UREA CYCLE DISORDERS ECHO *The Essentials*

National Urea Cycle Disorders Foundation

Project ECHO®

Welcome

Session 4

Long-term Management

Laura Konczal, MD, University Hospitals Cleveland Medical Center

UREA CYCLE DISORDERS ECHO

The Essentials

Session 4 : Long-term Management

Time	Content
5 minutes	Introductions and housekeeping
30 minutes	Didactic presentation and case study: Current treatment options, Laura Konczal, MD, University Hospitals Cleveland Medical Center
10 minutes	Group discussion
30 minutes	Didactic presentation and case study: Emerging treatment options, Laura Konczal, MD, University Hospitals Cleveland Medical Center
15 minutes	Group discussion

UREA CYCLE DISORDERS ECHO

The Essentials

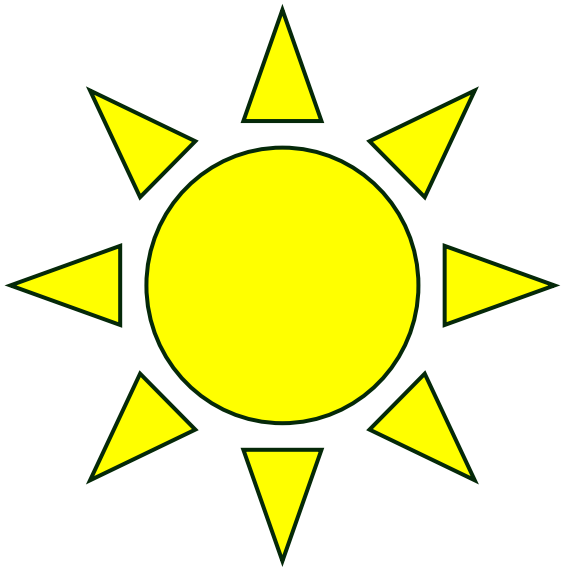
- If possible, please make sure to keep your camera turned on
- ECHO is intended for educational purposes only
- Presenters cannot and will not provide medical advice
- To receive CME credit, please remain in the session and complete the evaluation form using the link that will be provided after the presentation

Welcome

Introduce yourself in the chat

The Future is Bright

Current and Upcoming Therapies for UCD's on the Horizon



Laura L Konczal, MD
University Hospitals of Cleveland
Cleveland, Ohio

Urea Cycle Disorders Consortium

National Urea Cycle Disorders Foundation

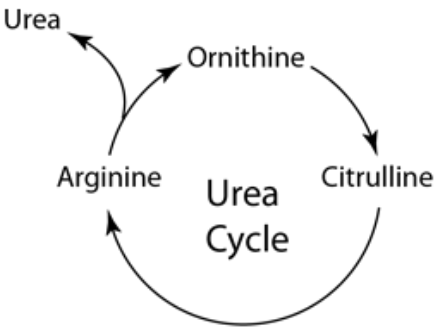
Disclosures

- Site PI for the Ultragenyx DTX301-CL301 and 301OTC02 AAV8 mediated OTCD gene therapy trials
- Site PI for the Moderna mRNA-3927-P101 and mRNA-3705-P101 studies for propionic acidemia
- Site PI for the Sanofi DFI7893_ 8400014 (AAV mediate gene therapy for PKU- now closed) and Sanofi Genzyme Rare Disease Registries

Objectives

- Review timeline of urea cycle and UCD discovery to current therapies
- Discuss current treatment options (acute and chronic management)
- Focus on liver transplantation
- Review limitations of current treatment options
- Discuss up and coming novel treatment options for UCD's pending approval or in clinical trials
- Future therapies in pre-clinical phase

Where did we begin?



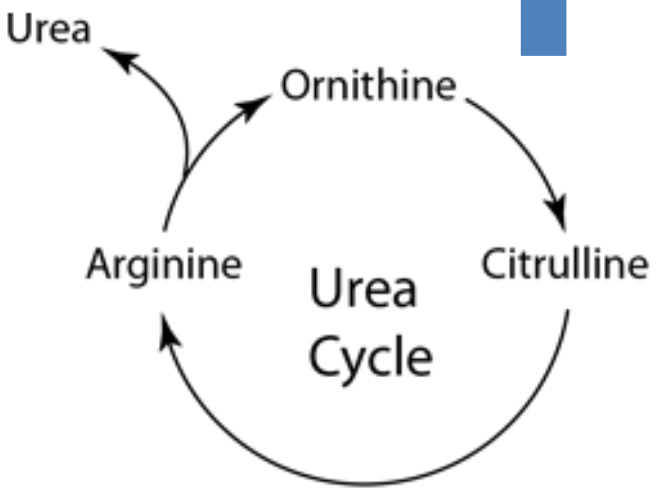
- **1932- Discovery of the Urea Cycle**
  - 1st metabolic cycle to be described
  - Also initially known as the "ornithine cycle of urea synthesis"
  - Hans Krebs and Kurt Henseleit (University of Freiburg in Breisgau, Germany)
- **1946-1957- Further Understanding of Urea Cycle Intermediates**
  - Carbamoyl phosphate, argininosuccinic acid added to the cycle as intermediates
  - Uncovered by researcher teams lead by Drs. Ratner and Cohen
  - Lead to the understanding of the modern understanding of the urea cycle
- **1958- Discovery of first Urea Cycle Disorder**
  - Argininosuccinic acid lyase deficiency
  - Reported by JD Allan, et al in Lancet (UK)
- **Early 1960's- Discovery of first proximal urea cycle disorder**
  - ornithine transcarbamylase deficiency

http://hyperphysics.phy-astr.gsu.edu/hbase/Organic/urea.html

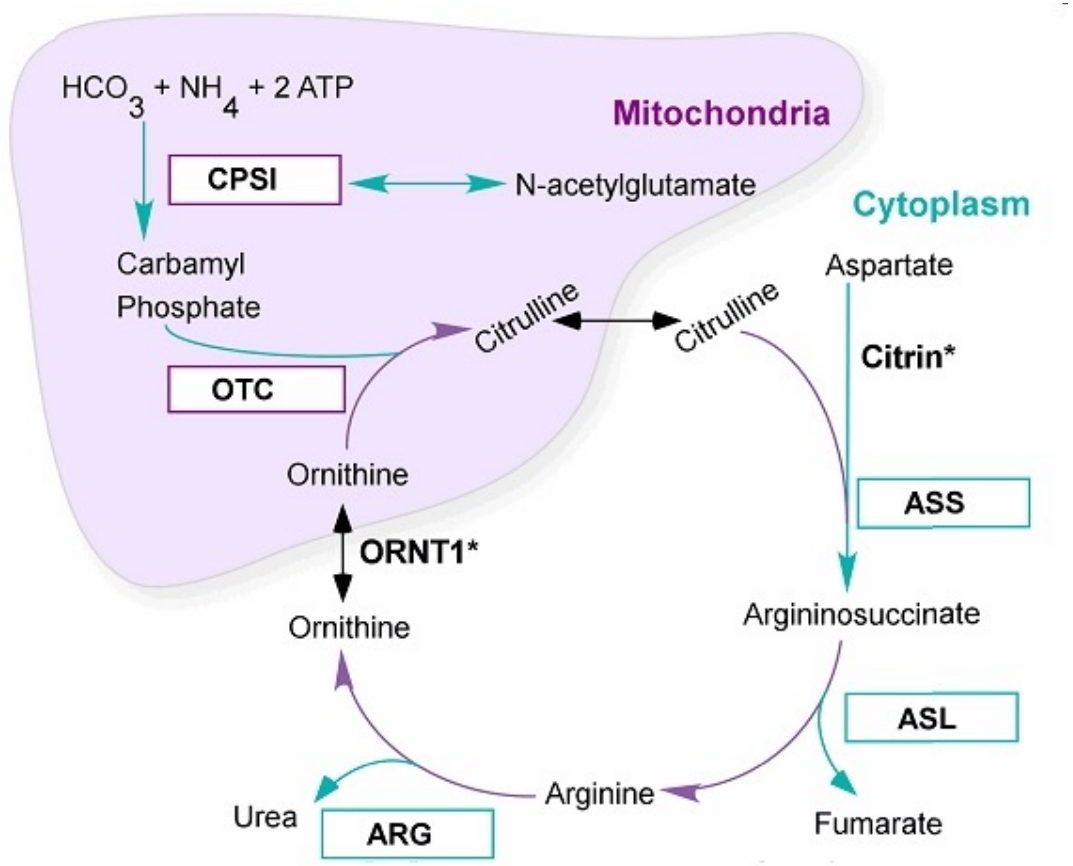
https://onlinelibrary.wiley.com/doi/pdf/10.1016/0307-4412(73)90048-4

A DISEASE, PROBABLY HEREDITARY, CHARACTERISED BY SEVERE MENTAL DEFICIENCY AND A CONSTANT GROSS ABNORMALITY OF AMINOACID METABOLISM. Allan, J.D. et al. The Lancet, Volume 271, Issue 7013, 182 - 187

Urea Cycle: discover to deeper understanding



http://hyperphysics.phy-astr.gsu.edu/hbase/Organic/urea.html



Nicholas Ah Mew, Maria Belen Pappa, Andrea L. Gropman, Chapter 57 - Urea Cycle Disorders, Editor(s): Roger N. Rosenberg, Juan M. Pascual, Rosenberg's Molecular and Genetic Basis of Neurological and Psychiatric Disease (Fifth Edition), Academic Press, 2015, Pages 633-647, ISBN 9780124105294, https://doi.org/10.1016/B978-0-12-410529-4.00057-7.

Early treatment of UCD's

Dietary restriction and amino acid use

- 1982- Michels et al reported very low protein diet supplemented with essential amino acids
  - lead to survival beyond 4 y of age in males with OTC deficiency
- Development of low protein/ low nitrogen essential amino acid metabolic formulas (developed later- 1980's and beyond)

Early Urea Cycle Management- Medication Development and Intervention

- 1983- Batshaw et al reported use of sodium benzoate as an ammonia scavenger and L-arginine supplementation to drive urea production
- 1984- Brusilow et al noted use of phenylacetate, sodium benzoate and L-arginine combination as ammonia scavengers, IV dextrose/lipids in combination to reduce ammonia in CPS1, OTC and citrullinemia
- 1992- First ammonia scavenger FDA approved for UCD treatment: **Phenylacetate (Ucephan)**
- 1996- Sodium phenylbutyrate FDA approved
- 1989- Korson et al reported the first liver transplant to treat a patient with OTC deficiency
- 1999- First gene therapy trial began (used adenovirus as a vector)

Today's Current Management Strategies for UCD's

- Dietary Protein Restriction
- Essential amino acid supplementation/ low nitrogen metabolic formulas
- L-arginine or L-citrulline to drive urea production (depending on disorder)
- Ammonia scavenger medications
- Liver transplantation in more severe cases

  Potential living donor liver transplants have made these more available
- Admission in times of crisis/ increased metabolic stress

  Ammunol, L-arginine, High dextrose containing IVF's Intralipids, Dialysis if needed (hemodialysis (preferred) or CVVH)

Historical Context & Impact on Current Treatment Options

- **Ammonia scavengers (early medications)**

  Acute (crisis) medications
  - Ammunol (sodium benzoate and sodium phenylacetate)
  - IV L-arginine

  Chronic (maintenance) medications
  - Sodium benzoate (enteral)
  - Sodium phenylbutyrate (Buphenyl)

- **Ammonia scavengers (recent developments)**

  Glycerol phenylbutyrate (Ravicti)

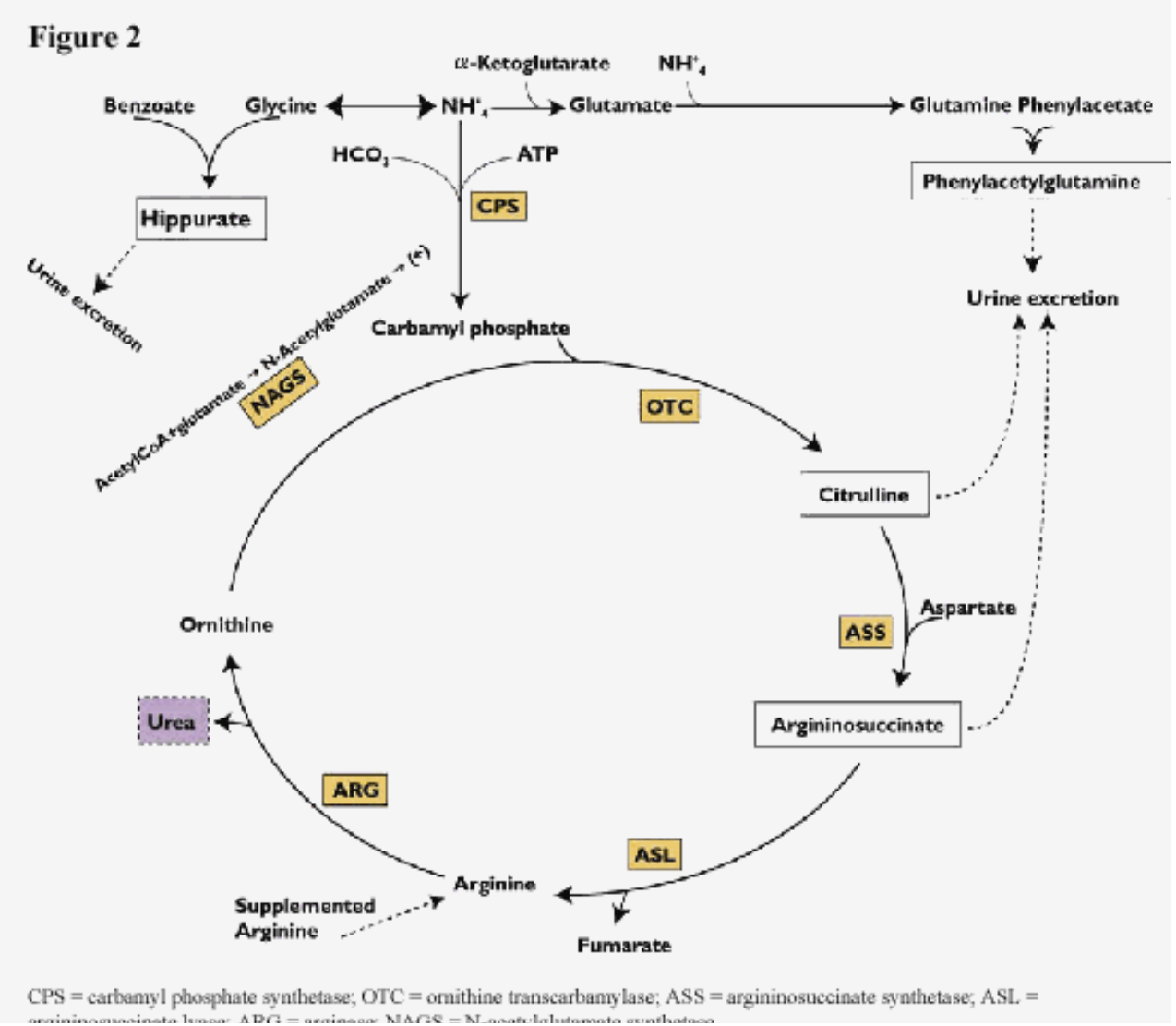
  Sodium phenylbutyrate (palatability enhanced)
  - Pheburane
  - Olpruva

Today's Ammonia Scavenger Options- Acute
Pros and Cons

- **Ammunol**
  - Combination of sodium benzoate and sodium phenylacetate (removes 3 N's)
  - IV medication
  - Acute crisis use only
  - Pregnancy class C
  - Risk of side effects from Ammunol (increases with higher doses)
    - Neurotoxicity (reversible)

      Somnolence, fatigue, lightheadedness, headache, neuropathy exacerbation, disorientation, altered taste, hearing loss, memory loss, cerebral edema
    - Hepatotoxicity
    - Marrow suppression
      - Anemia, cytopenias

- **IV L-arginine HCl**
  - Drives distal urea cycle to produce urea (removes 1 N)
  - Can be caustic to veins
    - Better via CVL
  - Vasodilator
    - decreased BP at high doses



CPS = carbamyl phosphate synthetase; OTC = ornithine transcarbamylase; ASS = argininosuccinate synthetase; ASL = argininosuccinate lyase; ARG = arginase; NAGS = N-acetylglutamate synthetase

Today's Ammonia Scavenger Options-Chronic
Pros and Cons

- Sodium phenylbutyrate- combines w glutamine to remove 2 N
  - **Buphenyl (original)**
    - Bitter noxious taste; large # tablets or powder
    - GI distress, decreased appetite
    - Irregular or cessation of menstrual periods (most common side effect)
    - Neurotoxicity (uncommon)
  - **Pheburane (new)**
    - Large amount of pellets w frequent dosing (5 or more times per day)
    - Can not dissolve coating (or bitter)
    - Neurotoxicity (rare)- somnolence, fatigue, lightheaded
    - Hypokalemia
    - Contains sucrose- avoid in diabetics, fructose intolerance, glucose-galactose malabsorption or sucrase-isomaltase insufficiency
  - **Olpruva (new)**
    - Frequent dosing (5 or more times per day)
    - Neurotoxicity- lightheadedness, fatigue, somnolence, N/V, headache, confusion
    - Hypokalemia

- Glycerol phenylbutyrate (Ravicti)
  - Removes 2 N (combines with glutamine)
  - Neurotoxicity- lightheadedness, fatigue, somnolence, headache, confusion, neuropathy
  - Hepatotoxicity
  - Pancreatic insufficiency or intestinal malabsorption can lead to reduced absorption
  - GI distress (N/V, discomfort), decreased appetite, diarrhea, gas
  - Marrow suppression (cytopenias, anemia)

- Sodium benzoate
  - Removes 1 N (combines w glycine)
  - Concentrated liquid w frequent dosing
  - Toxicity- somnolence
  - Gastrointestinal symptoms (nausea, vomiting, abdominal discomfort)
  - Allergic reactions (esp if allergic to aspirin)
  - Do not use with citric acid (or other acidic substances-> benzene)

Carbaglu for NAGS deficiency

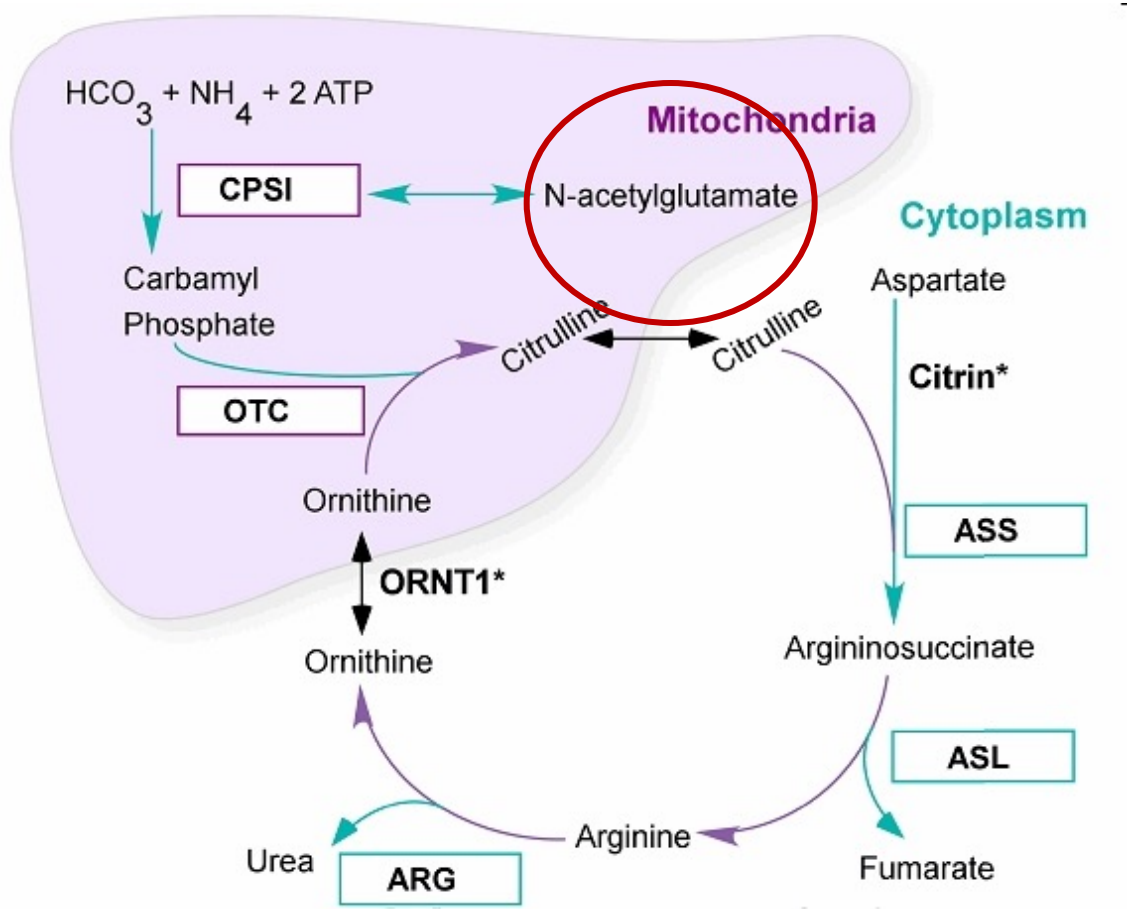
N-acetylglutamate synthase (NAGS) deficiency

N-acetylglutamate is the cofactor for CPS1 (first step of UC)

If body can not make N-acetylglutamate, CPS1 will not work effectively (missing cofactor)

Carbaglu is synthetic N-acetylglutamate

Treatment with Carbaglu is all that is needed in NAGS deficiency



Liver Transplantation

- 1989- First liver transplant for treatment of a UCD
- Over past ~10 years, liver transplant has become more common place as outcomes have proven to be excellent in patient with UCD's (and other hepatic based metabolic disorders)
- Living donor liver transplants have increased availability of livers for transplant
- Recent outcomes at experienced centers show >90% survival of patients and their transplanted liver graft
- Earlier transplant in more severe cases of UCD is more common

*Resources: National Urea Cycle Disorders Foundation (www.nucdf.org) STARZL Network (comprehensive liver transplant resources)

“Comparing Treatment Options for Urea Cycle Disorders” PCORI Funded Study

- Study proposed by NUCDF on behalf of UCD families, conducted in conjunction with Dr. Nicholas Ah Mew (PI) and team:
- Factors impacting decision regarding transplant vs medical management:
    - **Clinical:** Disease severity and/or stability
    - **Personal:** Burden on the family and the child
    - **Social:** Sharing experiences with other families impacting decision process & considerations for the patient’s independence
    - **System:** Access to quality metabolic care; cost and coverage of treatment; physician approach to guidance and treatment

Nate's Story

- Uncomplicated term pregnancy & delivery, nursing well for first 36h of life before d/c home
- By 48h- large emesis post feed then began unconsolably crying x 8-9h & stopped feeding
- Fell asleep by DOL 3, was difficult to wake, not eating.
- Saw PCP->sent home but still not eating so went to ED after 20h not eating & lethargy, no response to blood draw. Sent home but then PCP recalled to admit for observation due to mild hyperbilirubinemia
- Stayed in hospital x 6 days, lethargic, continually "sleeping" & not eating, for "observation" (but eventually did get some IV fluids)
- Legs began "cycling" motion (in retrospect suspected seizures)
- Nate became apneic on day 6 of admission and code called & transfer to ICU (9 days old)- but instead of labs tests, mother questioned about non-accidental trauma
- Stayed in coma in ICU until 11 days of age when ammonia finally sent- NH4 was >1500 umol/L (reference range <53 umol/L)
- Low protein diet, dextrose containing IVF's and Buphenyl started (no Ammunol, no dialysis)

Childhood/ Adolescence

- Generally stable until puberty but affected by significant developmental delays 2' to brain injury from very high ammonia
- At 12 y had gastroenteritis triggering first hyperammonemic crisis since infancy-> coma
- Instability of ammonia levels during puberty then stabilized
- Nate was stable until he was changed from Buphenyl to Ravicti then ammonia destabilized despite increasing doses w many hospital admissions; increasing liver transaminases while on Raviciti
- Transplant seriously considered for the first time at this point (age 18 y)

Crossroads for Nate's parents: continue medical treatment vs liver transplantation

Three Key Factors

1. Cost of ammonia scavenger and lifetime insurance cap
   - Ravicti was $120,000 per month
2. Future caregivers
   - Group home would be needed once parents could no longer care for him
   - Could a group home better manage:
     - specialized metabolic formulas, protein restrictions, ammonia scavenger medications, recognize signs and symptoms of high ammonia levels and understand when to seek emergency care when elevated ammonia is suspected or situations that may predispose to high ammonia

     OR

     - manage to give post transplant anti-rejection medications and get monitoring labs post transplant
3. Unstable on treatment regimen with Raviciti and feeling of loss of control over medical treatment decisions

Life After Transplant

18 y/o: went for liver transplant evaluation

20 y/o: Liver transplant (deceased donor) at UPMC

Nate is now nearly 10 years post transplant & doing well, transitioning to a group home

In his mother's words:

"Nate's transplant was the best thing we've done for him, and I mostly regret not doing it when he was 12 and became so unstable. One of the most telling things is after Nate woke up from transplant and began to recover he repeatedly told us how good he felt and how healthy he was. It makes me think that prior to transplant Nate probably never had a time when he wasn't feeling sick, nauseous, headachy etc."

"Our family stress level has decreased dramatically. First there isn't the constant worry of Nate getting really sick out of the blue. Nate was 20 years old when transplanted and for the first time ever I stopped checking in his room every night before we would go to bed. I always popped in and took a big breath to see if I smelled vomit - a first sign of high ammonia."

"The medications post transplant are common and easy to come by so no worries on shortages, problems with shipping, forgetting something necessary when traveling and not having access to it, etc."

"Nate's medical care is so much more mainstream. He can be seen by a wider array of physicians that know how to treat him and what to do when he has issues"

Downside to current treatments

- **Diet**-
  - difficult to follow, especially when not home or with peers (teens and young adults especially), feeling hungry, expensive especially if formula not covered in your state
- **Medications**-
  - expense, side effects
- **Liver transplant**-
  - risks of complications and even death, major surgery with long recover, must find a suitable donor, long term use of anti-rejection medications with subsequent immunosuppression, risk of rejection or failure of the graft (potentially requiring a second transplant), expense
- **Acute Treatment in crisis**-
  - side effects, expense, not available everywhere, complications from dialysis

Despite all of the above treatment options, ***death or permanent neurologic damage can still occur*** *as complications from a hyperammonemic crisis or in the case of transplant, complications/ rejection*

Break for Questions/Discussion

Current Treatment Options

10 minutes

Seeking better treatment options:

Recent Therapies in Development/ Current Clinical Trials

Seeking better treatment options:

Recent Therapies in Development/ Current Clinical Trials

- **Completed**
  - **Pegzilarginase for Arginase 1 deficiency**
    - Enzyme replacement therapy pending FDA approval
  - Hepatic liver cell/ hepatic stem cell treatment
    - 2 trials terminated in US/ 1 Completed in Europe
      - **Cytonet hepatocyte transplantation trial (US)**
        - From 3/2/10 through 2/8/16
        - Goal: infuse human liver cells (hepatocytes) from unrelated donors via the portal vein (multiple treatments) to bridge to liver transplant at ideal weight after 8 weeks while stabilizing the patient
        - Terminated after enrolling 10 patients
      - **Hepatocyte Transplantation for Liver Based Metabolic Disorders**
        - UPMC sponsored from 3/2011 through 3/31/2022 after enrolling 5 patients
        - Involved partial irradiation of liver in pt w metabolic disease & portal vein infusion of human hepatocytes (as an alternative to liver transplant)
        - Terminated
      - **Cellaion SA Study to Evaluate the Efficacy of HepaStem in Urea Cycle Disorders Paediatric Patients (HEP002)**
        - Study completed (10/2014 through 03/2017) after enrolling 5 patients in Belgium, France, Poland and Spain
        - No results posted

Companies Investing in
Clinical Programs for UCDC

National Urea Cycle Disorders Foundation

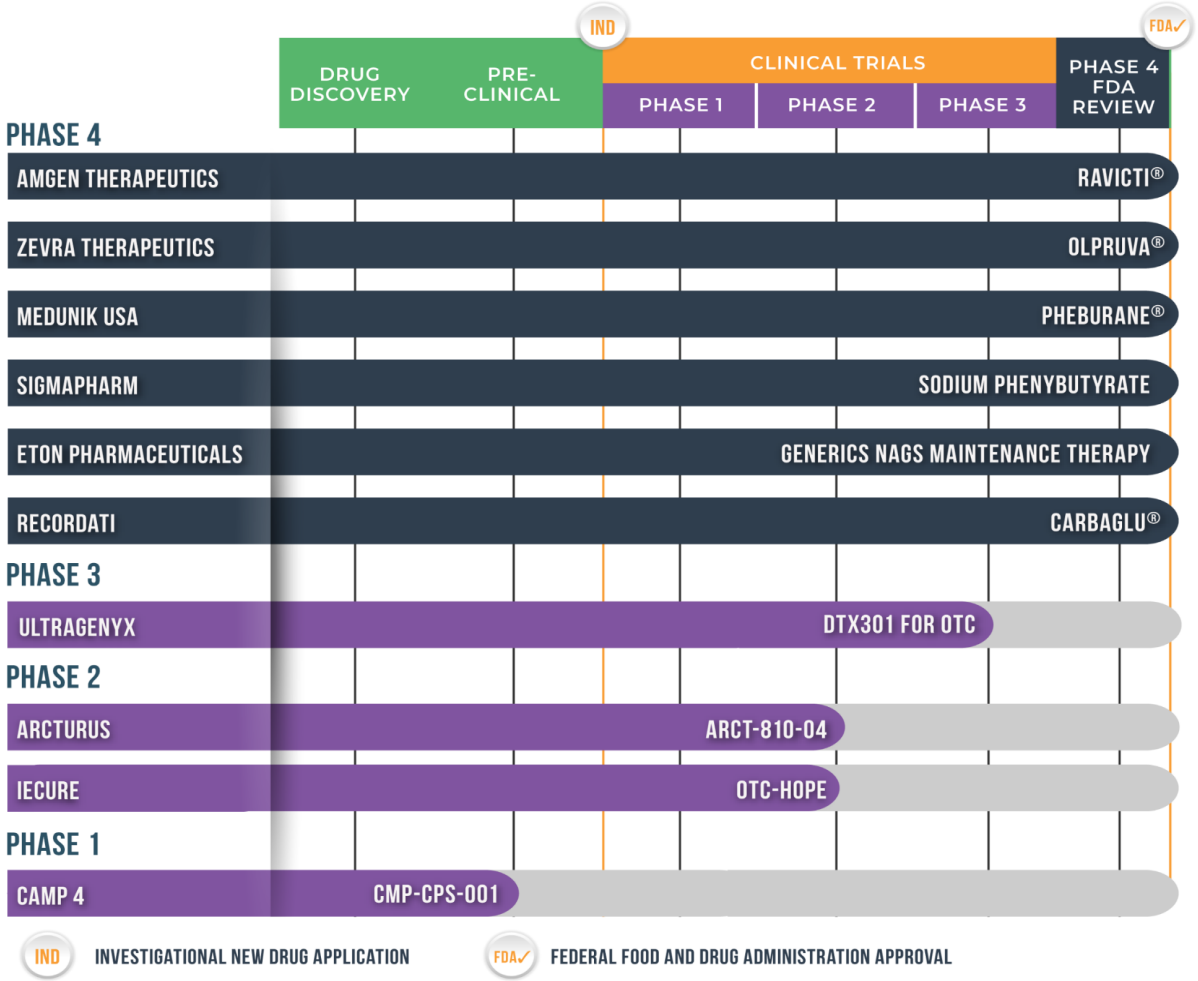


Image from NUCDF Website: https://ucdnews.org/

Pegzilarginase enzyme replacement therapy for Arginase 1 deficiency

Efficacy and safety of pegzilarginase in arginase 1 deficiency (PEACE): a phase 3, randomized, double-blind, placebo-controlled, multi-centre trial

Rossana Sanchez Russo,[a] Serena Gasperini,[b] Gillian Bubb,[c] Linda Neuman,[c] Leslie S. Sloan,[c] George A. Diaz,[d] and Gregory M. Enns,[e,*] on behalf of the PEACE Investigators

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[d]Department of Genetics and Genomic Sciences, Icahn School of Medicine at Mount Sinai, New York City, NY, United States
[e]Division of Medical Genetics, Department of Pediatrics, Stanford University School of Medicine and Lucille Packard Children's Hospital, Stanford, CA, United States

Summary

Background Arginase 1 Deficiency (ARG1-D) is a rare debilitating, progressive, inherited, metabolic disease characterized by marked increases in plasma arginine (pArg) and its metabolites, with increased morbidity, substantial reductions in quality of life, and premature mortality. Effective treatments that can lower arginine and improve clinical outcomes is currently lacking. Pegzilarginase is a novel human arginase 1 enzyme therapy. The present trial aimed to demonstrate efficacy of pegzilarginase on pArg and key mobility outcomes.

Methods This Phase 3 randomized, double-blind, placebo-controlled, parallel-group clinical trial (clinicaltrials.gov NCT03921541, EudraCT 2018-004837-34), randomized patients with ARG1-D 2:1 to intravenously/subcutaneously once-weekly pegzilarginase or placebo in conjunction with their individualized disease management. It was conducted in 7 countries; United States, United Kingdom, Canada, Austria, France, Germany, Italy. Primary endpoint was change from baseline in pArg after 24 weeks; key secondary endpoints were change from baseline at Week 24 in Gross Motor Function Measure part E (GMFM-E) and 2-min walk test (2MWT). Full Analysis Set was used for the analyses.

Findings From 01 May 2019 to 29 March 2021, 32 patients were enrolled and randomized (pegzilarginase, n = 21; placebo, n = 11). Pegzilarginase lowered geometric mean pArg from 354.0 μmol/L to 86.4 μmol/L at Week 24 vs 464.7 to 426.6 μmol/L for placebo (95% CI: −67.1%, −83.5%; $p < 0.0001$) and normalized levels in 90.5% of patients (vs 0% with placebo). In addition, clinically relevant functional mobility improvements were demonstrated with pegzilarginase treatment. These effects were sustained long-term through additional 24 weeks of subsequent exposure. Pegzilarginase was well-tolerated, with adverse events being mostly transient and mild/moderate in severity.

Interpretation These results support pegzilarginase as the first potential treatment to normalize pArg in ARG1-D and achieve clinically meaningful improvements in functional mobility.

Funding Aeglea BioTherapeutics.

eClinicalMedicine 2024;68: 102405
Published Online 12 January 2024
https://doi.org/10.1016/j.eclinm.2023.102405

The PEACE Phase 3 clinical trial showed a 76.7% plasma arginine reduction and 90.5% of pegzilarginase treated patients reached normal plasma arginine levels.

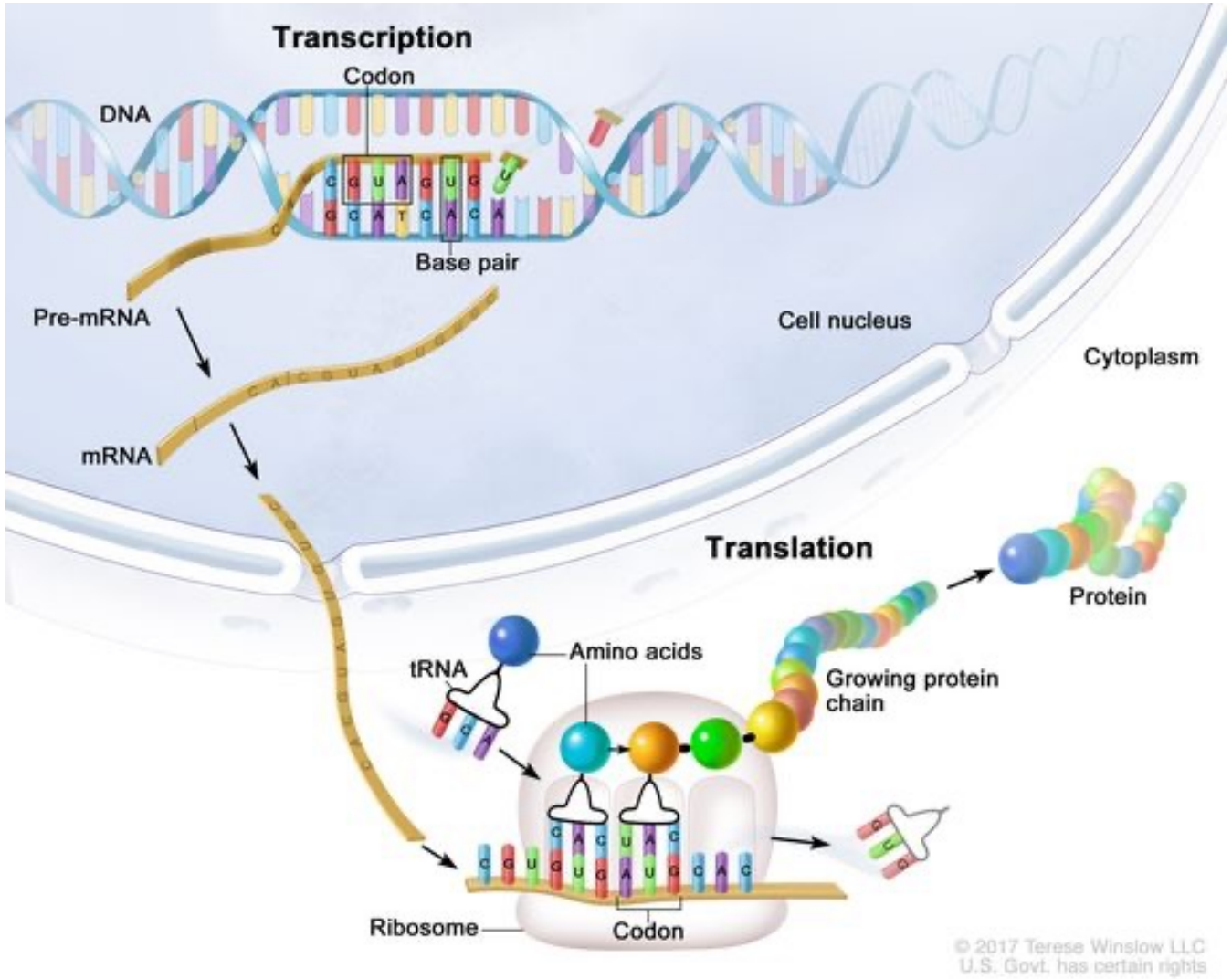
The arginine reduction was accompanied by a positive trend in Gross Motor Function Measure Part E, a measure of patient mobility.

Despite positive findings, the U.S. Food and Drug Administration (FDA) requested additional data to support Effectiveness including showing that plasma arginine and metabolite reduction predicts clinical benefit in patients with ARG1-D

Approved in Europe and manufactured by Immedica Pharma AB (Sweden) as Loargys

mRNA Therapies

Review of translation/ protein production



OTC deficiency mRNA therapy

Preclinical Mouse Data

Received: 14 December 2021 | Revised: 3 February 2022 | Accepted: 4 February 2022

DOI: 10.1002/ctd2.33

CLINICAL AND TRANSLATIONAL DISCOVERY

WILEY

RESEARCH ARTICLE

Restoring ornithine transcarbamylase (OTC) activity in an OTC-deficient mouse model using LUNAR-OTC mRNA

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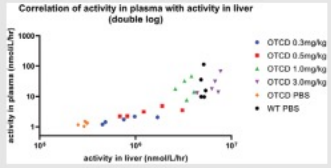
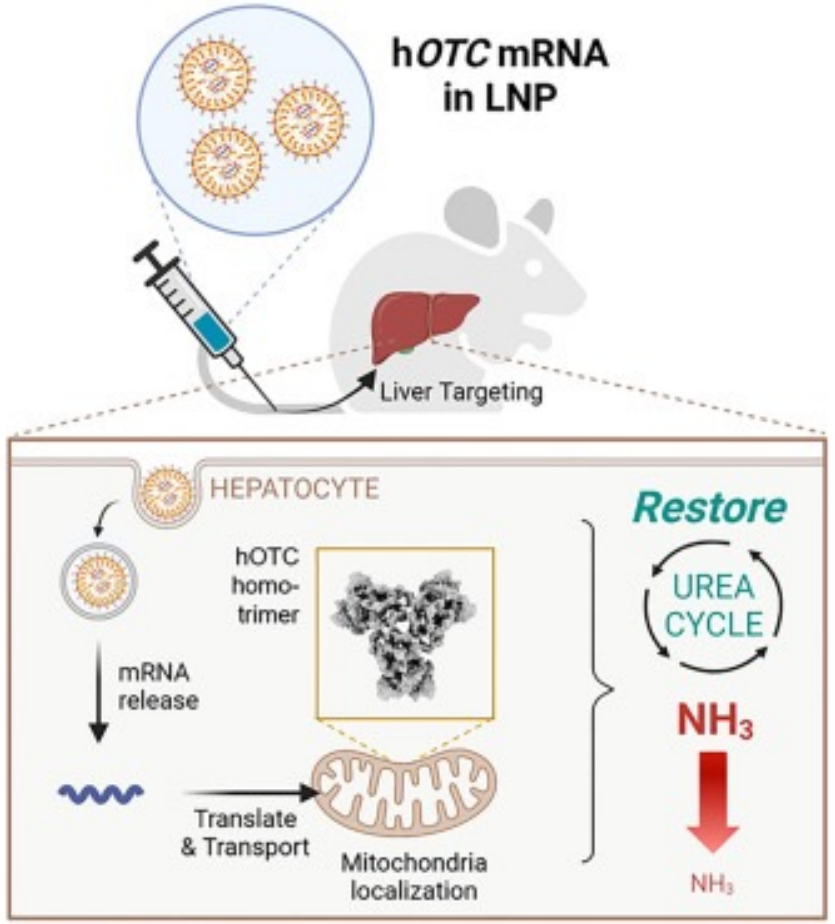
Graphical Abstract



Patients of ornithine transcarbamylase (OTC) deficiency lack the OTC activity in the liver. Using an OTC deficiency mouse model, we demonstrated that LUNAR-OTC, an OTC mRNA medicine, enables full restoration of OTC activity in mouse liver. Moreover, the OTC activity in the plasma is a surrogate assay for OTC activity in the liver, which can serve as a pharmacodymaic endpoint without the necessity of liver biopsies.



mRNA Clinical Trial for OTC deficiency Phase 1/2

Pipeline of Arcturus-Owned mRNA Therapeutic Candidates

Franchise	Product Name	Indication	Global Prevalence	Pre-Clinical	Phase 1	Phase 2	Phase 3	Anticipated Milestones
Hepatic	LUNAR®-OTC (ARCT-810)	Ornithine Transcarbamylase Deficiency	> 10,000					Phase 2 (EU+US) Interim Data Q2 2025

A Study to Evaluate the Pharmacodynamics and Safety of ARCT-810 in Participants with OTCD

- Currently Enrolling- start date 11/4/2024 with estimated completion 9/1/2026
- Goal of 9 patients
- Males and females = or >12 y/o who have hx of symptomatic hyperammonemia or elevated glutamine
- On treatment but no changes for 28d prior to enrolling
- Excluded if prior gene therapy or liver derived stem cell transfer in past 2y or if liver transplant has occurred

mRNA Clinical Trial for OTC deficiency Phase 1/2

- What is involved?
  - All participants will receive 5 doses of study drug (ARCT-810) via infusion at one of three dose levels
  - Ureagenesis via stable isotope ureagenesis assay will be performed to measure efficacy
  - Ammonia and glutamine levels will be monitored
  - Pharmacokinetics will be evaluated
  - Length of study is 85 days
  - Travel to site in Chevy Chase, MD

Restoration of Urea Cycle Function in OTC Deficiency: Glutamine Reduction Following ARCT-810 mRNA Therapy Across Two Clinical Studies*

Results:

- Mean glutamine levels declined significantly over time following initiation of mRNA therapy and increased following drug discontinuation.
- LMM analysis across all treated patients showed a mean reduction of 2.50 µmol/L/day ($p = 0.0055$)
- EU Study's slope (–1.82 µmol/L/day, $p = 0.016$) and US Study's slope (–4.38 µmol/L/day, $p = 0.004$).

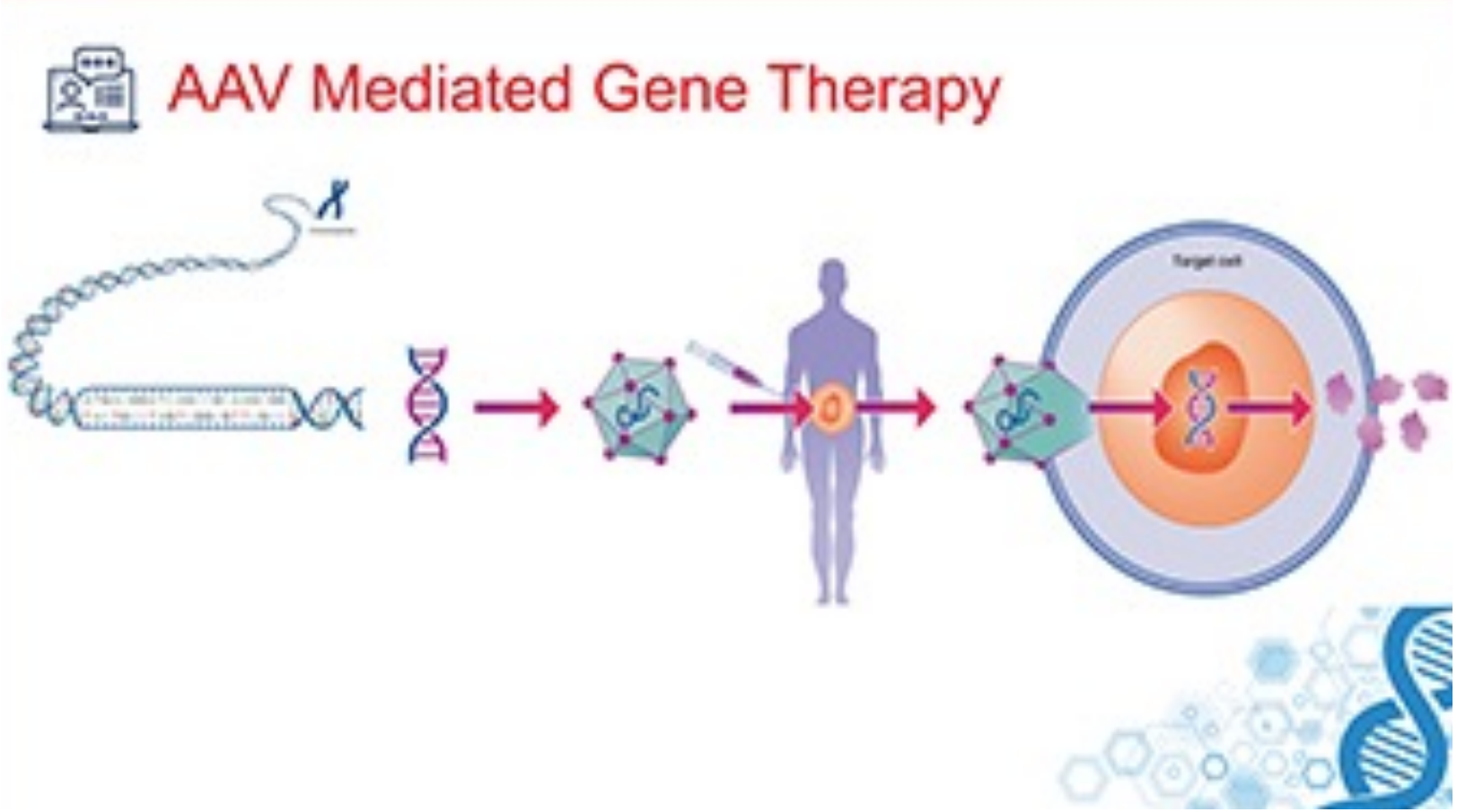
Conclusions:

- Shows preliminary biomarker evidence of mRNA-based enzyme replacement following ARCT-810 administration in a monogenic metabolic disease.
- Reduction in plasma glutamine following ARCT-810 administration supports the potential utility of glutamine as an indirect pharmacodynamic marker of improved urea cycle function with ARCT-810 in OTC deficiency.

*Benjamin Greener1, Rachael Batabyal2, Juergen Froehlich1, Constance Crowley1, David Geller1, Brock Pittenger1, Laura Reck Cechinel2, Laura Allan2, Kerri Gallagher2, Pad Chivukula1, Tamanna Roshan Lal2, Rob Freishtat2, Marshall Summar2

1 Arcturus Therapeutics, San Diego, CA, USA ; 2 Uncommon Cures, Chevy Chase, MD, USA

Gene Therapy
Nonintegrating Gene Addition



https://genetherapy.isth.org/education/interactive-modules/adeno-associated-viral-aav-vector-gene-therapy-application-to-hemophilia

DTX301 (avalotcagene ontaparvovec) Investigational Gene Therapy for OTC Deficiency

- OTC gene transfer may correct the OTC enzyme deficiency, improve patient quality of life and lengthen the lifespan by reducing:
  - Ammonia and glutamine levels at baseline
  - Reduce the burden of life-long medications (ammonia scavengers), dietary protein restriction and formulas
  - Risk of going into a catabolic state during times of physiologic stress
  - Risk of hyperammonemic crises or death during illness or infection
- DTX301 (avalotcagene ontaparvovec) is an adeno-associated virus serotype 8 (AAV8) vector containing a codon-optimized, human, wild-type *OTC* gene with a liver-specific promoter and enhancer
- DTX301 is dosed as a single peripheral intravenous infusion in conjunction with high dose prednisolone taken for 4 weeks before tapering off over the course of several weeks (to prevent secondary hepatic and systemic inflammatory response to the AAV8 vector)

CAPtivate (NCT02991144), a Global, Multicenter, Open-label Phase 1/2 Dose-finding Trial

- **Screening**
  (Day -35 to 0)

- **Study population:**
  Adults with late-onset, stable OTC deficiency and no active liver disease or signs/symptoms of hyperammonemia for 28 days before screening
- N = 11 patients

Single IV infusion of DTX301 on Day 1

Dosing

Cohort 1: **3.4 x 10^{12} GC/kg (n=3)**
Cohort 2: **1.0 x 10^{13} GC/kg (n=3)**
Cohort 3: **1.7 x 10^{13} GC/kg (n=3)**
Cohort 4: **1.7 x 10^{13} GC/kg with prophylactic oral steroid taper (n=2)**

52-week study period

- Blood monitoring every 4 days for first 12 weeks
- Clinical evaluations every 2-4 weeks

8-year extension study

Durable efficacy and safety of DTX301: Long-term follow up of a phase 1/2 trial in adults with ornithine transcarbamylase deficiency

Tarekegn Geberhiwot,[1] Janet A. Thomas,[2] Margo Sheck Breilyn,[3] Maria Luz Couce,[4] Nathalie Guffon,[5] Leticia Ceberio Hualde,[6] Aneal Khan,[7] Laura L. Konczal,[8] Melinda Peters,[9] Lipika Ghosh,[10] J. Lawrence Merritt, II,[10] on behalf of the CAPtivate DTX301 investigators

Goals of Phase 1/2

Main Endpoint:

Reduced incidence of adverse events (AEs)

Secondary Endpoints:

- 24-hour plasma ammonia levels
- Reduction in baseline disease management (reduced medication dose and increased protein intake)
- **Complete Responder:** complete discontinuation of ammonia-scavenging drugs and protein-restricted diet
- **Responder:** 50% reduction in baseline disease management
- The decision to titrate ammonia-scavenging drugs and liberalize diet was made upon review of all available data, including ammonia levels and changes in clinical signs and symptoms

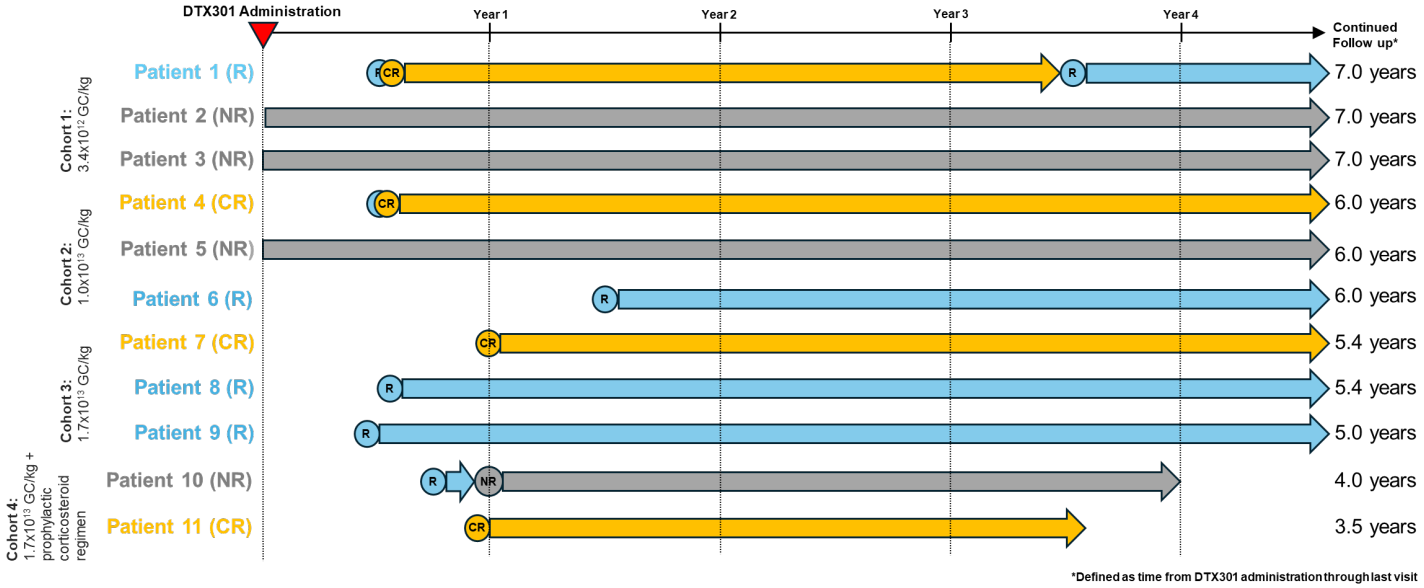
Safety DTX301

- To date, no treatment-related serious AEs, infusion-associated reactions, or dose-limiting toxicities have been reported
- All AEs were grade 1-2, except for 1 patient with serious grade 3 AEs
- Serious AEs included vomiting, nausea, compression fracture, hyperammonemia, hyperammonemic crisis, bone infarction, and osteonecrosis
- None of these AEs were considered related to DTX301
- During the phase 1/2 study, 8 patients experienced asymptomatic ALT increases possibly related to study drug
  - Consistent with those seen in other AAV gene transfer clinical trials
  - ALT increases lasted from 20-168 days with peak ALT levels from ~1.5-6.0 x ULN
  - ALT increases resolved with a protocol-specified tapering regimen of oral corticosteroids administered in the outpatient setting.
- During LTFU, 3 patients experienced mild ALT elevations (all considered by the investigator to be unrelated to DTX301)

Steroid-Related Adverse Events

- There were no corticosteroid-related hyperammonemic crises
- Five patients had non-serious corticosteroid-related AEs
- Most corticosteroid-related AEs were mild (grade 1)
  - Weight gain, emotional lability, increased appetite
- One patient had serious grade 3 AEs of bone infarction, osteonecrosis, and subcortical collapse
  - Bone changes are known potential complications of high dose steroid treatment

Clinical Response to DTX301



The overall mean (SD) rate of ureagenesis increased 77% from 137 (88) µmol*h/kg at Baseline to 224 (178) at Week 52

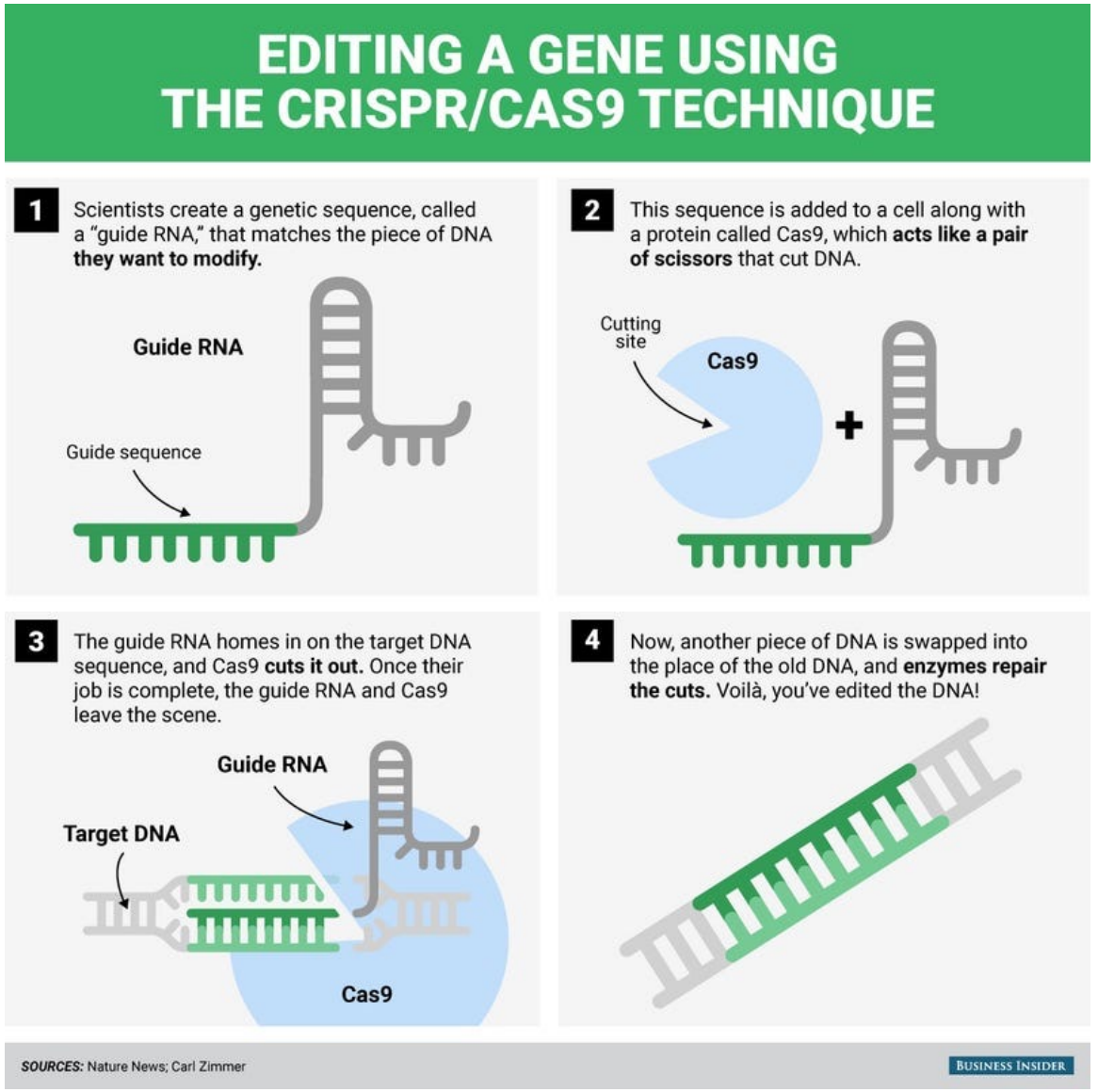
For most patients, plasma ammonia levels decreased towards normal within 6 weeks of DTX301 administration

- 7 of 11 patients have shown a meaningful clinical response to DTX301; 4 were Complete Responders and 3 were Responders
- 10 of 11 patients have remained clinically stable from the time of DTX301 administration

Phase 3- DTX301-CL301

- Double blinded, placebo controlled study with cross over
- Patients 12 y and older with OTC deficiency and at least one episode of symptomatic hyperammonemia
- Closed to enrollment at this time; patients are now proceeding to the unblinded phase of the study (although data is still blinded)

Gene Editing:
CRISPR/CAS9 Technique



https://www.businessinsider.com/crispr-gene-editing-explained-2015-12

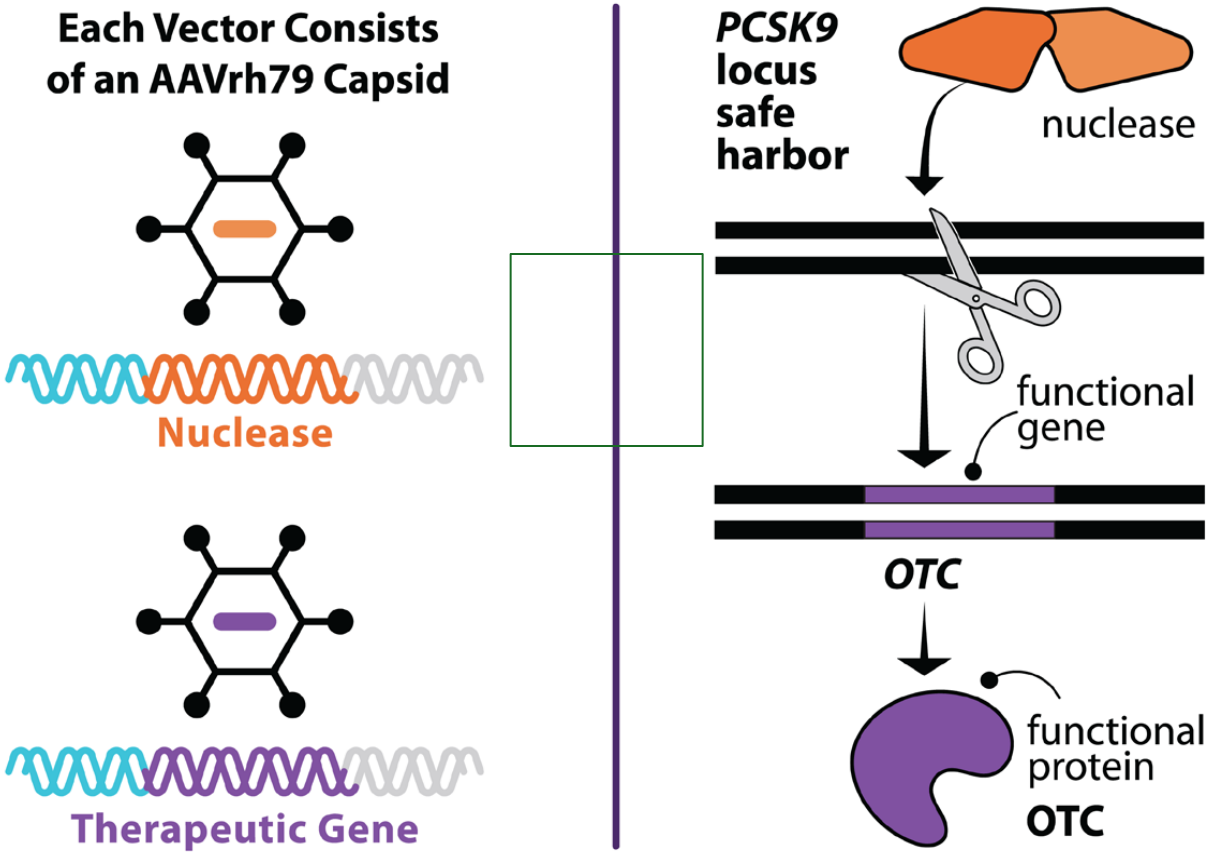
First UCD Gene Editing Clinical Trial

OTC-HOPE: The first in vivo, liver directed, AAV-mediated, gene insertion clinical trial in infants with Ornithine Transcarbamylase Deficiency

- An Open Label Study to Investigate ECUR-506 in Male Babies Less than 9 months of age with Neonatal Onset OTC Deficiency
- Phase 1/2/3 Open Label study for safety, efficacy and dose finding (Recruiting)
- Uses AAV vector to carry the CRSPR/CAS9 to the hepatocytes
- Start date: 4/8/24 (goal is to finish by 9/2026)
- 3 sites enrolling: Children's Hospital of Colorado, UCLA, Icahn School of Medicine at Mount Sinai (NYC)

Dual AAV Vectors:
ARCUS Nuclease and *OTC* Donor Gene/Gene Insertion

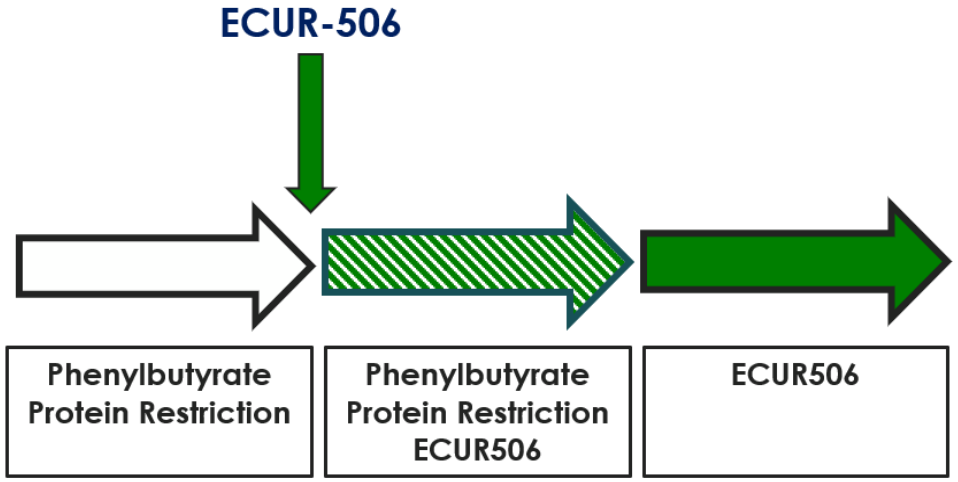
ECUR-506



G Cohn et al, 2025: OTC-HOPE: The first in vivo, liver directed, AAV-mediated, gene insertion clinical trial in infants with Ornithine Transcarbamylase Deficiency

Patient 1:
Safety over 10 months follow-up post dose

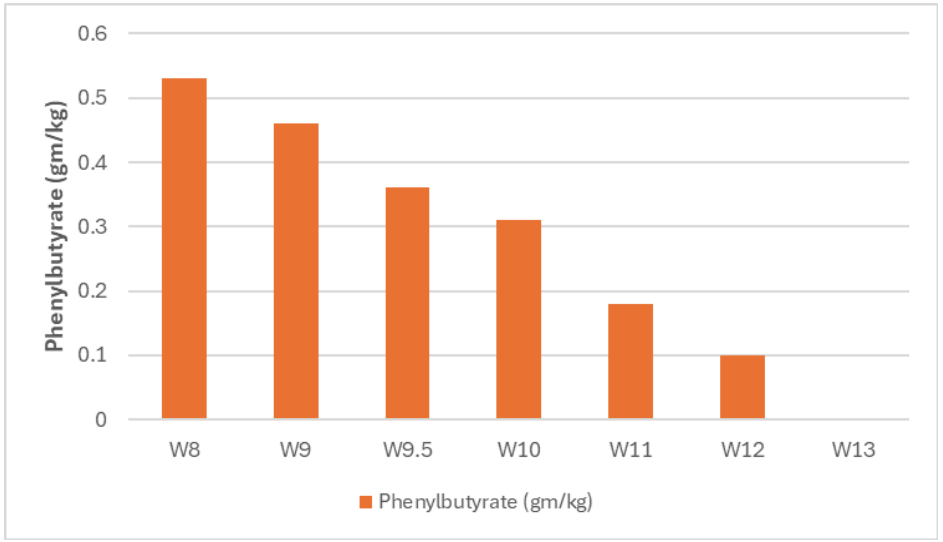
- Pt dosed at 6.5 months of age after two HAC related to neonatal onset OTC deficiency
- Low dose of ECUR-506 (1.3 x1013 GC/kg) in the OTC-HOPE study was well tolerated
- Asymptomatic, Grade 3 transaminase elevations at 4 weeks, managed with steroids & Tacrolimus-> resolved at 8 weeks
- Otherwise, unremarkable clinical course & safety labs throughout
  - (safety labs= chemistries, coags, total bili, alkaline phosphatase, GGT, hematology)
- Weight gain normal (50-75%ile)
- ECGs- normal



G Cohn et al, 2025: OTC-HOPE: The first in vivo, liver directed, AAV-mediated, gene insertion clinical trial in infants with Ornithine Transcarbamylase Deficiency

Patient 1: 10 Months Post-ECUR-506 Exposure-Complete Clinical Response

- Reduction in glutamine levels in weeks 6 & 7 to <lower limit of normal post-ECUR-506 prompted weaning of nitrogen scavenger therapy starting week 8 post-ECUR-506
- D/C of nitrogen scavenger therapy was achieved 12 weeks post ECUR-506 administration
- Protein intake liberalized (week 12) and normalized (week 15-Mo 10)
- Increased mean BUN & decreased mean ammonia indicates improved UC function
- No further hyperammonemic crises (off both medical and dietary treatment)
- Now off liver transplant list



G Cohn et al, 2025: OTC-HOPE: The first in vivo, liver directed, AAV-mediated, gene insertion clinical trial in infants with Ornithine Transcarbamylase Deficiency

**A complete clinical response is defined as the discontinuation of scavenger medication for a minimum duration of 28 da without any additional reductions in daily protein intake during this time period

Gene Editing

Base Editing Technique vs CRISPR Cas9 gene editing

CRISPR Cas9 gene editing

- Double stranded cut in DNA allows insertion, deletion or modification/ replacement of entire sequences of DNA
- Can be used in a variety of tissues
- Possible off target effects
  - unintended sites in the genome are cut and can introduce a clinically significant pathogenic variant that did not exist before like deletions and/or insertions to nontargeted genes

Base Editing

- No double stranded cuts in DNA
- Precise changes to individual DNA bases are made
- Two types of base editors
  - Cytosine Base Editors (CBEs)
    - Cytosine (C) > Thymine (T)
  - Adenine Base Editors (ABEs)
    - Adenine (A) > Guanine (G)
- More controlled and predictable gene edits
- Valuable for correcting point mutations
- Limited to only converting certain bases (C>T or A>G) in point mutations but not as applicable for large scale genome modifications

The NEW ENGLAND JOURNAL of MEDICINE

CURRENT ISSUE SPECIALTIES TOPICS

ORIGINAL ARTICLE | BRIEF REPORT

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

Authors: Kiran Musunuru, M.D., Ph.D. , Sarah A. Grandinette, B.S., Xiao Wang, Ph.D., Taylor R. Hudson, M.S., Kevin Briseno, B.S., Anne Marie Berry, M.S., Julia L. Hacker, M.S., +37 , and Rebecca C. Ahrens-Nicklas, M.D., Ph.D. Author Info & Affiliations

Published May 15, 2025 | N Engl J Med 2025;392:2235-2243 | DOI: 10.1056/NEJMoa2504747 | VOL. 392 NO. 22

Summary

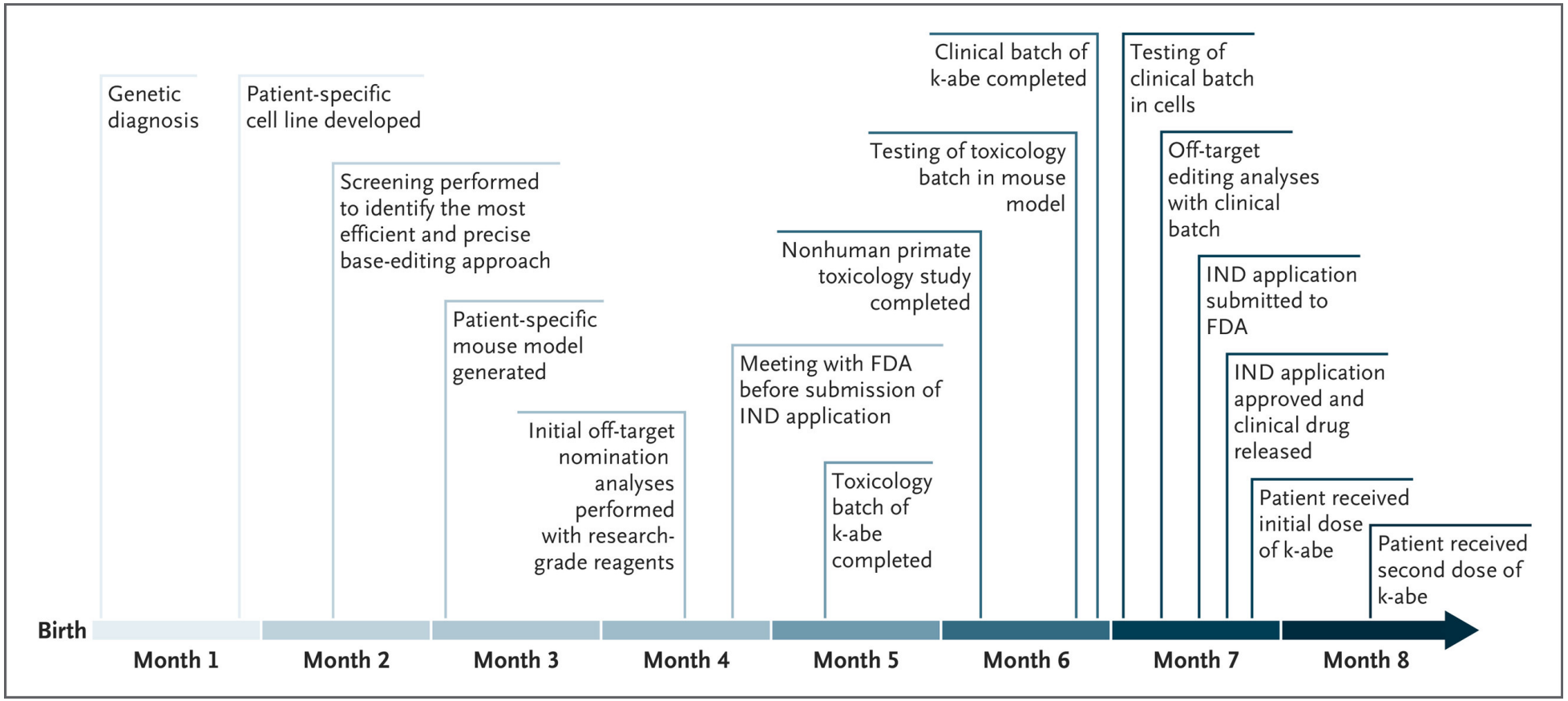
Base editors can correct disease-causing genetic variants. After a neonate had received a diagnosis of severe carbamoyl-phosphate synthetase 1 deficiency, a disease with an estimated 50% mortality in early infancy, we immediately began to develop a customized lipid nanoparticle–delivered base-editing 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

https://npr.brightspotcdn.com/dims3/default/strip/false/crop/5393x3595+0+0/resize/900/quality/85/format/webp/?url=http%3A%2F%2Fnpr-brightspot.s3.amazonaws.com%2F9b%2F6e%2F7a3d9ab645f1879a78fa39747b28%2Fkj-reaching-out-post-infusion.jpeg

Personalized Treatment of Infant with Neonatal onset Carbamoyl Phosphate Synthase (CPS1) deficiency

- Presentation in the neonatal period w/ severe hyperammonemia (1703 umol/L (ref range 9-33 umol/L), lethargy & respiratory distress in the first 48 h of life
- CRRT (dialysis) was initiated and ammonia normalized before initiating dietary protein restriction via specialized metabolic formula, supplementation w/ L-citrulline & ammonia scavenger therapy (glycerol phenylbutyrate)
- Diagnosis confirmed via molecular testing (two truncating pathogenic variants in trans in CPS1)
- Despite treatment and initial “honeymoon” phase of stability, patient had recurrent episodes of hyperammonemia beginning at 100 days of life
- Listed for liver transplantation at 5 m/o while his personalized gene editing therapy was being developed simultaneously

Timeline of diagnosis to customized treatment at U Penn/ CHOP



Patient-Specific In Vivo Gene Editing to Treat a Rare Genetic Disease
Author: Kiran Musunuru, Sarah A. Grandinette, Xiao Wang, et al
Publication: The New England Journal of Medicine
Publisher: Massachusetts Medical Society
Date: Jun 12, 2025

Follow-up so far

- Patient tolerated both doses without event
- Was able to reduce his glycerol phenylbutyrate dose by half
- Was able to get through two episodes of viral illness without decompensation
- Remains stable but still requires treatment with an ammonia scavenger medication and dietary protein restriction/ metabolic formula
- Still is affected with CPS1- but disease in milder post treatment than before treatment
- Long term follow-up will need to be performed to determine long term duration

Risks and Limitations of Gene Therapy & Gene Editing

Risks

- **AAV related hyperinflammatory response- especially acute hepatitis**
  - Managable with prophylactic immunosuppression (specifically high dose steroids)
- **Microangiopathy**
  - Has not been reported in UCD related studies
  - Has been seen in gene therapy trails for other metabolic diseases (including organic acidemias)
- **Death in a small number of patients**
  - Mainly in treatment of musculoskeletal disorders and using AAV other than AAV8
- **Steroid related side effects**
  - Weight gain, blood glucose elevation
  - Bone changes- decreased bone density, increased risk of fracture
  - prophylactic immunosuppression with sirolimus and tacrolimus was initiated at CHOP
- **Theoretic “Off target” effects**
  - Potential to alter other genes w editing (CRSPR/CAS9 technique minimizes this)
  - Integration into patient genome of vector sequence
  - Could tumor suppressor genes be altered?

Limitations

- **Cost/ Insurance coverage**
- **Any specific AAV vector can only be used one time (since antibodies will be formed after exposure)**
  - Note that this limitation is avoided with the use of lipid nanoparticles
- **Hepatocyte turn over**
  - How long will the effects of gene therapy or gene edit last?
- **No information available for use in pregnancy or breastfeeding**
  - Two methods of birth control must be used for the duration of the trial and for months after gene therapy dose is received
  - Could impact reproductive decisions in patients of childbearing potential

In the Preclinical Pipeline

- Gene Editing via CRISPR/CAS9 for citrullinemia type 1 (iECURE preclinical pipeline)

- Base Editing for all 7 urea cycle disorders through a more generalizable platform (through the CHOP group)

Thank you!

Thank you to the following people for sharing information, resources, personal stories and images to include in this ECHO presentation!

- Tresa Warner, Jill Williams and Lori Shockey- NUCDF
- George Diaz, MD, PhD- iECURE
- J. Lawrence Merritt II, MD- Ultragenyx
- Marshall Summar, MD- Uncommon Cures

Resources

- National Urea Cycle Disorders Foundation: http://www.nucdf.org/
- Urea Cycle Disorders Consortium: https://ucdc.rarediseasesnetwork.org/
- GeneReviews Urea Cycle Disorders https://www.ncbi.nlm.nih.gov/books/NBK1217/
- GeneReviews are also available for individual conditions
- STARZL Network: https://starzlnetwork.org/resources-landing/
- iECURE gene editing programs: https://iecure.com/programs/#pipeline
- Ultragenyx gene therapy programs: https://www.ultragenyx.com/our-research/pipeline/
- Uncommon Cures mRNA therapy programs: https://uncommoncures.com/
- https://www.ahrensnicklaslab.com/
- Musunuru K, Grandinette SA, Wang X, Hudson TR, Briseno K, Berry AM, Hacker JL, Hsu A, Silverstein RA, Hille LT, Ogul AN, Robinson-Garvin NA, Small JC, McCague S, Burke SM, Wright CM, Bick S, Indurthi V, Sharma S, Jepperson M, Vakulskas CA, Collingwood M, Keogh K, Jacobi A, Sturgeon M, Brommel C, Schmaljohn E, Kurgan G, Osborne T, Zhang H, Kinney K, Rettig G, Barbosa CJ, Semple SC, Tam YK, Lutz C, George LA, Kleinstiver BP, Liu DR, Ng K, Kassim SH, Giannikopoulos P, Alameh MG, Urnov FD, Ahrens-Nicklas RC. Patient-Specific In Vivo Gene Editing to Treat a Rare Genetic Disease. N Engl J Med. 2025 Jun 12;392(22):2235-2243. doi: 10.1056/NEJMoa2504747. Epub 2025 May 15. PMID: 40373211.

Questions / Discussion

Upcoming Treatment Options

15 minutes

UREA CYCLE DISORDERS ECHO

The Essentials

UREA CYCLE DISORDERS ECHO

The Essentials

Thank You

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Coming Soon!

Evaluation link / QR code