ProQR®

ADAR-MEDIATED RNA EDITING OF *SLC10A1* (NTCP) AS A THERAPEUTIC APPROACH TO REDUCE LIVER BILE ACID RE-UPTAKE IN CHOLESTATIC DISEASES

Gerard Platenburg

ASGCT May 16, 2025

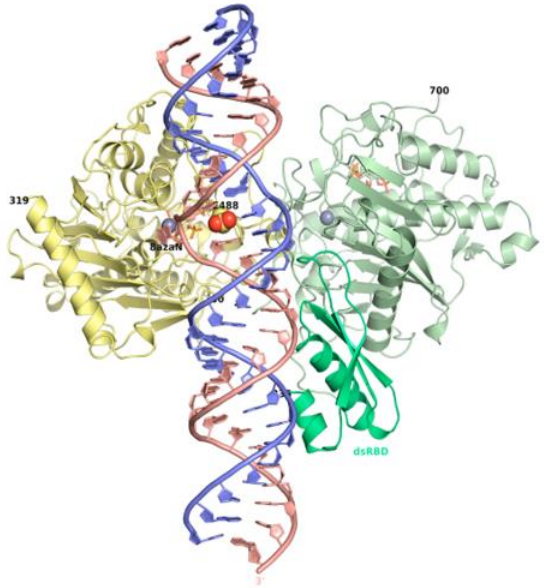
Disclosures

- I am an employee of ProQR Therapeutics

Axiomer™ EONs unlock cellular machinery potential to treat diseases

By attracting ADARs and allowing highly specific editing

ADAR *(Adenosine Deaminase Acting on RNA)*

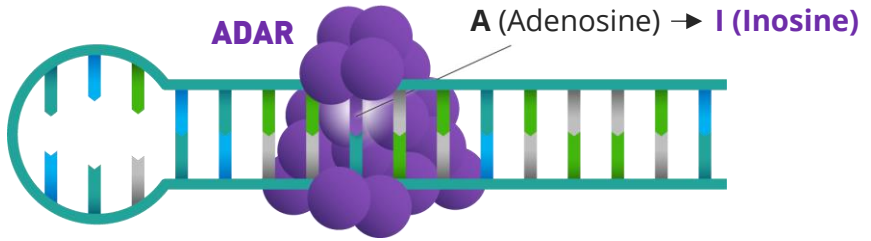


Enzyme that performs specific form of natural RNA editing, called **A-to-I editing.** During A-to-I editing an **A nucleotide (adenosine)** is changed into an **I nucleotide (inosine)**

ADAR editing *(A-to-I)*

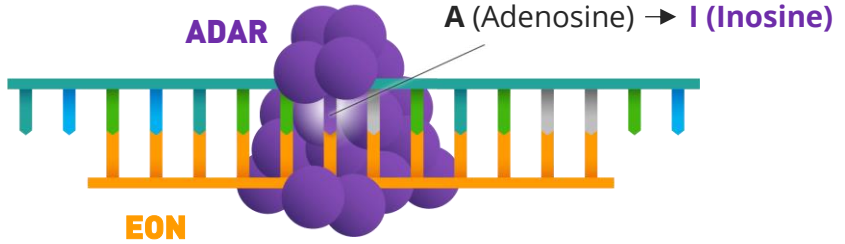
Natural ADAR editing *(A-to-I)*

RNA
Double stranded

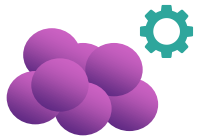
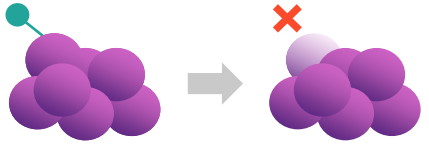


Editing Oligonucleotide (EON)-directed therapeutic editing *(A-to-I)*

RNA+EON
Double stranded



Creating a new class of medicines with broad therapeutic potential

Correction	Protein modulation		
			
Mutations correction Thousands of G-to-A mutations, many of them described in literature	**Alter protein function or include protective variants** Modified proteins achieving loss- or gain-of-functions that help addressing or preventing diseases	**Disrupt >400 different types of PTMs** Regulate protein activity, change localization, folding, preventing immune escape or slowing down degradation	**Change protein interactions** Changes localization, folding, protein function or prevents immune escape of glycosylated tumor antigens
Mutation correction leading to protein recovery	Variant resulting in a dominant negative effect	Reduction of protein phosphorylation altering protein function	Variant impacting protein interaction with sugar

AX-0810 RNA editing therapy targeting NTCP for cholestatic diseases

Cholestatic diseases have high unmet medical need. Patients accumulate bile acids in liver leading to fibrosis and ultimately liver failure.

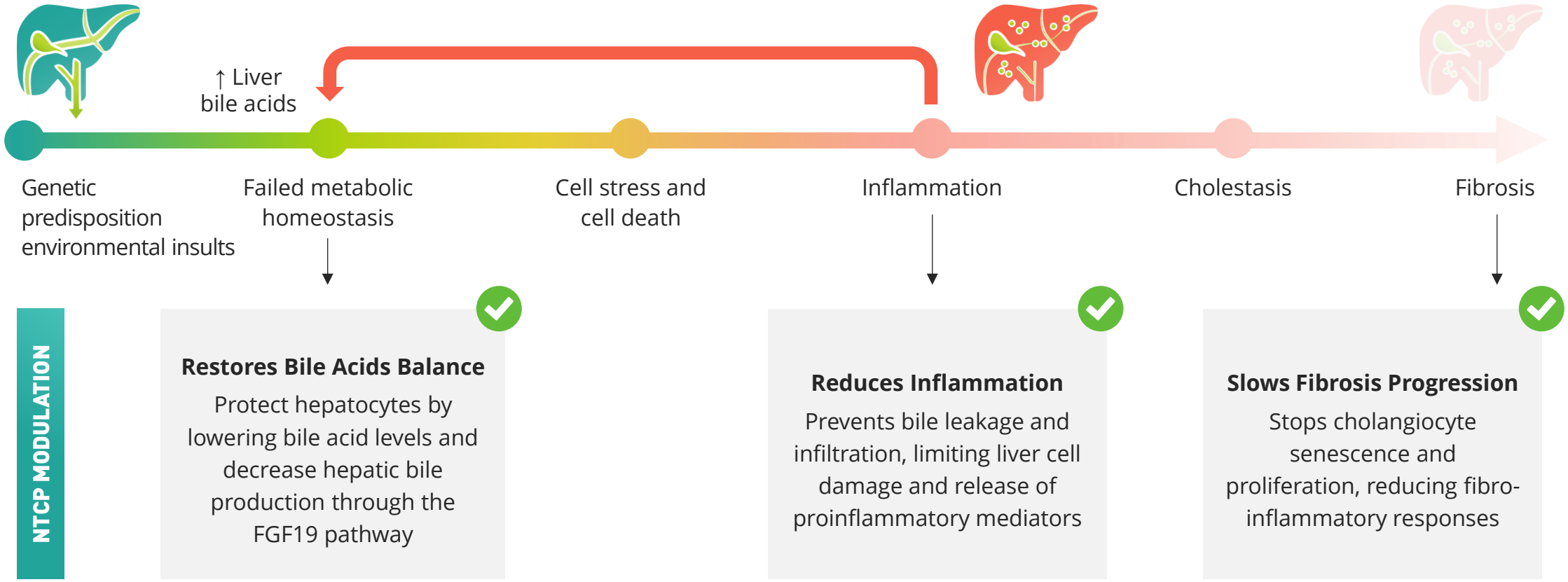
Initial indications are **Primary Sclerosing Cholangitis** affecting adults and Congenital **Biliary Atresia** affecting pediatrics early in life. Both conditions have no approved therapies and may require liver transplantation.[1,2]

- **Biliary Atresia** is projected to affect ~20,000 pediatric individuals in US and EU.
- **Primary Sclerosing Cholangitis** is projected to affect more than 80,000 individuals in US and EU.

AX-0810 is a unique therapeutic approach leading to a potentially disease modifying therapy by targeting the NTCP channel which is responsible for majority of bile acid re-uptake in liver cells.

[1]Trivedi PJ, et al. Clin Gastroenterol Hepatol. 2022 Aug;20(8):1687-1700.e4; [2]Schreiber RA, et al. J Clin Med. 2022 Feb 14;11(4):999

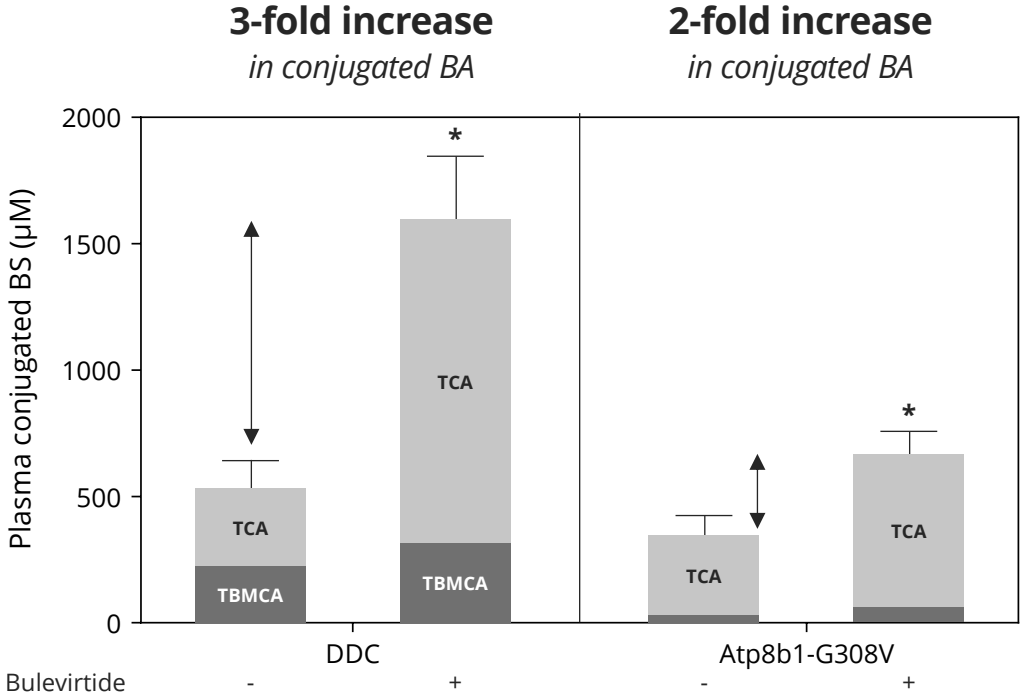
NTCP modulation leads to positive effect on different mechanism involved in cholestasis



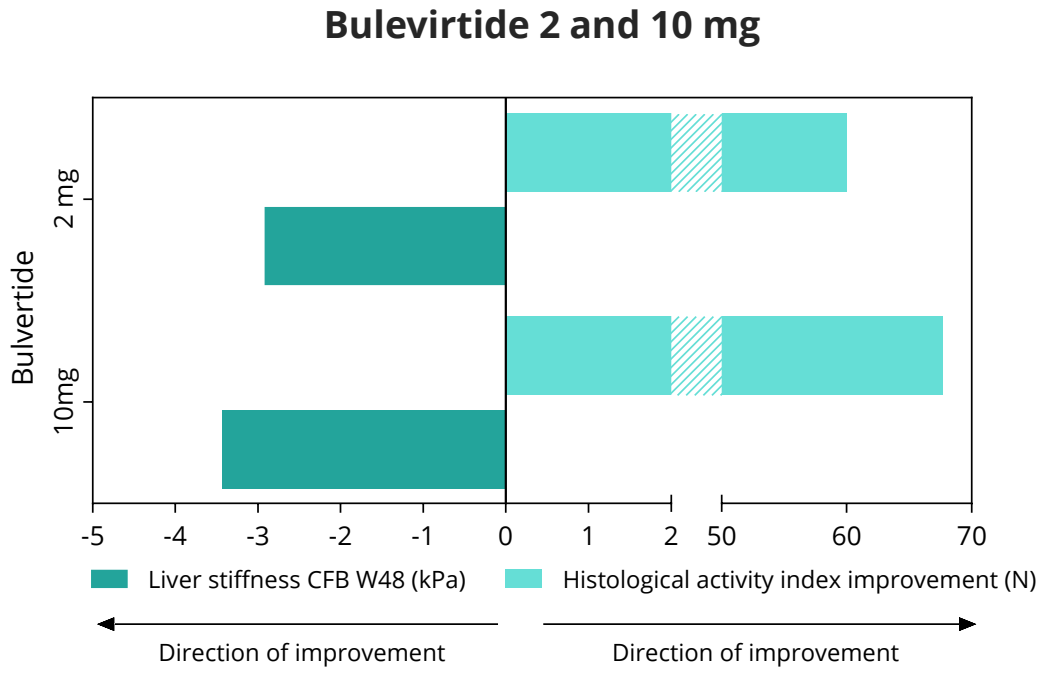
Zeng J, Fan J, Zhou H. Cell Biosci. 2023 Apr 29;13(1):77; Trauner M, Fuchs CD. Gut 2022;71:194–209; Halilbasic E, Claudel T, Trauner M. J Hepatol. 2013 Jan;58(1):155-68.

NTCP modulation validated in vivo and clinic

Reducing liver bile acids toxic overload via NTCP modulation is a key driver for hepatoprotective effects



NTCP modulation demonstrated effectivity in cholestatic disease model[1]



Clinical PoC with bulevirtide in Ph3 Hepatitis D trial. **Improvement** occur in patients, **even without virologic response**[2-4]

Bulevirtide (Hepcludex) is a daily SC injected NTCP inhibitor approved for Hepatitis D. NTCP channel is a known transporter for bile acids and hepatitis virus from bloodstream to the liver.

1. Slijepcevic D, et al. Hepatology. 2018 Sep;68(3):1057-1069; 2. Wedemeyer H, et al. N Engl J Med. 2023 Jul 6;389(1):22-32; 3. Wedemeyer H, J Hepatol. 2024 Oct;81(4):621-629.; 4. Dietz-Fricke C, JHEP Rep. 2023 Mar 15;5(4):100686.

NTCP variants reduced bile acids uptake into liver in health population research

Healthy population discovered with NTCP variants that reduces bile acids uptake into liver[1-4]

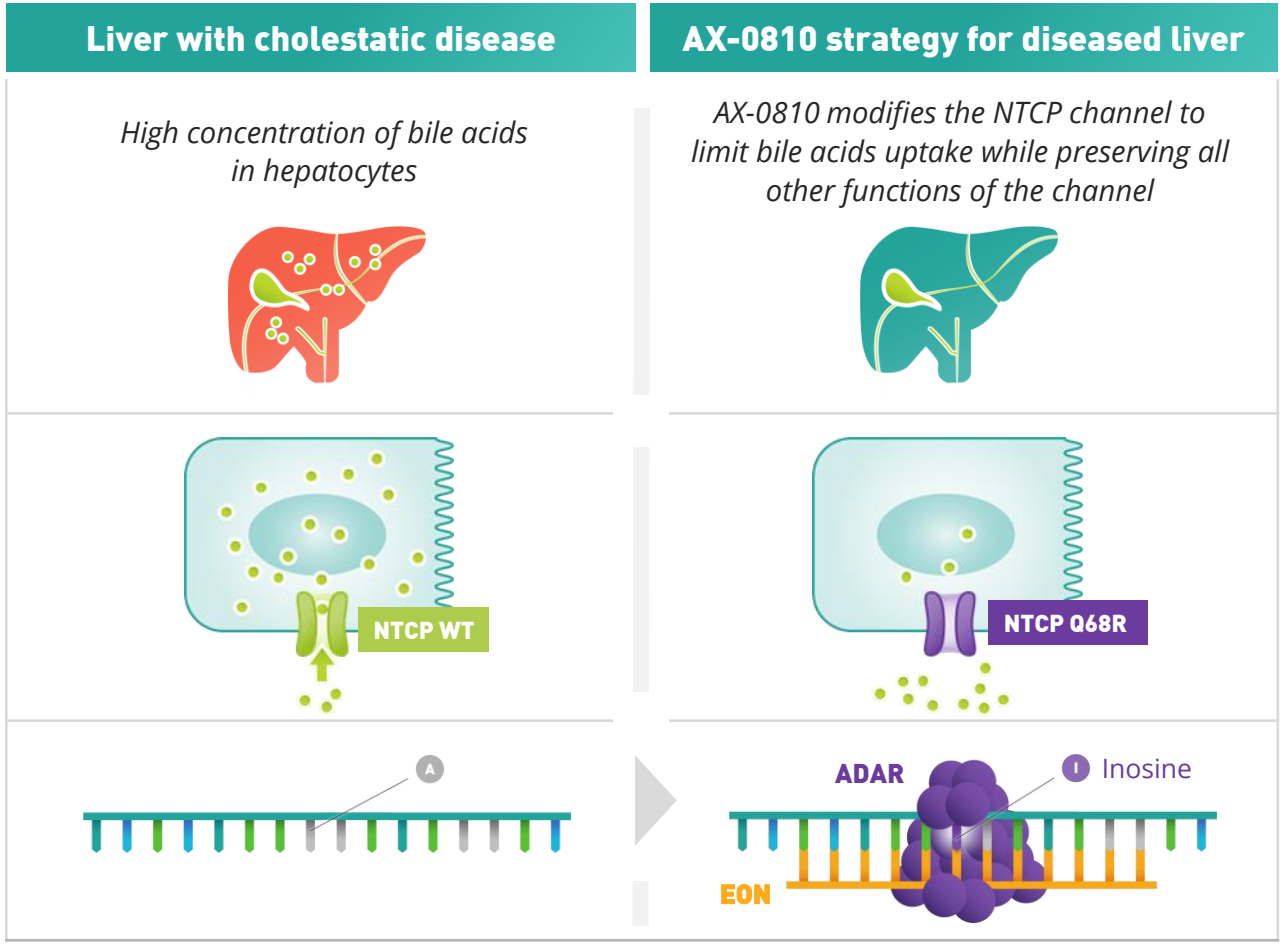
Ethnicity-dependent Polymorphism in Na+-taurocholate Cotransporting Polypeptide (*SLC10A1*) Reveals a Domain Critical for Bile Acid Substrate Recognition*

Richard H. Ho‡§¶, Brenda F. Leake‡, Richard L. Roberts‖, Wooin Lee‡, and Richard B. Kim‡**

d D Viruses and Bile Salts
on Molecular Determinants on Sodium
Polypeptide

[1]Salhab A, et al. Gut. 2022 Jul;71(7):1373-1385; [2]Ho RH, et al. J Biol Chem. 2004 Feb 20;279(8):7213-22; [3]Vaz FM, et al. Hepatology. 2015 Jan;61(1):260-7; [4]Schneider AL, et al. Clin Res Hepatol Gastroenterol. 2022 Mar;46(3):101824; [5]Slijepcevic D, et al. Hepatology. 2018 Sep;68(3):1057-1069; [6]Cai SY, et al. JCI Insight. 2017 Mar 9;2(5):e90780.

Human genetics validates NTCP modulation as strategy for cholestatic disease

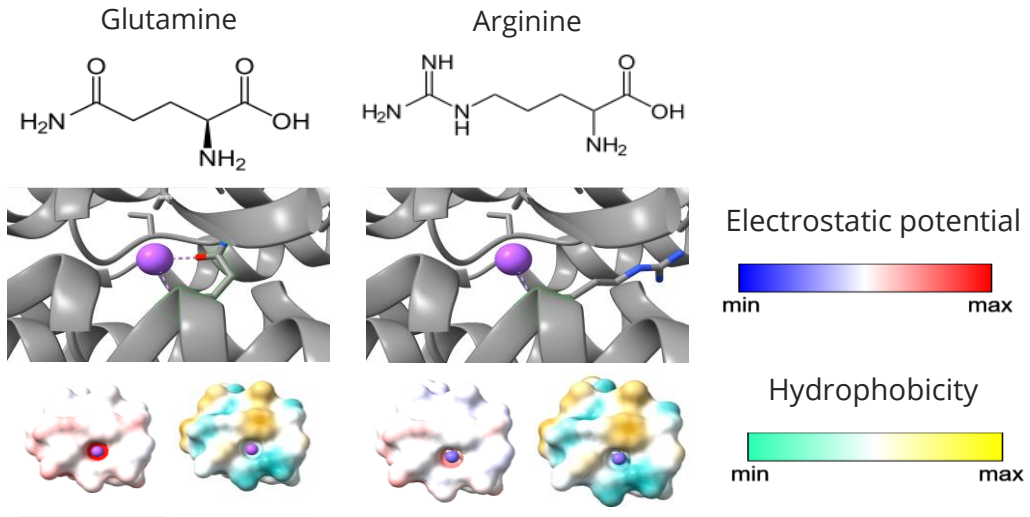


- The AX-0810 program introduces a variant in individuals with cholestatic disease to lower bile acids concentration in hepatocytes by a single A-to-I change
- The AX-0810 program is designed to be a disease modifying treatment
  - To alleviate symptoms in PSC and BA
  - To limit inflammation and fibrosis linked to bile acid toxicity
  - To prevent or delay the development of cirrhosis, organ failure and need for transplant

BA, Biliary atresia; PSC, Primary Sclerosing Cholangitis

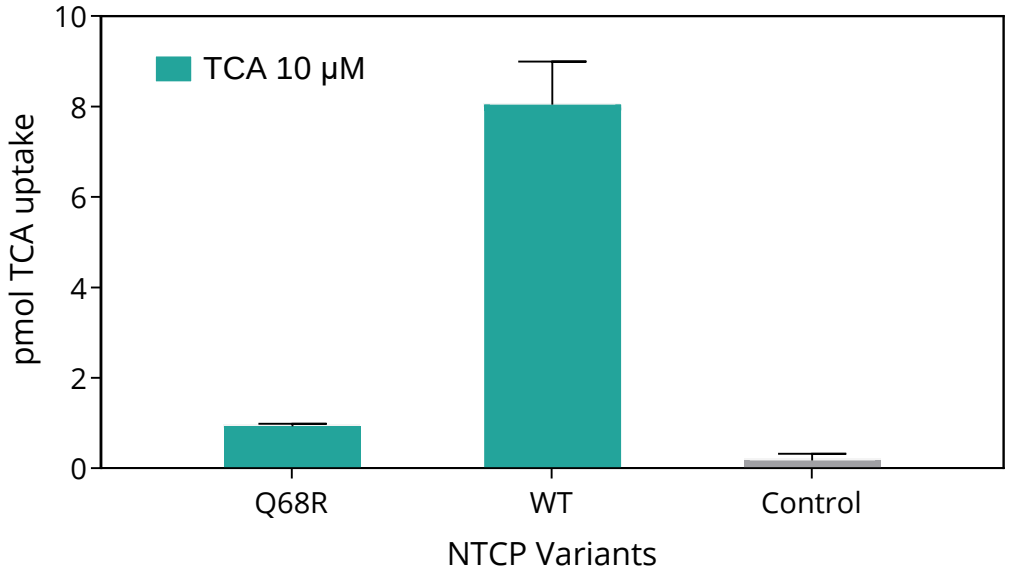
NTCP Q68R variant leads to modulation of bile acids re-uptake

3D Model of Q68R variant impact on Na^+ binding pocket of NTCP



BAs uptake (TCA) *in vitro**

n=3, mean±SEM



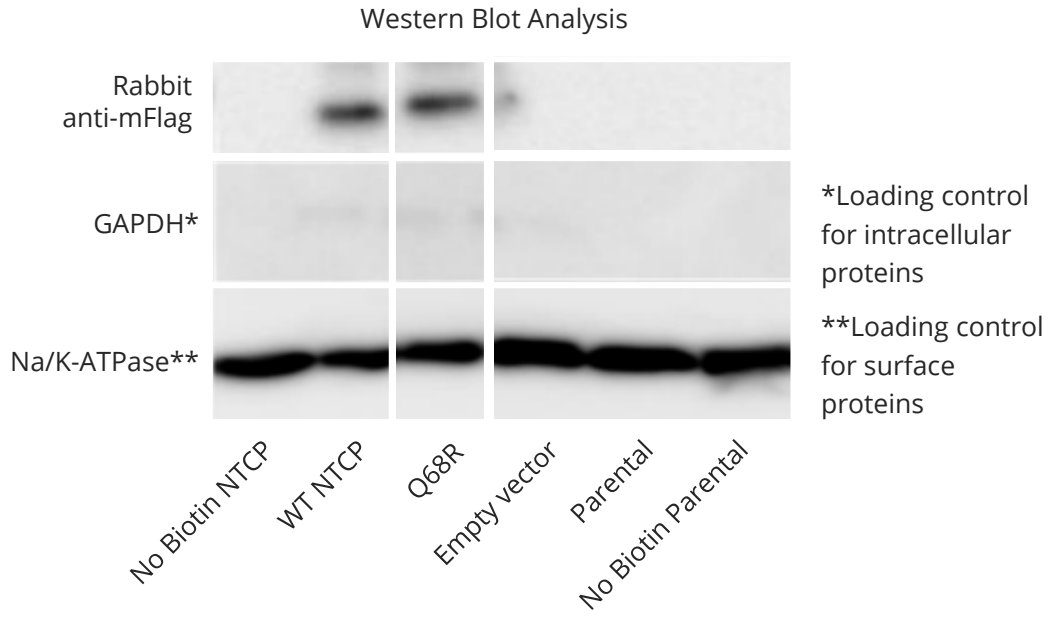
- The Q68R variant disrupts some hydrogen bonds and contacts in the Na^+ binding pocket.
- Clashes are inevitable since the Arg side chain is buried and likely to be found in one or another unfavorable rotamer state.

- Further assessment of Q68R variant in a bile acids uptake assay showed a near complete inhibition of BAs (specifically Taurocholic Acid or TCA) uptake *in vitro*, confirming findings from the 3D modeling

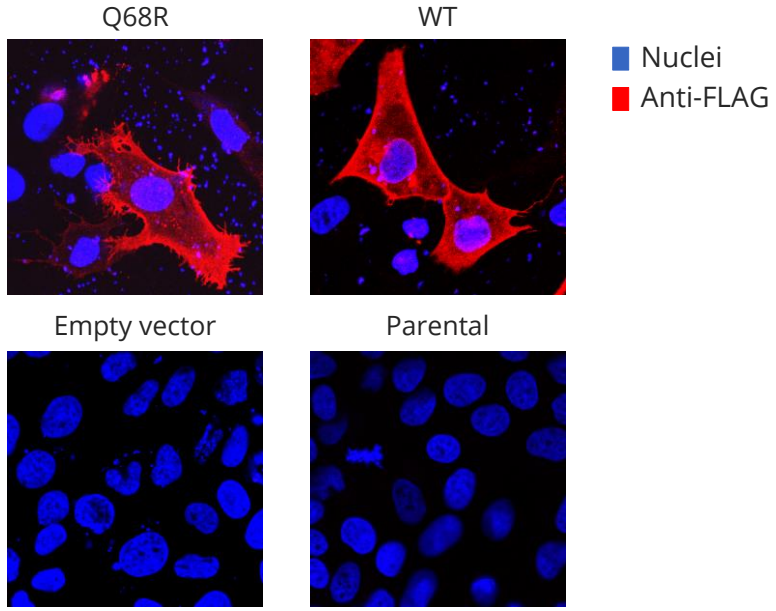
NTCP: Na-taurocholate cotransporting polypeptide, *Transiently transfected U2OS cells. Control is WT without TCA.

NTCP Q68R variant solely affects bile acids re-uptake function

NTCP protein expression was detected on western blot using the anti-FLAG antibody for all constructs



NTCP protein localization in vitro*



- No significant differences in NTCP RNA and protein levels were detected. The plasma membrane location of the Q68R variant was also unaffected.
- The Q68R variant solely affects NTCP bile acids reuptake function making it an approach of interest for Axiomer EON therapeutic application.

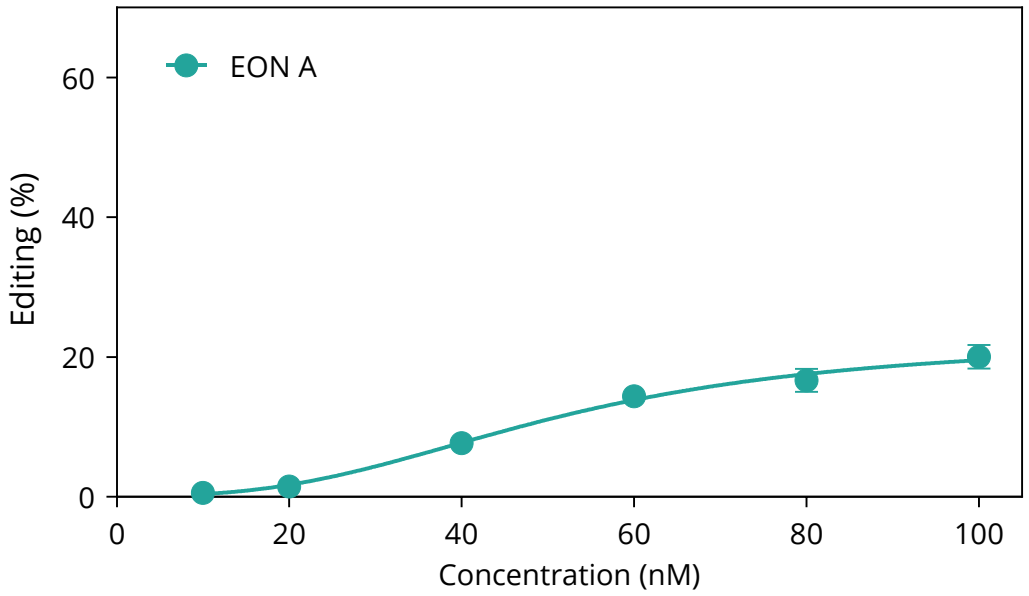
EON: editing oligonucleotide, NTCP: Na-taurocholate cotransporting polypeptide, *transiently transfected U2OS cells. S*LC10A1* is the gene that encodes for NTCP protein

EON mediated RNA editing leads to NTCP Q68R variant in WT hepatocytes

- Editing of NTCP RNA modulates bile acids reuptake in a dose dependent fashion

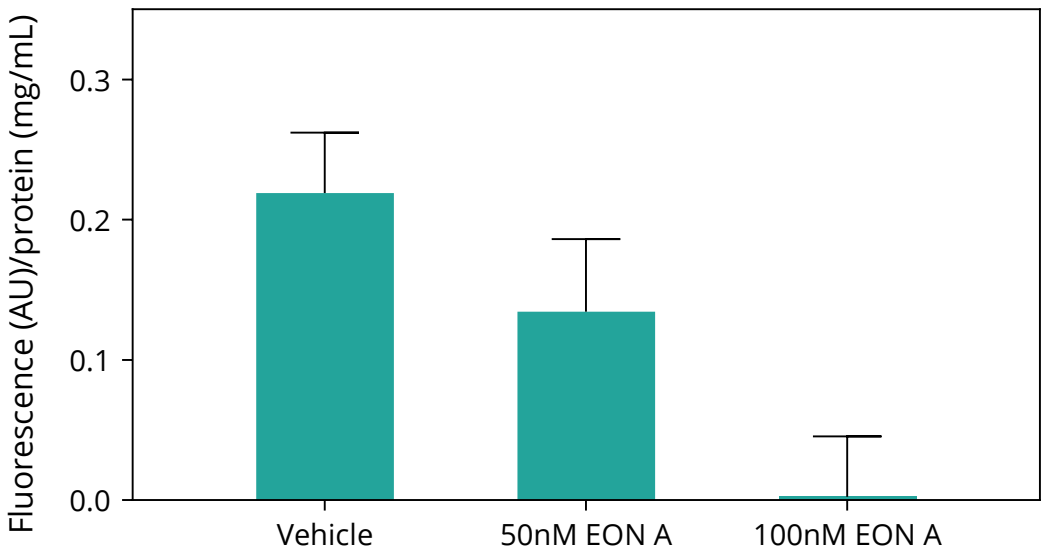
Early generation of EONs targeting NTCP RNA in PHH

Transfection, n=3, 72 hours, mean±SEM, dPCR



NTCP-mediated BAs uptake in HepaRG cells with Axiomer EON treatment

n=3, 50-100nM, 72 hours, mean±SEM



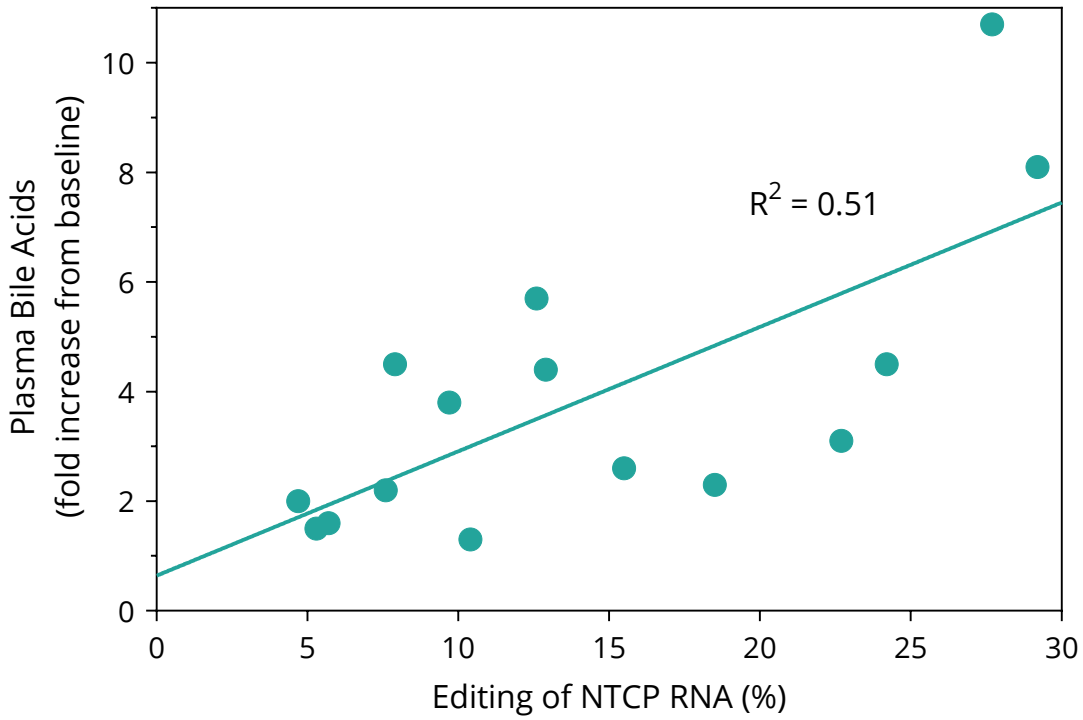
Early generation of EONs induces a dose-response inhibition of bile acids *in vitro* confirming its modulation by NTCP

NTCP: Na-taurocholate cotransporting polypeptide, BAs mentioned in this experiment are specifically Tauro-nor-THCA-24-DBD. *SLC10A1* is the gene that encodes for NTCP protein

EON mediated NTCP editing in NHP has linear correlation with bile acids plasma levels

Correlation between change in plasma BAs and editing of NTCP RNA in NHPs *in vivo*

n=6, Early generation EONs, IV, LNP formulation, 72 hours, dPCR



- NTCP target engagement with Axiomer EONs leads to the desired changes in biomarkers
- Correlation between plasma bile acids and early-generation EONs editing level in NHPs *in vivo* (linear regression R^2 = 0.51)

NTCP: Na-taurocholate cotransporting polypeptide, BAs mentioned in this experiment are specifically Tauro-nor-THCA-24-DBD. *SLC10A1* is the gene that encodes for NTCP protein

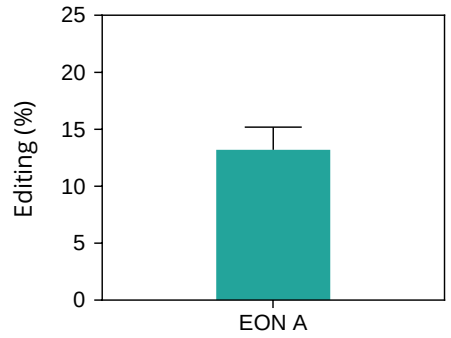
EON mediated editing demonstrates consistent editing of NTCP and impact on biomarker *in vivo*

MICE *in vivo*

EDITING EFFICIENCY

NTCP RNA Editing in Humanized Mice

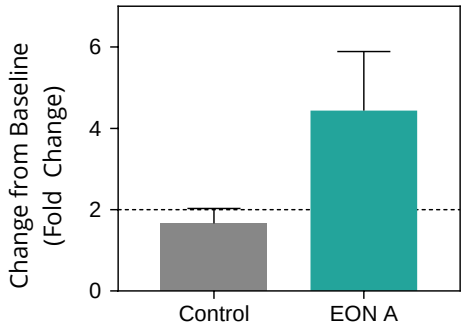
(N=4, 20mg/kg, 6 doses, GalNAc conjugation, SC, D25, ddPCR)



PLASMA TOTAL BILE ACIDS

Plasma TBA in Humanized Mice

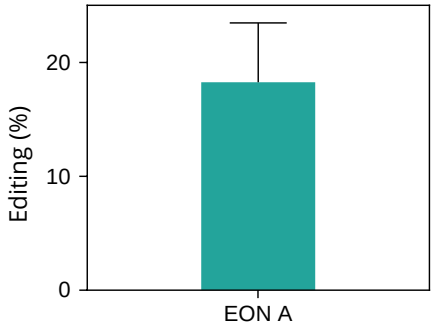
(N=4, 20mg/kg, 6 doses, GalNAc conjugation, SC, D25)



NHP *in vivo*

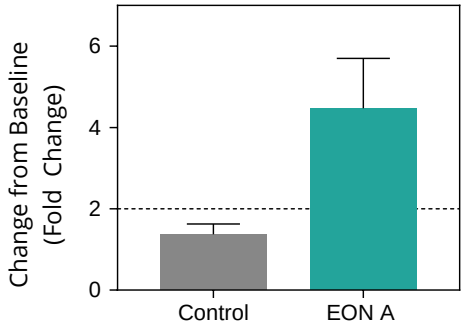
NTCP RNA Editing in NHP

(N=1, 1-4mg/kg, 4 doses, LNP formulation, IV, up to D46, ddPCR)



Plasma TBA in NHP

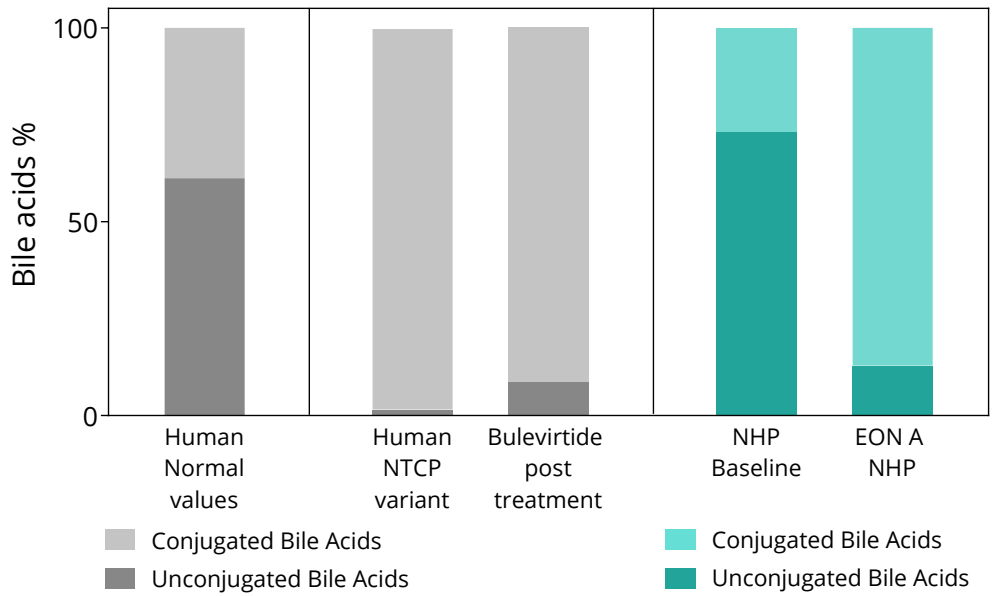
(N=1, 1-4mg/kg, 4 doses, LNP formulation IV, up to D39)



- EON A results in consistent editing data in humanized mouse model and NHP *in vivo* with approx. 15% editing reaching expected NTCP modulation
- Reaching >2-fold changes in biomarkers - expected impact on plasma bile acids levels following NTCP EON treatment

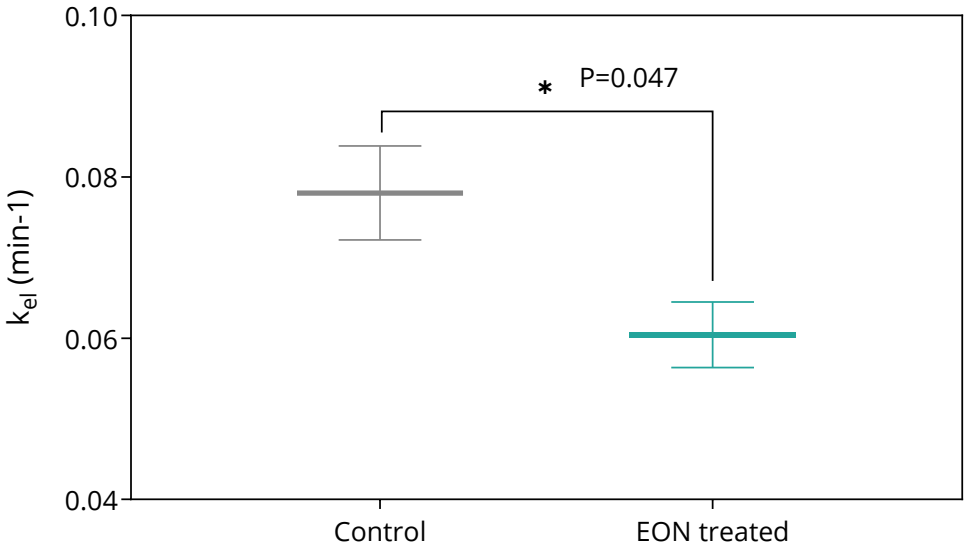
PoC in NHP on bile acid profile and TUDCA elimination

Change in Plasma BA Profile



TUDCA elimination rate from plasma in NHP

Exploratory study, early generation EON, n=5-7, 10mg/kg, 4 doses, SC, D51



- Conjugated bile acids are transported by NTCP back to the liver, change in plasma BA profile confirms NTCP specific modulation
- High confidence on NTCP EON treatment to positively impact BA toxic load in the liver

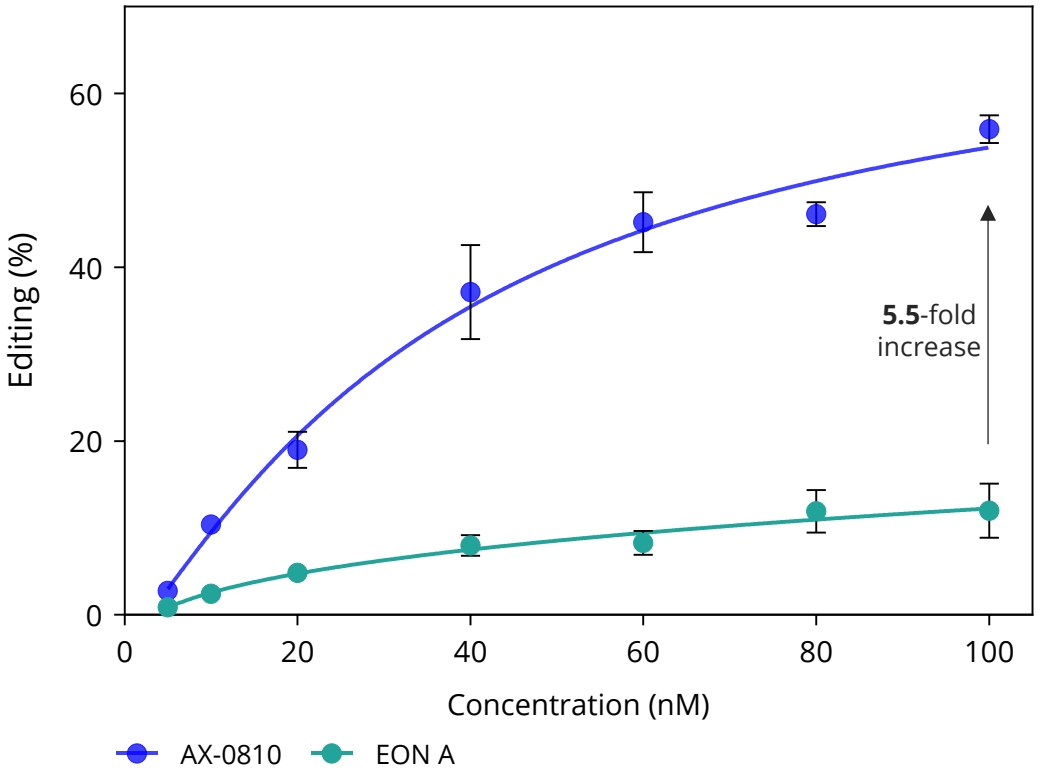
- TUDCA is a Tauro-conjugated bile acid specifically transported by NTCP from the plasma to the liver
- Decrease in TUDCA plasma clearance kinetics further confirm NTCP target engagement for EON treated NHP

Conditions in the NHP experiment on the left: N=1, 1-4mg/kg, 4 doses, LNP formulation, IV, up to D42, LC-MS/MS. Mao F, et al. J Biol Chem. 2019 Aug 2;294(31):11853-11862; Haag M, et al. Anal Bioanal Chem. 2015 Sep;407(22):6815-25.; Wedemeyer H, et al. N Engl J Med. 2023 Jul 6;389(1):22-32.

AX-0810 clinical candidate selected with enhanced potency and stability profile

AX-0810 clinical candidate has an enhanced potency profile over EON A in PHH

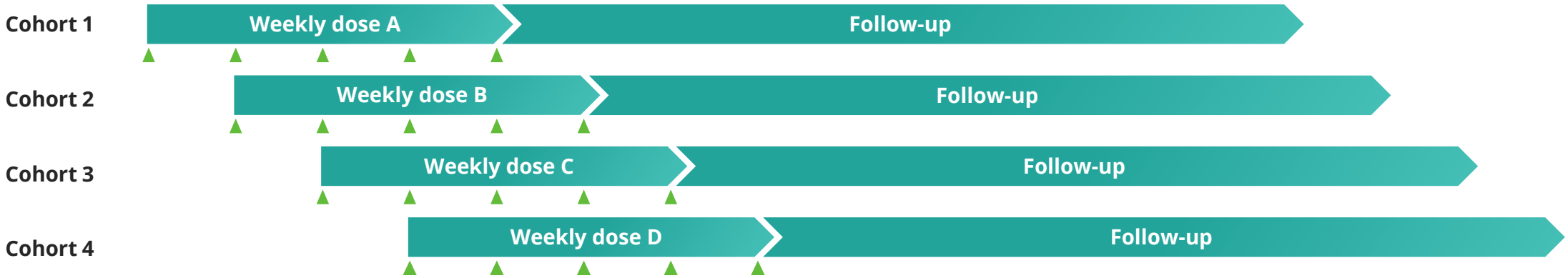
Transfection, n=3, 72 hours, dPCR, mean±SEM



- AX-0810 clinical candidate is a GalNAc conjugated EON
- 5.5-fold increase in potency over early generation NTCP editing oligonucleotide
- Improved stability profile *in vitro*
- Confirmed class safety, with no hepatotoxicity or immunostimulatory score

First in human trial of AX-0810 to establish target engagement

Integrated single/multiple ascending dose study design



Treatment

AX-0810 GalNAc conjugated editing oligonucleotide

Objectives

- Confirm target engagement as measured by biomarkers
- Assess safety, tolerability, and PK of AX-0810

Trial design

- Combined single and multiple ascending dose
- ≥60 heathy volunteers, 4 weeks dosing phase followed by 12 safety weeks follow-up
- 5 weekly subcutaneous injections
- Baseline and placebo-controlled design
- Standardized conditions for assessment of bile acids at multiple timepoints
- DMC safety reviews before proceeding to next dose and dose escalation

Key endpoints

- Change in bile acids levels and profile in plasma and urine, liver biomarkers
- Circulating RNA as exploratory endpoint

Top-line data in Q4 2025

Summary & next steps

AX-0810 for cholestatic diseases

Modulating NTCP activity to reduce hepatic bile acids load is a promising target for hepatoprotection in cholestatic diseases

Promising and consistent results reported to date in humanized mice and NHPs

- ✓ Meaningful impact on bile acid plasma level and bile acids profile build confidence for data readout in FIH clinical trial
- ✓ Axiomer NTCP EON impact on biomarkers in line with preclinical disease model and clinical data reported with NTCP inhibition
- ✓ Favorable safety profile observed
- ✓ AX-0810 GalNAc candidate with optimized potency and stability to enter clinic

CTA submission in Q2 2025

Top-line data from FIH expected in Q4 2025

Thank you!

Prof. Peter Beal, and his group

Professor in the Department of Chemistry at the University of California at Davis and Director of the NIH-funded UC Davis Chemical Biology Graduate Program

Prof. Stan van de Graaf, and his team

Full Professor; ACS - Atherosclerosis & Ischemic Syndromes, Amsterdam Gastroenterology Endocrinology Metabolism and Tytgat Institute for Liver and Intestinal Research

ProQR R&D Department

ProQR®

IT'S IN OUR RNA