BRIEF REPORT

Patient-Specific In Vivo Gene Editing to Treat a Rare Genetic Disease

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SUMMARY

Base editors can correct disease-causing genetic variants. After a neonate had received a diagnosis of severe carbamoyl-phosphate synthetase 1 deficiency, a disease with an estimated 50% mortality in early infancy, we immediately began to develop a customized lipid nanoparticle–delivered base-editing therapy. After regulatory approval had been obtained for the therapy, the patient received two infusions at approximately 7 and 8 months of age. In the 7 weeks after the initial infusion, the patient was able to receive an increased amount of dietary protein and a reduced dose of a nitrogen-scavenger medication to half the starting dose, without unacceptable adverse events and despite viral illnesses. No serious adverse events occurred. Longer follow-up is warranted to assess safety and efficacy. (Funded by the National Institutes of Health and others.)

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Programmable gene-editing technology based on clustered regularly interspaced short palindromic repeats (CRISPR)–CRISPR-associated protein 9 (Cas9)[1] has matured into therapeutic approaches that are improving the lives of patients with various diseases, such as sickle cell disease, β-thalassemia, and hereditary angioedema.[2-4] Precise, corrective CRISPR-Cas9 technology — namely, base editing (which can effect cytosine-to-thymine changes [cytosine base editing[5]] or adenine-to-guanine changes [adenine base editing[6]]) and prime editing[7] (which can produce any single-nucleotide change or small insertion or deletion) — can potentially address more than 90% of pathogenic variants in genetic diseases that, although rare individually, collectively affect hundreds of millions of people worldwide.[8] However, drug-development efforts have largely focused on recurrent variants in a few relatively common genetic diseases on account of the extensive resources needed to develop and bring to market any given therapy.[9]

We developed a workflow for the rapid development of customized, corrective gene-editing therapies for patients with ultrarare or unique "N-of-1" variants (Fig. 1). More specifically, we developed a base-editing therapy, delivered in vivo to hepatocytes through lipid nanoparticles, for a single patient who at birth received

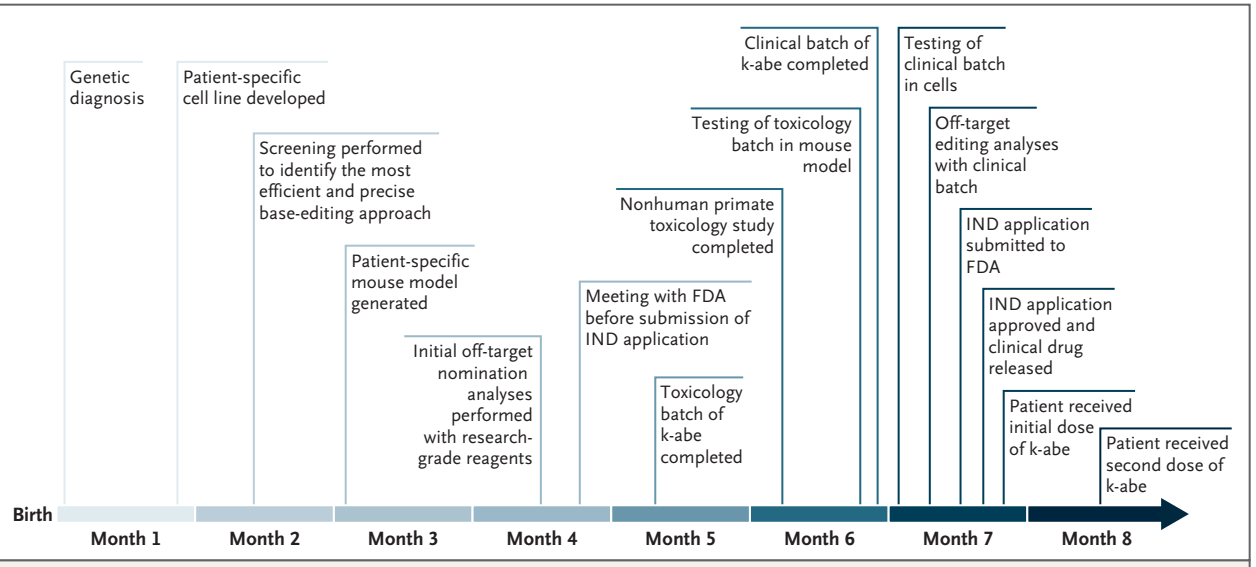

Figure 1. Timeline from Birth to Second Treatment with Kayjayguran Abengcemeran (K-abe).
FDA denotes Food and Drug Administration, and IND Investigational New Drug.

a diagnosis of neonatal-onset carbamoyl-phosphate synthetase 1 (CPS1) deficiency, an ultrarare inborn error of metabolism affecting the urea cycle. CPS1 deficiency affects 1 in 1,300,000 persons[10] and has an estimated mortality of 50% in early infancy.[11] Liver transplantation provides a functional urea cycle and improves outcomes.[12,13] However, hyperammonemic crises and irreversible neurologic injury often occur in infants before they grow large enough to undergo transplantation.[14-16] We administered the customized therapy to our patient twice, at approximately 7 and 8 months of age, with the goal of providing protection against hyperammonemia.

METHODS

STUDIES FOR INVESTIGATIONAL NEW DRUG APPLICATION

Full descriptions of cellular studies, studies in animals, and off-target assessments are provided in the Supplementary Appendix 1, available with the full text of this article at NEJM.org. The institutional animal care and use committee at the University of Pennsylvania and at AmplifyBio approved the studies in mice and nonhuman primates, respectively. Genome-sequencing data from the patient and blood-derived genomic DNA samples from the patient's father were obtained under a human subjects research protocol that was approved by the institutional review board at the University of California, Berkeley.

CLINICAL STUDY

Because the therapy (kayjayguran abengcemeran, or k-abe) was administered as part of clinical care under a single-patient expanded-access Investigational New Drug application, the clinical protocol was reviewed by the institutional review board at Children's Hospital of Philadelphia (CHOP) through alternative procedures. After the initial regulatory review by the Food and Drug Administration (FDA), we received authorization from the FDA to obtain concurrence by the CHOP chairperson of the institutional review board, who then reviewed and approved the study. The patient's parents provided written informed consent. Clinical activities were overseen by a multidisciplinary oversight committee comprising physicians from the CHOP metabolism, hepatology, immunology, gene therapy, and medical ethics services. The investigators vouch for the accuracy and completeness of the data and for the fidelity of the study to the protocol, available at NEJM.org.

RESULTS

CLINICAL PRESENTATION

Symptoms of CPS1 deficiency, including lethargy and respiratory distress, developed in the patient

(a male neonate) within the first 48 hours of life. Measurement of blood ammonia revealed a level greater than 1000 μmol per liter (1703 μg per deciliter; reference range, 9 to 33 μmol per liter [15 to 56 μg per deciliter]). Continuous renal-replacement therapy was initiated promptly. Plasma amino acid profiling revealed a critically elevated level of glutamine, undetectable citrulline, and a normal level of urinary orotic acid, findings suggestive of a proximal urea-cycle defect. Rapid targeted analysis of the patient's genome identified two truncating *CPS1* variants: c.1003C→T (p.Gln335Ter, referred to as Q335X, on the paternal allele) and c.2140G→T (p.Glu714Ter, referred to as E714X, on the maternal allele). The Q335X variant is absent in the Genome Aggregation Database but has been reported in a case of neonatal-onset CPS1 deficiency.[17]

The patient was weaned from continuous renal-replacement therapy and transitioned to long-term therapy that included nitrogen-scavenger medication (glycerol phenylbutyrate), citrulline supplementation (at a dose of 200 mg per kilogram of body weight per day, which remained unchanged throughout his clinical course), and a protein-restricted diet (given as a 1:1 mix of natural protein and essential amino acid formula). The patient had the expected infantile "honeymoon" period[14] from days 50 to 100, after which his biochemical status worsened, leading to a further reduction in protein intake and an increase in the dose of glycerol phenylbutyrate to manage the elevated ammonia and glutamine levels. Each hyperammonemic episode incurred a risk of permanent neurologic damage and death. Given the severity of his disease, the patient was listed for liver transplantation at 5 months of age.

PATIENT-SPECIFIC CUSTOMIZATION OF BASE-EDITING THERAPY

Reliable assessment of base editing of the *CPS1* Q335X variant would ideally use human hepatocytes with the variant; however, human hepatocytes were not available. Therefore, we used the cultured human HuH-7 cell line as a proxy. We synthesized a cassette harboring a 100-bp human genomic segment spanning the *CPS1* Q335X variant, as well as 100-bp segments spanning the patient's other *CPS1* variant and two reference variants in *PAH* to serve as positive controls (Fig. S2A in the Supplementary Appendix 1 [all supplementary figures and tables are available in Supplementary Appendix 1]). We transduced HuH-7 cells with a lentiviral vector containing the cassette, thereby inserting the cassette into the genome. This process was completed 1 month after the patient's birth.

To develop a patient-specific, bespoke gene editor, we screened various adenine base editors (ABEs) with guide RNAs (gRNAs) tiling the site of the Q335X variant in the lentivirus-transduced HuH-7 cells (Fig. S2B, S2C, and S2D and Fig. S3). We identified an ABE with a preference for NGC protospacer-adjacent motifs, termed NGC-ABE8e-V106W, and a gRNA with the target Q335X adenine in the eighth position of its protospacer sequence as the most efficient and precise base-editing approach; although there was bystander editing of neighboring adenines, all such edits were synonymous (Figs. S4 through S7). This process was completed 2 months after the patient's birth. We named the gRNA used in the lipid nanoparticle therapy (Table S1) "kayjayguran," the messenger RNA (mRNA) encoding the ABE (Fig. S1 and Table S2) "abengcemeran," and the therapy "k-abe" (for short).

PRECLINICAL STUDIES

After the initial regulatory review by the FDA, we manufactured a toxicology batch of k-abe (i.e., the batch used for toxicologic testing) and undertook a limited safety study in cynomolgus monkeys to characterize single-dose toxicity of the lipid nanoparticle therapy. A total RNA dose of 1.5 mg per kilogram of body weight was administered intravenously. No clinical signs of toxic effects were present, and there were transient elevations in alanine aminotransferase and aspartate aminotransferase levels to several times the upper limit of the normal range — findings that are consistent with the results of previous studies (Fig. S12).[18,19] Two weeks after treatment, plasma levels of lipid excipients had fallen more than 99.5% from the peak levels, a development that supported readministration of the therapy at intervals greater than 2 weeks (Fig. S13). The results of the study in cynomolgus monkeys (completed 5 months after the patient's birth) indicated that a dose of 0.1 mg per kilogram was a potentially safe initial clinical dose for the patient.

On learning of the patient's genetic diagnosis, we immediately started generating mouse models to assess the in vivo editing efficiency of k-abe. To maximize the chance of success, we used estab-

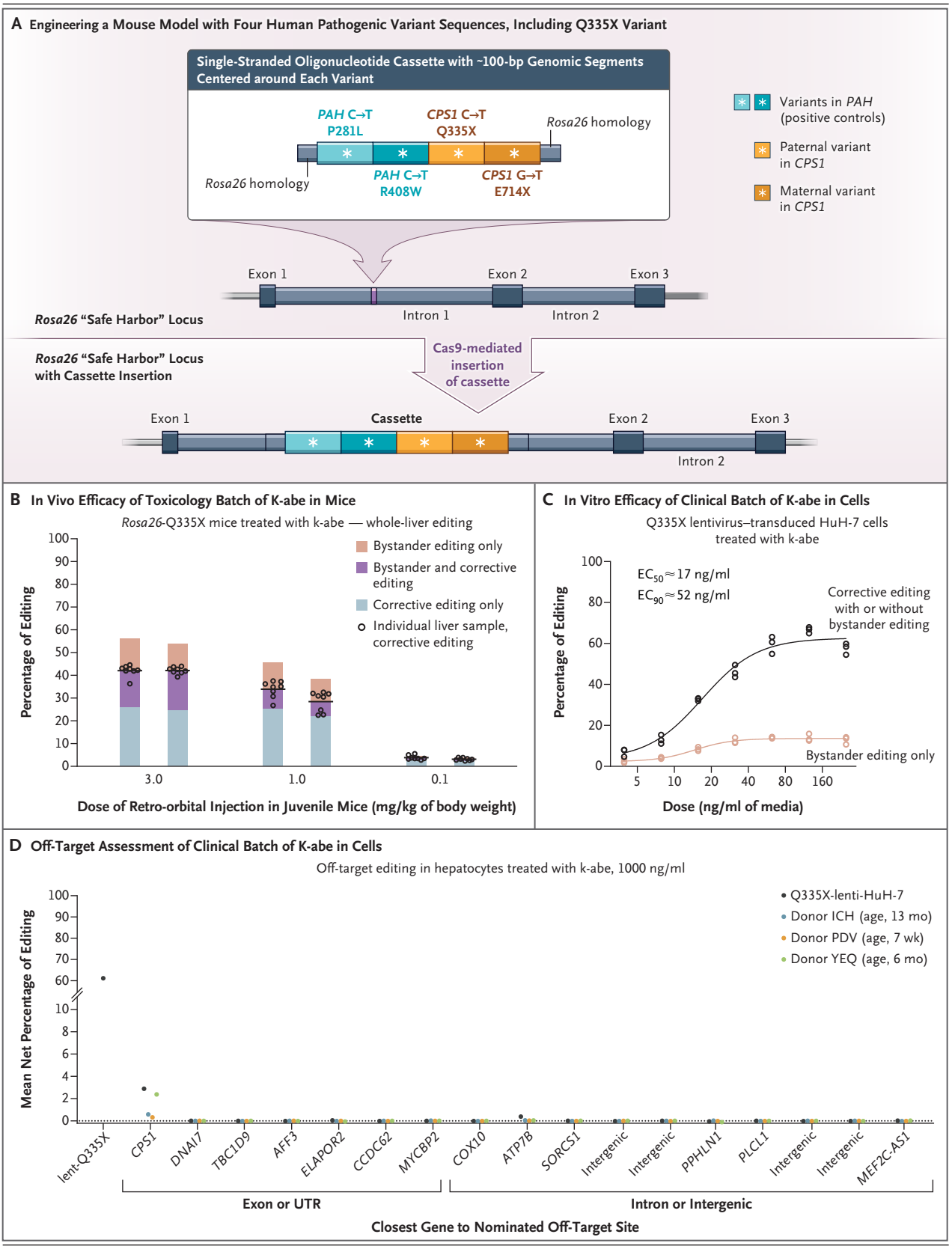
A Engineering a Mouse Model with Four Human Pathogenic Variant Sequences, Including Q335X Variant
Single-Stranded Oligonucleotide Cassette with ~100-bp Genomic Segments Centered around Each Variant
PAH C→T P281L
CPS1 C→T Q335X
Rosa26 homology
Rosa26 homology
PAH C→T R408W
CPS1 G→T E714X
Variants in PAH (positive controls)
Paternal variant in CPS1
Maternal variant in CPS1
Exon 1
Exon 2
Exon 3
Intron 1
Intron 2
Rosa26 "Safe Harbor" Locus
Rosa26 "Safe Harbor" Locus with Cassette Insertion
Cas9-mediated insertion of cassette
Exon 1
Cassette
Exon 2
Exon 3
Intron 2
B In Vivo Efficacy of Toxicology Batch of K-abe in Mice
Rosa26-Q335X mice treated with k-abe — whole-liver editing
Bystander editing only
Bystander and corrective editing
Corrective editing only
Individual liver sample, corrective editing
Percentage of Editing
100
90
80
70
60
50
40
30
20
10
0
3.0
1.0
0.1
Dose of Retro-orbital Injection in Juvenile Mice (mg/kg of body weight)
C In Vitro Efficacy of Clinical Batch of K-abe in Cells
Q335X lentivirus–transduced HuH-7 cells treated with k-abe
EC50≈17 ng/ml
EC90≈52 ng/ml
Corrective editing with or without bystander editing
Bystander editing only
Percentage of Editing
100
80
60
40
20
0
5
10
20
40
80
160
Dose (ng/ml of media)
D Off-Target Assessment of Clinical Batch of K-abe in Cells
Off-target editing in hepatocytes treated with k-abe, 1000 ng/ml
Q335X-lenti-HuH-7
Donor ICH (age, 13 mo)
Donor PDV (age, 7 wk)
Donor YEQ (age, 6 mo)
Mean Net Percentage of Editing
100
90
80
70
60
10
8
6
4
2
0
lent-Q335X
CPS1
DNAI7
TBC1D9
AFF3
ELAPOR2
CCDC62
MYCBP2
COX10
ATP7B
SORCS1
Intergenic
Intergenic
PPHLN1
PLCL1
Intergenic
Intergenic
MEF2C-AS1
Exon or UTR
Intron or Intergenic
Closest Gene to Nominated Off-Target Site

Figure 2 (facing page). Preclinical Studies.
Panel A shows the contents of the single-stranded DNA oligonucleotide cassette inserted into the endogenous mouse *Rosa26* locus in mouse zygotes. Panel B shows the extent of whole-liver corrective adenine base editing of the *CPS1* Q335X variant in *Rosa26*-Q335X mice. Several days after administration of a single dose of k-abe from the toxicology batch, we obtained multiple samples distributed throughout the liver of each juvenile mouse on necropsy. We assessed the extent of editing in eight samples per mouse by sequencing the *Rosa26*-Q335X cassette. The two bars at each dose level (3.0, 1.0, and 0.1 mg per kilogram of body weight) represent two mice. Across the three dose groups, no more than 1% insertional or deletional mutagenesis occurred at the target site. Panel C shows corrective adenine base editing of the *CPS1* Q335X variant in lentivirus-transduced HuH-7 cells treated with k-abe. Editing was determined 3 days after treatment at the stated dose (concentration after dilution with cell medium). The best-fit agonist response curve with variable slope (four-parameter logistic regression) and 50% effective concentration (EC_{50}) and 90% effective concentration (EC_{90}) values were calculated with GraphPad Prism. Panel D shows the evaluation of a high-priority subset of nominated off-target sites for any adenine-to-guanine editing through individual-site targeted amplicon sequencing in the Q335X lentivirus–transduced HuH-7 cells (Q335X-lenti-HuH-7) and in primary human hepatocytes from three male donors (donor ICH [13 months of age], donor PDV [7 weeks of age], and donor YEQ [6 months of age]) after treatment with k-abe at 1000 ng per milliliter of media, as compared with untreated cells. Of 21 high-priority nominated off-target sites, 16 were successfully sequenced and shown here. Cas9 denotes clustered regularly interspaced short palindromic repeats–associated protein 9, and UTR untranslated region.

lished CRISPR reagents in mouse zygotes to insert a cassette harboring a 100-bp human genomic segment spanning the *CPS1* Q335X variant into the *Rosa26* "safe harbor" locus (the same cassette used for the lentivirus-transduced HuH-7 cells) (Fig. 2A). At 5 months after the patient's birth, we performed a limited dose–response study in which the toxicology batch of k-abe was used in a small number of *Rosa26*-Q335X mice. In this study, we observed up to 42% whole-liver corrective editing, along with the expected synonymous bystander editing (Fig. 2B and Figs. S10 and S11A). Editing was evident at the lowest dose (0.1 mg per kilogram), which further supported that dose as the initial clinical dose for the patient. Subsequent validation of in vivo corrective editing in a second mouse model in which the Q335X variant was introduced into the endogenous mouse *Cps1* locus is described in Figures S9 and S11B.

With the clinical batch of k-abe that was produced 5 months after the patient's birth, we performed a dose–response potency assessment in lentivirus-transduced HuH-7 cells (Fig. 2C and Fig. S8). To assess off-target editing, we performed ONE-seq[18] and CHANGE-seq-BE assays[20] using recombinant NGC-ABE8e-V106W protein and kayjayguran, as well as a modified GUIDE-seq[21] assay using a nuclease version of the editor (Figs. S14 through S18, and see Supplementary Appendix 2), during months 4 and 5. The ONE-seq assay was performed with a synthetic library that had been designed with the patient's genome as the reference genome. The CHANGE-seq-BE assay was performed with genomic DNA obtained from the patient's father, who carried the Q335X variant (we were unable to obtain enough genomic DNA from the patient). We prioritized the on-target *CPS1* site and 21 nominated off-target sites for verification with individual-site targeted amplicon sequencing (Fig. 2D and Tables S3 through S6). We exposed lentivirus-transduced HuH-7 cells and primary human hepatocytes from three donors to a supersaturating dose of k-abe. Low-level synonymous bystander editing was evident at the endogenous wild-type *CPS1* genomic site in all four cell lots, a finding that is consistent with the gRNA (kayjayguran) having a 1-base mismatch to the wild-type sequence (the HuH-7 cells retained endogenous wild-type *CPS1* alleles in addition to the transduced *CPS1* Q335X variant sequence). We observed a low level of off-target editing at an intronic site in *ATP7B* in the HuH-7 cells but not in the three primary human hepatocyte lots. *ATP7B* encodes a copper transporter and was not considered to represent biologic risk because its loss of function has not been linked to carcinogenesis.[22] Subsequent analysis of a larger set of nominated off-target sites detected no off-target editing in the treated primary human hepatocytes (Fig. S19).

TREATMENT OF THE PATIENT

A single-patient, expanded-access Investigational New Drug application was submitted to the FDA when the patient was 6 months of age, which was approved 1 week later. The patient was presumed to have no cross-reactive immunologic material, and out of concern for the potential development

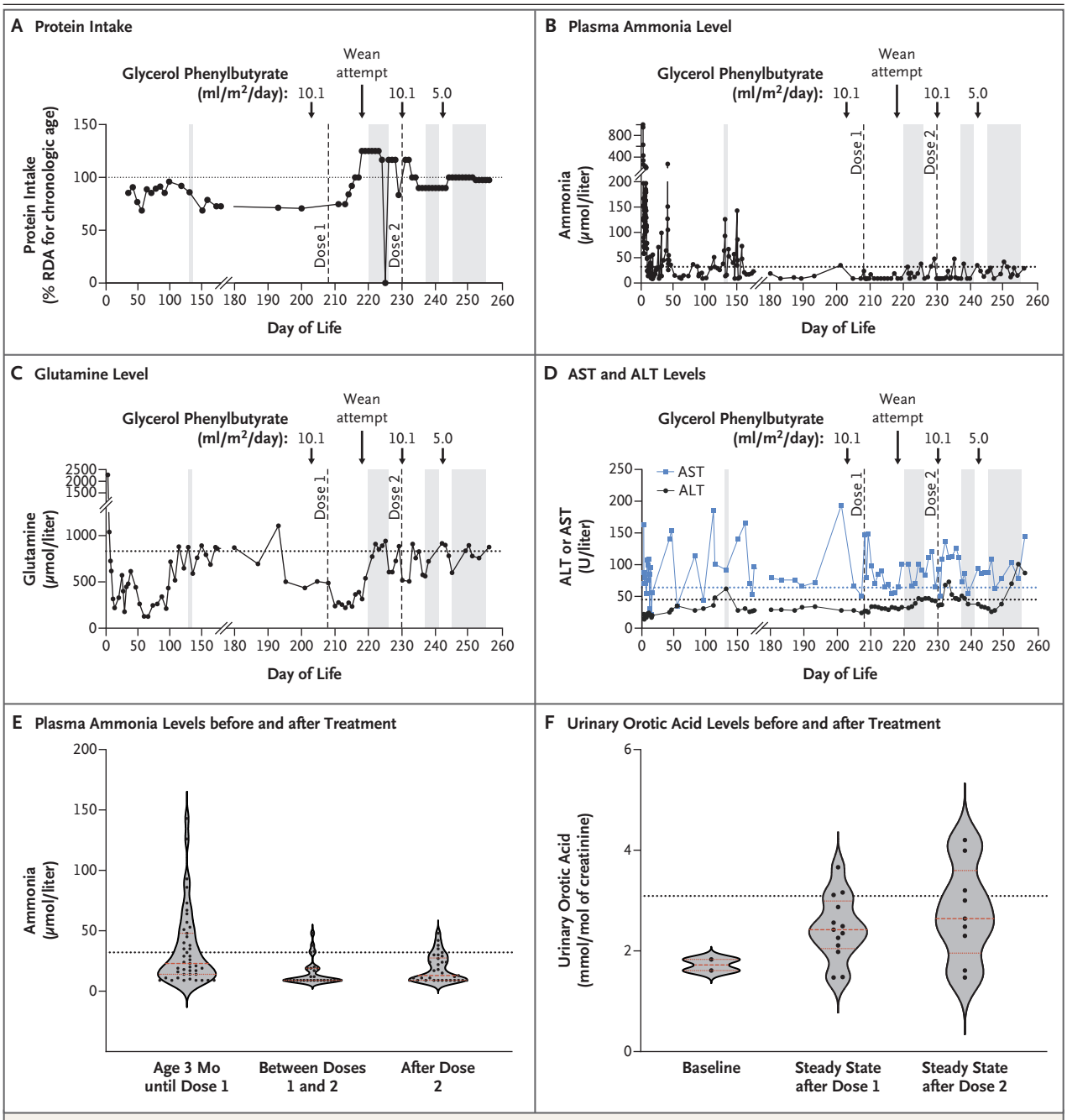

Figure 3. Biochemical Profile before and after Treatment with K-abe.

Shown are the timelines of protein intake (Panel A) and levels of plasma ammonia (Panel B), glutamine (Panel C), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) (Panel D). The gray bars from left to right indicate periods of rotavirus-positive gastroenteritis before treatment, rhinovirus-positive upper respiratory tract infection after dose 1, and two viral illnesses after dose 2 (gastroenteritis followed by a new rhinovirus or enterovirus infection with associated viral transaminitis). In Panels B through F, the dotted horizontal lines indicate upper limits of the normal range for the laboratory value. Panels E and F show violin plots of plasma ammonia levels and urinary orotic acid levels, respectively, before and after treatment. Inside the plots, the red dashed line indicates the median, and the red dotted lines indicate the upper and lower quartiles. The clusters of dots indicate the individual data points. To convert the values for ammonia to micrograms per deciliter, divide by 0.5872. To convert the values for glutamine to micrograms per deciliter, divide by 68.42. RDA denotes recommended dietary allowance.

of an immune response to full-length CPS1 protein, prophylactic immunosuppression with sirolimus and tacrolimus was initiated on days 205 and 209, respectively, after the patient's birth. We selected this steroid-sparing regimen because corticosteroids can trigger hyperammonemia in patients with CPS1 deficiency. On day 208 after birth, the patient received an intravenous infusion of k-abe at a total RNA dose of 0.1 mg per kilogram. After treatment, it was possible to increase his dietary protein intake. Because the patient was born at 35 weeks' gestation, his prescribed protein goal was sometimes above the recommended daily allowance for chronologic age (Fig. 3A). The patient recovered from a viral respiratory infection without the occurrence of an illness-associated hyperammonemic crisis; however, he received intravenous fluids, which is standard during illness, and was on a protein-free diet for 1 day (day 225 after birth). We were unable to wean him from glycerol phenylbutyrate; we had reduced the dose from 10.1 to 8.1 ml per square meter of body-surface area per day but then restored the original dose because of rising glutamine levels.

Given the incomplete biochemical correction in the patient — and according to the clinical protocol — he received a second dose of k-abe (0.3 mg per kilogram) 22 days after receipt of the first k-abe infusion. The patient had a coughing episode during the second infusion that resolved with nasal suctioning. Transient elevations in alanine aminotransferase and aspartate aminotransferase levels occurred a few days after the second k-abe infusion and recurred a few weeks later during the course of viral illness (Fig. 3D). At 2 weeks after the second infusion, the patient was able to receive a reduced dose of glycerol phenylbutyrate to half the starting dose (from 10.1 to 5.0 ml per square meter per day) without unacceptable adverse effects.

During the 4 weeks after the second infusion of k-abe, two viral infections, each with accompanying vomiting and diarrhea, developed in the patient. In contrast to a gastroenteritis infection that had occurred in the patient before the administration of k-abe, he recovered from the viral infections without a hyperammonemic crisis and was able to continue his full-protein diet during the course of his illnesses. The median blood ammonia levels before the first k-abe dose (23 μmol per liter [39 μg per deciliter]; interquartile range, 14 to 48 μmol per liter [24 to 82 μg per deciliter]), between the first and second doses (9 μmol per liter [15 μg per deciliter]; interquartile range, 9 to 19 μmol per liter [15 to 32 μg per deciliter]), and after the second dose (13 μmol per liter [22 μg per deciliter]; interquartile range, 9 to 28 μmol per liter [15 to 48 μg per deciliter]) support the occurrence of a treatment-related reduction (Fig. 3E). CPS1 contributes to orotic acid synthesis, and patients with CPS1 deficiency often have urinary orotic acid levels at the lower end of the normal range (median level in our patient before the first k-abe dose, 1.7 mmol per mole of creatinine; interquartile range, 1.6 to 1.8); after receipt of the two doses of k-abe, the levels in our patient were often at the high end of the normal range (2.4 mmol per mole of creatinine; interquartile range, 2.0 to 3.0) or above the normal range (2.6 mmol per mole of creatinine; interquartile range, 2.0 to 3.6) (Fig. 3F). The patient's weight increased from 7.14 kg (the 9th percentile) at 207 days after birth (before the first dose), to 8.17 kg (the 26th percentile) at 256 days after birth (the end of the 7-week follow-up period), and his neurologic status was stable.

DISCUSSION

In this study, we describe a personalized base-editing therapy wholly developed in the 6-month span after a patient's birth. The patient was able to receive an increased amount of dietary protein and a reduced dose (to half the starting dose) of a nitrogen-scavenger medication, despite the "stress tests" presented by consecutive viral infections. The short follow-up is a limitation of this study; longer follow-up is needed to assess the safety and efficacy of k-abe, as well as the patient's neurologic health. Liver biopsy to assess for corrective *CPS1* editing was deferred because it posed an unacceptable risk to the infant. The potential for germline editing with k-abe could not be evaluated, although a study of a different lipid nanoparticle gene-editing drug did not detect editing in sperm samples from nonhuman primates nor germline transmission of gene edits in female mice to offspring.[19]

An advantage of lipid nanoparticle therapies is the potential for readministration,[23] which is contraindicated with adeno-associated virus–delivered therapies, given the immunogenicity of the vec-

tor. We opted to start with a very low initial dose of k-abe to evaluate safety, which was followed by a moderately higher second dose. In principle, the patient could receive additional and higher doses of k-abe in the future, if needed.

Therapies similar to k-abe could be developed for hundreds of hepatic inborn errors of metabolism. Similar to antisense oligonucleotide therapy,[24,25] corrective gene editing lends itself to rapid customization for individual patients owing to the platform nature of the technology.[9] Shared components among gene-editing therapies could include the same lipid nanoparticle formulation and mRNA, with the gRNA customized to each patient's variant.

We assessed k-abe for editing efficiency in mice and for safety in nonhuman primates. Such studies might not be necessary for future patient-specific treatments; perhaps cell-based studies would be sufficient. Although k-abe was developed under emergency conditions for a devastating neonatal-onset metabolic disorder, we anticipate that rapid deployment of patient-specific gene-editing therapies will become routine for many genetic diseases.

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Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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