Antisense oligonucleotide therapeutic candidates targeting regulatory RNAs to treat urea cycle disorders

Bryan Matthews, Yun Joon Jung, Gabby Bedard, Eric Coughlin, Brynn Akerberg, Rachana Kelkar, Preeti Sathe, Henry Moukheiber, Shraddha Kamath, Amy Jiang, Brian Schwartz, Jiaqi Huang, Abeer Almutairy, Yuting Liu, Jesse Lugus, Shubh Roy, Yuchun Guo, Alla Sigova, Alfica Sehgal, and David Bumcrot

CAMP4 Therapeutics Corp., Cambridge, MA, USA

Abstract

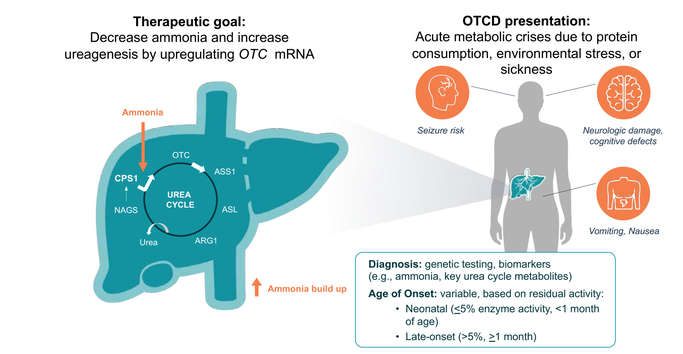
Diseases caused by haploinsufficiencies or hypomorphic mutations could be potentially addressed by elevating expression of the healthy alleles of the affected disease-associated genes. Even relatively modest increases (less than 2-fold) can be highly impactful on disease phenotype. Regulatory RNAs (regRNAs), including enhancer RNAs and promoter-associated RNAs, are key regulators of gene transcription. Our RAP Platform™ enables both the identification of regRNAs associated with a target gene and upregulation of the target gene's expression using antisense oligonucleotides (ASOs). Here, we describe how we are applying this approach to develop and characterize therapeutic product candidates targeting regRNAs associated with the human ornithine transcabamylase gene (OTC) for the potential treatment of Urea Cycle Disorders (UCDs) such as OTC deficiency (OTCD).

Using our RAP Platform, we identified key cis-elements regulating the transcription of OTC gene in human and mouse hepatocytes and candidate regRNAs transcribed from these cis-elements. We then designed and screened ASOs targeting the OTC regRNAs and discovered several ASOs that specifically upregulated OTC mRNA in human hepatocytes in vitro in a dose-dependent manner. We further demonstrated the efficacy of the selected ASOs in a humanized mouse model, in which ASO treatment resulted in OTC upregulation, significant reduction in plasma ammonia levels as well as comcommitant increase in urea levels. To investigate the mechanism by which the selected ASOs induce OTC gene expression upregulation, we evaluated ASO target engagement activities and their ability to modify the secondary structure of regRNAs. We show that selected ASOs modulated the binding of negative transcriptional regulators resulting in increased OTC transcription.

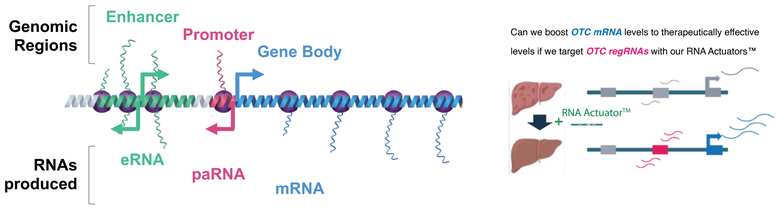
Our results support the therapeutic potential of ASOs targeting *OTC* regRNAs for the treatment of UCDs.

Background

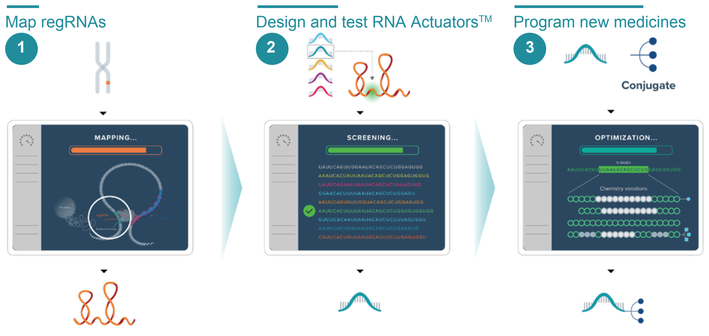
Ornithine Transcarbamylase Deficiency (OTCD) is an X-linked urea cycle disorder resulting in an inability to adequately process ammonia to urea



Regulatory RNAs (regRNAs) are a broad class of cis-acting noncoding RNAs

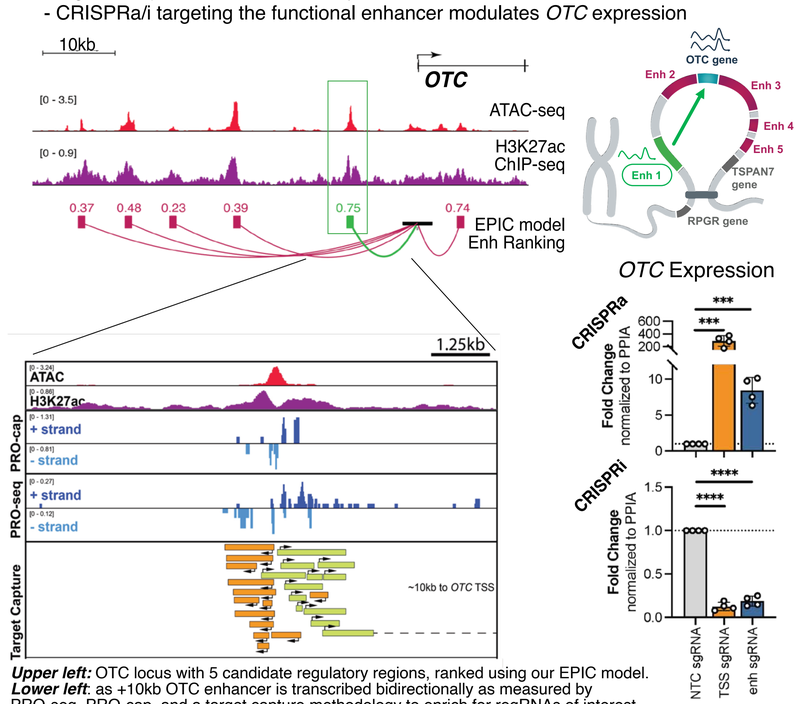


RAP Platform™: Programming medicines targeting regRNAs



Mapping *OTC* locus in primary human hepatocytes

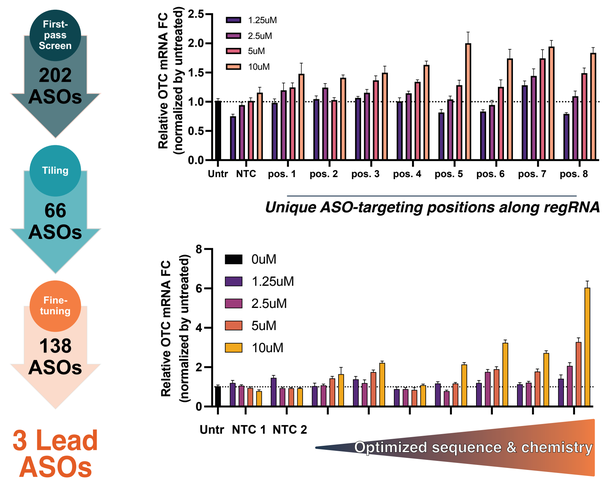
Upstream OTC enhancer is a functionally-validated regulatory region
- OTC enhancer exhibits hallmark open chromatin and histone modification
- regRNA is produced bidirectionally from enhancer
- CRISPRa/i targeting the functional enhancer modulates *OTC* expression



Upper left: OTC locus with 5 candidate regulatory regions, ranked using our EPIC model.
Lower left: as +10kb OTC enhancer is transcribed bidirectionally as measured by PRO-seq, PRO-cap, and a target capture methodology to enrich for regRNAs of interest.
Right: Guide RNAs targeting the OTC enhancer in HepG2 cells expressing CRISPRa (VPH) or CRISPRi (KRAB) effectors validate that the enhancer is functional. This effect is specific to OTC (data not shown).

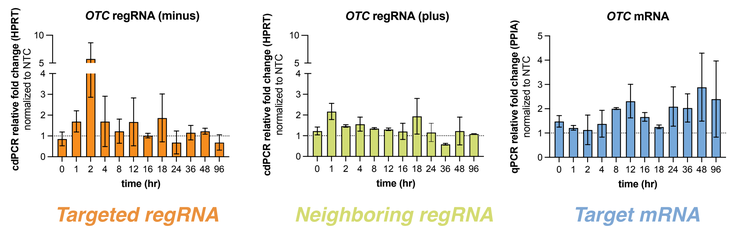
ID and optimization of *OTC* regRNA-targeting ASOs

ASO screening identifies lead dose-responsive ASOs



Above: Top Graph- ASO screening of ASOs targeting different positions along the regRNA for their ability to upregulate *OTC* mRNA, resulting in multiple hits with >1.5 Fold-change (FC). ***Bottom graph***- Optimization of lead ASO chemistry and sequence improves its ability to upregulate OTC mRNA in vitro.
Below: Treatment with an ASO targeting the minus strand regRNA results in upregulation of the target *OTC* regRNA within 2h followed by a later upregulation of *OTC* mRNA at 12 and 24h.
All: FC by quantitative or digital PCR relative to a housekeeper gene (HPRT or PPIA, as indicated) and sample indicated on the y-axis

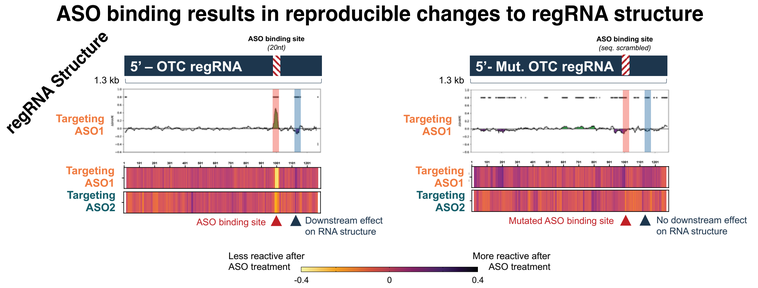
Upregulation of targeted regRNA precedes *OTC* mRNA changes



OTC regRNA-targeting ASOs: mechanism of action

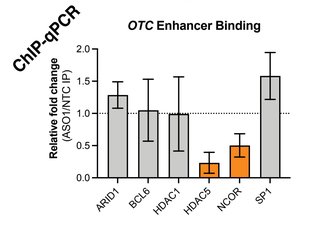
Working Model

ASO binding results in reproducible changes to regRNA structure

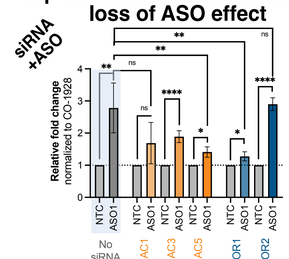


Targeting ASO1 binding alters regRNA secondary structure as measured by SHAPE-MaP (RNA nucleotide reactivity relative to an NTC ASO). Effect was not observed for mutant regRNA lacking the ASO binding site (i.e., ASO2).

Treatment with ASO reduces repressor binding at enhancer



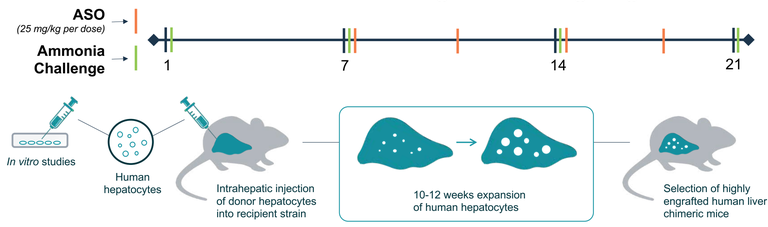
Repressor knockdown results in loss of ASO effect



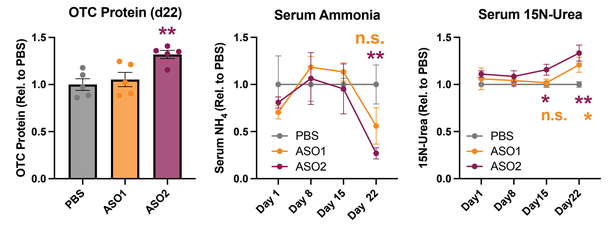
Left: Treatment with targeting ASO1 results in a decrease in binding of HDAC5 and NCOR1 (ChIP-qPCR)
Right: Knockdown of HDAC1,3,5 or NCOR1 prior to ASO treatment (48h) ablates ASO1 effect on OTC mRNA

In vivo efficacy of *OTC* regRNA-targeting ASOs

Humanized liver mouse model allows testing of hOTC-targeting ASOs *in vivo*



regRNA-targeting ASO leads to increased OTC protein and ureagenesis



Left: OTC protein levels were measured at final endpoint, day 22, using capillary-based Western.
Center: Serum ammonia was measured from fresh serum at the indicated day. Data is normalized to the matched timepoint PBS control +/- SEM for n=5 mice per group.
Right: Labeled serum urea following a challenge with 15N-NH_4 was measured by LC-MS/MS. Data is normalized to the matched timepoint PBS. P values are one way ANOVA. * p<0.05, **p<0.01

Takeaways

ASOs targeting *OTC* regRNAs upregulate *OTC* mRNA in a dose-dependent

OTC regRNA upregulation preceded mRNA upregulation after ASO treatment

ASOs modulate repressor binding leading to increased *OTC* transcription

ASOs targeting *OTC* regRNAs increase OTC protein levels and decrease ammonia levels in humanized liver mouse model

CAMP4's RAP Platform™ facilitates the development of precise, potent therapeutic candidates that can be programmed to treat a broad range of diseases