A platform genome editing medicinal product for inborn errors of metabolism

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ADVANCING
KNOWLEDGE
FOR
GOOD

Perelman School of Medicine at the University of Pennsylvania

Children's Hospital of Philadelphia

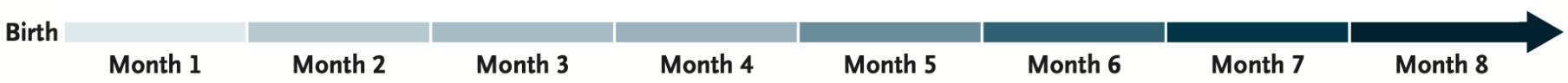
Relationships with Verve Therapeutics, Capstan Therapeutics, Lexeo Therapeutics, Beam Therapeutics, Nava Therapeutics (K.M.), AskBio and LatusBio (R.A.-N.)

New case of neonatal-onset urea cycle disorder

A 2-day-old male infant, named KJ, became lethargic and had respiratory distress.

The blood ammonia level was significantly elevated >1000 µmol/L (normal for age is <33 µmol/L).

He was transferred to the neonatal intensive care unit and rapidly started on life-saving dialysis.



Musunuru, Grandinette … Ahrens-Nicklas. *N Engl J Med* 2025; 392:2235-43

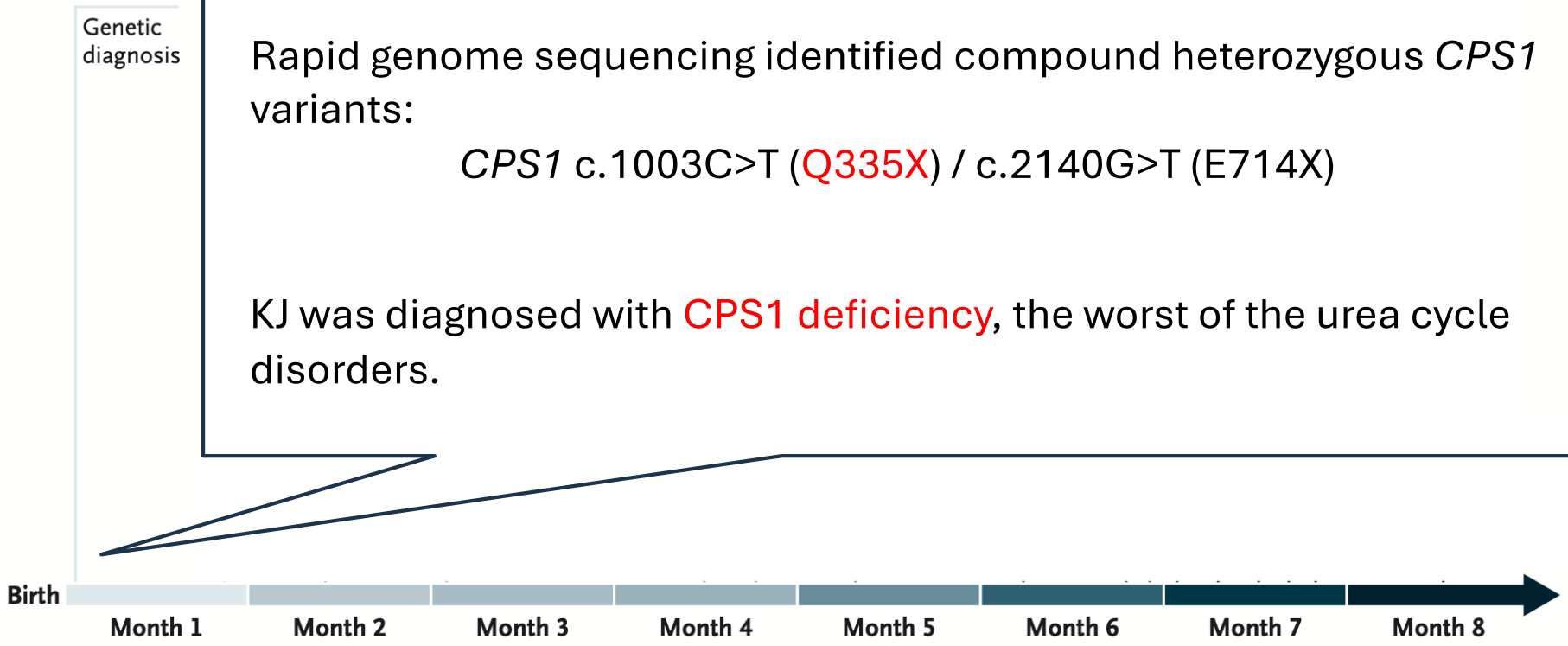
Genetic diagnosis

Metabolic testing confirmed a proximal urea cycle defect.

Rapid genome sequencing identified compound heterozygous *CPS1* variants:

CPS1 c.1003C>T (Q335X) / c.2140G>T (E714X)

KJ was diagnosed with CPS1 deficiency, the worst of the urea cycle disorders.

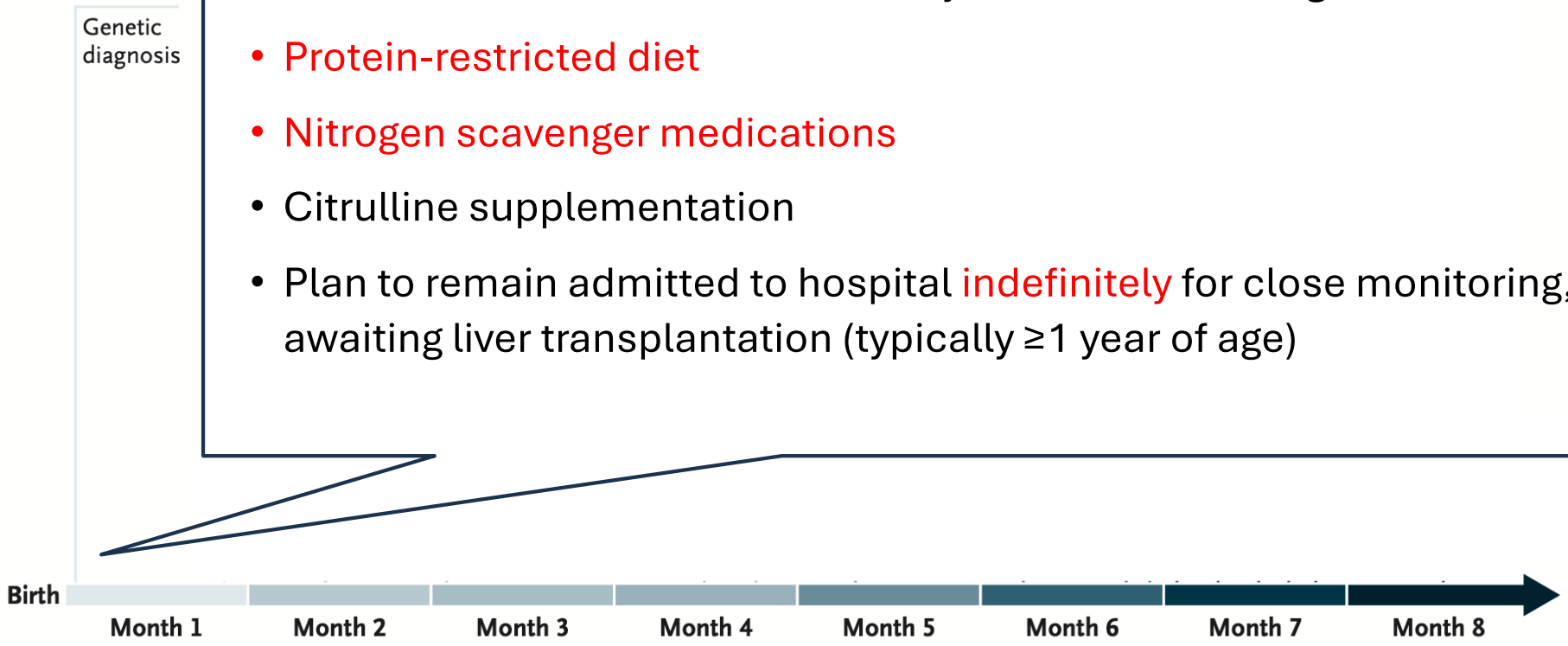


Musunuru, Grandinette … Ahrens-Nicklas. *N Engl J Med* 2025; 392:2235-43

Genetic diagnosis

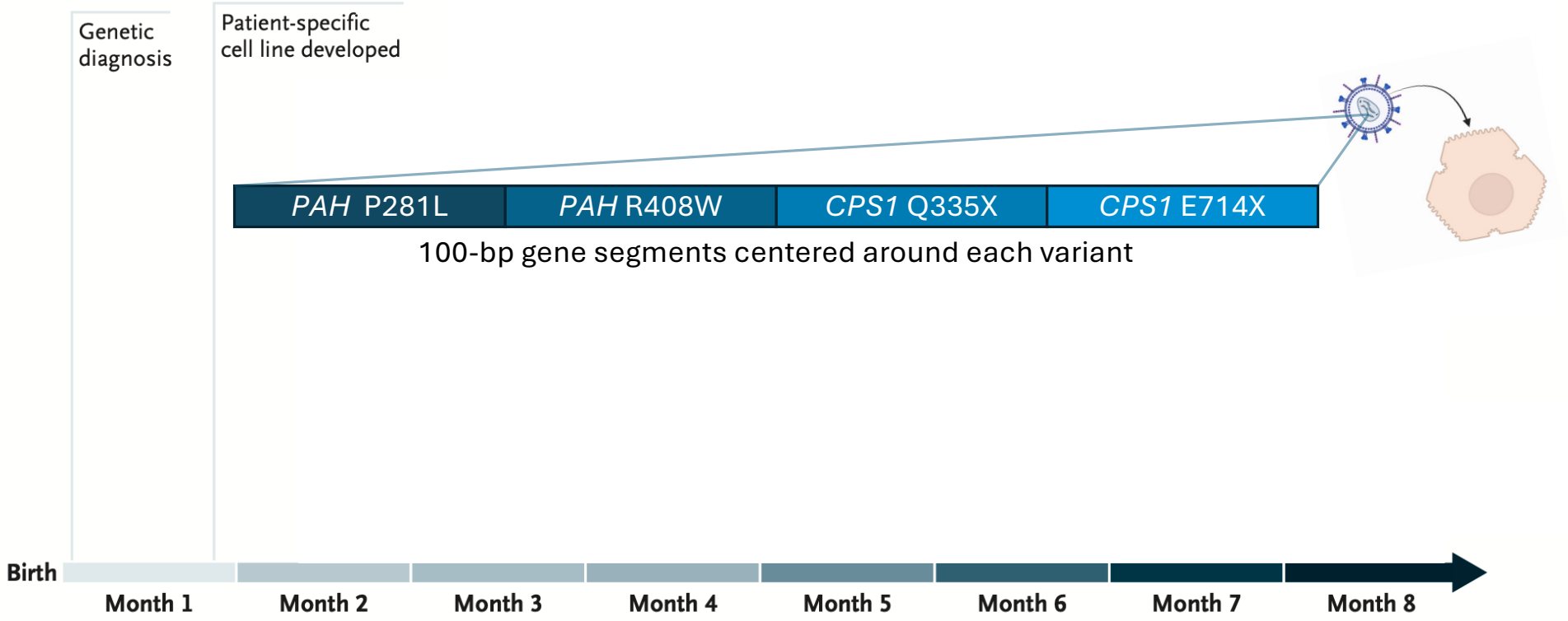
KJ was transitioned to chronic urea cycle disorder management:

- Protein-restricted diet
- Nitrogen scavenger medications
- Citrulline supplementation
- Plan to remain admitted to hospital indefinitely for close monitoring, awaiting liver transplantation (typically ≥1 year of age)



Musunuru, Grandinette … Ahrens-Nicklas. *N Engl J Med* 2025; 392:2235-43

Screening of base editors for correction of *CPS1* Q335X variant



Musunuru, Grandinette … Ahrens-Nicklas. *N Engl J Med* 2025; 392:2235-43

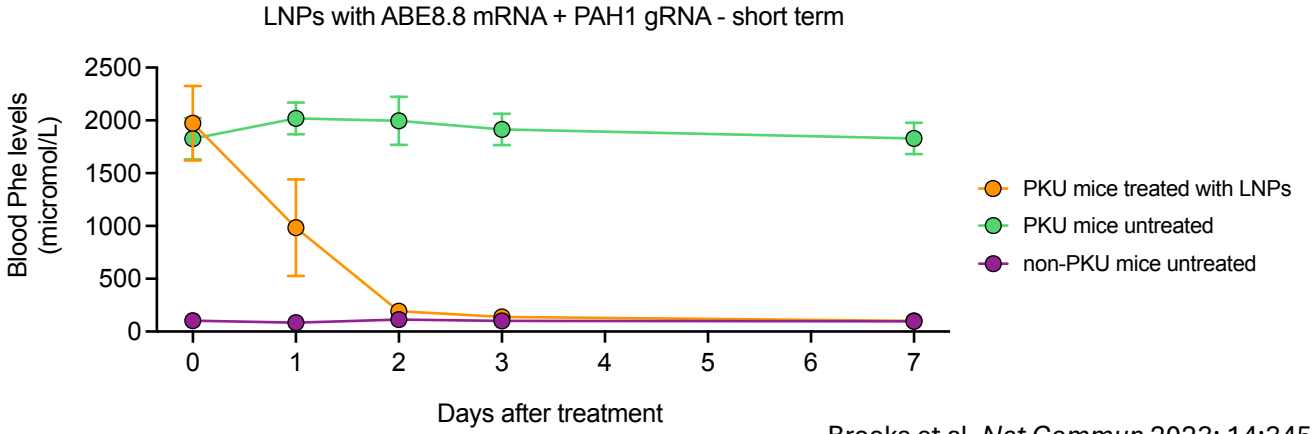
Lentivirus-transduced cell lines

- Need to rapidly insert variants into a relevant cell line (e.g., HuH-7 cells as proxy for human hepatocytes), but need not be in endogenous gene loci
- Various options—lentivirus, transposase, integrase—with lentivirus being the most accessible to research laboratories
- Can use cell lines for:
  - screening for editing solutions
  - potency assays
- Need to include variant(s) that serve as validated positive reference controls

LNP adenine base editing in “humanized” PKU mice (P281L or R408W)

PAH P281L mice
positive reference control #1
ABE8.8 + “PAH1” gRNA

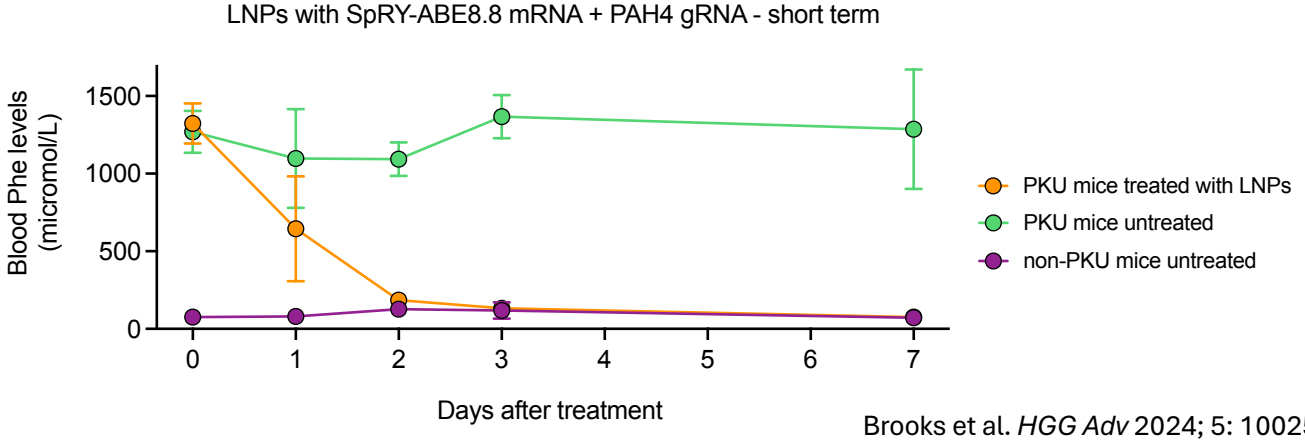
<u>whole-liver editing</u>
on-target correction = 40%



Brooks et al. *Nat Commun* 2023; 14:3451

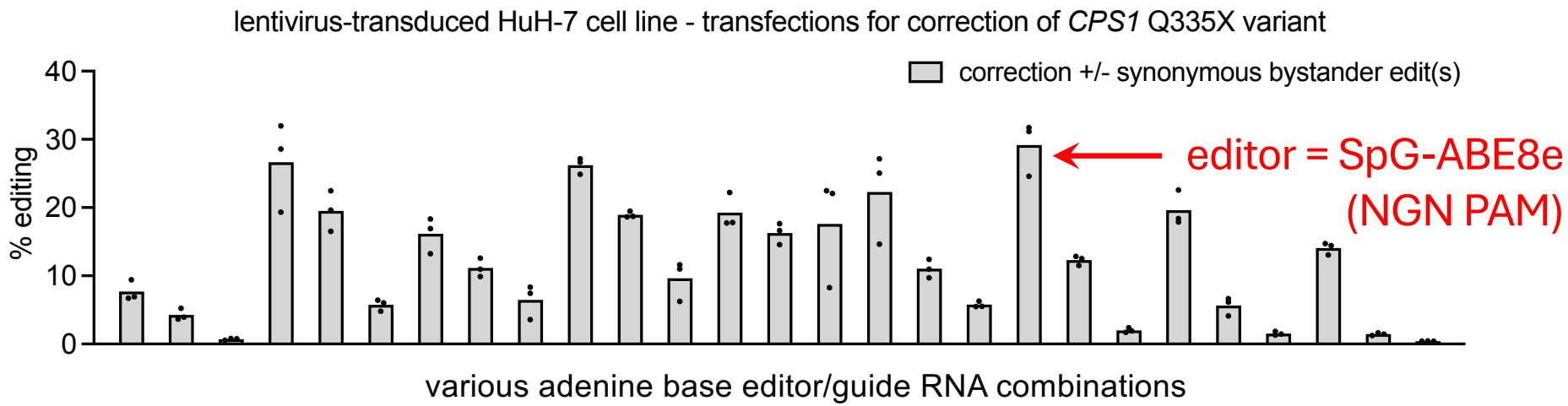
PAH R408W mice
positive reference control #2
SpRY-ABE8.8 + “PAH4” gRNA

<u>whole-liver editing</u>
on-target correction = 26%



Brooks et al. *HGG Adv* 2024; 5: 100253

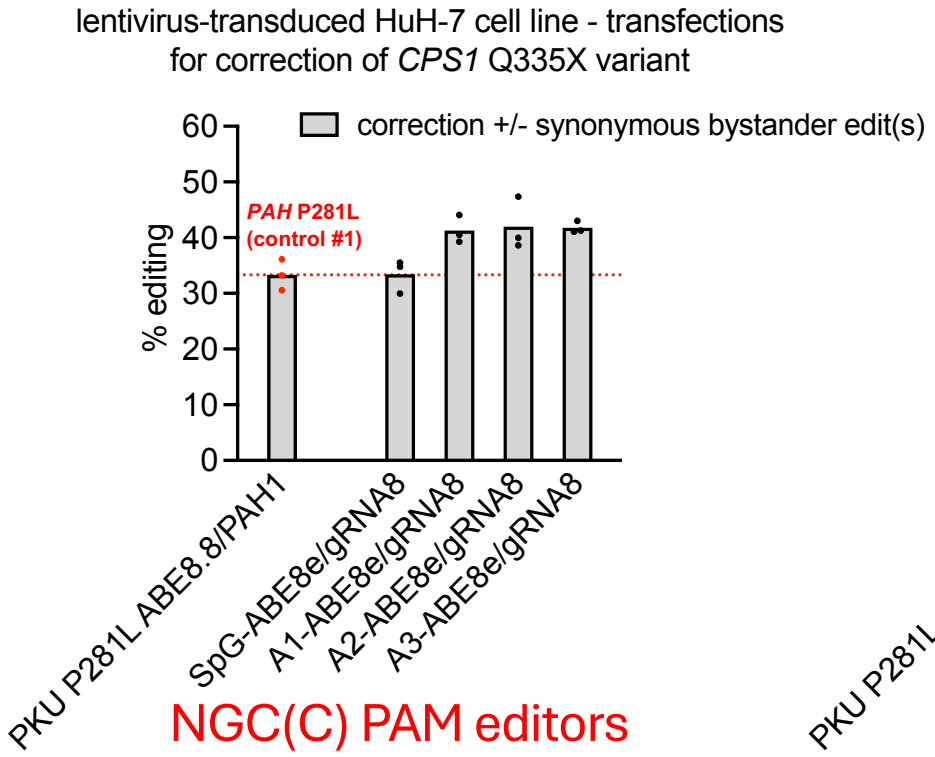
Screening of base editors for correction of *CPS1* Q335X variant



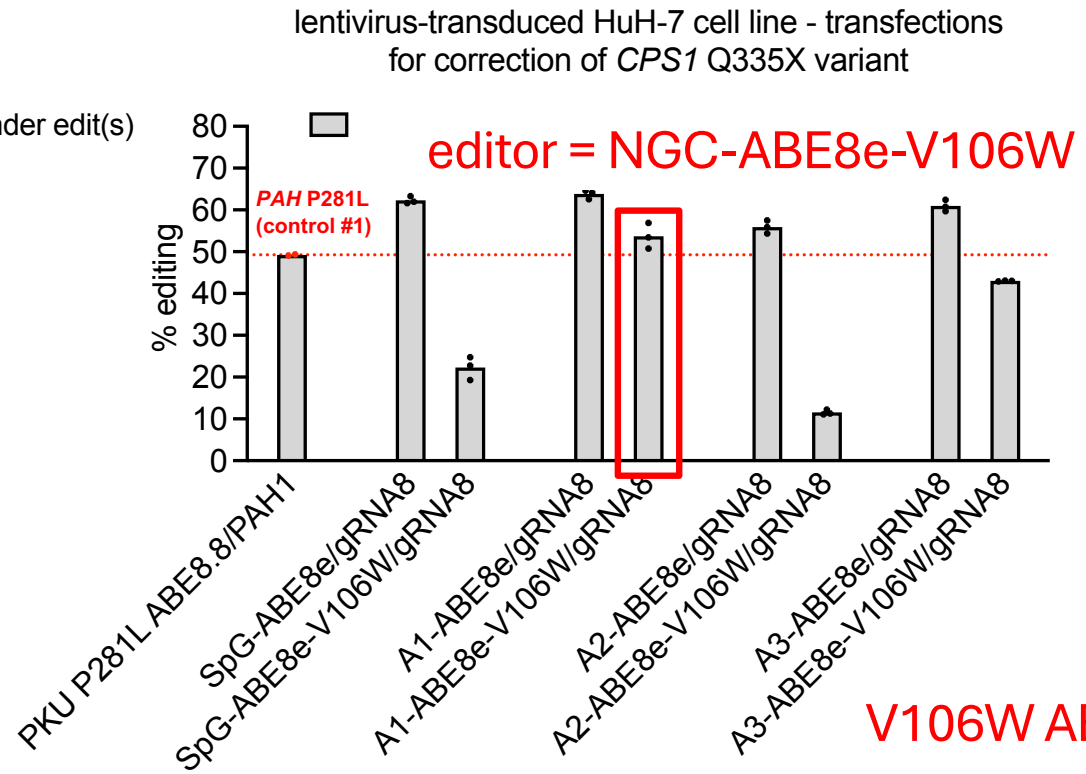
Results in less than 4 weeks

Musunuru, Grandinette … Ahrens-Nicklas. *N Engl J Med* 2025; 392:2235-43

Screening of base editors for correction of *CPS1* Q335X variant



NGC(C) PAM editors

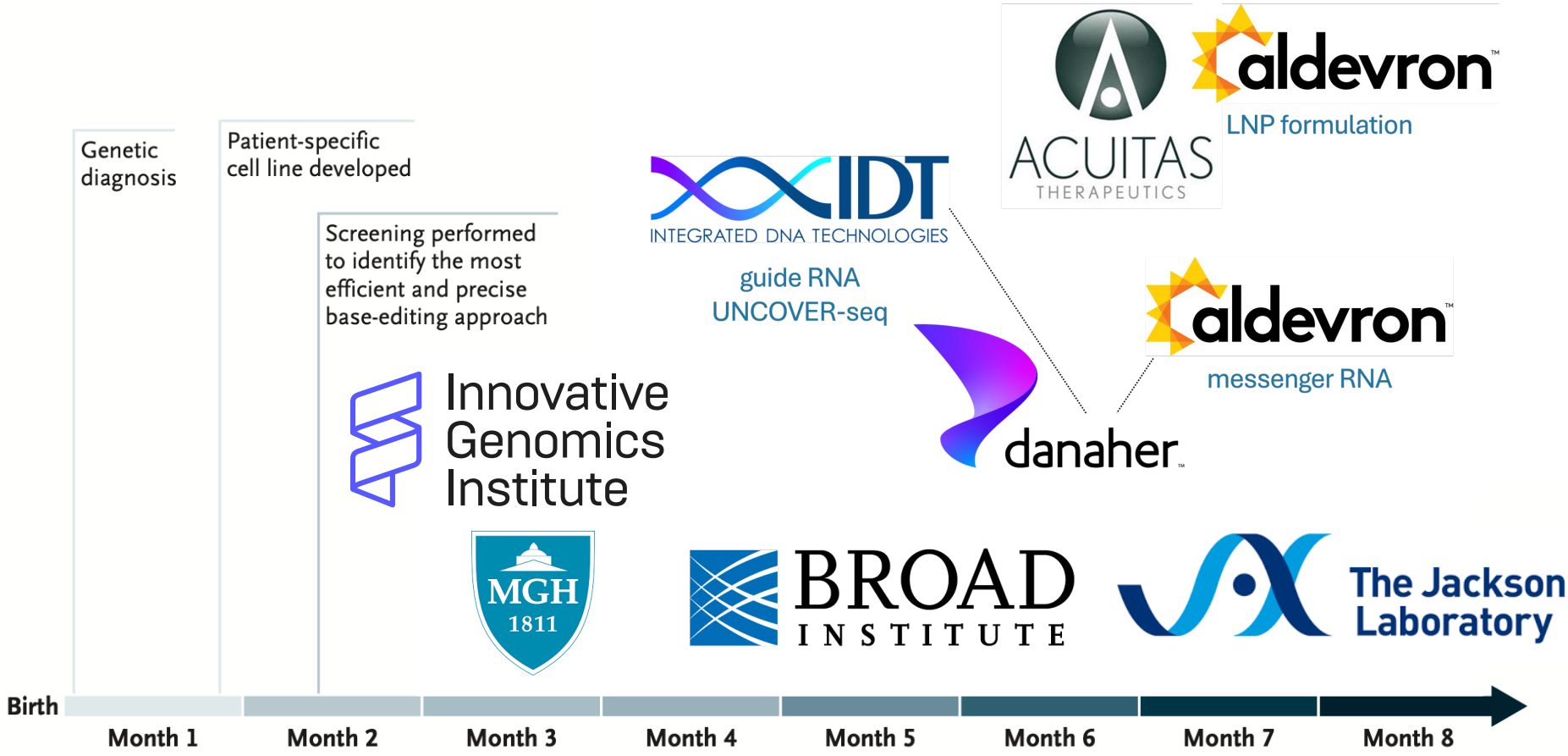


V106W ABEs

Completed in 6 weeks

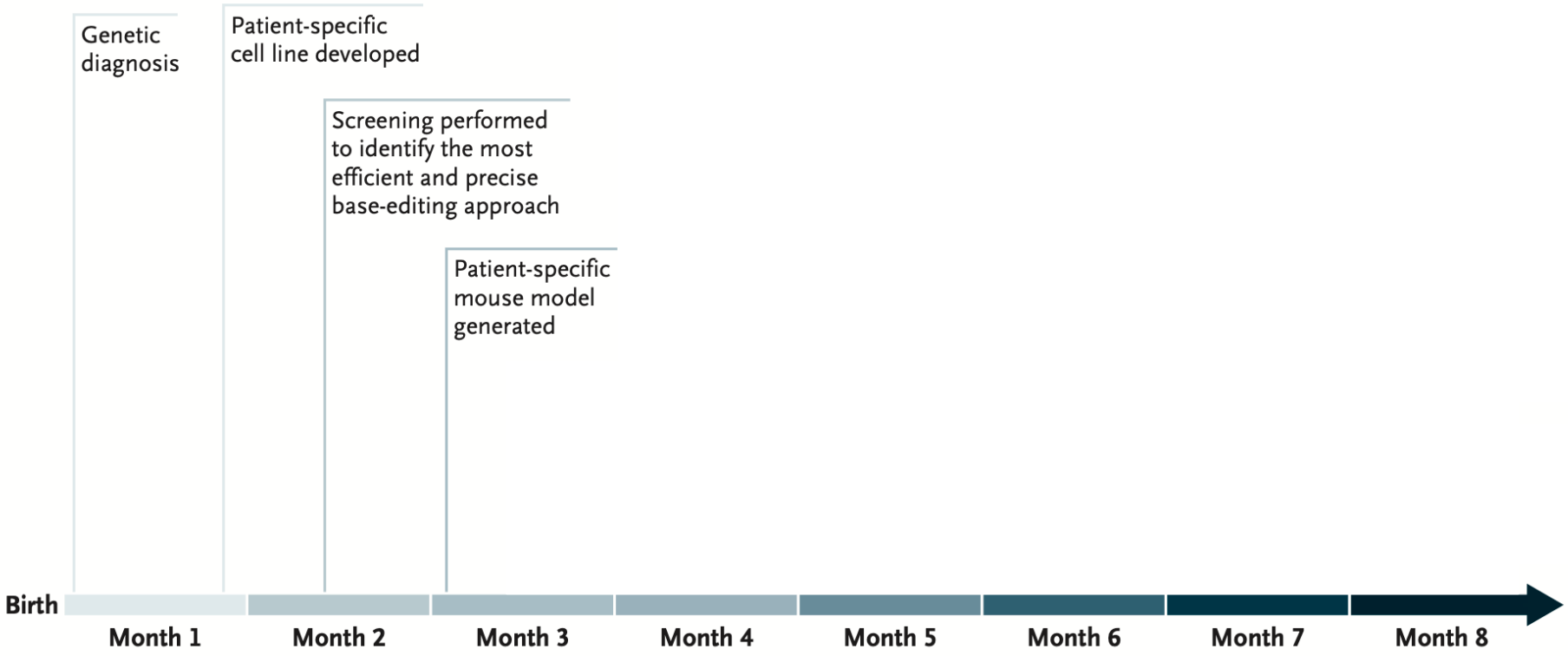
Musunuru, Grandinette … Ahrens-Nicklas. *N Engl J Med* 2025; 392:2235-43

Assembly of a team of academic and industry partners



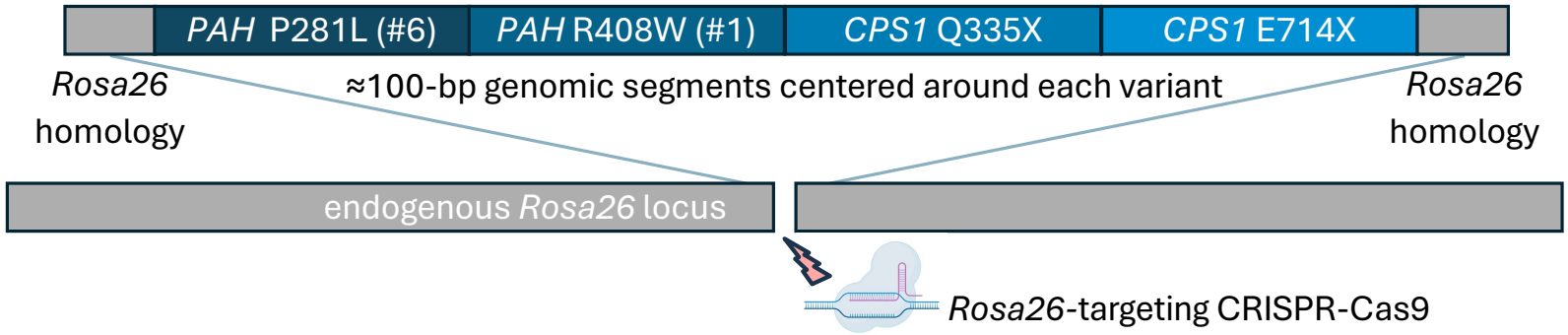
Musunuru, Grandinette … Ahrens-Nicklas. *N Engl J Med* 2025; 392:2235-43

Generation of patient-specific Q335X mice for *in vivo* testing



Musunuru, Grandinette … Ahrens-Nicklas. *N Engl J Med* 2025; 392:2235-43

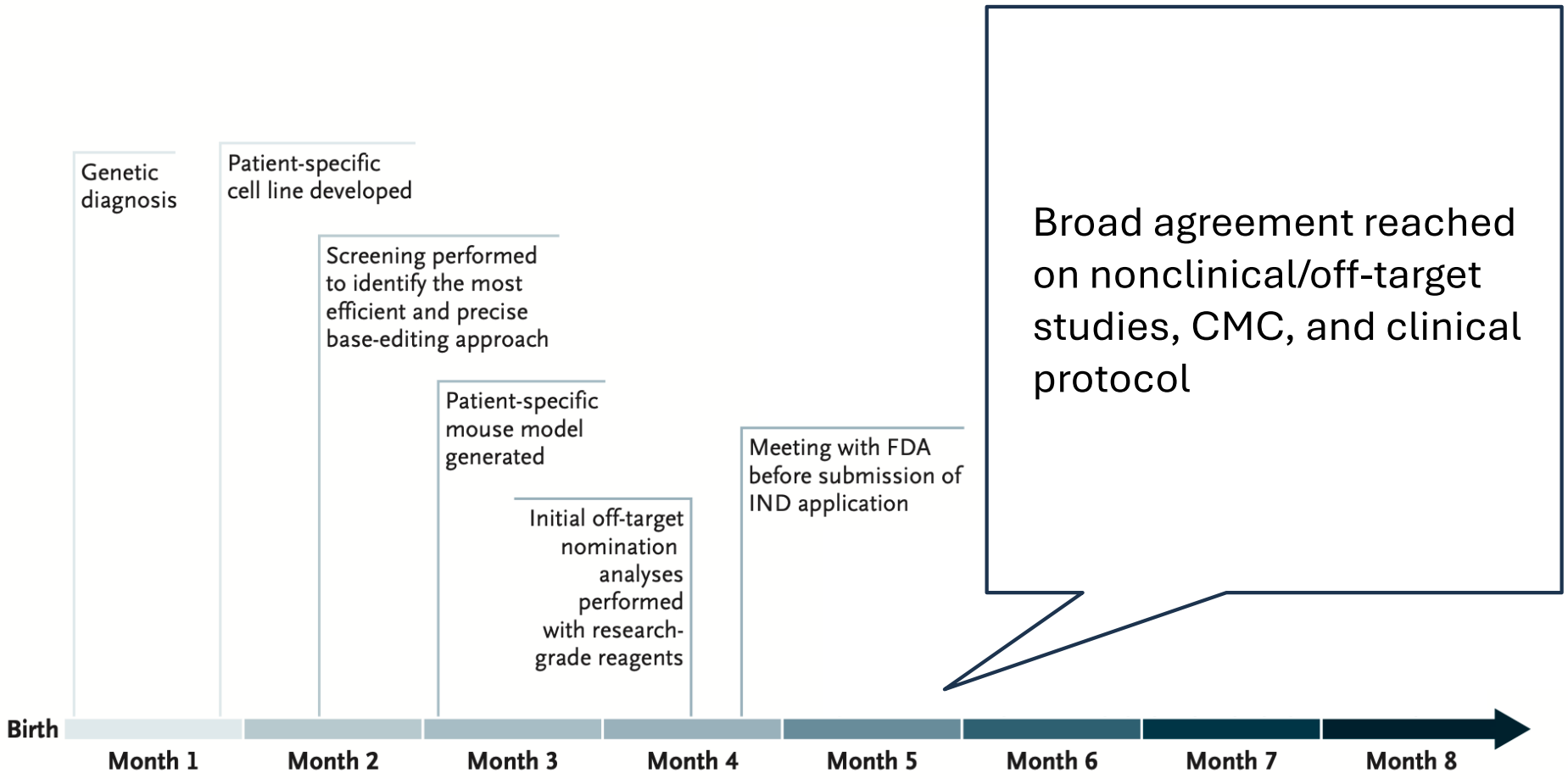
Rosa26 multi-variant mice



Obtained two founder Rosa26 *multi-variant mice in 2 months*

Musunuru, Grandinette … Ahrens-Nicklas. *N Engl J Med* 2025; 392:2235-43

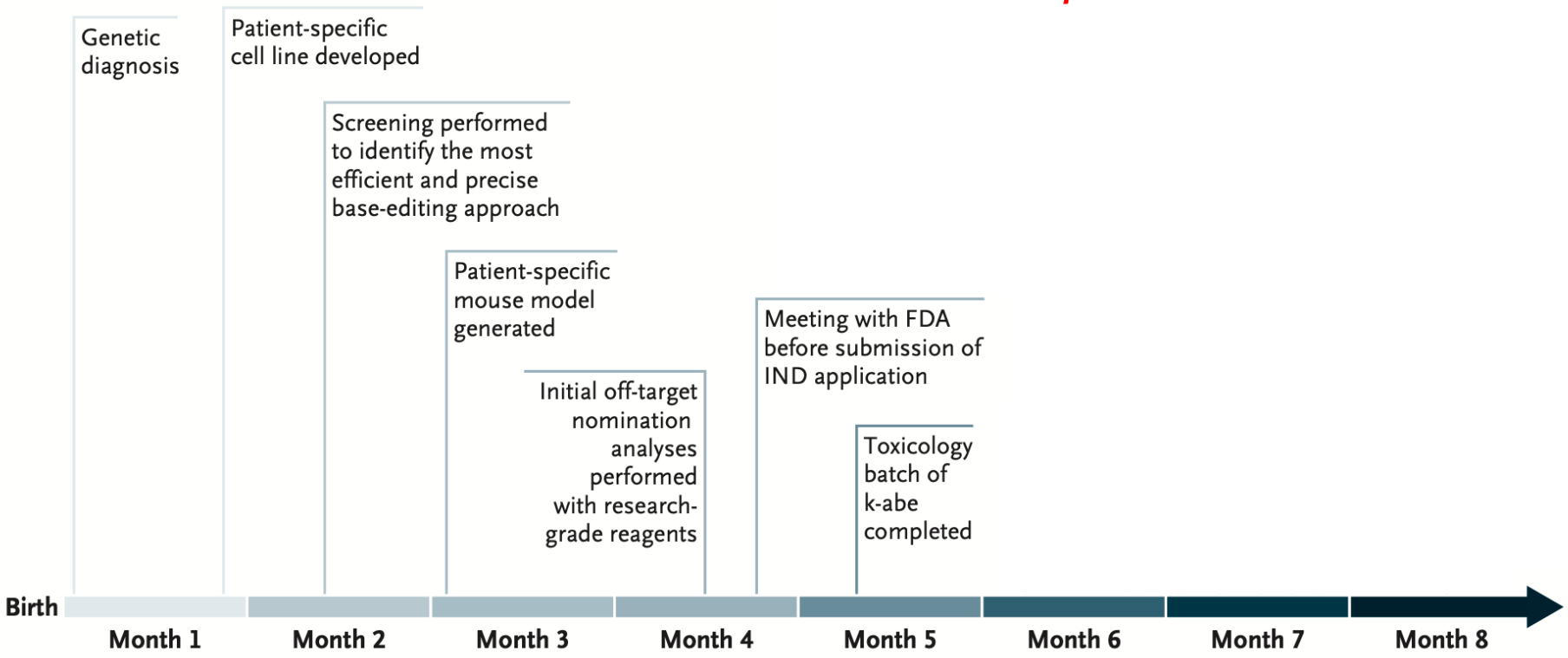
Pre-IND meeting with U.S. Food and Drug Administration (FDA)



Musunuru, Grandinette … Ahrens-Nicklas. *N Engl J Med* 2025; 392:2235-43

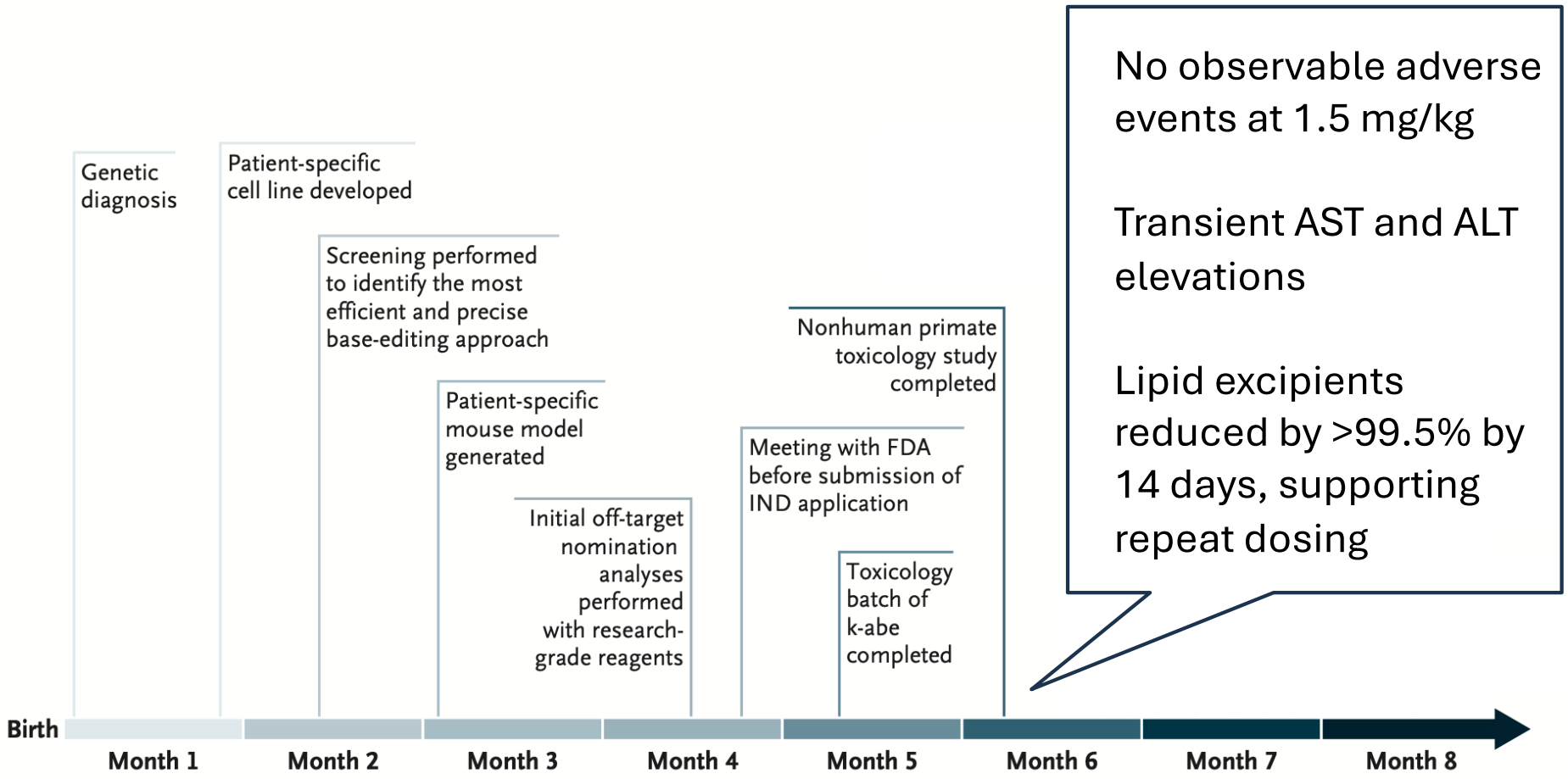
Toxicology batch of kayjayguran abengcemeran (k-abe)

Completed in 4 months



Musunuru, Grandinette … Ahrens-Nicklas. *N Engl J Med* 2025; 392:2235-43

Nonhuman primate toxicology study



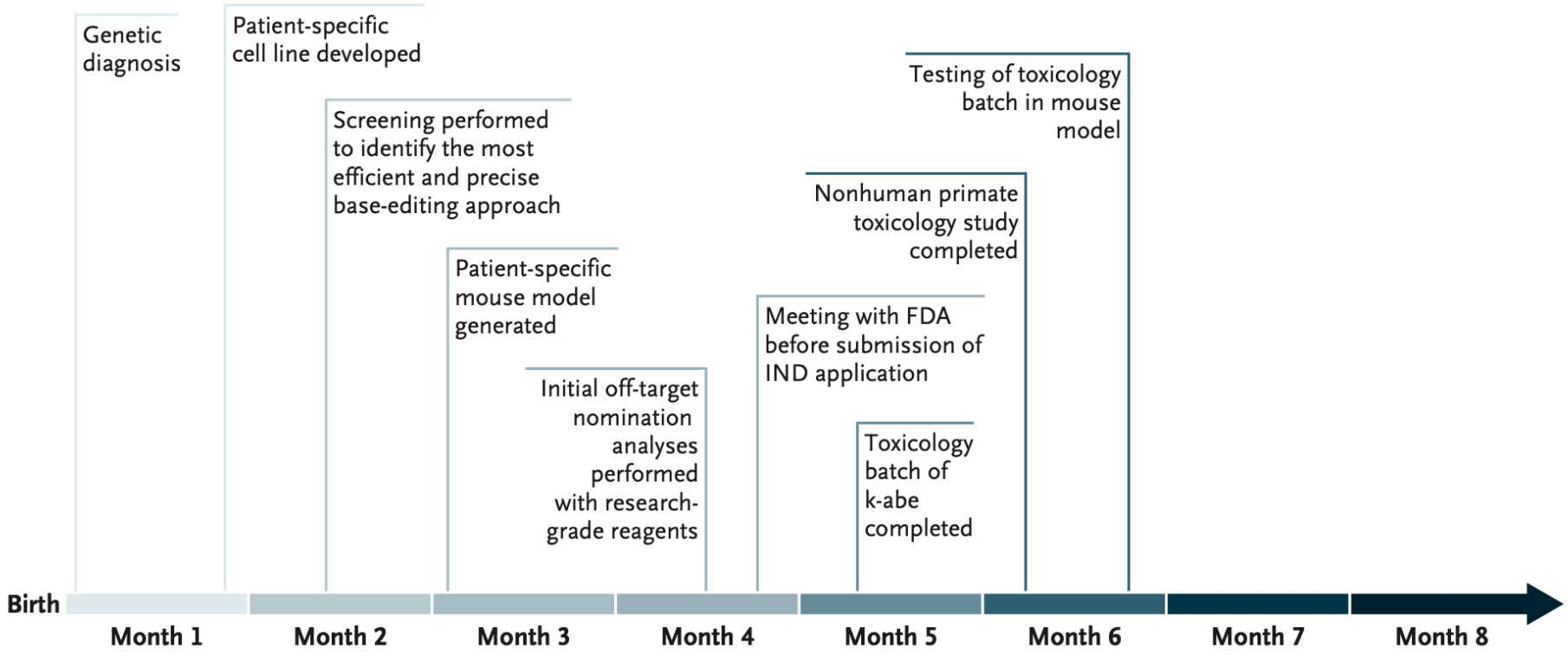
No observable adverse events at 1.5 mg/kg

Transient AST and ALT elevations

Lipid excipients reduced by >99.5% by 14 days, supporting repeat dosing

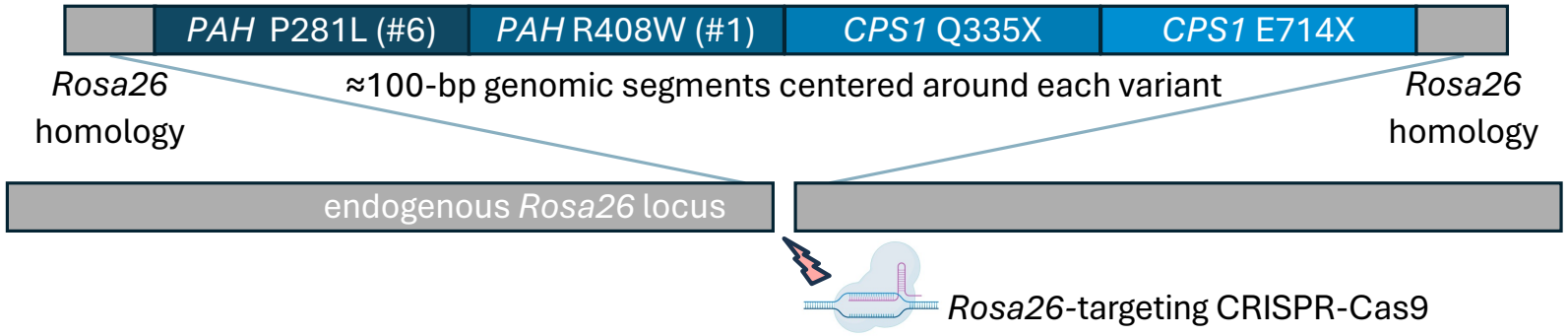
Musunuru, Grandinette … Ahrens-Nicklas. *N Engl J Med* 2025; 392:2235-43

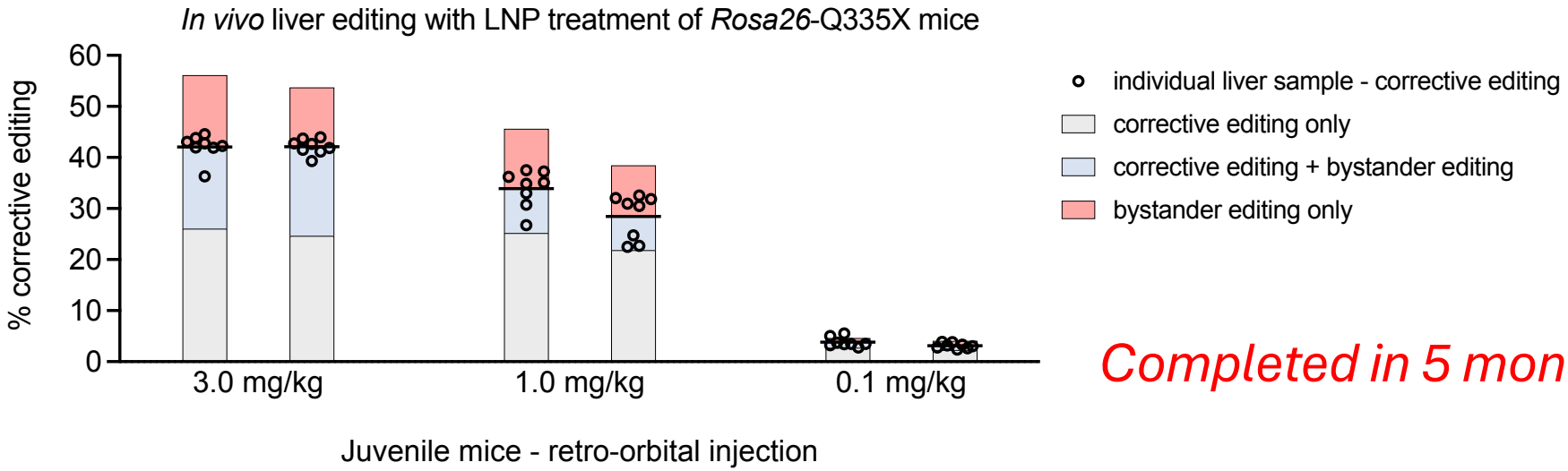
Testing of toxicology batch of k-abe in *Rosa26* multi-variant mice



Musunuru, Grandinette … Ahrens-Nicklas. *N Engl J Med* 2025; 392:2235-43

Correction of *CPS1* Q335X variant in *Rosa26* multi-variant mice

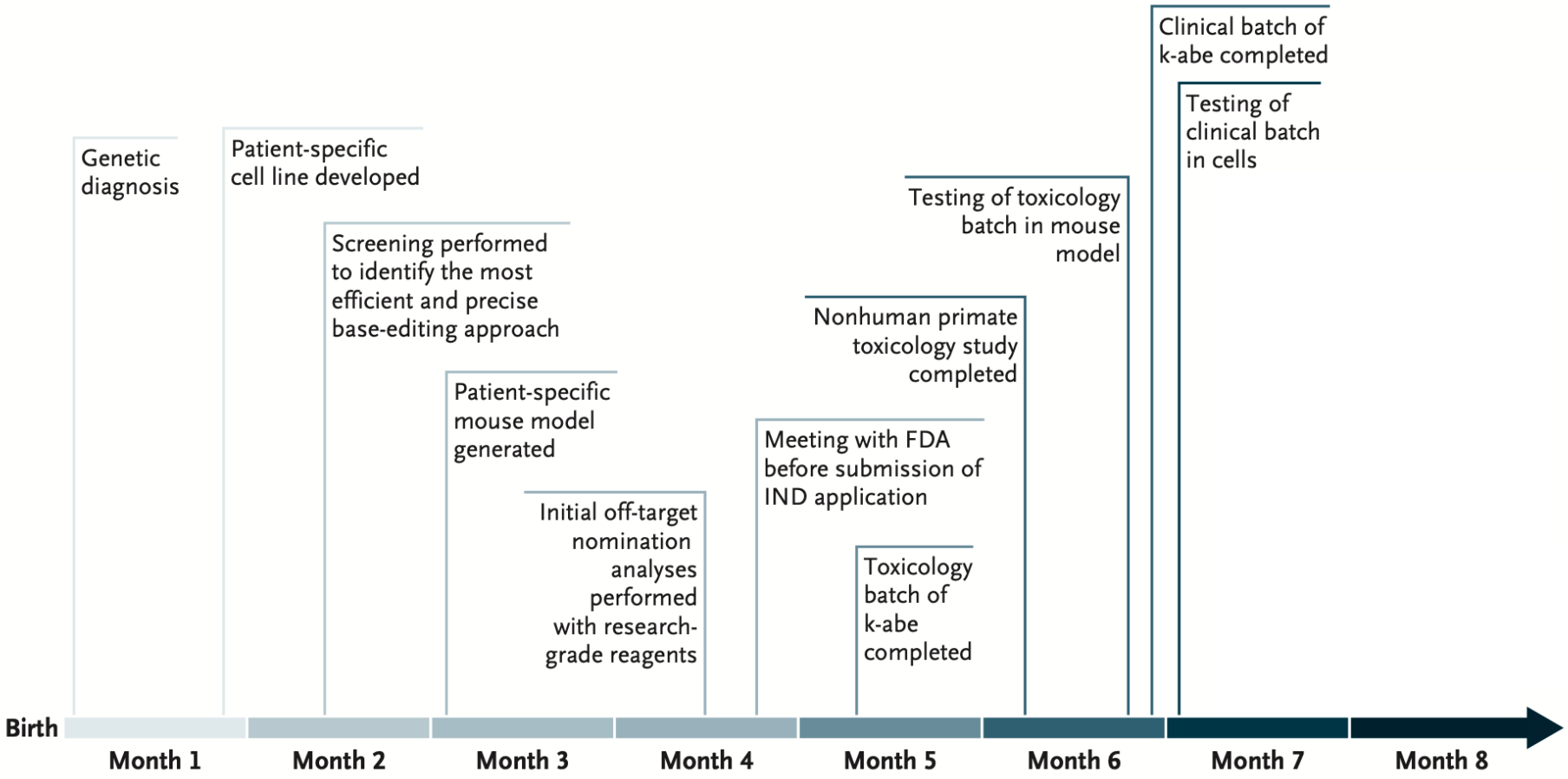




Completed in 5 months

Musunuru, Grandinette … Ahrens-Nicklas. *N Engl J Med* 2025; 392:2235-43

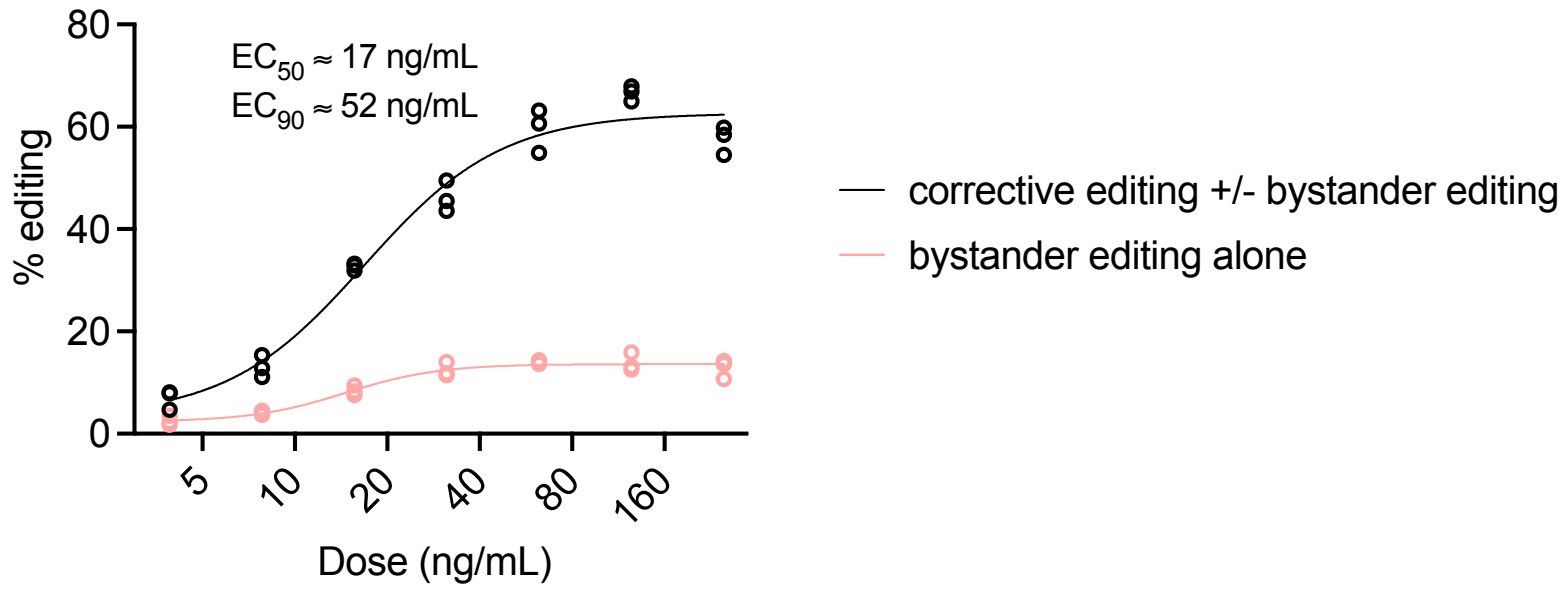
Manufacturing of clinical batch of k-abe and testing in cells



Musunuru, Grandinette … Ahrens-Nicklas. *N Engl J Med* 2025; 392:2235-43

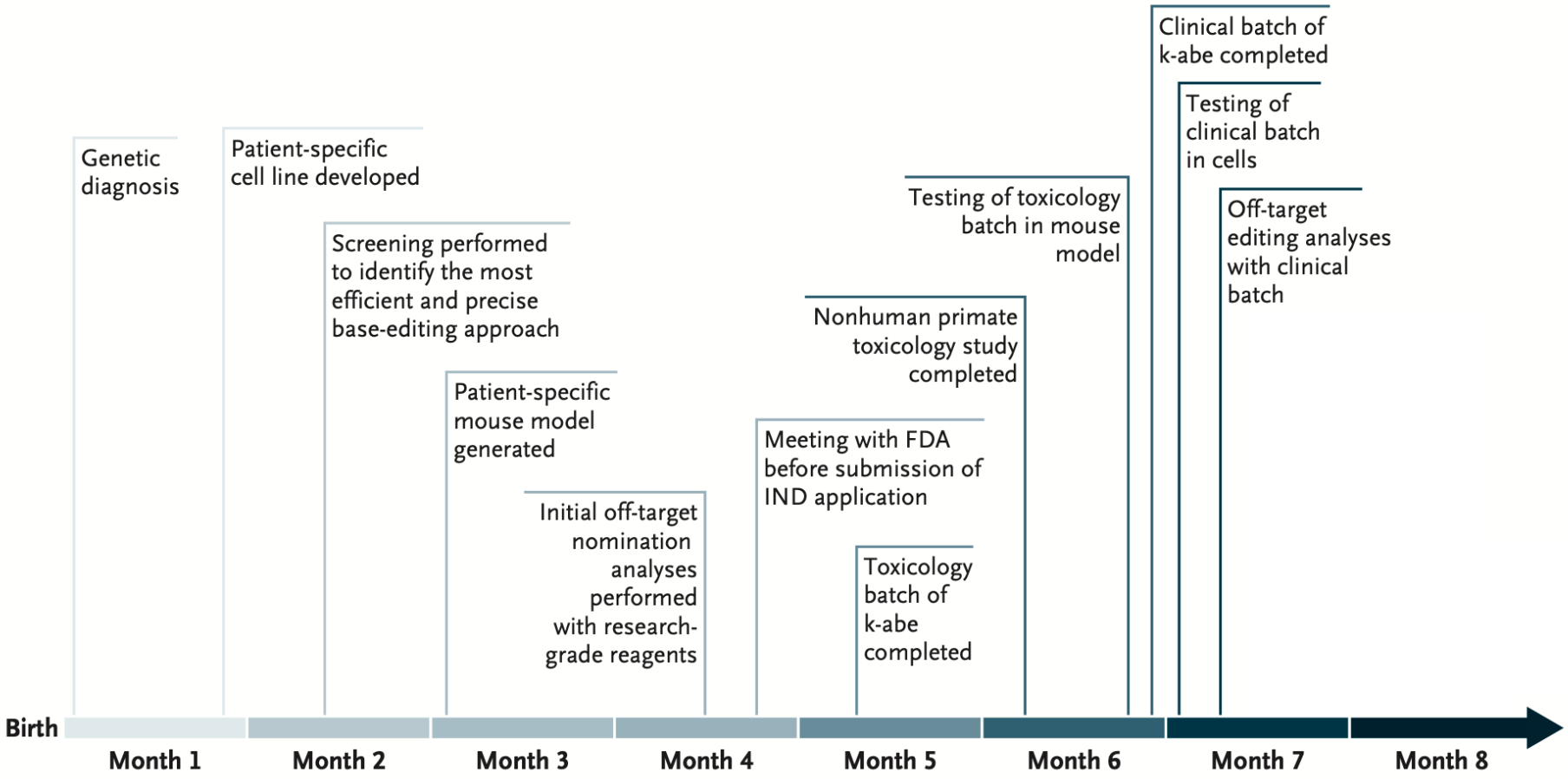
Manufacturing of clinical batch of k-abe and testing in cells

Q335X lentivirus-transduced HuH-7 cells treated with k-abe



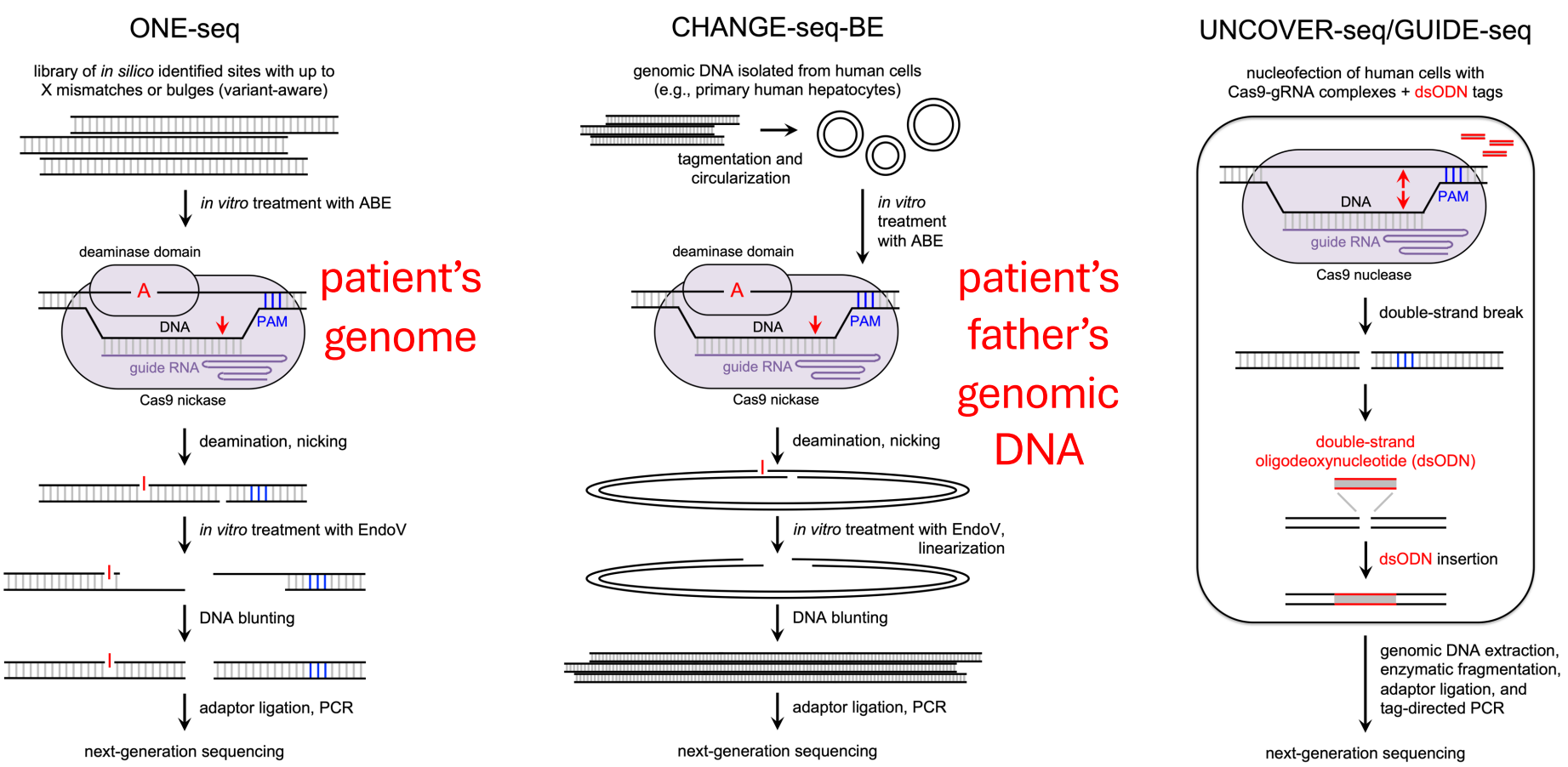
Musunuru, Grandinette … Ahrens-Nicklas. *N Engl J Med* 2025; 392:2235-43

Off-target analyses with clinical batch of k-abe



Musunuru, Grandinette … Ahrens-Nicklas. *N Engl J Med* 2025; 392:2235-43

Patient-specific off-target analyses



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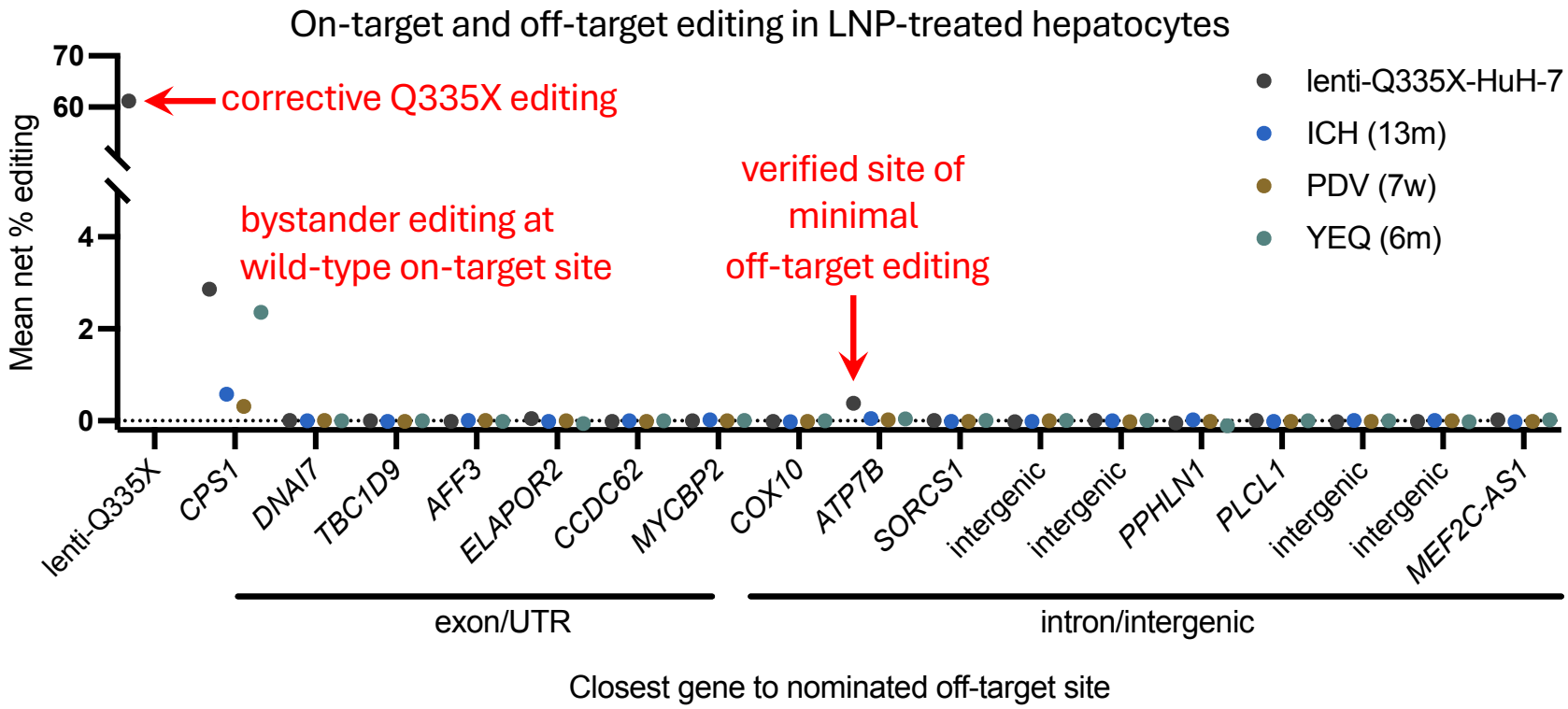
Patient-specific off-target analyses



Completed in 6 months

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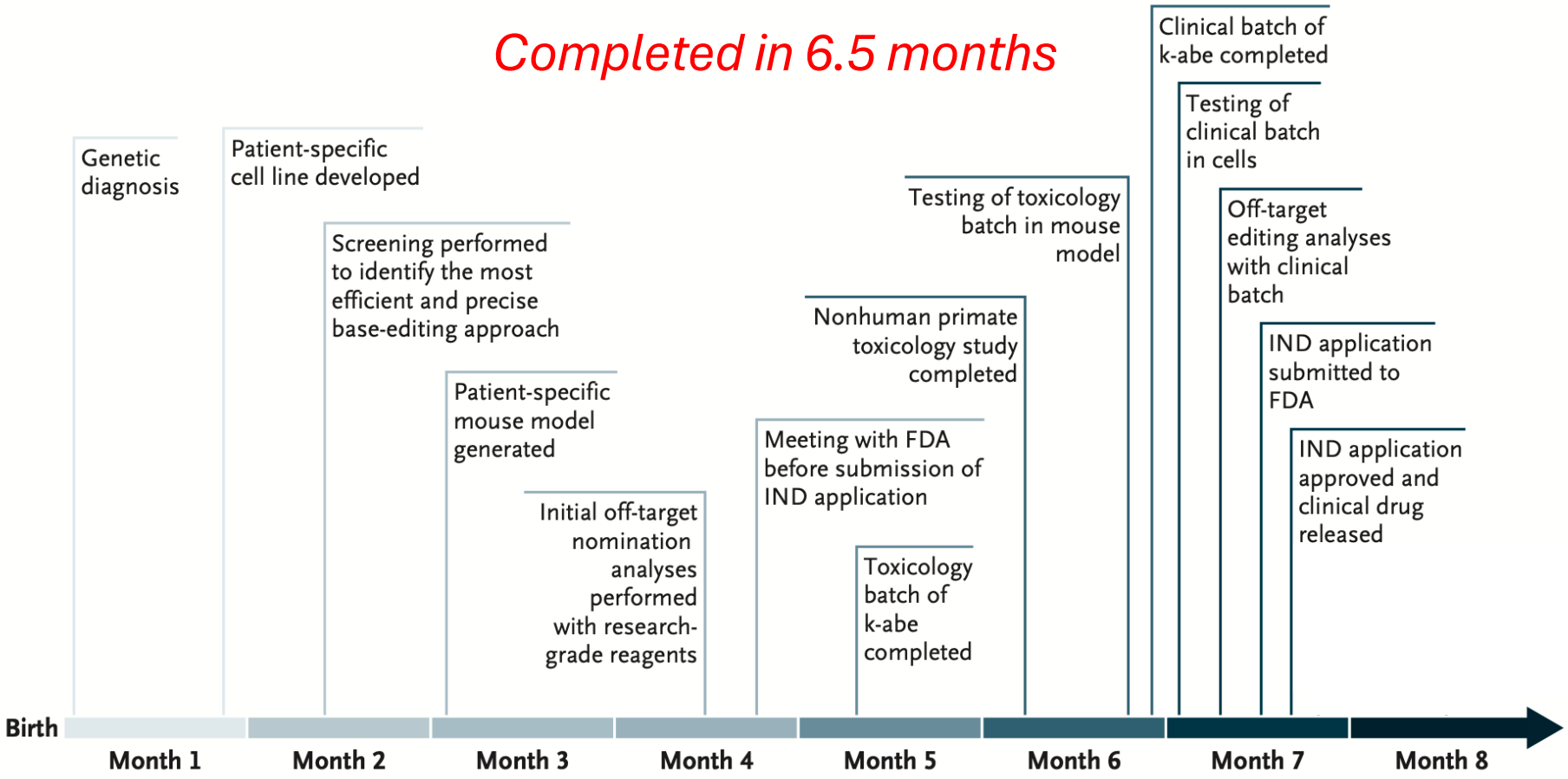
Single patient expanded access IND application to FDA

Completed in 6.5 months



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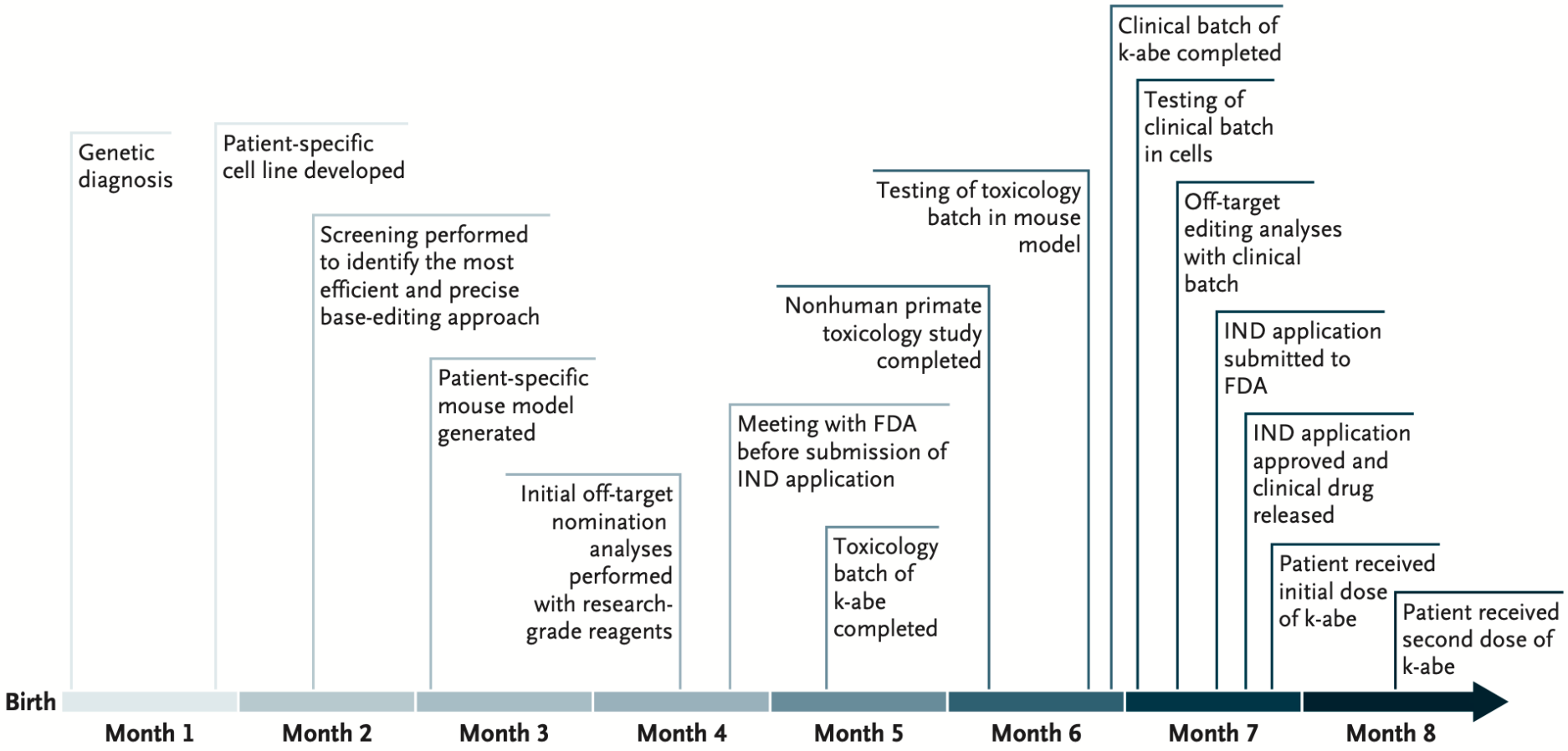
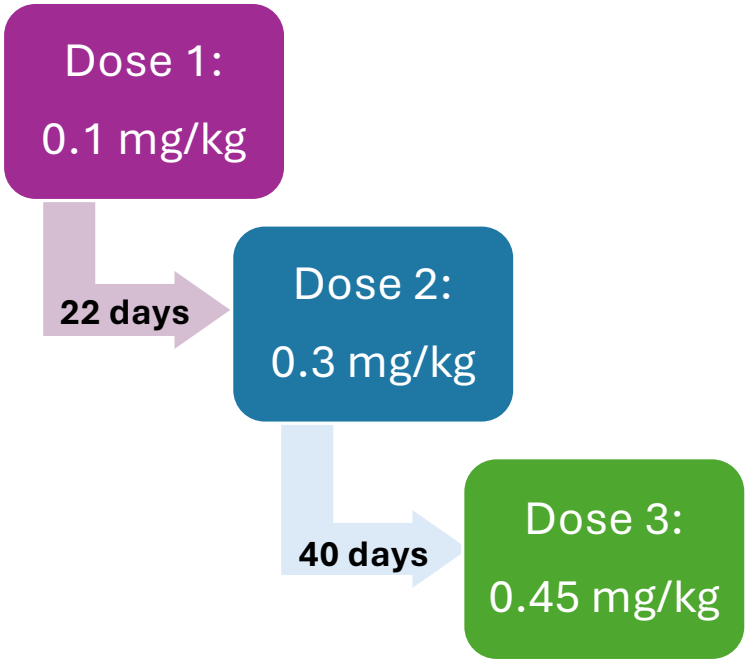
Initial treatment with k-abe (day 208 after birth)



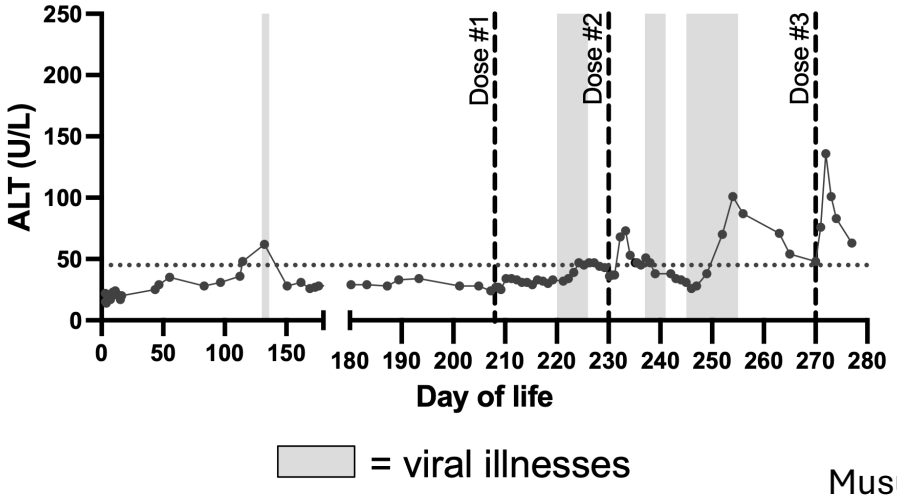
Musunuru, Grandinette … Ahrens-Nicklas. *N Engl J Med* 2025; 392:2235-43

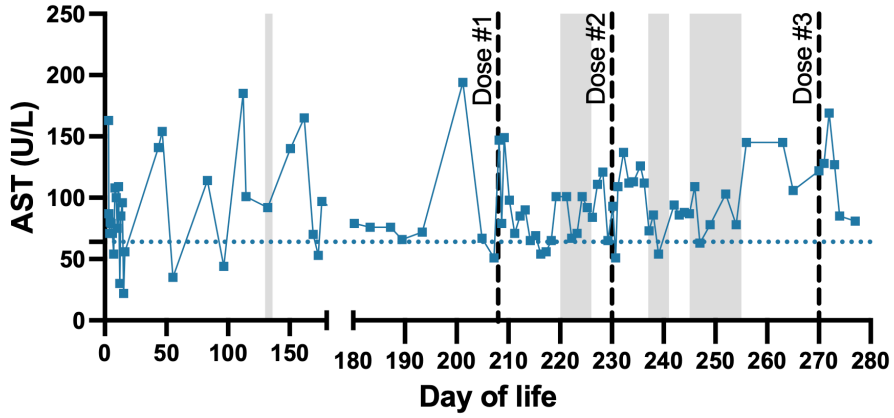
Dosing schedule



Treatment with k-abe resulted in no serious adverse events

- Brief coughing episode at beginning of dose 2 and of dose 3; after initial episode, no further cough and able to tolerate full rate of infusion
- Low-grade fever and transient rash after dose 3
- Mild, transient, asymptomatic, dose-dependent increases in ALT with no other liver function abnormalities





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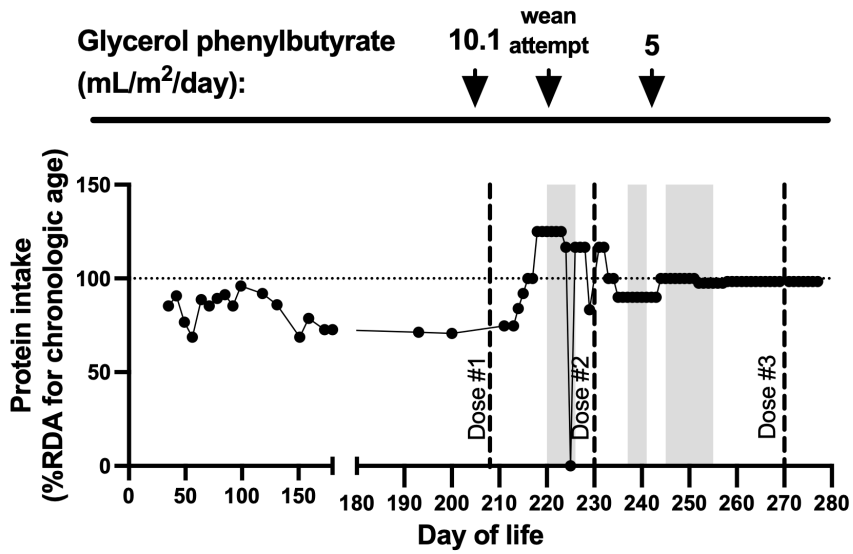
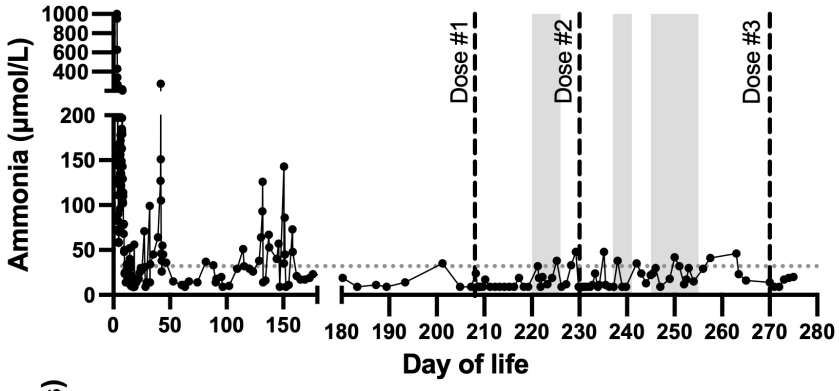
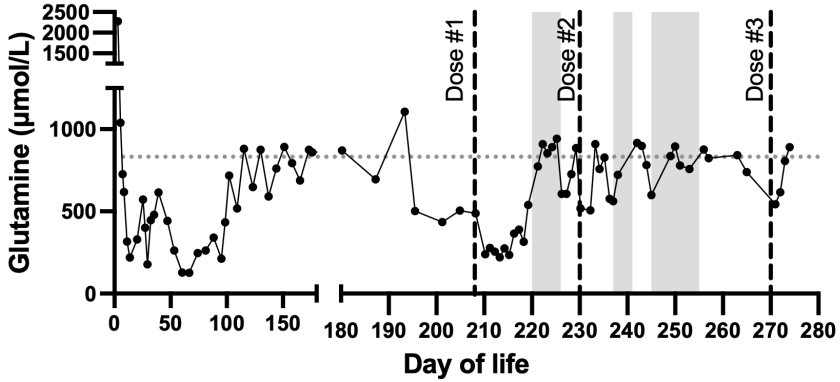
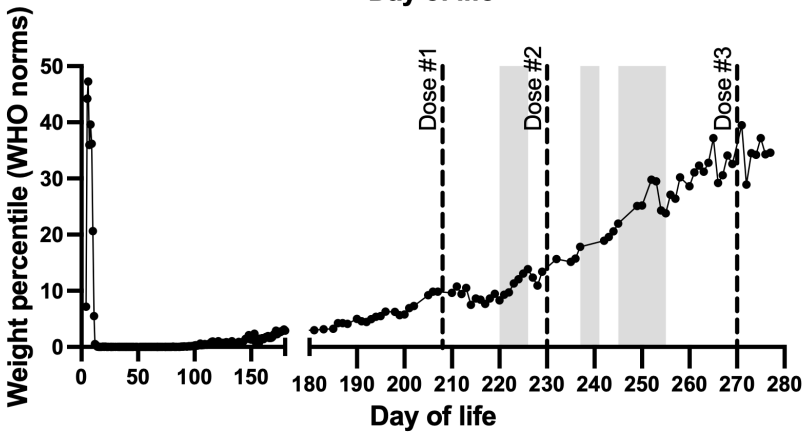
Higher protein tolerance, weaning of nitrogen scavenger medication









= viral illnesses

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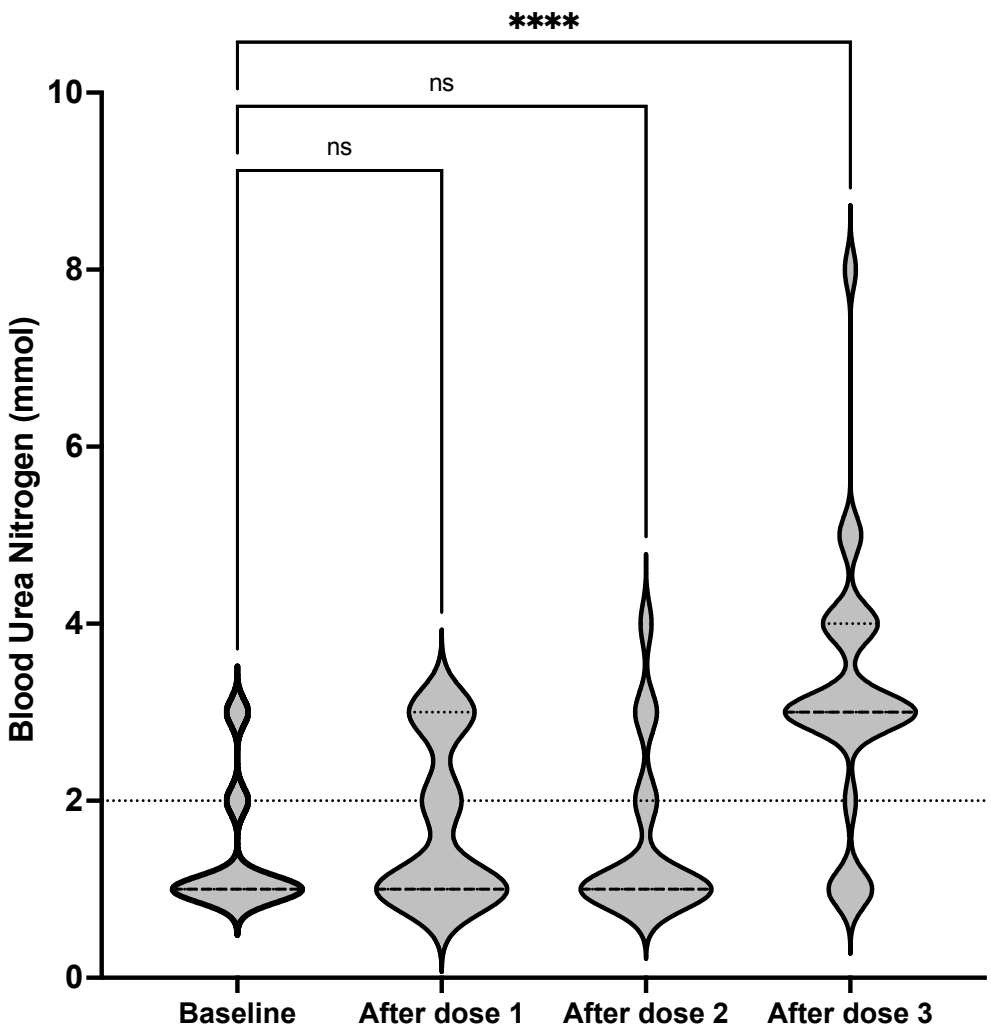
Increased blood urea nitrogen levels after treatment



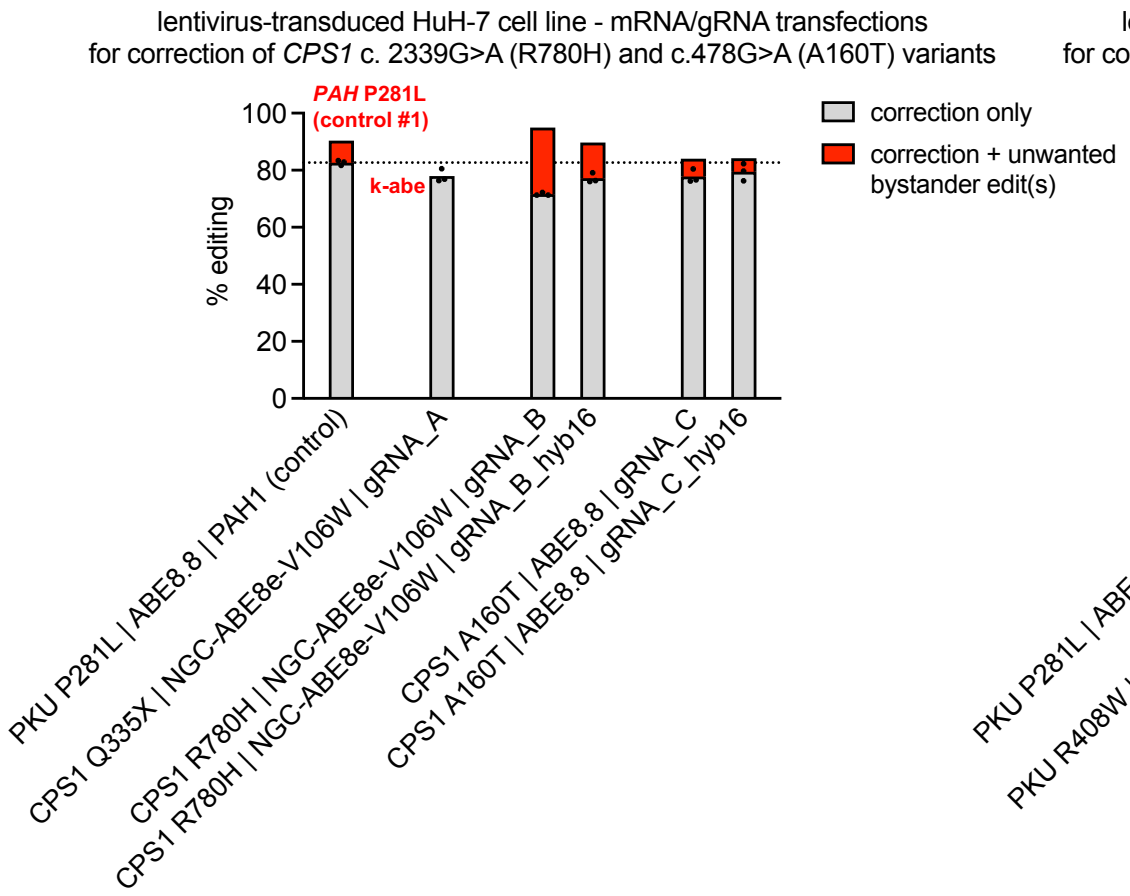
Conclusions, challenges, and opportunities

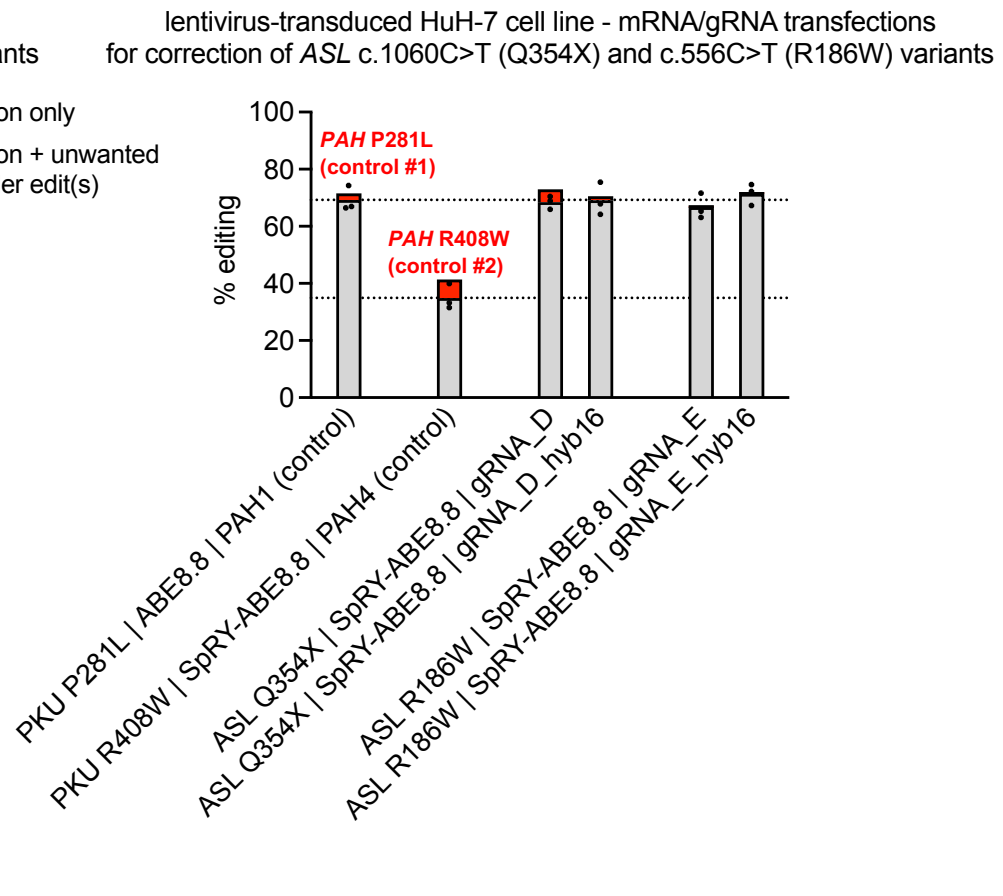
- KJ will likely continue to need some urea cycle management, but early signs suggest that his disease may be less severe
- It is possible to develop a personalized gene-editing therapy in 6 months
- Repeated doses of an LNP base-editing therapy can be safely given to an infant
- Longer follow-up and studies of additional non-invasive markers are needed to quantify potential benefit and durability
- Ultimately, we need to move from *N*-of-1 studies to platform trials

Conclusions, challenges, and opportunities

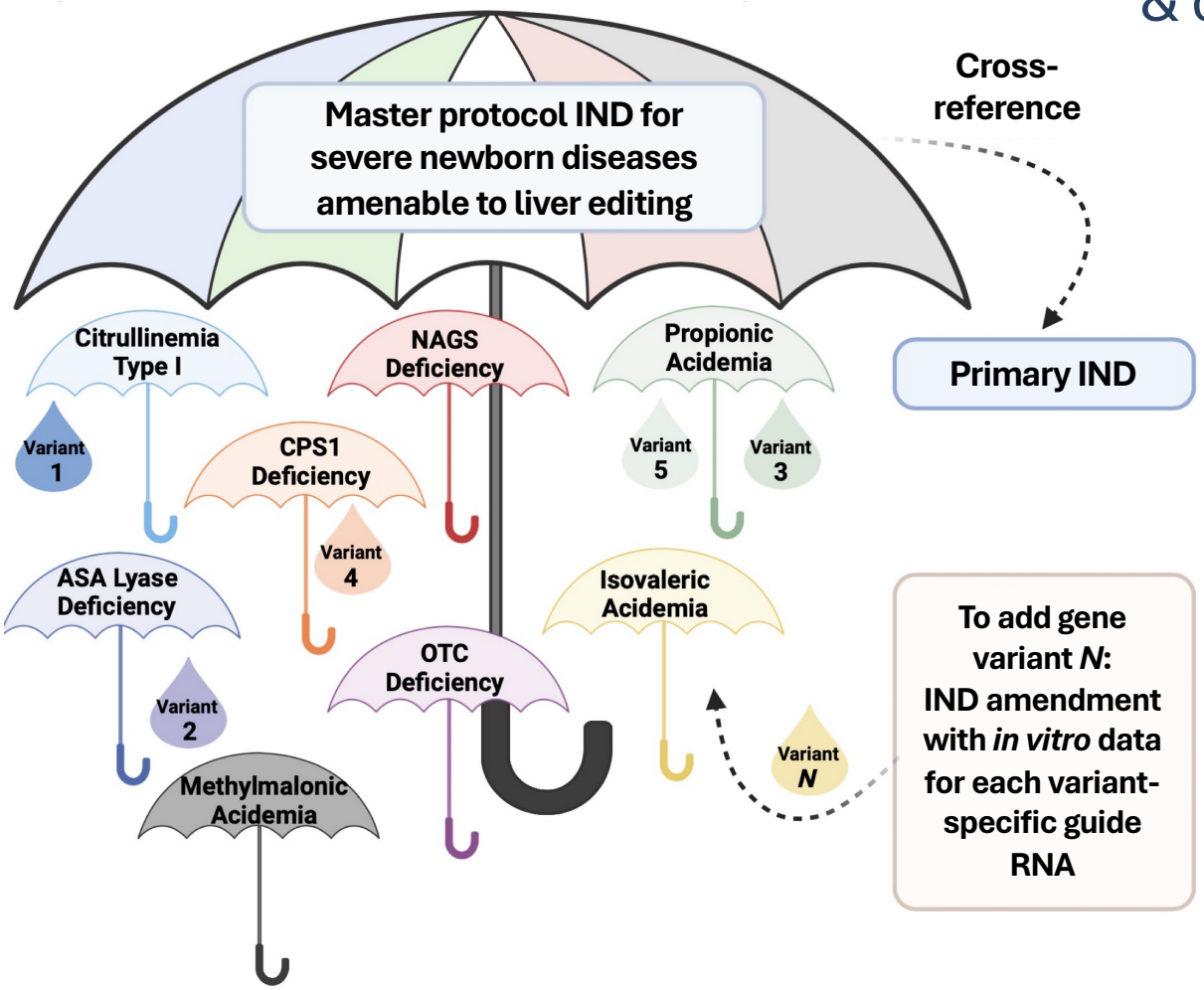
- Going forward, if using the same LNP formulation and adenine base editor to correct a pathogenic variant to wild-type in the liver:
  - Are additional nonhuman primate studies necessary?
  - Are additional animal toxicology studies necessary?
  - Are any animal studies necessary at all?
- When would cellular studies alone (on-target and off-target assessment) be sufficient? When does the severity and unmet need of a disease create a favorable balance of benefit and risk?

Screening of base editors for correction of other urea cycle disorder variants





Master protocol for urea cycle disorders & organic acidemias



NIH CGE
Somatic Cell Genome Editing

KJ and his parents

University of Pennsylvania
Xiao Wang
Sarah Grandinette
Dominique Brooks
Madelynn Whittaker
Aidan Quigley
Anne Marie Berry
Julia Hacker
Lauren Testa
Elena Kahn
Ananya Talikoti
Emily Feierman
Delaney Rutherford
Ping Qu
Nataliya Petrenko

Innovative Genomics Institute
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Petros Giannikopoulos
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Nicholas Tougas
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Michael Jepperson
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Helen Velishek
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Morgan Sturgeon
Christian Brommel
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Gavin Kurgan
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He Zhang
Kyle Kinney
Garrett Rettig

The Jackson Laboratory
Cathleen Lutz

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Chris Boshoff

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