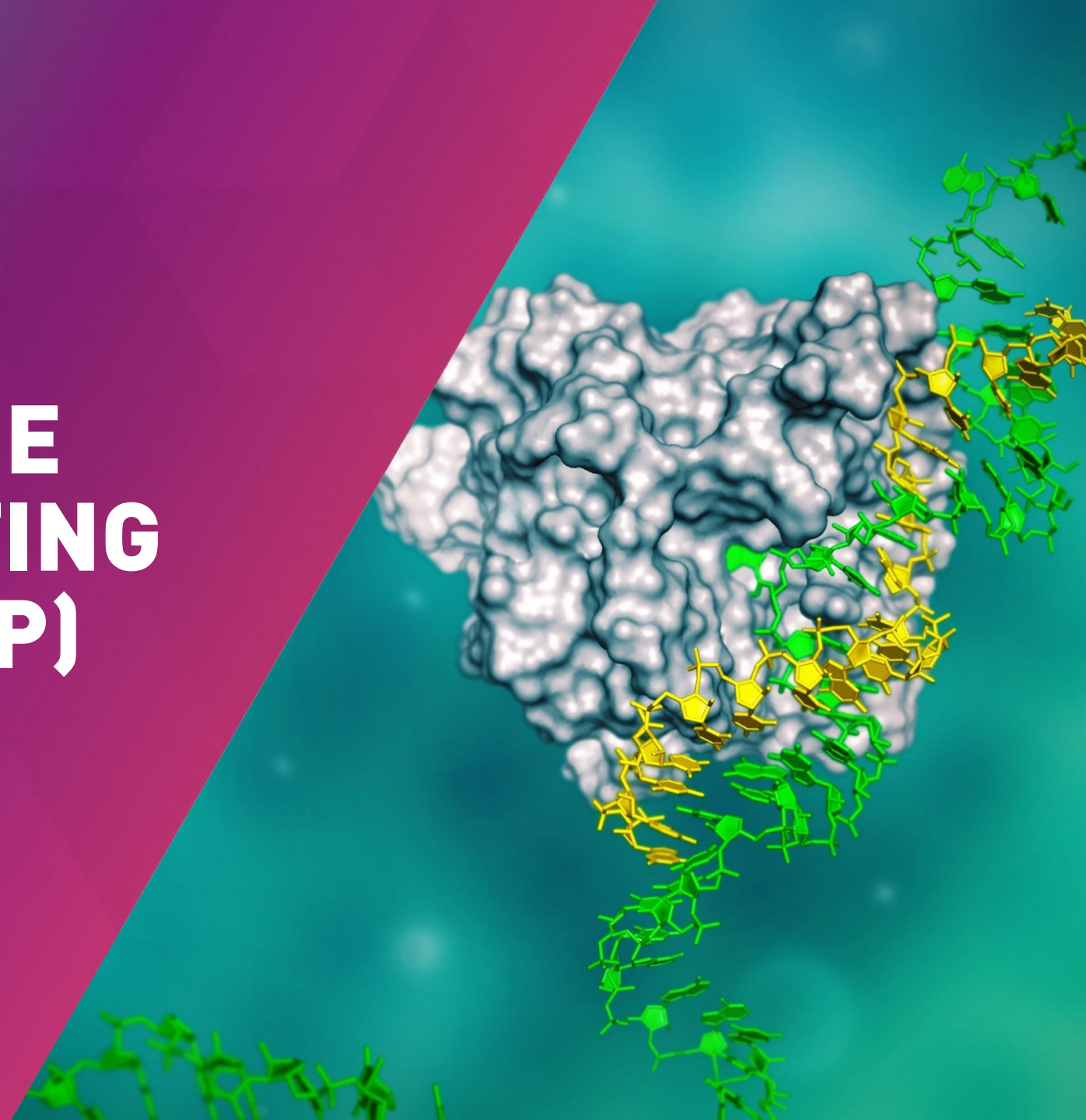
OLIGONUCLEOTIDE GUIDED RNA EDITING OF SLC10A1 (NTCP)

as a therapeutic approach to lower bile acid re-uptake in cholestatic diseases

Gerard Platenburg, CSO and Co-founder of ProQR

20th Annual OTS Meeting, October 8, 2024

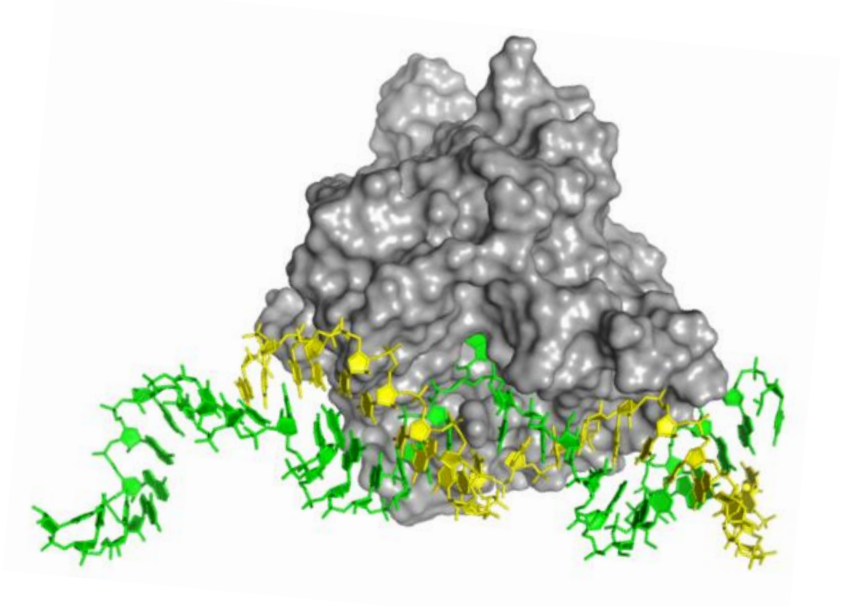


Forward-looking statements

This presentation contains forward-looking statements. All statements other than statements of historical fact are forward-looking statements, which are often indicated by terms such as "anticipate," "believe," "could," "estimate," "expect," "goal," "intend," "look forward to", "may," "plan," "potential," "predict," "project," "should," "will," "would" and similar expressions. Such forward-looking statements include, but are not limited to, statements regarding our strategy and future operations, statements regarding the potential of and our plans with respect to our technologies and platforms (including Axiomer™), our preclinical model data, our pipeline targets, our other programs and business operations, our current and planned partnerships and collaborators and the intended benefits thereof, including the collaboration with Lilly and the intended benefits thereof, including the upfront payment, equity investment, and milestone and royalty payments from commercial product sales, if any, from the products covered by the collaboration, as well as the potential of our technologies and product candidates; our updated strategic plans and the intended benefits thereof, our plans to seek strategic partnerships for our ophthalmology assets, and our financial position and cash runway. Forward-looking statements are based on management's beliefs and assumptions and on information available to management only as of the date of this presentation. Our actual results could differ materially from those anticipated in these forward-looking statements for many reasons, including, without limitation, the risks, uncertainties and other factors in our filings made with the Securities and Exchange Commission, including certain sections of our annual report filed on Form 20-F. These risks and uncertainties include, among others, the cost, timing and results of preclinical studies and other development activities by us and our collaborative partners whose operations and activities may be slowed or halted due to shortage and pressure on supply and logistics on the global market; our reliance on contract manufacturers to supply materials for research and development and the risk of supply interruption from a contract manufacturer; the ability to secure, maintain and realize the intended benefits of collaborations with partners, including the collaboration with Lilly; the possible impairment of, inability to obtain, and costs to obtain intellectual property rights; possible safety or efficacy concerns that could emerge as new data are generated in research and development; general business, operational, financial and accounting risks; and risks related to litigation and disputes with third parties. Given these risks, uncertainties and other factors, you should not place undue reliance on these forward-looking statements, and we assume no obligation to update these forward-looking statements, even if new information becomes available in the future, except as required by law.

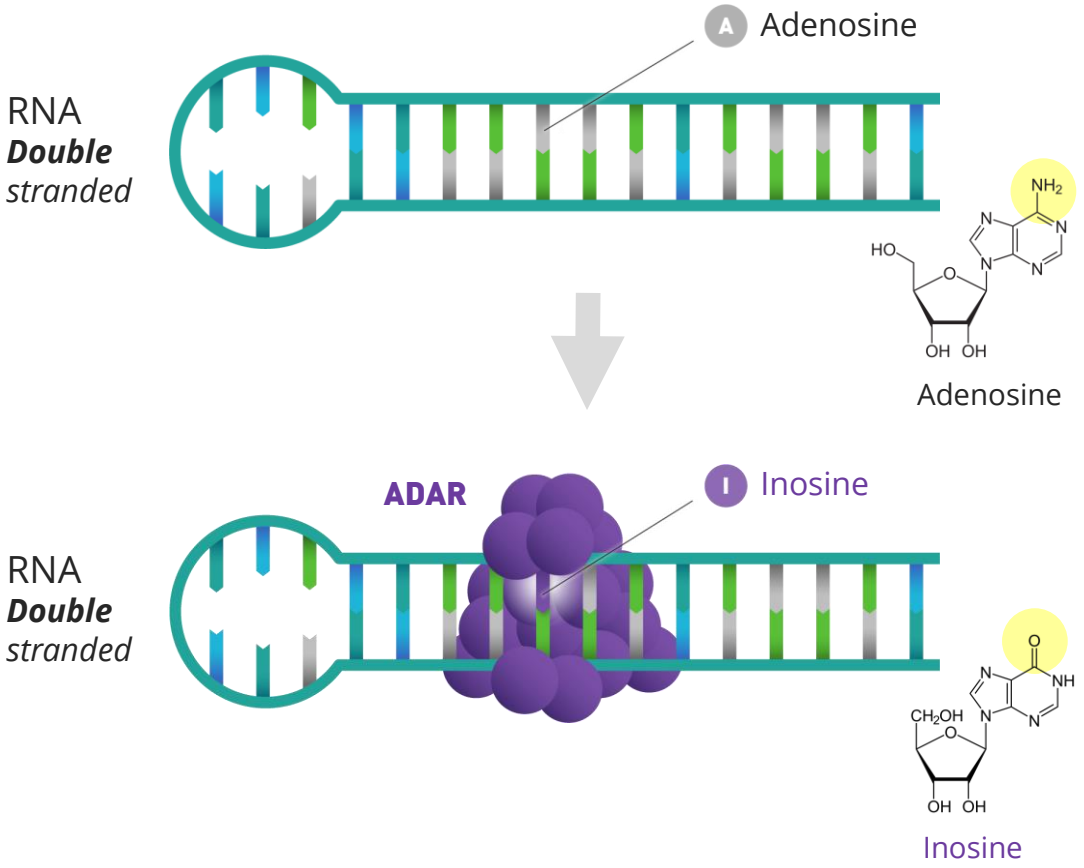
What is ADAR editing?

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)**

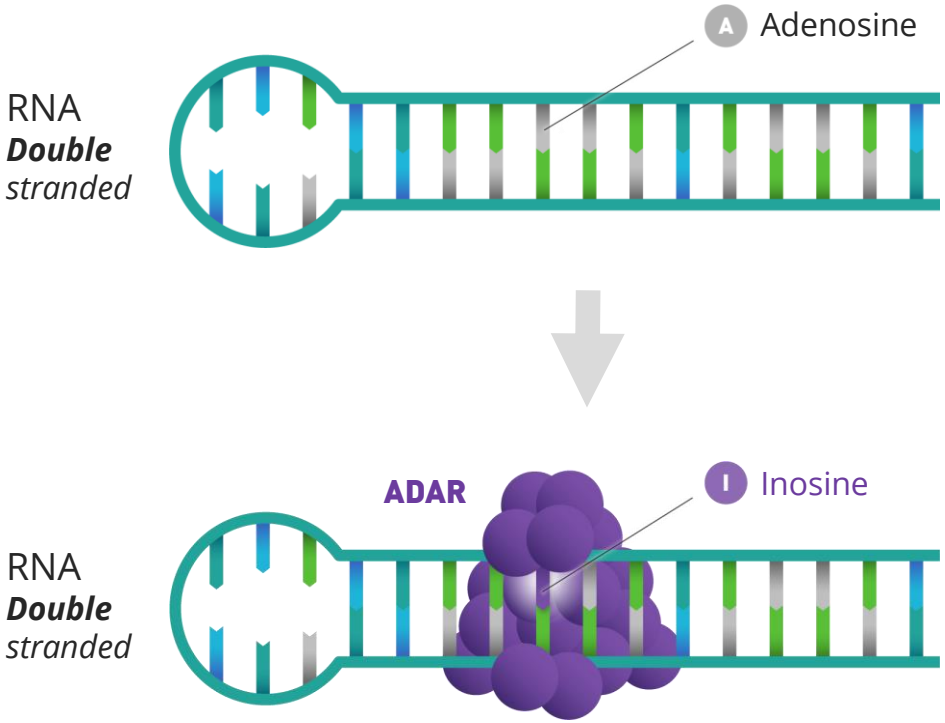
Natural ADAR editing *(A-to-I)*



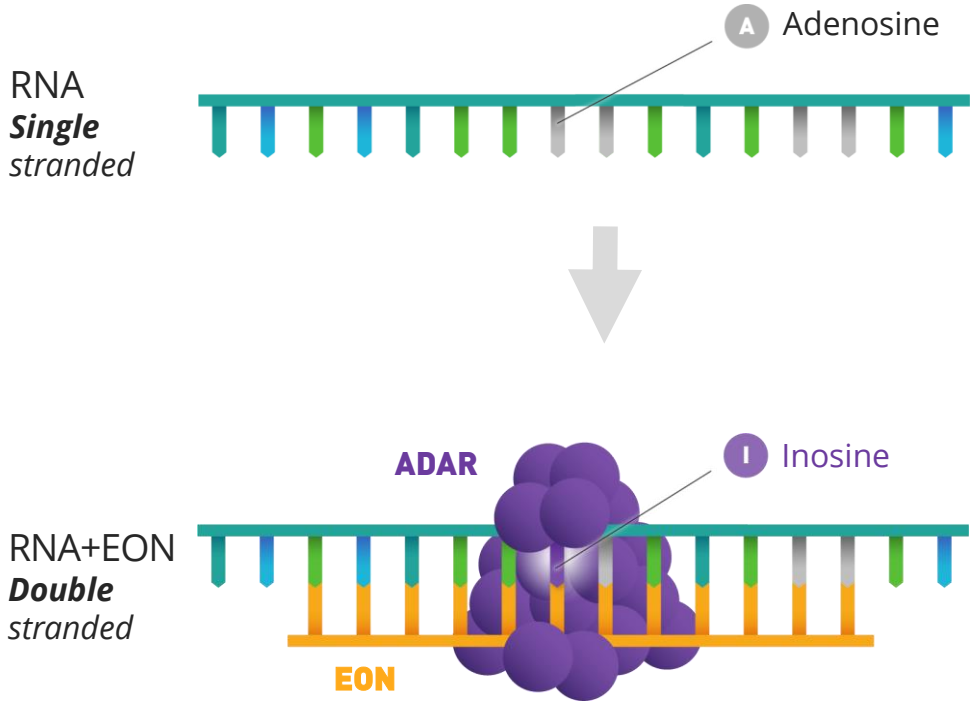
Axiomer™ EONs unlock cellular machinery potential to treat diseases

By attracting ADARs and allowing highly specific editing

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



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

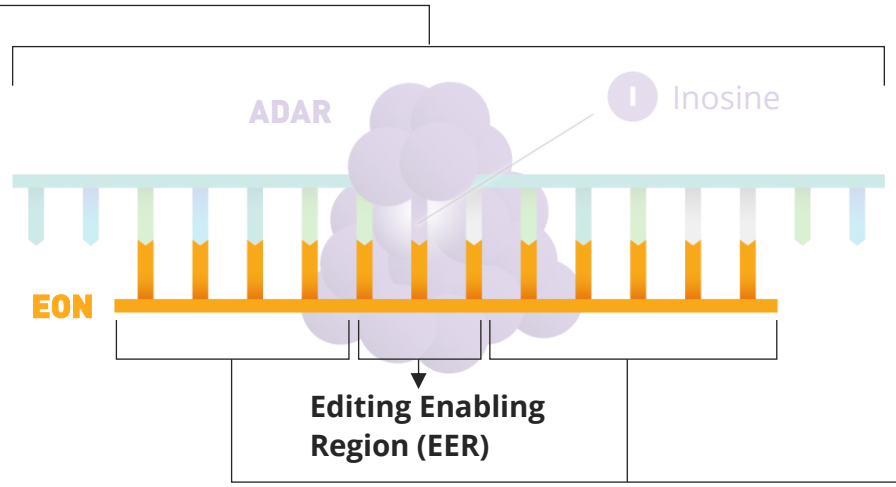


ADARs: Adenosine deaminases acting on RNA.

Driving the development of optimized EONs for therapeutic use

Sequence defines target
RNA binding and provides stability

EON total length
Between ~25 - 30 nt



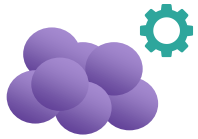
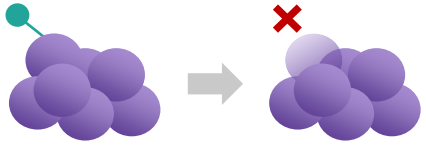
ADAR-binding region (ABR)
Backbone modifications enable ADAR binding, and disable off-target editing

Optimized sequence and chemistry define functionality

- Increase editing efficacy
- Bring metabolic stability
- Prevent off-target ('bystander') editing
- Ensure bioavailability (cell and tissue uptake)
- Offer safety and tolerability at therapeutic doses

ADAR: Adenosine deaminase acting on RNA, EON: Editing oligonucleotide, Nt: nucleotides

Axiomer™ 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

BROAD THERAPEUTIC POTENTIAL

- Common diseases
- Rare diseases
- Target a wide variety of organs
- Treat so-far undruggable targets

PTMs: Post-translational modifications.

AX-0810 for cholestatic diseases

RNA-editing therapy

for Primary Sclerosing Cholangitis and Congenital Biliary Atresia

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

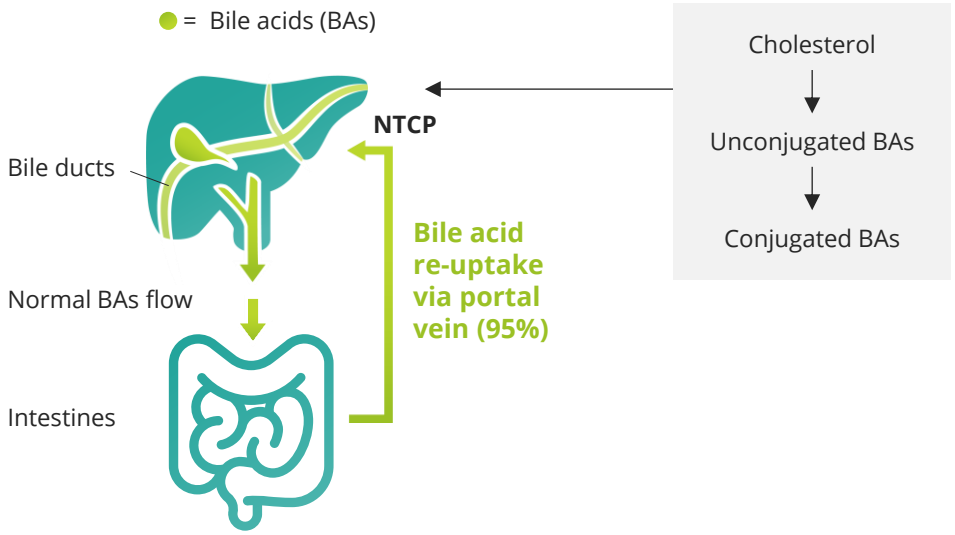
Initial indications are **Primary Sclerosing Cholangitis** affecting adults and Congenital **Biliary Atresia** affecting pediatrics early in life. Both conditions have no approved therapies and require liver transplantation.

- **Biliary Atresia** is projected to affect ~24,000 pediatric individuals in US, EU and JP.
- **Primary Sclerosing Cholangitis** is projected to affect more than 80,000 individuals in EU, US and JP.

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.

Bile acids are produced in the liver to facilitate digestion of lipids

Bile acids metabolism



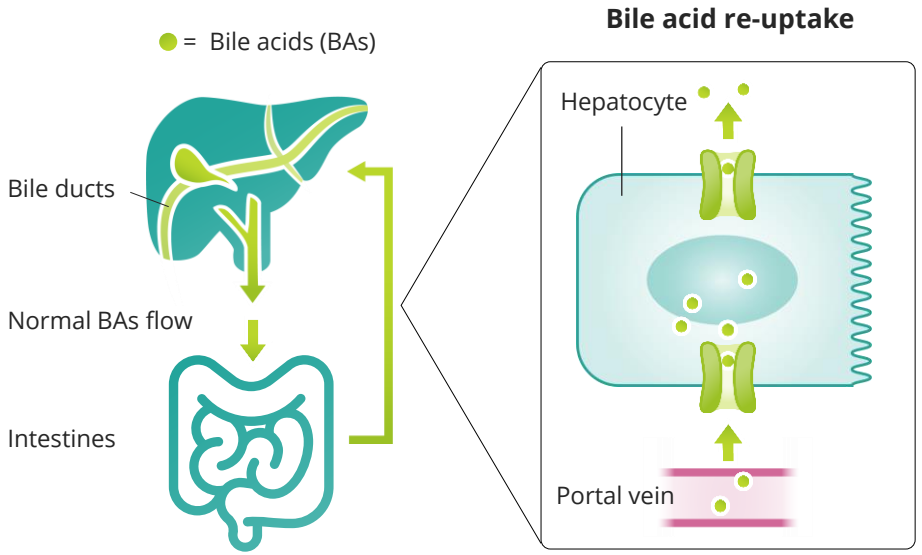
- Bile acids help the digestion of fats and absorption of certain vitamins.
- They are either glycine or taurine conjugated in the liver to make them easier to dissolve in water and excreted with urine.
- Around 95% of the conjugated bile acids are then reabsorbed by the liver for reuse through **Na-taurocholate cotransporting polypeptide (NTCP).**
- NTCP is selective for the re-uptake of conjugated BAs

NTCP: Na-taurocholate cotransporting polypeptide.

Excessive accumulation of bile acids in the liver leads to cell stress and damage

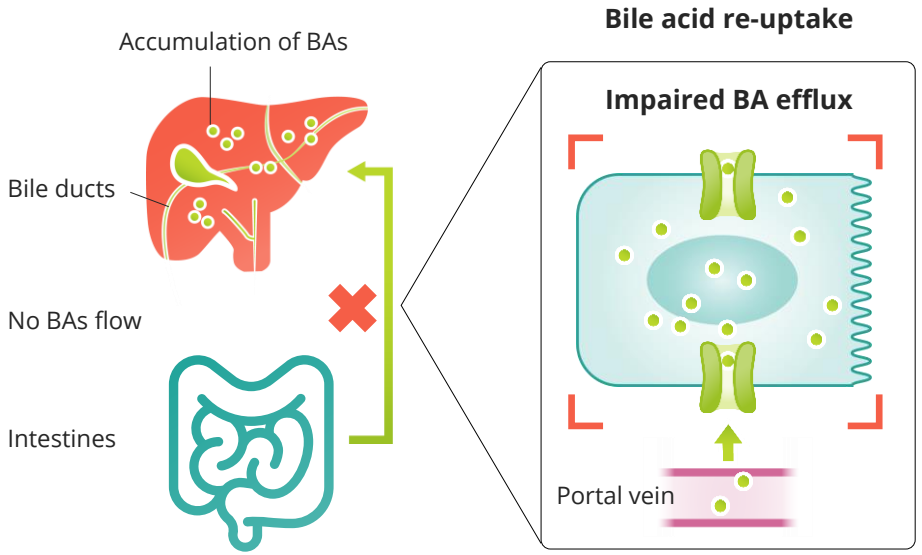
Healthy individuals

Normal BA levels in the hepatocytes



Individuals with cholestatic diseases

Excessive BA accumulation in the hepatocytes



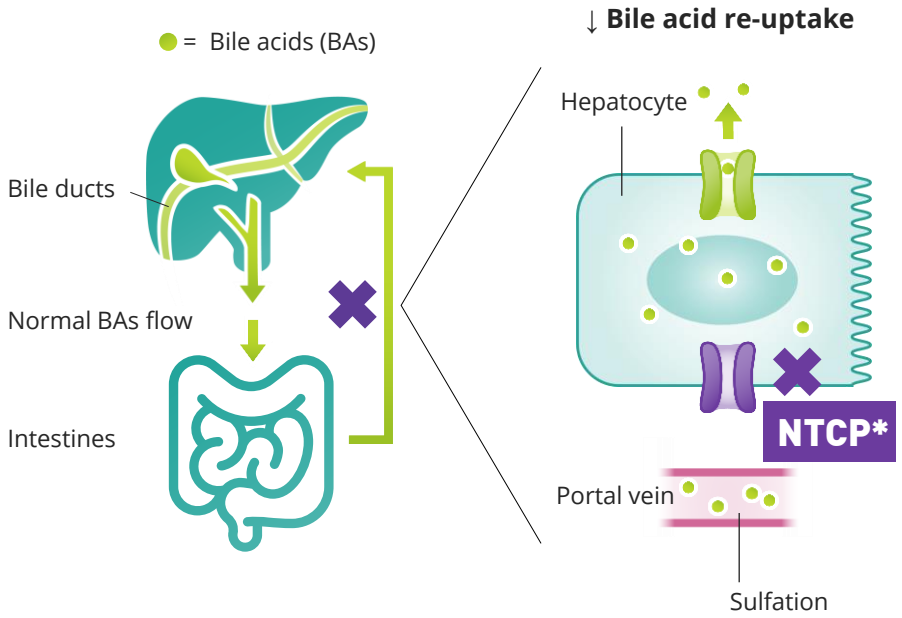
Cell stress and damage leading to cell death and inflammation

Dysfunctional bile ducts lead to a toxic buildup of bile acids in the liver of individuals with cholestatic diseases

References: Cai SY, et al. JCI Insight. 2017;2(5):e90780 and Cai SY, Boyer JL. Ann Transl Med. 2021 Apr;9(8):737

Natural variants affect NTCP bile acid reuptake function without symptoms

Bile acids metabolism with variants affecting NTCP BAs reuptake function



* Bile acids re-uptake modulated function

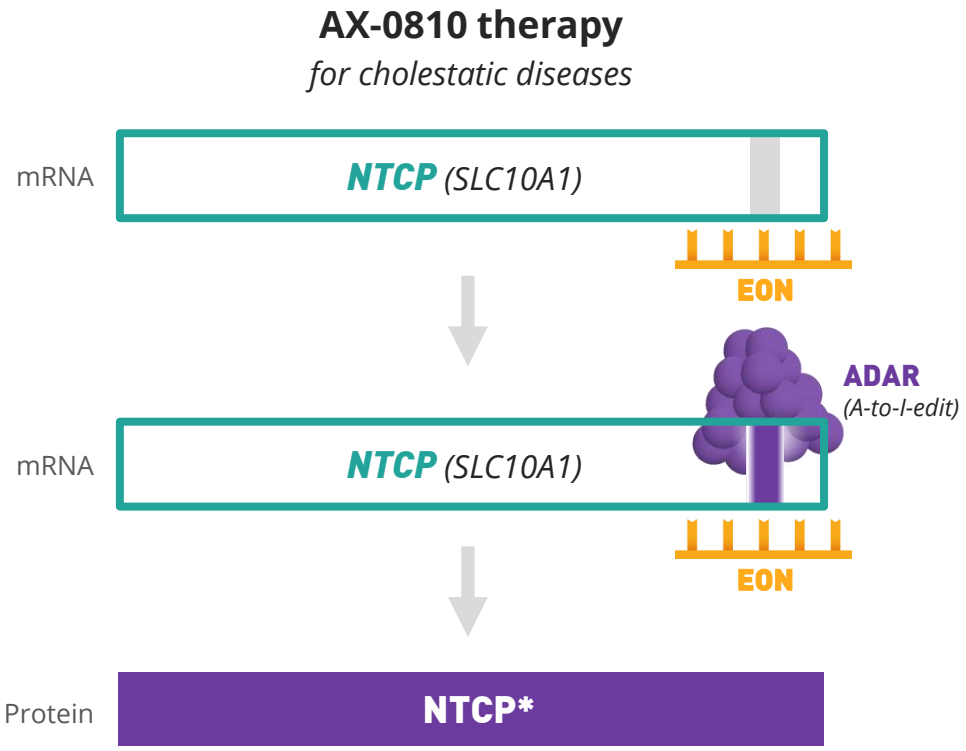
In early infancy
individuals with NTCP variants showed mild clinical symptoms with elevated total bile acids (tBA) levels (up to 1500µM).

By mid-childhood
tBA levels are decreased. Human body use a process called sulfation to facilitate elimination of bile acids in plasma.

Adult individuals
with NTCP bile acid reuptake function deficiency do not present with any significant clinical symptoms or signs of liver disease.

NTCP: Na-taurocholate cotransporting polypeptide, BA: Bile acids, References: Vaz, F et al. *Hepatology* vol. 61,1(2015):260-7; Mao, F et al. *The Journal of biological chemistry* vol. 294,31 (2019): 11853-11862.

AX-0810 a novel therapeutic strategy to reduces bile acids re-uptake into liver



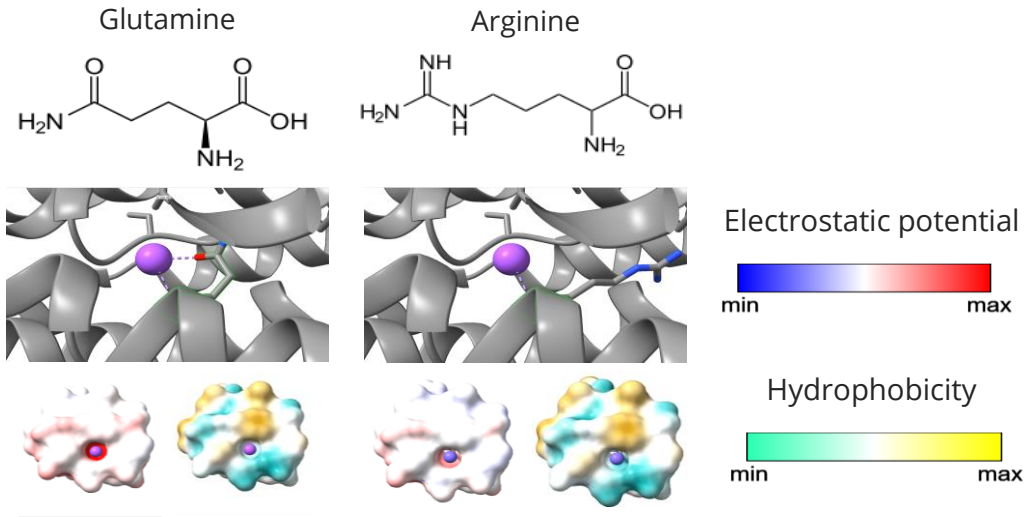
* Bile acids re-uptake modulated function

- **AX-0810 program is a novel and "on target" approach reducing bile acid re-uptake into the hepatocytes**
  - Transient and controlled approach leading to a modulated NTCP function
- **AX-0810 program can reduce bile acid toxic load in the liver**
  - To slow-down the disease progression and alleviate the symptoms in PSC and BA individuals
  - To prevent or delay the development of cirrhosis, organ failure and the need for transplant

BAs: Biliary atresia, NTCP: Na-taurocholate cotransporting polypeptide, PSC: Primary Sclerosing Cholangitis. *SLC10A1* is the gene that encodes for NTCP protein.

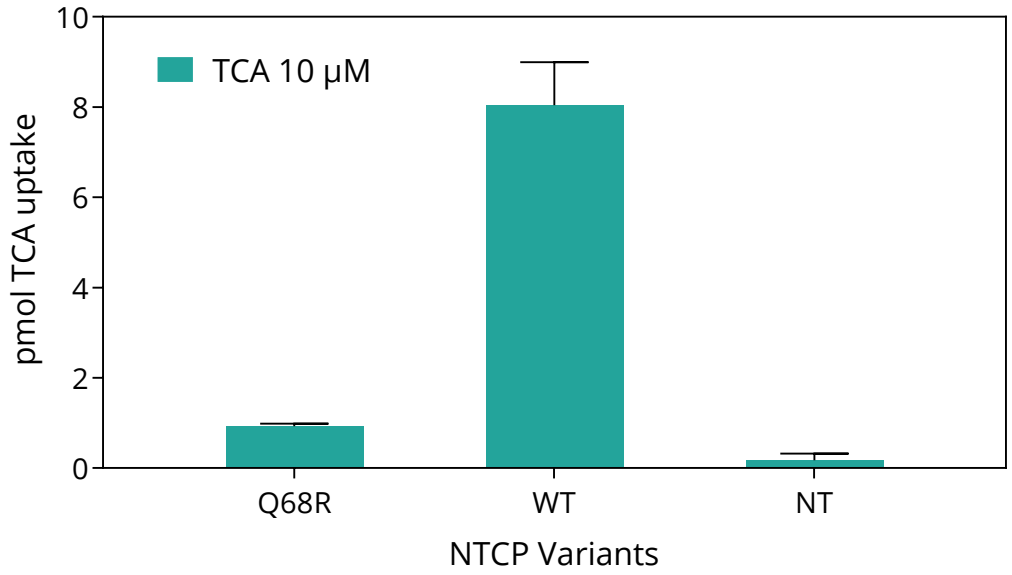
Introducing the Q68R variant leads to modulation of bile acids reuptake

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



BAs uptake (TCA) *in vitro**

n=3, mean±SEM



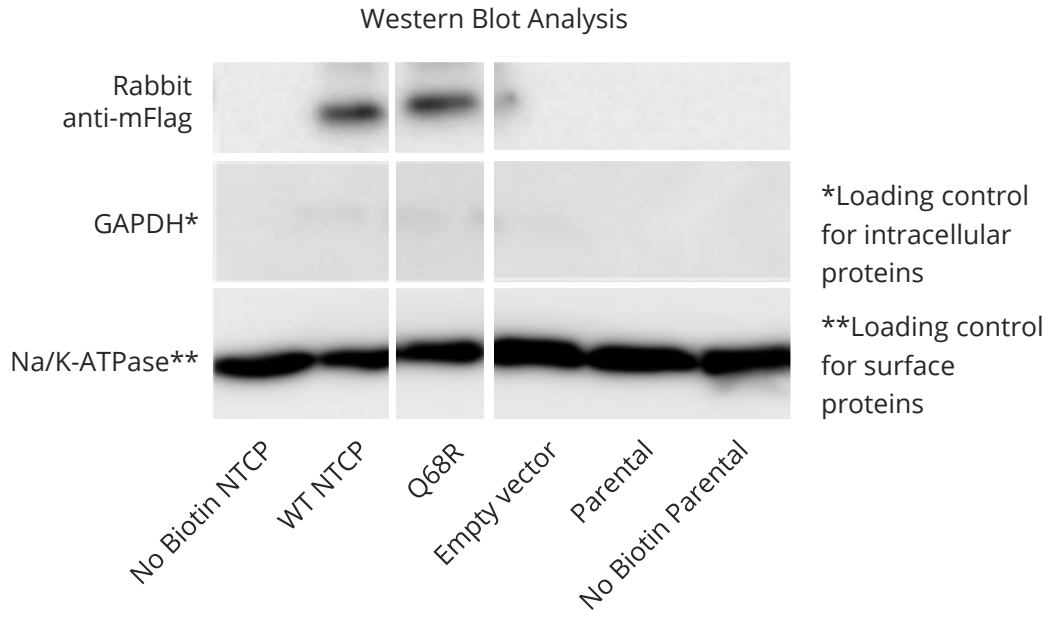
- The Q68R variant disrupts some hydrogen bonds and contacts in the Na^+ binding pocket.
- Clashes are inevitable since the Arg side chain is buried and likely to be found in one or another unfavorable rotamer state.

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

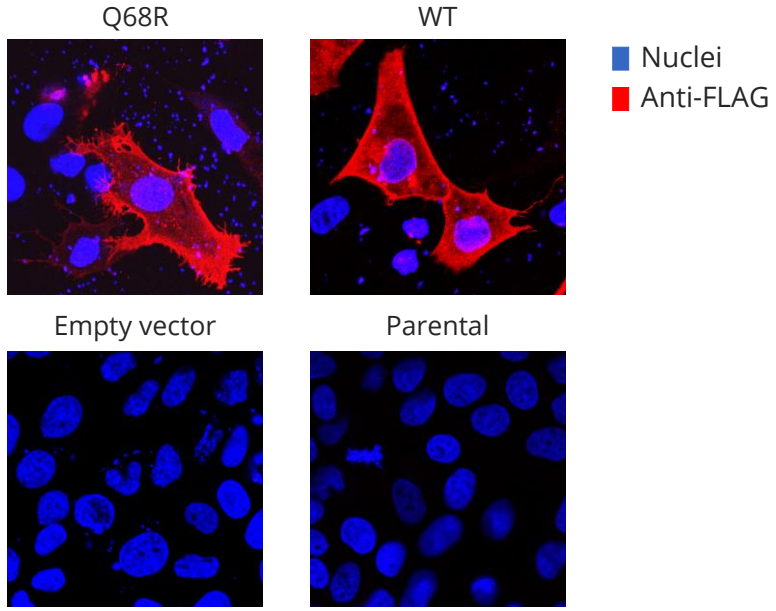
BAs: Bile acids, NTCP: Na-taurocholate cotransporting polypeptide, *transiently transfected U2OS cells.

The Q68R variant solely affects NTCP bile acids reuptake 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 acid reuptake function making it an approach of interest for Axiomer EON therapeutic application.

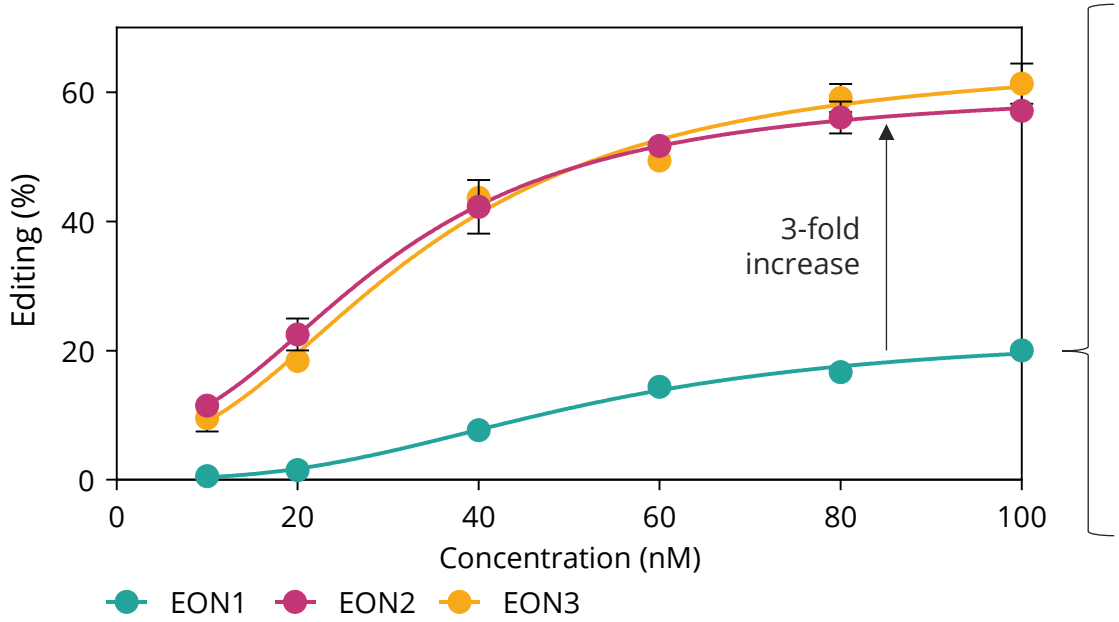
BAs: Bile acids, EON: editing oligonucleotide, NTCP: Na-taurocholate cotransporting polypeptide, *transiently transfected U2OS cells. *SLC10A1* is the gene that encodes for NTCP protein

Axiomer™ EON treatment leads to NTCP Q68R variant in WT hepatocytes

Editing of NTCP RNA modulates BAs reuptake in a dose dependent fashion

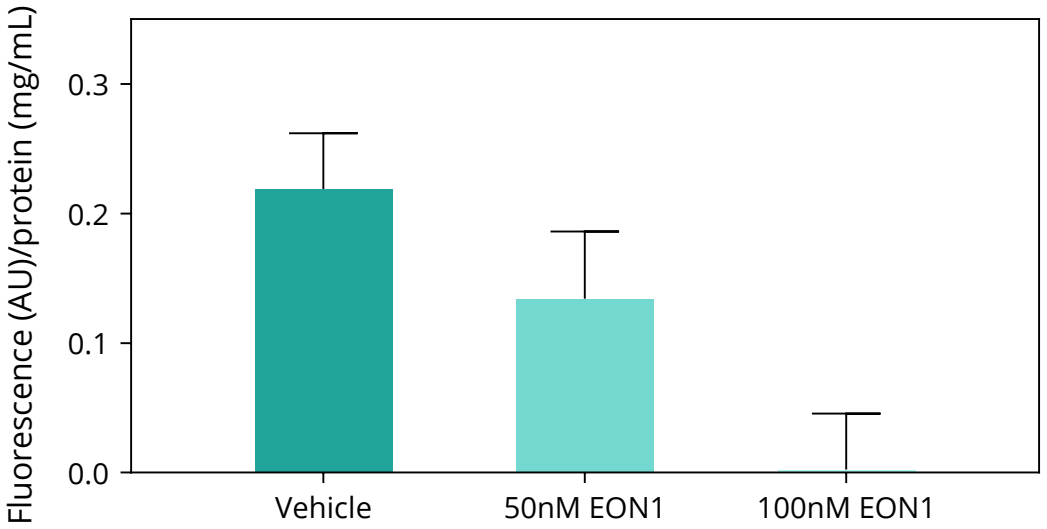
EONs targeting NTCP RNA optimization in PHH

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



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

n=3, 50-100nM, 72 hours, mean±SEM



Leveraging expertise in EONs optimization, including adjustment of sequence and chemistry, lead to increased potency of EONs targeting NTCP RNA.

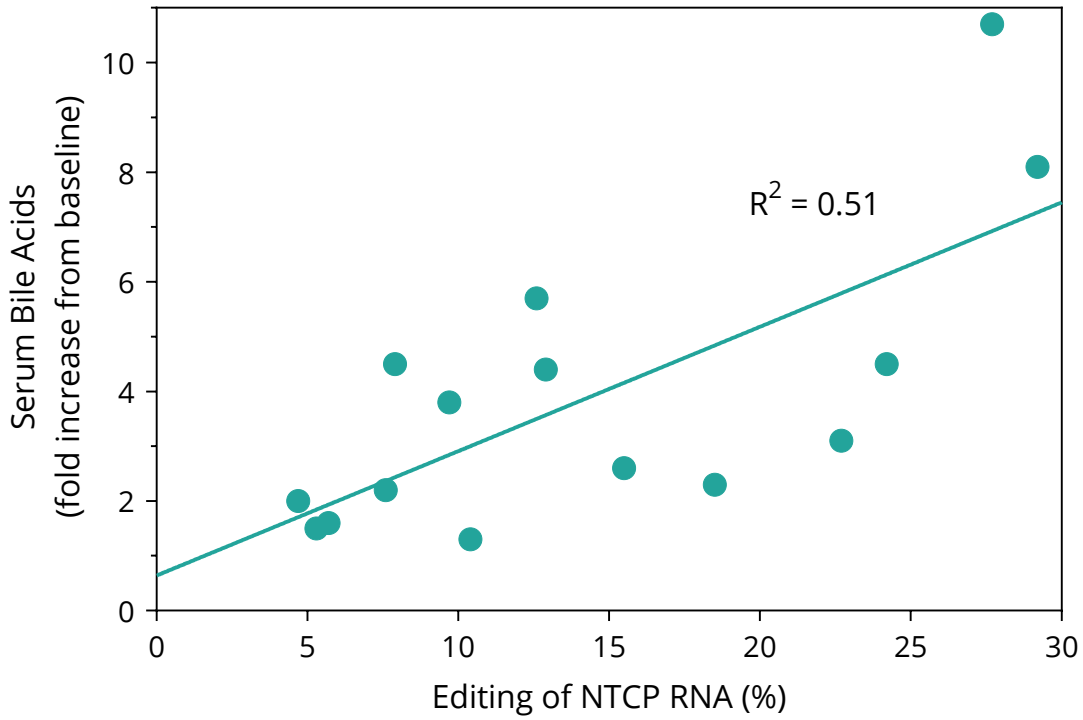
Early generation of EONs (EON1) induces a dose-response inhibition of BAs in vitro confirming its mediation by NTCP

BAs: Bile acids, 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

Proof of concept with Axiomer EONs targeting NTCP in liver of NHPs

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

n=6, EON1, IV, LNP formulation, 72 hours



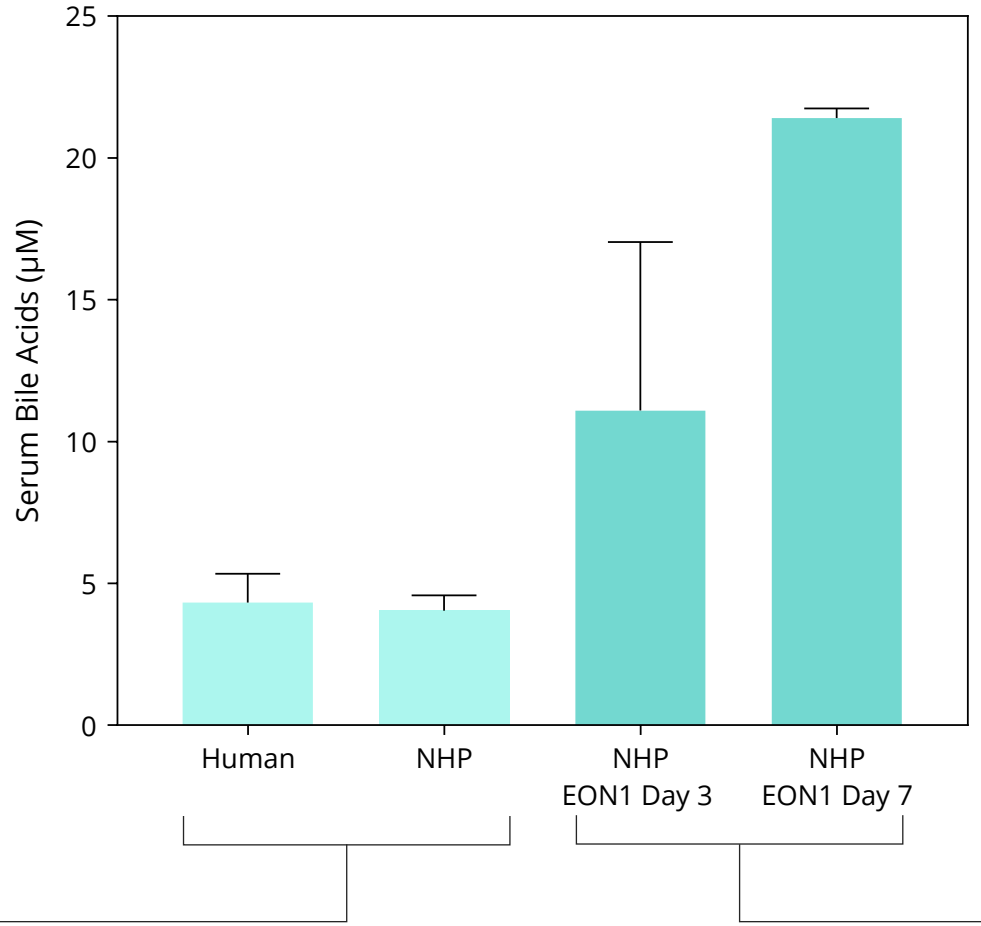
- NTCP target engagement with Axiomer EONs leads to the desired changes in biomarkers
- High correlation between serum bile acids and EON1 editing level in NHPs in vivo (linear regression R2 = 0.51)

BAs: Bile acids, EON1: early generation editing oligonucleotides targeting SCL10A1 (NTCP) mRNA, 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

NTCP editing oligonucleotides lead to the desired changes in biomarkers in NHPs

NHP is a predictive model for humans

Translatability between NHP and human confirmed with human sequence homology and equivalent level of serum bile acids



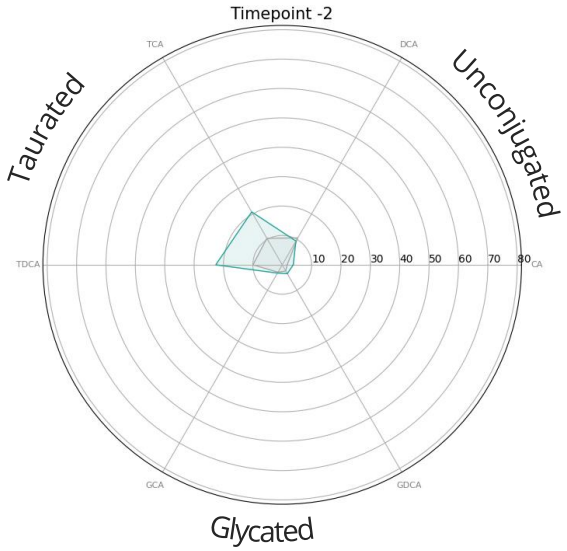
NTCP editing oligonucleotides induce the desired change in biomarkers

Above the 2-fold change of serum bile acids considered as the threshold to reach clinically meaningful improvement in disease progression in patients suffering from cholestatic liver disease

Axiomer™ EONs induce a change in serum bile acid level and profile

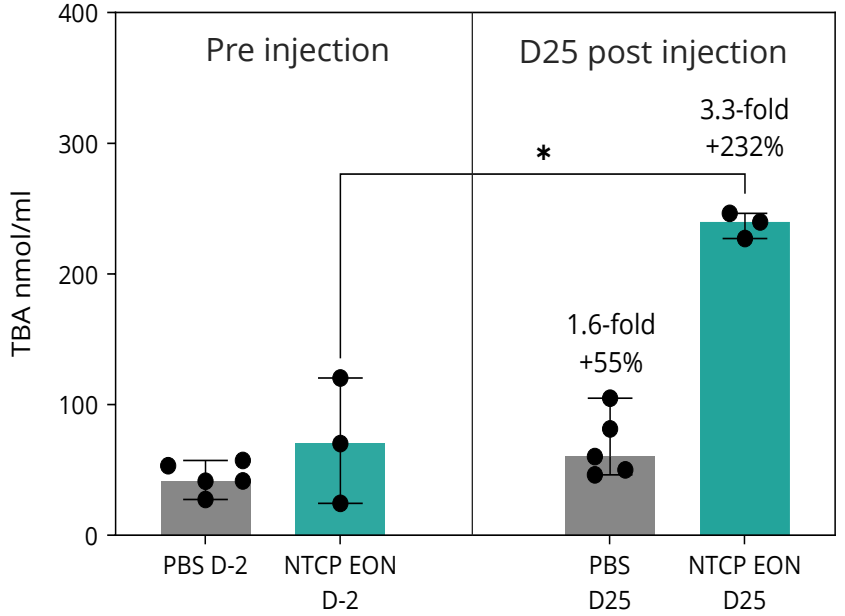
Confirming NTCP bile acids reuptake function modulation

Bile acid profile
Pre injection

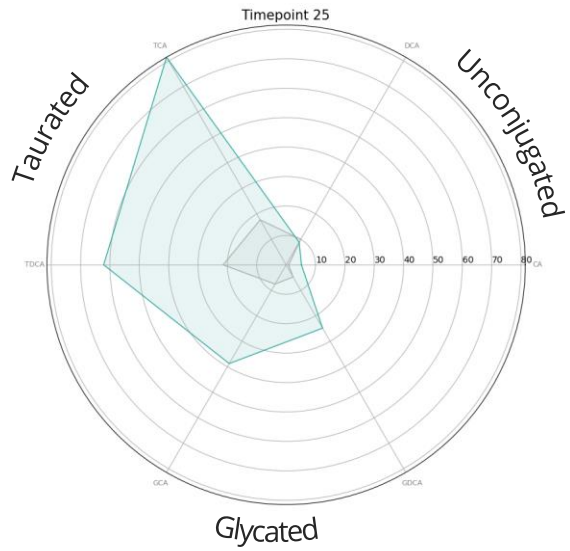


Change in serum bile acids with Axiomer EONs targeting NTCP RNA in humanized liver of mice *in vivo*

n=3-5, 20mg/kg, NTCP EON, SC, GalNAc conjugation, 25 days

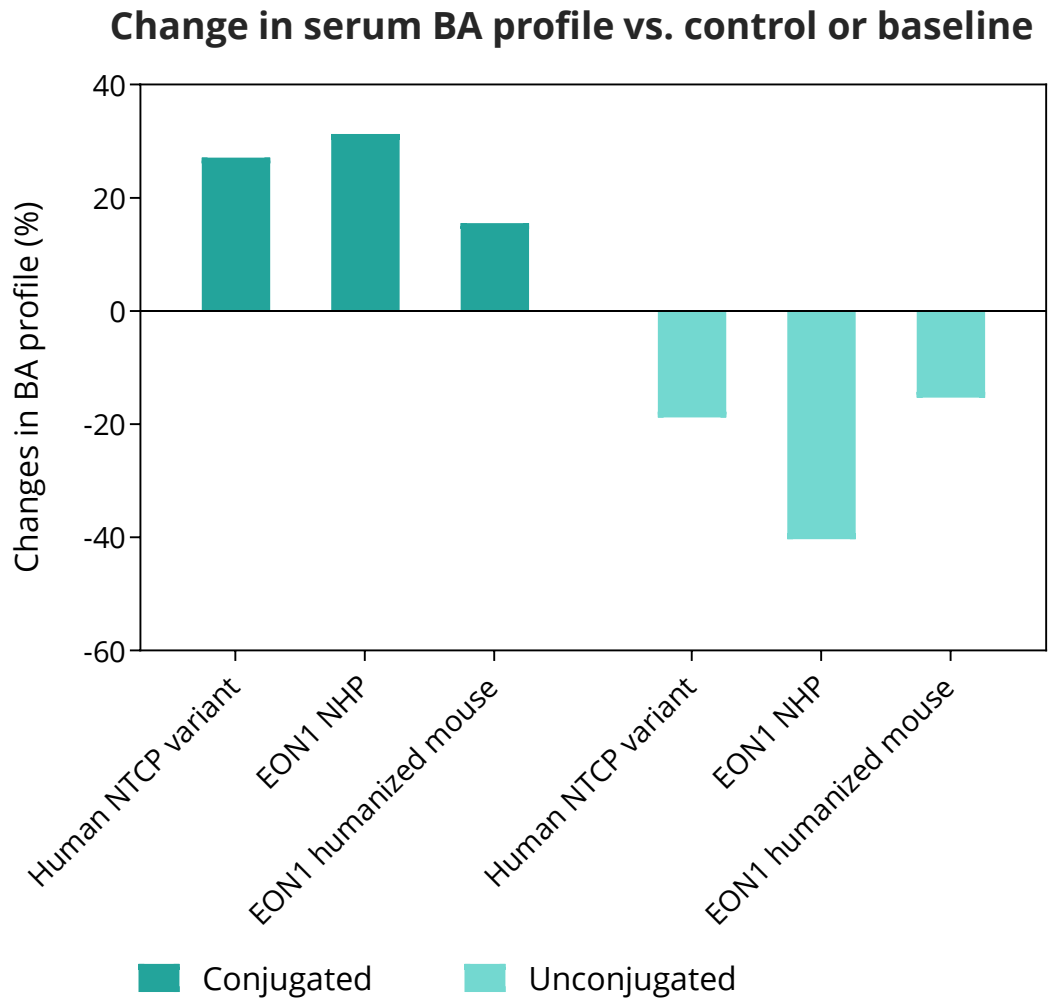


Bile acid profile
D25 post injection



The increased levels of conjugated bile acids in the serum confirm the targeted modulation of NTCP bile acids reuptake function with Axiomer EON in humanized mouse model

Axiomer™ EONs induce a change in BA profile consistent with NTCP natural variant



- In serum, EON1 shows a comparable change in bile acid profile (conjugated and unconjugated) to NTCP natural variant
- Trend towards increase in conjugated BA in serum confirming NTCP specific modulation
- Impact on BA secretion pathways as expected and no changes in NTCP levels show by proteomics data

AX-0810 early evidence generation approach on safety and target engagement

Phase 1 on healthy volunteers

Objectives

- Assess safety, tolerability, PK and PD of AX-0810 without interference by concomitant pathological conditions
- Establish target engagement by biomarkers

Trial design

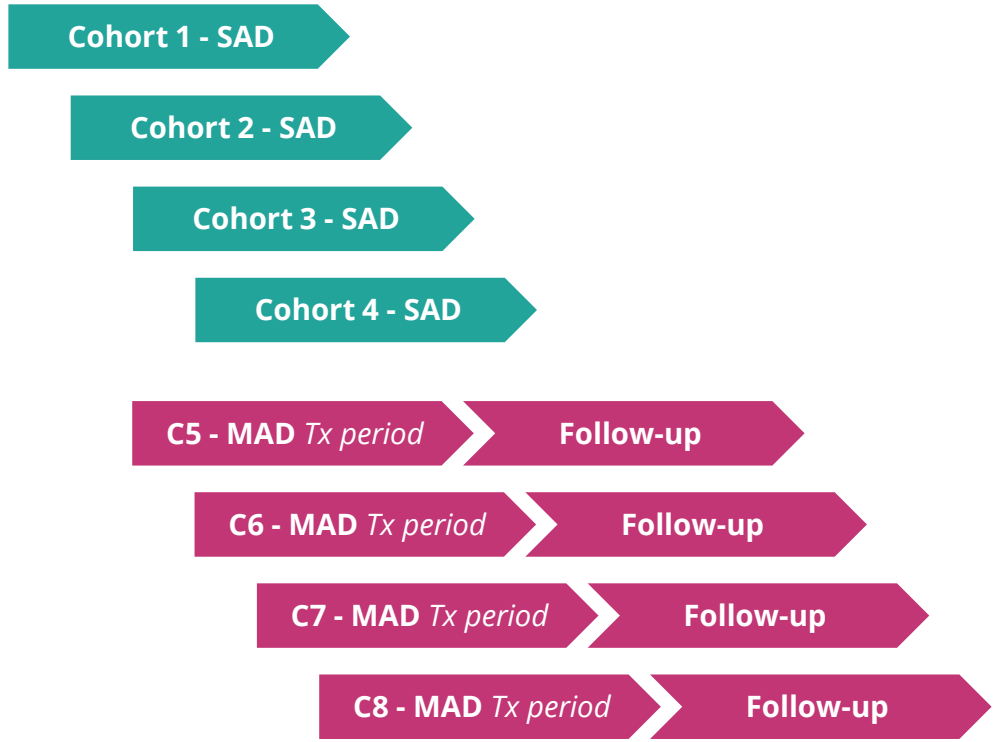
- Single and multiple dose ascending trial
- Single trial site: timely recruitment and data generation

Endpoints will include

- Safety, tolerability, PK and PD of AX-0810
- Change in bile acids in serum, urine and feces, liver enzymes and serum cholesterol
- Change in disease specific biomarkers: ALP and bilirubin
- Measure RNA editing in circulating exosomes in plasma

Entry into clinical trials in late 2024 / early 2025

Preliminary study design



ALP, Alkaline phosphatase; MAD, multiple ascending dose; PD, Pharmacodynamic; PK, Pharmacokinetics; SAD, single ascending dose.

Summary
and next steps

Proof of concept with NTCP Axiomer EONs in *in vivo*

in vivo proof of target engagement (NTCP) with relevant changes in biomarkers *in vivo* in NHPs and humanized mouse models using Axiomer EONs

A targeted approach modulating NTCP for Cholestatic diseases

Axiomer EONs have the potential to specifically modulate NTCP protein BAs reuptake function in cholestatic diseases

AX-0810 program is moving towards the clinic

These findings support the new therapeutic application of Axiomer EONs targeting NTCP for cholestatic diseases

Thank you slide

ProQR® THERAPEUTICS

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ProQR®

IT'S IN OUR RNA