male patients with a single pathogenic allele manifest the disease, whereas female patients with a single pathogenic allele have variable penetrance.

- Citrullinemia type 1, which has an estimated incidence of 1:250,000 (Summar et al., 2013). In the cytoplasm, ASS catalyzes the third step of the urea cycle, conversion of citrulline and aspartate into argininosuccinate. There is a recurrent pathogenic variant in the *ASS1* gene that has been reported in multiple populations around the world, the G390R variant (Engel et al., 2009). For the leader IND application, the Sponsor proposes a version of the **LNP1.UCD.ABE2** DP that can correct the *ASS1* [redacted] variant [redacted].

- Argininosuccinate lyase (ASL) deficiency, also known as argininosuccinic aciduria, which has an estimated incidence of 1:218,750 (Summar et al., 2013). In the cytoplasm, ASL catalyzes the fourth step of the urea cycle, conversion of argininosuccinate into arginine and fumarate. There are no highly recurrent pathogenic variants in the *ASL* gene, although several local founder variants have been reported (Balmer et al., 2014). For the follower IND application, the Sponsor proposes versions of the **LNP1.UCD.ABE1** DP that can correct the *ASL* [redacted] and [redacted] variants [redacted].

- Arginase deficiency, which has an estimated incidence of 1:950,000 (Summar et al., 2013). In the cytoplasm, arginase catalyzes the fifth step of the urea cycle, conversion of arginine into urea and ornithine, the former of which is excreted in urine and the latter of which is transported into the mitochondria. While at a population level arginase deficiency patients have a lower risk of hyperammonemic crises, there are reports of severe neonatal-onset cases. There are no highly recurrent pathogenic variants in *ARG*.

- *N*-acetylglutamate synthetase (NAGS) deficiency, which has an estimated incidence of <1:2,000,000 (Summar et al., 2013). In the mitochondria, NAGS synthesizes *N*-acetylglutamate, which is an essential cofactor of CPS1. There are no highly recurrent pathogenic variants in *NAGS*.

- Hyperornithinemia-hyperammonemia-homocitrullinuria (HHH) syndrome, also known as ornithine translocase deficiency, which has an estimated incidence of <1:2,000,000 (Summar et al., 2013). HHH arises from pathogenic variants in *SLC25A15*, which encodes the transporter that shuttles ornithine from the cytoplasm into the mitochondria.

The Sponsor is proposing to treat patients [redacted] with infantile-onset UCDs presenting with hyperammonemic crises.

[redacted]

Representative Case 1: CPS1 deficiency caused by the* CPS1 *Q335X variant

The Sponsor recently reported the case of an infant with the *CPS1* c.1003C>T (Q335X) variant who was treated with a base editing DP, CHOP-LNP.CPS1.Q335X, under a single patient expanded access IND application (**IND #31438**) (Musunuru et al., 2025). The LNP formulation to be used in the LNP1.UCD.ABE2 DP differs from that used in the CHOP-LNP.CPS1.Q335X DP. However, if a new CPS1 deficiency subject were to present with the Q335X variant, the Sponsor anticipates they would have an equally severe infantile-onset UCD.

The original infant with the *CPS1* Q335X variant presented with lethargy and poor feeding in the neonatal period. On day of life 2, he was observed to have a blood ammonia level above the quantification range of the clinical laboratory assay (>1,000 µmol/L, normal range 9–33 µmol/L). He was found to have elevated plasma glutamine, undetectable plasma citrulline, and absent urine orotic acid, consistent with a biochemical diagnosis of CPS1 deficiency. Subsequently he was found to harbor biallelic pathogenic variants in *CPS1*, a maternally inherited c.2140G>T (E714X) allele and a paternally inherited Q335X allele. Despite receiving standard-of-care therapy, prior to treatment with the CHOP-LNP.CPS1.Q335X DP, he had multiple episodes of hyperammonemia requiring urgent escalation of care including initiation of a sick day diet and administration of IV ammonia scavenger medications. The CHOP-LNP.CPS1.Q335X DP comprised the **NGC-ABE8e-V106W** mRNA DS (see **Table 3**) and the **CPS1-001** gRNA DS (see **Table 1**), which were identified as having optimal corrective editing efficiency for the *CPS1* Q335X variant (see **Status of Product Development** subsection, **Figure 8**). Since receiving the CHOP-LNP.CPS1.Q335X DP, the patient has been able to tolerate 100% of the recommended dietary allowance (RDA) for protein for his age and a reduction in his nitrogen scavenger medication dosage (Musunuru et al, 2025).

[redacted]

[redacted]

Therapeutic Rationale

Rationale for the liver as the target organ

The urea cycle is largely active in hepatocytes, especially periportal hepatocytes, and correction of the primary genetic defect in a UCD patient solely within the liver via organ transplantation is curative for hyperammonemia (see **Figure 3**). Natural history studies of UCD patients demonstrate two clinical presentations: infantile-onset (often neonatal-onset) disease, in which hyperammonemia that can cause irreversible brain injury can occur within a few days of birth, with high morbidity and mortality; and late-onset disease, with moderate or mild symptoms that emerge later in childhood or adulthood and are elicited by protein-rich meals or situations of high metabolic demand.

Infantile-onset CPS1 deficiency is generally associated with less than 5% of the normal CPS1 activity in liver, whereas late-onset CPS1 disease is associated with higher residual activity (Martínez et al, 2010). Among a cohort of Japanese patients with CPS1 deficiency, one patient with late-onset disease (13 years of age) was observed to have 4.8% residual hepatic enzymatic activity, and another patient with genetically confirmed late-onset disease (13 years of age) had 17% residual activity (Kurokawa et al., 2007). In the same cohort, three patients with genetically confirmed infantile-onset disease had 6%, 6.25%, and 11% residual activity, respectively. By extrapolation, achieving correction of ≈10-15% of the *CPS1* alleles in the hepatocytes of infantile-onset CPS1 deficiency patients with no residual activity (i.e., complete loss of function) should ameliorate disease phenotypes in these patients.

Similar analyses of correlations between residual enzyme activity and severity of disease, performed by the Urea Cycle Disorders Consortium (UCDC) and the European Registry and Network for Intoxication Type Metabolic Diseases (E-IMD) Consortia Study Group, have been informative for other UCDs. For male patients with OTC deficiency, a threshold of 4.3% residual OTC enzymatic activity distinguishes severe disease from attenuated disease (Scharre et al., 2022). For patients with citrullinemia type 1, a threshold of 8.1% residual ASS enzymatic activity distinguishes severe disease from attenuated disease (Zielonka et al., 2019). For patients with ASL deficiency, a threshold of 7.9% residual ASL enzymatic activity distinguishes severe disease from attenuated disease (Zielonka et al., 2020).

An independent body of evidence for therapeutic editing thresholds in UCDs comes from preclinical studies, i.e., functional rescue of phenotypes in UCD mouse models. In a floxed *Cps1* mouse model in which liver-directed adeno-associated virus (AAV)-Cre was used to achieve gene knockdown, resulting in hyperammonemia and death soon after treatment, liver-directed AAV expression of CPS1 that achieved 15% of the wild-type level of protein activity resulted in reduced plasma ammonia levels and survival (Nitzahn et al., 2020). These observations are concordant with the therapeutic editing threshold of ≈10-15% extrapolated from human genetics.

In the *spf^{ash}* mouse model, which has a hypomorphic *Otc* allele and which experiences hyperammonemia and death when challenged with a high-protein diet, liver-directed AAV-

mediated homology-directed repair (HDR) corrective editing of the pathogenic allele in neonatal mice resulted in reduced plasma ammonia levels and survival on a high-protein diet (Yang et al., 2016). The mean HDR editing of 10% resulted in ≈15-20% of the wild-type level of OTC protein activity, with the baseline level of enzymatic activity in *spf*^{ash} being ≈5%, suggesting a therapeutic hepatocyte allelic editing threshold of ≈15% to ameliorate disease phenotypes even in patients with complete OTC deficiency.

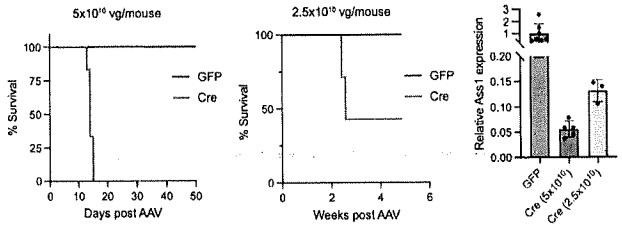


Figure 4. *Ass1* expression and mouse survival. 5% expression is lethal; 10-15% keeps some mice alive.

Using a floxed *Ass1* mouse model in which liver-directed AAV-Cre was used to tune the level of gene knockdown, the Sponsor found that 5% residual expression of *Ass1* is lethal, whereas 10-15% residual expression keeps some mice alive (**Figure 4**), suggesting a therapeutic hepatocyte allelic editing threshold of ≈10-15% to ameliorate disease phenotypes in citrullinemia type 1 patients.

In an *Asl* mouse model that dies shortly after birth, liver-directed AAV expression of ASL that achieved 5-10% of the wild-type level of enzymatic activity significantly improved life expectancy (Ashley et al., 2018), suggesting a therapeutic hepatocyte allelic editing threshold of ≈10% to ameliorate disease phenotypes in ASL deficiency patients.

In an *Arg* knockout mouse model of arginase deficiency that has hyperammonemia and dies shortly after birth, adenoviral expression of arginase normalized plasma ammonia levels and promoted survival when the liver arginase enzymatic activity was ≈20% that of control heterozygous mice but not when the activity was ≈10% that of heterozygous mice (Gau et al., 2009). These observations suggest a therapeutic hepatocyte allelic editing threshold of ≈10% to ameliorate disease phenotypes in arginase deficiency patients.

In summary, the clinical and preclinical evidence point to **≈10-15% corrective editing in hepatocytes generally being of therapeutic value across the UCDs**.

Rationale for a base editing strategy

With the leader **LNP1.UCD.ABE2** DP, the Sponsor plans to use corrective adenine base editing that introduces an A>G change in the genome, in a highly specific manner, at the site of any of a collection of pathogenic variants in the *CPS1*, *OTC*, *ASS1*, *ASL*, *ARG*, *NAGS*, and *SLC25A15* genes. The Sponsor plans the same with the follower **LNP1.UCD.ABE1** DP. The effect of reverting one of these variants to wild-type would be to restore functionality to the protein product, i.e., the enzyme or transporter, durably reducing and even normalizing blood ammonia levels in patients with at least one copy of the variant in question. The DP will comprise LNPs encapsulating an mRNA encoding an ABE and a single gRNA targeting the site of the target UCD gene variant, in liquid form for IV infusion and delivery to the hepatocytes in the liver:

- The leader **LNP1.UCD.ABE2** DP will contain NGC-ABE8e-V106W mRNA, ABE8e-V106W mRNA, SpG-ABE8e-V106W mRNA, or SpRY-ABE8e-V106W mRNA (see **Table 3**), as well as a gRNA matched to the target UCD gene variant to be corrected (see **Table 1**), e.g., the NGC-ABE8e-V106W mRNA and CPS1-001 gRNA are matched to the *CPS1* Q335X variant, the NGC-ABE8e-V106W mRNA and CPS1-002 gRNA are matched to the *CPS1* [redacted] variant, etc.

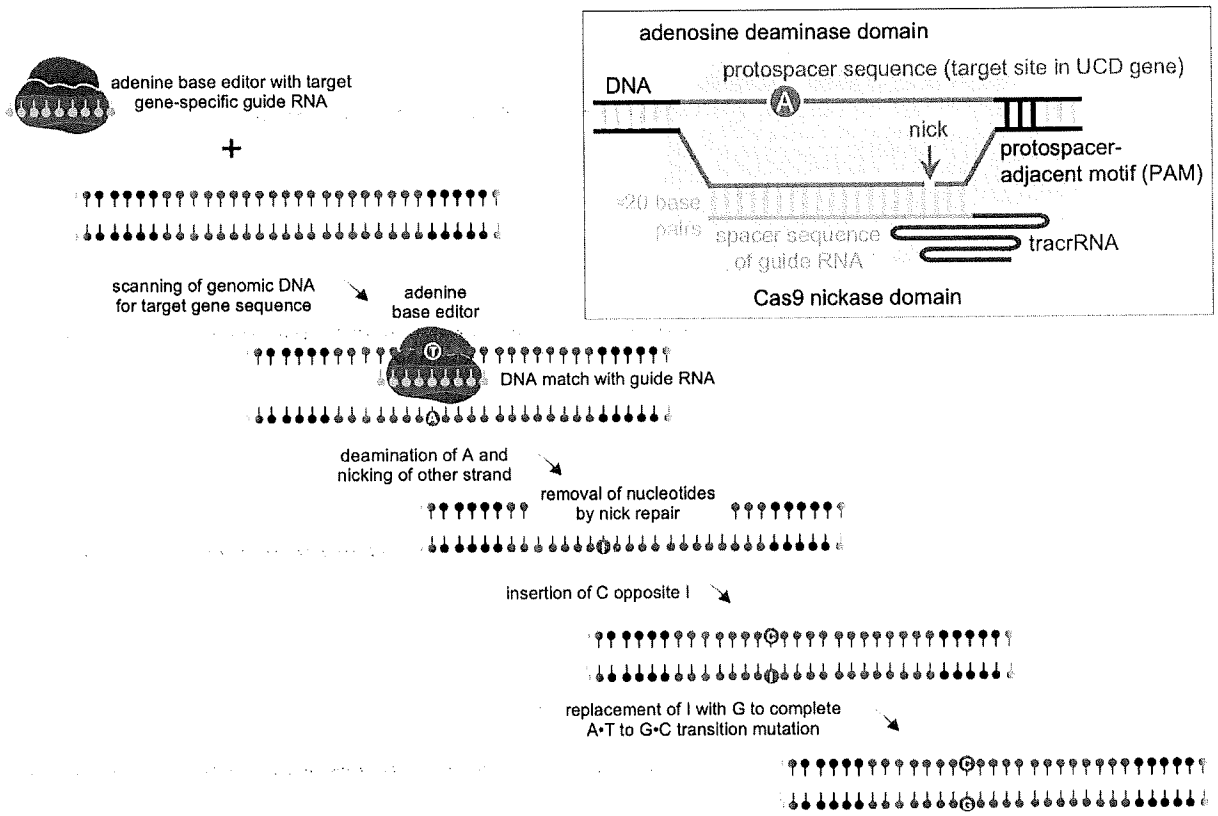


Figure 5. Schematic of the stepwise mechanism of adenine base editing. The adenine base editor (ABE) combines with a guide RNA (gRNA), engages with double-strand genomic DNA, and scans through the DNA to find the unique site in the genome that matches the spacer sequence (first 20 bases) of the gRNA. Deamination of the target adenine base on one strand, nicking of the other strand, and the ensuing cellular DNA repair process results in conversion of the original adenine base to guanine. The inset shows a close-up of the ABE in a complex with the gRNA and the specific target gene sequence.

- The follower **LNP1.UCD.ABE1** DP will contain ABE8.8 mRNA, SpG-ABE8.8 mRNA, or SpRY-ABE8.8 mRNA (see **Table 4**), as well as a gRNA matched to the target UCD gene variant to be corrected (see **Table 2**), e.g., the ABE8.8 mRNA and CPS1-003 gRNA are matched to the *CPS1* [redacted] variant, the SpRY-ABE8.8 mRNA and ASL-001 gRNA to the *ASL* [redacted] variant, etc.
- Each version of the leader **LNP1.UCD.ABE2** DP will contain **identical lipid excipients** (see **Table 5**) and will be formulated in the same way. Thus, only the gRNA DS and, in some cases, the mRNA DS will vary.
- Similarly, each version of the follower **LNP1.UCD.ABE1** DP will contain **identical lipid excipients** (see **Table 5**) and will be formulated in the same way. Thus, only the gRNA DS and, in some cases, the mRNA DS will vary.
- For the leader **LNP1.UCD.ABE2** DP, the mRNA DS varies in its sequence in only 23 positions of the >5-kb sequence at most, i.e., ≥99.6% identity.

- For the follower **LNP1.UCD.ABE1** DP, the mRNA DS varies in its sequence in only 28 positions of the >5-kb sequence at most, i.e., ≥99.5% identity.
- For either DP, the gRNA DS varies in its sequence in only 20 of 100 positions at most.

The mechanism of action of each DP is as follows:

(1) The LNPs will be internalized by the hepatocytes following binding of endogenous apolipoprotein E (apoE) to the LNP, leading to engagement of low-density lipoprotein receptor (LDLR) on the plasma membrane and subsequent receptor-mediated endocytosis of the LNP carrying the mRNA and gRNA. Comprehensive nonclinical data have shown that **the LNP characteristics of the DP drive the biodistribution profile of the DP**.

(2) After LNP-bound apoE engages with LDLR, endocytosis of the LNP occurs. The subsequent reduction in endosomal pH results in a charge-based interaction of the ionizable lipid with the endosomal membrane, which ultimately results in the release of the mRNA and gRNA cargoes into the cytoplasm (Akinc et al., 2010; Kulkarni et al, 2018).

(3) The mRNA will be translated into an ABE protein, which comprises a catalytically impaired clustered regularly interspaced short palindromic repeats (CRISPR)-associated protein 9 (Cas9) nuclease domain (that functions as a single-strand nickase and minimizes the production of double-strand breaks) fused with an adenosine deaminase domain (Gaudelli et al., 2017; Gaudelli et al., 2020; Richter et al., 2020) (**Figure 5**). The gRNA comprises a tracrRNA domain that complexes with the Cas9 nickase domain and a spacer sequence that corresponds to a protospacer DNA sequence matching the region of the UCD gene spanning the targeted variant, which includes the target adenosine nucleotide to be corrected. The protospacer is located immediately upstream of the protospacer-adjacent motif (PAM), which is required for ABE activity. **The protospacer is chosen to be unique in the genome, enabling a highly specific gRNA that would not bind efficiently elsewhere in the genome and that would thus minimize off-target editing.**

(4) Base pairing between the gRNA and the target DNA sequence will result in displacement of the PAM-containing genomic DNA strand to form a single-stranded DNA R-loop, i.e., an editing window, that exposes the target adenosine nucleotide to the deaminase activity of the ABE (**Figure 5**).

(5) ABEs with the **ABE8.8** deaminase domain will generate a narrower editing window, in which adenine bases in positions 4 through 7 of the protospacer sequence will generally have much higher efficiency of editing than adenine bases in the nearby surrounding positions. This property is advantageous if, for the best performing gRNA, the target adenine base is within the narrow window, and there are nearby adenine bases just outside the window for which there is the possibility of "bystander" editing—the ABE8.8 deaminase domain limits the amount of bystander editing.

(6) ABEs with the **ABE8e-V106W** deaminase domain will generate a broader editing window, in which adenine bases in positions 1 through 10 of the protospacer sequence will generally have much higher efficiency of editing than adenine bases in the nearby surrounding positions. This property is advantageous if, for the best performing gRNA,

the target adenine base lies outside of the center of the window; whereas the ABE8.8 deaminase domain would be less likely to support efficient editing of the target adenine base, the ABE8e-V106W deaminase domain might support high-efficiency editing. However, bystander editing of nearby adenine bases is much likelier, and if bystander editing results in a nonsynonymous coding change or a splice site change, it might limit the usefulness of ABEs with the ABE8e-V106W deaminase domain.

(7) Deamination of adenosine will produce inosine, which is read as guanosine by DNA polymerase during DNA repair.

(8) To increase efficiency of the DNA repair process, the Cas9 domain will nick the unedited DNA strand to induce a DNA repair mechanism that uses the edited strand as the template for DNA repair, resulting in an adenine to guanine substitution. For a G>A variant, this substitution on the sense strand will revert the variant to wild-type. For a C>T variant, this substitution on the antisense strand will result in a thymine to cytosine substitution on the sense strand, reverting the variant to wild-type.

(9) Corrected alleles will produce functional protein, which will stimulate the urea cycle and would be expected to reduce ammonia levels in the blood.

Rationale for clinical trial design

As the study design and major endpoints would be identical for an individual trial of any of the versions of the leader **LNP1.UCD.ABE2** DP, regardless of the UCD gene variant targeted for correction, the Sponsor is proposing an **umbrella clinical trial design** (Woodcock & LaVange, 2017) with the LNP1.UCD.ABE2 DP. Specifically, the Sponsor's proposed Phase I/II first-in-human (FIH) clinical study plans to enroll infantile-onset UCD patients [redacted], with at least one copy of one of the targetable UCD gene variants (e.g., *CPS1* [redacted] variant, *ASS1* [redacted] variant) resulting in hyperammonemia, in an umbrella trial design. The proposed design is provided in **Section 12, Clinical Program Overview**. The same considerations apply to the follower **LNP1.UCD.ABE1** DP, and so the Sponsor proposes an **essentially identical umbrella trial design** with the LNP1.UCD.ABE1 DP, albeit under **a separate IND application**.

Status of Product Development

The Sponsor has established that certain combinations of an ABE and a gRNA can efficiently and specifically correct each of several UCD gene variants (*CPS1* Q335X, [redacted], and [redacted] variants; *ASL* [redacted] and [redacted] variants; *ASS1* [redacted] variant) to wild-type in human hepatocytes *in vitro* and, in the exemplary case of the *CPS1* Q335X variant, variant-humanized mice *in vivo*, along with clinical evidence supporting corrective editing of the *CPS1* Q335X variant *in vivo* in a patient with neonatal-onset CPS1 deficiency (Musunuru et al., 2025). A description of the studies performed to date are provided in this section, below. The design of the proposed IND-enabling studies, including the definitive biodistribution and toxicology animal study and the off-target analyses, are provided in **Section 10, Proposed Nonclinical Studies**. The planned chemistry, manufacturing, and controls (CMC) are described in **Section 11, Chemistry, Manufacturing, and Controls**. The proposed clinical study design is described in **Section 12, Clinical Program Overview**.

Adenine base editor and guide RNA screening in cell models with UCD gene variants

A challenge in developing corrective base editing therapies is the lack of readily available *in vitro* models harboring rare patient-specific variants in which to test the efficacy of drug candidates. Accordingly, the Sponsor sought to generate human hepatocyte cell lines bearing patient-specific variants, using cultured HuH-7 hepatoma cells, a commonly used proxy for primary human hepatocytes (which can only be maintained in culture for several days).

The Sponsor found that techniques like nuclease-mediated HDR editing and prime editing do **not** reliably allow for generation of such cell lines for all variants. Moreover, one disadvantage of using clonal cell lines derived from single cells of a transformed cell line like HuH-7 is that there can be substantial heterogeneity among different clonal cell lines in various characteristics, including transfectability with either plasmids or LNPs. Thus, the ability to make head-to-head comparative assessments of editing efficiencies of different variants, across different cell lines, is compromised. (For example, it would not be possible to reliably compare the efficiency of a corrective editing solution for the *CPS1* Q335X variant in one cell line with the efficiency of a corrective editing solution for the *CPS1* [redacted] variant in another cell line, to determine which editing solution is more potent.) Another disadvantage is that even when successful, generating a clonal edited HuH-7 cell line can take several months, time that cannot be afforded for a patient with an infantile-onset UCD who is at ongoing risk for permanent neurologic injury and even death with any hyperammonemic crisis.

Accordingly, the Sponsor has adopted an alternative approach, taking only a few weeks, in which multiple variants are introduced into the same HuH-7 cells. This is achieved using a lentiviral vector with genomic sequences spanning individual variants. For example, the lentiviral vector might have a ≈100-bp *CPS1* genomic sequence spanning the Q335X variant, a ≈100-bp *CPS1* genomic sequence spanning the [redacted] variant, a ≈100-bp *ASS1* genomic sequence spanning the [redacted] variant, and ≈100-bp genomic sequences spanning additional UCD gene variants (**Figure 6**). The vector might also include variants that can serve as positive reference controls, such as the *PAH* P281L variant and the *PAH* R408W variant, which have well-validated corrective editing solutions that definitively treat phenylketonuria (PKU) in humanized mouse models (Brooks et al., 2023; Brooks et al., 2024). The lentivirus is used to transduce HuH-7 cells.

To expeditiously identify a base editing solution for the *CPS1* Q335X variant (**Representative Case 1**), the Sponsor generated a lentivirus-transduced HuH-7 cell line harboring the Q335X variant as well as the control *PAH* variants (Musunuru et al., 2025). (Multiple attempts to use prime editing to introduce the Q335X variant into the endogenous *CPS1* locus in HuH-7 cells

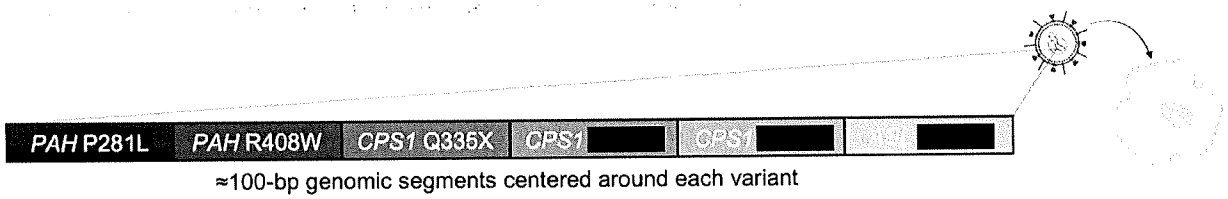


Figure 6. Lentivirus-transduced HuH-7 cell line. Schematic showing how an **example** lentiviral vector might be used to transduce human HuH-7 hepatoma cells with a cassette comprising six adjacent 100-bp genomic segments with four UCD gene variants, with two additional variants (*PAH* P281L, *PAH* R408W) serving as positive reference controls.

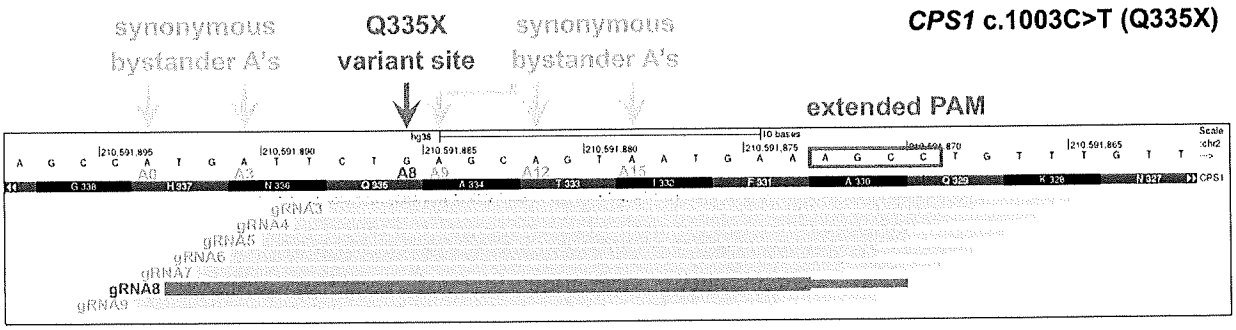


Figure 7. Schematic of genomic site of *CPS1* Q335X variant. Adapted from the UCSC Genome Browser (GRCh38/hg38). The red arrow and the vertical yellow bar indicate the position of the G altered to A (A8, in red) by the Q335X variant on the antisense strand. The grey arrows indicate the sites of potential bystander editing (A0, A3, A9, A12, and A15, in grey). The horizontal bars indicate protospacer (thick) and PAM (thin) sequences targeted by the gRNA3 through gRNA9 guide RNAs; the number in the name indicates the position of the Q335X variant adenine in that gRNA's protospacer sequence. The red horizontal bar indicates gRNA8, which demonstrated the highest corrective editing activity (see **Figure 8**). The red box indicates the extended PAM sequence for gRNA8 (AGCC).

were unsuccessful.) Using the Q335X lentivirus-transduced HuH-7 cell line, the Sponsor screened a variety of ABEs in combination with individual candidate gRNAs in **plasmid transfection** experiments. Seven gRNAs (designated gRNA3 through gRNA9) tiling the site of the Q335X variant, such that the variant adenine base ranged from positions 3 through 9 of the protospacer sequence that spanned the ABE editing window (**Figure 7**), were tested with ABEs compatible with the PAMs associated with each of the protospacer sequences: SpG for NGN PAMs, SpRY for all other PAMs (i.e., near-PAMless) (Walton et al., 2020). ABEs with three different deaminase domains were tested: ABE8.8, with the narrowest editing window, ABE8.20, with an intermediate editing window, and ABE8e, with the broadest window (Gaudelli et al., 2020; Richter et al., 2020). In the initial screen (**Figure 8A**), the SpG-ABE8e/gRNA8 combination had the highest corrective editing efficiency for the Q335X variant.

In a secondary transfection screen (**Figure 8B**), the Sponsor assessed three engineered ABEs with a preference for NGC PAMs (the extended PAM sequence for gRNA8 is AGCC), designated A1, A2, and A3. All versions of the ABE8e editor with gRNA8 displayed highly efficient corrective editing. In the same transfection experiment, the well-established solution for the *PAH* P281L variant (ABE8.8 with the P281L-specific gRNA, designated "PAH1") had similar or less corrective editing efficiency for the P281L variant compared to the ABE8e editors with gRNA8 for the Q335X variant.

Because ABE8e has been reported to have gRNA-independent off-target RNA and DNA editing, the ABE8e-V106W variant, which largely eliminates this off-target editing of ABE8e (Richter et al., 2020) was used in a final transfection screen (**Figure 8C**). The SpG, A1, A2, and A3 ABE8e editors, without and with the V106W variant, in combination with gRNA8 were tested. Among the V106W-containing editors, A1-ABE8e-V106W most closely matched the efficiency of the corresponding editor without the V106W variant, with the other V106W-containing editors showing reduced efficiency. In the same transfection experiment, the validated solution for the *PAH* P281L variant (ABE8.8 with "PAH1") had similar corrective editing efficiency for the P281L variant compared to the A1-ABE8e-V106W editor with gRNA8 for the Q335X variant.

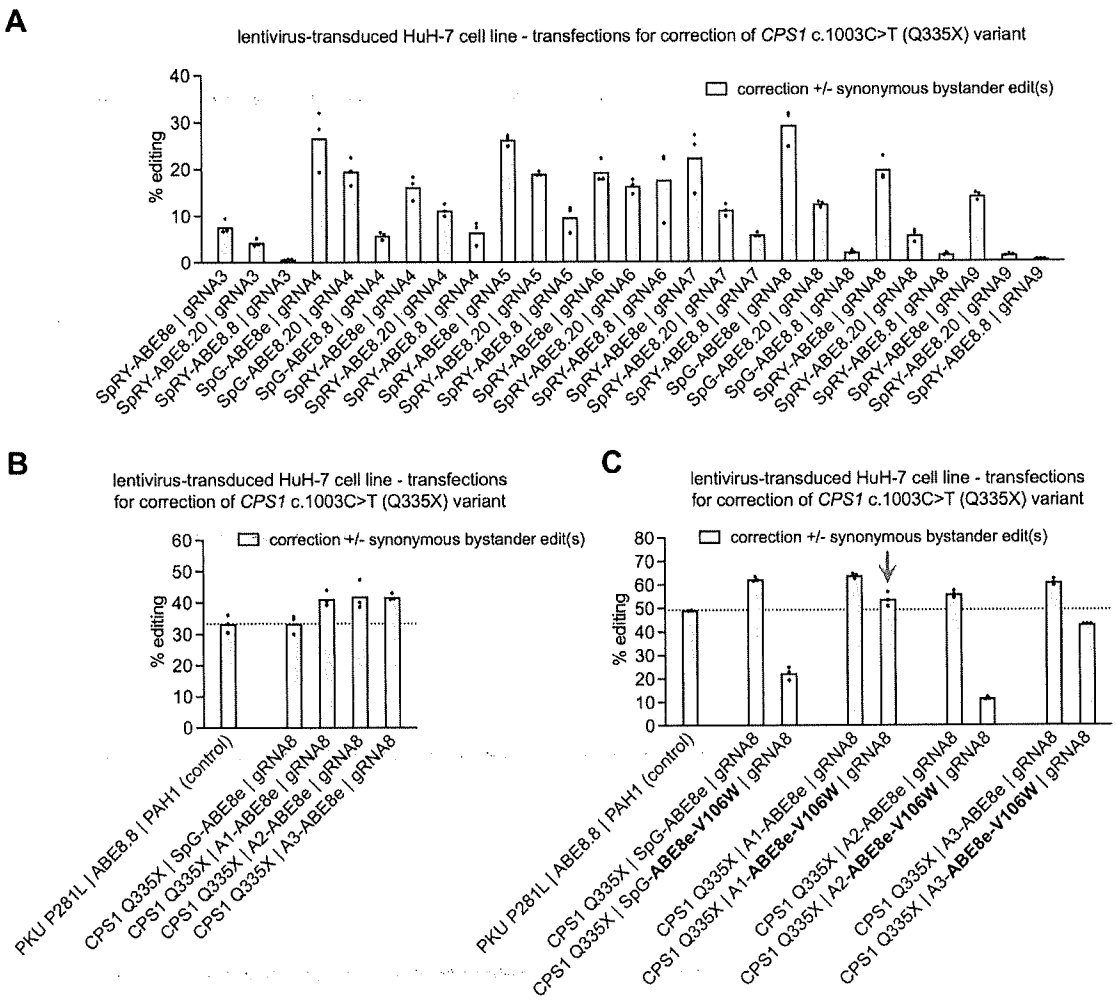


Figure 8. Screening of ABE/gRNA combinations for correction of *CPS1* Q335X variant. (A–C) Corrective *CPS1* c.1003C>T (Q335X) editing (determined from genomic DNA) following transfection of Q335X/P281L lentivirus-transduced HuH-7 cells with plasmids encoding ABE/gRNA combinations (n = 3 biological replicates), calculated as the proportion of aligned sequencing reads with the indicated type of edits. "Correction +/– synonymous bystander edit(s)" refers to reads in which the *CPS1* Q335X adenine variant, or the *PAH* P281L variant, is edited to guanine, with or without base editing of one or more nearby synonymous adenines, with no base editing of any other adenines. The horizontal dotted lines in (B) and (C) indicate the editing level for the validated PKU P281L reference control condition, and the red arrow in (C) indicates the ABE/gRNA combination—designated NGC-ABE8e-V106W/gRNA8—chosen for further development.

The A1-ABE8e-V106W editor is also designated **NGC-ABE8e-V106W**. NGC-ABE8e-V106W has a more restricted PAM preference relative to SpG: NGC(A/C) versus NGN (Silverstein et al., 2025; in this reference, the Cas9 domain is called "LWKYQS"). The Sponsor performed individual amplicon sequencing and next-generation sequencing (NGS) of the lentiviral cassette with a genomic DNA sample from NGC-ABE8e-V106W/gRNA8 plasmid-treated Q335X lentivirus-transduced HuH-7 cells (**Figure 9**). Although there was substantial bystander editing, it predominantly resulted in synonymous changes not expected to affect protein function. A version of gRNA8 with full chemical modifications was designated **CPS1-001** (see **Table 1**).

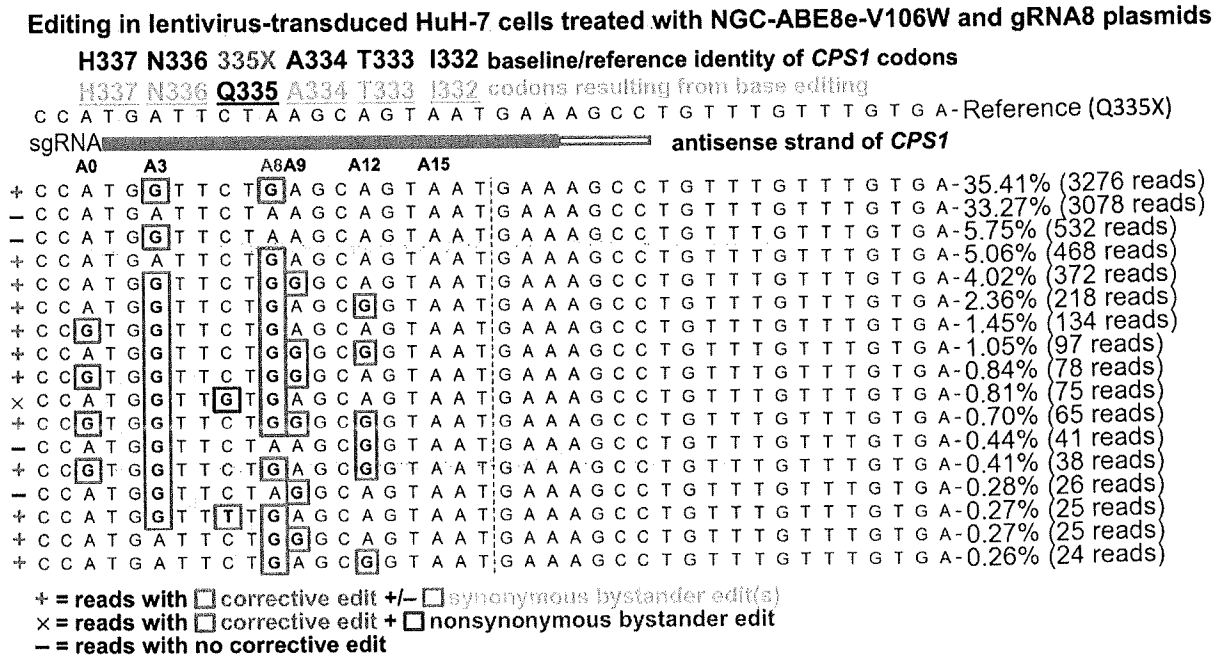
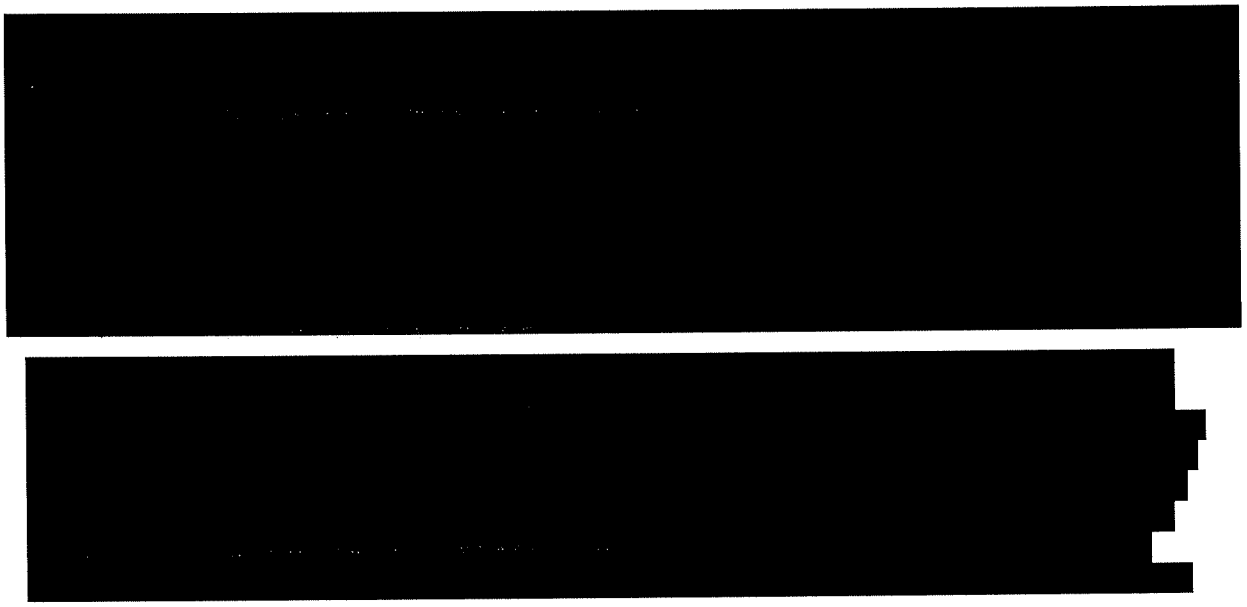


Figure 9. Empirical corrective adenine base editing of *CPS1* Q335X variant. Standard CRISPResso (http://crispresso2.pinellolab.org/submission) next-generation sequencing (NGS) output for the editing in the sample from the NGC-ABE8e-V106W/gRNA8 plasmid-treated HuH-7 cells that displayed the highest level of editing (the condition marked by the red arrow in **Figure 8C**, also designated A1-ABE8e-V106W/gRNA8). The codons in the vicinity of the Q335X site are indicated; the top-listed amino acid is the baseline/reference identity of the codon, and the bottom-listed amino acid is the one that results from base editing of the adenine in the codon. The red horizontal bar indicates the gRNA8 protospacer sequence, and the adjacent thin red box indicates the extended PAM sequence.



[redacted]

[redacted]

[As described in the following subsections, this NGC-ABE8e-V106W/CPS1-001 combination was used for a DP that was administered to a patient under a single patient expanded access IND (**Representative Case 1**).]

To identify a base editing solution for the *CPS1* [redacted] variant [redacted], the Sponsor used a lentivirus-transduced HuH-7 cell line harboring the *CPS1* [redacted] variant, as well as the PKU *PAH* P281L variant that serves as a positive reference control.

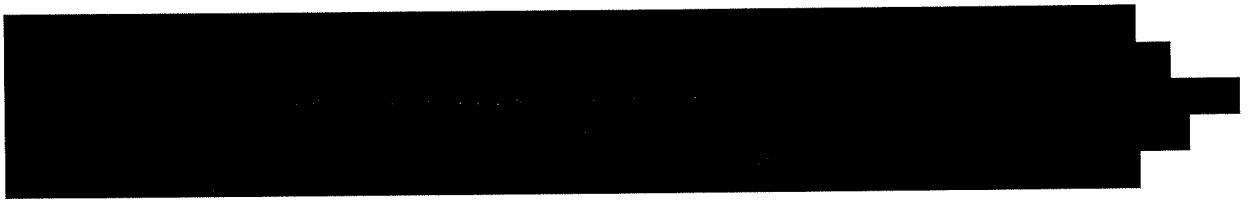
[redacted]

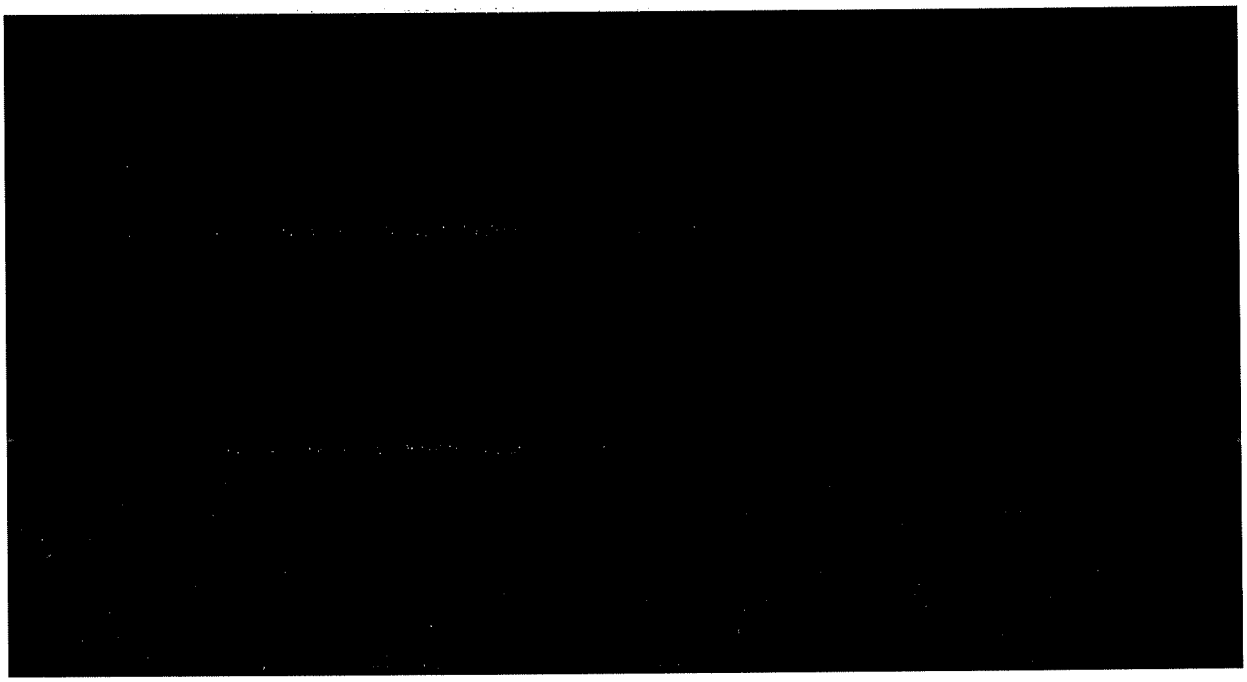
Accordingly, transfections were performed with ***in vitro* transcribed** ABE mRNAs and chemically **synthesized** gRNAs (i.e., **RNA transfection**) (**Figure 11A**). Although some nonsynonymous bystander editing was evident (red portions of bars in **Figure 11A**), the desired corrective editing (grey portions of bars in **Figure 11A**) of the *CPS1* [redacted] variant matched that of the desired corrective editing of the PKU P281L positive reference control.

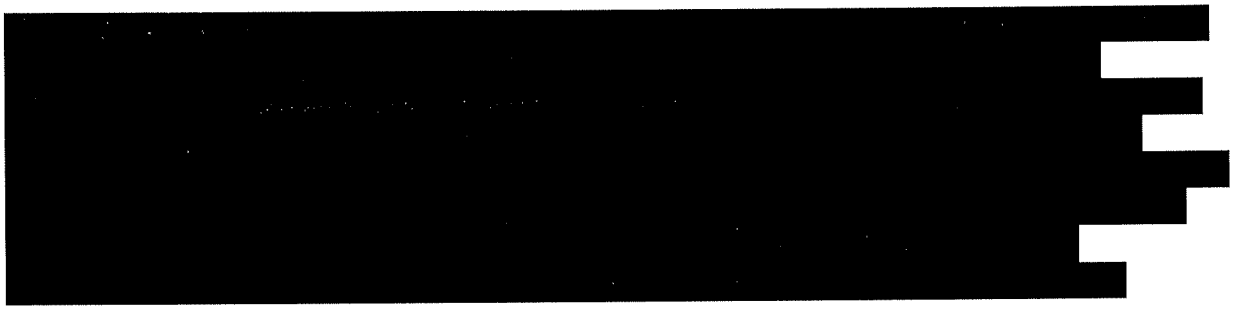
[redacted]

In a separate experiment, the Sponsor used a lentivirus-transduced HuH-7 cell line harboring the *CPS1* Q335X, [redacted], and [redacted] variants, as well as the PKU *PAH* R408W variant that serves as an alternative positive reference control (SpRY-ABE8.8 with the R408W-specific gRNA, designated "PAH4"). Transfections were performed with ABE mRNAs and chemically **synthesized** gRNAs (**Figure 11B**). The desired corrective editing of the *CPS1* [redacted] variant (using NGC-ABE8e-V106W mRNA) matched that of the desired corrective editing of the *CPS1* Q335X variant (using the same NGC-ABE8e-V106W mRNA) **in the same cells**, predicting that the version of the **LNP1.UCD.ABE2** DP specific for the [redacted] variant would have similar *in vivo* potency as the version of the **LNP1.UCD.ABE2** DP specific for the Q335X variant. [redacted]

To identify a base editing solution for the *CPS1* [redacted] variant [redacted], the Sponsor used the same lentivirus-transduced HuH-7 cell line harboring the *CPS1* Q335X, [redacted], and [redacted] variants and the PKU *PAH* R408W positive reference control. A variety of ABE/gRNA combinations were screened (data not shown), with the ABE8.8 editor and the gRNA [redacted] having a similar degree of desired corrective editing of the *CPS1* [redacted] variant as the desired corrective editing of the *CPS1* Q335X variant and the desired corrective editing of the *CPS1* [redacted] variant **in the same cells** (albeit with a different mRNA encoding an ABE with a different deaminase domain), predicting that the version of the follower **LNP1.UCD.ABE1** DP specific for the [redacted] variant would have similar *in vivo* potency to the versions of the leader **LNP1.UCD.ABE2 DP** specific for the Q335X variant or the [redacted] variant (**Figure 11B**).







To identify base editing solutions for the *ASL* [redacted] variant [redacted] and the *ASL* [redacted] variant, the Sponsor used a lentivirus-transduced HuH-7 cell line harboring the [redacted] and [redacted] variants, as well as the PKU *PAH* P281L and R408W variants that serve as positive reference controls. The Sponsor screened a variety of ABEs in combination with individual candidate gRNAs (**Figure 13**) in plasmid transfection experiments and then verified the leads in a mRNA/gRNA transfection experiment; [redacted] chemically **synthesized** gRNAs were assessed in the verification experiment (**Figure 14**). For each variant, the SpRY-ABE8.8 mRNA [redacted] performed best, with the desired corrective editing matching that of the desired corrective editing of the PKU P281L positive reference control. [redacted]

To identify a base editing solution for the *ASS1* [redacted] variant [redacted], the Sponsor generated an HuH-7 cell line harboring the *ASS1* [redacted] variant via prime editing. A variety of ABE/gRNA combinations were screened via plasmid transfection, using the SpRY-ABE8e and SpRY-ABE8.8 editors since no NGG or NGN PAMs were available (**Figure 16A**); SpRY-ABE8e[redacted] had the highest desired corrective editing. In a lentivirus-transduced HuH-7 cell line expressing the SpRY-ABE8e-V106W editor and harboring the *ASS1* [redacted] variant and the PKU *PAH* R408W positive reference control, editing of the [redacted] variant [redacted] was similar to that seen of R408W with chemically synthesized "PAH4". [redacted]

Based on these data, the Sponsor has provisionally chosen the following configurations for the mRNA and gRNA DSs to be used in the leader **LNP1.UCD.ABE2** DP (correlating with the sequences shown in **Table 1** and **Table 3**):

- *CPS1* Q335X: **CPS1-001** = [redacted]; **NGC-ABE8e-V106W** mRNA
- *CPS1* [redacted]: **CPS1-002** = [redacted]; **NGC-ABE8e-V106W** mRNA
- *ASS1* [redacted]: **ASS1-001** = [redacted]; **SpRY-ABE8e-V106W** mRNA

Also based on these data, the Sponsor has provisionally chosen the following configurations for the mRNA and gRNA DSs to be used in the follower **LNP1.UCD.ABE1** DP (correlating with the sequences shown in **Table 2** and **Table 4**):

- *CPS1* [redacted]: **CPS1-003** = [redacted]; **ABE8.8** mRNA
- *ASL* [redacted]: **ASL-001** = [redacted]; **SpRY-ABE8.8** mRNA
- *ASL* [redacted]: **ASL-002** = [redacted]; **SpRY-ABE8.8** mRNA

Validation in humanized mouse models

To perform *in vivo* testing of an investigational LNP DP (CHOP-LNP.CPS1.Q335X) prior to administration of the DP to the patient in **Representative Case 1**, who had neonatal-onset CPS1 deficiency caused by the *CPS1* c.1003C>T (Q335X) variant and the *CPS1* c.2140G>T (E714X) variant, the Sponsor wished to generate humanized mice with the *CPS1* Q335X variant. This allowed the **NGC-ABE8e-V106W** mRNA DS and **CPS1-001** gRNA DS, rather than mouse-specific surrogates, to be directly tested *in vivo*. However, the Sponsor was operating within a **limited timeframe** (several months) presented by the acuity of the patient's CPS1 deficiency and his continuing hyperammonemic episodes. Accordingly, the Sponsor attempted to rapidly generate two genetically modified mouse models that could serve the purpose of *in vivo* testing.

First, the Sponsor microinjected mouse zygotes with Cas9, a gRNA targeting the endogenous mouse *Cps1* gene near the orthologous site of the *CPS1* Q335X variant, and a single-strand DNA oligonucleotide intended to knock in a humanized segment spanning the Q335X variant and the protospacer/PAM sequences matched to the CPS1-001 gRNA. The Sponsor obtained a single female founder mouse in which close to 50% of the alleles in a sample of genomic DNA had the desired humanized segment (**Figure 17**). The first litter born from this founder mouse did not have any F1 offspring with the humanized Q335X allele. The second litter had multiple offspring heterozygous for the humanized Q335X allele (these offspring were designated ***Cps1*-Q335X**

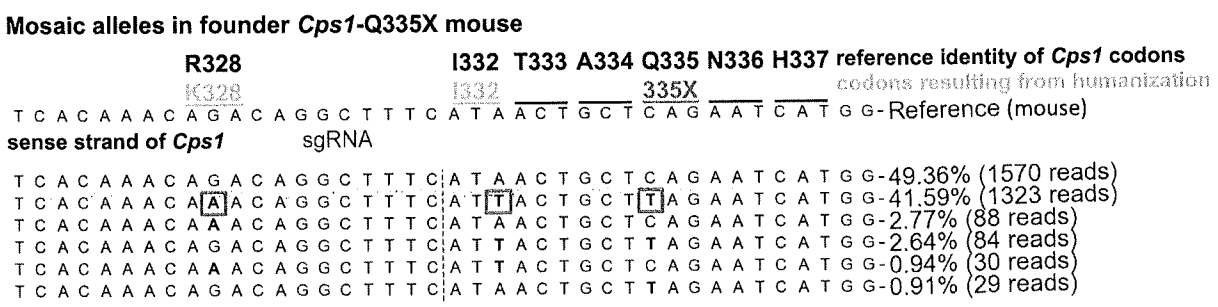


Figure 17. Founder mouse with humanized *Cps1*-Q335X allele. Standard CRISPResso2 NGS output for the editing in a genomic DNA sample from a founder pup derived from a mouse zygote into which CRISPR-Cas9 and a single-strand DNA oligonucleotide with the human Q335X target sequence with homology arms matched to the endogenous *Cps1* gene were injected. The codons in the vicinity of the Q335X site are indicated; the top-listed amino acid is the reference identity of the codon (wild-type mouse ortholog), and the bottom-listed amino acid is the one that results from CRISPR-Cas9-mediated editing (humanized sequence). The grey horizontal bar indicates the span of the humanized sequence targeted by the CPS1-001 gRNA, with almost half of the alleles in the mouse sample bearing the humanized sequence in that span. The red square indicates the human pathogenic variant, and the grey squares indicate additional changes that humanize the sequence.

knock-in mice); however, these mice were not born by the time the patient was 6 months of age, when the single patient expanded access IND application (**IND #31438**) was submitted to the Agency, and thus it was not possible to include any *in vivo* editing data from these mice in the IND application. The Sponsor subsequently performed a limited dose-response study of the CHOP-LNP.CPS1.Q335X DP. There was substantial corrective editing in two mice treated at a dose of 3 mg/kg (mean 52% whole-liver editing) and in two mice treated at a dose of 1 mg/kg (mean 35% whole-liver editing) (**Figure 18**). Attempts at breeding heterozygous mice to generate homozygous *Cps1*-Q335X mice have been unsuccessful; all homozygotes have either passed away pre-term or on day of life 0, consistent with past efforts to generate *Cps1* knockout mice (Schofield et al., 1999; Khoji et al., 2019) as well as other mouse models of neonatal-onset UCDs (Patejunas et al., 1994; Reid Sutton et al., 2003; Senkevitch et al., 2012; Wang et al., 2017). This phenomenon of perinatal lethality across UCD genes **makes it prohibitive to use UCD mice to model biological responses to DPs** with respect to functional endpoints (i.e., prolonged survival, reduced blood ammonia levels, etc.). Accordingly, the Sponsor holds that the only use of mouse models would be to demonstrate ***in vivo* hepatic editing efficiency** of DPs.

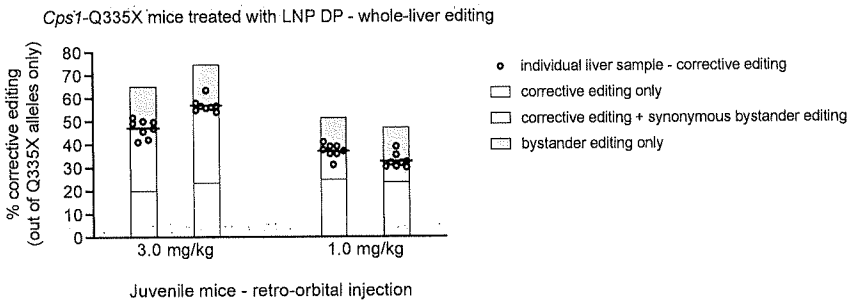


Figure 18. Whole-liver corrective editing in *Cps1*-Q335X mice. Corrective and/or bystander editing of the endogenous Q335X variant sequence in F1 *Cps1*-Q335X mice, following a single treatment with the CHOP-LNP.CPS1.Q335X DP at the indicated dose (n = 8 liver samples per mouse in juvenile mice treated at 1 month of age, with necropsy several days after treatment).

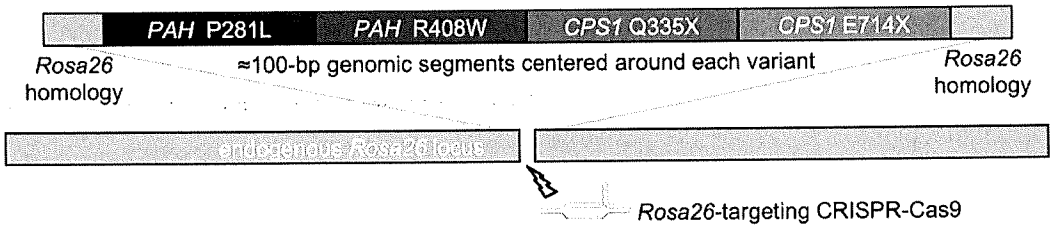


Figure 19. Generation of *Rosa26*-Q335X mice. Schematic showing how a single-strand DNA oligonucleotide cassette harboring the *CPS1* Q335X variant sequence and three other variants was inserted into the endogenous mouse *Rosa26* locus in mouse zygotes using CRISPR-Cas9 to introduce a double-strand break in the *Rosa26* locus, followed by homology-directed repair with the cassette.

Second, the Sponsor microinjected mouse zygotes with Cas9, a well-validated gRNA targeting the *Rosa26* safe harbor locus, and a single-strand DNA oligonucleotide with ≈100-bp genomic sequences spanning the patient's *CPS1* Q335X and E714X variants, as well as the *PAH* P281L and R408W variants (the two PKU positive reference controls) (**Figure 19**). The Sponsor obtained two founder mice, both female, in which the genomic segment was introduced into the *Rosa26* locus in some alleles. Both *Rosa26* founder mice achieved germline transmission in their first litters, with multiple F1 offspring in each litter harboring the transgenic allele (these offspring were designated ***Rosa26*-Q335X** mice). The Sponsor used these heterozygous offspring for a limited dose-response study of the CHOP-LNP.CPS1.Q335X DP prior to submission of the single patient expanded access IND application (**IND #31438**). Corrective editing was observed in two mice treated at a dose of 3 mg/kg, two mice treated at a dose of 1 mg/kg, and two mice treated at a dose of 0.1 mg/kg (**Figure 20A**); these data were generated in time (within 6 months of the patient's birth) to be included in the single patient expanded access IND application. A dose-response study was also performed in lentivirus-transduced HuH-7 cells (**Figure 20B**), thereby establishing an *in vitro–in vivo* correlation. Of note, although these *Rosa26*-Q335X mice could be considered a patient-specific model because they harbored one copy each of the patient's two variants (Q335X and E714X), the two variants were on the same *Rosa26* allele, and the two copies of the endogenous wild-type *Cps1* locus were intact. Thus, these mice would not have had a CPS1 deficiency phenotype even if bred to homozygosity, and so they could be used only to model ***in vivo* hepatic editing efficiency** of DPs.

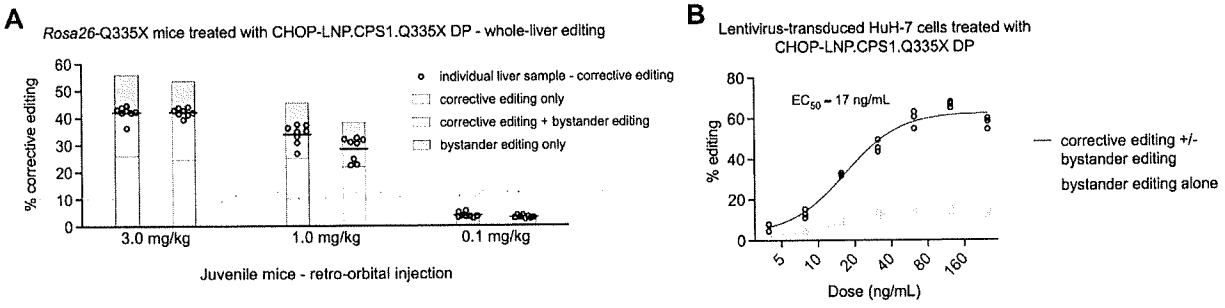


Figure 20. Corrective editing in *Rosa*-Q335X mice and Q335X HuH-7 cells. (A) Whole-liver corrective and/or bystander editing of the *CPS1* Q335X variant sequence incorporated into the *Rosa26* locus in F1 *Rosa26*-Q335X mice, following a single treatment with the CHOP-LNP.CPS1.Q335X DP at the indicated dose (n = 8 liver samples per mouse, from juvenile mice treated at 1-2 months of age, with necropsy several days after treatment). **(B)** Corrective and/or bystander editing of the Q335X variant sequence incorporated into HuH-7 cells treated with the CHOP-LNP.CPS1.Q335X DP at a dose range (n = 3 biological replicates per dose).

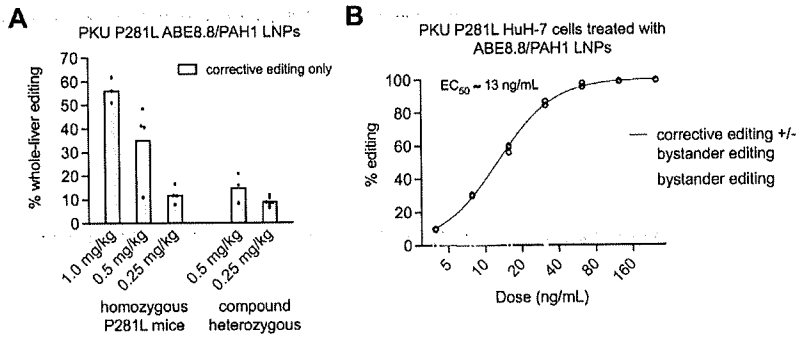


Figure 21. Corrective editing in *Pah*-P281L mice and P281L HuH-7 cells. (A) Whole-liver corrective editing of the endogenous *PAH* P281L variant sequence in homozygous or compound heterozygous *Pah*-P281L mice, following a single treatment with ABE8.8/PAH1 LNPs at the indicated dose (each point is the mean of n = 8 liver samples per mouse, from mice treated at 2-3 months of age, with necropsy 1 week after treatment). **(B)** Corrective and/or bystander editing of the P281L variant sequence incorporated into HuH-7 cells treated with the ABE8.8/PAH1 LNPs at a dose range (n = 3 biological replicates per dose).

In separate work, the Sponsor generated a humanized PKU mouse model in which the *PAH* P281L variant (which is serving as a positive reference control in cellular experiments) was introduced into the orthologous position in endogenous mouse *Pah* exon 7 (designated *Pah*-P281L mice) (Brooks et al., 2023). Homozygous mice have elevated blood phenylalanine levels (typically in the 1500-2000 µmol/L range), (2) hypopigmentation of the fur, (3) reduced weight, and (4) a variety of neurochemical and neurobehavorial phenotypes. Upon treatment with LNPs with ABE8.8 mRNA and a P281L-targeting gRNA ("PAH1"), these PKU phenotypes either fully correct or partially correct within a few weeks. The Sponsor has performed dose-response studies in the *Pah*-P281L mice as well as in HuH-7 cells harboring the *PAH* P281L variant (**Figure 21**) (Brooks et al., 2023), thereby establishing an *in vitro–in vivo* correlation.

Clinical validation

The CHOP-LNP.CPS1.Q335X DP, comprising the **NGC-ABE8e-V106W** mRNA DS (see **Table 3**) and the **CPS1-001** gRNA DS (see **Table 1**), was administered by the Sponsor to the patient in **Representative Case 1** under a single patient expanded access IND application (**IND #31438**) (Musunuru et al., 2025). [redacted]

The Sponsor submitted the single patient expanded access IND application to the Agency, and it was approved 1 week later, before the patient was 7 months old. On day of life 208, the patient received an intravenous infusion of the CHOP-LNP.CPS1.Q335X DP, comprising a total RNA dose of 0.1 mg/kg. After treatment, it was possible to liberalize his dietary protein intake (as he was born at 35 weeks gestation, at times his prescribed protein goal was above the chronological recommended dietary allowance) (**Figure 22A–C**). He recovered from a viral respiratory infection without experiencing an illness-associated hyperammonemic crisis; however, he received intravenous fluids as is standard during illness and was on a protein-free diet for one

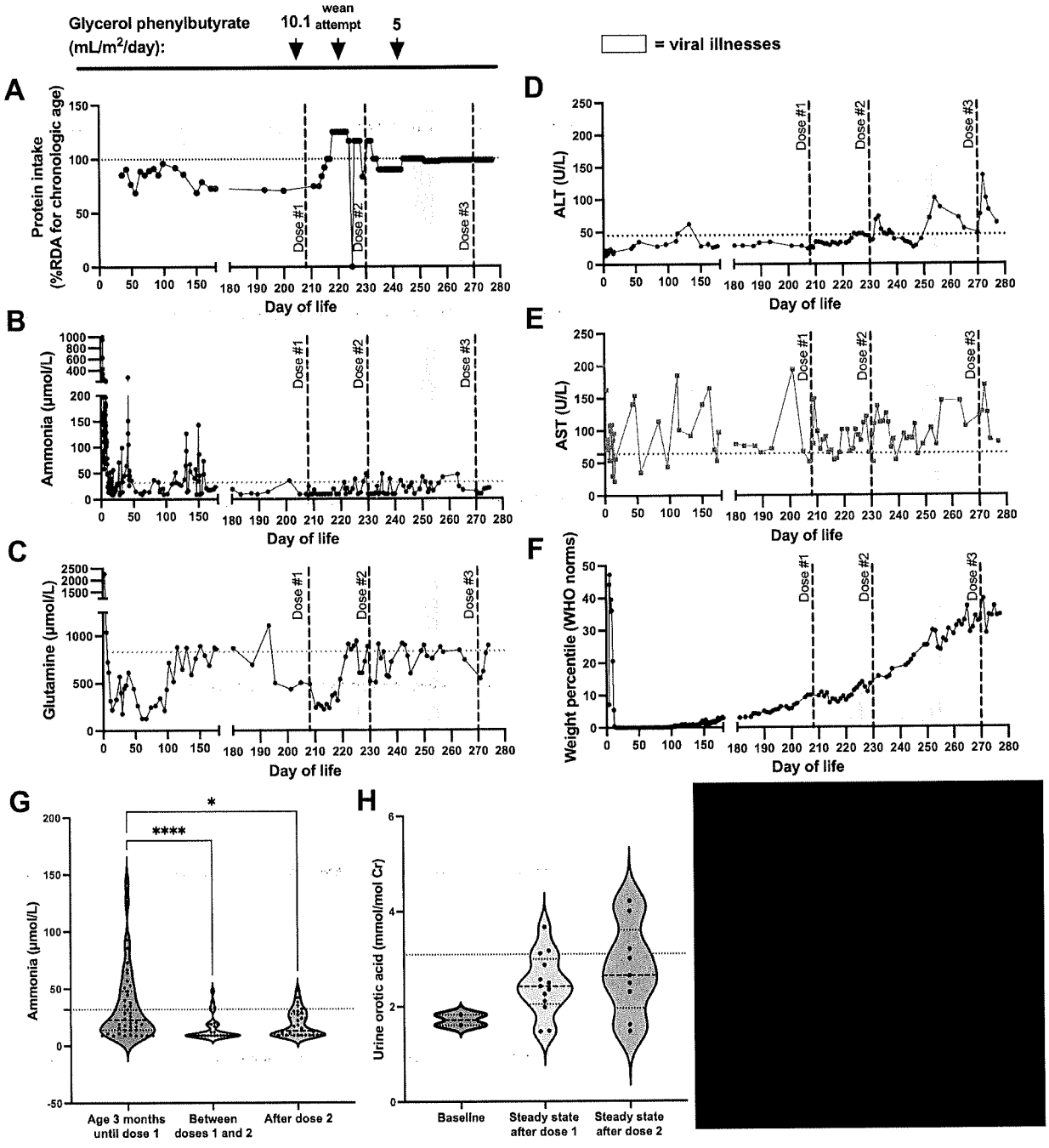


Figure 22. Biochemical profile of the patient in Representative Case 1 before and after treatment with the CHOP-LNP.CPS1.Q335X DP. **(A)** Protein intake, plasma levels of **(B)** ammonia, **(C)** glutamine, **(D)** alanine aminotransferase (ALT), and **(E)** aspartate aminotransferase (AST), and **(F)** weight percentile during the patient's lifetime through three treatments with the LNP DP (up to day of life 280). The grey bars from left to right indicate a rhinovirus-positive upper respiratory infection after dose 1 and two viral illnesses after dose 2 (gastroenteritis followed by a new rhinovirus/enterovirus infection with associated viral transaminitis). The horizontal dotted lines indicate upper limits of normal laboratory value ranges. **(G, H)** Pre-treatment and post-treatment ammonia levels and urine orotic acid levels, respectively. The horizontal dotted lines indicate upper limits of normal laboratory value ranges. [redacted] The horizontal dotted line indicates the lower limit of the normal BUN range. * $P<0.05$; **** $P<0.0001$.

day (day of life 225). The nitrogen scavenger medication, glycerol phenylbutyrate, could not be weaned (reduced from 10.1 to 8.1 mL/m^2/day but restored to the original dose due to rising glutamine levels).

Given the incomplete biochemical correction, and according to the clinical protocol, the patient received a second dose of the CHOP-LNP.CPS1.Q335X DP, 0.3 mg/kg, 22 days after the first dose (day of life 230). The only adverse event was a coughing episode during the second infusion that resolved with nasal suctioning. Transient ALT and AST elevations occurred a few days following the second dose, with recurrence a few weeks later in the setting of viral illness (**Figure 22D–E**). He tolerated a halving of glycerol phenylbutyrate (5 mL/m^2/day) 2 weeks after the second dose. In the 4 weeks following the second infusion, the patient developed two viral infections, each with vomiting and diarrhea. In contrast to a gastroenteritis infection that occurred prior to treatment with k-abe, he recovered from the illnesses without suffering a hyperammonemic crisis and was able to continue his full-protein diet during the illnesses. Blood ammonia levels before the first dose (median 23 μmol/L), between the first and second doses (9 μmol/L), and after the second dose (13 μmol/L) support a treatment-related difference (**Figure 22G**). CPS1 contributes to orotic acid synthesis, and CPS1 deficiency patients often have low-normal urine orotic acid levels (pre-treatment median 1.7 mmol/mol-Cr); following the two treatments, levels were often high-normal or above-normal (median 2.4 mmol/mol-Cr; median 2.6 mmol/mol-Cr) (**Figure 23H**).

In light of the continued need for glycerol phenylbutyrate, and according to the clinical protocol, the patient received a third and final dose of the CHOP-LNP.CPS1.Q335X DP, 0.45 mg/kg, 40 days after the second dose (day of life 270). The only adverse events were a coughing episode during the third infusion, similar to the cough that occurred with the second infusion, and then mild rash and fever in the evening following the infusion, which resolved by the following morning. There were slightly higher though still transient ALT and AST elevations a few days after the third dose, compared to the second dose. The patient subsequently had an uncomplicated hospital course, and he was discharged home on day of life 306, for the first time since his birth. The patient's weight increased from <10th percentile for his age prior to the first dose to >40th percentile for his age at the time of discharge (**Figure 22F**). [redacted]

Pilot study of intended clinical LNP composition in nonhuman primates

The Sponsor undertook a pilot study of the intended clinical LNP composition for the leader **LNP1.UCD.ABE2** DP (and the follower **LNP1.UCD.ABE1** DP) in nonhuman primates (NHPs). [redacted]

[redacted]

For the pilot study of this LNP composition, the Sponsor used the ABE8.8 mRNA [redacted] and a gRNA targeting the *PCSK9* gene [redacted], using a published, validated gRNA sequence (Musunuru et al., 2021). The Sponsor formulated LNPs [redacted] and administered them at a 2.5 mg/kg dose to four ≈2-year-old cynomolgus monkeys [redacted]. Over the two-week period following LNP treatment, there were no clinical events, and the liver function tests (blood levels of ALT, AST, alkaline phosphatase, and total bilirubin) had minimal changes (**Figure 23A**). Compared to pre-treatment LDL cholesterol levels, there were reductions in LDL cholesterol ranging from 46% to 58% (**Figure 23B**). After necropsy, whole-liver editing was assessed, with *PCSK9* editing proportions ranging from 42% to 55%, quite concordant with the reductions in LDL cholesterol (Musunuru et al., 2021) (**Figure 24A**). A wide variety of other organs were also assessed to determine the biodistribution of editing (**Figure 24C**). There were low levels of editing in the spleen, adrenal glands, and skin around the IV infusion site; in all the other organs, any signal was indistinguishable from background. These results are consistent with those of previous studies of LNP biodistribution (Gillmore et al., 2021; Musunuru et al., 2021; Lee et al., 2023).

[redacted]

[redacted]

7. MEETING PURPOSE

The purpose of the meeting is to seek input on the summarized nonclinical proof-of-concept and efficacy data; the proposed definitive animal study; the proposed assessment of potential off-target editing; the proposed chemistry, manufacturing, and controls; and the proposed clinical study. The objectives of the meeting are to receive advice from the Agency as summarized in the enclosed questions.

8. PROPOSED AGENDA

The Sponsor's proposed agenda is presented below.

Topic	Estimated Duration
Introductions	5 minutes
Discussion of questions	50 minutes
Summary and review of action items	5 minutes

9. LIST OF QUESTIONS, GROUPED BY DISCIPLINE

Nonclinical

Question #1: Does the Agency agree that cellular studies, rather than humanized mouse studies, will provide sufficient proof-of-concept (POC) data to support the administration of the LNP1.UCD.ABE2 DP to infantile-onset urea cycle disorder (UCD) patients?

Question #2: Does the Agency agree that the proposed definitive biodistribution/toxicology study of one version of the LNP1.UCD.ABE2 DP in wild-type rats will provide sufficient data to support an IND application for all versions of the LNP1.UCD.ABE2 DP?

Question #3: Does the Agency agree that the proposed definitive biodistribution/toxicology study of one version of the LNP1.UCD.ABE2 DP in wild-type rats will provide sufficient data to support re-dosing of patients with the LNP1.UCD.ABE2 DP?

[redacted]

Question #5: Does the Agency agree that the proposed off-target editing studies of a given version of the LNP1.UCD.ABE2 DP will provide sufficient data to support the administration of that version of the LNP1.UCD.ABE2 DP to infantile-onset UCD patients?

Question #6: Does the Agency agree that the overall nonclinical development plan is sufficient to support an IND application for all versions of the LNP1.UCD.ABE2 DP, as well as a separate IND application for all versions of the follower LNP1.UCD.ABE1 DP?

Chemistry, Manufacturing, and Controls (CMC)

[redacted]

Question #8: Does the Agency agree that the proposed potency assay for the LNP1.UCD.ABE2 DP is acceptable to support an IND application for all variant-specific versions of the LNP1.UCD.ABE2 DP?

Clinical

Question #9: Does the Agency agree that the general design, including the proposed safety and exploratory efficacy outcome measures, enrollment criteria, and long-term follow-up plan are appropriate for the Phase I/II umbrella trial protocol outlined in the protocol synopsis?

Question #10: Does the Agency agree that a Phase III extension of the Phase I/II umbrella trial protocols in the LNP1.UCD.ABE2 DP IND application and the follower LNP1.UCD.ABE1 DP IND application, combining the efficacy studies of the two DPs into a single clinical trial conducted under a master protocol IND, would be appropriate?

10. PROPOSED NONCLINICAL STUDIES

Question #1: Does the Agency agree that cellular studies, rather than humanized mouse studies, will provide sufficient proof-of-concept (POC) data to support the administration of the LNP1.UCD.ABE2 DP to infantile-onset urea cycle disorder (UCD) patients?

Question #2: Does the Agency agree that the proposed definitive biodistribution/toxicology study of one version of the LNP1.UCD.ABE2 DP in wild-type rats will provide sufficient data to support an IND application for all versions of the LNP1.UCD.ABE2 DP?

Question #3: Does the Agency agree that the proposed definitive biodistribution/toxicology study of one version of the LNP1.UCD.ABE2 DP in wild-type rats will provide sufficient data to support re-dosing of patients with the LNP1.UCD.ABE2 DP?

[redacted]

Sponsor Position: The proposed definitive animal study (**Figure 25**, **Table 7**, and **Appendix 1 – Definitive Animal Study Synopsis**) has been designed specifically in accordance with the recommendations of the **FDA Guidance for Industry on Human Gene Therapy Products Incorporating Human Genome Editing (2024)**, particularly **Section IV: Considerations for Nonclinical Studies.** In light of the Agency's feedback for a Pre-IND meeting for a similar LNP base editing drug product (CHOP-LNP1.PAH.ABE1, **PTS #PS008879/4**), the Sponsor is proposing to assess the biodistribution and toxicology of the DP in a Good Laboratory Practice (GLP)-like definitive study in wild-type rats (**Figure 25** and **Table 7**).

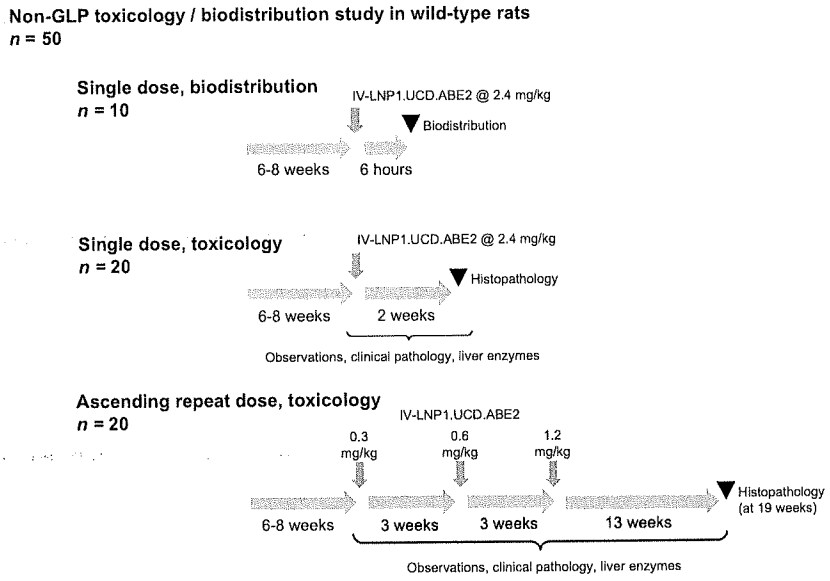


Figure 25. Schematic of proposed definitive rat biodistribution and toxicology study. Green arrows indicate dosing; black arrowheads indicate necropsy.

Table 7. Proposed definitive animal study for the LNP1.UCD.ABE2 DP.

Study Design	Dose Groups	Primary Assessments
GLP-like biodistribution/toxicology study in wild-type rats, with a version of the DP [redacted]	Single 2.4 mg/kg dose biodistribution and toxicology groups, and repeat escalating dose groups separated by 3 weeks—0.3 mg/kg, 0.6 mg/kg, 1.2 mg/kg—or vehicle control, n = 10 rats per group (treated at 6-8 weeks of age); equal numbers of females and males in each group, otherwise random, blinded assignment	General safety, clinical observations including cardiac and respiratory rates, clinical pathology including liver function tests, lipid excipient levels, and anti-PEG and anti-Cas9 antibodies in blood at various timepoints up to necropsy; gross and histological pathology, and lipid excipient and mRNA quantification in liver and other organs at necropsy and for unscheduled deaths

A more comprehensive summary of the proposed definitive rat biodistribution/toxicology study is available in **Appendix 1 – Definitive Animal Study Synopsis**.

Because there are no rat or NHP models of UCDs, particularly models with any of the targeted UCD variants, studies with wild-type rats or NHPs would have limited utility for assessing on-target editing efficiency of the LNP1.UCD.ABE2 DP. The Sponsor proposes to assess **biodistribution in rats only**. As the biodistribution of editing observed in the pilot NHP study of the intended clinical LNP composition (see **Figure 24C**) is highly concordant with the LNP biodistribution observed in other studies (Gillmore et al., 2021; Musunuru et al., 2021; Lee et al., 2023), the Sponsor holds that an additional NHP biodistribution study of the same LNP would be redundant. As such, the proposed rat biodistribution study **respects the Replacement, Reduction, and Refinement framework to minimize animal use**.

The Sponsor proposes to assess **toxicology in rats**. The Sponsor holds that performing the proposed definitive rat biodistribution/toxicology study with **one version** of the LNP1.UCD.ABE2 DP [redacted] is sufficient to support the IND application for **all versions** of the LNP1.UCD.ABE2 DP.

All variant-specific versions of the LNP1.UCD.ABE2 DP will be nearly identical. All will be formulated in the same way using **identical lipid excipients, which drive the biodistribution and toxicology**. The only distinctions will be in the mRNA and gRNA components. The ABE8e-V106W, NGC-ABE8e-V106W, SpG-ABE8e-V106W, and SpRY-ABE8e-V106W mRNA components, shown in **Table 3**, are very similar. The full-length mRNAs, spanning the coding sequence, the 5′ and 3′ untranslated regions, and the 3′ polyadenylate sequence, are identical in length at about 5.2 kilobases. The mRNAs differ within the Cas9 coding portion by at most 23 bases distributed throughout the Cas9 sequence; the remainder of the sequence, including the adenosine deaminase portion, is identical. Thus, a maximum of 23 out of ≈5200 positions in the mRNAs differ (≥99.6% identity). The difference in the mRNAs is not expected to affect the toxicology of the DP.

Across all variant-specific versions of the DP, the gRNA components will be identical in the tracrRNA portions (the final 80 nucleotides of the 100-nucleotide RNA molecule), with the differences being in the spacer portions (the first 20 nucleotides). The sequences of representative gRNAs are shown in **Table 1,** although the Sponsor anticipates that, after the initial clearance of the IND for the LNP1.UCD.ABE2 DP, **additional gRNA DSs targeting other variants will be added by amendment to the IND in real time as new subjects are born and diagnosed**. Even accounting for DNA nucleotide substitutions for RNA nucleotides in order to reduce off-target editing (Whittaker et al., 2025), a maximum of 20 out of 100 positions differ among the gRNAs, which are not expected to affect the toxicology of the DP.

The version of the DP [redacted] will be the only version tested in the definitive rat biodistribution/toxicology study. Because minor differences in the mRNA and gRNA components are not expected to affect the biodistribution and toxicology of the DP, the Sponsor holds that it is unnecessary to test additional versions of the DP. In addition, the Sponsor holds that **the experimental design of the definitive rat biodistribution/toxicology study respects the Replacement, Reduction, and Refinement framework to minimize animal use**.

The definitive rodent study (**Figure 25** and **Table 7** above) comprises a group that undergoes **single dosing** and a group that undergoes **repeat dosing** with three consecutive treatments with the LNP1.UCD.ABE2 DP, with the doses separated by 3 weeks, followed by a long-term necropsy endpoint matched with a control group (13 weeks following the third treatment). For the repeat dosing group, the first dose is 0.3 mg/kg, the second dose is 0.6 mg/kg, and the third dose is 1.2 mg/kg. [redacted] Additional goals of this nonclinical study design are to (1) demonstrate ≥95% clearance of LNP components [redacted] from the blood within 3 weeks after each dosing, (2) establish that the expected sequelae of LNP treatment, e.g., ALT elevations, are either absent or rapidly self-resolve within the 3-week intervals following each of the three doses, (3) evaluate if repeat dosing generates an anti-Cas9 or anti-PEG antibody response, and (4) document that long-term toxicological effects with repeat dosing are absent.

Because (1) the pilot study of the intended clinical LNP composition [redacted] has already shown no adverse clinical events and minimal changes in liver function test (LFTs) at a dose of 2.5 mg/kg (see **Figure 23A**), [redacted] the Sponsor proposes not to test higher than 2.4 mg/kg in the single-dose protocols and 1.2 mg/kg in the repeat-dose protocols of the definitive rat biodistribution/toxicology study. Of note, **the Sponsor has previously established the clinical safety of a single-patient ascending dose protocol in the subject of a recent single patient expanded access IND (IND #31438)** (Musunuru et al., 2025).

[redacted]

In regard to specific subsections of the FDA Guidance:

"The use of in vitro models … should be considered for evaluating the activity of a human GE product in the target cell type(s) for genomic modification."

The Sponsor will use lentivirus-transduced HuH-7 cells harboring the patient's disease-causing UCD variant(s), the *CPS1* Q335X variant as a positive reference control, and two positive reference control PKU *PAH* variants (P281L and R408W), as outlined in **Figure 6**, to assess for on-target editing activity (specific single-nucleotide changes) by the variant-specific versions of the LNP1.UCD.ABE2 DP, as exemplified in **Section 6, History of the Project/Background, Status of Product Development**, especially **Figures 6–16**. The Sponsor holds that the strong correlation between *in vitro* and *in vivo* editing efficiency demonstrated for the *CPS1* Q335X variant and the *PAH* P281L variant (see **Figures 20–21**) in support of the prior single patient expanded access IND (**IND #31438**) for the patient in **Representative Case 1** is sufficient to justify the use of ***in vitro* studies**, **without the need for additional *in vivo* studies**, to design and evaluate additional variant-specific versions of the LNP1.UCD.ABE2 DP.

The Sponsor has previously made use of lentivirus-transduced HuH-7 cells harboring the *CPS1* Q335X variant and the two reference *PAH* variants (P281L and R408W) (Musunuru et al., 2025), conceptually similar to the example outlined in **Figure 6**. In a dose-response study using the LNP DP administered to the patient in **Representative Case 1**, there was greater than 60% desired corrective editing at higher doses, with an EC_{50} of ≈17 ng/mL (**Figure 20B**). This experiment will serve as the basis for a **potency assay** for the LNP1.UCD.ABE2 DP, with the EC_{50} for corrective editing of the target variant [as measured by next-generation sequencing (NGS)] serving as a quantitative measure of potency.

Specifically, the Sponsor proposes to use lentivirus-transduced HuH-7 cells harboring target UCD variants in addition to the *CPS1* Q335X, *PAH* P281L, and *PAH* R408W reference control variants as the basis of the potency assay. (Refer to **Section 11, Chemistry, Manufacturing, and Controls, LNP1.UCD.ABE2 Drug Product, Potency Assay** for a comprehensive description of the proposed potency assay.) This potency assay would allow for determination of whether a particular clinical batch of the DP meets a minimum potency threshold that would make it appropriate for use in the patient dosing scheme laid out in the clinical protocol (**Section 12, Clinical Program Overview**).

"The animal species and/or models selected for in vivo studies should demonstrate a biological response to the human GE product … Given the differences in the genomic sequences between humans and animals, analysis of the biological activity may be done in a species-specific context (e.g., using a surrogate product), as appropriate."

The Sponsor holds that, for several reasons, humanized animal models are **not** suitable for demonstration of biological responses to variant-specific versions of the LNP1.UCD.ABE2 DP:

- Because of the high degree of **personalization** that will be needed to make different versions of the DP suitable for individual patients with unique/near-unique UCD variants, and the **limited timeframe** to administer the DP (ideally within several months after the birth or initial diagnosis of a patient), it will **not** be possible to generate a new homozygous knock-in mouse model—with a patient's variant in the endogenous UCD gene locus—needed to demonstrate a biological response to treatment with the DP.

- Even if a new homozygous knock-in UCD mouse model could be fully generated in a reasonable timeframe, the high likelihood of **perinatal lethality** (Schofield et al., 1999; Khoji et al., 2019; Patejunas et al., 1994; Reid Sutton et al., 2003; Senkevitch et al., 2012; Wang et al., 2017) would make it prohibitive to demonstrate a biological response to treatment with the DP.
- The utility of a heterozygous UCD mouse model (whether knock-in of the variant into the endogenous locus or into the *Rosa26* safe harbor locus) would be limited to demonstrating *in vivo* hepatic editing efficiency of DPs—which can instead be modeled with *in vitro* studies (e.g., **Figures 20–21**).
- The **Replacement, Reduction, and Refinement framework to minimize animal use** should be respected as much as possible.

"In vivo nonclinical safety studies for a human GE product (or surrogate product) should incorporate elements of the planned clinical trial (e.g., dose level range, ROA, delivery device, dosing schedule, study endpoints, concomitant therapies, etc.), to the extent feasible."

The definitive rat biodistribution/toxicology study (**Figure 25** and **Table 7**) has been designed with the planned clinical trial in mind. [redacted] **The Sponsor will measure the concentration of the LNP1.UCD.ABE2 DP after passage through the needle and syringe system used in the toxicology study to ensure device compatibility.**

"Assessment of biodistribution should be conducted to characterize the distribution, persistence, and clearance of the GE product, any expressed GE components in vivo, editing activity in target and non-target tissues, and the potential for inadvertent germline modification. These evaluations may be conducted independently or in conjunction with POC and/or safety studies."

In prior studies with LNPs, it has been observed that LNPs predominantly distribute to the liver, with minor distribution to the spleen and adrenal glands (Gillmore et al., 2021; Musunuru et al., 2021; Lee et al., 2023; see **Figure 24C**). The Sponsor will assess distribution, persistence, and clearance of GE components in rats (**Figure 25** and **Table 7**). The Sponsor proposes a tiered approach, wherein lipid excipients [redacted] will be assessed in a broad selection of tissues first, and the expressed GE component [redacted] will then be assessed only in tissues that are positive for lipid excipients.

"The intended clinical GE product should be evaluated in the definitive POC and safety studies, as feasible."

An engineering batch of the LNP1.UCD.ABE2 DP, made [redacted] with the intended clinical manufacturing process (as described in **Section 11, Chemistry, Manufacturing, and Controls**) will be used for the definitive rat toxicology study.

"Editing efficiency required to achieve the desired biological activity or therapeutic effect."

The necessary editing threshold (10-15% whole-liver corrective editing) has been established by prior studies (summarized in **Section 6, History of the Project/Background, Therapeutic Rationale**, e.g., **Figure 4**).

"Assessment of immunogenicity of the GE components and expressed transgene(s)."

The definitive rat biodistribution/toxicology study (**Figure 25** and **Table 7**) will monitor the development of anti-drug antibodies, specifically against the Cas9 component of the ABE and the PEG-lipid component of the LNP.

"Evaluation of the potential for inadvertent germline modification."

The definitive rat biodistribution/toxicology study (**Figure 25** and **Table 7**) will assess lipid excipients [redacted] and the expressed GE component [redacted] in the gonads. The Sponsor holds that a germline transmission mouse study with the LNP1.UCD.ABE2 DP is unnecessary if the proposed definitive rat biodistribution/toxicology study documents a lack of detectable mRNA delivery in gametes, within the limit of detection of available assays.

If the Agency deems it to be necessary for the Sponsor to perform a germline transmission mouse study with the LNP1.UCD.ABE2 DP, the Sponsor proposes that if the study with a single version of the LNP1.UCD.ABE2 DP shows no transmission, it would be unnecessary to perform a germline transmission study for any of the other versions of the LNP1.UCD.ABE2 DP. As explained above, all variant-specific versions of the LNP1.UCD.ABE2 DP will be nearly identical. All will be formulated in the same way using the same lipid excipients. The only distinctions will be in the extremely similar gRNA and mRNA components. Given the near identity of the gRNA and mRNA components, which are entirely enclosed within the LNPs and are not released until internalization of the LNPs into cells, the distribution of the DP and its components *in vivo* into gametes is not expected to differ, **due to the identical lipid excipients, which drive biodistribution and toxicology**.

Table 8 summarizes the germline transmission mouse study that would be performed if biodistribution studies document lipid excipient and mRNA delivery in gametes. The version of the LNP1.UCD.ABE2 DP [redacted] would be used for this study, should the study be required. As most homozygous UCD mouse models harboring the pathogenic variant in the endogenous locus are **perinatal lethal**, the Sponsor proposes to utilize the *Rosa26*-Q335X mouse model (**Section 6, History of the Project/Background, Status of Product Development, Validation in humanized mouse models, Figures 19–20**) for a germline transmission study, if it is necessary.

Table 8. Provisional germline transmission study for the LNP1.UCD.ABE2 DP.

Study Design	Dose Groups	Primary Assessments
Non-GLP germline transmission study in homozygous *Rosa26*-Q335X mouse disease model	Single administration, 1.2 mg/kg LNP dose, with dosing timed to allow for a full cycle of gametogenesis before mating; numbers of females and males chosen to generate ≈250 viable offspring of LNP-treated female and LNP-treated male mice	Genotype at the site of the Q335X variant in the *Rosa26* locus by NGS of genomic DNA samples of offspring

Question #5: Does the Agency agree that the proposed off-target editing studies of a given version of the LNP1.UCD.ABE2 DP will provide sufficient data to support the administration of that version of the LNP1.UCD.ABE2 DP to infantile-onset UCD patients?

Sponsor Position: The Sponsor notes the recommendations of the **FDA Guidance for Industry on Human Gene Therapy Products Incorporating Human Genome Editing (2024)** related to off-target editing, contained in **Section IV: Considerations for Nonclinical Studies**.

In regard to specific subsections of the FDA Guidance:

"Identification of on- and off-target editing events, including the type, frequency, and location ... Multiple methods (e.g., in silico, biochemical, cellular-based assays) that include a genome-wide analysis are recommended to reduce bias in identification of potential off-target sites."

The Sponsor is planning to use **three orthogonal methods** to **nominate** candidate sites of **gRNA-dependent off-target editing** for all variant-specific versions of the LNP1.UCD.ABE2 DP. Of note, these same methods were used to support a recent single patient expanded access IND (**IND #31438**) (Musunuru et al., 2025).

The **first nomination method** is Circularization for High-throughput Analysis of Nuclease Genome-wide Effects by sequencing adapted for adenine base editing (CHANGE-seq-BE) (Lazzarotto et al., 2024), a **homology-independent** biochemical assay that provides an **unbiased genome-wide analysis** (**Figure 26**). CHANGE-seq uses Tn5 tagmentation to fragment genomic DNA obtained from cells (e.g., human hepatocytes), followed by circularization of the DNA fragments via intramolecular ligation. After enzymatic degradation of any remaining linear DNA, the circular DNA will be mixed *in vitro* with a ribonucleoprotein (RNP) comprising recombinant ABE protein (ABE8e-V106W, NGC-ABE8e-V106W, SpG-ABE8e-V106W, or SpRY-ABE8e-V106W, as appropriate) complexed with the variant-specific synthetic gRNA. The RNP will nick certain oligonucleotide sequences on one strand and deaminate an adenine base on the other strand. EndoV will be used to cleave the other strand adjacent to the deaminated base, resulting in the equivalent of a double-strand break that linearizes the circular DNA molecule. After end-repair and adaptor ligation to the ends of linearized DNA molecules, next-generation sequencing (NGS) will identify the sequences that were edited *in vitro* and the frequency of editing, generating a rank-ordered list of candidate (i.e., potential) off-target sites.

The **second nomination method** is bioinformatic prediction, which relies on sequence similarity between genomic sites and the protospacer/PAM sequence specified by the ABE/gRNA set [redacted]. This method uses bioinformatic tools such as Cas-OFFinder and GuideScan (Bae et al., 2014; Perez et al., 2017) to identify sites in the reference human genome with up to three mismatches, or up to two mismatches plus up to one RNA or DNA bulge. These sites will automatically be regarded as candidate (potential) off-target sites.

The **third nomination method** is OligoNucleotide Enrichment and sequencing (ONE-seq), a **homology-dependent** biochemical assay that uses a synthetic human genomic library selected by sequence similarity to the protospacer/PAM sequence specified by the ABE/gRNA (Petri et al., 2021; Musunuru et al., 2021) (**Figure 26**). Thus, ONE-seq is an extension of the second method (bioinformatic prediction). The Sponsor will design a library with sites in the reference genome with up to five mismatches, or up to three mismatches plus up to one RNA or DNA bulge, to the on-target protospacer/PAM sequence. After synthesis by a commercial vendor, the

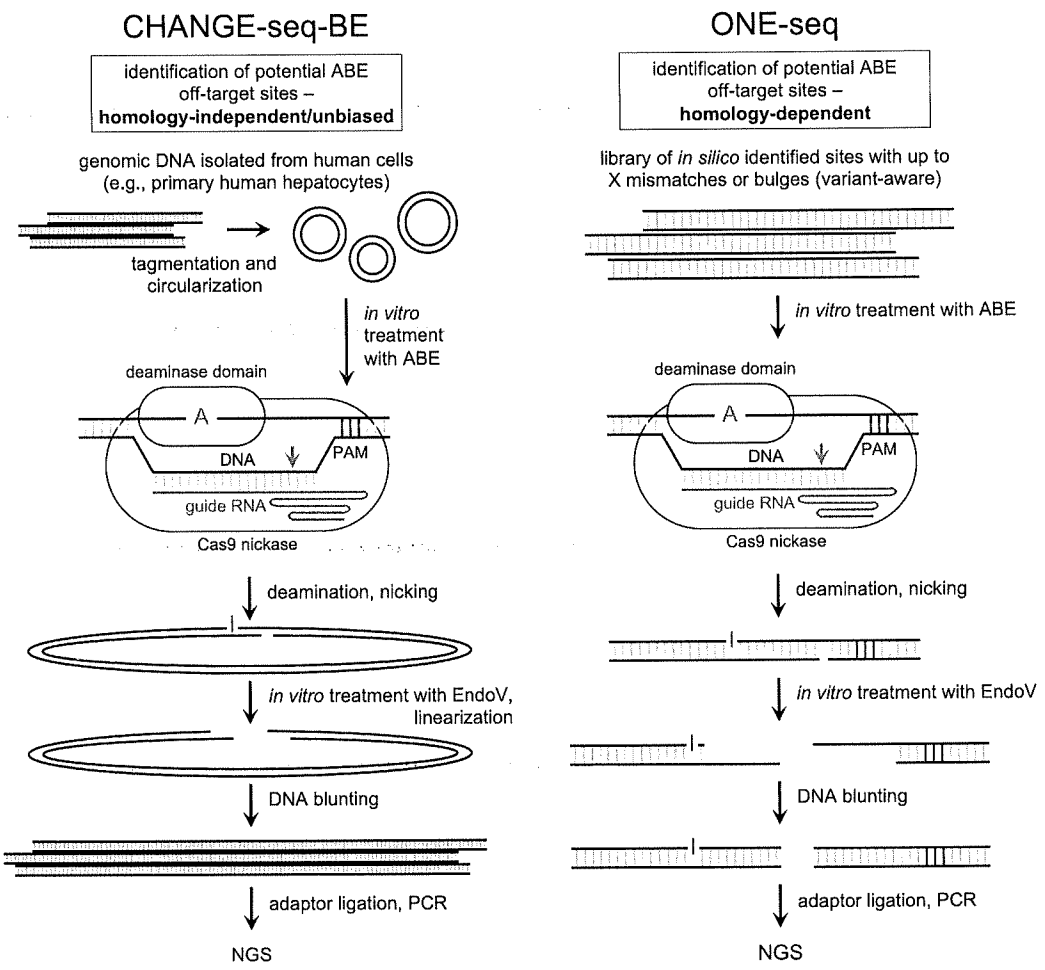


Figure 26. Off-target nomination methods. These methods nominate candidate off-target sites, which are subsequently evaluated to verify whether or not off-target editing genuinely occurs at the sites in target cells.

library will be mixed *in vitro* with an RNP comprising recombinant ABE protein complexed with the synthetic gRNA. The RNP will nick certain oligonucleotide sequences on one strand and deaminate an adenine base on the other strand. EndoV will be used to cleave the other strand adjacent to the deaminated base, resulting in the equivalent of a double-strand break. NGS will then quantify the frequency with which each unique oligonucleotide sequence was edited *in vitro*, generating a rank-ordered list of candidate (potential) off-target sites (typically, the on-target site is at or near the top of the list).

Standard off-target assessment techniques share a critical limitation: each is tied to the specific individual genome represented by the cells or the genomic DNA sample used for analysis. For this reason, most off-target analyses have overlooked the potential for naturally occurring human genetic variation to create novel off-target editing sites in some patients. Furthermore, even if one were to predict that a common or rare genetic variant might create an off-target editing site, it can be challenging to evaluate whether editing actually occurs at that site in the therapeutically relevant cells (e.g., hepatocytes) if there is no way to obtain such cells from a patient with that variant. The Sponsor proposes to use the ONE-seq methodology to empirically identify

candidate off-target sites created by genetic variation. **Variant-aware** ONE-seq uses oligonucleotide libraries designed not just using the reference human genome but also incorporating data from the 1000 Genomes Project, the Human Genome Diversity Project, etc., with bioinformatic tools like CRISPRme (Cancellieri et al., 2023).

"Verification of off-target sites should be conducted using methods with adequate sensitivity to detect low frequency events … For in vivo GE products, the analysis should also include the major cell types in which editing events are detected. Appropriate controls should be included to confirm the quality of the assay and to assure interpretability of the results and its suitability for the intended use."

The Sponsor is planning to use **a primary approach** and, if needed, **a secondary approach** to **verify** candidate sites as *bona fide* off-target sites, i.e., sites where off-target editing genuinely occurs in hepatocytes.

The **primary verification approach** is the rhAmpSeq system (Integrated DNA Technologies), which uses highly multiplexed, targeted amplicon sequencing. rhAmpSeq can readily accommodate hundreds or even thousands of candidate sites. Any sites that either (1) are flagged by rhAmpSeq as having off-target editing, or (2) are dropouts with rhAmpSeq, can be reassessed with individual targeted amplicon sequencing (PCR followed by NGS). Regarded as the gold standard, this approach typically has a lower limit of detection of ≈0.1% editing.

The Sponsor proposes to assess off-target editing by the LNP1.UCD.ABE2 DP in three groups of cell types:

- Lentivirus-transduced HuH-7 cell line bearing the targeted UCD variant (as shown in the example in **Figure 6**), untreated vs. treated with a supersaturating dose of the LNP1.UCD.ABE2 DP (e.g., 20× the EC_{90} value calculated from a dose-response study of the DP in the HuH-7 cell line)
- Primary human hepatocytes (PHHs) from at least three donors (obtained via a commercial vendor), untreated vs. treated with a supersaturating dose of the LNP1.UCD.ABE2 DP, with ABE expression confirmed via comparison of treated PHHs to treated lentivirus-transduced HuH-7 cells by quantitative reverse transcription PCR (RT-PCR) of the ABE mRNA
- Additional cultured or primary cell types nominated by the proposed definitive rat biodistribution/toxicology study (**Figure 25** and **Table 7**), due to substantial on-target editing and/or mRNA delivery, and untreated vs. treated with a supersaturating dose of the LNP1.UCD.ABE2 DP, with ABE expression confirmed with quantitative RT-PCR of the ABE mRNA

The lentivirus-transduced HuH-7 cells have the advantage that they can be used to directly assess for on-target editing efficiency **simultaneously** with off-target editing, confirming that the LNP1.UCD.ABE2 DP has successfully transfected the cells and has exposed them to supersaturating amounts of the mRNA and gRNA components. HuH-7 cells are highly proliferative, reflecting a distinct cellular state from the quiescent PHHs. In all cases, genomic analysis of DP-treated versus untreated cells will be performed three days after DP treatment.

The **secondary verification approach**, termed Lenti-seq, would involve only high-priority candidate off-target sites that are created by human genetic variants and are not present in readily

available hepatocytes. HuH-7 cells will be transduced with a lentivirus bearing a concatenated sequence bearing 100-bp fragments spanning (1) the on-target UCD variant sequence and (2) each of the high-priority candidate variant off-target sites, like the scheme showed in **Figure 6**. Treatment of these off-target lentivirus-transduced HuH-7 cells with a supersaturating dose of the LNP1.UCD.ABE2 DP will be followed three days later by genomic analysis for on-target corrective editing of the UCD variant and for off-target editing in each of the candidate variant off-target sites.

"Assessment of genomic integrity, including chromosomal abnormalities, insertions or deletions, and potential oncogenicity or insertional mutagenesis."

Besides gRNA-dependent off-target editing, base editors have the potential for **gRNA-independent off-target editing** incurred by activity of the TadA deaminase domain independent of the Cas9 component of the ABE. Although ABEs have proven to be relatively inert compared to cytosine base editors, the Sponsor will use two methods to rule out gRNA-independent **DNA editing** and gRNA-independent **RNA editing** by the ABE8e-V106W TadA deaminase domain. **For the former**, WGS will be performed in PHHs treated with supersaturating amounts of the LNP1.UCD.ABE2 DP, to assess for evidence of genome-wide DNA editing above background levels observed in untreated PHHs. **For the latter**, RNA sequencing (RNA-seq) will be used to assess for evidence of **RNA editing** (above background levels) in PHHs treated with supersaturating amounts of the LNP1.UCD.ABE2 DP. The goal of this analysis is **to detect RNA editing** occurring at rates above background, **not to quantify changes in expression in specific genes**. Comparison of gene expression patterns in LNP-treated cells and untreated cells will be heavily confounded by short-term exposure to LNP lipids. **Therefore, the Sponsor holds it is not necessary to complete full differential gene expression and pathway analysis.**

The Sponsor proposes that this analysis be performed **with only one version** of the LNP1.UCD.ABE2 DP [redacted], since gRNA-independent off-target editing is determined by the TadA deaminase domain alone (shared by all versions of the DP) and not by PAM preference or gRNA. Based on the negative findings of a previous study of LNP-delivered NGC-ABE8e-V106W mRNA expression in PHHs (included in the Sponsor's previous single patient expanded access IND, **IND #31438**), the Sponsor does not expect to detect gRNA-independent off-target editing.

Aligned WGS reads from pre- and post-treated PHH genomic DNA will be analyzed with the bioinformatics tool Manta (Chen et al., 2016) to detect rare **structural variants**. Briefly, Manta identifies split reads (i.e., single read that spans a structural variation breakpoint such as in inversions or translocation) to precisely locate the breakpoints of SVs and then performs local *de novo* assembly of the regions surrounding the breakpoints. Based on the negative findings of a previous study of LNP-delivered NGC-ABE8e-V106W mRNA expression in PHHs (included in the Sponsor's previous single patient expanded access IND, **IND #31438**), the Sponsor does not expect to detect treatment-related structural variants.

Because there are no DNA elements in the LNP1.UCD.ABE2 DP, there is no concern for insertional mutagenesis.

For the **overall testing plan** (**Figure 27**) the Sponsor proposes to do the CHANGE-seq-BE and ONE-seq **nomination methods** with a **development batch** of each variant-specific gRNA. **Verification approaches** will be undertaken with the **clinical batch** of each of the variant-specific versions of the LNP1.UCD.ABE2 DP. The initial verification approaches will be

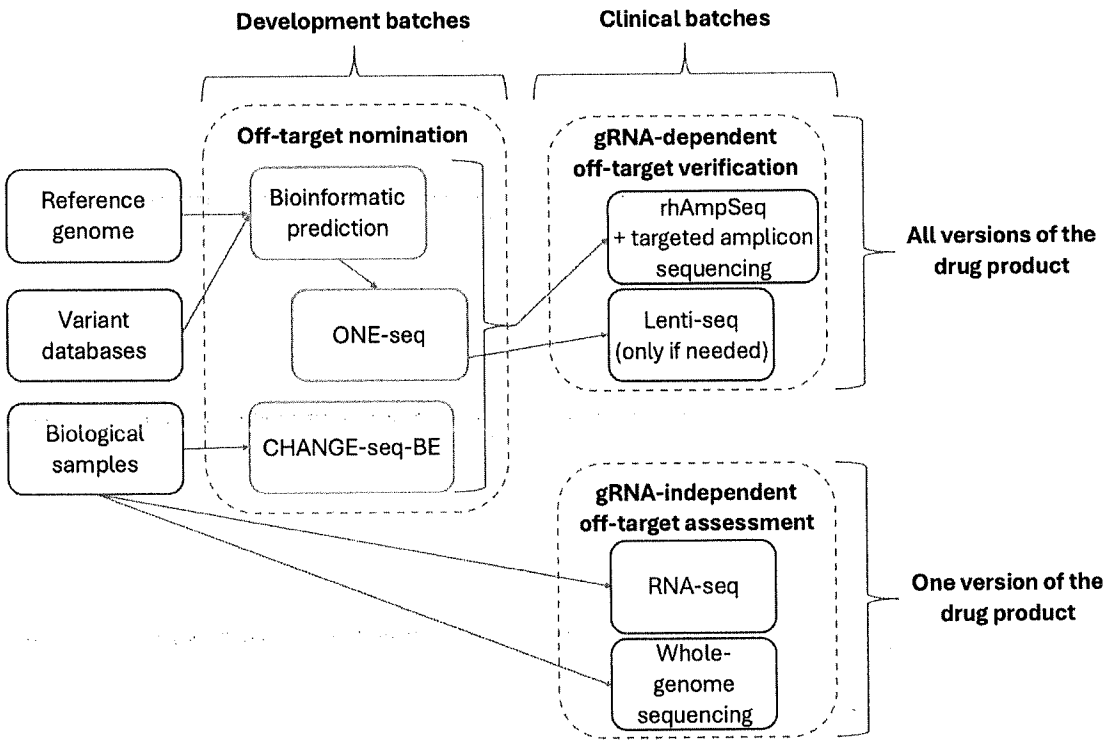


Figure 27. Overview of testing plan for off-target analyses.

performed with a supersaturating dose of the DP (e.g., 20× the EC_{90} value calculated from a dose-response study of the DP). For any verified site of off-target editing at the supersaturating dose, the Sponsor will then perform a dose-response study **to verify if off-target editing occurs at lower, clinically relevant doses of the DP**.

"Evaluation of the biological consequences associated with on- and off-target editing, including, but not limited to, viability and function of the edited cells (e.g., differentiation capacity of progenitor cells)."

Because on-target editing entails the correction of a disease-causing variant to wild-type, it is expected to have only favorable biological effects or neutral effects on the edited cells, even non-target cells.

For any site for which off-target editing by an LNP DP has been **verified** by rhAmpSeq and/or targeted amplicon sequencing, the Sponsor will apply a **risk assessment framework** to assess the biological risk of the edit(s) at the site:

(1) Is the edit in or near a cancer gene, e.g., in the Catalogue of Somatic Mutations in Cancer (COSMIC) database (Tate et al., 2019)?

(2) Does the edit affect a genomic site that is likely to have functional impact: e.g., coding versus non-coding, Ensembl Variant Effect Predictor analysis (McLaren et al., 2016), and Combined Annotation-Dependent Depletion (CADD) score (Rentzsch et al., 2019)?

(3) Is the edit likely to affect gene expression in the target tissue (hepatocytes) or other tissues in which on-target editing is evident?

(4) Do structural variants involving the site of the edit occur?

(5) Is the edit likely to occur at pharmacological doses of an LNP DP administered to patients (rather than a supersaturating dose of an LNP DP used in off-target assays)?

Should any off-target sites be identified in the studies described above with any of the versions of the LNP1.UCD.ABE2 DP, and if a particular off-target edit is deemed to be of high risk by the framework above, the Sponsor will seek guidance from the Agency on the proposed experimental path to address this risk directly.

Additional genotoxicity assessment.

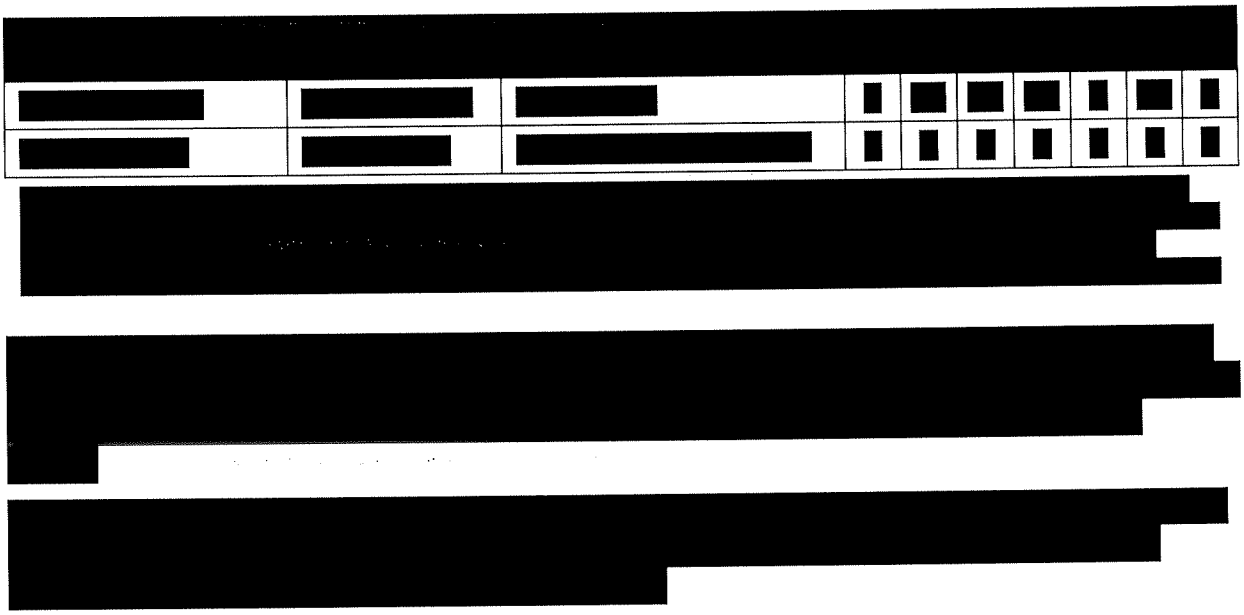
Question #6: Does the Agency agree that the overall nonclinical development plan is sufficient to support an IND application for all versions of the LNP1.UCD.ABE2 DP, as well as a separate IND application for all versions of the follower LNP1.UCD.ABE1 DP?

Sponsor Position: The overall nonclinical development plan is outlined in this section, above, and addresses each relevant recommendation of the **FDA Guidance for Industry on Human Gene Therapy Products Incorporating Human Genome Editing (2024)**.

The Sponsor holds that all the nonclinical studies proposed above for the leader **LNP1.UCD.ABE2** DP are equally relevant to the follower **LNP1.UCD.ABE1** DP, and if the Agency considers the **development plan proposed herein** for the **LNP1.UCD.ABE2** DP (not just the nonclinical studies, but also CMC and the clinical program) to be provisionally acceptable for a leader IND application, then a **parallel, identical development plan** for the **LNP1.UCD.ABE1** DP should be provisionally acceptable for a separate, follower IND application. The sole distinction between the leader **LNP1.UCD.ABE2** DP and the follower **LNP1.UCD.ABE1** DP is the deaminase domain contained in the ABEs encoded by the mRNA DSs—the ABE8e TadA deaminase domain with a V106W variant (ABE8e-V106W), versus the ABE8.8 TadA deaminase domain.

The Sponsor recognizes that the Agency considers the ABE8e-V106W TadA deaminase domain and the ABE8.8 TadA deaminase domain to be sufficiently different to warrant separate IND applications, and the Sponsor is prepared to duplicate the entirety of the studies performed for the leader **LNP1.UCD.ABE2** DP, for the follower **LNP1.UCD.ABE1** DP as well.

The Sponsor requests that the Pre-IND guidance provided by the Agency for this IND application (i.e., for the LNP1.UCD.ABE2 DP) also apply to a follower IND application (i.e., for the LNP1.UCD.ABE1 DP).



Potency assay

Question #8: Does the Agency agree that the proposed potency assay for the LNP1.UCD.ABE2 DP is acceptable to support an IND application for all variant-specific versions of the LNP1.UCD.ABE2 DP?

Sponsor Position: The Sponsor notes the recommendations of the **FDA Guidance for Potency Tests for Cellular and Gene Therapy Products (2011)** and the recommendations of the **FDA Guidance for Industry on Human Gene Therapy Products Incorporating Human Genome Editing (2024)** related to potency assays, contained in **Section III.B.3.i: In vivo-administered Human Genome Editing Drug Products**, specifically the following points:

- *"For early phase studies, potency assays evaluating the ability of the GE components to perform the desired genetic sequence modification may be adequate."*
- *"We recommend that, whenever possible, the potency assays be performed in the target cells or tissues (or a representative surrogate)."*
- *"We also recommend inclusion of such a potency assay in the DP stability studies."*

The Sponsor proposes an early-phase-appropriate potency assay for the LNP1.UCD.ABE2 DP, using a lentivirus-transduced HuH-7 cell line harboring a patient's target disease-causing variant(s) along with **at least three additional variants** to serve as **positive reference controls**:

- *CPS1* Q335X variant, for which there is a **clinically validated** base editing solution, comprising the NGC-ABE8e-V106W mRNA and CPS1-001 gRNA (refer to **Section 6, History of the Project/Background, Status of Product Development, Clinical validation**) (Musunuru et al., 2025)
- *PAH* P281L variant, for which there is a well-validated base editing solution, comprising the ABE8.8 mRNA and "PAH1" gRNA, that definitively treats PKU in a humanized mouse model (see **Figure 21**) (Brooks et al., 2023)

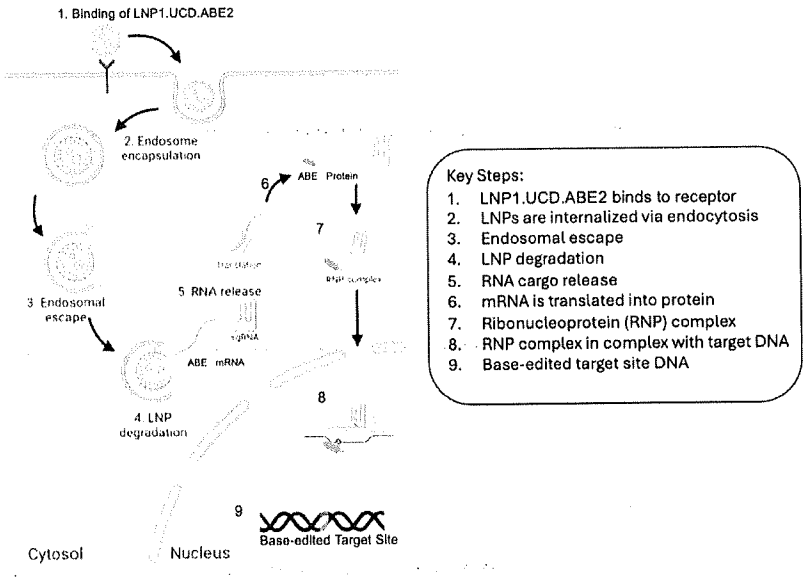


Figure 31. Mechanism of action of the LNP1.UCD.ABE2 LNP. Figure adapted from Lee & Han, 2024.

- *PAH* R408W variant, for which there is a well-validated base editing solution, comprising the SpRY-ABE8.8 mRNA and "PAH4" gRNA, that definitively treats (PKU) in a humanized mouse model (Brooks et al., 2024)

Refer to **Figure 6** for a schematic of an example of this kind of lentivirus-transduced HuH-7 cell line. The version of the cell line tailored to a patient's variant(s) will be used to assess for the desired genetic sequence modification—namely, the on-target corrective base editing activity (specific single-nucleotide changes) appropriate to the version of the LNP1.UCD.ABE2 DP manufactured for the patient, as determined by amplicon-based sequencing, i.e., NGS.

The mechanism of action of LNP1.UCD.ABE2 is illustrated in **Figure 31**. The Sponsor proposes to assess potency by evaluating the final stage of the mechanism of action, i.e., the efficiency of corrective base editing at the site of the variant targeted by the DP. In the potency assay, lentivirus-transduced HuH-7 cells will be transfected with LNP1.UCD.ABE2 DP samples and, ultimately, the level of corrective base editing measured by amplicon-based sequencing with an eight-point dose range to establish an EC_{50} (see **Figures 20–21** for examples of this kind of assay). In parallel, the HuH-7 cells will also be transfected with a reference standard, i.e., a previously characterized lot of the LNP1.UCD.ABE2 DP (e.g., targeting the *CPS1* Q335X variant) or a different LNP DP (e.g., targeting the PKU *PAH* P281L or R408W variant). The editing efficiency of the investigational DP will be reported in relation to the reference standard.

The HuH-7 cell-based assay is now being optimized, and for the proposed Phase I/II clinical trial under this IND, the Sponsor will use this assay with a minimum threshold of editing efficiency—vis-à-vis a reference standard, to be defined in the IND application—as the acceptance criterion for DP release and stability testing [redacted]. The Sponsor will continue to develop and qualify the assay as a quantitative relative potency assay.

The Sponsor holds that although the CMC plan and the potency assay described above are being proposed for the leader IND application for the LNP1.UCD.ABE2 DP, they are equally relevant to the LNP1.UCD.ABE1 DP and would be appropriate for a separate, follower IND application.

12. CLINICAL PROGRAM OVERVIEW

Question #9: Does the Agency agree that the general design, including the proposed safety and exploratory efficacy outcome measures, enrollment criteria, and long-term follow-up plan are appropriate for the Phase I/II umbrella trial protocol outlined in the protocol synopsis?

Question #10: Does the Agency agree that a Phase III extension of the Phase I/II umbrella trial protocols in the LNP1.UCD.ABE2 DP IND application and the follower LNP1.UCD.ABE1 DP IND application, combining the efficacy studies of the two DPs into a single clinical trial conducted under a master protocol IND, would be appropriate?

Sponsor Position: The Sponsor notes the recommendations of the **FDA Guidance for Industry on Human Gene Therapy Products Incorporating Human Genome Editing (2024)**, particularly **Section V: Considerations for Clinical Studies**: "*Clinical trial design should include an appropriately-defined patient population, an efficient and safe approach to product administration (including data-based dosing, dose schedule, and treatment plan), adequate safety monitoring, and appropriate safety and efficacy endpoints.*"

In accordance with the Guidance, the Sponsor proposes a Phase I/II open-label umbrella clinical trial designed to evaluate the safety and efficacy of **LNP1.UCD.ABE2** (**Table 22**). The full clinical protocol and informed consent form will be included in the IND submission for the **LNP1.UCD.ABE2** DP.

The Sponsor also proposes an essentially identical Phase I/II open-label umbrella clinical trial designed to evaluate the safety and efficacy of **LNP1.UCD.ABE1**, under a separate, follower IND submission (substitute "LNP.UCD.ABE1" for every mention of "LNP.UCD.ABE2" in **Table 22** and in the subsequent study-related text sections).

Table 22. Clinical synopsis.	
Study title:	A Phase I/II open-label safety and efficacy study of LNP1.UCD.ABE2, a lipid nanoparticle-delivered base editing therapy, in patients with urea cycle disorders due to variants amenable to corrective editing by LNP1.UCD.ABE2
Clinical phase:	Phase I/II
Number of subjects:	[redacted]
Study rationale:	To date, there are no one-time, disease-modifying medical therapies that durably correct neurotoxic ammonia elevations in patients with severe urea cycle disorders (UCDs). The goal of this study is to restore expression and activity of a deficient urea cycle enzyme or related transporter and reduce ammonia levels through corrective adenine base editing of a causative variant in a UCD gene.
Study objectives:	The primary outcome is safety. The secondary and exploratory outcomes explore efficacy.

Study design:	Open-label clinical trial of LNP1.UCD.ABE2 injected via intravenous (IV) infusion
Study dose:	[redacted]
Inclusion criteria:	[redacted]
Exclusion criteria:	[redacted]
Primary outcome:	[redacted]

Secondary outcome:	[redacted]
Exploratory outcomes:	[redacted]
Immunosuppression medications:	[redacted]
Study duration:	[redacted]
Long-term follow-up:	[redacted]

Prescreening period to assess amenability of a variant to corrective editing by the LNP1.UCD.ABE2 DP

The LNP1.UCD.ABE2 DP is designed to treat patients with severe infantile-onset UCDs who are homozygous or compound heterozygous for a pathogenic variant targeted by a version of the DP with a gRNA specific for the subject's variant. While it is possible that a subject will present

with one of the variants described in **Table 1**, it is likely that most subjects will have unique disease-causing variants not included in **Table 1**.

If a subject's variant has not already been evaluated for amenability to corrective editing by the LNP1.UCD.ABE2 DP, a referring provider can request a prescreening evaluation in which the provider will give the investigators the following de-identified information about the potential subject:

- Disease-causing variant(s)

[redacted]

The investigators will then complete *in vitro* analysis to assess the amenability of the variant(s) to corrective editing by the LNP1.UCD.ABE2 DP as exemplified in **Section 6, History of the Project/Background, Status of Product Development**, especially **Figures 6–16**.

If a variant is found to be amenable, the investigators will provide this information to the subject's referring provider, and the subject will be eligible for screening.

If a subject's variant has previously been adjudicated to be amenable to corrective editing by the LNP1.UCD.ABE2 DP, a prescreening period is not required.

Subject screening and assignment to genotype arm

At screening, all subjects will have confirmatory review of their clinical UCD gene sequencing results by a clinical geneticist. If there is any uncertainty regarding the molecular diagnosis, subjects may undergo repeat confirmatory molecular testing.

[redacted]

Once a subject's genotype is confirmed, they will be assigned to the appropriate genotype arm for the target UCD variant that they harbor. Given that most UCD patients have private or ultra-rare variants, it is likely that each subject will be in a unique genotype arm. [redacted]

If a subject is compound heterozygous for two alleles that are amenable to corrective editing by the LNP1.UCD.ABE2 DP, the subject will be assigned to the arm targeting the variant that, if corrected, provides the highest potential benefit to the subject, in the opinion of the investigators. Greater potential benefit could be due to one variant being more severe or one variant having a gRNA that yields higher editing efficiency *in vitro*.

To ensure there is an appropriate benefit-to-risk ratio for each subject, during screening the data safety and monitoring board (DSMB, see details below) will review the data needed to support the determination of inclusion/exclusion criteria and the genotype arm assignment for each subject that passes the investigator screening. In addition, the DSMB will review the *in vitro* data to support the amenability of the variant to corrective editing by the LNP1.UCD.ABE2 DP, as well as the variant-specific off-target assessments.

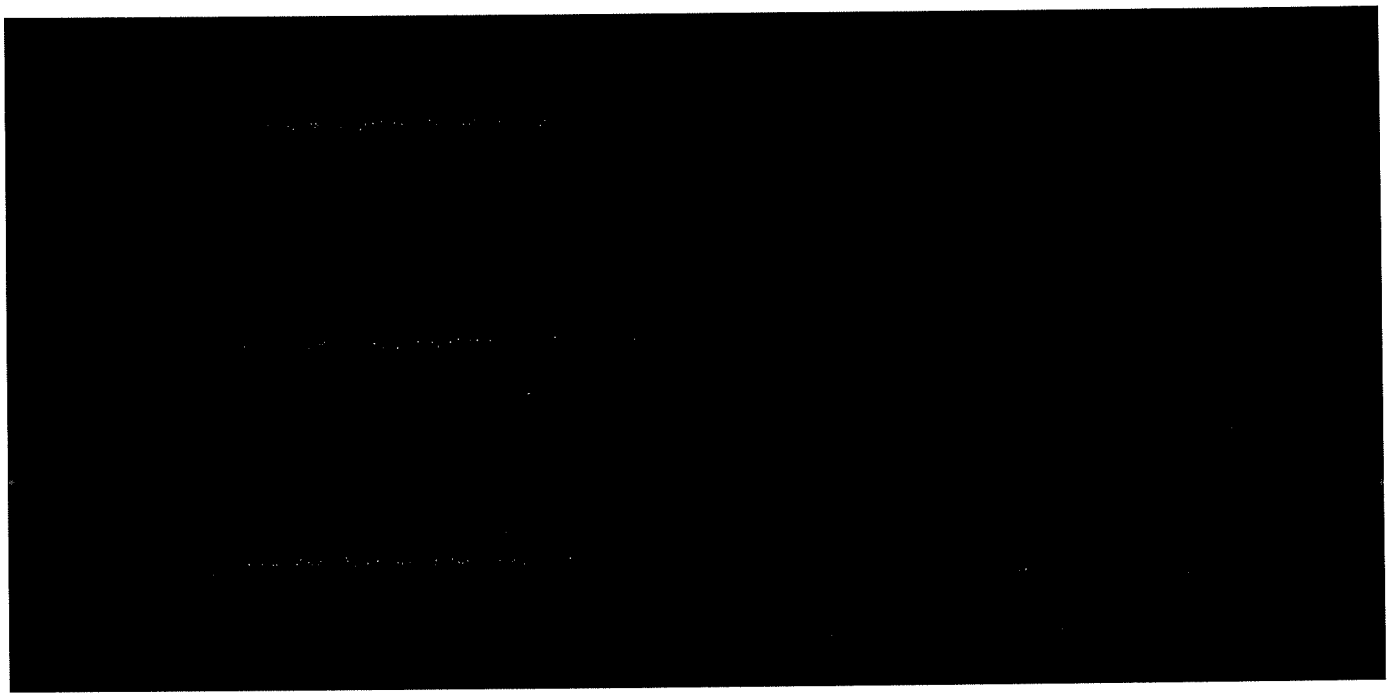
Once the DSMB has confirmed that a subject has successfully passed screening, the subject will be eligible for dosing if the variant is already included in the IND. If the variant is not included in the IND, an IND amendment will be submitted to the Agency that includes:

- The certificate of release for the subject-specific version of the LNP1.UCD.ABE2 DP
- *In vitro* data supporting the amenability of the variant to corrective editing by the LNP1.UCD.ABE2 DP
- *In silico* and *in vitro* off-target editing data for the subject-specific version of the LNP1.UCD.ABE2 DP

The subject will be eligible for dosing after the Agency has approved the IND amendment.

Data safety monitoring board and subject enrollment timeline

A DSMB will be established, comprising at least 4 people who are experts in UCDs, gene editing therapies, and safety/pharmacovigilance. The DSMB will review screening data, safety data, and exploratory efficacy data from all study participants at predetermined intervals and as any concerns arise (**Figure 32**). Communications or meetings will occur at a minimum of quarterly intervals each year, including the occasions of the following milestones for each subject:



To prioritize the safety of subjects, the dosing plan will begin with a low dose that is still predicted to provide benefit. This dose will be finalized after completion of the proposed definitive rat biodistribution/toxicology study (**Figure 25** and **Table 7**).

Rationale for immunosuppression plan

[redacted]

Plan for liberalization of dietary protein and nitrogen scavenger medications

[redacted]

Proposed Phase III extension of the Phase I/II umbrella trial protocols

The Sponsor proposes to enroll [redacted] in the Phase I/II clinical trial of the **LNP1.UCD.ABE2** DP under the leader IND. In parallel, upon clearance of a separate, follower IND, the Sponsor proposes to enroll [redacted] in an analogous Phase I/II clinical trial of the **LNP1.UCD.ABE1** DP. If the safety and efficacy findings of both Phase I/II clinical trials are favorable, the Sponsor envisions submitting a master protocol IND, under which there would be a Phase III extension of both Phase I/II umbrella trial protocols, combined into a single clinical trial. Subjects would be recruited into either the **LNP1.UCD.ABE2** or **LNP1.UCD.ABE1** arm depending on the amenability of their pathogenic UCD variants to corrective editing by either DP, with the goal of accruing an additional [redacted]. If the efficacy findings in both arms are favorable, the Sponsor would consider submitting Biologics License Applications for approval of both DPs.

13. A LIST OF FDA STAFF ASKED TO PARTICIPATE IN THE REQUESTED MEETING

The participation of members of the Center for Biologics Evaluation and Research is requested.

14. TELECONFERENCE DATE/TIME

September 19, 2025, at 3:00 pm ET.

15. FORMAT OF THE MEETING

A face-to-face webinar has been requested and scheduled.

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