Targeting regRNAs with oligonucleotides to treat OTC deficiency

Yun Joon Jung, Mario Gamboa, Rachana Kelkar, Preeti Sathe, Henry Moukheiber, Evan Cohick, Shraddha Kamath, Amy Jiang, Brian Schwartz, Bryan Matthews, Jiaqi Huang, Abeer Almutairy, Chris Brynczka, Brynn Akerberg, Yuting Liu, Jesse Lugus, Shubh Roy, Yuchun Guo, David Bumcrot, Alfica Sehgal

CAMP4 Therapeutics, Cambridge, MA, USA

Abstract

Urea cycle disorders (UCD) are inborn errors of metabolism that affect the ammonia detoxification process, which results in hyperammonemia, causing devastating neurological damage. The most common type of UCD is ornithine transcarbamylase (OTC) deficiency, a complete or partial lack of OTC enzyme activity.

CAMP4 has developed an RNA Actuating Platform (RAP™) that enables us to increase gene expression by targeting regulatory RNAs (regRNAs) with antisense oligonucleotides. regRNAs are non-coding RNAs expressed from regulatory regions of the genome, including enhancers and promoters. RAP™ utilizes Next Generation Sequencing powered by proprietary machine learning technology to identify and map novel regRNAs that act as rheostats to precisely control the transcription of individual genes. Antisense oligonucleotides (ASOs) that target the regRNAs are then designed and screened to identify those that induce the desired effect on gene expression.

Here we describe the identification of a regulatory elements and the corresponding regRNAs that control OTC gene expression. We designed and screened ASOs targeting OTC regRNAs and discovered several that specifically upregulate OTC mRNA in a dose-dependent manner across multiple primary donors.

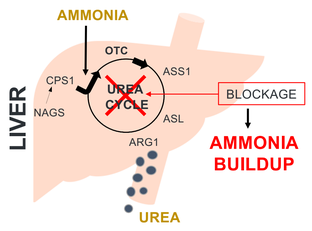
To test active ASOs in a disease model, we identified the mouse regulatory elements and regRNAs controlling Otc gene expression and screened for ASOs that upregulate Otc in mouse hepatocytes. We then assessed *in vivo* efficacy of ASO treatment in the well-established Otc$^{spf/ash}$ mouse model. We demonstrate a therapeutically relevant reduction in plasma ammonia following the ammonium chloride challenge in ASO-treated mice versus controls.

Furthermore, we tested ASOs upregulating human OTC in a humanized liver mouse model and in cynomolgus monkeys. In both cases, we show that human OTC regRNA-targeting ASOs decrease ammonia and increase urea levels in treated animals.

These results validate the potential of CAMP4's RAP™ platform to identify precise, potent therapeutic candidates to upregulate genes to address a range of diseases and support the development of regRNA-targeted ASOs to treat the urea cycle disorders.

Background

Partial loss of function mutations cause OTC deficiency

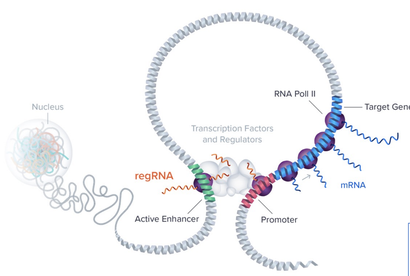


- **Caused by genetic defects in enzymatic components of the urea cycle**
- **Excessive ammonia tied to cognitive defects, neurological effects (e.g., seizure)**
- **Disease severity and age of onset varies with residual OTC activity and ammonia levels**

Therapeutic goal: increase OTC gene product to reach 10-20% of normal

Fig 1. Schematic diagram of urea cycle disorder

Enhancer activity is the critical regulatory step in mRNA production



In the nucleus, genes and their regulatory elements are organized into conserved 3D DNA structures known as Insulated Neighborhoods to control gene expression

regRNAs are uniquely transcribed within neighborhoods and act as rheostats– fine-tuning mRNA levels for precise genomic control

mRNA: "Coding" RNA that is converted to protein, which has a specific functional role in the cell

regRNA: "Noncoding" RNA that is not converted to protein but rather regulates the level of nearby mRNA

Fig 2. Schematic diagram of gene regulatory elements in 3D structure

CAMP4's RAP™ charts an efficient path from RNA targets to drugs

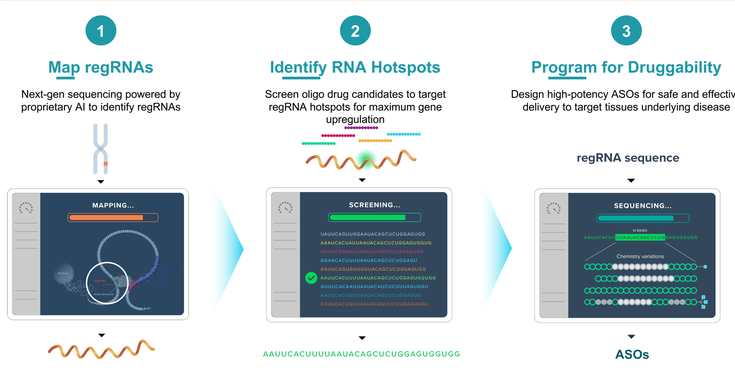
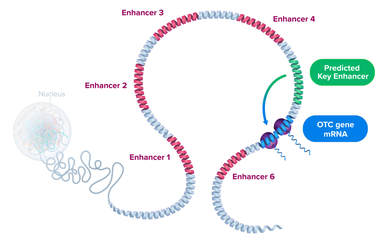
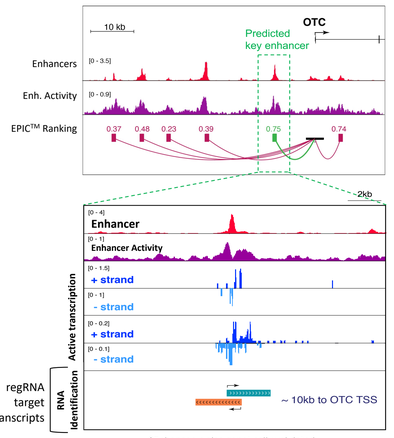


Fig 3. Schematic diagram of RAP™ stages

Results

Applied EPIC™ model to predict key regRNA controlling OTC





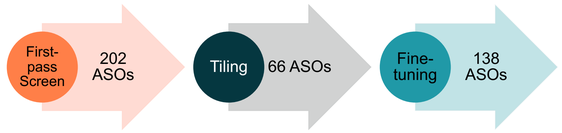
- There are 6 active enhancers in the same neighborhood as OTC
- Prioritize relevant enhancers using a proprietary AI engine called EPIC™.
- Inducible enhancer is genetically validated (Jang *et al.*, 2017)
- Identified and sequenced the regRNA associated with the enhancer

EPIC™: Enhancer-Promoter Interaction Characterization

*Enhancer is also genetically validated

Fig 4. Gene tracks of regRNAs in OTC regRNA regions

Developing regRNA-based therapeutics



473 unique ASO sequences screened for OTC

Fig 5. Multiple stages of developing regRNA-based therapeutics

Lead ASO targeting OTC regRNA shows dose-dependent increase in OTC mRNA in hepatocytes across multiple donors

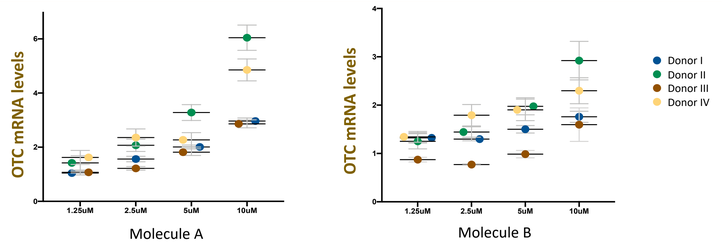


Fig 6. Dose-dependent upregulation of OTC mRNA in multiple donors. Primary human hepatocytes were treated for 48 hours with ASO at desired conc, mRNA levels were measured by qPCR. The data is a mean of 3 biological replicates.

Upregulation of OTC mRNA increases ureagenesis in human OTC-deficient hepatocytes

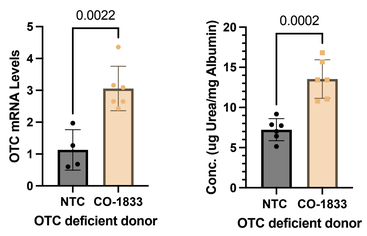


Fig 7. Upregulation of OTC mRNA and ureagenesis in human OTC-deficient hepatocytes. Primary human OTC-deficient hepatocytes (OTC c.-106C>A (Allele ID 480410, late-onset OTC deficiency) - pathogenic (dbSNP: rs749748052), were treated for 48 hours with ASO at desired conc, mRNA levels were measured by qPCR. The data is a mean of 3 biological replicates.

EPIC™ predicts key regRNA controlling mouse Otc, which is validated by *in vitro* screenings

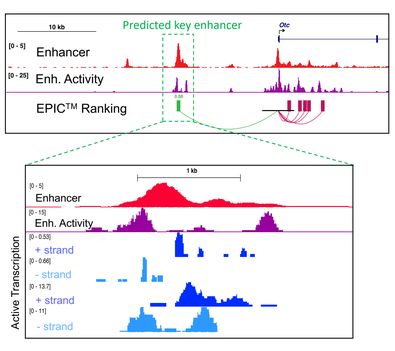


Fig 8. Key regRNA identification through primary mouse hepatocyte mapping and EPIC™

in vitro

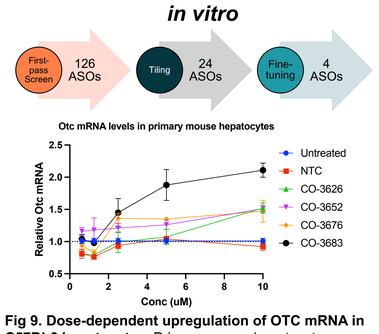


Fig 9. Dose-dependent upregulation of OTC mRNA in C57BL6 hepatocytes. Primary mouse hepatocytes were treated for 48 hours with ASO at desired conc, mRNA levels were measured by qPCR. The data is a mean of 3 biological replicates.

mouse surrogate model: *in vivo* efficacy

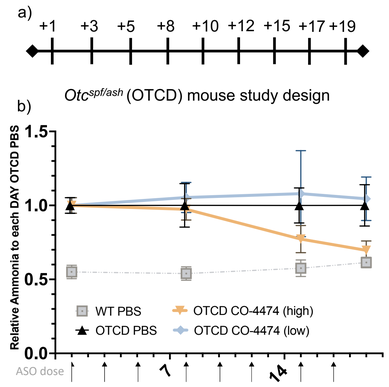


Fig 10. Ammonia reduction by mouse Otc regRNA targeting ASO in *Otc$^{spf/ash}$* mouse model The *Otc$^{spf/ash}$* mouse model (OTCD) carries a variant (c.386G>A, p.Arg129His) in the last nucleotide of exon 4 of the *Otc* gene. This transition causes inefficient splicing and decreased mRNA levels (5-12% of WT levels). *Otc$^{spf/ash}$* livers and have a mild biochemical phenotype with 5-10% Otc activity.

Mice were treated via SC injection of GalNAc-conjugated ASOs for 19 days a) Schematic diagram of experimental design. b) Ammonia challenge was given to measure impact of ASO on ammonia reduction. n=5

Safety profile of lead ASOs

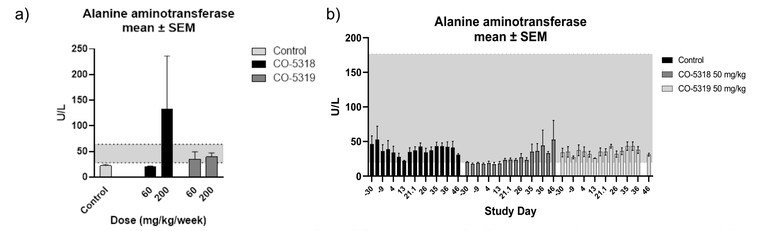
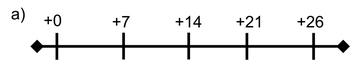


Figure 11. Acceptable acute tolerability for ASOs targeting OTC regRNA. a) Mouse treated via SC injection Q3D for one week at the specified dose levels. There were no toxicologically significant changes for either ASO through 60 mg/kg/week. Elevated ALT levels for CO-5318 only at highest tested dose of 200 mg/kg/week. b) Cynomolgus monkeys were treated via SC injection Q2W at the indicated dose. There were no toxicologically significant findings in any liver function test or in other antemortem observations (data not shown). Reference interval shown in bracketed gray bar.

in vivo efficacy: NHP



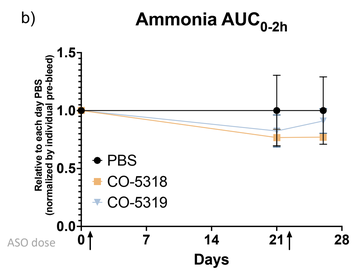


Fig 12. Ammonia reduction by human OTC regRNA targeting ASO in *wildtype* Cynomolgus monkeys (NHP) a) Schematic diagram of experimental design. NHPs treated via SC injection of GalNAc-conguated ASOs for one months at the specified dose levels on Day 0 and 21 b) Ammonia challenge was given to measure impact of ASO on ammonia reduction and plasma ammonia levels were measured over 2 hours

in vivo efficacy: Humanized mouse model

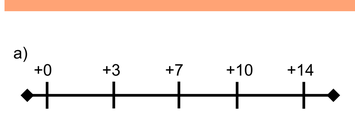
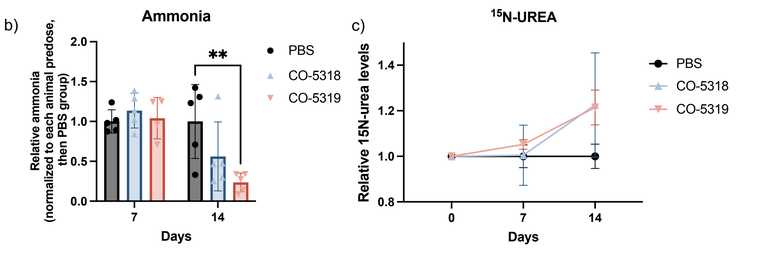


Fig 13. Ammonia reduction by human OTC regRNA targeting ASO in *wildtype* humanized mouse model a) Schematic diagram of experimental design. Female liver-humanized *Fah$^{-/-}$Rag2$^{-/-}$Il2rg$^{-/-}$* (FRG) mice were tested with GalNAc-ASO on Day 0, 3, 7, and 10. Animals were challenged with ^{15}N-NH$_3$ to measure b) ammonia and c) urea.



Summary

CAMP4's RAP™ platform can identify precise, potent therapeutic candidates to upregulate genes to address a range of diseases and support the development of regRNA-targeted ASOs to treat the urea cycle disorders

- Identified regRNA that controls OTC gene expression
- Identified regRNA targeting ASOs that can upregulate human OTC mRNA in a dose-dependent manner → increase ureagenesis in WT and OTC deficient patient cells
- ASOs targeting mouse regRNA increase Otc mRNA in both *in vitro* and *in vivo* model
- Lead ASOs show acceptable safety profile in mice and NHP
- Lead ASOs show efficacy in both NHP and humanized mouse models
- This approach offers a novel way of treating a disease caused by a hypomorphic allele by upregulating the endogenous gene expression using ASOs.

Our results indicate that CAMP4's RAP™ technology may provide precise, potent therapeutics that can be programmed to treat thousands of diseases.