ProQR®

ASGCT 2024 – PRECLINICAL PROOF OF CONCEPT FOR THE AX-0810 PROGRAM TARGETING NTCP FOR CHOLESTATIC DISEASES

Investment Community Webinar

May 9, 2024

Agenda

Welcome, Agenda, and FLS
Sarah Kiely

Introduction
Daniel A. de Boer

ASGCT presentation summary
Preclinical proof of concept for the AX-0810 program targeting NTCP for Cholestatic Diseases

Gerard Platenburg

Next Steps and Upcoming Milestones
Daniel A. de Boer

Q&A
Daniel A. de Boer, Gerard Platenburg

Speakers

Sarah Kiely
VP Investor Relations and Corporate Affairs

Daniel A. de Boer
Founder & CEO

Gerard Platenburg
Chief Scientific Officer

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.

ProQR Therapeutics

Short overview

Focus on Axiomer™

Exclusively focused on the development of proprietary Axiomer™ RNA editing platform across multiple therapeutic areas; initial focus on liver and CNS diseases

Novel mechanism of action, leading patent estate

Axiomer™ was discovered in ProQR labs in 2014 and uses well-proven modality of oligonucleotides to recruit a novel mechanism of action

Validated across multiple genes

Preclinical data demonstrate Axiomer™ is broadly validated across multiple genes

ADAR

Axiomer™ is ADAR-mediated RNA editing, recruiting endogenous adenosine deaminase acting on RNA (ADAR)

Two pillars underlie strategy

ProQR developing wholly owned pipeline with initial targets in liver-originated diseases

- AX-0810 program preclinical proof of concept at ASGCT 2024
- AX-0810 for cholestatic diseases and AX-1412 for cardiovascular disease rapidly advancing to the clinic late 2024/early 2025

Selectively enter into partnerships: initial partnership with Lilly in September 2021, expansion announced December 2022

Cash-runway into mid-2026

Cash position of €102.7 M as of end of Q1 2024 provides runway to mid 2026, beyond multiple clinical data readouts

ProQR development pipeline

	TARGET	DISCOVERY	NON-CLINICAL	CLINICAL	GUIDANCE	ESTIMATED POPULATION
PROQR PROGRAMS						
CHOLESTATIC DISEASES	**AX-0810** *for* **NTCP**				Entry into clinical trials in late 2024 / early 2025	~ 100K[1]
CARDIOVASCULAR DISEASES	**AX-1412** *for* **B4GALT1**				Entry into clinical trials in late 2024 / early 2025	~ 200M[2]
	AX-1005 *for CVD*					
RARE NEURODEVELOPMENT DISORDER	**AX-2402** *for Rett syndrome*					~ 20K
METABOLIC DISEASES	**AX-2911** *for NASH*					~ 16M
	AX-0601 *for obesity and T2D*					~ 650M
	AX-9115 *for rare metabolic condition*					~ 20K
OTHERS	*Multiple targets in discovery pipeline*					
PARTNERED PROGRAMS						
Lilly	*Initial **5** undisclosed targets*	*Progress undisclosed*				
	*Next **5** undisclosed targets*	*Progress undisclosed*				
	*Up to **5** potential additional targets*					

[1]Approximately 100K people affected with Primary Sclerosing Cholangitis and Biliary Atresia in US and EU5. [2]Approximately 200 million people suffer from too high a level of cholesterol in US and EU5. *SLC10A1* is the gene that encodes for NTCP protein.
CVD: Cardiovascular Diseases, NASH: Nonalcoholic steatohepatitis, T2D: Type 2 Diabetes.
References: Boonstra K, Beuers U, Ponsioen CY. J Hepatol. 2012 May;56(5):1181-1188; Karlsen TH, et al. J Hepatol. 2017 Dec;67(6):1298-1323; Dyson JK, et al. Lancet. 2018 Jun 23;391(10139):2547-2559; Sundaram SS, et al. Liver Transpl. 2017 Jan;23(1):96-109. Raghu VK, et al. Liver Transpl. 2021 May;27(5):711-718; NORD, 2019. Tsao CW, et al. Circulation. 2022;145(8):e153–e639. World Health Organization, World Gastroenterology Organization

High probability of success strategy for translation of Axiomer™

Objectives for translational Axiomer trials

- Generate **robust translational dataset** with proper sample size
- Demonstrate dose dependent **target engagement** and downstream disease relevant **biomarkers**
- Achieve **human PoC** for product development **AX-0810 and AX-1412**

Translational philosophy

- Select targets that introduce **a variant in WT sequence**
- This allows to study **target engagement** (editing) and **biomarkers in healthy volunteers**
- Execute translational trials **rapidly, cost-effectively and with proper sample size** leading to **high-value dataset** for platform, translation and product development

Targets selected for initial clinical trials

- Selected A-to-G variants that were **discovered in human genetics research**, which are associated with health benefits
- These targets allow to **introduce variants in WT sequence**, allowing to extend these health benefits to the broader population and patients

These trials are designed to demonstrate

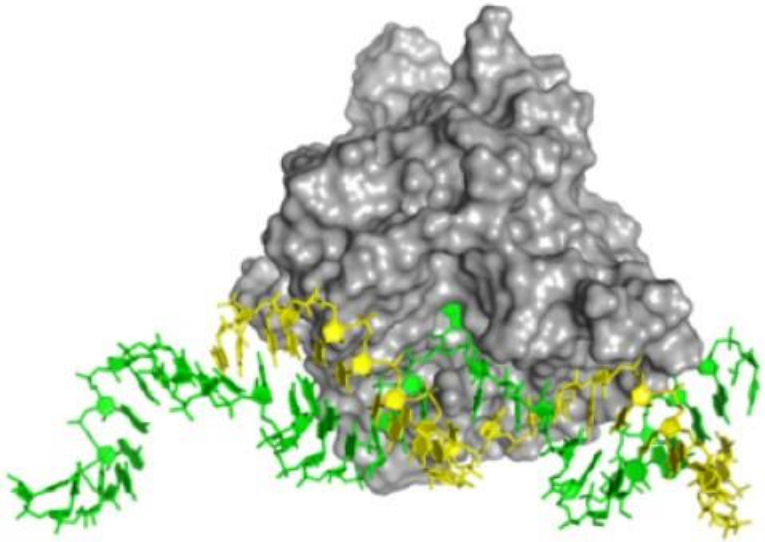
- ✓ Safety and tolerability
- ✓ PK, dose levels and dose frequency
- ✓ Target engagement
- ✓ Biomarkers

First Proof of Concept with Changes in Biomarkers in NHPs using ADAR RNA Editing Technology

Nicole H. P. Cnubben[1], Marko Potman[1], Tsinatkeab Hailu[1], Francesco De Chiara[1], Katarzyna E. Kolodziej[1], Tessa Schoon[1], Seda Yilmaz-Elis[1], Sjef de Kimpe[1], Stan F. J. van de Graaf[2], Gerard Platenburg[1] | [1]ProQR Therapeutics, [2]Amsterdam University Medical Centers

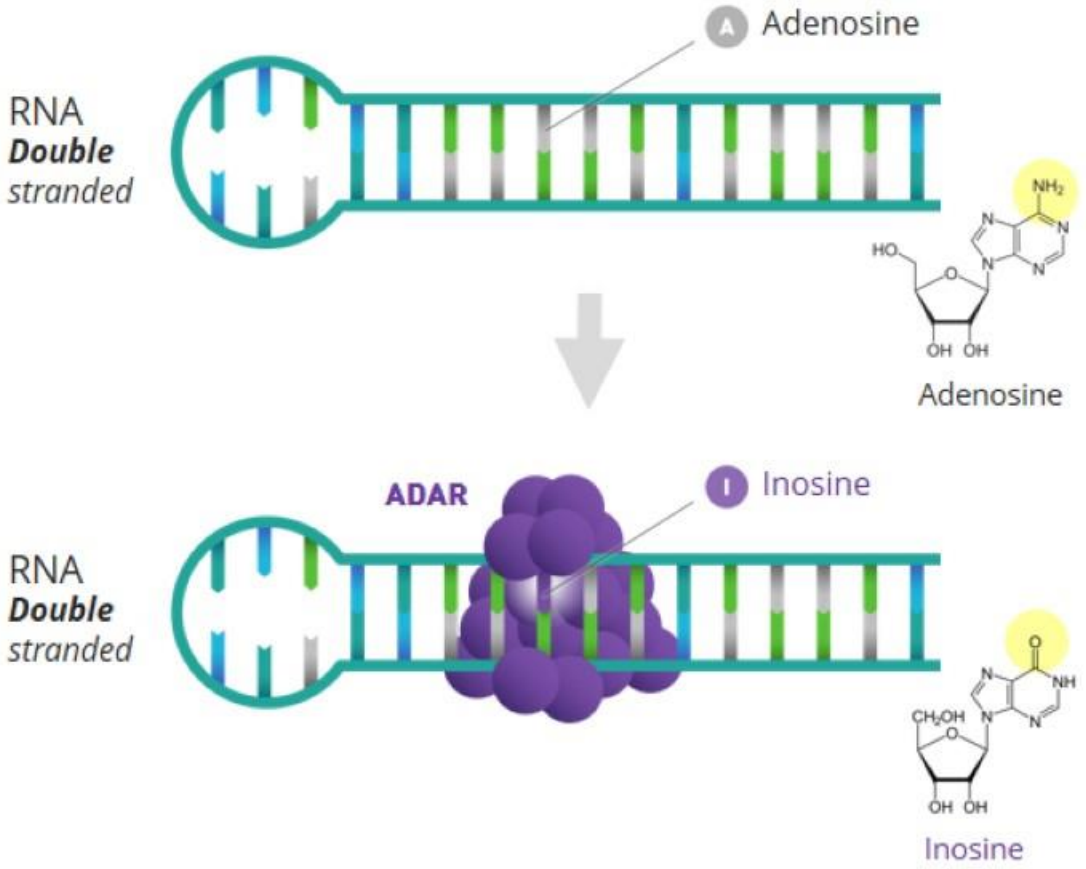
What is ADAR editing?

ADAR *(Adenosine Deaminase Acting on RNA)*



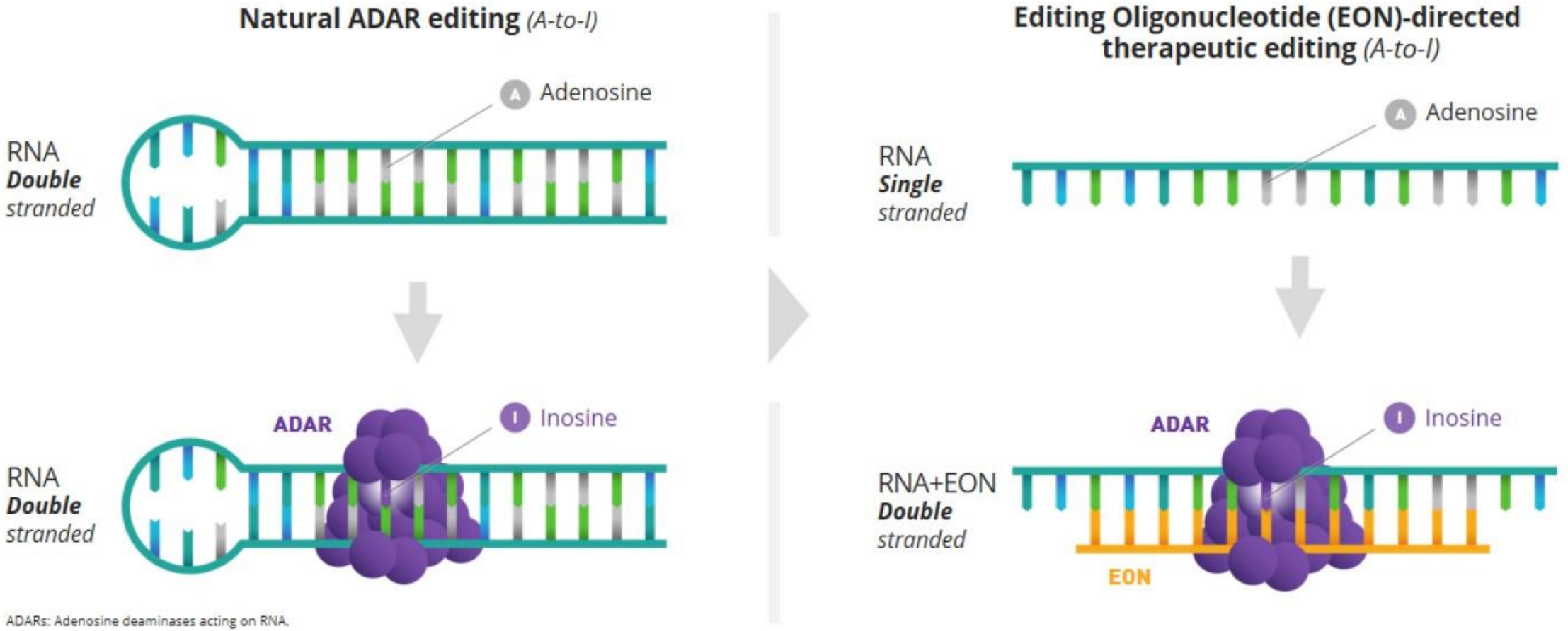
Enzyme that performs specific form of natural RNA editing, called **A-to-I editing.** During A-to-I editing an **A nucleotide (adenosine)** is changed into an **I nucleotide (inosine)**

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



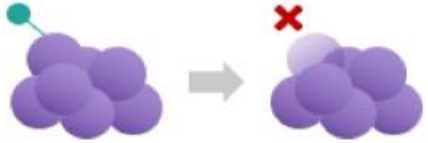
Axiomer™ EONs unlock cellular machinery potential to treat diseases

By attracting ADARs and allowing highly specific editing



ADARs: Adenosine deaminases acting on RNA.

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.

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

AX-0810 is a novel and "on target" approach reducing bile acid re-uptake into the hepatocytes

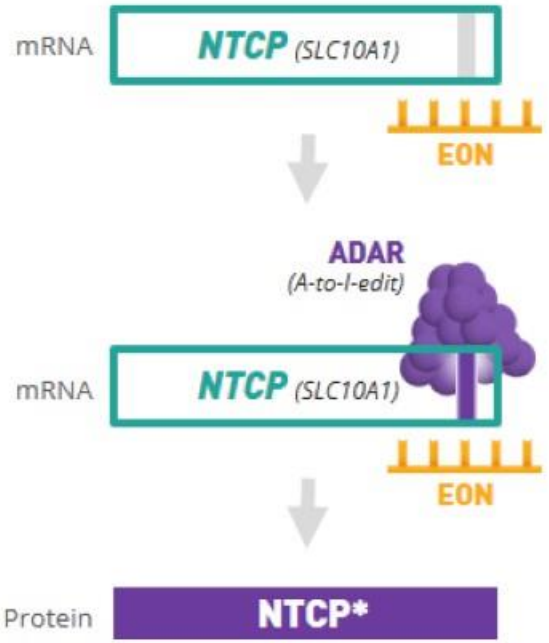
- Transient and controlled approach leading to a modulated NTCP function

AX-0810 can reduce bile acid toxic load in the liver

- To alleviate associated pathology and symptoms in PSC and BA
- To prevent or delay the development of cirrhosis, organ failure and need for transplant

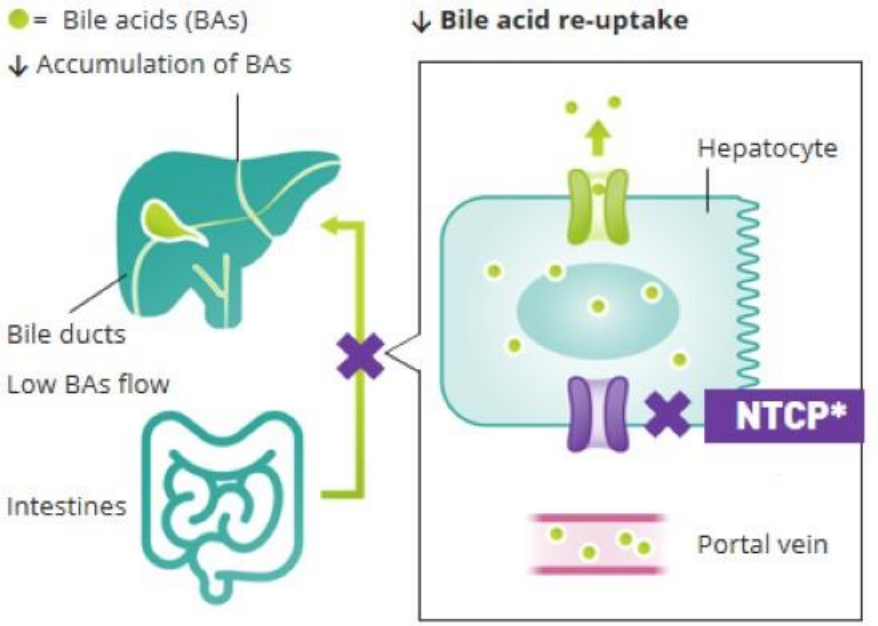
AX-0810 therapy

for cholestatic diseases



*Bile acids re-uptake modulated function

Reduced BAs levels in the hepatocytes

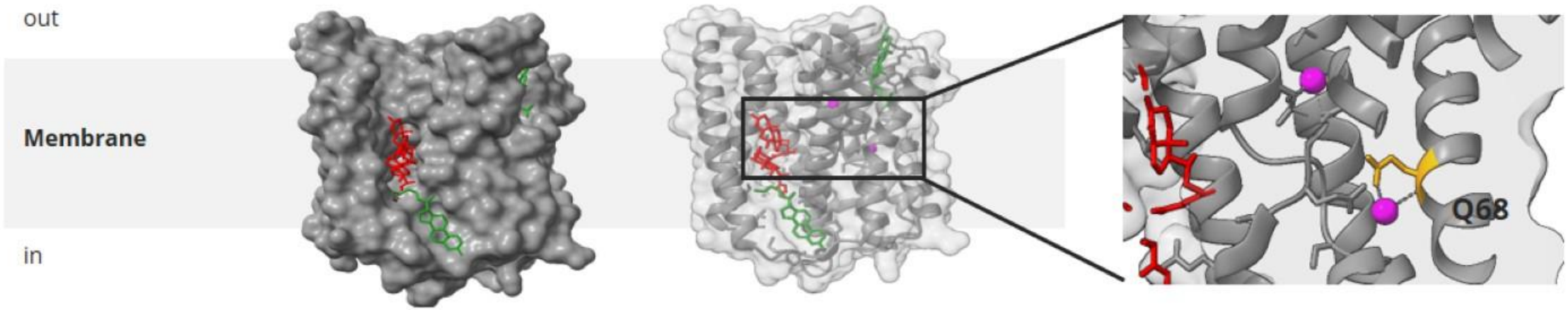


* Bile acids re-uptake modulated function

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

NTCP Q68 an amino acid of interest located in the Na^+ binding pocket

Structure of the human sodium/bile acid cotransporter (NTCP) and position of Q68 in the Na binding pocket

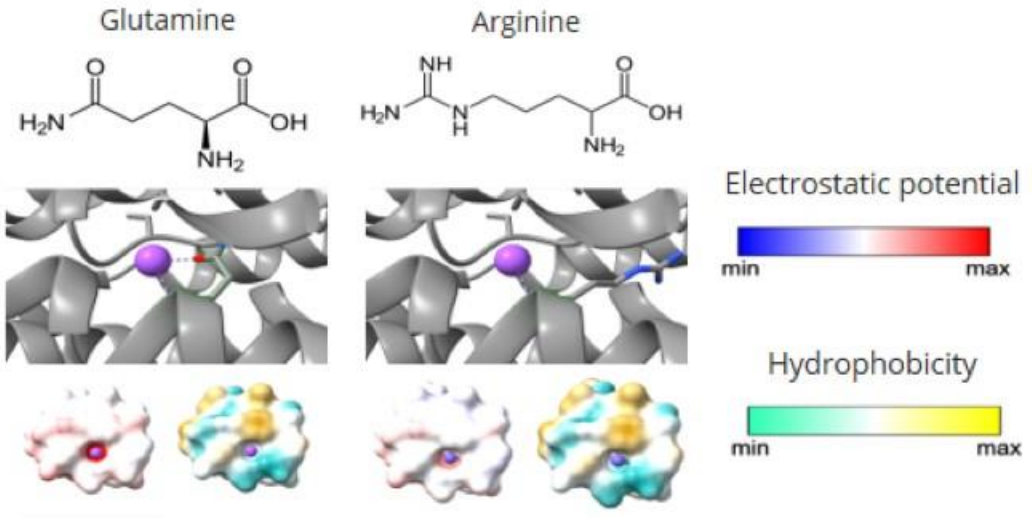


NTCP Q68 is located inside of a Na^+ binding pocket and an amino acid change can indirectly affect NTCP function by altering the precise geometry required for Na^+ binding

BAs: Bile acids, NTCP: Na-taurocholate cotransporting polypeptide.

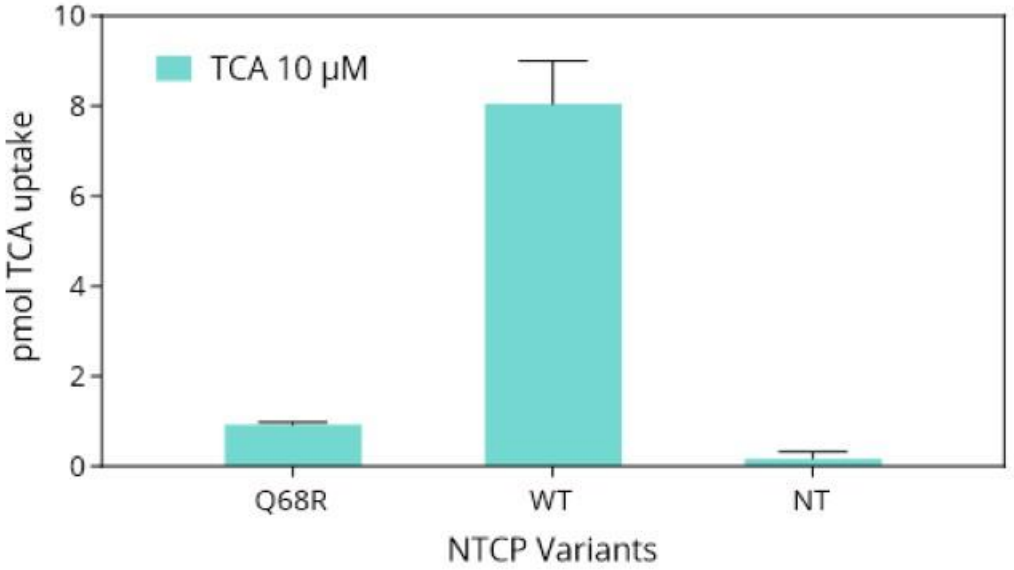
Introducing the Q68R variant leads to modulation of bile acids reuptake

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.

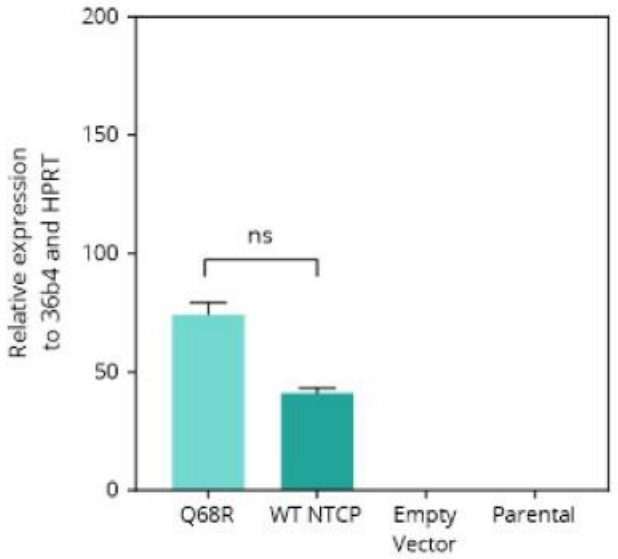
Further assessment of Q68R variant in a BAs uptake assay showed a near complete inhibition of BAs (specifically Taurocholic Acid or TCA) uptake *in vitro*, confirming findings from the 3D modeling

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

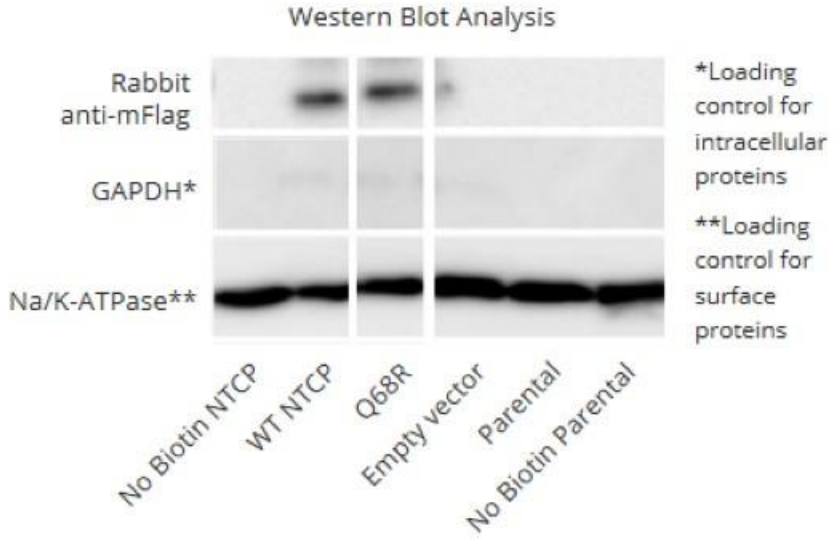
The Q68R variant solely affects NTCP bile acids reuptake function

NTCP RNA expression

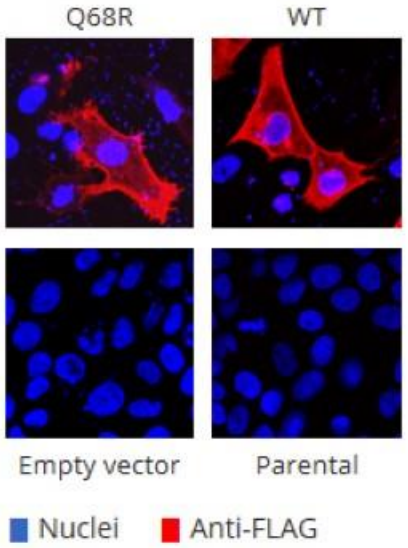
n=3, mean±SEM



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 expression and protein levels was 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.

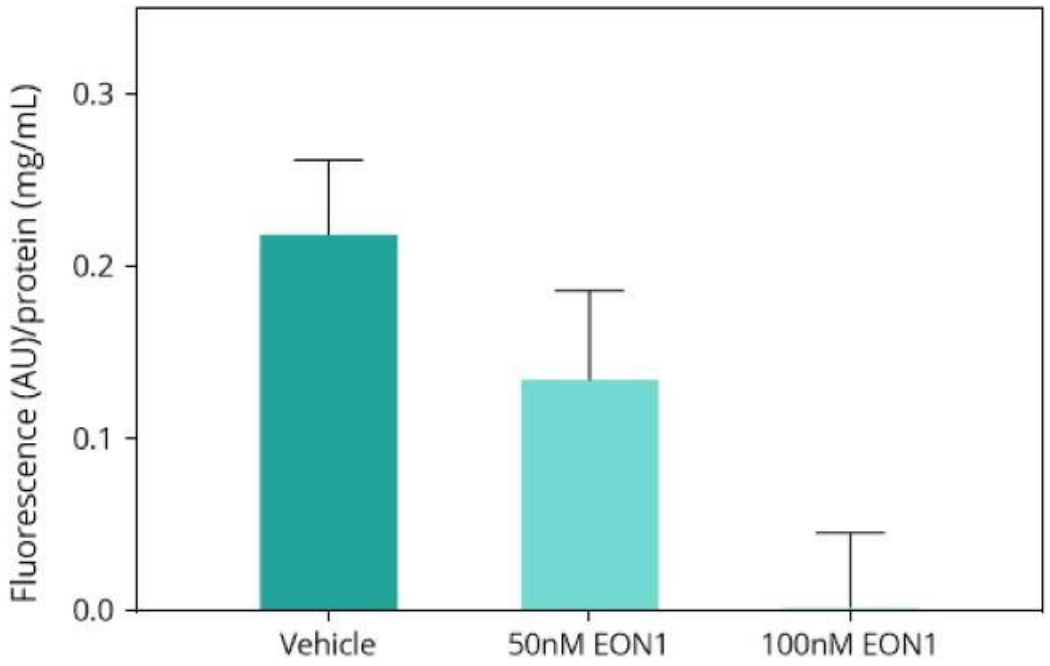
BAs: Bile acids, EON: editing oligonucleotide, NTCP: Na-taurocholate cotransporting polypeptide, *transiently transfected U2OS cells. *SLC10A1* is the gene that encodes for NTCP protein

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

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

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

n=3, 50-100nM, 72 hours, mean±SEM



- Early generation of EONs were designed to lead to NTCP Q68R variant (EON1)
- EON1 induces a dose-response inhibition of BAs *in vitro* confirming its mediation by NTCP.
- Linear regression analysis between EON concentration and TCA uptake showed a negative coefficient ($R^2 = 0.56$)

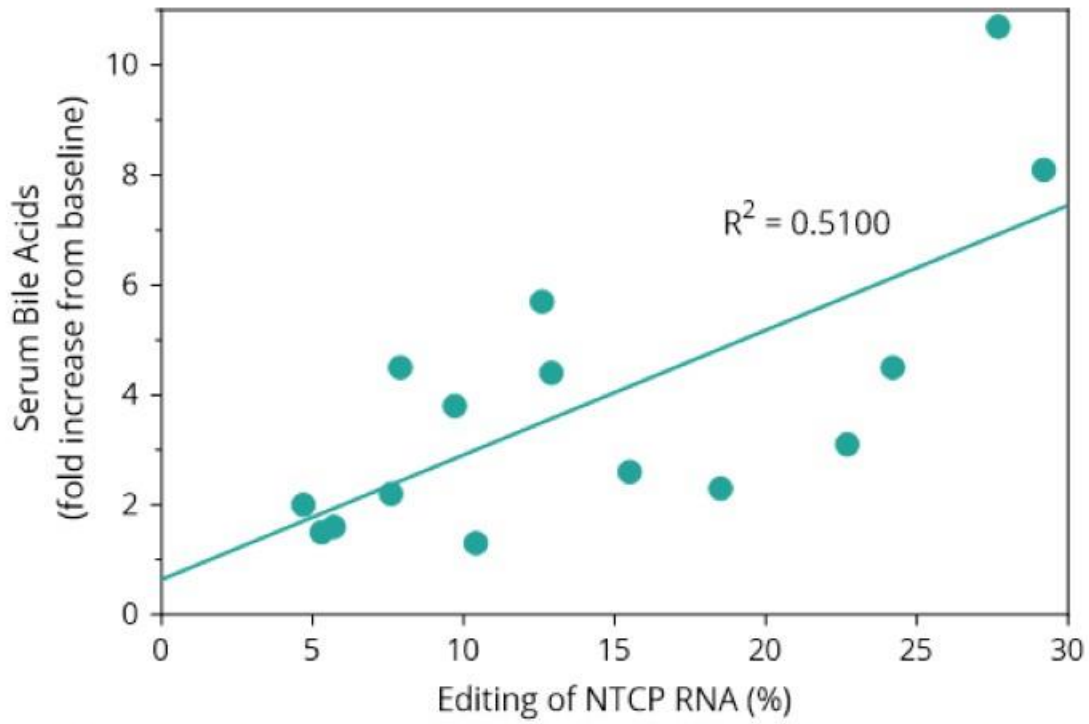
BAs: Bile acids, NTCP: Na-taurocholate cotransporting polypeptide. BAs mentioned in this experiment are specifically Tauro-nor-THCA-24-DBD. *SLC10A1* is the gene that encodes for NTCP protein

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

NTCP target engagement with Axiomer EONs achieves desired changes in serum bile acids

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

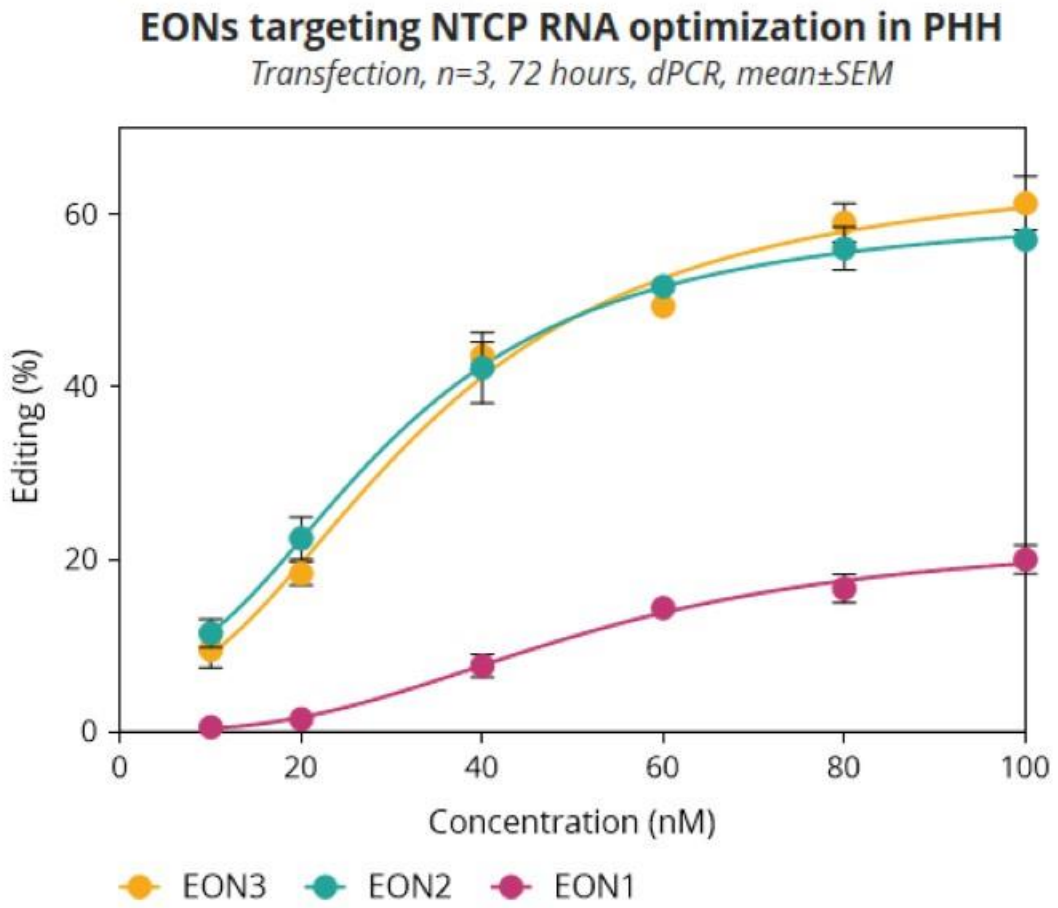
n=6, EON1, IV, LNP formulation, 72 hours



- High correlation between change in serum BAs and EON1 editing level in NTCP RNA reported in NHPs *in vivo* (linear regression $R^2 = 0.51$)
- With EON1, 29% editing in NHP led to a change in serum BAs of 8-fold 72 hours after treatment

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

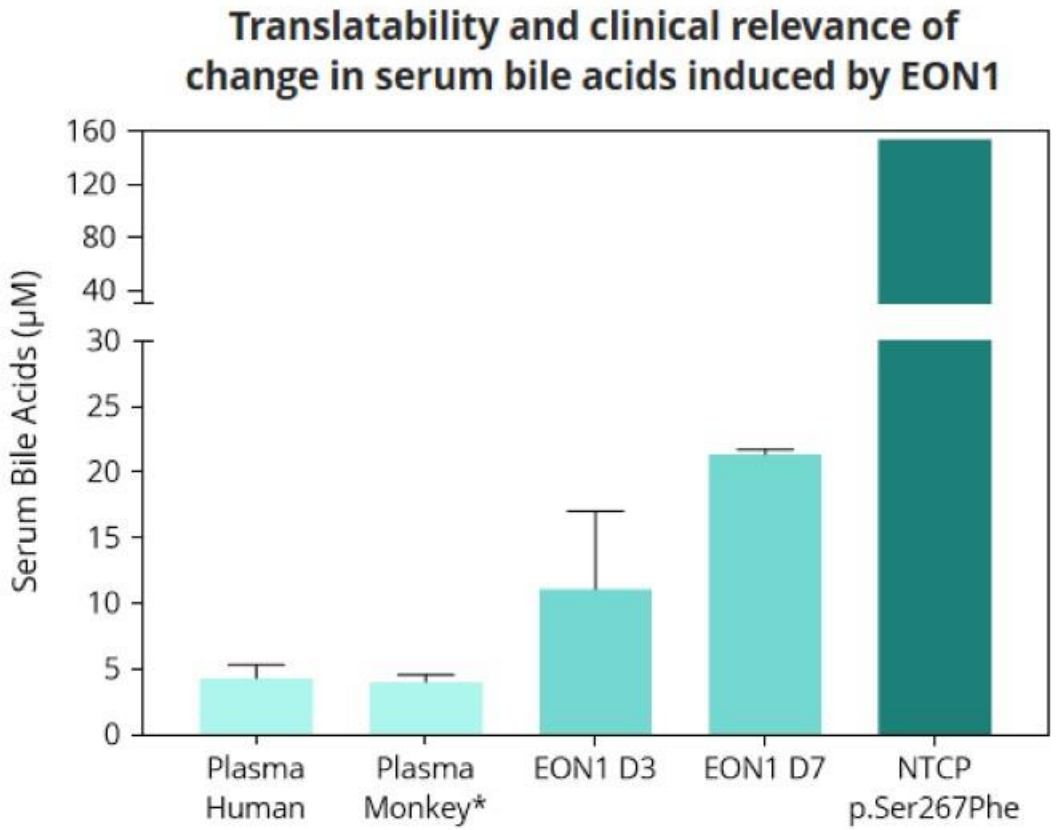
Later generation EONs further optimizing potency



- Leveraging expertise in EONs optimization, including adjustment of sequence and chemistry, lead to increased potency of EONs targeting NTCP RNA.
- Further optimizations have enabled achievement of up to 60% editing, representing an increase in 3-fold versus EON1.

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

Translatability and clinical relevance of serum bile acids changes in liver fibrosis



*Cynomolgus monkey and Rhesus macaques.

- **Translatability and derisking of FIH in healthy volunteers**
  Translatability between NHP and human confirmed with human sequence homology and equivalent level of serum bile acids
- **Potential for clinically meaningful improvement**
  Expected that a change in 2-fold of serum bile acids could lead to clinically meaningful improvement in disease progression in patients suffering from chronic liver disease
- **No harmful effects from elevated total bile acids**
  High total bile acids levels are not harmful, as evidenced by natural variants in some individuals (NTCP p.Ser267Phe) who exhibit much higher levels of sBA without clinical symptoms.

Summary
and next steps

First proof of concept with NTCP in NHPs *in vivo*

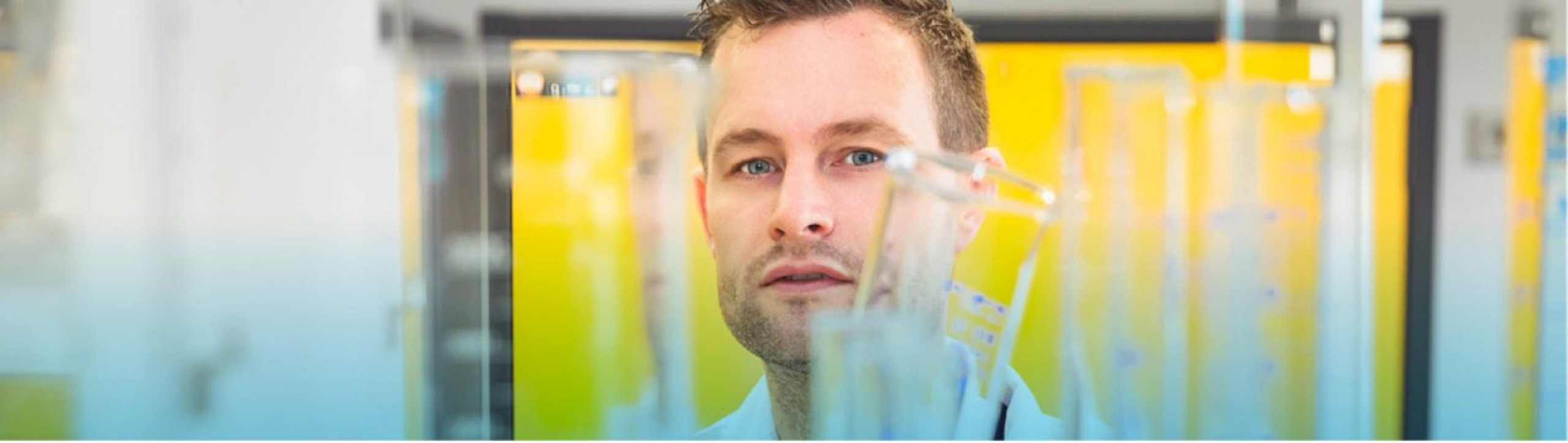
For the first time in ADAR RNA editing field, we reported *in vivo* proof of target engagement (NTCP) with changes in biomarkers in NHPs using Axiomer EONs

A targeted approach modulating NTCP for Cholestatic diseases

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

Moving towards the clinic

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



Next Steps and Upcoming Milestones

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

Phase 1 on healthy volunteers for cholestatic diseases

Objectives

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

Trial design

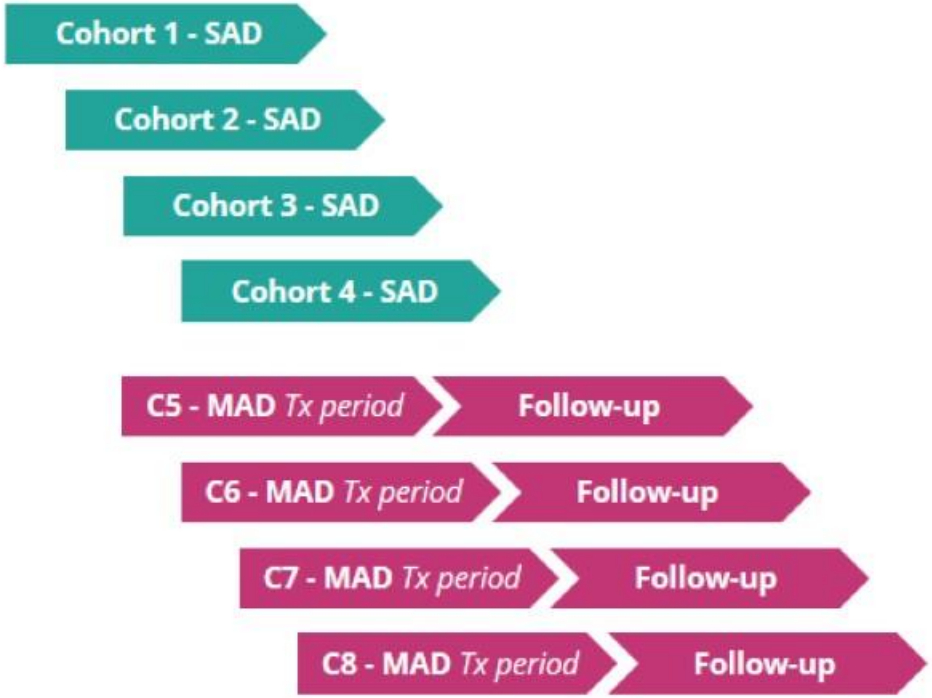
- Single and multiple dose ascending trial
- Single trial site: timely recruitment and data generation

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.

2024 and beyond outlook

Building momentum toward development

Pipeline

AX-0810 targeting NTCP for cholestatic diseases

- 2024 – announce clinical development candidate translational data, and clinical development plans
- Late 2024/early 2025 – advance to clinic

AX-1412 targeting B4GALT1 for cardiovascular disease

- 2024 – report preclinical proof of concept data; announce clinical development candidate; report translational data; announce clinical trial design
- Late 2024/early 2024 – advance to clinic

New pipeline program announcement(s)

Potential in 2024 and beyond

IP

Leading patent estate

Continued expansion of leading IP portfolio supporting that applying endogenous ADAR by administering antisense oligonucleotides for RNA editing is proprietary to ProQR

Partnerships

Eli Lilly

- Potential additional data updates
- Potential additional milestone income from existing partnership
- Potential option to exercise for expansion of deal to 15 targets, which would result in a $50 million opt-in payment to ProQR

Rett Syndrome Research Trust

- Partnership announced January 2024

Potential new

- Potential to electively form new partnerships, which could include multi-target discovery alliances, or product alliances on specific programs

Cash

Strong cash runway

Cash position of €102.7 M as of end of Q1 2024 provides runway to mid 2026, beyond multiple clinical data readouts

Q&A

IT'S IN OUR RNA