Upregulation of a bile acid regulator by targeting regulatory RNAs can enhance hepatic bile acid efflux in PBC

Brian E. Schwartz, Keerthana Gnanapradeepan, Mateo Rodriguez, Rachana Kelkar, Preeti Sathe, Mario Gamboa, Jesse Lugus, Cynthia Smith, Yuting Liu, Rutuja Pai, Scott Waldron, David Bumcrot, Alfica Sehgal

CAMP4 Therapeutics, Cambridge, MA, USA

Abstract

PBC (Primary Biliary Cholangitis) is an autoimmune disease characterized by the progressive destruction of bile duct cells, leading to accumulation of bile acids and ultimately intrahepatic cholestasis. There is no cure for PBC, and 30-40% of patients will not respond to the first line treatment, ursodeoxycholic acid (UDCA). Currently, the only approved second line therapy is obeticholic acid (OCA), which has demonstrated limited efficacy and a poor tolerability profile. Therefore, there is significant unmet need for new PBC therapeutics. We hypothesize that increasing the expression of a specific hepatic bile acid regulator would enhance bile flow from the liver and restore enterohepatic circulation of bile acids in the context of PBC.

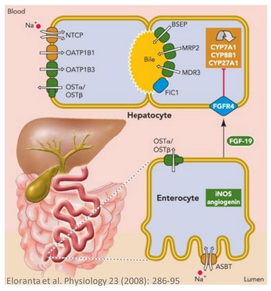
CAMP4 has developed an RNA Actuating Platform (RAP™) that enables us to increase gene expression by targeting regulatory RNAs (regRNAs) with antisense oligonucleotides (ASOs). regRNAs are non-coding RNAs expressed from regulatory regions of the genome, including enhancers and promoters. RAP™ utilizes 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. ASOs which 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 the bile acid regulator gene expression. We designed and screened ASOs targeting regRNAs associated with this gene and discovered several that specifically upregulate it 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 the bile acid regulator gene expression and screened for ASOs that upregulate it in mouse hepatocytes. We then assessed in vivo activity upregulation in the well-established ANIT-induced mouse model. We demonstrate a therapeutically relevant increase in target gene expression in ASO-treated mice versus controls.

Furthermore, we tested ASOs upregulating the human bile acid regulator in a humanized liver mouse model. We show that these regRNA-targeting ASOs likewise increase target gene expression and decrease hepatic bile acids.

Background

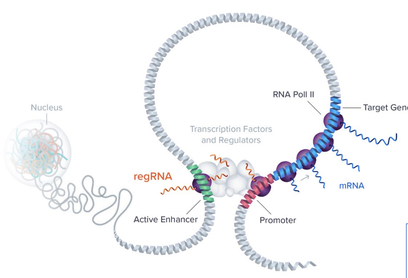
Bile acid regulation involves communication between intestine and liver



- Identified a key regulator of bile acid metabolism ("BA Regulator")
- BA Regulator overexpression in mice can decrease intracellular bile acid load
- We predict that a ~1.5x increase in BA Regulator expression should have a therapeutic effect

Therapeutic goal: increase target gene expression ~1.5x

Enhancer activity is the critical regulatory step in mRNA production



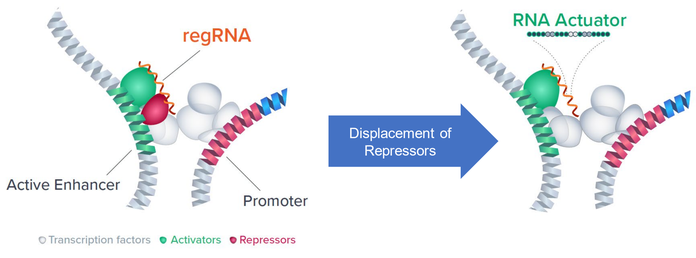
Schematic diagram of gene regulatory elements in 3D structure

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

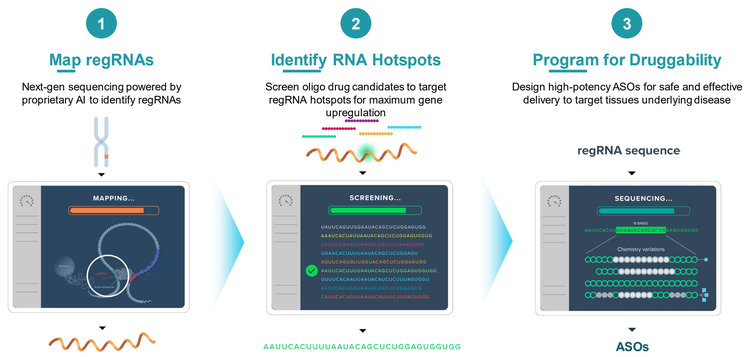
RNA Actuators (ASOs) bind regRNAs and may prevent the recruitment of repressors, thus allowing gene transcription



regRNA: enhancer RNA (eRNA), promoter RNA (paRNA) , Naturally occurring antisense (NAT)

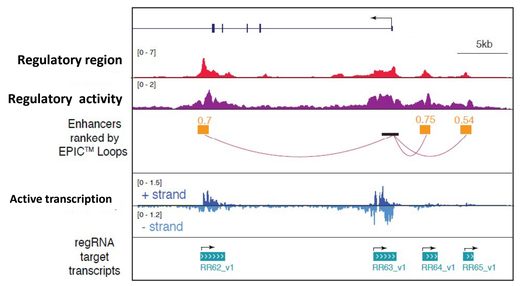
Methods and Screening Results

Overview of drug development at CAMP4



Schematic diagram of RAP™ stages

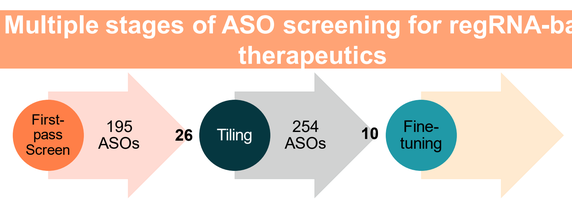
Application of EPIC™ model to infer regulatory regions and regRNAs controlling expression of target gene in hepatocytes



Gene tracks of regRNAs for the BA Regulator gene

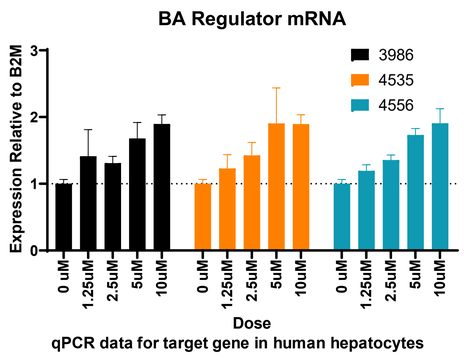
- Three (out of 9) putative regulatory regions were prioritized using EPIC™
- ASOs were designed and screened against regRNAs associated with the regulatory regions
- Hits (>1.5x upregulation) were tested in dose-response assays

Multiple stages of ASO screening for regRNA-based therapeutics



Ten human-specific ASOs were selected for fine-tuning

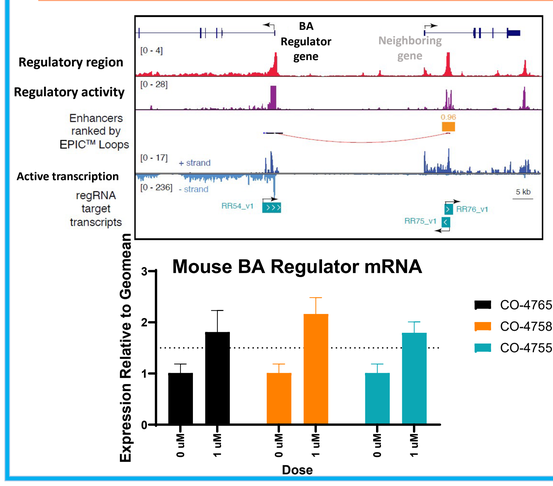
Initial screen identified several sequences across 3 different regRNAs



qPCR data for target gene in human hepatocytes

- Dose-responsive upregulation by a subset of hit ASOs in human primary hepatocytes
- Hepatocytes were maintained in sandwich culture and treated by free-uptake with ASOs for 3 days
- Each ASO targets a different regRNA, suggesting that all three have a role in target gene expression

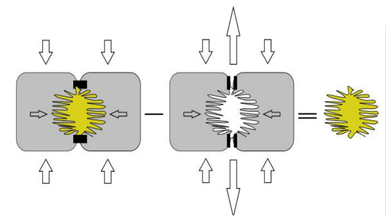
EPIC™ predicts key regRNA controlling the mouse BA Regulator, which is validated by *in vitro* screenings



- Three regRNAs were prioritized using EPIC™ in mouse liver
- ASOs were designed and screened against regRNAs associated with the regulatory regions
- Hits (>1.5x upregulation) were identified in mouse primary hepatocytes

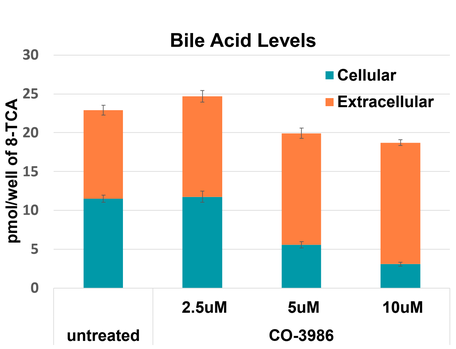
In vitro Results

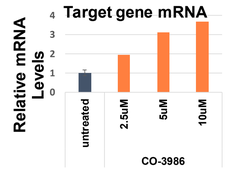
Biliary clearance assay "B-CLEAR" shows enhanced BA efflux with human ASO leads

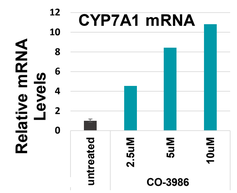


Total BA - cellular BA = bile pocket BA

- Labeled bile acid (d8-TCA) was spiked into ASO-treated hepatocyte cultures
- Total and cellular levels of d8-TCA were used to estimate bile pocket BA, which can be used to measure efflux



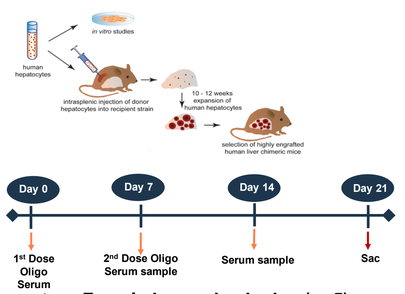


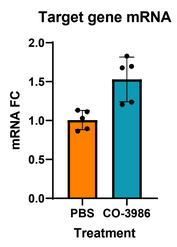


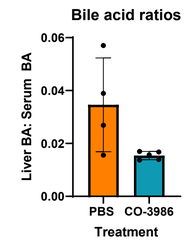
- Human-specific ASO, CO-3986, increases the efflux of bile acids from the intracellular compartment to the bile cannuliculi (left)
- Target gene mRNA was measured in duplicate wells by qRT-PCR (right). The target gene was upregulated in a dose-responsive manner as expected. *CYP7A1* was also upregulated as a compensatory response to decreased intracellular bile acid levels

In vivo Results

Target gene upregulation reduces hepatic bile acid levels in a humanized mouse

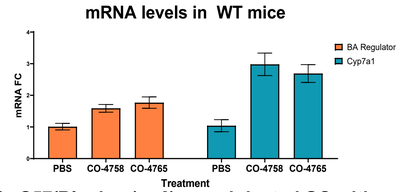


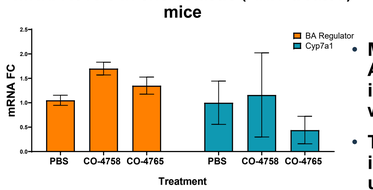




- Female humanized mice (n=5) were treated with GalNAc-ASO CO-3986 or PBS control on Days 0 and 7, then sacrificed on Day 21
- Target gene mRNA was increased, which promoted efflux of hepatic bile acids

Target gene upregulation in an induced-cholestasis model





- Male C57/Bl mice (n=6) were injected SC with ASO (or PBS) on Days 0 and 5. Cholestasis was induced (left) by 75mg/kg ANIT or mock-treated with olive oil (above)
- Target gene upregulation was achieved by ASO in both conditions. Compensatory *Cyp7a1* upregulation occurred only in non-cholestatic animals

Summary

We have applied our RNA Actuator platform to identify and prioritize non-coding regulatory RNAs (regRNAs) that control the expression of a BA regulator in hepatocytes. By targeting these regRNAs with RNA Actuators, we can selectively upregulate the expression of this gene in the liver. Upregulation enhances bile acid efflux from primary human hepatocytes as well as in a humanized mouse liver model. Likewise, a mouse-specific RNA Actuator can upregulate BA regulator expression in a mouse model of PBC. These proof-of-concept studies serve to validate the upregulation of this target gene as a potential treatment for PBC, and may be more broadly applicable to other diseases which result in intrahepatic cholestasis.