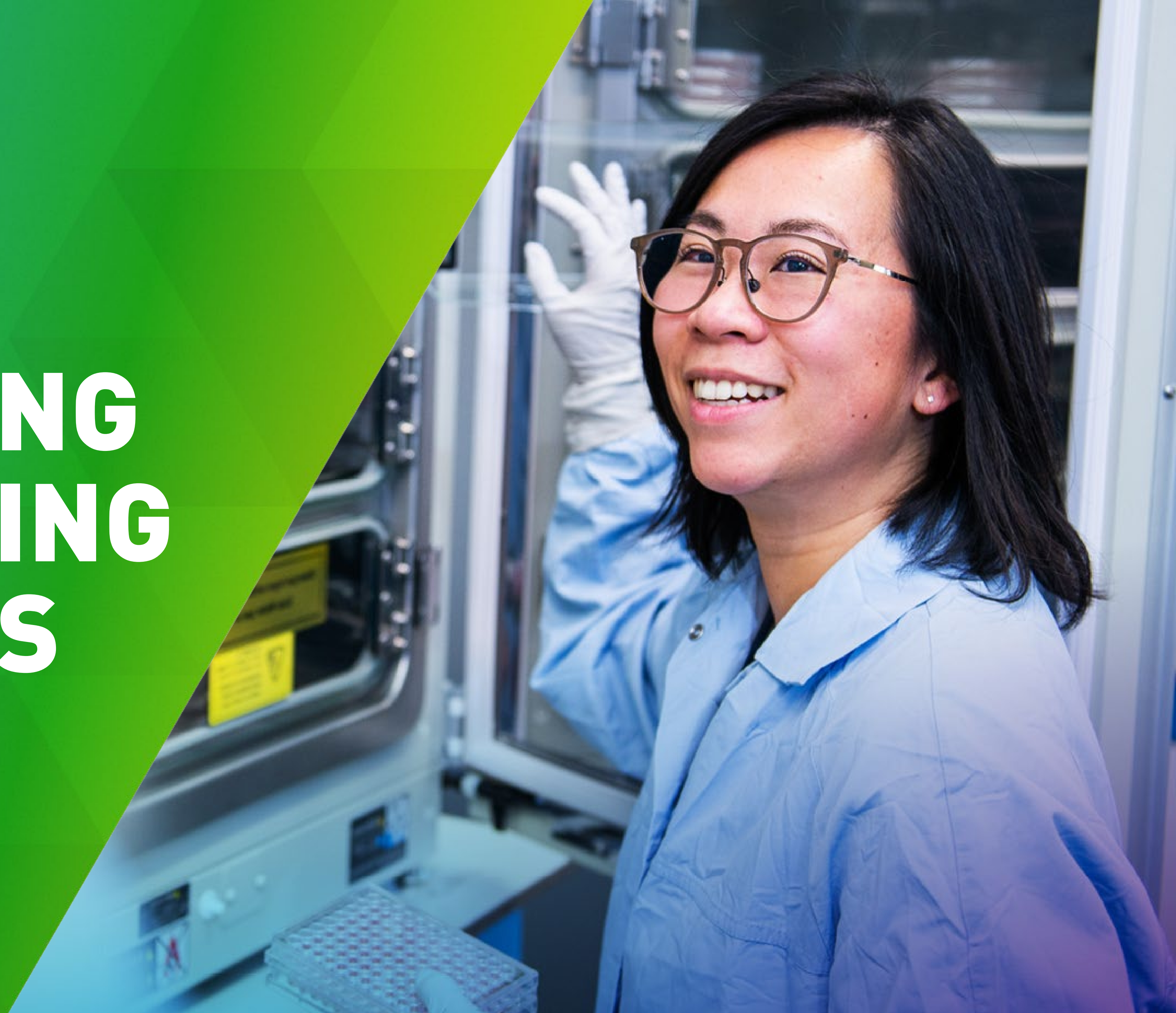
DEVELOPING RNA-EDITING MEDICINES

for patients in need

Nasdaq: PRQR

December 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.

Axiomer™ advancing to value inflection

Innovative ADAR-enabled RNA editing science driving advancement of Axiomer

supported by robust IP estate

High impact strategic partnerships

Eli Lilly, Rett Syndrome Research Trust

Pipeline with transformative potential for diseases with high unmet medical needs

Across rare and common liver and CNS disease

Experienced team driving execution

Runway into mid 2027

***€89.4 million cash** and cash equivalents as of end of Q3, **plus $82.1 million** gross proceeds from October financing providing runway into mid-2027*

ProQR's Axiomer™ ADAR journey since 2014

ProQR invents oligo mediated RNA Editing recruiting endogenous ADAR

2014

Key ADAR patents get granted in EU and US

2020-2023

ProQR pivots to solely focus on ADAR editing

2022

ProQR's ADAR patents win opposition cases filed by strawmen across the world

2023-2024

2014-2018+

ProQR files key patents that protect ADAR mediated RNA editing broadly

2015-2021

ProQR optimizes the ADAR platform in stealth

2021

ProQR and Eli Lilly enter into first 5 target partnership worth $1.25B

2022

ProQR and Eli Lilly expand partnership to 10 targets worth ~$3.9B

2023

ProQR demonstrates >50% editing in CNS and liver in NHP and announces pipeline

2024

- ProQR first in the field to report a disease relevant biomarker effect using Axiomer in NHP. Initial indication of good safety profile.
- Initial clinical validation of ADAR editing

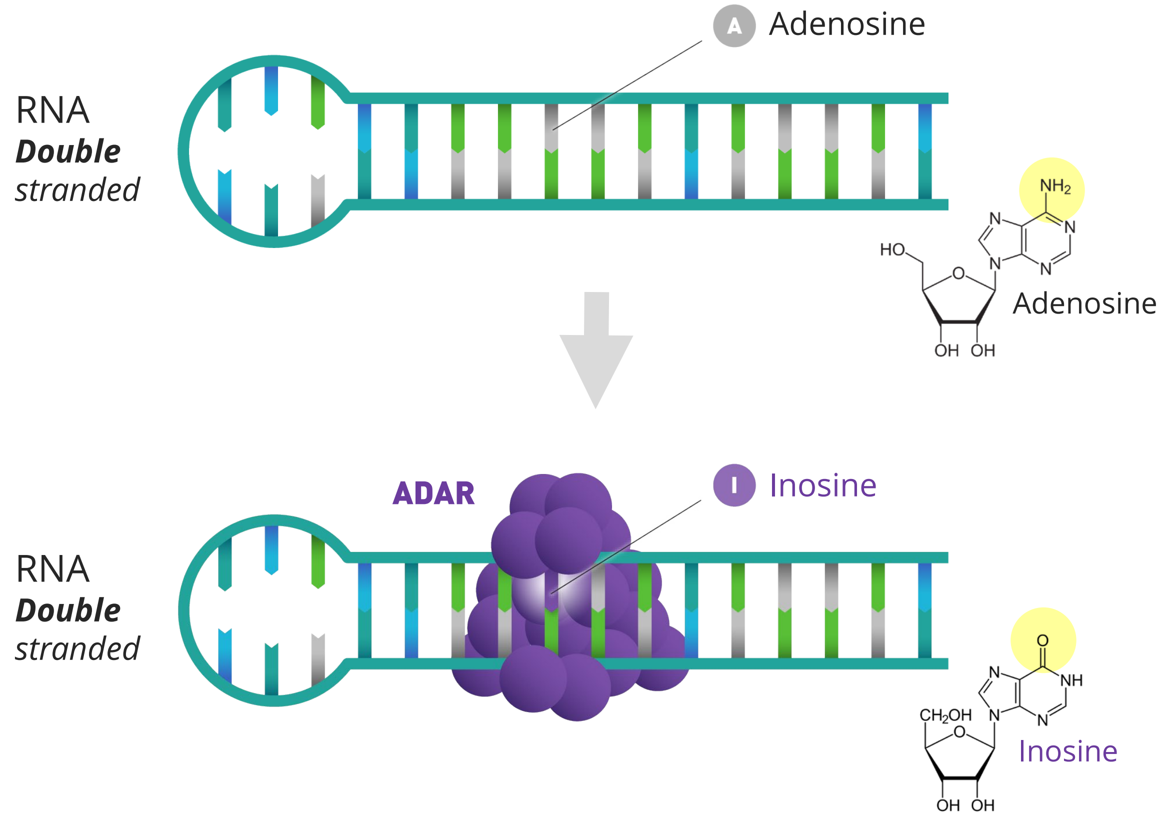
ADARs: Adenosine deaminases acting on RNA, EONs: Editing oligonucleotides

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)**

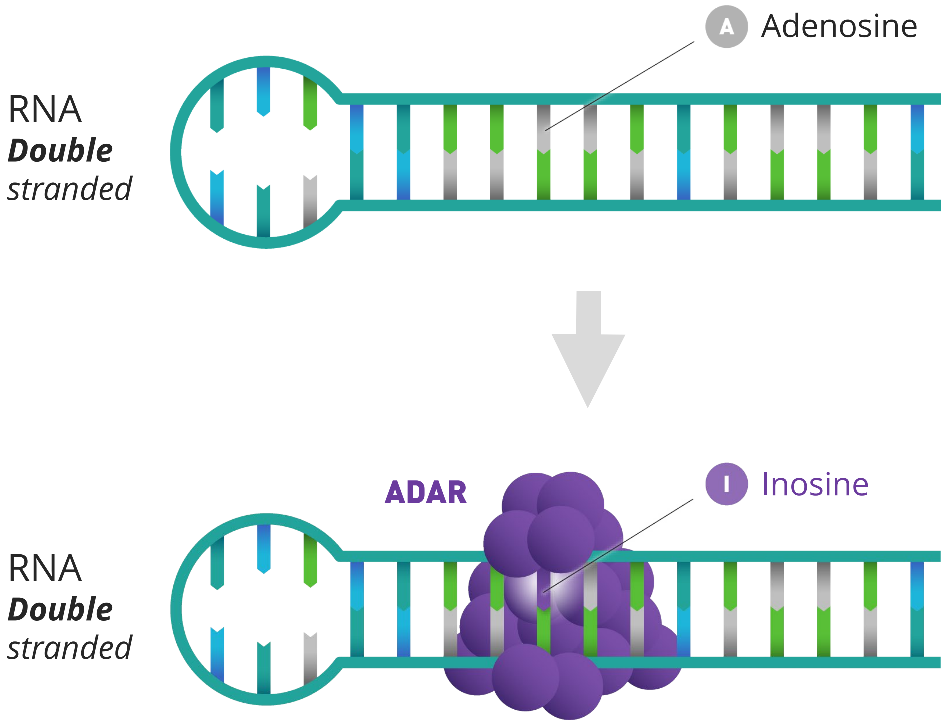
Natural ADAR editing *(A-to-I)*



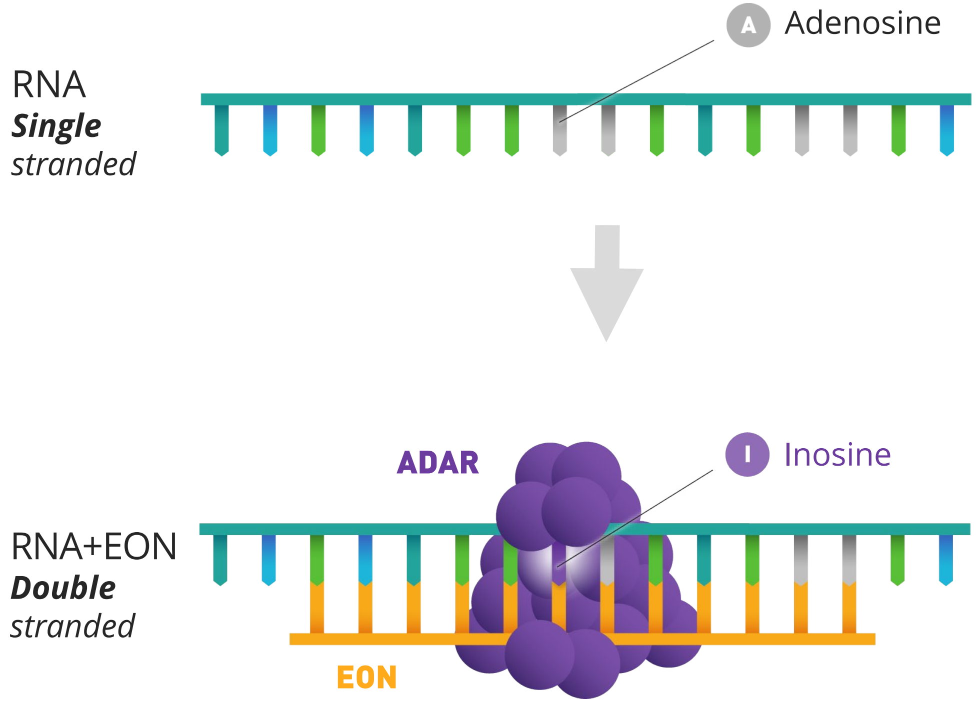
Axiomer™ EONs unlock cellular machinery potential to treat diseases

By attracting ADARs and allowing highly specific editing

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

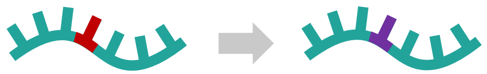
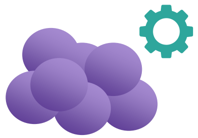
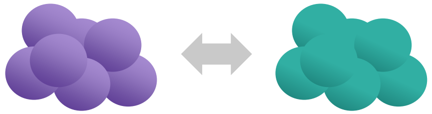
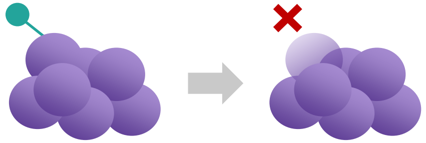


Editing Oligonucleotide (EON)-directed therapeutic editing *(A-to-I)*



ADARs: Adenosine deaminases acting on RNA.

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 Program

Targeting NTCP to address cholestatic diseases unmet medical need at the root cause

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.

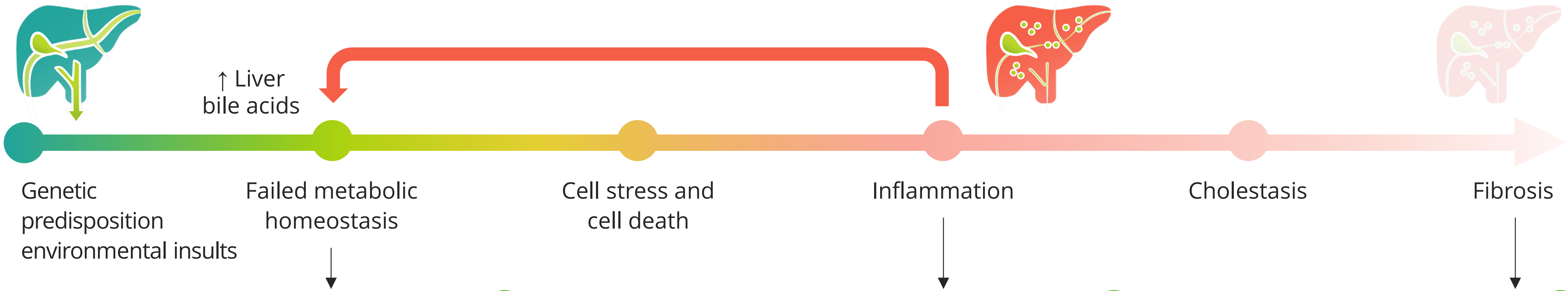
Initial indications are **Primary Sclerosing Cholangitis** affecting adults and Congenital **Biliary Atresia** affecting pediatrics early in life. Both conditions have no approved therapies and may require liver transplantation.[1,2]

- **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



NTCP MODULATION

Restores Bile Acids Balance
Protect hepatocytes by lowering bile acid levels and decrease hepatic bile production through downregulating effects

Reduces Inflammation
Prevents bile leakage and infiltration, limiting liver cell damage and release of proinflammatory mediators

Slows Fibrosis Progression
Stops cholangiocyte senescence and proliferation, reducing fibro-inflammatory responses

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 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]

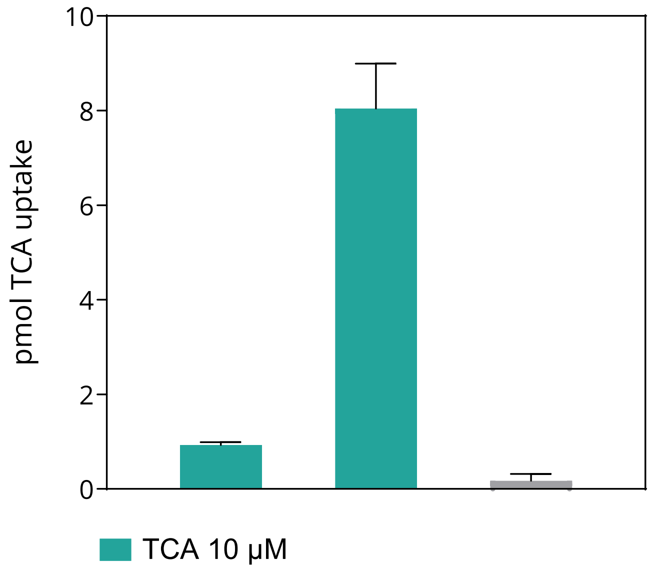
[1]Salhab A, et al. Gut. 2022 Jul;71(7):1373-1385; [2]Ho RH, et al. J Biol Chem. 2004 Feb 20;279(8):7213-22; [3]Vaz FM, et al. Hepatology. 2015 Jan;61(1):260-7; [4]Schneider AL, et al. Clin Res Hepatol Gastroenterol. 2022 Mar;46(3):101824; [5]Slijepcevic D, et al. Hepatology. 2018 Sep;68(3):1057-1069;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

NTCP modulation validated *in vitro, vivo* and clinic

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

BAs uptake (TCA) in vitro*

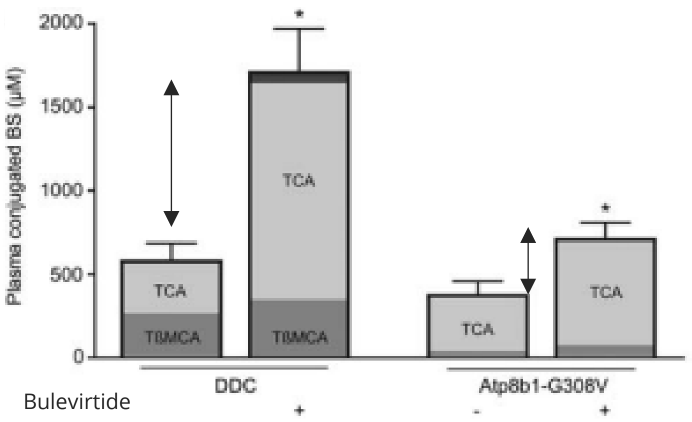
n=3, mean±SEM



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

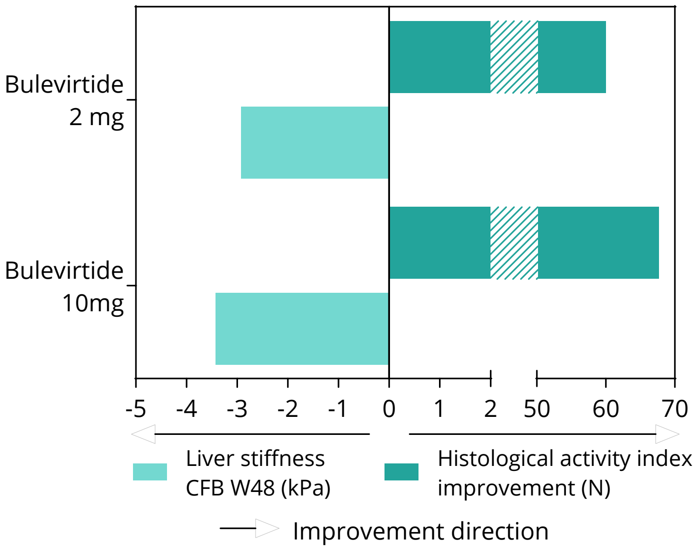
3-fold increase in conjugated BA

2-fold increase in conjugated BA



NTCP modulation demonstrated effectivity in cholestatic disease model[1]

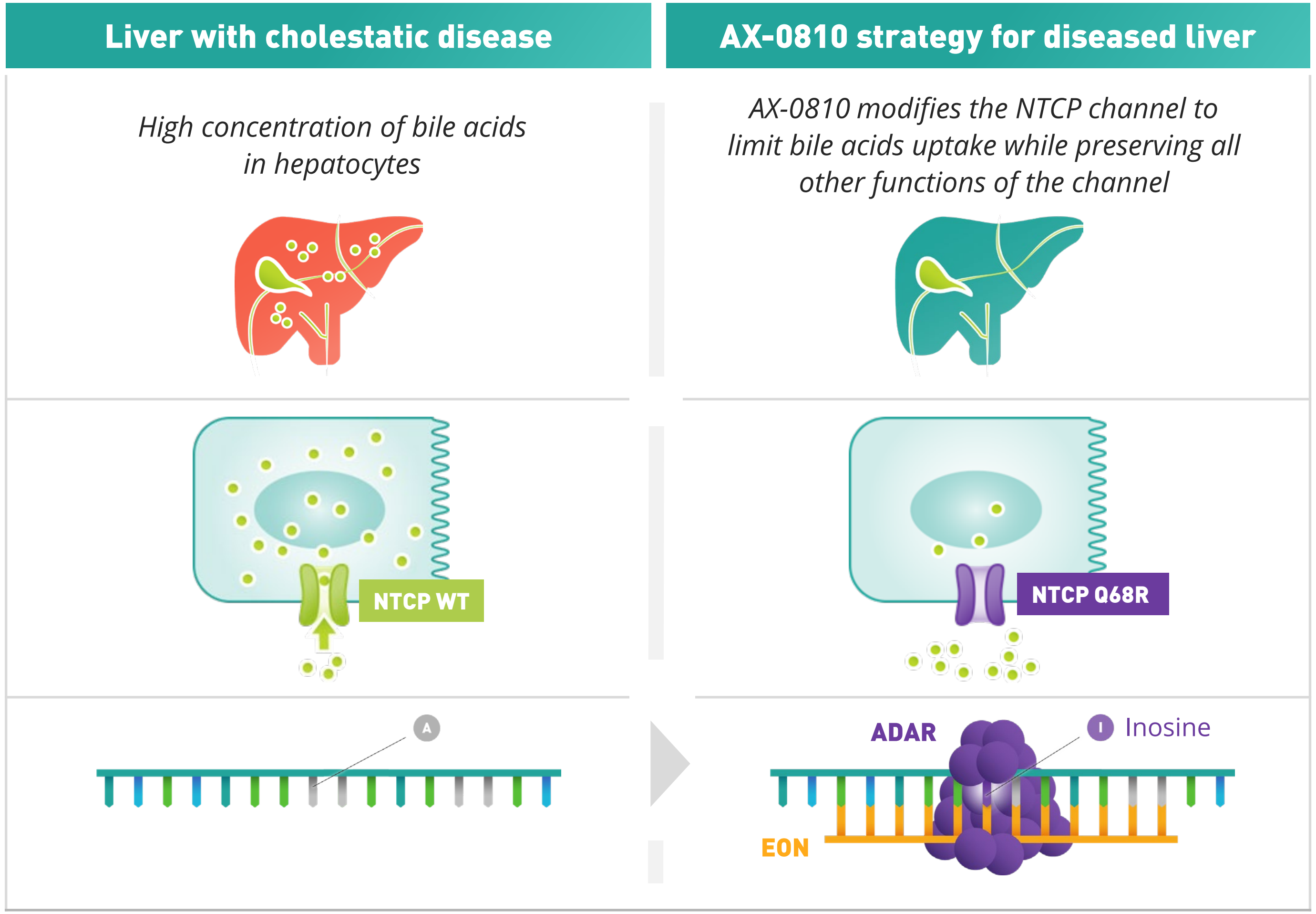
Bulevirtide 2 and 10 mg



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.

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

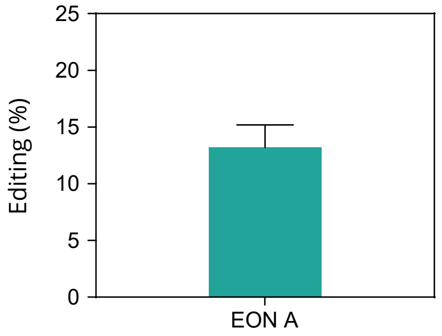
BA, Biliary atresia; PSC, Primary Sclerosing Cholangitis

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

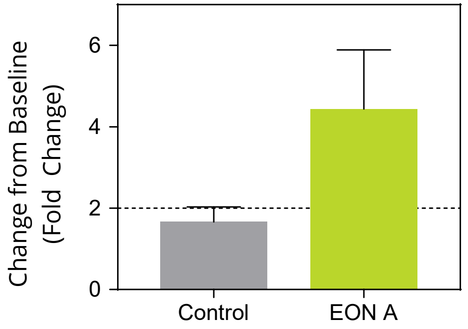
EDITING EFFICIENCY | **PLASMA TOTAL BILE ACIDS**

MICE *in vivo*

NTCP RNA Editing in Humanized Mice
(N=4, 20mg/kg, 6 doses, GalNAc conjugation, SC, D25, ddPCR)

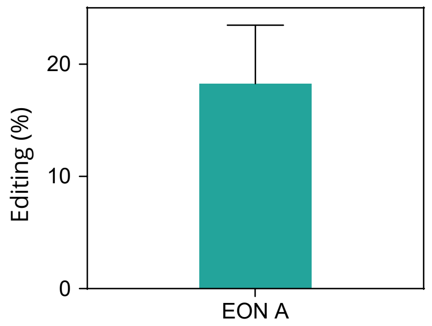


Plasma TBA in Humanized Mice
(N=4, 20mg/kg, 6 doses, GalNac conjugation, SC, D25)

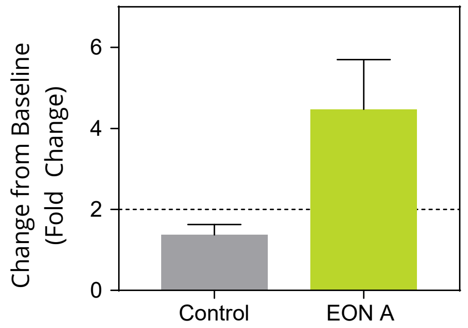


NHP *in vivo*

NTCP RNA Editing in NHP
(N=1, 1-4mg/kg, 4 doses, LNP formulation, IV, up to D46, ddPCR)



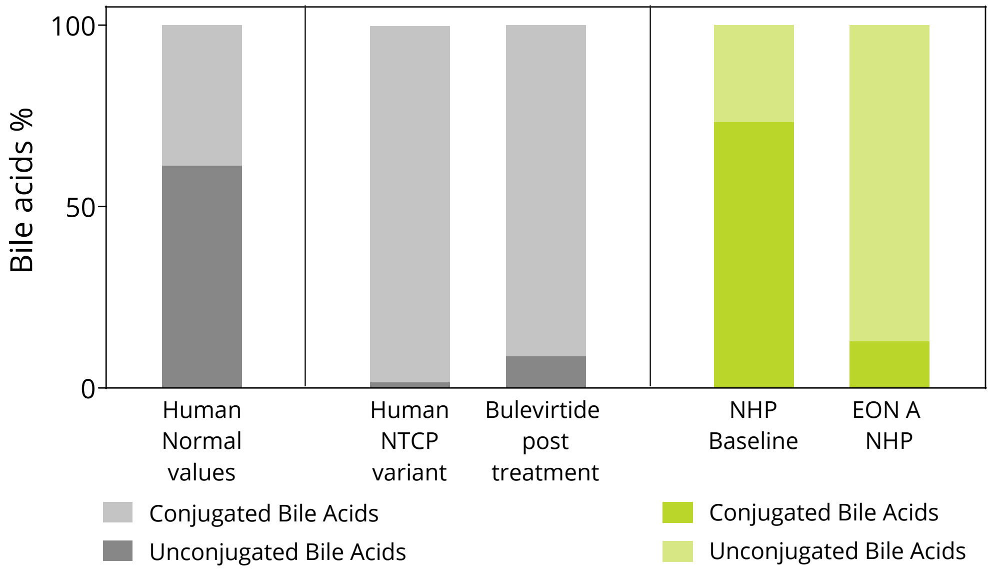
Plasma TBA in NHP
(N=1, 1-4mg/kg, 4 doses, LNP formulation IV, up to D39)



- 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

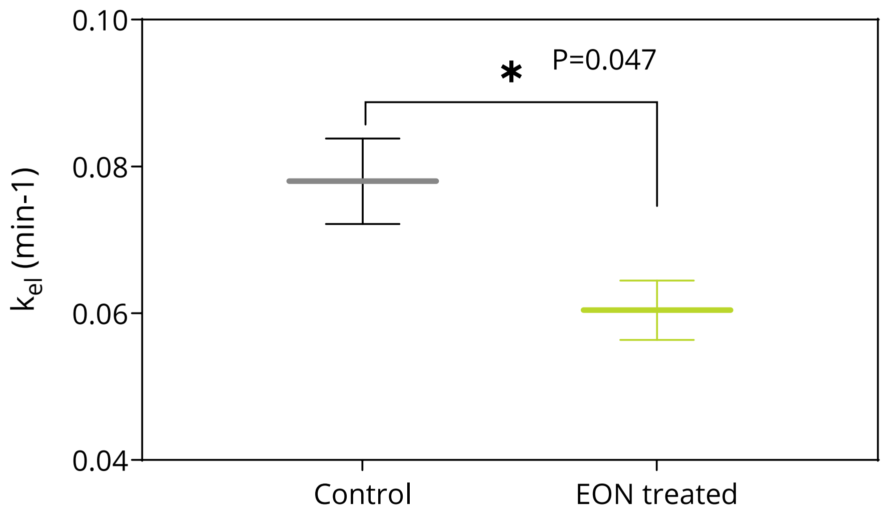
Change in Plasma BA Profile



- Conjugated bile acids are transported by NTCP back to the liver
- The observed change in plasma BA profile confirms NTCP specific modulation
- In view of the preclinical data, high confidence on NTCP EON treatment to positively impact BA toxic load in the liver

TUDCA elimination rate from plasma in NHP

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



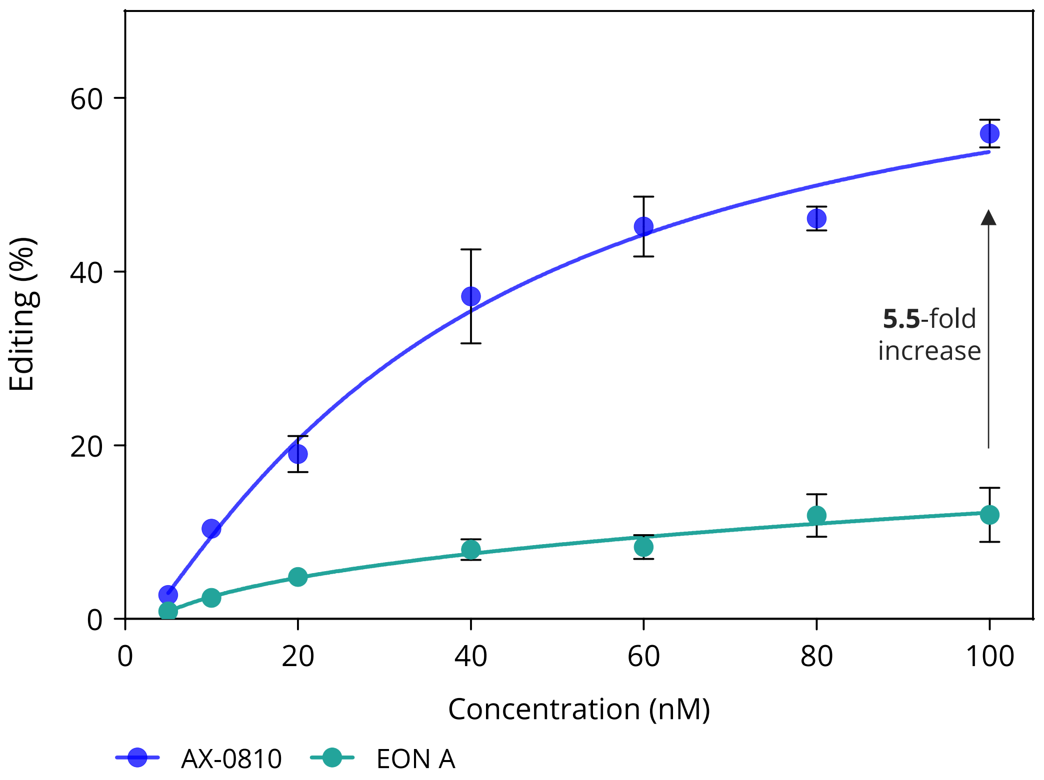
- TUDCA is a Tauro-conjugated bile acids specifically transported by NTCP from the plasma to the liver
- In an NHP experiment using administration of TUDCA following NTCP EON treatment, TUDCA plasma clearance into the liver was assessed
- Decrease in plasma clearance kinetics further confirm NTCP target engagement for EON treated NHP

Conditions in humanized mice: N=4, 20mg/kg, 6 doses, GalNAc conjugation, SC, D25, ddPCR; Conditions in the NHP experiment N=1, 1-4mg/kg, 4 doses, LNP formulation, IV, up to D42, ddPCR. 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

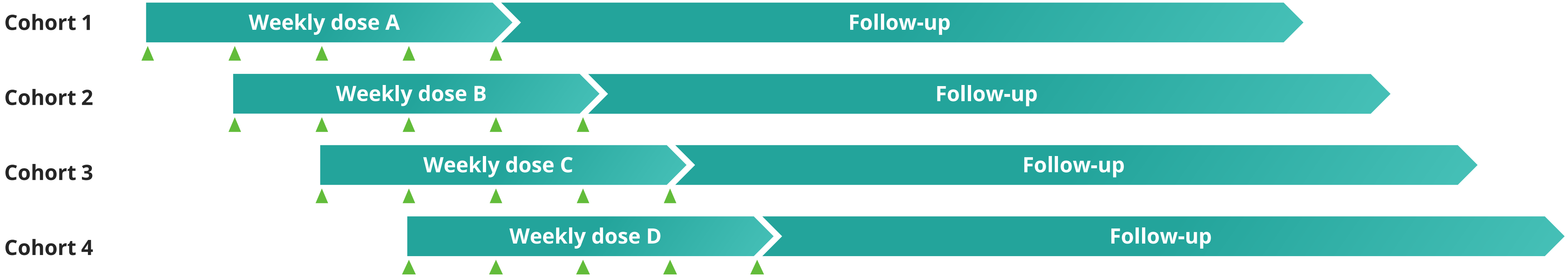
Transfection, n=3, 72 hours, dPCR, mean±SEM



- 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 oligo-nucleotide

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

CTA submission in Q2 2025

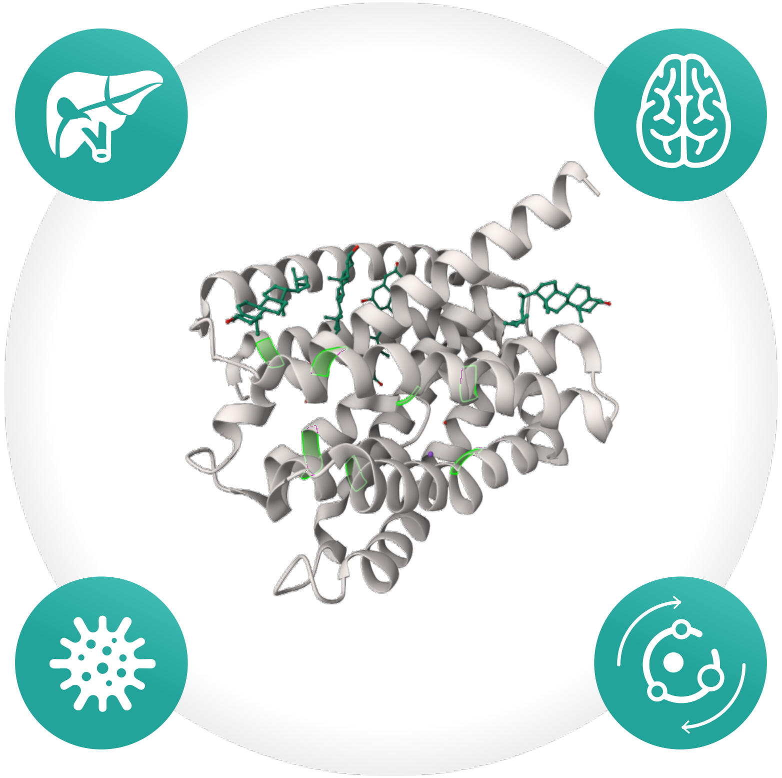
Top-line data in Q4 2025

NTCP and bile acids are involved in a variety of therapeutic areas

Providing opportunity across multiple indications

Cholestatic diseases

- Primary Sclerosing Cholangitis (PSC)
- Biliary Atresia
- Primary Biliary Cholangitis (PBC)
- Alagille syndrome
- Dubin-Johnson Syndrome
- Progressive Familial Intrahepatic Cholestasis (PFIC)
- Drug-Induced Cholestasis
- Alcoholic Liver Disease
- Secondary Biliary Cirrhosis
- Rotor syndrome
- Neonatal cholestasis



Neurological diseases

- Multiple Sclerosis
- Amyotrophic Lateral Sclerosis
- Neurological diseases
- Epilepsy
- Parkinson's Disease

Infectious disease

- Parasitic Infections
- Sepsis-Associated Cholestasis
- Viral Hepatitis: Hepatitis A, B, C, D, E

Metabolic diseases

- Hyperlipidemia
- Hypertension
- MASH
- Obesity
- Diabetes
- Lysosomal storage diseases
- Hyper-cholesterolemia
- ASCVD

AX-2402 Program

Targeting MECP2 to restore protein functionality in Rett Syndrome, a severe neurodevelopmental disorder

AX-2402 RNA editing therapy targeting MECP2 for Rett Syndrome

RETT SYNDROME RESEARCH TRUST

- Rett Syndrome is a **devastating and progressive neurodevelopmental disorder** caused by variants in the transcription factor Methyl CpG binding protein 2 (*MECP2*). There is a **high unmet need for a disease modifying therapy**.
- Nonsense variants lead to **severe phenotypes.** They represent more than one third **of Rett Syndrome** cases and are projected to affect **20,000 individuals** in US and EU.[1,2]
- Rett Syndrome is **not a neurodegenerative disorder** and restoring levels of the MECP2 protein has shown to **reverse symptoms** in mice.[3]
- Axiomer has the potential to **restore the precise level of MECP2 protein regulatory function,** which is lacking in Rett Syndrome, and become a disease modifying therapy.
- Rett Syndrome Research Trust partnership includes $9.1 M in funding; collaboration established in January 2024, expanded in December 2024

[1]Krishnaraj R, et al. Hum Mutat. 2017 Aug;38(8):922-93; [2]RSRT 2023 conference; [3]Guy J, et al. Science. 2007 Feb 23;315(5815):1143-7.

Axiomer™ has the potential to restore physiological levels of functional MECP2

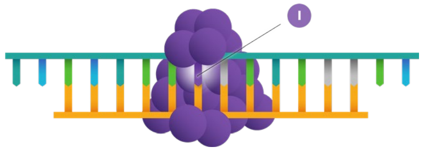
AX-2402 correcting MECP2 R270X into WT-like R270W

GGGGCC>UGAAAGCCG

EXON 3 | EXON 4

RETT syndrome

Postnatal microcephaly, stereotypic hand movements, ataxia, abnormal breathing, and growth retardation, social withdrawal, loss of speech, seizures



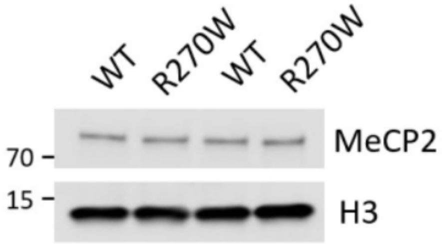
GGGGCUGA>GAAGCCG

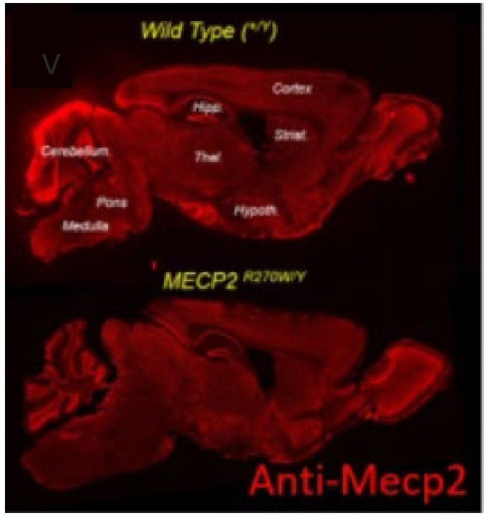
EXON 3 | EXON 4 | EXON 5

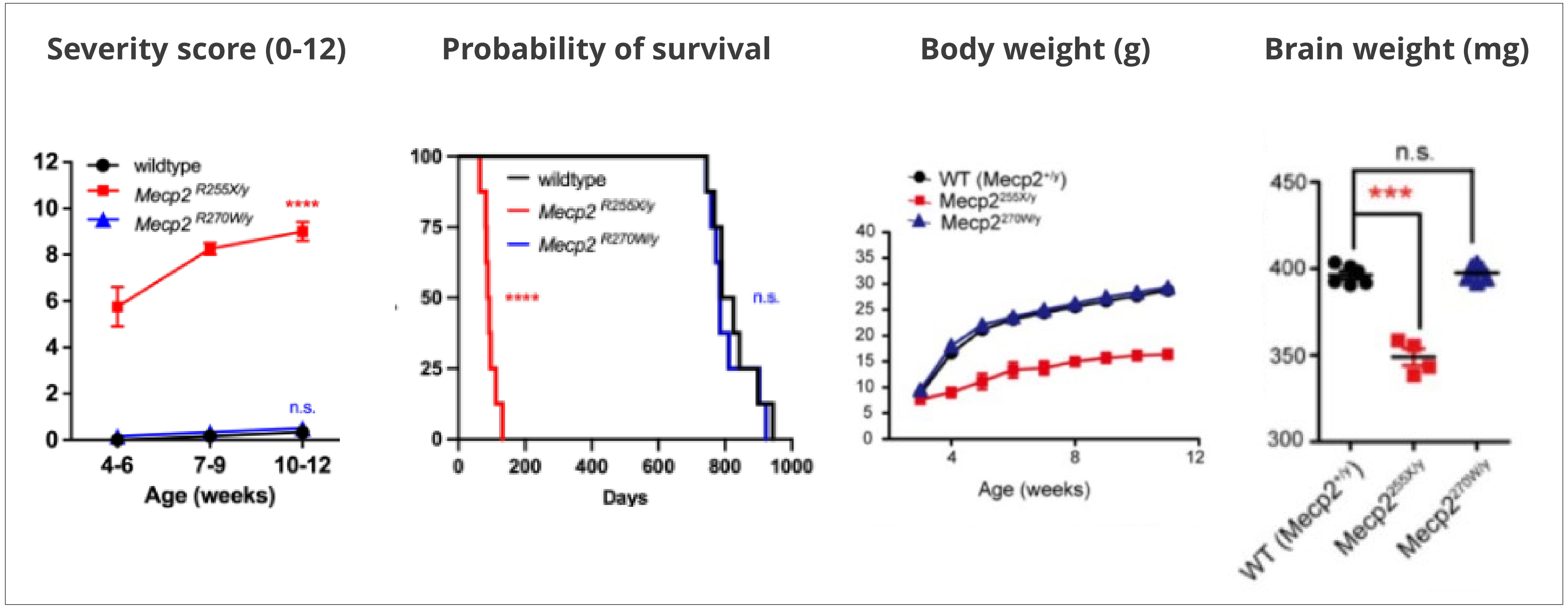
WT like phenotype

- MeCP2 protein restoration/recovery
- MeCP2 R270W (Arg > Trp) mouse model indistinguishable from wild type mice

R270W variant demonstrates wild-type like profile





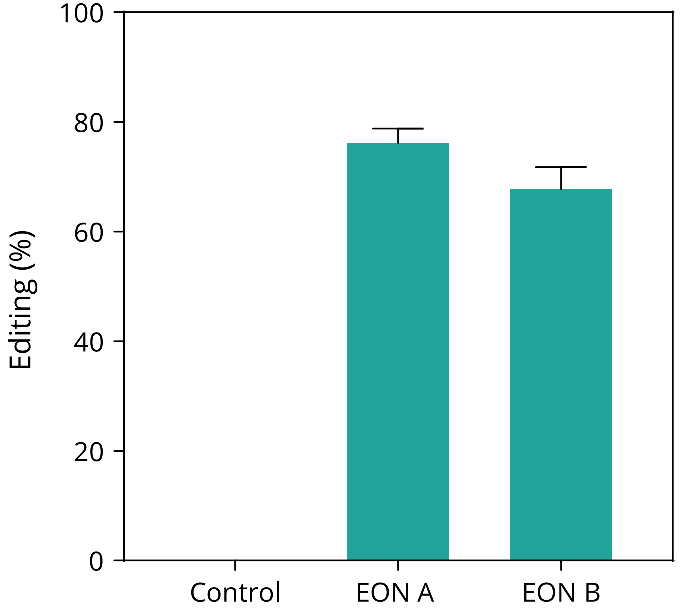


AX-2402 can restore physiological levels of functional MECP2 potentially reverting Rett syndrome into a WT like phenotype[1]

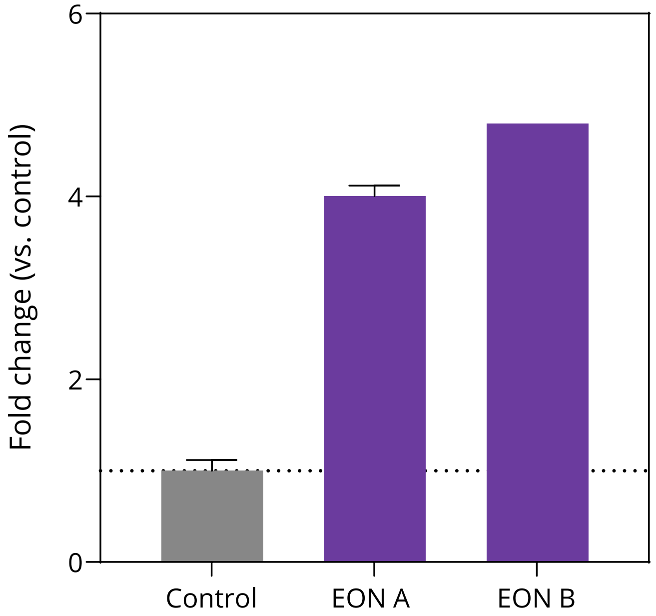
[1]Colvin, S. (2023) thesis. Massachusetts Institute of Technology. Figures adapted from: Colvin, S. (2023) thesis. Massachusetts Institute of Technology

EON mediated editing in patient's cells increases mRNA levels and restores protein expression

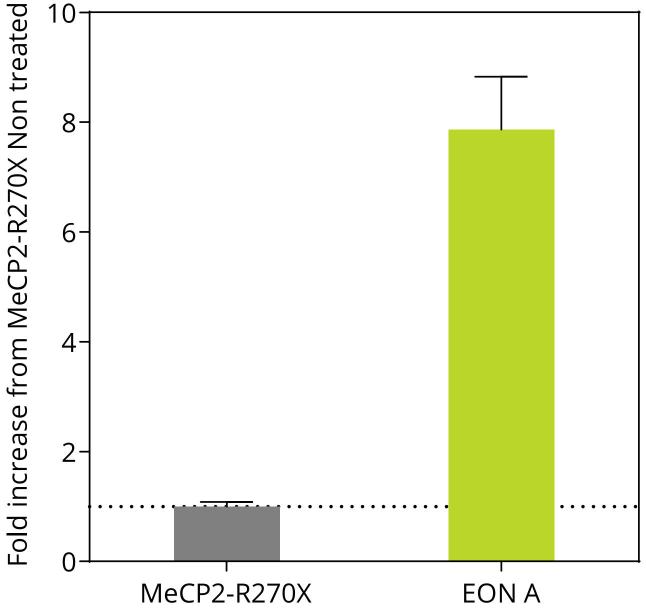
PTC recoding leading to absent NMD mediated RNA degradation



Up to 80 % editing of R270X MECP2 in patient fibroblasts



Increased MECP2 RNA levels due to PTC recoding and NMD inhibition



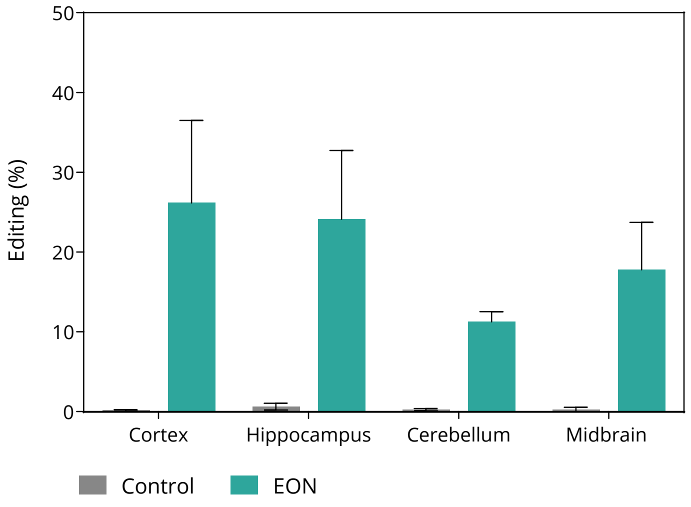
Increased R270W MECP2 protein levels

EON, Editing oligonucleotide; NT, Non-treated; TF, transfection, Conditions panel on the left and middle: 100 nM EON, transfection, 48h, N=2, mean±SEM. Conditions panel on the right: MeCP2-R270X-NanoLuc activity; 100 nM EON, transfection, 48h, N=8, mean±SEM.

Consistent CNS editing demonstrated across species

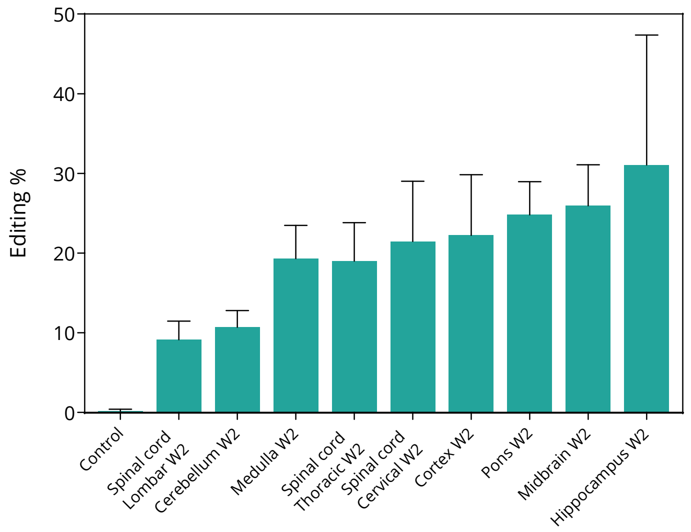
Mice *in vivo*

ICV, 250µg, undisclosed target, single dose, n=6, 4 weeks, ddPCR, mean, SD



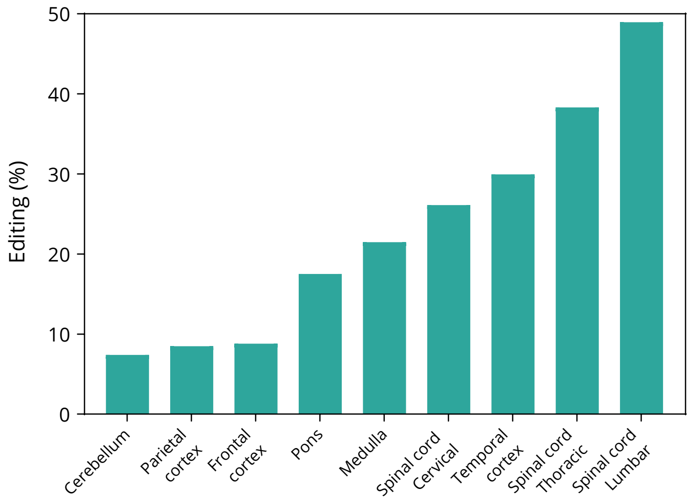
Rat *in vivo*

ICV, 500µg, APP, single dose, n=5, 2 weeks, ddPCR, mean, SD



NHP *in vivo*

IT administration, undisclosed target 12mg, single dose, n=3, 7 days, ddPCR*

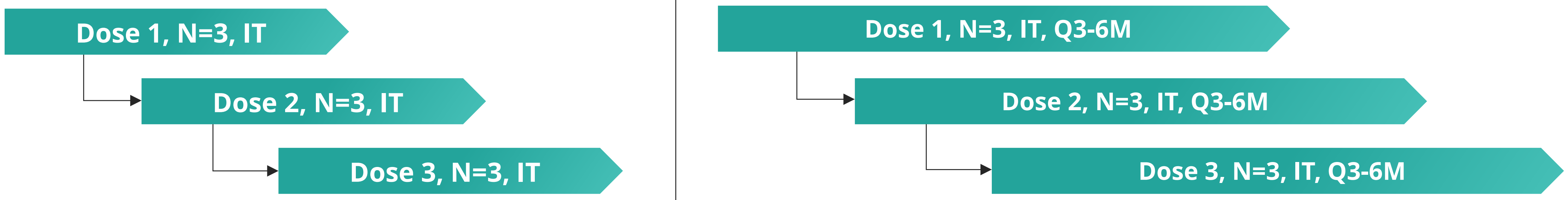


- Up to 40% editing *in vivo* leading to 26-fold change in protein function recovery in brain tissues of interest at 4 weeks with a single dose in mice model
- In rat, Axiomer EONs demonstrated up to 50% editing *in vivo* with sustained editing between W2 and W4 after single dose
- Up to 30% RNA editing reported in brain and approx. 50% in spinal cord in NHP in vivo

* Data of 2 NHPs not analyzable due to human error during injection procedure.

Preliminary clinical trial design

Single dose | **Repeated dose**



- Preliminary Phase 1/2 SAD & MAD design
- Up to 18 subjects with the R270X mutation
- Primary objective: safety, tolerability and pharmacokinetics
- Secondary objectives: target engagement and biomarkers
- Financially supported by $8.1M funding provided by Rett syndrome Research Trust
- **Clinical candidate selection in 2025**
- **Top-line data expected in 2026**

AX-1412 Program

Targeting B4GALT1 to reduce the risk of cardiovascular diseases

AX-1412 RNA editing therapy targeting B4GALT1 for cardiovascular diseases

Leading causes of death in the world
~18 million people die from CVDs every year (**32% of all global deaths**) Despite therapies, the unmet medical need remains.

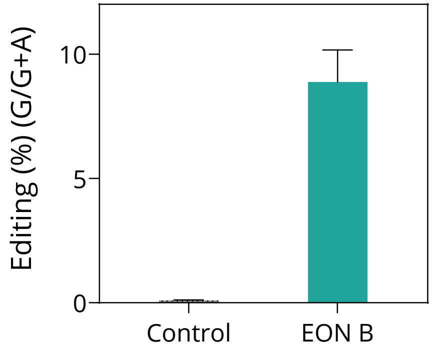
AX-1412 is designed to provide people with a protective genetic variant of B4GALT1 that is associated with **36%[1] reduction in the risk of cardiovascular disease**.

AX-1412 may become a **stand-alone cardiovascular therapy** that may also work **synergistically with standard of care** to further reduce risk of CVDs.

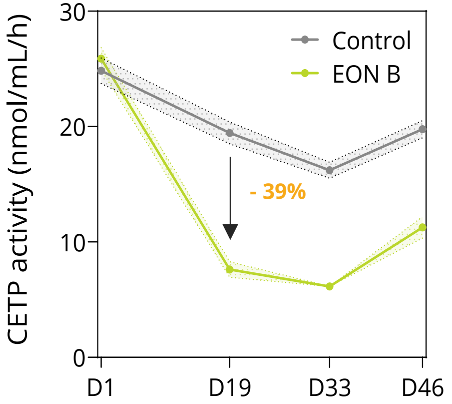
[1]Montasser ME, et al. Science. 2021 Dec 3;374(6572):1221-1227

EON-mediated editing of B4GALT1 leads to meaningful effect on key biomakers in E3L.CETP Mice

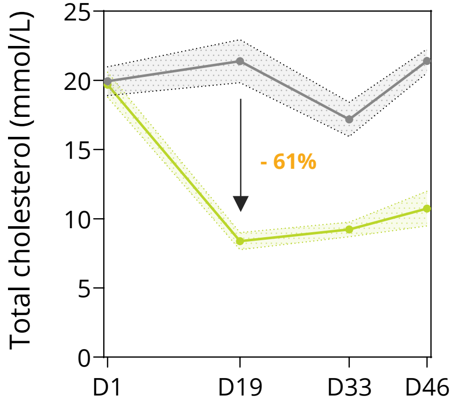
B4GALT1 editing and biomarkers in E3L.CETP mice *(N=10, 2mg/kg, LNP formulation, IV Q1W, D46, ddPCR)*



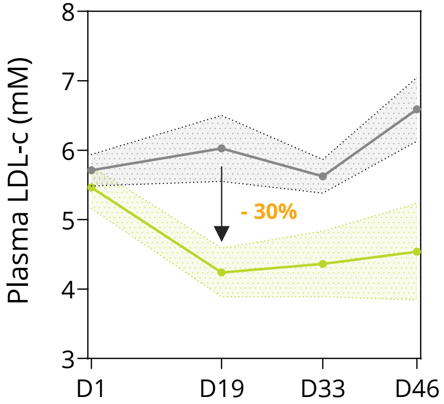
CETP activity



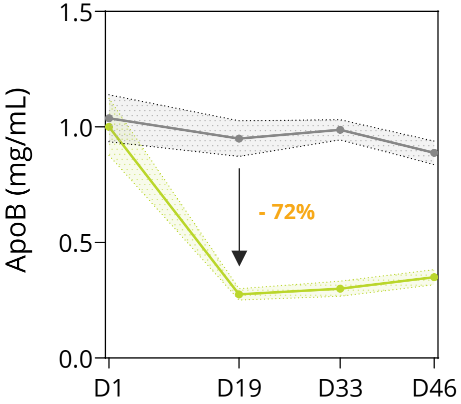
Total Cholesterol



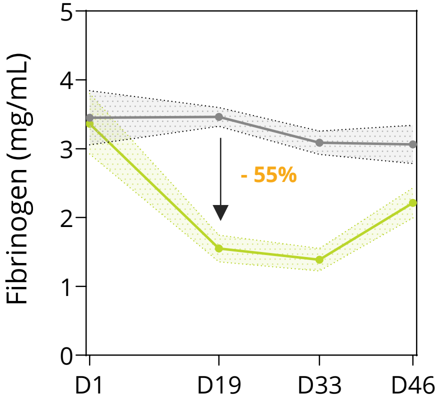
Plasma LDL-c



ApoB



Fibrinogen

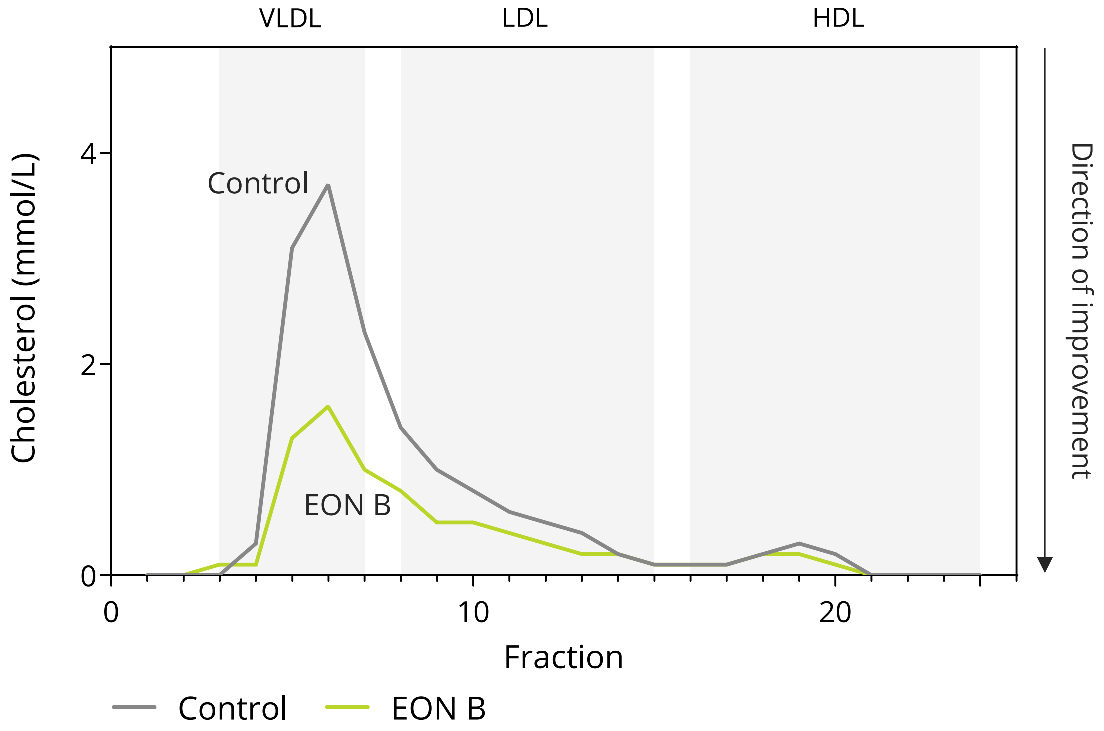


Following treatment with EON B, a marked reduction in total cholesterol, ApoB, and LDL-c by observed already at Day 19 confirms our approach to address cardiovascular diseases

B4GALT1 EON leads to a positive shift in lipoprotein profiles

Specifically targeting atherogenic lipoproteins

Impact on lipoprotein profile following editing of B4GALT1 in E3L.CETP mice
(N=10, 2mg/kg, LNP formulation, IV Q1W, D46)



- Treatment with EON B significantly decreases VLDL and LDL cholesterol compared to control
- These lipoproteins are associated with increased cardiovascular risk due to their role in atherosclerotic plaque formation
- HDL cholesterol which supports reverse cholesterol transport and is associated with reduced cardiovascular risk, remains unchanged

Summary & next steps

AX-1412 for CVD

EON-mediated RNA editing of B4GALT1 leads to the required reduction in galactosylation

reflecting the human genetics observed effect

LNP-delivered EON editing B4GALT1 leads to editing and meaningful changes

in biomarker effect on LDLC, CEPT, cholesterol and fibrinogen in an industry-standard in vivo disease model

Further optimization of a GalNAc delivered EON ongoing

to achieve a TPP desirable for CVD

Expected to provide an update on the optimization efforts in mid 2025

AX-2911 Program

Targeting PNPLA3 to address unmet medical needs in MASH

AX-2911 RNA-editing therapy to address Metabolic dysfunction associated steatohepatitis (MASH)

MASH and subsequent stages of liver disease **are very prevalent and still on the rise worldwide**. MASH individuals have a high unmet medical needs due to the **progressive** nature of the disease (cirrhosis and hepatocellular carcinoma) and **limited therapeutic options** available[1]

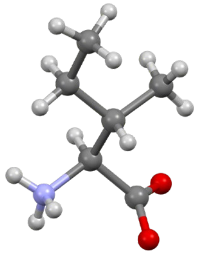
PNPLA3 (patatin-like phospholipase domain-containing 3) I148M is a variant **commonly reported** in the MASH population worldwide (20-60% of the patients) and is known as **associated risk factor**.[2,3] Approximately 8 million individuals in US and EU are homozygous for the 148M variant.

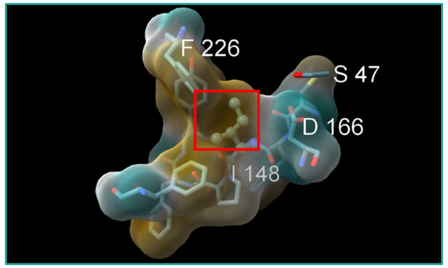
Axiomer EONs have the potential to change the Methionine into a Valine bringing the **PNPLA3 protein back to a WT-like functional conformation**.

[1]Sandireddy R, et al. Front Cell Dev Biol. 2024 Jul 16;12:1433857; [2]Romeo S, et al. Nat Genet. 2008 Dec;40(12):1461-5; [3]Salari N, et al. BMC Endocr Disord. 2021 Jun 19;21(1):125.

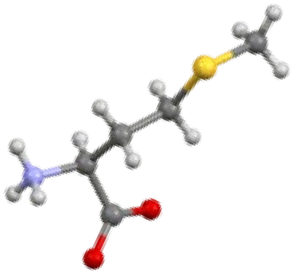
Axiomer™ creates a PNPLA3 protein with WT-like functionality

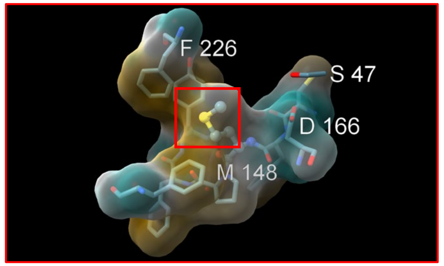
PNPLA3 148I (WT)
Isoleucine (ATC)



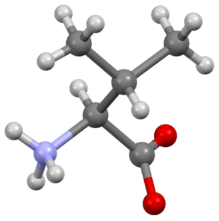


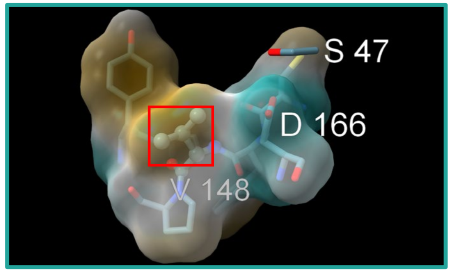
PNPLA3 148M (Mutant)
*Methionine (<u>**A**</u>TG)*





PNPLA3 148V
(Axiomer *de-novo* variant)
*Valine (<u>**G**</u>TG)*





F226 not included here

Electrostatic potential

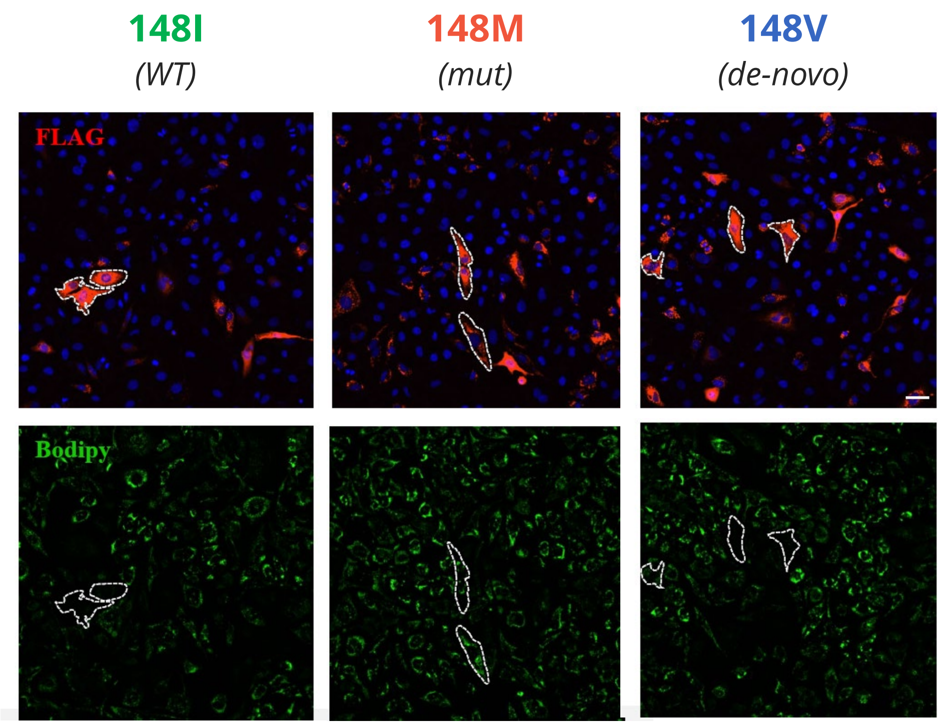
Hydrophobicity

***In silico* analysis of variants**

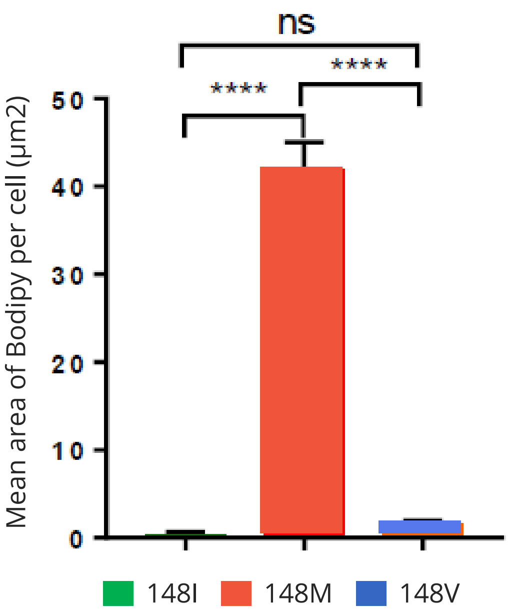
- 148M shows a non-conservative substitution with predicted **functional consequences, with change in binding cavity volume limiting access of substrate to the active site**
- Equivalent potential between Isoleucine (WT) or Valine (Axiomer correction) at location 148 in 3D models
- 148I and 148V predict **no functional consequences** for PNPLA3, with valine expected to behave like isoleucine

PNPLA3 148V variant has WT-like lipid metabolizing properties

148I and 148V reports equivalence in lipid droplet sizes



Hoechst (nuclei), Bodipy (Lipids) and M2 anti-flag (PNPLA3)



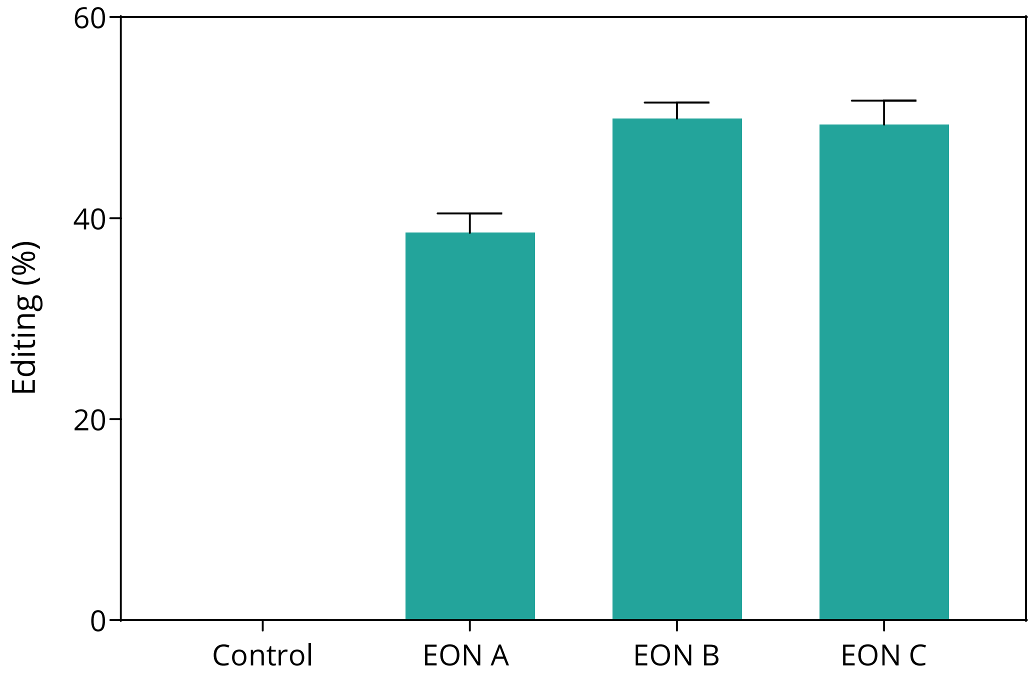
- The wild-type 148I shows smaller lipid droplets, reflecting normal lipid metabolism
- The 148M variant induces significantly larger lipid droplets, consistent with its pathogenic role in lipid metabolism disorders
- The corrected variant 148V results in wild-type like droplet sizes, suggesting a corrective effect on lipid accumulation, similar to 148I

Treatment conditions: HeLa cells, plasmid, transfection, 250uM linoleic acids, 24h, cell lipase activity by IF One-way ANOVA, ****, P<0.0001; Mean, SEM.

EON mediated PNPLA3 editing leads to over 50% RNA editing and change in lipid droplet

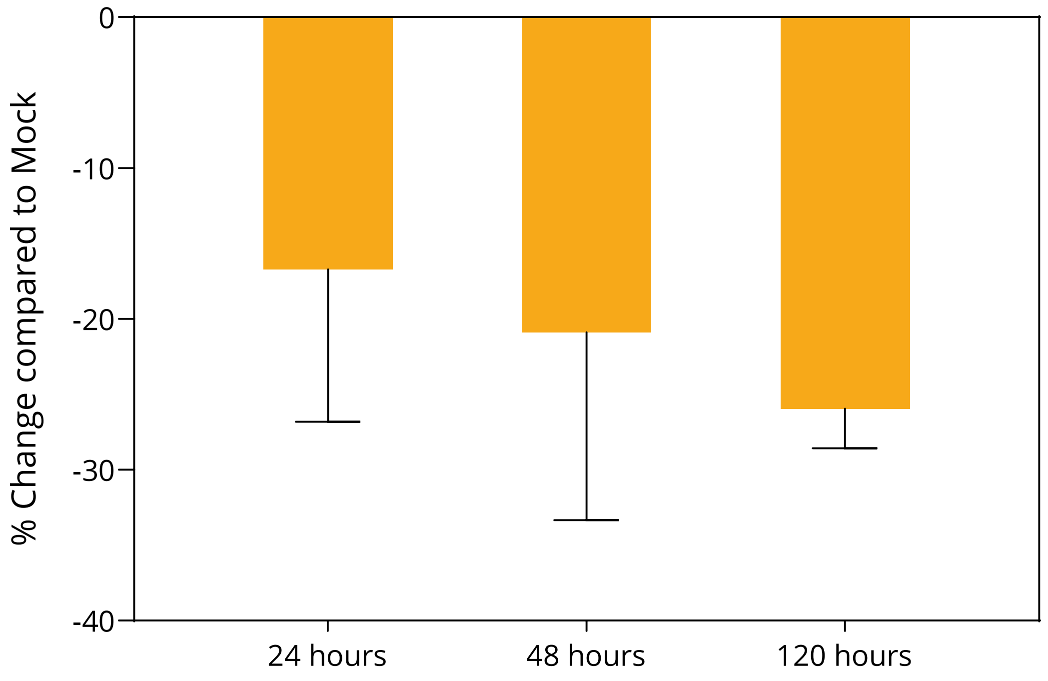
Editing of PNPLA3 in PHH

100nM EON, transfection, 72h, dPCR, mean, SEM, n=3



Change in intracellular lipid droplets post PNPLA3 148V EON treatment

Bodipy/DAPI stainings, 5μM EON, transfection, exposure to linoleic acid, mean, SEM, n=2



Summary & next steps

AX-2911 for MASH

Final optimization of AX-2911 EONs ongoing for clinical candidate selection in 2025

Expected 3-6 monthly dosing interval subcutaneous GalNAc-delivered

Development activities to start in 2025

Start clinical trial in 2026

ProQR development pipeline

	TARGET	DISCOVERY	NON-CLINICAL	CLINICAL	NEXT MILESTONE	ESTIMATED POPULATION
DEVELOPMENT PIPELINE						
AX-0810 *for Cholestatic diseases*	NTCP				CTA filing in Q2 2025	~100K patients
AX-2402 *for Rett syndrome*	MECP2 R270X				Candidate selection in 2025	~5K patients
AX-1412 *for Cardiovascular disease*	B4GALT1				Scientific update in mid 2025	~200M patients
AX-2911 *for MASH*	PNPLA3				Candidate selection in 2025	~8M patients
DISCOVERY PIPELINE						
AX-1005 *for CVD*	Undisclosed					~200M patients
AX-0601 *for obesity and T2D*	Undisclosed					~650M patients
AX-9115 *for rare metabolic condition*	Undisclosed					
AX-2403 *for Rett syndrome*	MECP2 R168X					~6K patients
AX-2404 *for Rett syndrome*	MECP2 R255X					~5K patients
AX-2405 *for Rett syndrome*	MECP2 R294X					~6K patients
AX-2406 *for Rett syndrome*	MECP2 R133H					
AX-3875 *for rare metabolic & CNS disorder*	Undisclosed					
AX-4070 *for rare CNS disorder*	Undisclosed					
PARTNERED PIPELINE						
10 targets (option to expand to 15)	Undisclosed	*Progress undisclosed*				Lilly

[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: Trivedi PJ, et al. Clin Gastroenterol Hepatol. 2022 Aug;20(8):1687-1700.e4; Schreiber RA, et al. J Clin Med. 2022 Feb 14;11(4):999; Tsao CW, et al. Circulation. 2022;145(8):e153–e639. World Health Organization, World Gastroenterology Organization

Catalyst overview

4 trial readouts expected in 2025-2026, cash runway into mid-2027

AX-0810 for Cholestatic disease

- CTA submission Q2 2025
- Top-line data Q4 2025

AX-2402 for Rett Syndrome

- Clinical candidate selection in 2025
- Anticipated trial start and top-line data in 2026

AX-1412 for Cardiovascular disease

- Non-clinical data update in mid 2025

AX-2911 for MASH

- Clinical candidate selection in 2025
- Anticipated trial start and top-line data in 2026

Partnerships

- Opportunity to earn up to $3.75B in milestones in the Lilly partnership
- Opportunity to receive a $50 M opt-in fee from Lilly for expansion to 15 targets
- Opportunity for other strategic partnerships

Well positioned

to advance Axiomer™

Clinical trial results across 4 trials in 2025 and 2026 expected

- Clinical PoC data of NTCP trial in 2025
- Up to 4 clinical trials with data readouts in 2025/2026

Rich discovery pipeline with potential for broad pipeline expansion

- Large number of potential therapeutic applications in discovery pipeline
- Broad applicability beyond current discovery pipeline

Leading IP position

- Axiomer™ is protected by >20 published patent families
- Continuously investing in expanding IP estate

Validating Strategic Partnerships

- Eli Lilly collaboration valued up to $3.9B, with opportunity for near-term milestones
- Rett Syndrome Research Trust cofinancing of AX-2402 program
- Selectively form additional partnerships

Strong balance sheet

- €89.4 million cash and cash equivalents as of end of Q3, plus $82.1 million gross proceeds from October financing
- Cash runway to mid-2027, excluding potential for additional BD-related upside

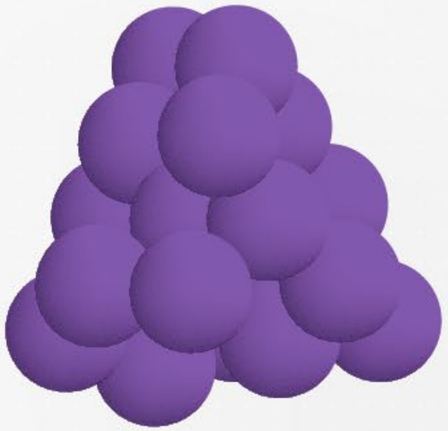
ProQR®

IT'S IN OUR RNA

Resource slides

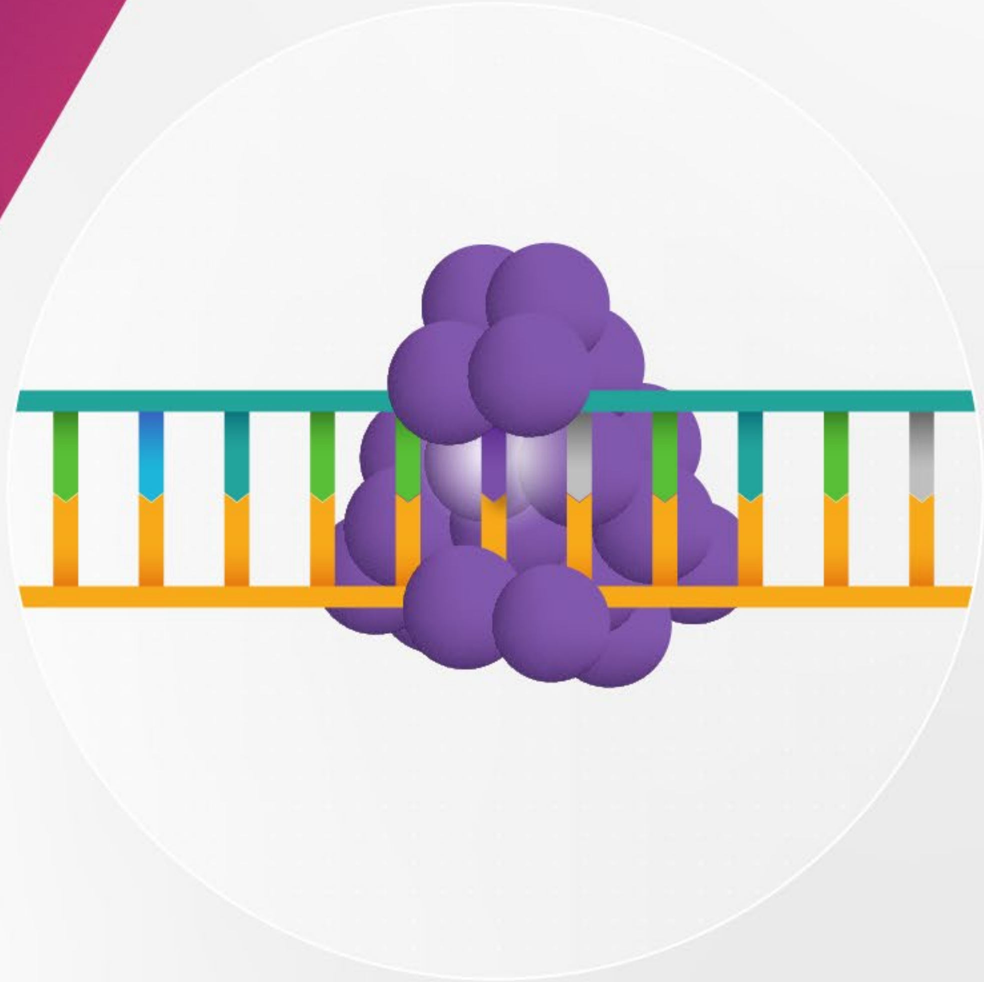
HOW DOES ADAR WORK?

Explained in 5 minutes



WHAT IS AXIOMER™?

Explained in 5 minutes



ProQR Leadership Team

Management Team

Daniel de Boer
Founder & CEO, Board Executive Director

avanzanite bioscience, Hybridize Therapeutics, Termeer Foundation, RunningIT, FAIR Therapeutics, Amylon, XINVENTO

Gerard Platenburg
Chief Scientific Officer, Board Executive Director

Prosensa, ISA Pharmaceuticals, Pharming

René Beukema
Chief Corporate Development Officer, Board Executive Director

frame cancer therapeutics, Galapagos, Crucell, mytomorrows, GE Capital, Fibriant

Jurriaan Dekkers
Chief Financial Officer

AstraZeneca, Acerta Pharma (A member of the AstraZeneca Group), DaVita Medical Group

Sheila Sponselee
VP, Head of People and Operations

mytomorrows

Board of Directors

James Shannon, MD
Chair

Novartis, gsk

Alison Lawton

genzyme, Kaleido Biosciences

Begoña Carreño

Novartis, almirall

Martin Maier, PhD

IONIS Pharmaceuticals, Alnylam Pharmaceuticals

Bart Filius

Galapagos

Dinko Valerio

Crucell, AescAp

Theresa Heggie

Alnylam Pharmaceuticals, FREELINE

Key Advisors

John Maraganore, PhD
Board advisor

Alnylam Pharmaceuticals

Peter A. Beal, PhD
ProQR Chief ADAR Scientist

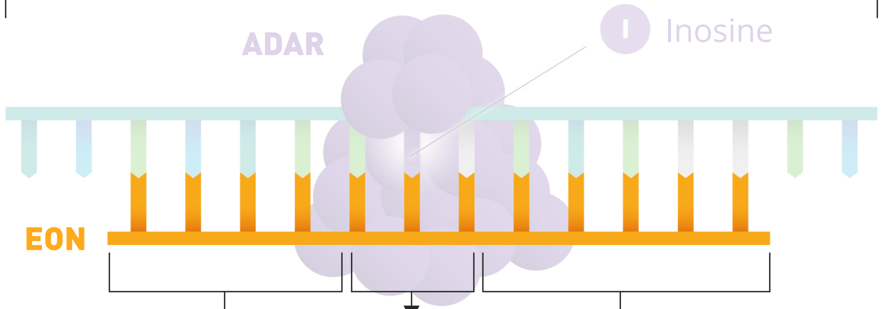
Phillip D. Zamore, PhD
Scientific Advisory Board

hhmi Howard Hughes Medical Institute, UMASS Medical School, Voyager Therapeutics, Alnylam Pharmaceuticals

Driving the development of optimized EONs for therapeutic use

Sequence defines target RNA binding and provides stability

EON total length
Between ~25 - 30 nt



Editing Enabling Region (EER)

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

Leading IP supporting ADAR-mediated RNA editing platform technology

- Axiomer™ IP strategy commenced in 2014 with first patent application filings
- Currently 24 published patent families, comprising 31 national/regional patents
- Axiomer™ IP portfolio is constantly expanding
- Oppositions/appeals and several Third-Party Observations have been filed against a variety of applications and patents in the Axiomer™ IP portfolio, all by strawmen

ProQR Axiomer™ leading IP estate for ADAR-mediated RNA editing

- ProQR's Axiomer™ IP contains 3 early RNA editing platform patent families covering single-stranded oligonucleotides that recruit **endogenous** ADAR
- Oppositions/appeals and Third-Party Observations have been filed throughout these three patent families
- First (2014): oligonucleotides with a complementary (**targeting**) and a stem-loop (**recruiting**) portion
- Second (2016): oligonucleotides **without a stem-loop structure** but with **one or more mismatches** and chemical modifications
- Third (2016): oligonucleotides **without a stem-loop structure** but with specific chemical modifications in the '**Central Triplet**'

Overview of Axiomer™ related patents

Docket	Priority	Feature	Status	Remarks
1 (0004)	17DEC2014	Targeted RNA Editing using endogenous ADARs	Granted AU BR CA CN EP IL IN JP NZ US US ZA	Platform IP
2 (0013)	22JUN2016	Short EONs with wobble and/or mismatch base pairs	Granted AU IL JP KR US US US	Platform IP
3 (0014)	01SEP2016	Chemically modified short EONs	Granted AU CN EP JP KR NZ US US US ZA	Platform IP
4 (0016)	19JAN2017	EONs + protecting SONs (heteroduplex formation)	Granted US	Platform IP
5 (0023)	18MAY2018	PS linkages / chiral linkages (*e.g.*, PS, PN)	Published	Platform IP
6 (0025)	28JAN2019	Editing of PTC in exon 61 USH2A	Published	Target
7 (0026)	11FEB2019	Phosphonacetate linkages / UNA modifications	Published	Platform IP
8 (0029)	03APR2019	MP linkages	Published	Platform IP
9 (0031)	24APR2019	Editing inhibition	Published	Platform IP
10 (0032)	13JUN2019	Benner's base (dZ)	Published Granted ZA	Platform IP – with UC Davis (P Beal)
11(0035)	23DEC2019	Editing in exon 35 of ABCA4 for Stargardt disease	Published	Target
12 (0039)	23JUL2020	Split EONs	Published	Platform IP
13 (0045)	14FEB2022	PCSK9	Published	Target
14 (0046)	15JUL2022	5'-GA-3' editing	Published	Platform IP – with UC Davis (P Beal)
15 (0048)	15JUL2022	diF modification	Published	Platform IP
16 (0051)	21OCT2022	Heteroduplex Editing Oligonucleotide (HEON) complexes	Published	Platform IP
17 (0052)	24NOV2022	HFE	Published	Target
18 (0053)	09DEC2022	B4GALT1	Published	Target
19 (0054)	01DEC2022	ALDH2	Published	Target
20 (0055)	20JAN2023	AG1856 + (H)EONs	Published	Platform IP – with FU Berlin (A Weng)
21 (0057)	20FEB2023	ANGPTL3	Published	Target
22 (0058)	24MAR2023	KCC2	Published	Target
23 (0059)	24MAR2023	PNms linkages	Published	Platform IP
24 (0060)	27MAR2023	NTCP	Published	Target

ProQR Axiomer™ IP

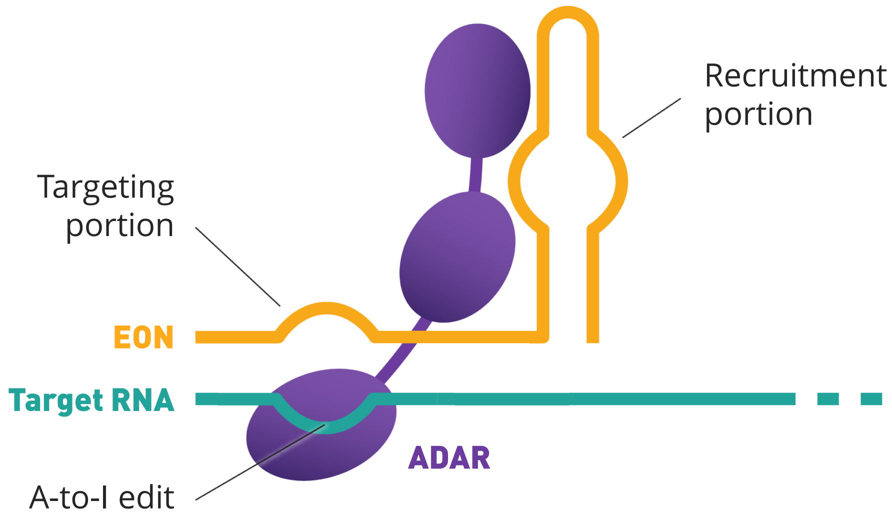
Broad coverage

- Axiomer™ patent claims are broad and cover:
  - **Any type of chemically modified oligonucleotide** aimed at RNA editing of **any possible target** and **any possible disease** using **endogenous** ADAR
  - Specific targets
  - Oligonucleotides with chirally-controlled linkages
  - Oligonucleotides with all sorts of chemistries (also in the 'Central Triplet'), including **DNA**
- To note: claims directed to chemically modified oligonucleotides **do not cover viral delivery** of the oligonucleotide

Overview of key claims – 1

Granted claims in the 1st Axiomer™ patent family relate to (chemically modified) oligonucleotides that comprise:

- **A targeting portion** for binding to a target RNA incl. target adenosine
- **A recruitment portion** (hairpin structure) for recruiting **endogenous** ADAR to edit the target adenosine



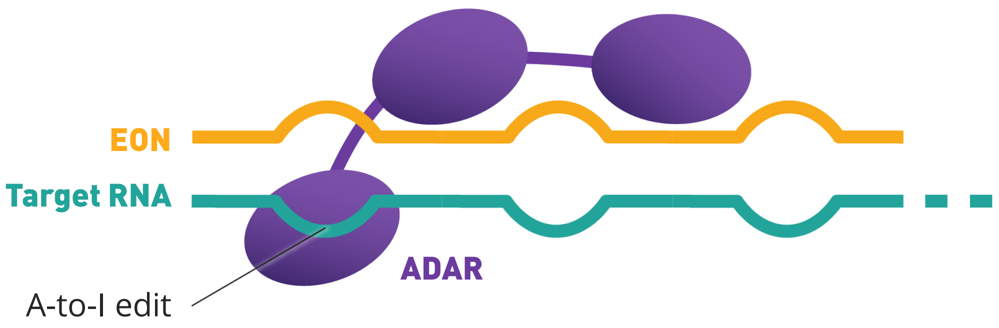
EP 3 234 134 B1 - Granted; appeal pending
US 10,676,737 - Granted
US 11,781,134 - Granted

Claim 17. A method for making a change in a target RNA sequence in a human cell, comprising the steps of:

- introducing into the cell an oligonucleotide construct that is **sufficiently complementary** to bind by nucleobase pairing to the target RNA sequence, wherein the target RNA sequence comprises a target adenosine;
- allowing the formation of a double-stranded structure of the oligonucleotide construct with the target RNA sequence upon base pairing;
- allowing the double-stranded structure of the oligonucleotide and the target RNA sequence to recruit **an hADARl or hADAR2 enzyme naturally present in the cell;**
- allowing the hADARl or hADAR2 enzyme to perform deamination of the target adenosine to an inosine in the target RNA sequence.

Overview of key claims – 2

Granted claims in the 2nd Axiomer™ patent family relate to oligonucleotides that do **not** have a hairpin structure, but instead have one or more wobbles and/or mismatches, and chemical modifications in the base, ribose sugar and/or linkage to increase stability and are still able to recruit **endogenous** ADAR to edit the target adenosine.



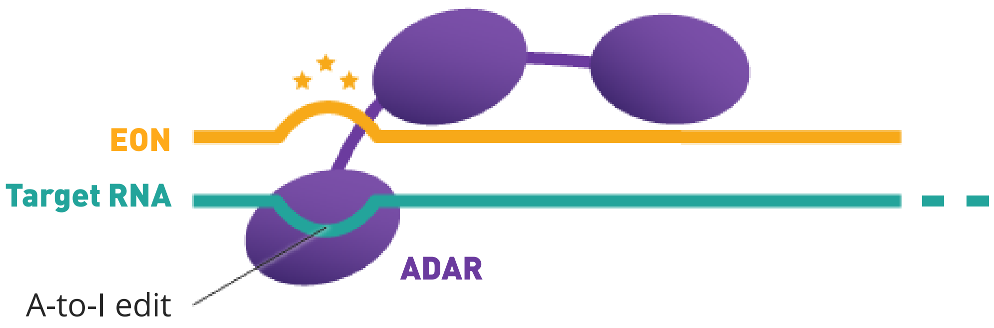
US 10,941,402 - Granted
US 11,851,656 - Granted
US 12,018,257 - Granted

Target-specific claims

- An AON capable of forming a double stranded complex with a target RNA in a cell, wherein: the target RNA encodes CFTR, CEP290**, alpha1- antitrypsin (A1AT)**, LRRK2, or BDNF, or the target RNA is encoded by the IDUA gene
- The AON is complementary to a target RNA region comprising a target adenosine
- The AON comprises one or more nucleotides with **one or more sugar modifications**
- The AON does **not** comprise a portion that is capable of forming an intramolecular stem-loop structure that is capable of binding an ADAR enzyme
- The AON is shorter than 100 nucleotides
- The AON **optionally comprises 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10** mismatches, wobbles and/or bulges with the complementary target RNA region, and, wherein formation of the double stranded complex between the AON and the target RNA results in the deamination of the target adenosine by an ADAR enzyme **present in the cell**

Overview of key claims – 3

Granted claims in the 3rd Axiomer™ patent family relate to oligonucleotides that do **not** have a hairpin structure, but have **chemical modifications** in the base, ribose sugar and/or linkage to increase stability and are still able to recruit **endogenous** ADAR to edit the target adenosine.



US 10,941,402 - Granted
US 11,851,656 - Granted
EP 3 507 366 B1 - Granted; appeal pending

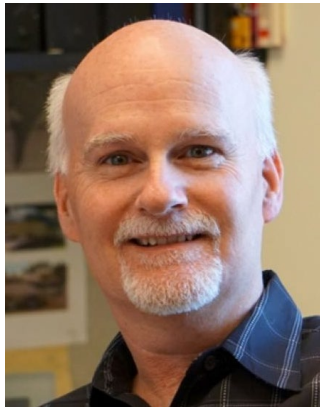
An antisense oligonucleotide (AON) capable of forming a double stranded complex with a target RNA sequence in a cell, preferably a human cell, for the deamination of a target adenosine in the target RNA sequence by an ADAR enzyme present in the cell, said AON comprising **a Central Triplet** of 3 sequential nucleotides, wherein the nucleotide directly opposite the target adenosine is the middle nucleotide of the Central Triplet, wherein 1, 2 or 3 nucleotides in said Central Triplet comprise **a sugar modification and/or a base modification** to render the AON more stable and/or more effective in inducing deamination of the target adenosine; with the proviso that the middle nucleotide does not have a 2’-O-methyl modification.

INVESTOR & ANALYST EVENT

December 11, 2024

Peter Beal, PhD

ProQR Chief ADAR Scientist & SAB member, Professor UC Davis



- 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
- Advanced understanding of the structures and mechanism of action for the ADAR enzymes responsible for adenosine to inosine RNA editing in humans
- Led in the development of structure-guided methods for optimizing chemically modified oligonucleotides for recruitment of RNA-binding proteins including ADARs
- Teaches organic chemistry at the undergraduate level and several classes in nucleic acids chemistry and chemical biology at the graduate level
- Over 100 peer-reviewed publications in the field of RNA chemical biology and mentored over 50 Ph.D. and M.S. degree students
- ProQR Chief ADAR Scientist, Scientific Advisory Board

Axiomer™ advancing to value inflection

Innovative ADAR-enabled RNA editing science driving advancement of Axiomer

supported by robust IP estate

High impact strategic partnerships

Eli Lilly, Rett Syndrome Research Trust

Pipeline with transformative potential for diseases with high unmet medical needs

work at root cause

Experienced team driving execution

Runway into mid 2027

€89.4 million cash and cash equivalents as of end of Q3, plus $82.1 million gross proceeds from October financing providing runway into mid-2027

Axiomer™ Platform

Driving innovation in the ADAR RNA editing field

Presenter: Peter Beal, PhD

Axiomer™ RNA-editing platform technology

Versatile

- Ability to target multiple organs and a wide range of diseases with numerous applications
- Potential to include protective variants
- Designed to target a variety of RNA species (mRNA, miRNA, lncRNA)

Safety

- No permanent changes
- No irreversible DNA damages and less risk of permanent side effects

High specificity

- Highly targeted therapeutic with potential to minimize off-target effects and reduce the risk of adverse reactions

Transient

- Provide a long-lasting therapeutic effect that does not require frequent dosing
- Potential to target diseases for which permanent changes would be deleterious

No viral vector

- No risk of immunogenicity or capacity limitation due to the vector
- Efficient development and faster production increase the chance to reach market

Endogenous ADARs

- Leverage body's potential to treat disease
- Less risk of off-target effect vs. exogenous ADARs

ADAR: Adenosine deaminase acting on RNA, mRNA: messenger RNA, miRNA: microRNA, lcRNA: long non-coding RNA

ProQR's Axiomer™ ADAR journey since 2014

ProQR invents oligo mediated RNA Editing recruiting endogenous ADAR

2014

Key ADAR patents get granted in EU and US

2020-2023

ProQR pivots to solely focus on ADAR editing

2022

ProQR's ADAR patents win opposition cases filed by strawmen across the world

2023-2024

2014-2018+

ProQR files key patents that protect ADAR mediated RNA editing broadly

2015-2021

ProQR optimizes the ADAR platform in stealth

2021

ProQR and Eli Lilly enter into first 5 target partnership worth $1.25B

2022

ProQR and Eli Lilly expand partnership to 10 targets worth ~$3.9B

2023

ProQR demonstrates >50% editing in CNS and liver in NHP and announces pipeline

2024

- ProQR first in the field to report a disease relevant biomarker effect using Axiomer in NHP. Initial indication of good safety profile.
- Initial clinical validation of ADAR editing

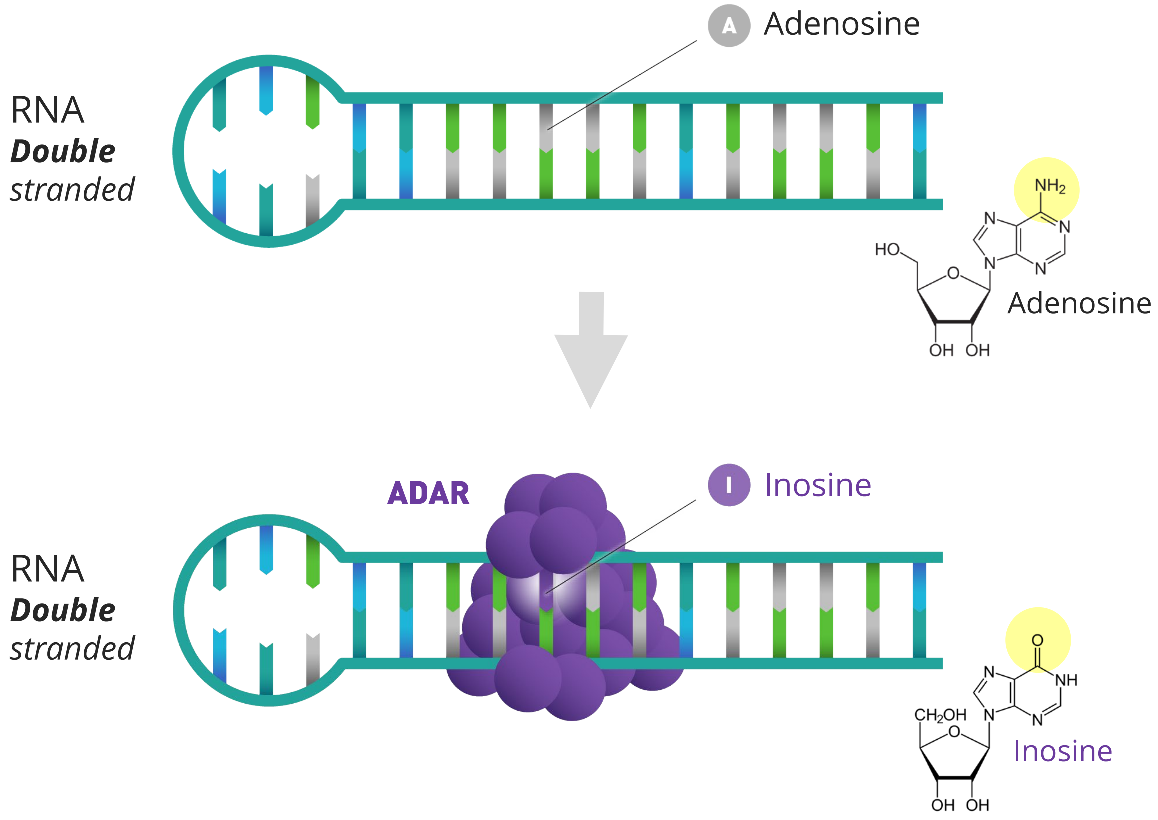
ADARs: Adenosine deaminases acting on RNA, EONs: Editing oligonucleotides

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)**

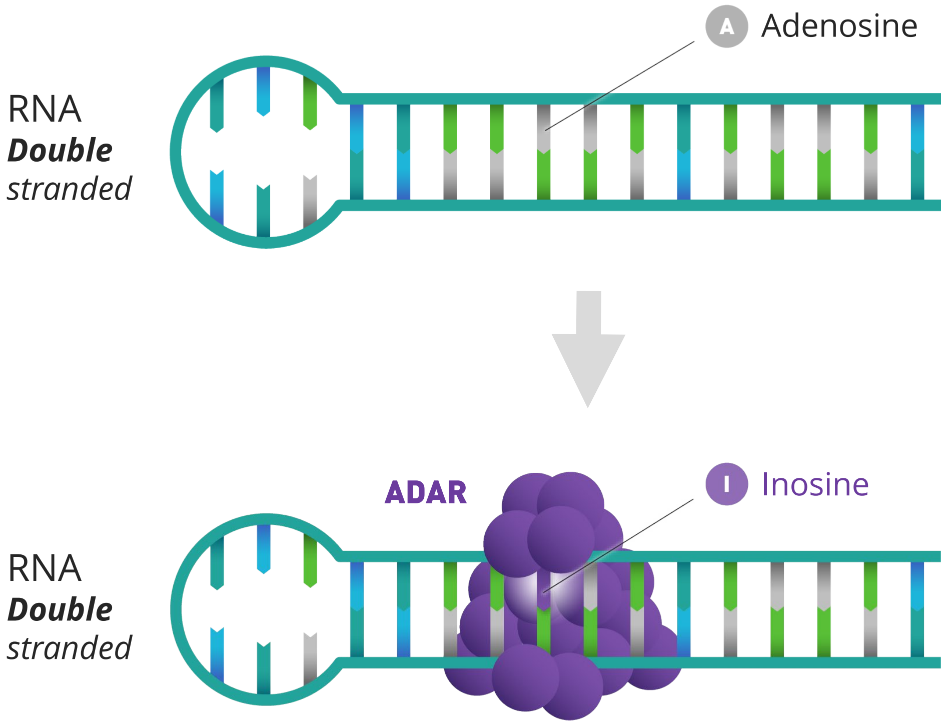
Natural ADAR editing *(A-to-I)*



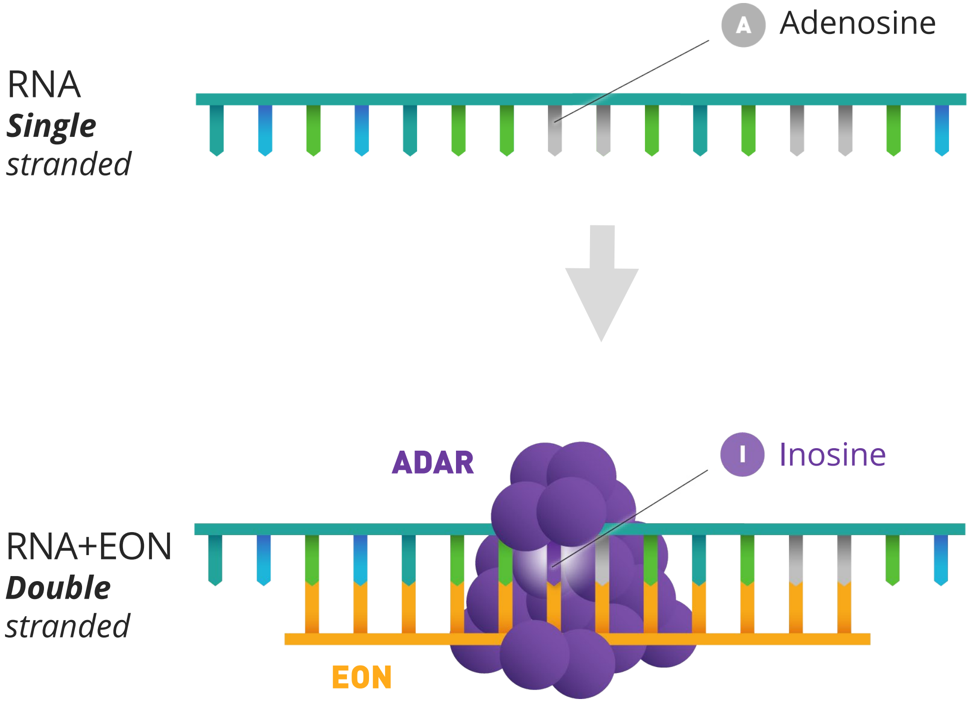
Axiomer™ EONs unlock cellular machinery potential to treat diseases

By attracting ADARs and allowing highly specific editing

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

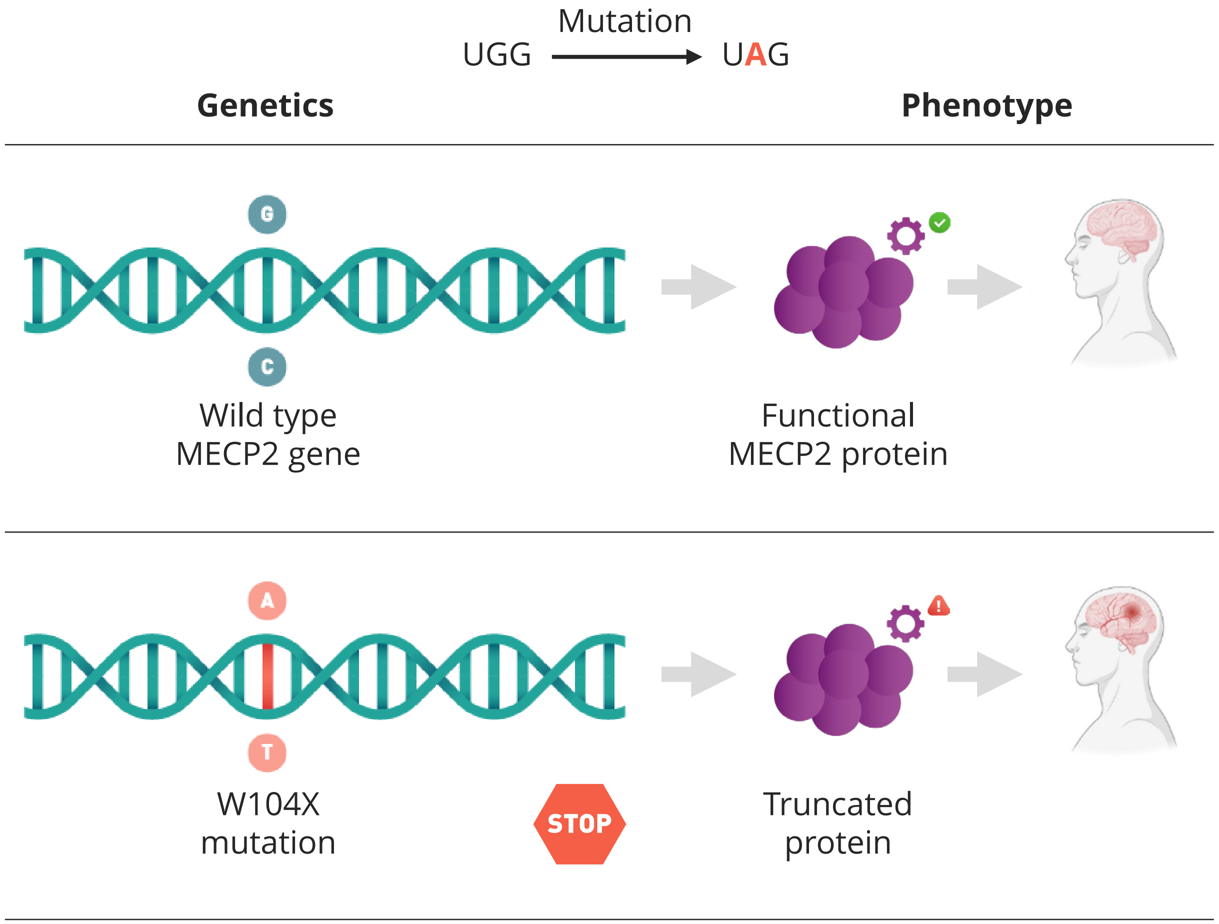


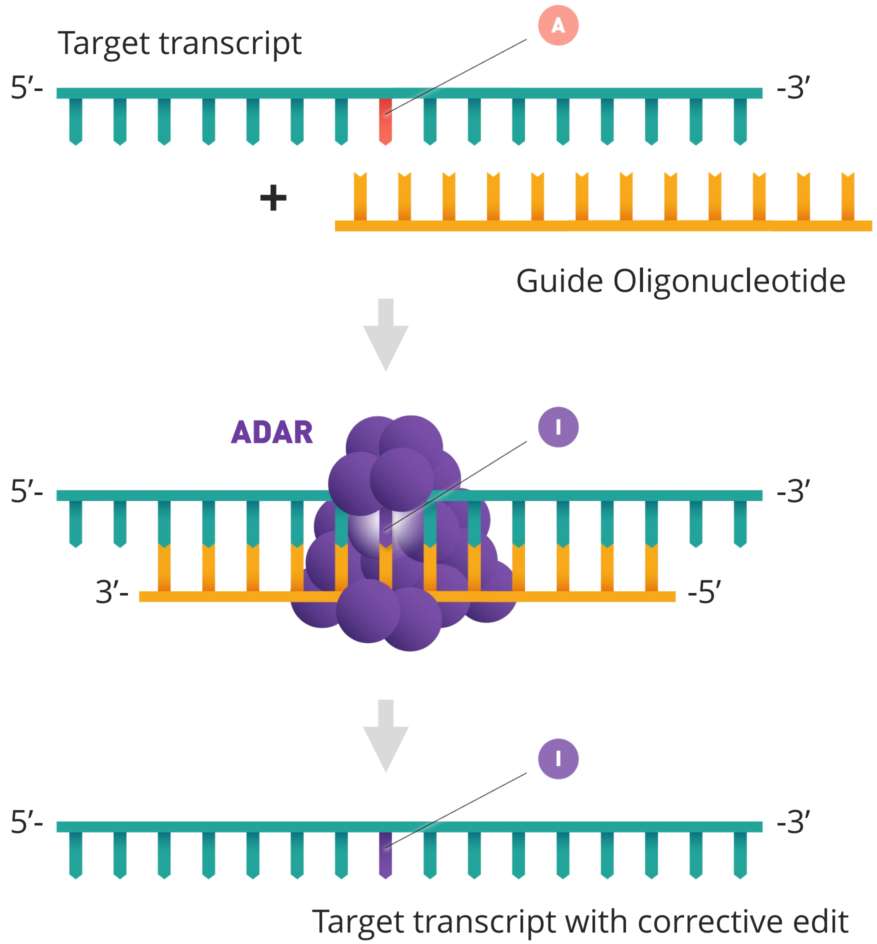
Editing Oligonucleotide (EON)-directed therapeutic editing *(A-to-I)*



ADARs: Adenosine deaminases acting on RNA.

Oligonucleotide-directed RNA editing



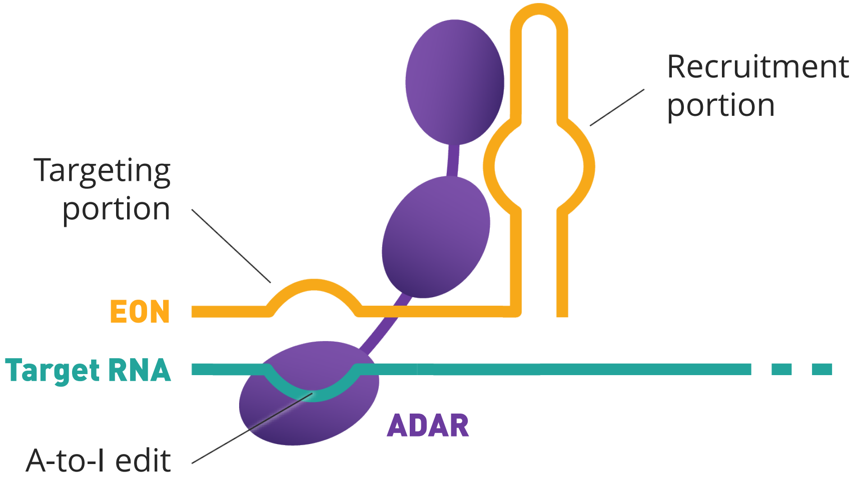


Reference: Doherty EE, Beal PA. Mol Ther. 2022 Jun 1;30(6):2117-2119.

Driving innovation in the RNA field with Axiomer™ editing oligonucleotides

1st Axiomer EONs generation

relate to (chemically modified) oligonucleotides that comprise

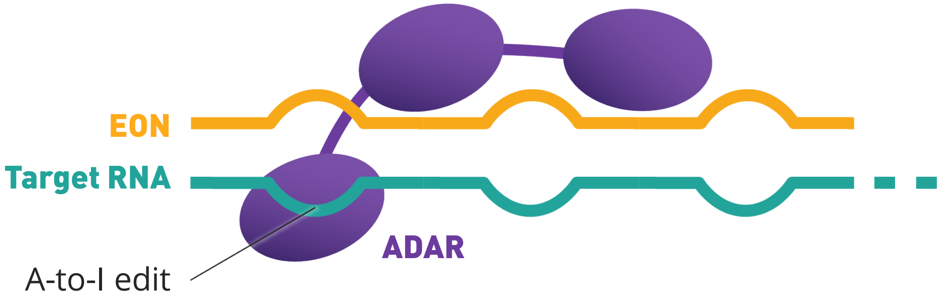


- **A targeting portion** for binding to a target RNA incl. target adenosine
- **A recruitment portion** (hairpin structure) for recruiting **endogenous** ADAR to edit the target adenosine

Patents: Granted appeal pending EP 3 234 134 B1; Granted US 10,676,737; Granted US 11,781,134

2nd Axiomer EONs generation

relate to oligonucleotides that comprise



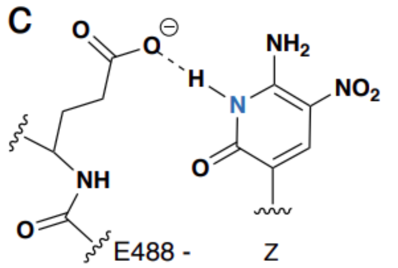
- **No hairpin structure**
- One or more wobbles and/or mismatches, and chemical modifications in the base, ribose sugar and/or linkage to **increase activity as well as stability** and are still able to recruit **endogenous** ADAR to edit the target adenosine.

Patents: Granted US 10,941,402; Granted US 11,851,656; Allowed US 18/296,912

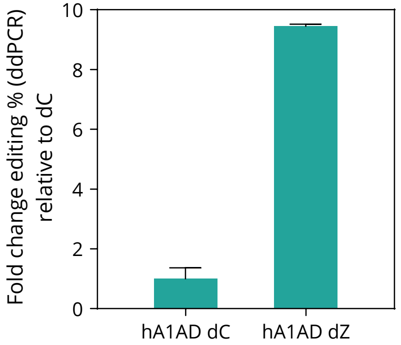
ProQR leading research to optimize editing oligonucleotides for therapeutic use

Modification of the orphan base

dZ in EER to increase ADAR activity

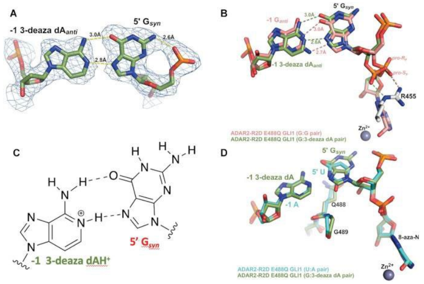


RNA editing of *SERPINA1* E366K in A1AD patient hepatocytes
Transfection of 100nM EON, N=2, 48 hours

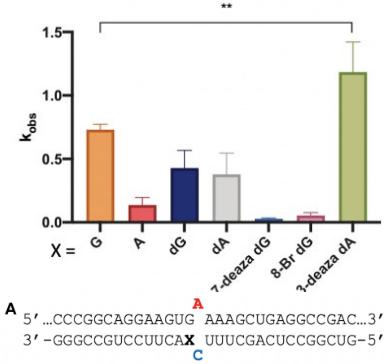


Modification of the base opposite to 5'g

3-deaza-dA in EER to increase editing activity in 5'G unfavorable context



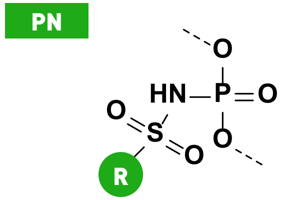
In vitro deamination kinetics for ADAR2 and duplex RNAs derived from *hMECP2* R255X
100nM ADAR2, 3 technical replicates, mean, SD



Linkage modifications in the ABR

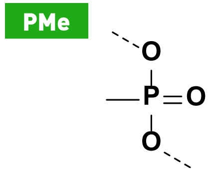
PN and PMe linkages in the ABR to increase stability, EON liver concentration and target engagement

Phosphoroamidate linkage

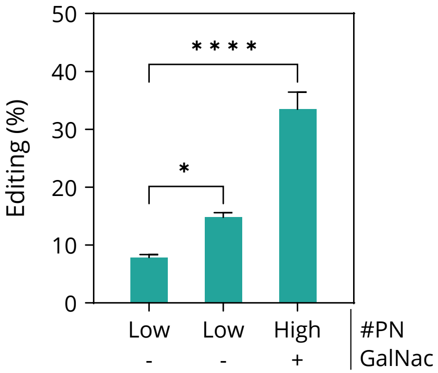


R = Me: Mesyl-PN

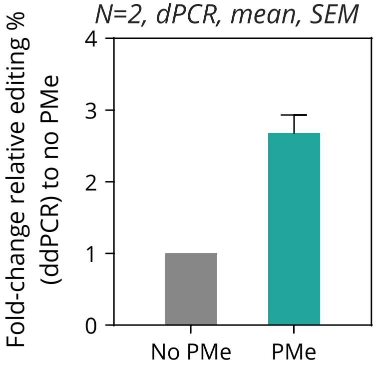
Methylphosphonate linkage



RNA editing of *ActB* in liver
C57Bl/6J mice, 7d, 3x10mg/kg, SC, N=4, dPCR, mean, SEM



RNA editing of *APP* in HepG2 cells
Gymnosis, 5d, 5µM single dose, N=2, dPCR, mean, SEM



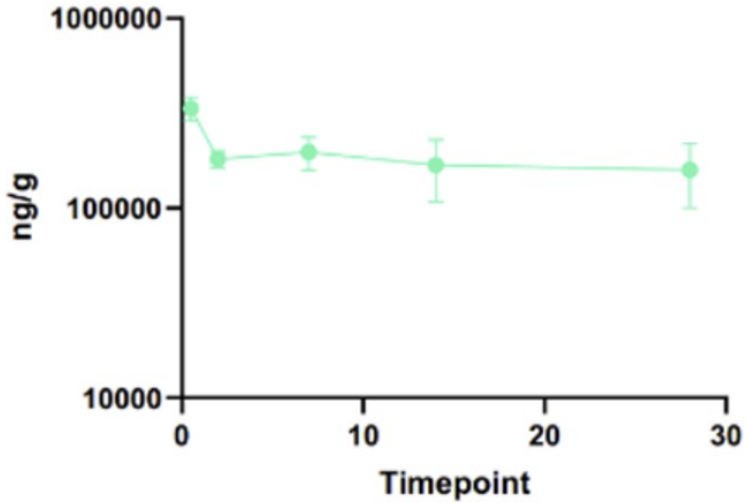
Adapted from Doherty EE, et al. Nucleic Acids Res. 2022;50(19):10857-10868. Statistical significance between groups was determined using one-way ANOVA with Tukey's multiple comparisons test or an unpaired t-test with Welch's correction; **P < 0.01; ***P < 0.001; ****P < 0.0001.

Sequence optimization enables stable editing oligonucleotides with prolonged PK

Learnings from advanced programs inform editing optimization

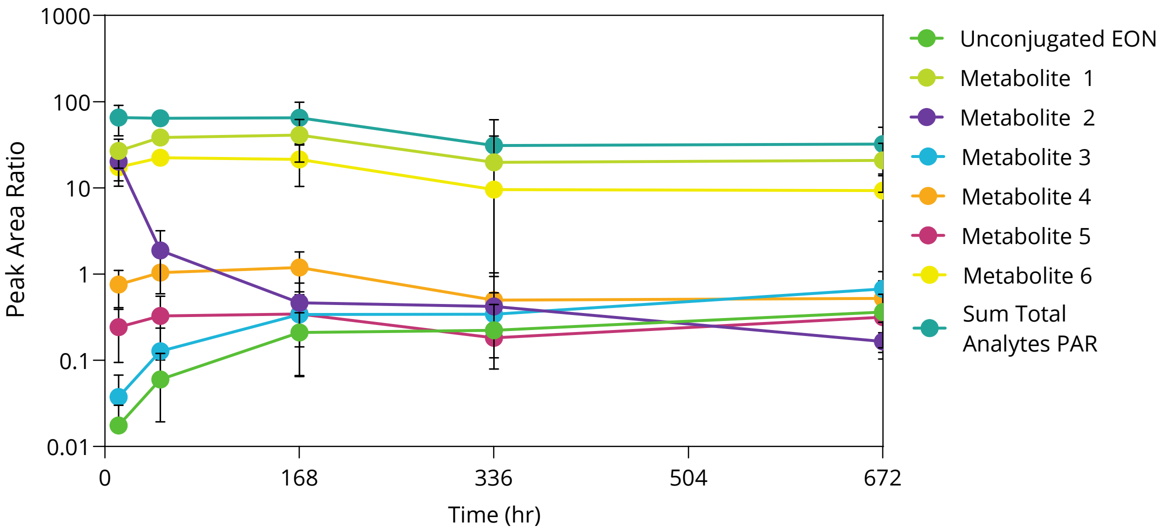
EON11 concentration in liver of mice disease model

Hybridization-HPLC, n=6, 30mg/kg, EON11, SC, GalNAc conjugation, up to 4 weeks



EON11 metabolites in liver of mice disease model

LC-MS, n=6, 30mg/kg, EON11, SC, GalNAc conjugation, up to 4 weeks

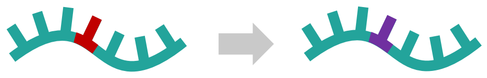
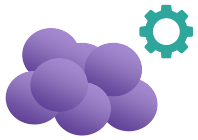
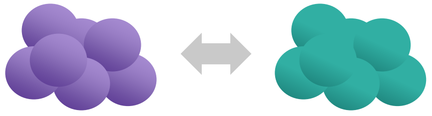
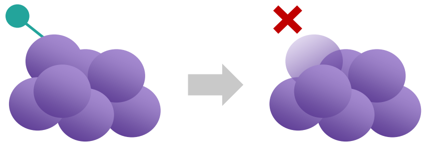


- Rapid absorption in the liver and long half-life of EON11 in liver measured – around 80 days
- EON show high stability with no metabolites observed for oligonucleotide itself

- Up to six metabolite were identified and all were the metabolite of the GalNAc entity
  - Most represented is linker between EON and GalNAc moiety
  - Others were a combination of different cleavages of different GalNAc arms or within the linker

Perkins E. 726. Complex Metabolism and Prolonged PK/PD of a GalNAc-Conjugated Editing Oligonucleotide (EON) in Mice. ASGCT 27th Annual Meeting Abstracts; *Molecular Therapy*, Volume 32, Issue 4, 1 - 889

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

Axiomer™ RNA editing science translating toward therapeutic applications

Science

- Harnessing advanced knowledge of ADAR and oligonucleotide science
- Pioneering the optimization of editing oligonucleotides (EONs) to achieve best-in-class therapeutic solutions

Versatile applicability

- Demonstrating proven success in correcting genetic mutations and enabling diverse protein modulation strategies
- Platform with potential to address diverse conditions rooted in human genetics

Leadership position

- Driving innovation in the ADAR RNA editing science with Axiomer EONs since 2014
- Dominant IP position to drive ADAR-mediated RNA editing platform innovation

AX-0810 Program

Targeting NTCP to address cholestatic diseases unmet medical need at the root cause

Presenters: Prof. Gideon Hirschfield, Gerard Platenburg

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

Prof. Gideon Hirschfield MA (Oxon), MB BChir (Cantab), PhD, FRCP

Professor of Gastroenterology and Hepatology, Toronto, Ontario, Canada

- Lily and Terry Horner Chair in Autoimmune Liver Disease Research
- Director, The Autoimmune and Rare Liver Disease Programme, Toronto General Hospital
- Professor, Division of Gastroenterology and Hepatology, University of Toronto
- Prof. Gideon M. Hirschfield is an experienced and highly focused clinician-scientist specialising in autoimmune and cholestatic liver diseases. He holds the Lily and Terry Horner Chair in Autoimmune Liver Disease Research at the Toronto Centre for Liver Disease, Toronto General Hospital, and serves as a Professor of Medicine in the Division of Gastroenterology and Hepatology at the University of Toronto.
- Prof. Hirschfield completed undergraduate studies in Medicine from the Universities of Oxford and Cambridge and subsequently was awarded a PhD from the University of London in 2006. He completed specialist training in Internal Medicine, Gastroenterology and Hepatology in London, Cambridge and Toronto.
- An internationally recognised expert, Prof. Hirschfield has published over 350 peer-reviewed articles, including lead authorship in high-impact journals such as the New England Journal of Medicine, The Lancet, and Nature Genetics.
- His research focuses on advancing therapies for autoimmune and cholestatic liver diseases with the clear goal of preventing the need for transplantation alongside improving patient quality of life

State of the art in cholestatic liver disease

Gideon Hirschfield

Toronto, Canada

THE AUTOIMMUNE & RARE LIVER DISEASE PROGRAMME

PBC, PSC, AIH, Hepato-biliary IgG4-RD & Genetic Cholestasis

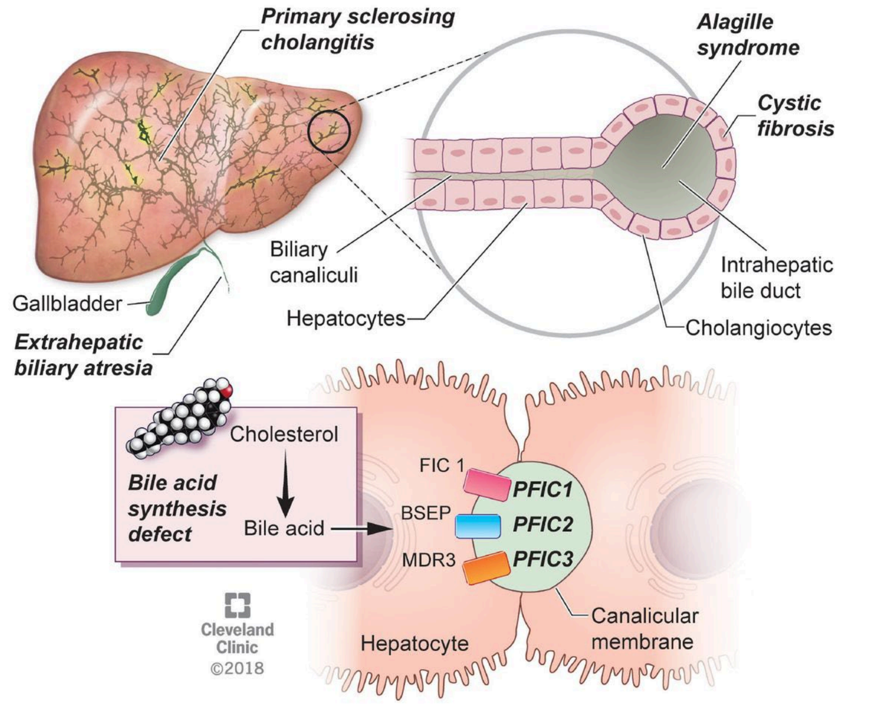
Care Teaching Research

Disclosures

- Intercept Pharmaceuticals, Inc./Advanz
- Ipsen
- CymaBay Therapeutics/Gilead Sciences
- Pliant Therapeutics
- Escient
- Mirum
- GSK
- Kowa
- Chemomab
- Falk
- ProQR

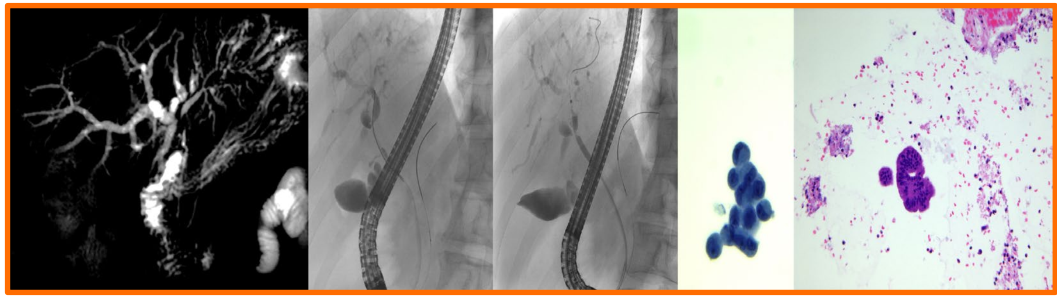
Cholestatic liver disease

Where unmet need in Hepatology practice remains



BSEP = bile salt export pump; FIC 1 = familial intrahepatic cholestasis protein 1; MDR3 = multidrug resistance protein 3; PFIC = progressive familial intrahepatic cholestasis

Praveen Kumar Conjeevaram Selvakumar et al. CCJM 2019;86:454-464



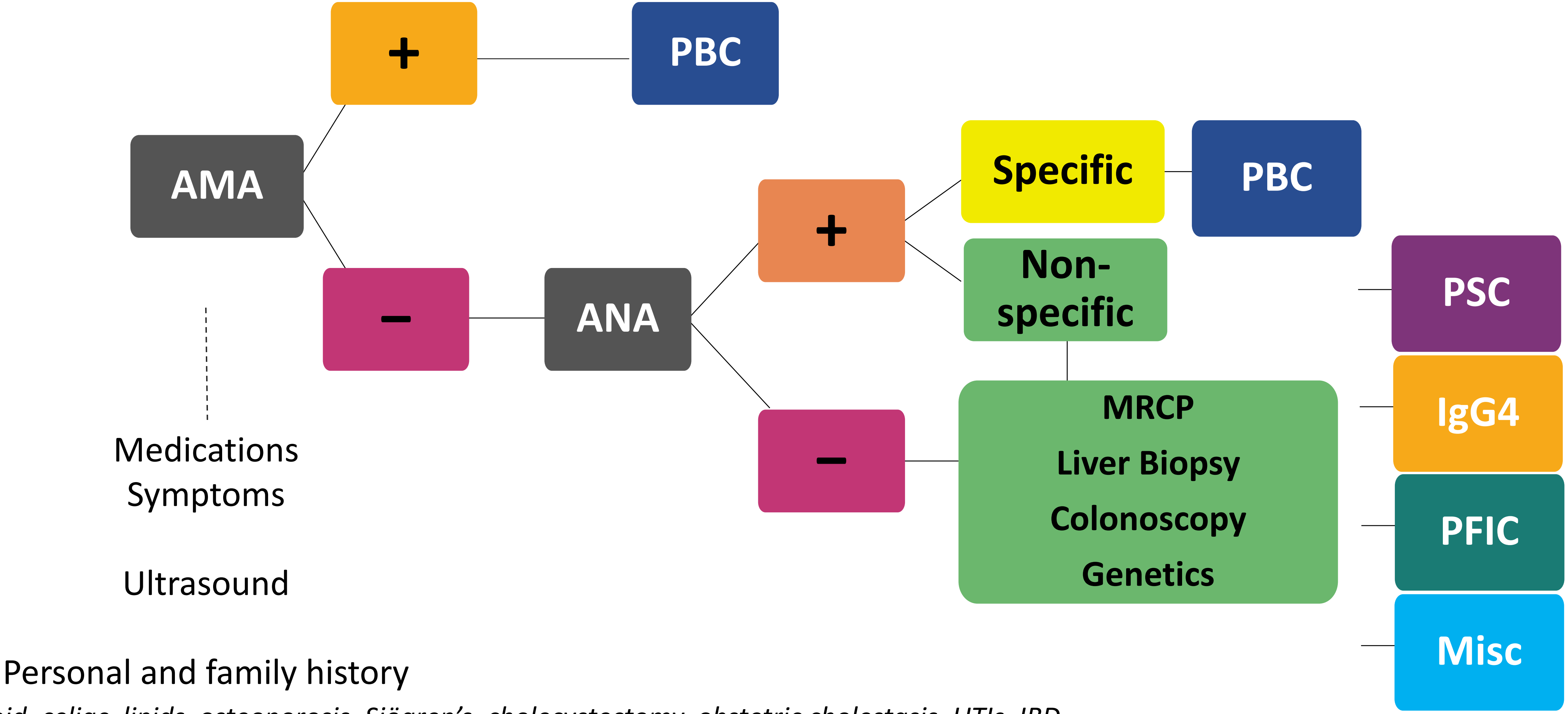
Paediatric/genetic cholestatic liver disease incl. biliary atresia

Primary biliary cholangitis

Primary sclerosing cholangitis

https://www.researchgate.net/figure/Scratch-lesions-Pruritus-of-different-origins-may-lead-to-scratch-lesions-Two-examples_fig3_308094035

Investigating the adult patient with cholestasis



e.g. thyroid, celiac, lipids, osteoporosis, Sjögren's, cholecystectomy, obstetric cholestasis, UTIs, IBD

Investigations

Albumin (g/L):	**33** (L)
Bilirubin, Total (mg/dl):	**2**(H)
Bilirubin, Direct:	1(H)
AST (U/L):	82 (H)
ALT (U/L):	82 (H)
Alkaline Phosphatase (U/L):	**330** (H)
GGT (U/L):	171 (H)

Hb (g/L):	89 (L)
WBC (10*9/L):	3.9 (L)
Plt (10*9/L):	**104** (L)
MCV (fL):	79.9 (L)

Liver Stiffness

Result: **17.0 kPa**
Valid measurements: 10
IQR: 2.7 kPa. IQR/Median: 16 %.

India (May 2023):
Grade 1 varices / cirrhosis & splenomegaly

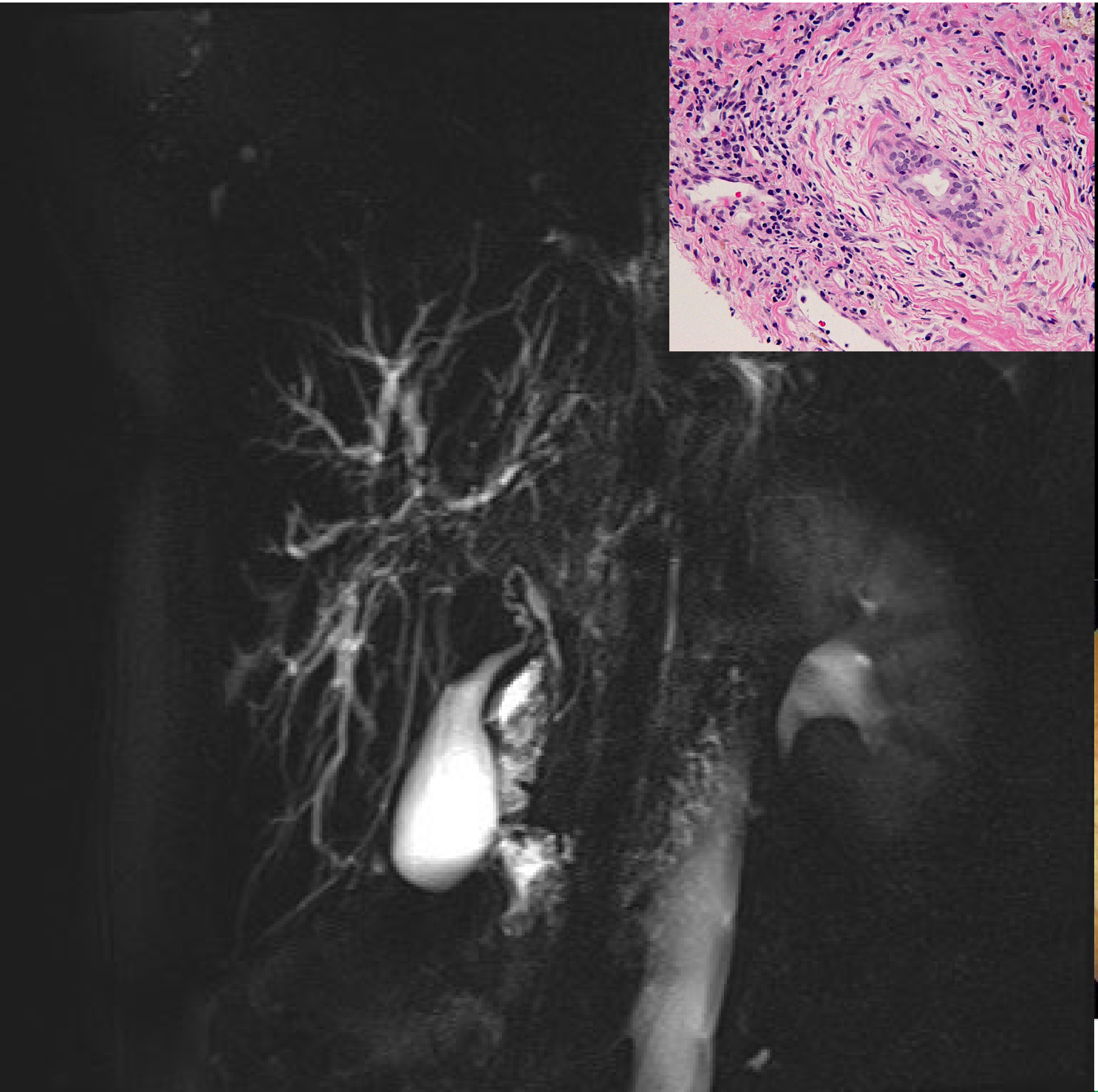
Immunology

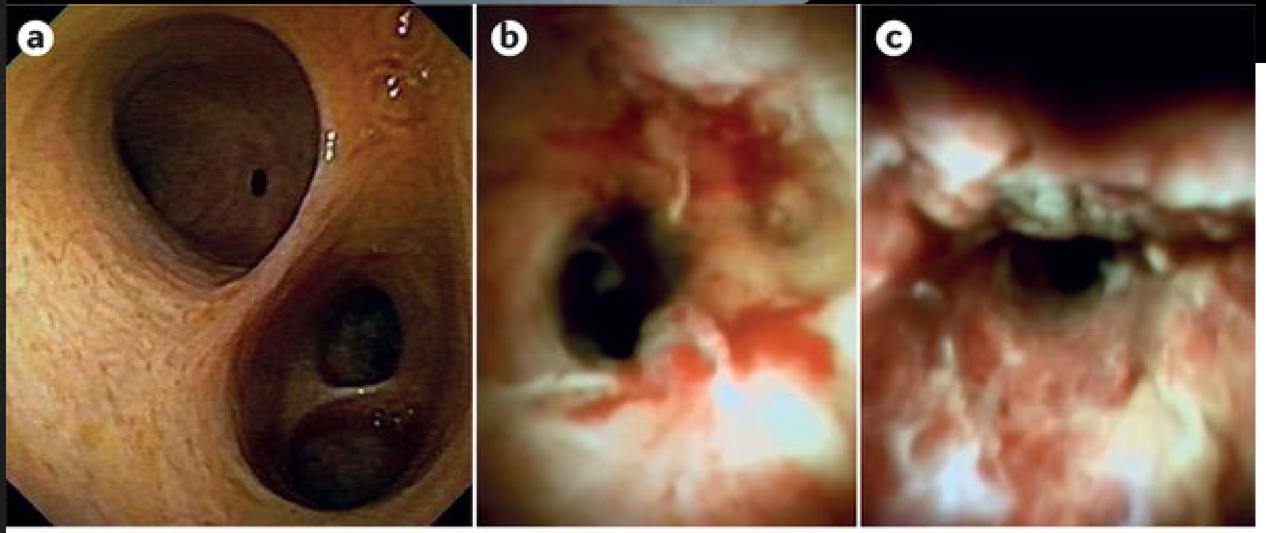
ANA Pattern 1	**Centromere**
ANA Titre 1	**1:640 or greater**

IgG 14.0 g/L
IgA 3.62 g/L
IgM **3.41** g/L

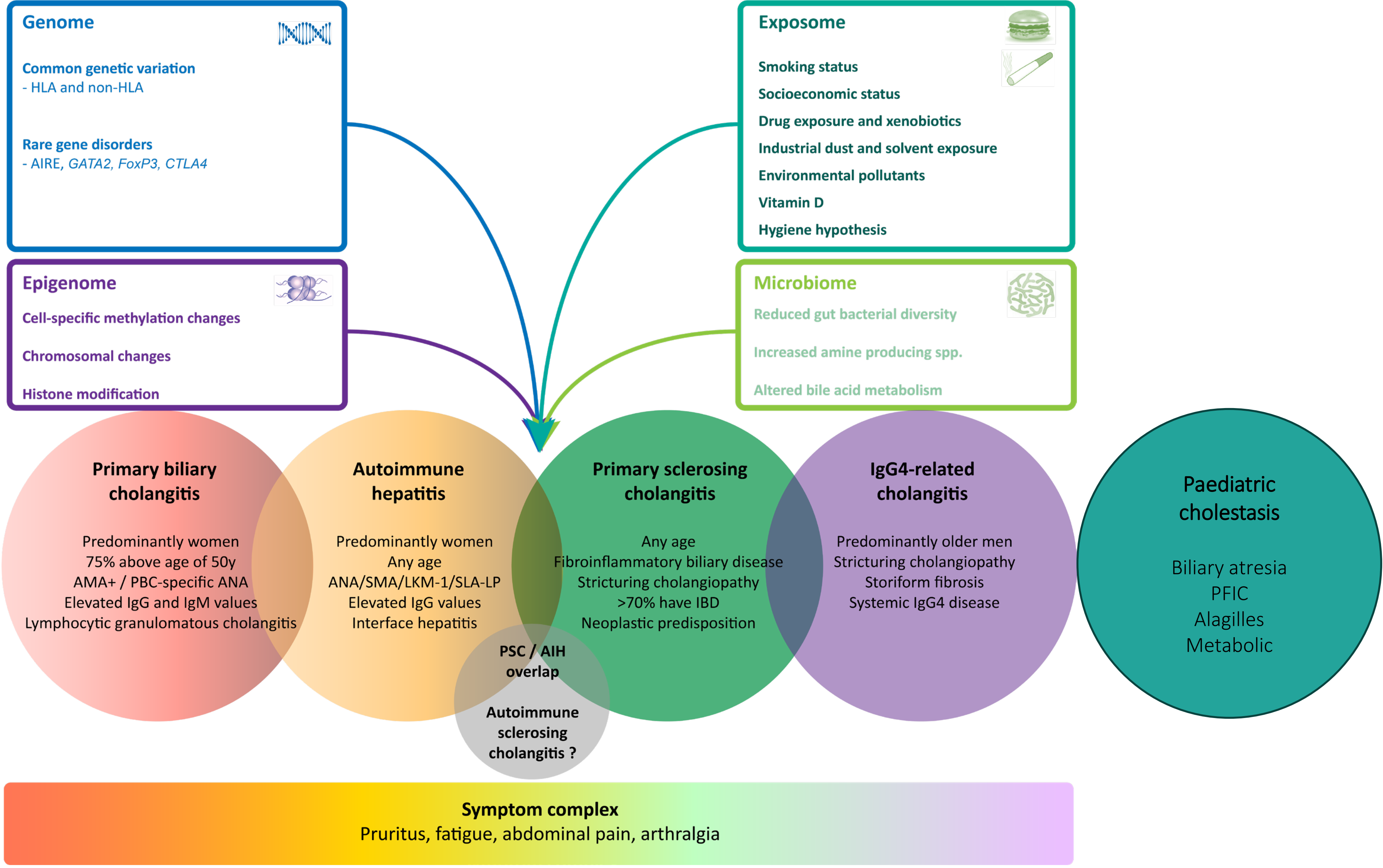
Anti AMA-M2	**1+**
Anti M2-3E (BPO)	Negative
Anti Sp100	**3+**
Anti PML	**2+**
Anti gp210	Negative

Living with uncertainty....





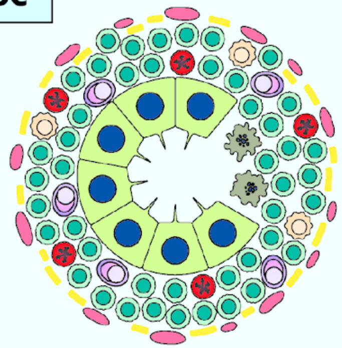
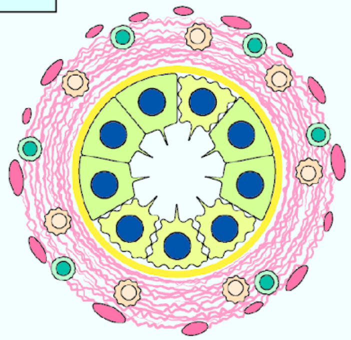
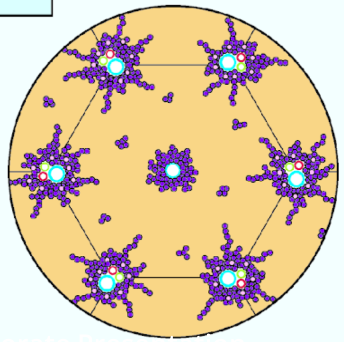
Nature Reviews | Gastroenterology & Hepatology



Family...
Children to adult

Incidence per 100,000:	0.8-2.8	0.4-2.4	0.4-2.3	??	Rare but leading cause of paediatric transplant
Prevalence per 100,000:	1.9-40.2	4.8-42.9	0.8-31.7	??	

Trivedi and Hirschfield Gut 2021

Initiation	Pre-Clinical	Early Clinical	Advanced Clinical
PBC 	**Histopathology:** ▪ Lymphoplasmacytic Portal Infiltrates ▪ Lymphocytic Cholangitis ± Florid Duct Lesions ▪ Displaced BM and PCP ▪ ± Granulomas **Autoantibodies:** ▪ AMA, ANA (gp210, sp100), SMA	**Laboratory Tests:** ▪ Elevated ALP and ggt ▪ Variable elevation ALT, AST ▪ Elevated IgM **Signs or Symptoms:** ▪ Cholestatic pruritus ▪ Fatigue ▪ Hyperpigmentation **SOC Therapy:** ▪ UDCA; OCA; Fibrates	**Intolerance or Inadequate Response to UDCA:** ▪ Progressive Ductopenia ↓ ▪ Progressive Ductular Reaction ↓ ▪ Biliary Fibrosis ↓ ▪ Biliary Cirrhosis
PSC 	**Histopathology:** ▪ Focal Fibrous Obliterative Cholangitis ▪ Peribiliary Fibrosis ▪ Lymphocyte-Macrophage Portal Infiltrates ▪ Displaced PCP **Autoantibodies:** ▪ pANCA (pANNA), ANA	**Laboratory Tests:** ▪ Elevated ALP and ggt ▪ ± Elevation ALT, AST **Signs or Symptoms:** ▪ Asymptomatic ▪ Associated IBD ▪ Cholestatic pruritus ▪ Fatigue **SOC Therapy:** ▪ None	▪ Progressive Biliary Strictures ↓ ▪ Fibro-Obliterative Ductopenia ↓ ▪ Progressive Biliary Obstruction ↓ ▪ Progressive Ductular Reaction ↓ ▪ Biliary Fibrosis ↓ ▪ Biliary Cirrhosis
AIH 	**Histopathology:** ▪ Lymphoplasmacytic Portal Infiltrates ▪ Interface Hepatitis ▪ ± Central Perivenulitis **Autoantibodies:** ▪ ANA, SMA, LKM1, SLA	**Laboratory Tests:** ▪ Elevated ALT, AST ▪ Elevated IgG **Signs or Symptoms:** ▪ Asymptomatic ▪ Fatigue ▪ Other AI Diseases **SOC Therapy:** ▪ 1st Line Steroids ± Thiopurine ▪ 2nd Line CNI or MMF	**Intolerance or Inadequate Response to 1st or 2nd Line Immunosuppression:** ▪ Progressive portal fibrosis ↓ ▪ Bridging fibrosis ↓ ▪ Post-necrotic cirrhosis

Trivedi et al. Gastroenterology 2024

So many questions in PSC and reasons to do better for our shared patients

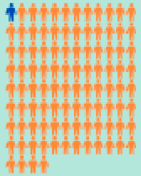
Effective treatments will only come with understanding what causes disease

Epidemiology/Genetics/Exposome	Basic science: cells, animals, human	Clinical science
Why me? Why children? Why men more than women? Why the symptoms?	Why the biliary tree? Why the association with IBD? Why is cancer an issue? Why does PSC recur post-transplant?	Why so difficult to treat? Who to study? How to cross the treatment goalpost? What to treat: liver or bowel?

Primary Sclerosing Cholangitis-Inflammatory Bowel Disease: Epidemiology, Mortality, and Impact of Diagnostic Sequence

Population ~15 million
26% self-identify as a visible minority

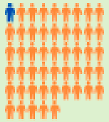
PREVALENCE



1 person living with PSC-IBD per 100 living with IBD

PSC-IBD: 5.5 per 100,000 PY
IBD: 588 per 100,000 PY

INCIDENCE



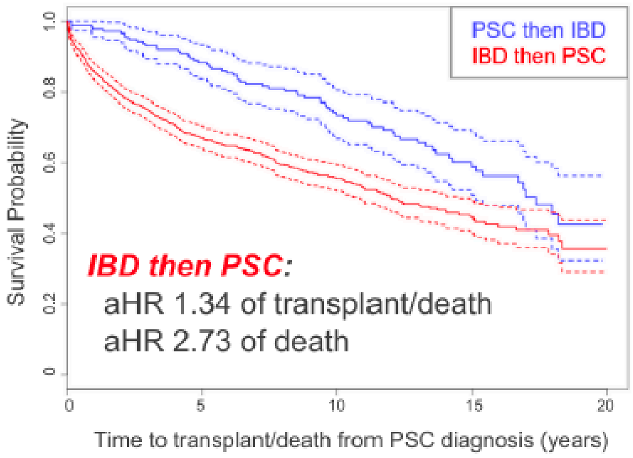
1 PSC-IBD diagnosis per 50 IBD diagnoses

PSC-IBD: 0.47 per 100,000 PY
IBD: 24.6 per 100,000 PY

PSC-IBD COHORT CHARACTERISTICS

$ Higher socioeconomic status

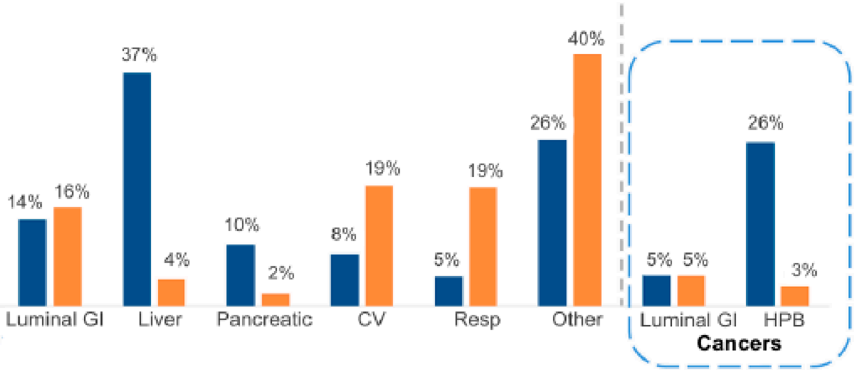
83% IBD then PSC
17% PSC then IBD



INCIDENT COHORT MORTALITY

- PSC-IBD: 30% mortality, median 65 y.o.
- IBD: 8.6% mortality, median 74 y.o.

Predominant Underlying Cause of Death



Leung et al. J Hep Reports 2024

Genetic phenocopies?

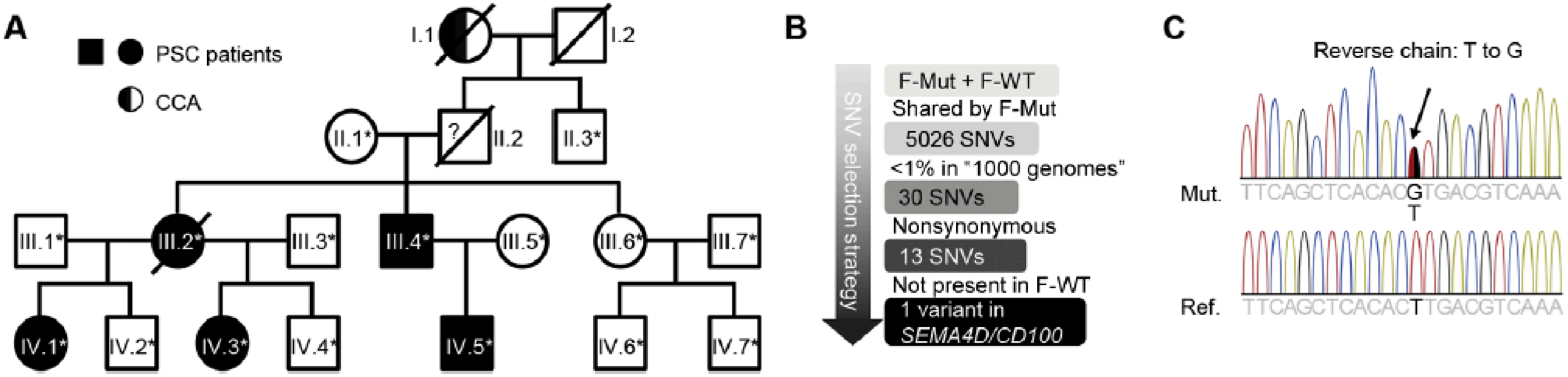
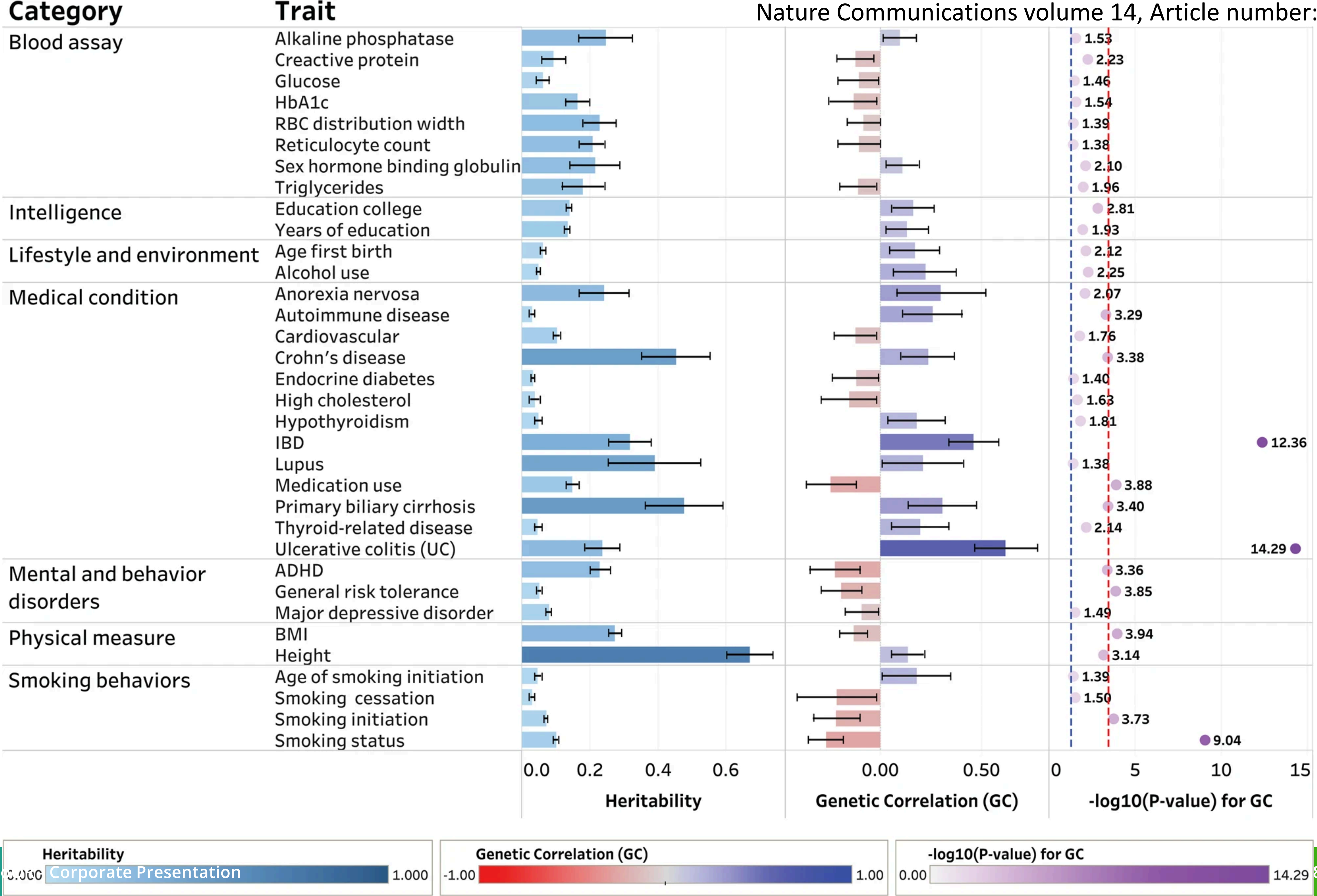


Fig. 1. Identification of a missense mutation in *SEMA4D/CD100* in a family with PSC. (**A**) Pedigree of a family with PSC. Squares, male participants; circles, female participants; black filled symbols, patients with PSC; half-filled symbol, patient with cholangiocarcinoma (CCA) but without a confirmed diagnosis of PSC; crossed-out symbols, deceased participants. Whole-exome sequencing was carried out on participants with an asterisk. (**B**) Single-nucleotide variant (SNV) selection strategy. (**C**) Confirmation of the CD100^{K849T} mutation by Sanger sequencing. F-Mut, family members with PSC; F-WT, healthy family members.

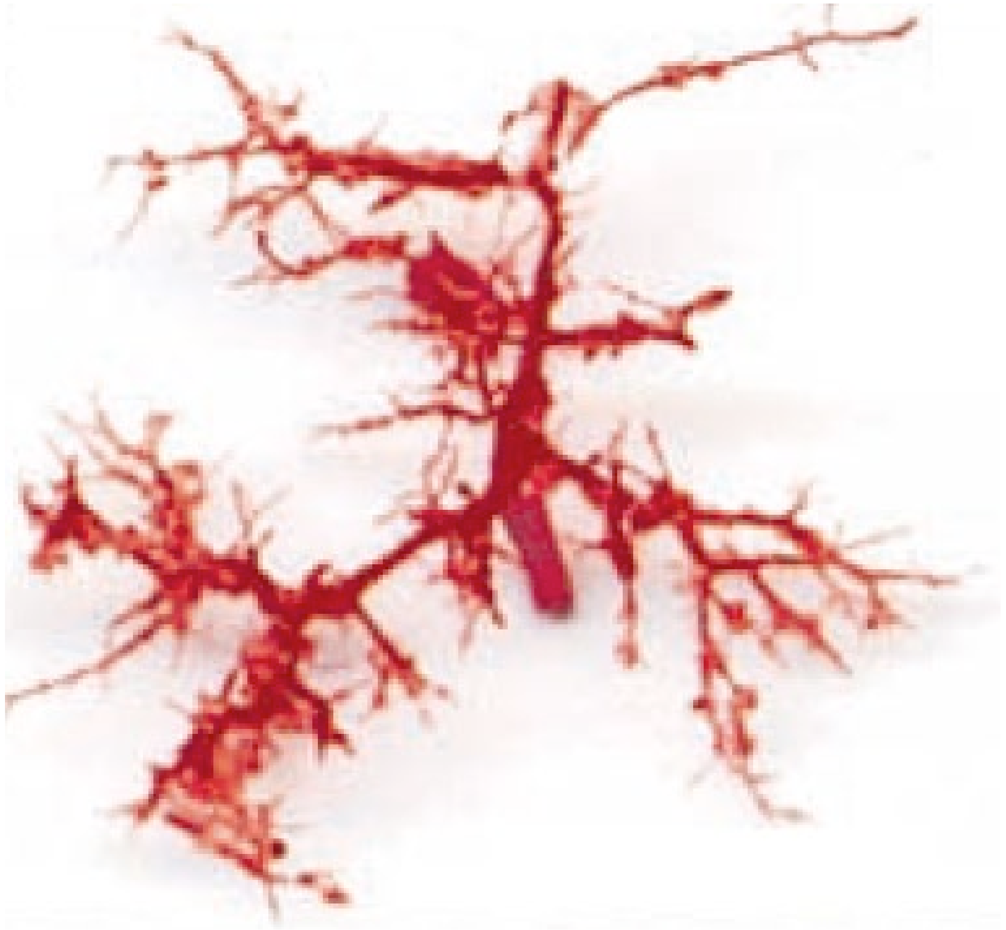
"However, this mutation is not a common risk factor for PSC in general because our examination of 3178 patients did not identify any other carriers, and, to the best of our knowledge, it has not been reported in PSC elsewhere."

Jiang et al., Sci. Transl. Med.13, eabb0036 (2021)

Nature Communications volume 14, Article number: 1069 (2023)



Mdr2 deficient *mice develop* cholangiopathy



Gastroenterology 2002;123:1238–1251

Article

Bile acid metabolites control T_H17 and T_{reg} cell differentiation

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Saiyu Hang[1,12], Donggi Paik[1,12], Lina Yao[2], Eunha Kim[1], Trinath Jamma[3], Jingping Lu[4], Soyoung Ha[1], Brandon N. Nelson[5], Samantha P. Kelly[5], Lin Wu[6], Ye Zheng[7], Randy S. Longman[8], Fraydoon Rastinejad[4], A. Sloan Devlin[2], Michael R. Krout[5], Michael A. Fischbach[9]*, Dan R. Littman[6,10]* & Jun R. Huh[1,11]*

Bile acids are abundant in the mammalian gut, where they undergo bacteria-mediated transformation to generate a large pool of bioactive molecules. Although bile acids are known to affect host metabolism, cancer progression and innate immunity, it is unknown whether they affect adaptive immune cells such as T helper cells that express IL-17a (T_H17 cells) or regulatory T cells (T_{reg} cells). Here we screen a library of bile acid metabolites and identify two distinct derivatives of lithocholic acid (LCA), 3-oxoLCA and isoalloLCA, as T cell regulators in mice. 3-OxoLCA inhibited the differentiation of T_H17 cells by directly binding to the key transcription factor retinoid-related orphan receptor-γt (RORγt) and isoalloLCA increased the differentiation of T_{reg} cells through the production of mitochondrial reactive oxygen species (mitoROS), which led to increased expression of FOXP3. The isoalloLCA-mediated enhancement of T_{reg} cell differentiation required an intronic *Foxp3* enhancer, the conserved noncoding sequence (CNS) 3; this represents a mode of action distinct from that of previously identified metabolites that increase T_{reg} cell differentiation, which require CNS1. The administration of 3-oxoLCA and isoalloLCA to mice reduced T_H17 cell differentiation and increased T_{reg} cell differentiation, respectively, in the intestinal lamina propria. Our data suggest mechanisms through which bile acid metabolites control host immune responses, by directly modulating the balance of T_H17 and T_{reg} cells.

Article

Human gut bacteria produce T_H17-modulating bile acid metabolites

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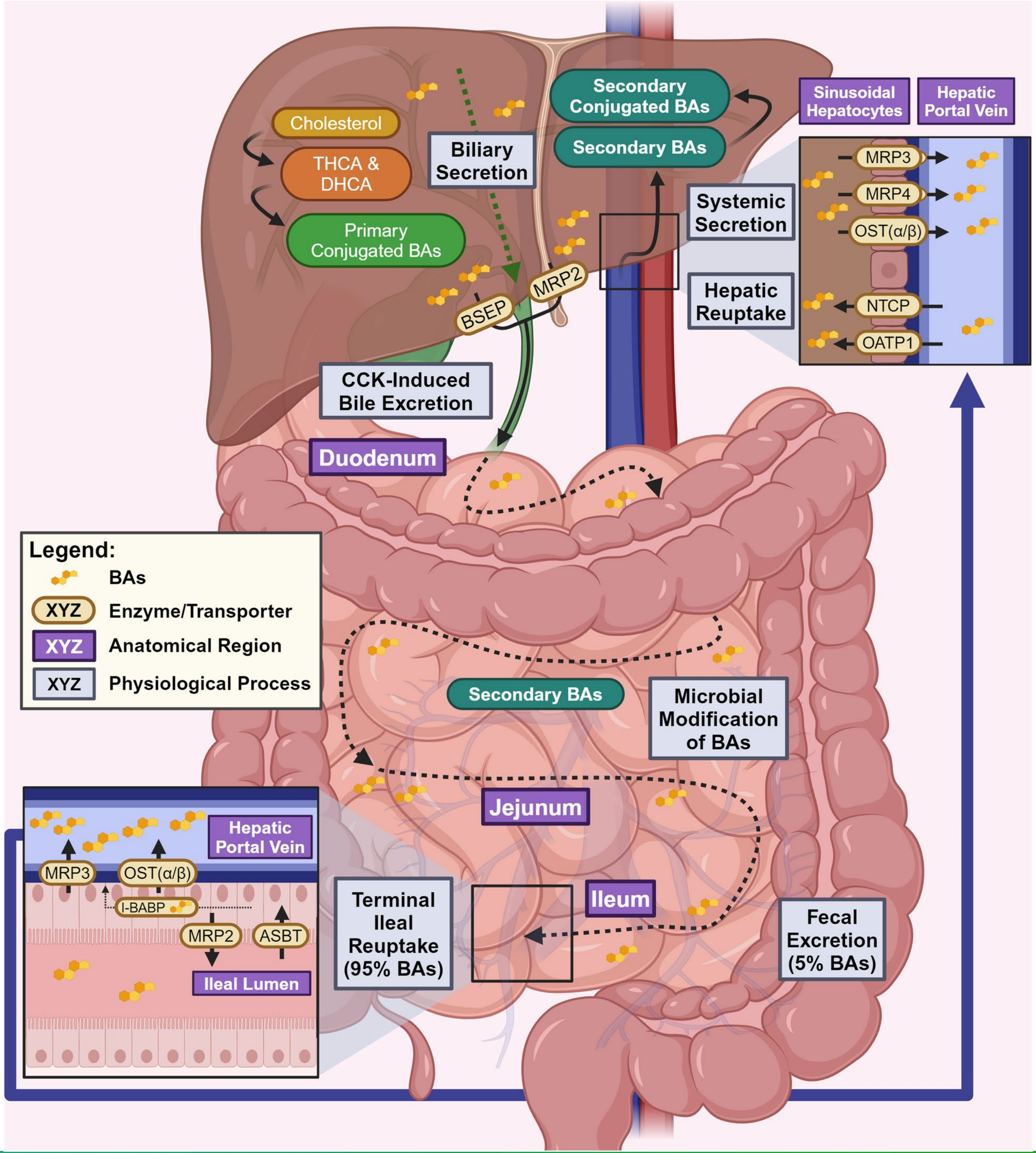
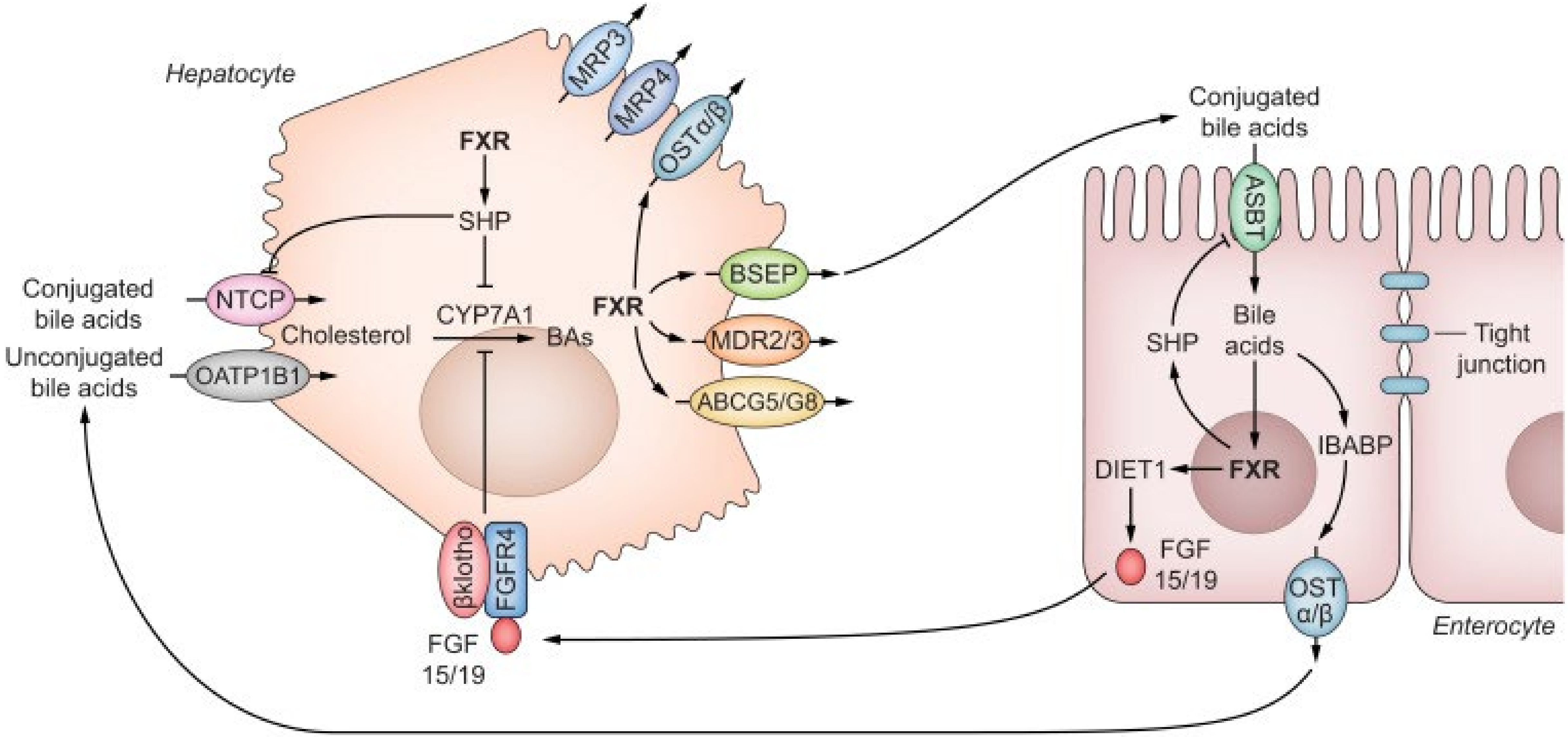
Donggi Paik[1,15], Lina Yao[2,15], Yancong Zhang[3,4], Sena Bae[4,5], Gabriel D. D'Agostino[2], Minghao Zhang[6], Eunha Kim[1], Eric A. Franzosa[4,5], Julian Avila-Pacheco[3], Jordan E. Bisanz[7], Christopher K. Rakowski[8], Hera Vlamakis[3,9], Ramnik J. Xavier[3,9,10,11], Peter J. Turnbaugh[7,12], Randy S. Longman[13], Michael R. Krout[8], Clary B. Clish[3], Fraydoon Rastinejad[6], Curtis Huttenhower[3,4,5], Jun R. Huh[1,14,15] & A. Sloan Devlin[2,15]

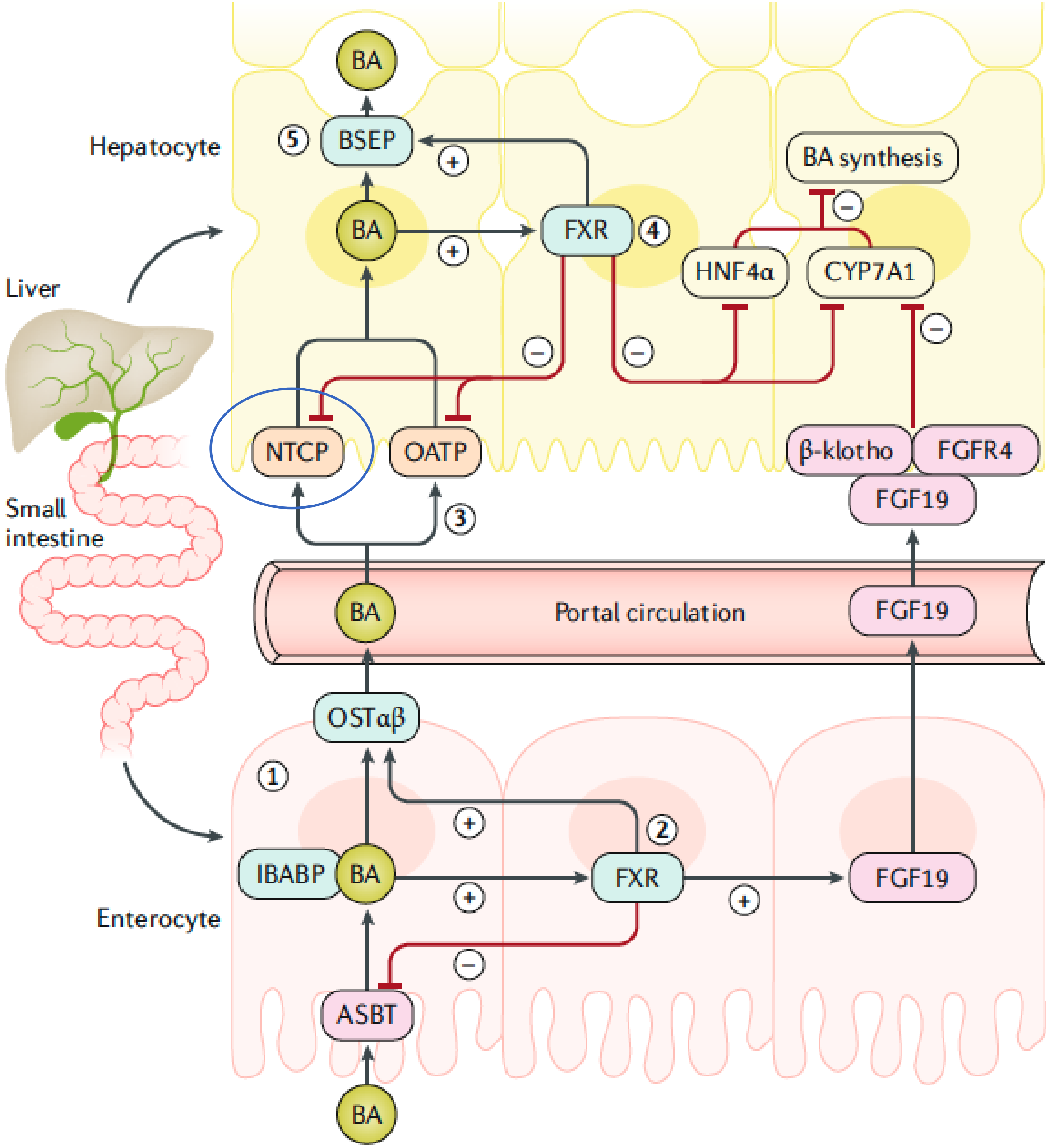
The microbiota modulates gut immune homeostasis. Bacteria influence the development and function of host immune cells, including T helper cells expressing interleukin-17A (T_H17 cells). We previously reported that the bile acid metabolite 3-oxolithocholic acid (3-oxoLCA) inhibits T_H17 cell differentiation[1]. Although it was suggested that gut-residing bacteria produce 3-oxoLCA, the identity of such bacteria was unknown, and it was unclear whether 3-oxoLCA and other immunomodulatory bile acids are associated with inflammatory pathologies in humans. Here we identify human gut bacteria and corresponding enzymes that convert the secondary bile acid lithocholic acid into 3-oxoLCA as well as the abundant gut metabolite isolithocholic acid (isoLCA). Similar to 3-oxoLCA, isoLCA suppressed T_H17 cell differentiation by inhibiting retinoic acid receptor-related orphan nuclear receptor-γt, a key T_H17-cell-promoting transcription factor. The levels of both 3-oxoLCA and isoLCA and the 3α-hydroxysteroid dehydrogenase genes that are required for their biosynthesis were significantly reduced in patients with inflammatory bowel disease. Moreover, the levels of these bile acids were inversely correlated with the expression of T_H17-cell-associated genes. Overall, our data suggest that bacterially produced bile acids inhibit T_H17 cell function, an activity that may be relevant to the pathophysiology of inflammatory disorders such as inflammatory bowel disease.

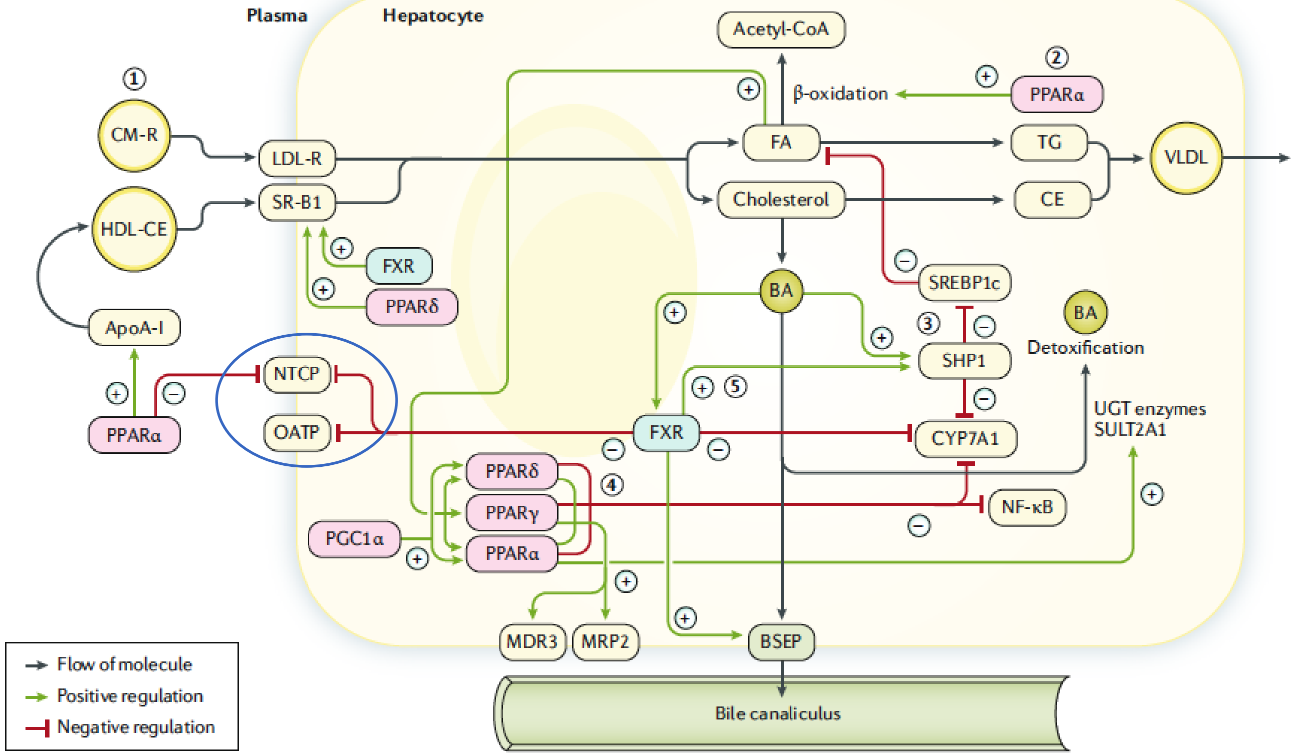


Signal Transduction and Targeted Therapy (*Sig Transduct Target Ther*) ISSN 2059-3635 (online)

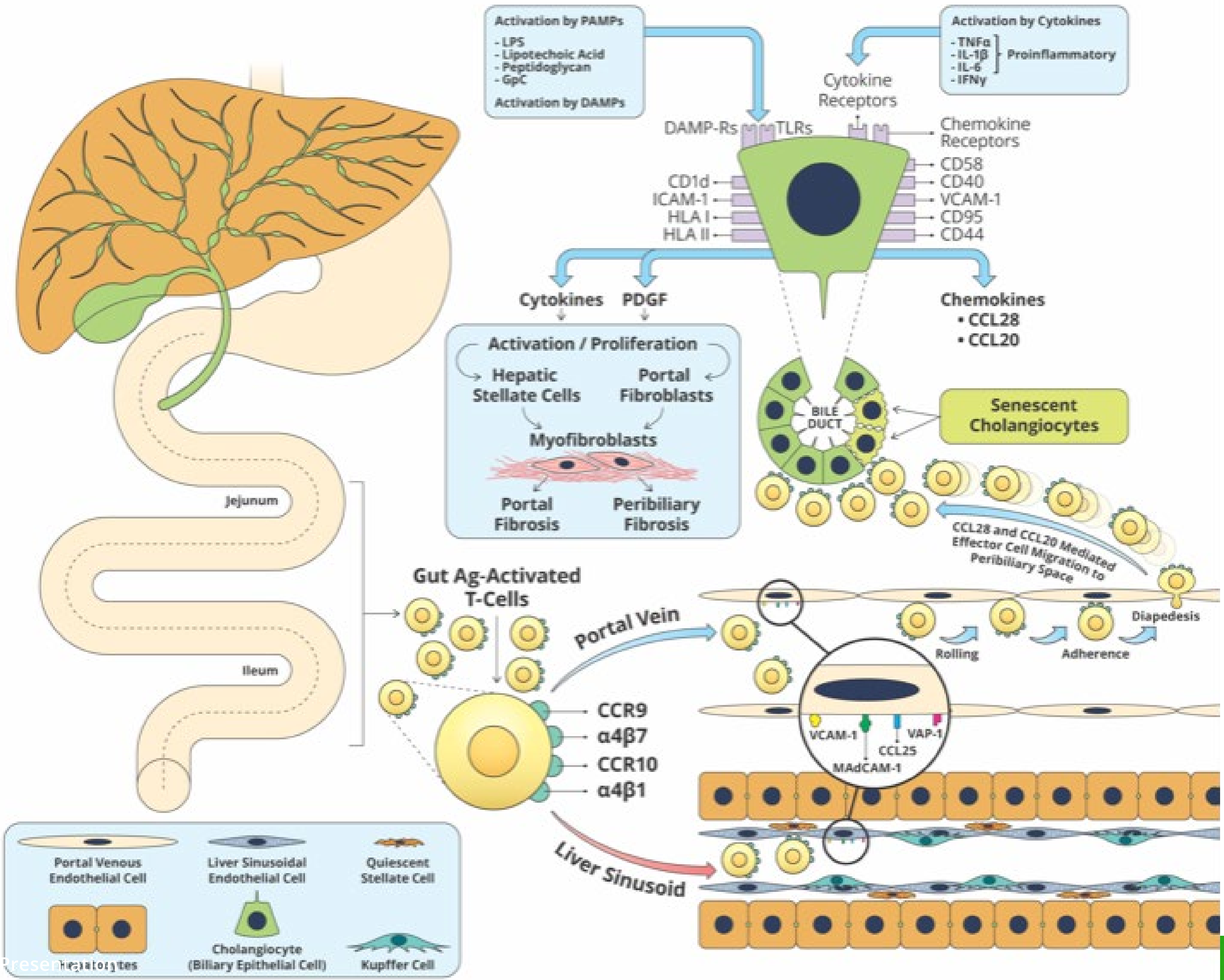


Fuchs et al. Hep 2024





Gulamhusein and Hirschfield Nat Rev Gastro Hep 2020



Trivedi, Hirschfield, Adams, Vierling Gastroenterology 2024

C COVERT

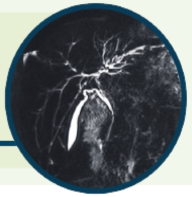
PSC affects both sexes and occurs at all ages; however, the majority of patients are male and the median age at onset is 30–40 years. Up to 80% of cases are associated with IBD. Approximately 50% of patients with PSC are asymptomatic at diagnosis.

PSC SYMPTOMS

When symptomatic, PSC is insidious. Patients most often complain of abdominal pain, pruritus, and fatigue.

PSC DIAGNOSIS

Diagnosis is usually based on: 1 Serum ALP elevation, 2 multi-focal biliary strictures with intervening dilatations on cholangiography (usually MRCP), 3 exclusion of secondary sclerosing cholangitis, and 4 liver biopsy when small-duct PSC or PSC-AIH is suspected.



Typical MRCP in PSC

HOLISTIC APPROACH

PSC care must integrate disease monitoring, treatment and research with psychosocial support that addresses the fear, uncertainty, and social isolation many patients experience. PSC support societies are an excellent resource.

PSC Care: Monitoring & Treatment · Research · Psychosocial Support

C CHOLANGITIS

Genetic and environmental factors interact to establish the pathogenesis of PSC, which involves the gut microbiota, impaired bile acid composition and cholestatis, and autoimmunity.

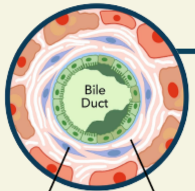
GENETICS — > 20 HLA and non-HLA loci have been linked to PSC, establishing it as an autoimmune disease.

MICROBIOTA — Altered gut and biliary microbiota may drive the immune response in PSC.

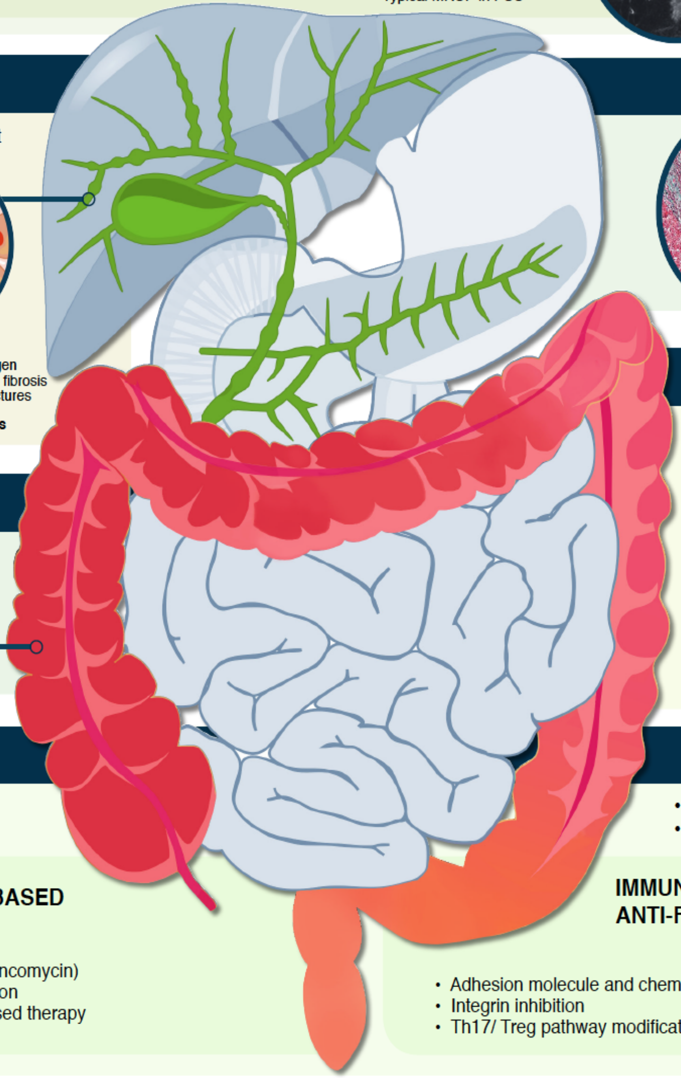
IMMUNE RESPONSE — The predominant cells identified in the vicinity of bile ducts are T cells, macrophages and neutrophils.

ENVIRONMENT — Multiple environmental exposures have been associated with PSC.

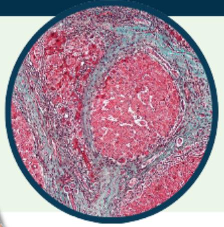
BILE ACIDS — Bile acid homeostasis is impaired and biliary epithelium is activated.



"Onion-skinning" fibrosis



CIRRHOSIS C



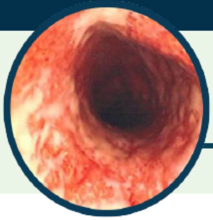
The end-point of PSC is cirrhosis.

The extent of inflammation and fibrosis observed does not necessarily correlate with the risk of biliary dysplasia or malignancy.

C COLITIS

PSC-IBD is phenotypically distinct from IBD without PSC.

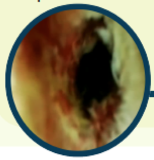
- Majority of IBD in PSC patients is UC and presents earlier than in those without PSC
- Frequently presents with pancolitis, predominantly right-sided, with "back-wash ileitis" and rectal sparing



CANCER C

CANCER RISK

PSC patients are at increased risk of **colorectal** and **hepatopancreatobiliary cancers**, including cholangiocarcinoma, hepatocellular carcinoma, pancreatic cancer, and gallbladder cancer.



Cholangiocarcinoma

PSC SURVEILLANCE

- Colonoscopy with screening biopsies at diagnosis and every 1-2 years
- Annual US
- Annual MRI/MRCP

Non-cancer screening:

- If cirrhosis: US and AFP every 6 months, screening for complications per guidelines
- Screening for osteoporosis and malnutrition

C CURE

Liver transplantation is indicated per regional guidelines, including for decompensated cirrhosis, intractable pruritus, recurrent bacterial cholangitis, and HCC.

- 5-year survival post-transplantation exceeds 80%
- PSC recurrs at a rate of approximately 20% post-transplantion

BILE ACID-BASED THERAPY

- UDCA and experimental analogues
- *Nor*UDCA
- FXR and FGF19 analogues
- PPAR agonists
- ASBT inhibitors

MICROBIOTA-BASED THERAPY

- Antibiotics (e.g. vancomycin)
- Fecal transplantation
- Bacteriophage-based therapy

IMMUNE-MODULATING & ANTI-FIBROTIC THERAPY

- Adhesion molecule and chemokine inhibition
- Integrin inhibition
- Th17/ Treg pathway modification

BIOMARKERS

Biomarkers are important for prognostication & evaluating treatment effect.

- ?ALP
- ?ELF
- ?PRO-C3

- Liver biopsy

- Quantitative MRI
- Elastography

Deeb et al. J Hep 2020

THE AUTOIMMUNE & RARE LIVER DISEASE PROGRAMME

PBC, PSC, AIH, Hepato-biliary IgG4-RD & Genetic Cholestasis

Care Teaching Research

THANK YOU

Gideon Hirschfield

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.

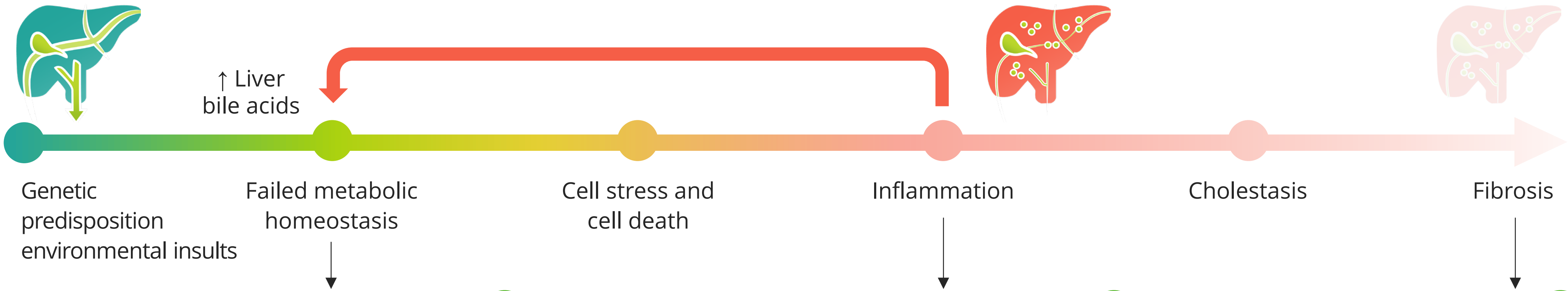
Initial indications are **Primary Sclerosing Cholangitis** affecting adults and Congenital **Biliary Atresia** affecting pediatrics early in life. Both conditions have no approved therapies and may require liver transplantation.[1,2]

- **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



NTCP MODULATION

Restores Bile Acids Balance

Protect hepatocytes by lowering bile acid levels and decrease hepatic bile production through downregulating effects

Reduces Inflammation

Prevents bile leakage and infiltration, limiting liver cell damage and release of proinflammatory mediators

Slows Fibrosis Progression

Stops cholangiocyte senescence and proliferation, reducing fibro-inflammatory responses

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 variants reduced bile acids uptake into liver in health population research

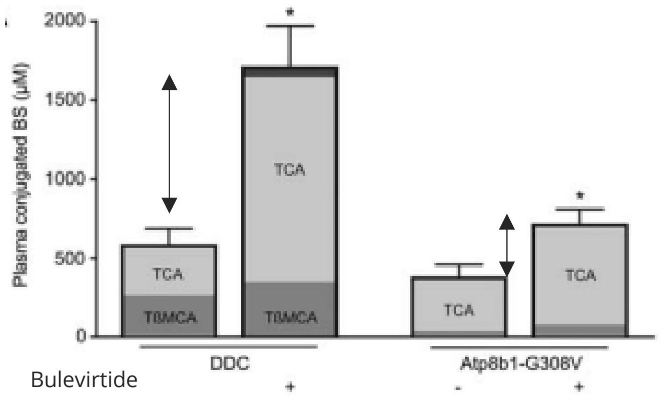
Healthy population discovered with NTCP variants that reduces bile acids uptake into liver[1-4]

[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.

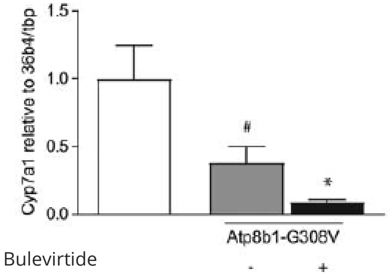
NTCP modulation has hepato-protective effects *in vivo* in disease models

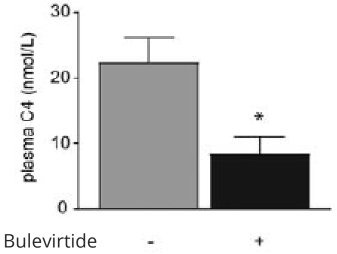
3-fold increase in conjugated BA

2-fold increase in conjugated BA

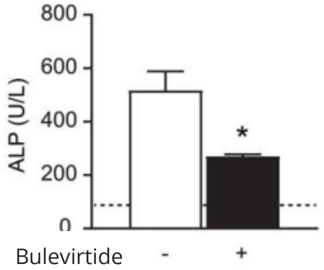


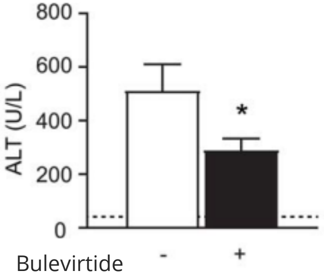
NTCP inhibition **increases plasma bile acids concentrations** (2- to 3-fold in mouse models)

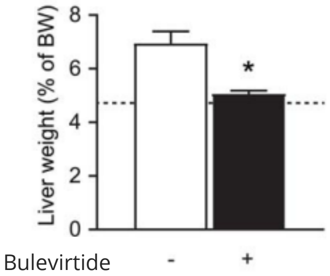




Reduced bile acid production during cholestasis (expected to decrease intrahepatic bile acids load)





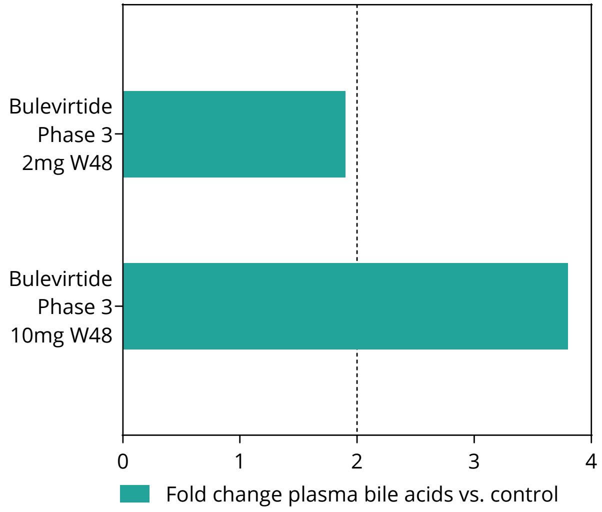


Reduced cholestatic liver injury via **improvement in liver enzymes**

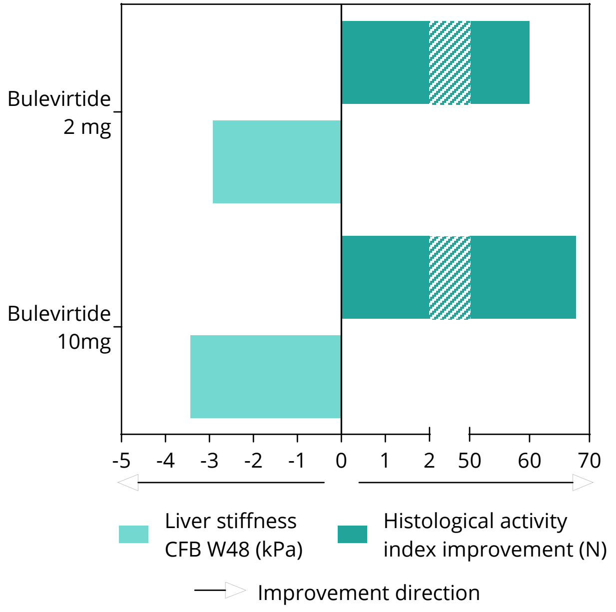
Bulevirtide (Hepcludex) is a daily SC injected NTCP inhibitor approved for Hepatitis D. Slijepcevic D, et al. Hepatology. 2018 Sep;68(3):1057-1069.

NTCP modulation leads to clinically meaningful impact in patients

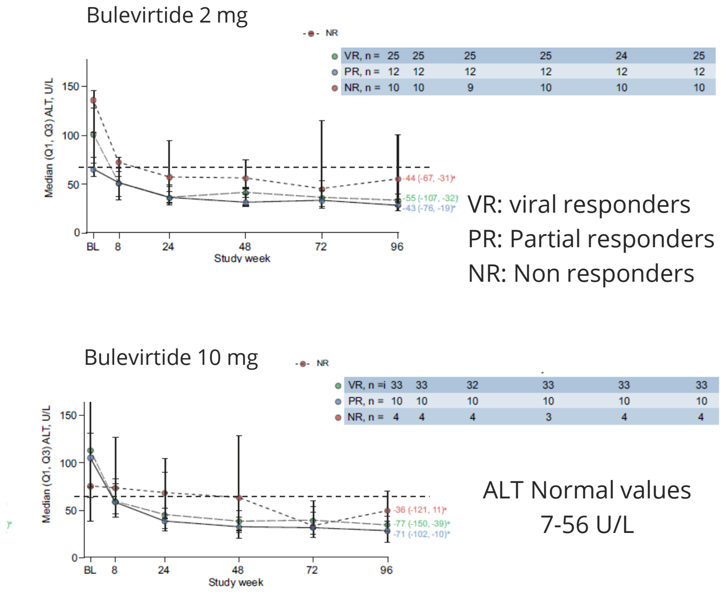
Reducing liver bile acids toxic overload via NTCP modulation is a key driver for hepatoprotective effects



NTCP inhibition **increases plasma bile acids concentrations in humans (2- to 4-fold)**



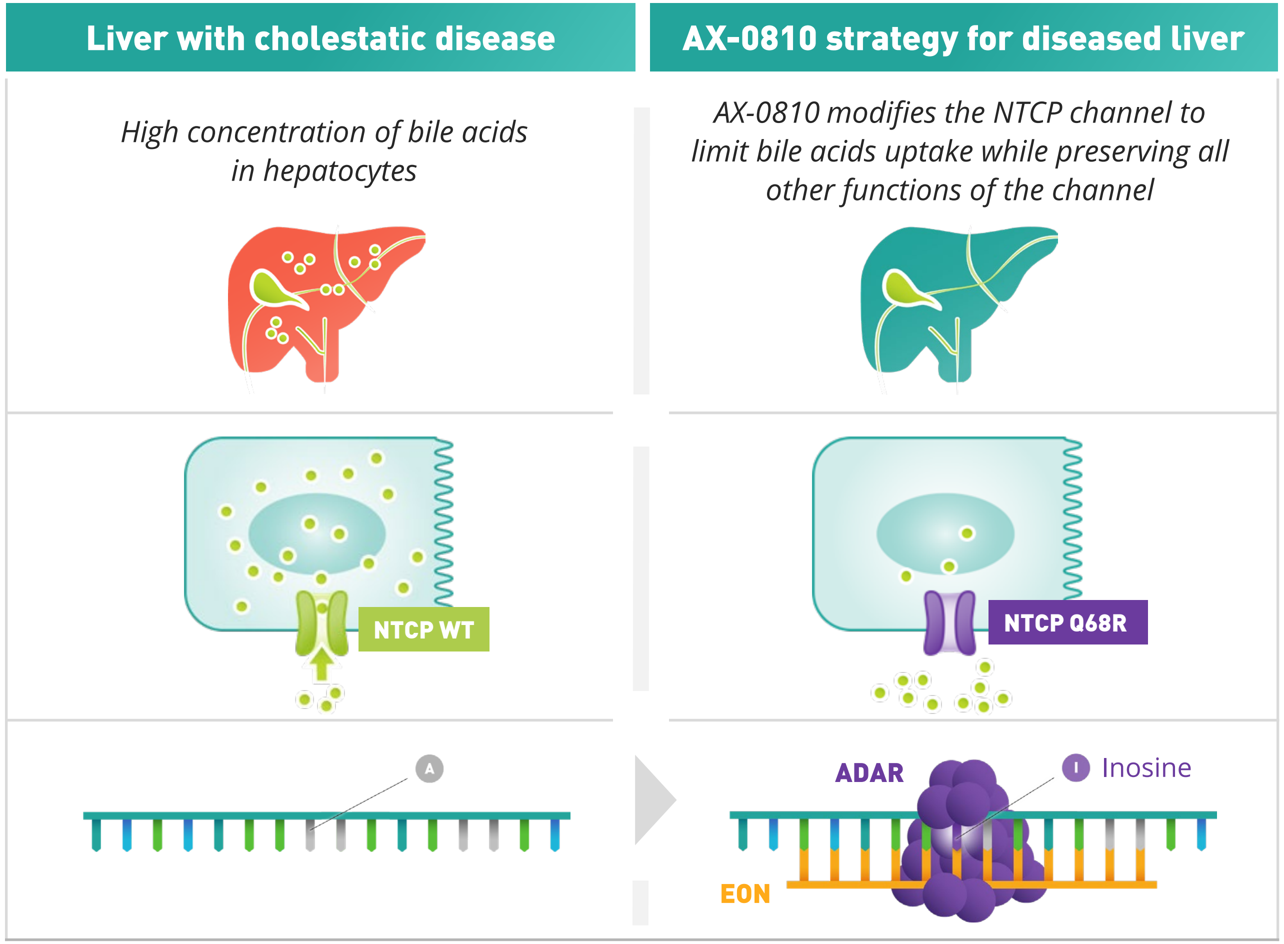
Treatment with NTCP inhibitor led to **improvement in liver fibrosis (stiffness and histology)**



Liver enzyme improvement occur in patients, **even without virologic response***

*NTCP channel is a known transporter for bile acids and hepatitis virus from bloodstream to the liver. Bulevirtide (Hepcludex) is a daily SC injected NTCP inhibitor approved for Hepatitis D. Wedemeyer H, et al. N Engl J Med. 2023 Jul 6;389(1):22-32; Wedemeyer H, J Hepatol. 2024 Oct;81(4):621-629.; Dietz-Fricke C, JHEP Rep. 2023 Mar 15;5(4):100686.

Human genetics validates NTCP modulation as strategy for cholestatic disease

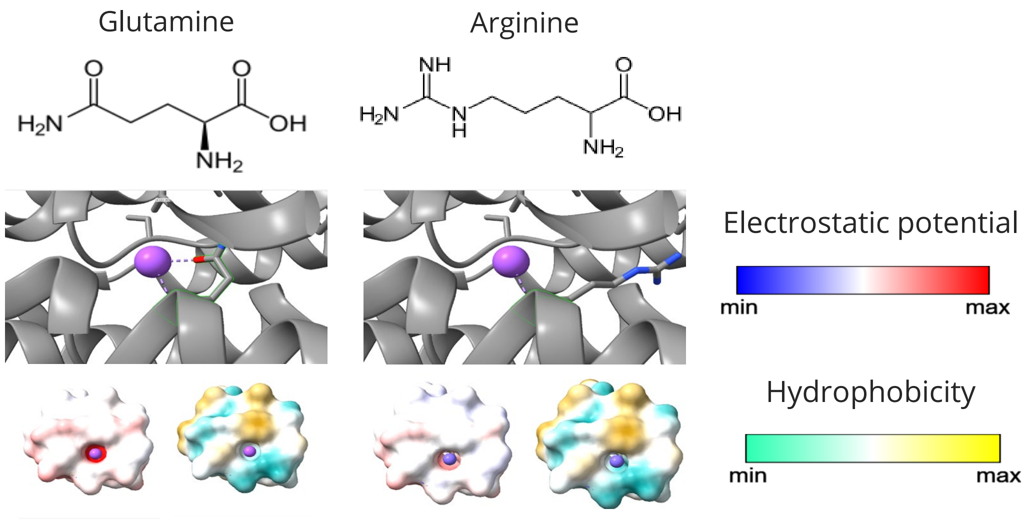


- The AX-0810 program introduces a variant in individuals with cholestatic disease to lower bile acids concentration in hepatocytes by a single A-to-I change
- The AX-0810 program is designed to be a disease modifying treatment
  - To alleviate symptoms in PSC and BA
  - To limit inflammation and fibrosis 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

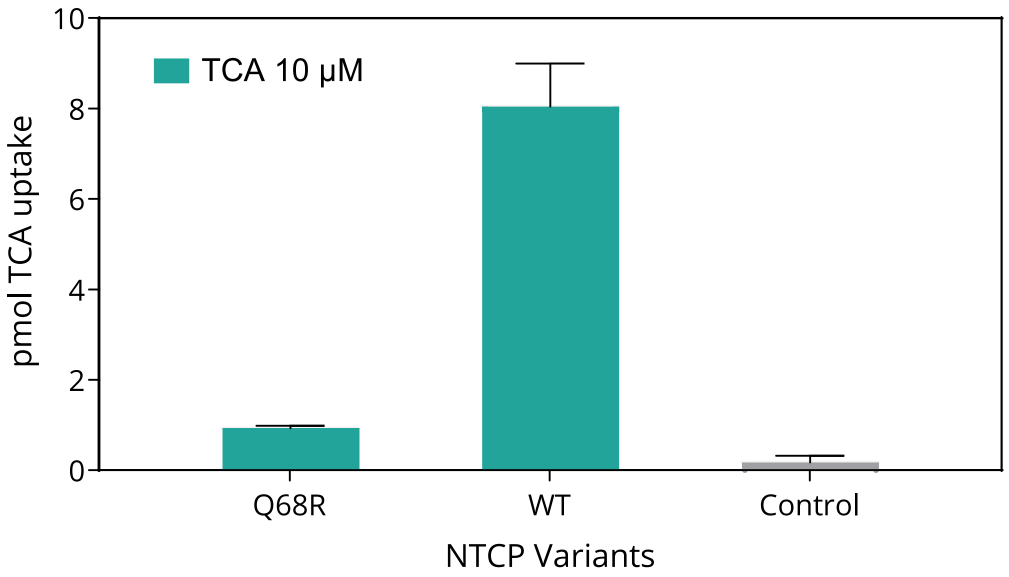
Q68R NTCP variant leads to modulation of bile acids re-uptake

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



BAs uptake (TCA) *in vitro**

n=3, mean±SEM



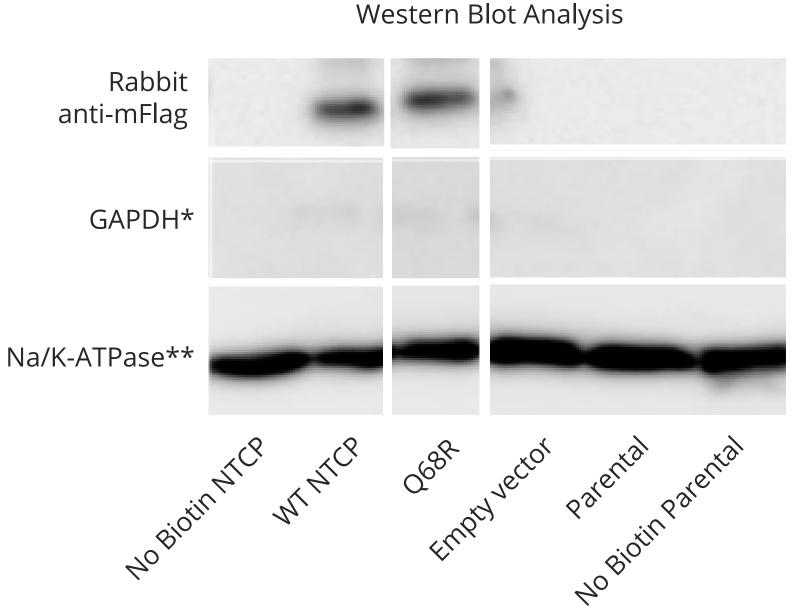
- The Q68R variant disrupts some hydrogen bonds and contacts in the Na^+ binding pocket.
- Clashes are inevitable since the Arg side chain is buried and likely to be found in one or another unfavorable rotamer state.

- 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.

Q68R NTCP variant solely affects bile acids re-uptake function

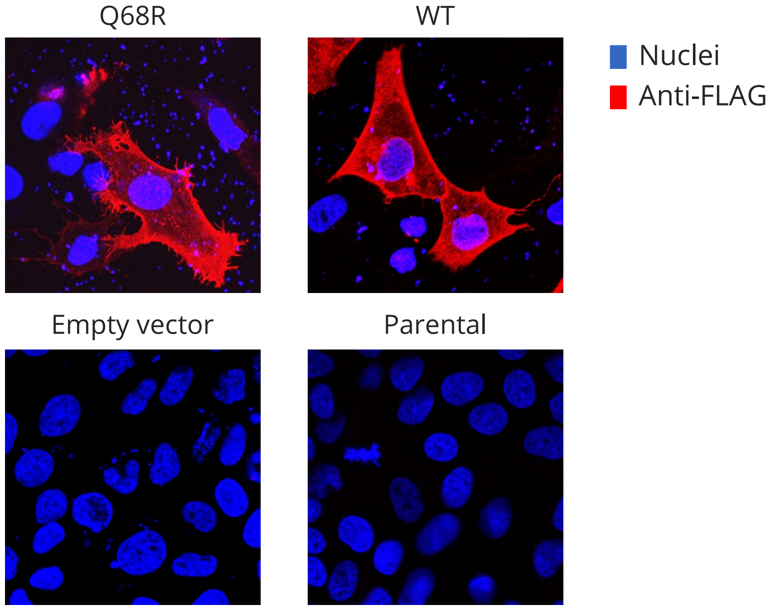
NTCP protein expression was detected on western blot using the anti-FLAG antibody for all constructs



*Loading control for intracellular proteins

**Loading control for surface proteins

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.

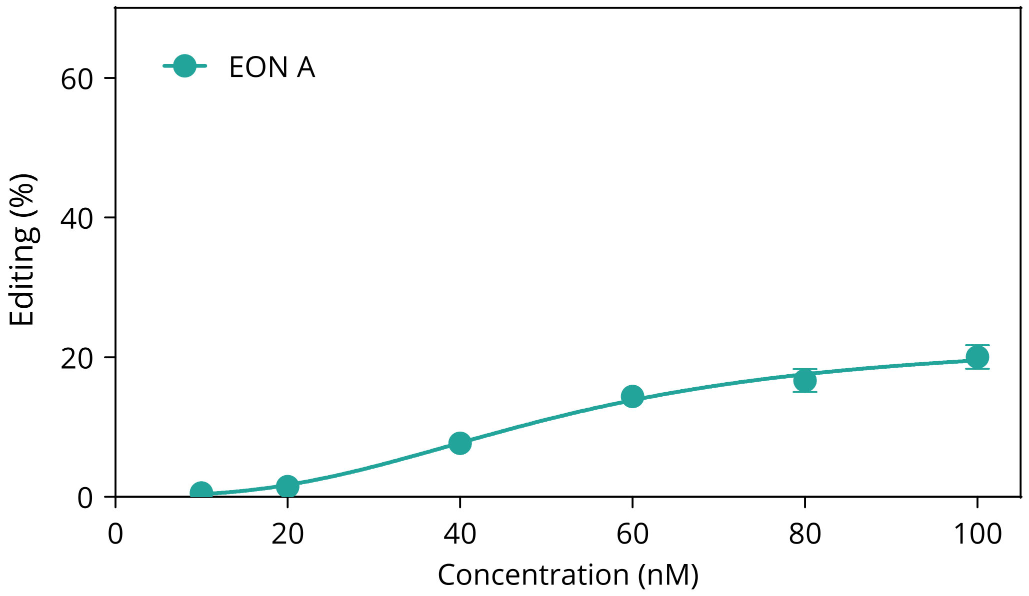
EON: editing oligonucleotide, NTCP: Na-taurocholate cotransporting polypeptide, *transiently transfected U2OS cells. *SLC10A1* is the gene that encodes for NTCP protein

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

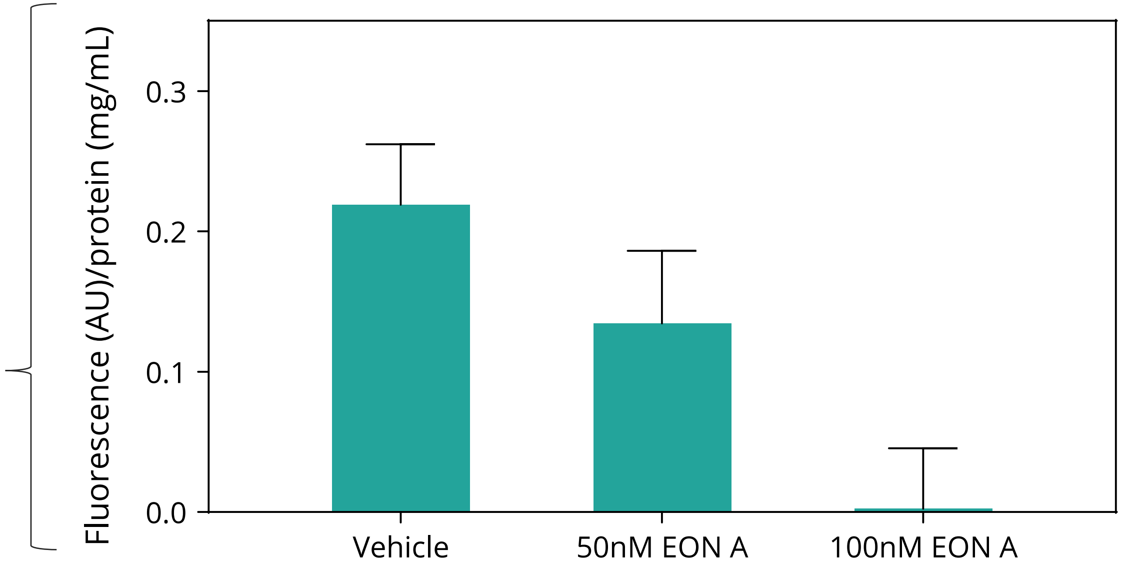
Early generation of EONs targeting NTCP RNA in PHH

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



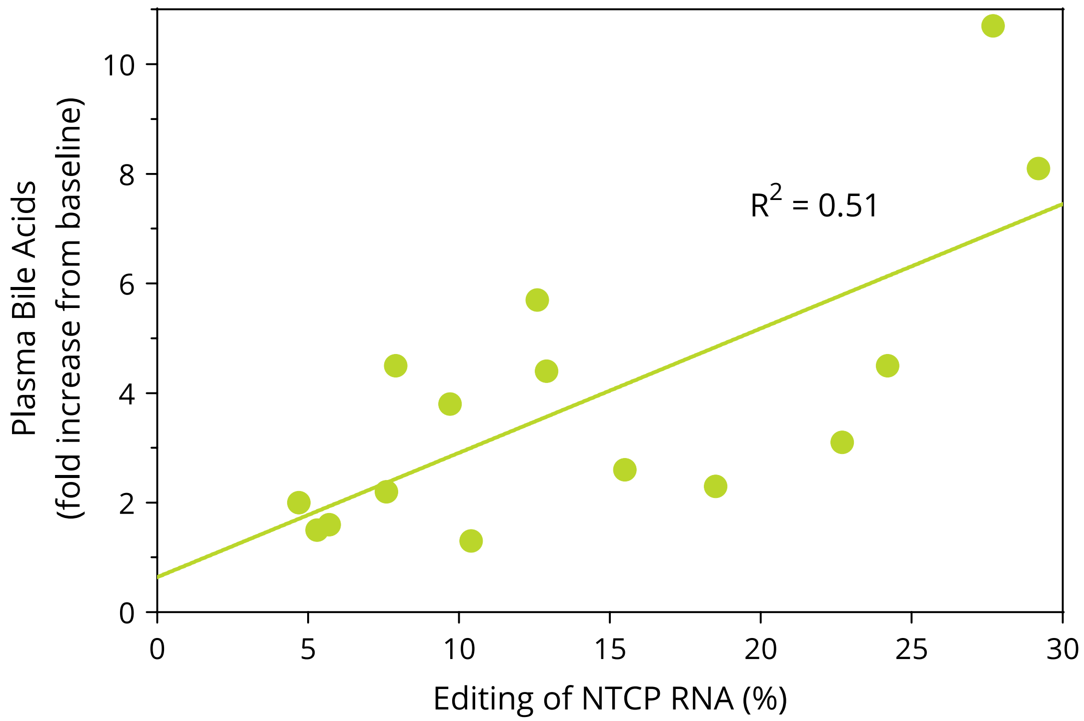
Early generation of EONs induces a dose-response inhibition of bile acids *in vitro* confirming its modulation by NTCP

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

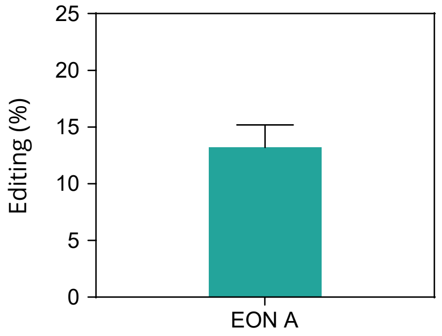
EON mediated editing demonstrates consistent editing of NTCP and impact on biomarker *in vivo*

EDITING EFFICIENCY

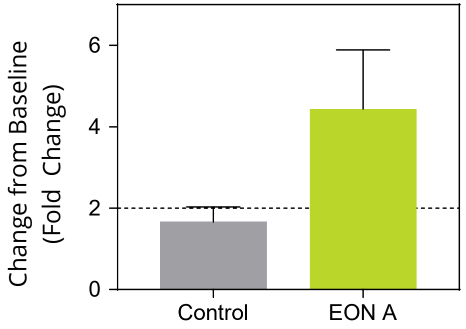
PLASMA TOTAL BILE ACIDS

MICE *in vivo*

NTCP RNA Editing in Humanized Mice
(N=4, 20mg/kg, 6 doses, GalNAc conjugation, SC, D25, ddPCR)

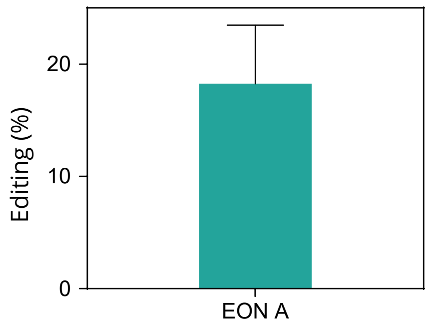


Plasma TBA in Humanized Mice
(N=4, 20mg/kg, 6 doses, GalNac conjugation, SC, D25)

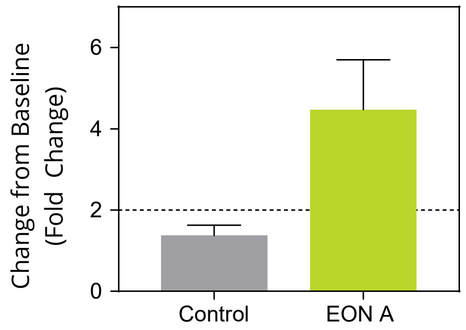


NHP *in vivo*

NTCP RNA Editing in NHP
(N=1, 1-4mg/kg, 4 doses, LNP formulation, IV, up to D46, ddPCR)



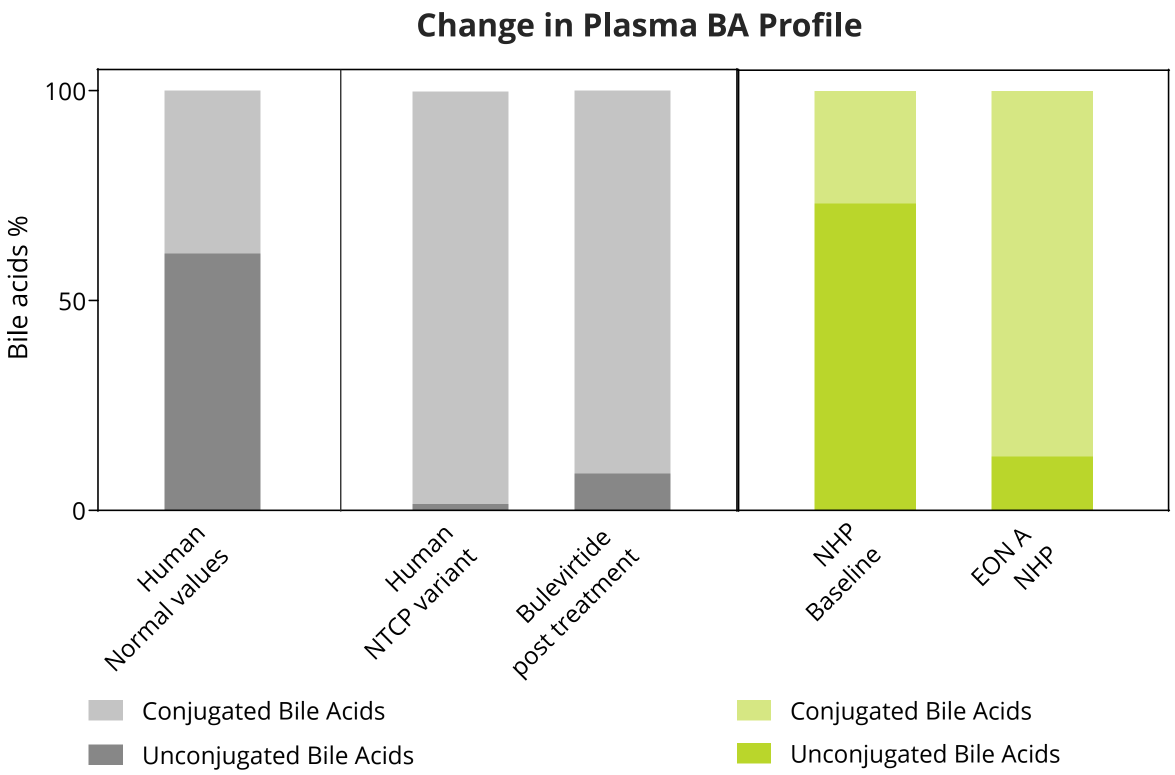
Plasma TBA in NHP
(N=1, 1-4mg/kg, 4 doses, LNP formulation IV, up to D39)



- 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

NTCP editing demonstrates favorable composition of bile acids profile in NHP

Increase in conjugated bile acids confirms NTCP engagement in vivo

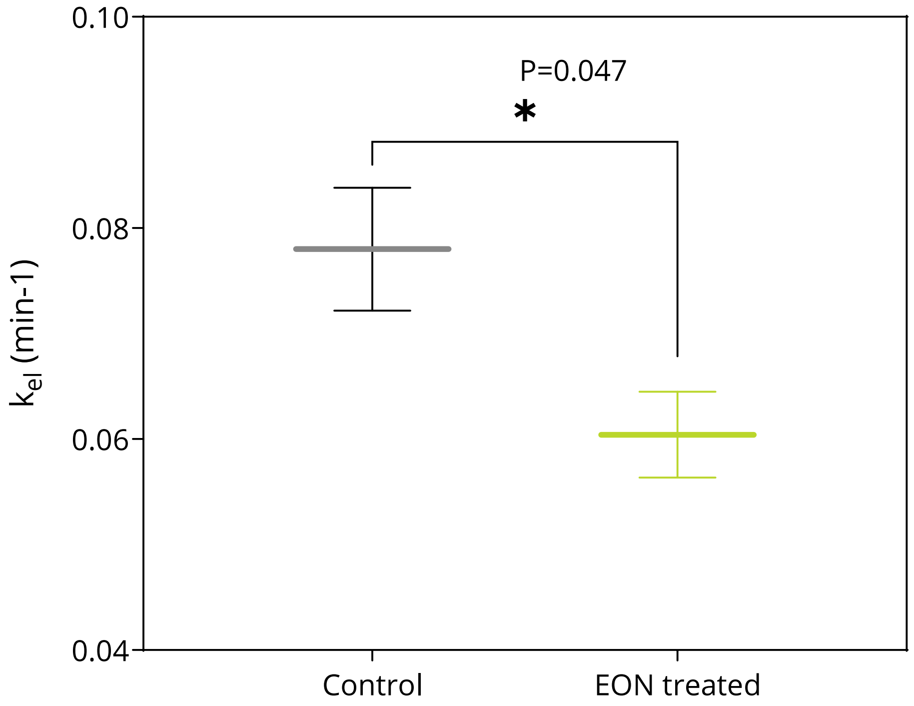


- Conjugated bile acids are transported by NTCP back to the liver
- The observed change in plasma BA profile confirms NTCP specific modulation
- In view of the preclinical data, high confidence on NTCP EON treatment to positively impact BA toxic load in the liver

Conditions in humanized mice: N=4, 20mg/kg, 6 doses, GalNAc conjugation, SC, D25, ddPCR; Conditions in the NHP experiment N=1, 1-4mg/kg, 4 doses, LNP formulation, IV, up to D42, ddPCR. 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.

EON mediated NTCP editing demonstrates reduced clearance in bile acids challenge assay in NHP

TUDCA elimination rate from plasma in NHP
(Exploratory study, early generation EON, n=5-7, 10mg/kg, 4 doses, SC, D51)

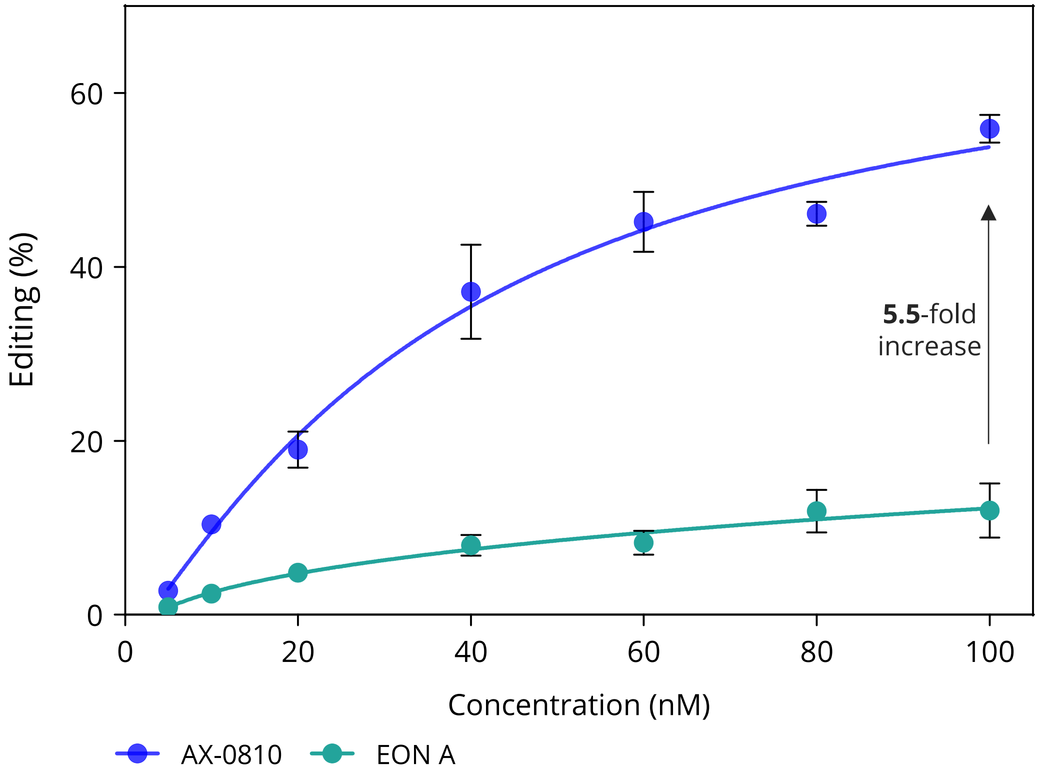


- TUDCA is a Tauro-conjugated bile acids specifically transported by NTCP from the plasma to the liver
- In an NHP experiment using administration of TUDCA following NTCP EON treatment, TUDCA plasma clearance into the liver was assessed
- Decrease in plasma clearance kinetics further confirm NTCP target engagement for EON treated NHP

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



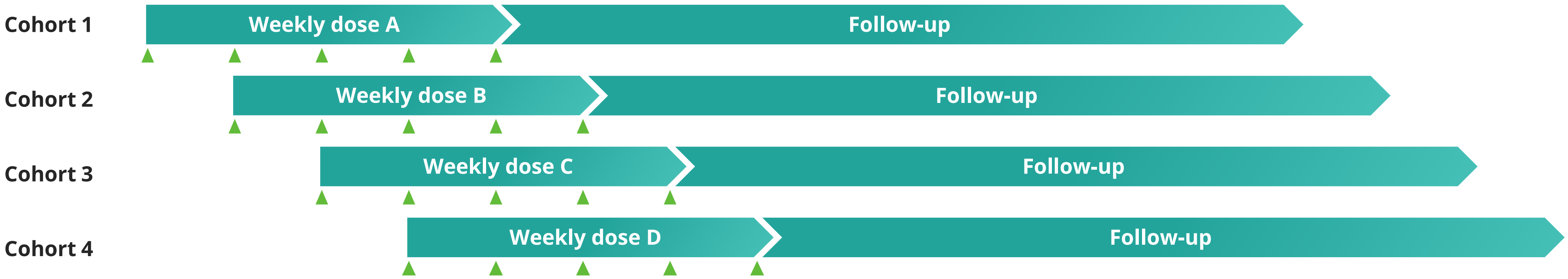
- AX-0810 clinical candidate is a GalNAc conjugated EON
- 5.5-fold increase in potency over early generation NTCP editing oligonucleotide
- Improved stability profile *in vitro*
- Confirmed class safety, with no hepatotoxicity or immunostimulatory score

CTA enabling activities ongoing for AX-0810

***In vitro* safety screening**	**Delivery method**	**GLP tox studies**	**Manufacturing**	**Regulatory**
• AX-0810 clinical candidate passed in vitro screening for class toxicities • Chemical modifications and Z-base derisked in genotoxicity tests	Preferential distribution of GalNAc conjugated EONs confirmed	• Dose ranges and margins established for GLP toxicity studies, ongoing studies in two species • Bioanalytical methods to measure clinical candidates in plasma and tissue established	Scale-up of EON manufacturing process successfully completed, stability of formulated EON confirmed, and favorable shelf life achieved	Interactions with regulatory authorities ongoing

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

Integrated single/multiple ascending dose study design



Treatment

AX-0810 GalNAc conjugated editing oligo-nucleotide

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

- ✓ Favorable safety profile observed
- ✓ AX-0810 GalNAc candidate with optimized potency and stability to enter clinic

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

CTA submission in Q2 2025

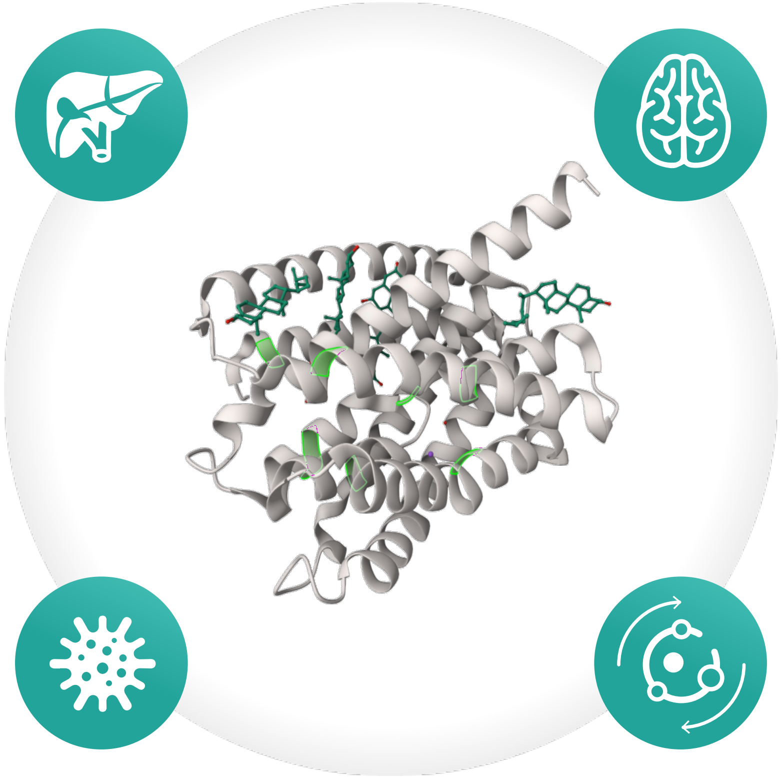
Top-line data from FIH expected in Q4 2025

NTCP and bile acids are involved in a variety of therapeutic areas

Providing opportunity across multiple indications

Cholestatic diseases

- Primary Sclerosing Cholangitis (PSC)
- Biliary Atresia
- Primary Biliary Cholangitis (PBC)
- Alagille syndrome
- Dubin-Johnson Syndrome
- Progressive Familial Intrahepatic Cholestasis (PFIC)
- Drug-Induced Cholestasis
- Alcoholic Liver Disease
- Secondary Biliary Cirrhosis
- Rotor syndrome
- Neonatal cholestasis



Neurological diseases

- Multiple Sclerosis
- Amyotrophic Lateral Sclerosis
- Neurological diseases
- Epilepsy
- Parkinson's Disease

Infectious disease

- Parasitic Infections
- Sepsis-Associated Cholestasis
- Viral Hepatitis: Hepatitis A, B, C, D, E

Metabolic diseases

- Hyperlipidemia
- Hypertension
- MASH
- Obesity
- Diabetes
- Lysosomal storage diseases
- Hyper-cholesterolemia
- ASCVD

AX-2402 Program

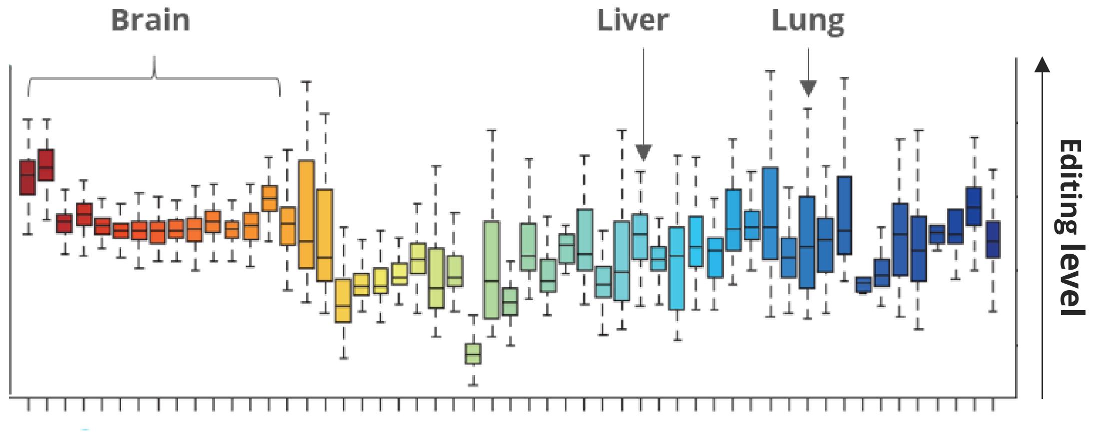
Targeting MECP2 to restore protein functionality in Rett Syndrome, a severe neurodevelopmental disorder

Presenters: Monica Coenraads, MBA and Gerard Platenburg

CNS is a prime target organ for Axiomer RNA editing technology

- Numerous neurological disorders lack effective therapies, urge for new therapeutic approaches
- ADAR enzymes are highly expressed in the brain with very active editing capacity
- EONs have shown broad distribution, durability and were observed to have a favorable safety profile making them a well-suited approach for CNS indications

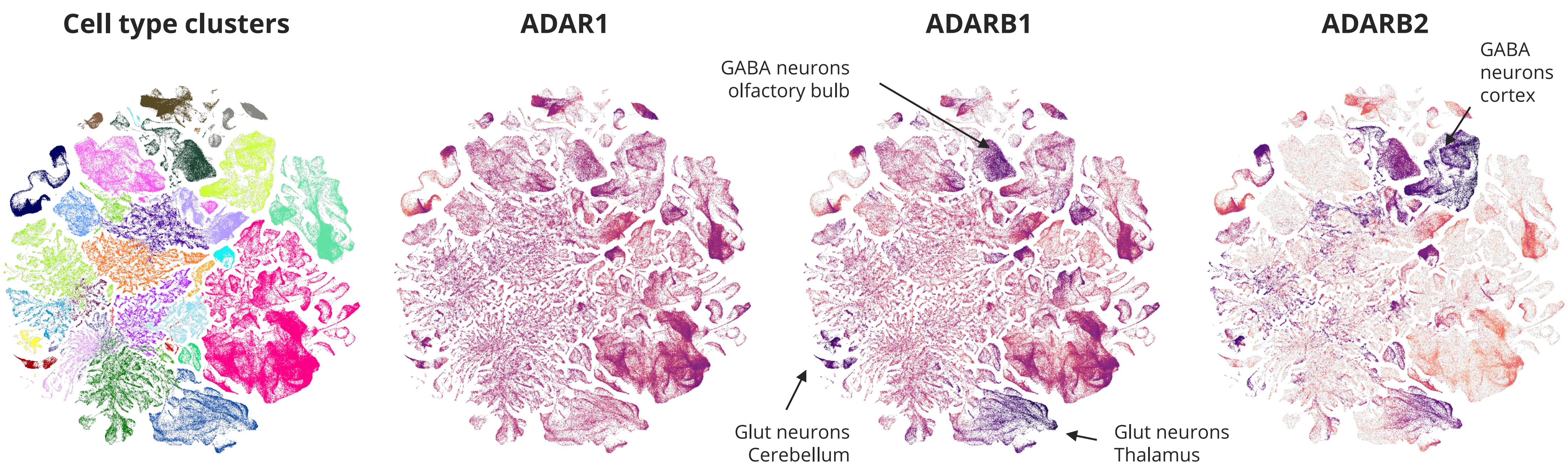
ADAR mediated A-to-I editing in human tissues[1]



[1]Figure adapted from Tan et al. Nature. 2017 Oct 11;550(7675):249-254

Robust ADAR expression across cell type and regions in mouse brain

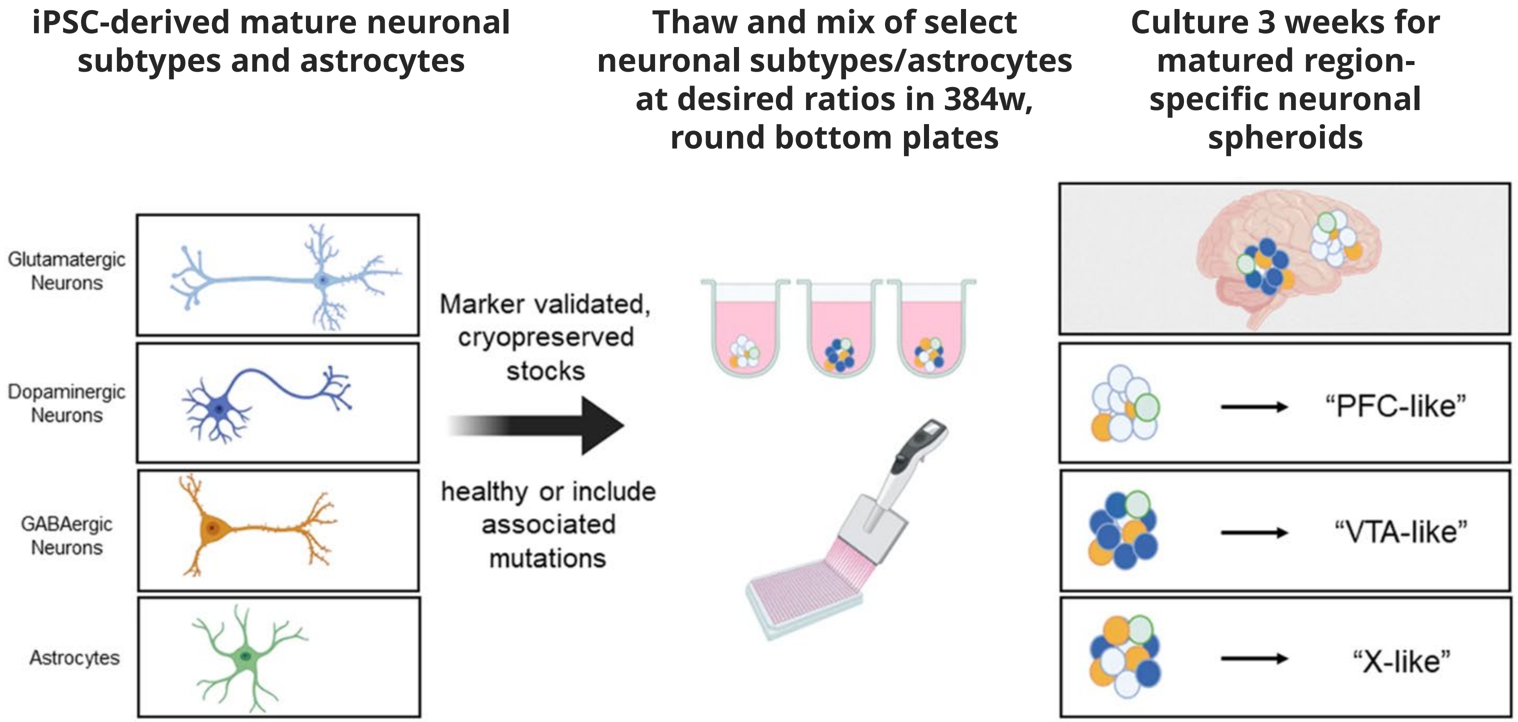
Cell type specific expression of Adarb1 and Adarb2 genes



High expression of Adar genes in different cell type and regions in the mouse brain

Whole Mouse Brain Transcriptomic Cell Type Atlas – Allen brain atlas

Predictive CNS models to inform development of RNA editing

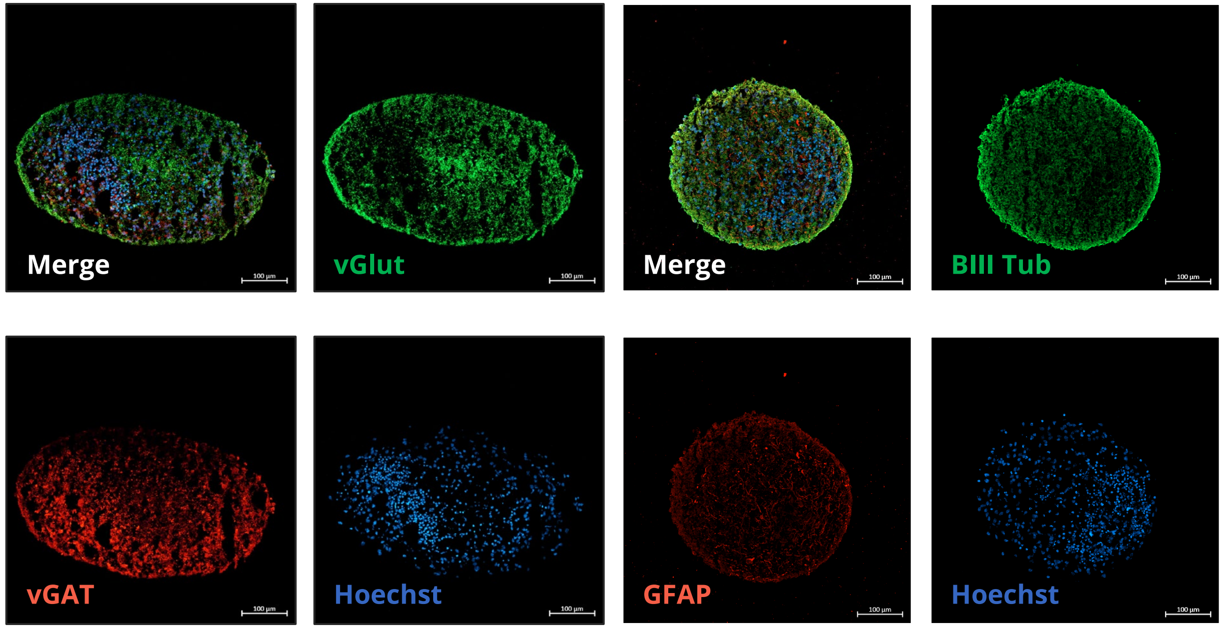


- Spheroids are 3D cultures that model specific brain regions depending on the mix of hIPSC-derived neuronal subpopulations used
- They can give rise to pre-frontal cortex-like (PFC`) or ventral tegmental area (VTA)-like 3D structures
- Uniformly-shaped PFC, form within 24-48 hours with size yield of ~400 µm

Development of reproducible, region-specific neural stem cell (NSC)-derived spheroids addresses limitations of 3D iPSC-derived organoids, offering a robust and predictive tool for accelerating drug discovery in neurodegenerative diseases, substance abuse, and pain management.

Highly efficient RNA editing in brain organoid recapitulating human cortex

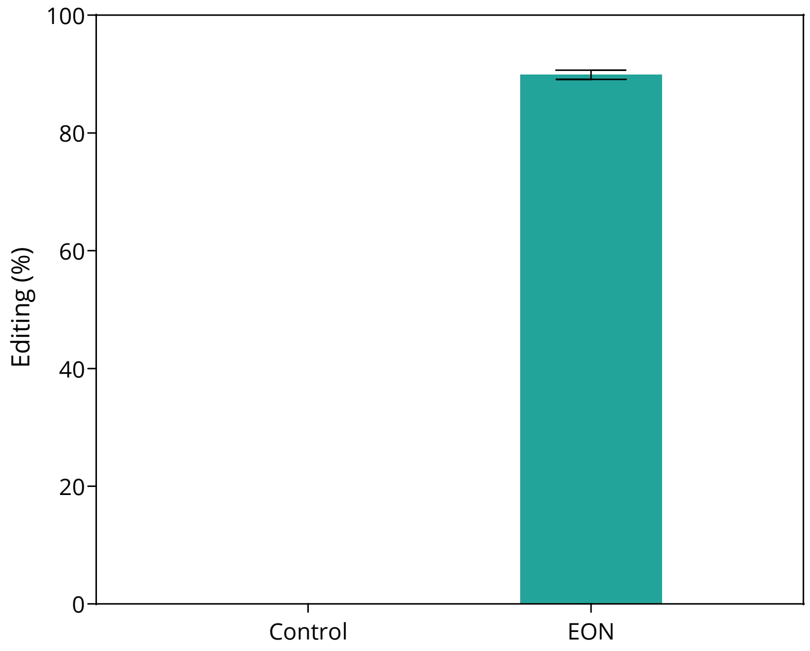
Reaching 90% editing in neurospheroids



PFC-like spheroids are composed by 90% neurons and 10% astrocytes and exhibit a 70:30 ratio of excitatory (Glutamatergic) and inhibitory (GABAergic) neurons recapitulating the cellular composition of the human cortex

RNA editing of APP in human PFC-like spheroids

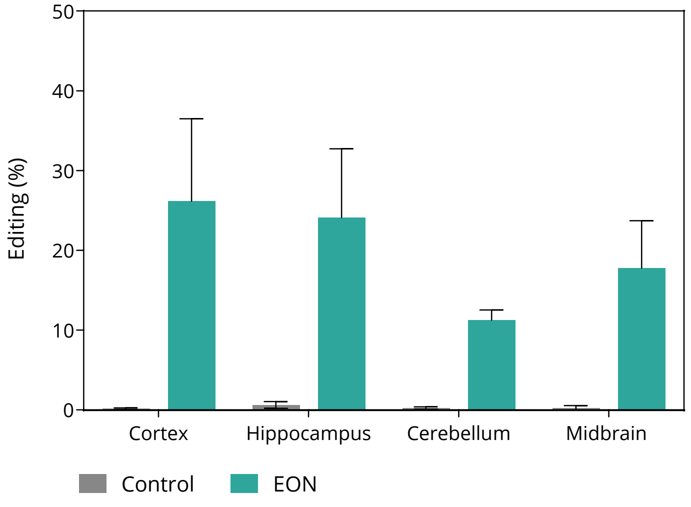
Transfection, 5 µM, single dose, n=3, 7 days, mean, SD, ddPCR



Consistent CNS editing demonstrated across species

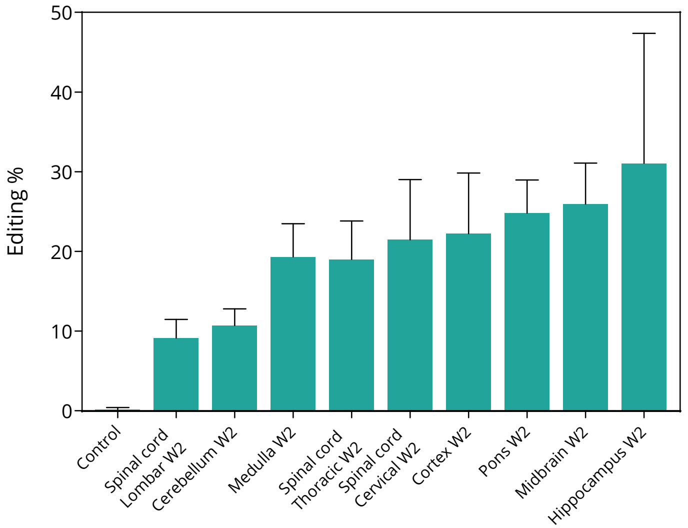
MICE *in vivo*

ICV, 250µg, undisclosed target, single dose, n=6, 4 weeks, ddPCR, mean, SD



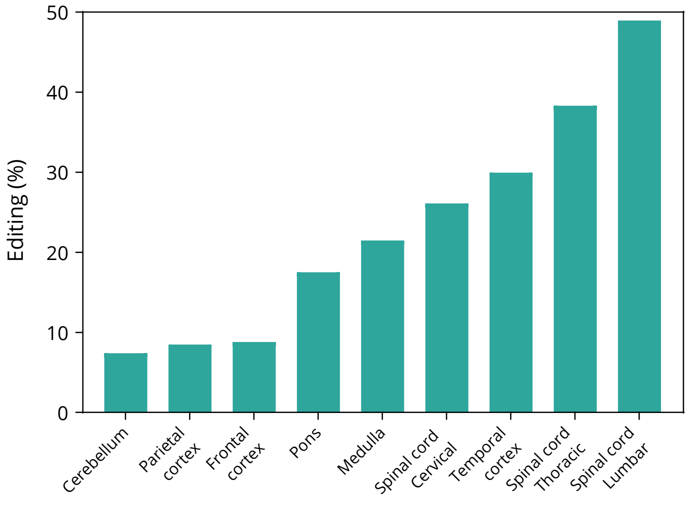
RAT *in vivo*

ICV, 500µg, APP, single dose, n=5, 2 weeks, ddPCR, mean, SD



NHP *in vivo*

IT administration, undisclosed target 12mg, single dose, n=3, 7 days, ddPCR*



- Up to 40% editing *in vivo* leading to 26-fold change in protein function recovery in brain tissues of interest at 4 weeks with a single dose in mice model
- In rat, Axiomer EONs demonstrated up to 50% editing *in vivo* with sustained editing between W2 and W4 after single dose
- Up to 30% RNA editing reported in brain and approx. 50% in spinal cord in NHP in vivo

* Data of 2 NHPs not analyzable due to human error during injection procedure.

Axiomer™ holds strong potential to make a meaningful impact to CNS diseases

Strong RNA Editing Performance

- Robust RNA editing in critical CNS regions validating the efficiency of Axiomer platform in CNS indications

Broad Applicability Across CNS Regions

- RNA editing was successfully achieved in multiple regions of the nervous system, indicating the platform's broad applicability across different CNS regions

Consistent and Durable Results with Well Understood Safety Profile

- Consistent RNA editing across species, with durable effects observed
- EONs have been observed to have a favorable safety profile in CNS

RETT SYNDROME RESEARCH TRUST

AX-2402 RNA editing therapy targeting MECP2 for Rett Syndrome

- Rett Syndrome is a **devastating and progressive neurodevelopmental disorder** caused by variants in the transcription factor Methyl CpG binding protein 2 (*MECP2*). There is a **high unmet need for a disease modifying therapy**.
- Nonsense variants lead to **severe phenotypes.** They represent more than one third **of Rett Syndrome** cases and are projected to affect **20,000 individuals** in US and EU.[1,2]
- Rett Syndrome is **not a neurodegenerative disorder** and restoring levels of the MECP2 protein has shown to **reverse symptoms** in mice.[3]
- Axiomer has the potential to **restore the precise level of MECP2 protein regulatory function,** which is lacking in Rett Syndrome, and become a disease modifying therapy.
- Rett Syndrome Research Trust partnership includes $9.1 M in funding; collaboration established in January 2024, expanded in December 2024

[1]Krishnaraj R, et al. Hum Mutat. 2017 Aug;38(8):922-93; [2]RSRT 2023 conference; [3]Guy J, et al. Science. 2007 Feb 23;315(5815):1143-7.

Monica Coenraads, MBA

Founder, Chief Executive Officer at Rett Syndrome Research Trust

- Monica Coenraads' involvement with Rett syndrome began the day her then-two-year-old daughter was diagnosed with the disorder. A year later, in 1999, she co-founded the Rett Syndrome Research Foundation (RSRF) and held the position of scientific director during the eight years of the Foundation's drive to stimulate scientific interest and research in Rett syndrome, culminating with the groundbreaking work in 2007 which demonstrated the first global reversal of symptoms in preclinical models of the disorder. Monica launched the Rett Syndrome Research Trust in late 2008 to pursue the next steps from that milestone.
- As chief executive officer she oversees all aspects of the organization, including day-to-day operations, strategic direction, fundraising, and communications. Together with her colleagues and with input from advisors and the scientific community at large, Monica sets and executes RSRT's research agenda.
- Under Monica's leadership at RSRF and RSRT, $117 million has been raised for Rett syndrome.

AX-2402 RNA editing therapy targeting MECP2 for Rett Syndrome

RETT SYNDROME RESEARCH TRUST

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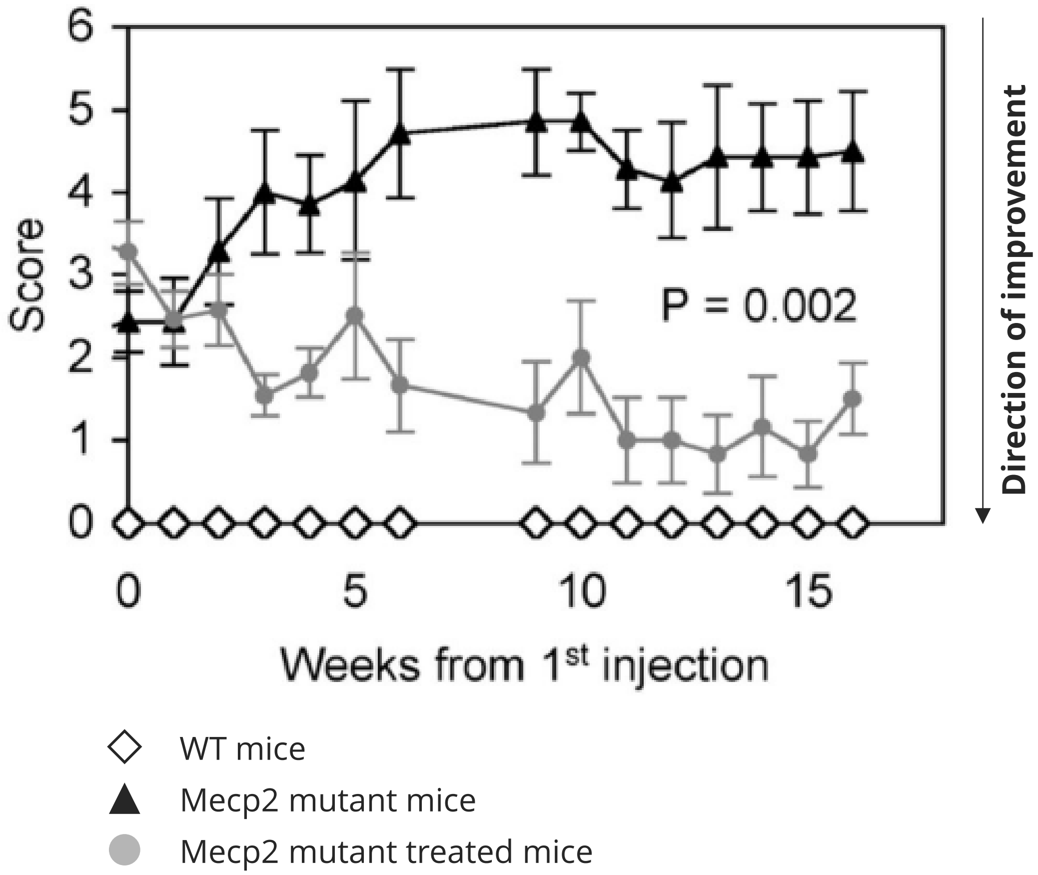
[1]Krishnaraj R, et al. Hum Mutat. 2017 Aug;38(8):922-93; [2]RSRT 2023 conference; [3]Guy J, et al. Science. 2007 Feb 23;315(5815):1143-7.

MECP2 gene is frequently mutated in Rett syndrome (RTT)

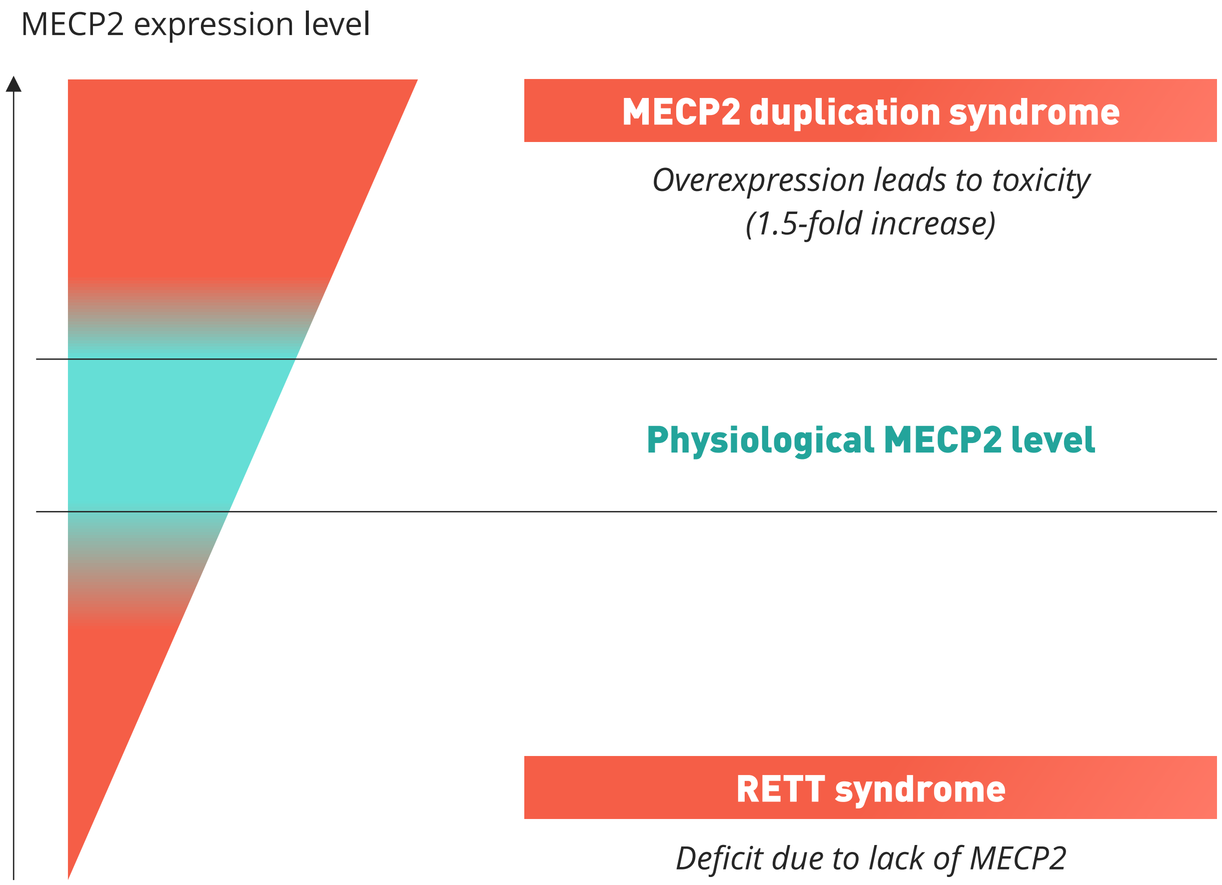
- MECP2 gene, encoding methyl-CpG binding protein 2 (MeCP2):
  - Master epigenetic modulator of gene expression and plays a vital role in neuronal maturation and function
  - Mutations lead to misfolded, truncated or absent protein and loss of function
  - This loss of MECP2 regulating function leads to Rett syndrome and 35% of point mutations cause a premature termination codon (PTC)
- In 2007, Adrian Bird's lab demonstrated that Rett syndrome symptoms are reversible in mice[1]



[1]Guy J, et al. Science. 2007 Feb 23;315(5815):1143-7. Figure adapted from Guy J, et al. Science. 2007 Feb 23;315(5815):1143-7.

MECP2 expression level tightly regulated in neurons

Axiomer is a well-suited approach to restore physiological levels of MECP2



- Axiomer approach makes use of ADAR endogenous system to restore physiological levels of functional MECP2
- Axiomer avoid the risk of expressing unsafe levels of MECP2, potentially leading to MECP2 duplication syndrome

Axiomer™ has the potential to restore physiological levels of functional MECP2

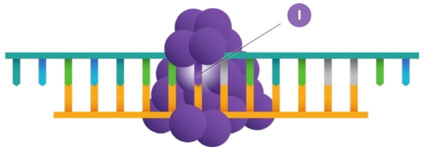
AX-2402 correcting MECP2 R270X into WT-like R270W

GGGGC**C>UGA**AAGCCG

EXON 3 | EXON 4 //

RETT syndrome

Postnatal microcephaly, stereotypic hand movements, ataxia, abnormal breathing, and growth retardation, social withdrawal, loss of speech, seizures



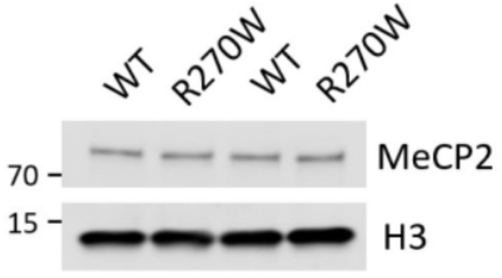
GGGGC**UGA>G**AAGCCG

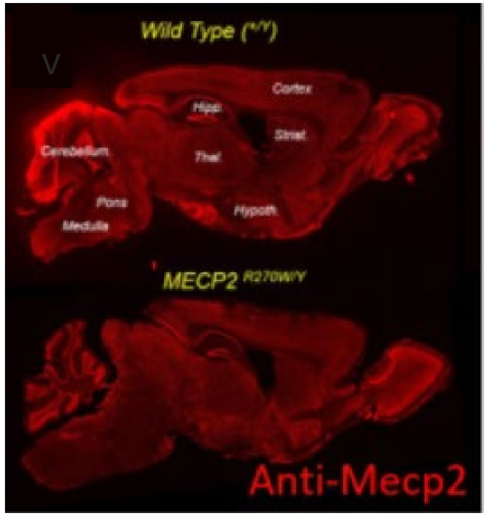
EXON 3 | EXON 4 | EXON 5

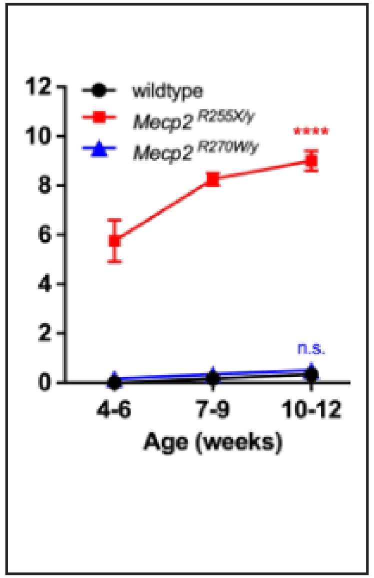
WT like phenotype

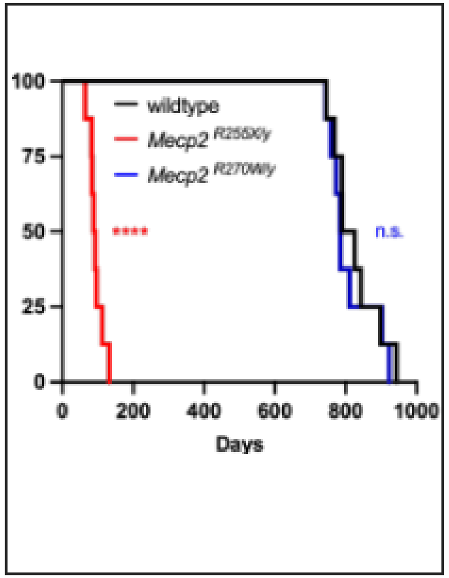
- MeCP2 protein restoration/recovery
- MeCP2 R270W (Arg > Trp) mouse model indistinguishable from wild type mice

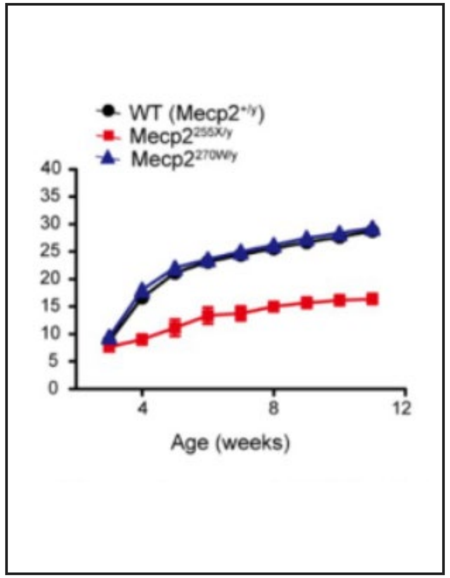
R270W variant demonstrates wild-type like profile

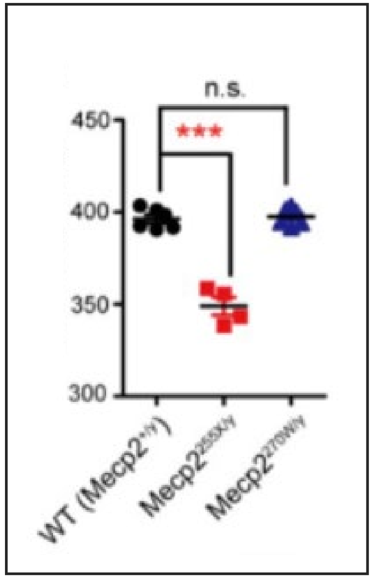










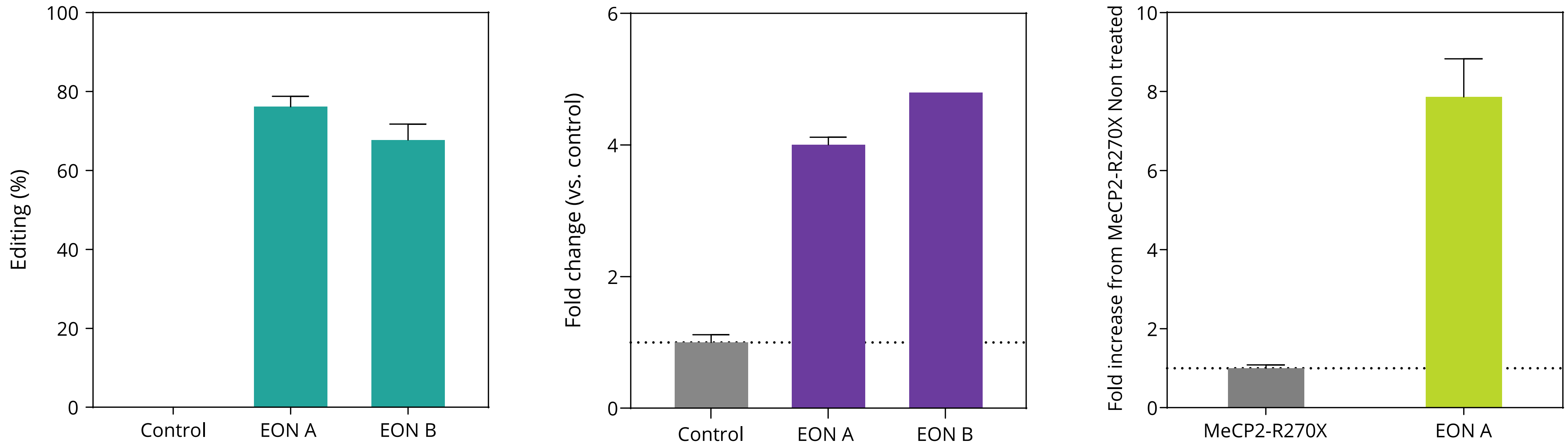


AX-2402 can restore physiological levels of functional MECP2 potentially reverting Rett syndrome into a WT like phenotype[1]

[1]Colvin, S. (2023) thesis. Massachusetts Institute of Technology. Figures adapted from: Colvin, S. (2023) thesis. Massachusetts Institute of Technology

EON mediated editing in patient's cells increases mRNA levels and restores protein expression

PTC recoding leading to absent NMD mediated RNA degradation



Up to 80 % editing of R270X MECP2 in patient fibroblasts

Increased MECP2 RNA levels due to PTC recoding and NMD inhibition

Increased R270W MECP2 protein levels

EON, Editing oligonucleotide; NT, Non-treated; TF, transfection, Conditions panel on the left and middle: 100 nM EON, transfection, 48h, N=2, mean±SEM. Conditions panel on the right: MeCP2-R270X-NanoLuc activity; 100 nM EON, transfection, 48h, N=8, mean±SEM.

Preliminary clinical trial design

Single dose

Dose 1, N=3, IT

Dose 2, N=3, IT

Dose 3, N=3, IT

Repeated dose

Dose 1, N=3, IT, Q3-6M

Dose 2, N=3, IT, Q3-6M

Dose 3, N=3, IT, Q3-6M

- Preliminary Phase 1/2 SAD & MAD design
- Up to 18 subjects with the R270X mutation
- Primary objective: safety, tolerability and pharmacokinetics
- Secondary objectives: target engagement and biomarkers
- Financially supported by $8.1M funding provided by Rett syndrome Research Trust
- **Clinical candidate selection in 2025**
- **Top-line data expected in 2026**

AX-1412 Program

Targeting B4GALT1 to reduce the risk of cardiovascular diseases

Presenter: Gerard Platenburg

AX-1412 RNA editing therapy targeting B4GALT1 for cardiovascular diseases

Leading causes of death in the world
~18 million people die from CVDs every year (**32% of all global deaths**) Despite therapies, the unmet medical need remains.

AX-1412 is designed to provide people with a protective genetic variant of B4GALT1 that is associated with **36%[1] reduction in the risk of cardiovascular disease**.

AX-1412 may become a **stand-alone cardiovascular therapy** that may also work **synergistically with standard of care** to further reduce risk of CVDs.

[1]Montasser ME, et al. Science. 2021 Dec 3;374(6572):1221-1227

B4GALT1 p.Asn352Ser variant reduces CVD risk

- It is described that people who carry missense variants like the p.Ans352Ser in B4GALT1, **have 36% lower chance of the development of coronary artery disease.**[1] This variant is known as the “old Amish order variant”
- This variant reduces CVD risk through 2 independent risk factors, fibrinogen and LDL-C, through independent pathways from PCSK9
- This protective variant is a A-to-G variant, on that can be introduced by Axiomer mediated ADAR editing
- B4GALT1 is not suitable for knockdown technologies, as leads to semi-lethality and severe development abnormalities in mouse studies

Science
HUMAN GENOMICS

Genetic and functional evidence links a missense variant in *B4GALT1* to lower LDL and fibrinogen

May E. Montasser[1]*†, Cristopher V. Van Hout[2,3]†, Lawrence Miloscio[2]†, Alicia D. Howard[1,4], Avraham Rosenberg[5], Myrasol Callaway[5], Biao Shen[5], Ning Li[5], Adam E. Locke[2], Niek Verweij[2], Tanima De[2], Manuel A. Ferreira[2], Luca A. Lotta[2], Aris Baras[2], Thomas J. Daly[5], Suzanne A. Hartford[5], Wei Lin[5], Yuan Mao[5], Bin Ye[2], Derek White[5], Guochun Gong[5], James A. Perry[1], Kathleen A. Ryan[1], Qing Fang[5], Gannie Tzoneva[2], Evangelos Pefanis[5], Charleen Hunt[5], Yajun Tang[5], Lynn Lee[5], Regeneron Genetics Center Collaboration‡, Carole Sztalryd-Woodle[1,6], Braxton D. Mitchell[1,7], Matthew Healy[8], Elizabeth A. Streeten[1,9], Simeon I. Taylor[1], Jeffrey R. O’Connell[1], Aris N. Economides[2,5], Giusy Della Gatta[2]§, Alan R. Shuldiner[2]§

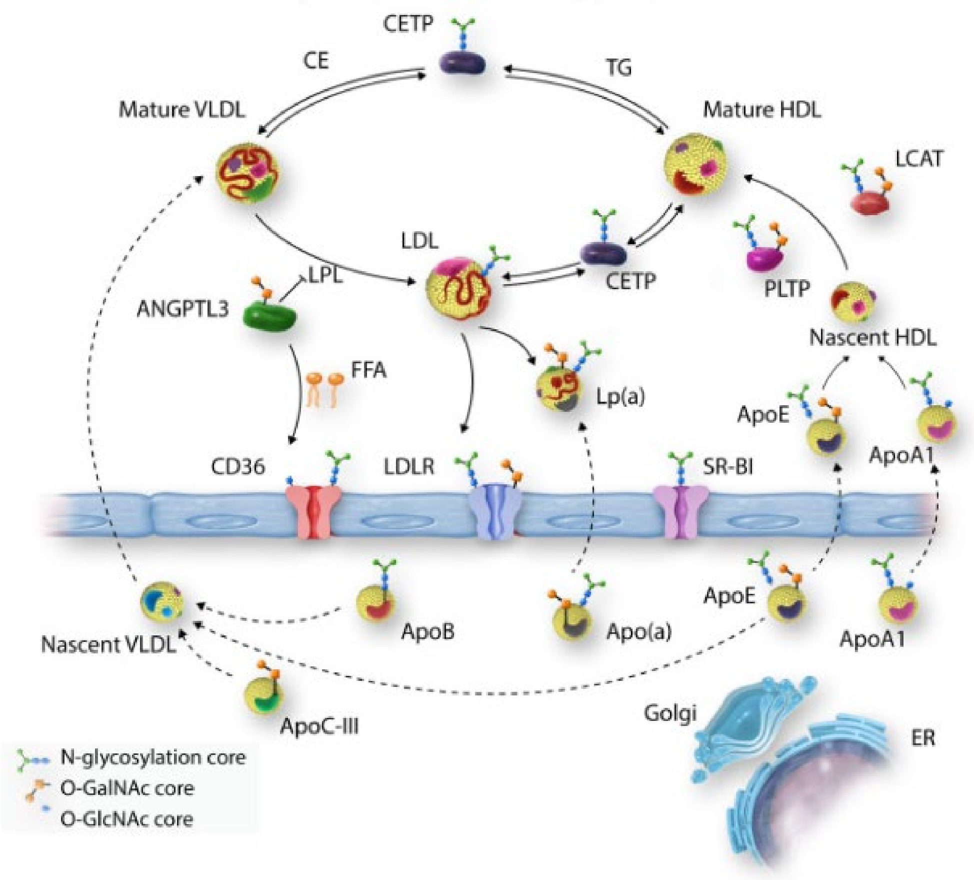
Increased blood levels of low-density lipoprotein cholesterol (LDL-C) and fibrinogen are independent risk factors for cardiovascular disease. We identified associations between an Amish-enriched missense variant (p.Asn352Ser) in a functional domain of beta-1,4-galactosyltransferase 1 (*B4GALT1*) and 13.9 milligrams per deciliter lower LDL-C ($P = 4.1 \times 10^{-19}$) and 29 milligrams per deciliter lower plasma fibrinogen ($P = 1.3 \times 10^{-5}$). *B4GALT1* gene–based analysis in 544,955 subjects showed an association with decreased coronary artery disease (odds ratio = 0.64, $P = 0.006$). The mutant protein had 50% lower galactosyltransferase activity compared with the wild-type protein. N-linked glycan profiling of human serum found serine 352 allele to be associated with decreased galactosylation and sialylation of apolipoprotein B100, fibrinogen, immunoglobulin G, and transferrin. *B4galt1* 353Ser knock-in mice showed decreases in LDL-C and fibrinogen. Our findings suggest that targeted modulation of protein galactosylation may represent a therapeutic approach to decreasing cardiovascular disease.

Montasser *et al.*, *Science* **374**, 1221–1227 (2021)

[1]Montasser ME, et al. Science. 2021 Dec 3;374(6572):1221-1227

Glycosylation is a key process in lipid metabolism

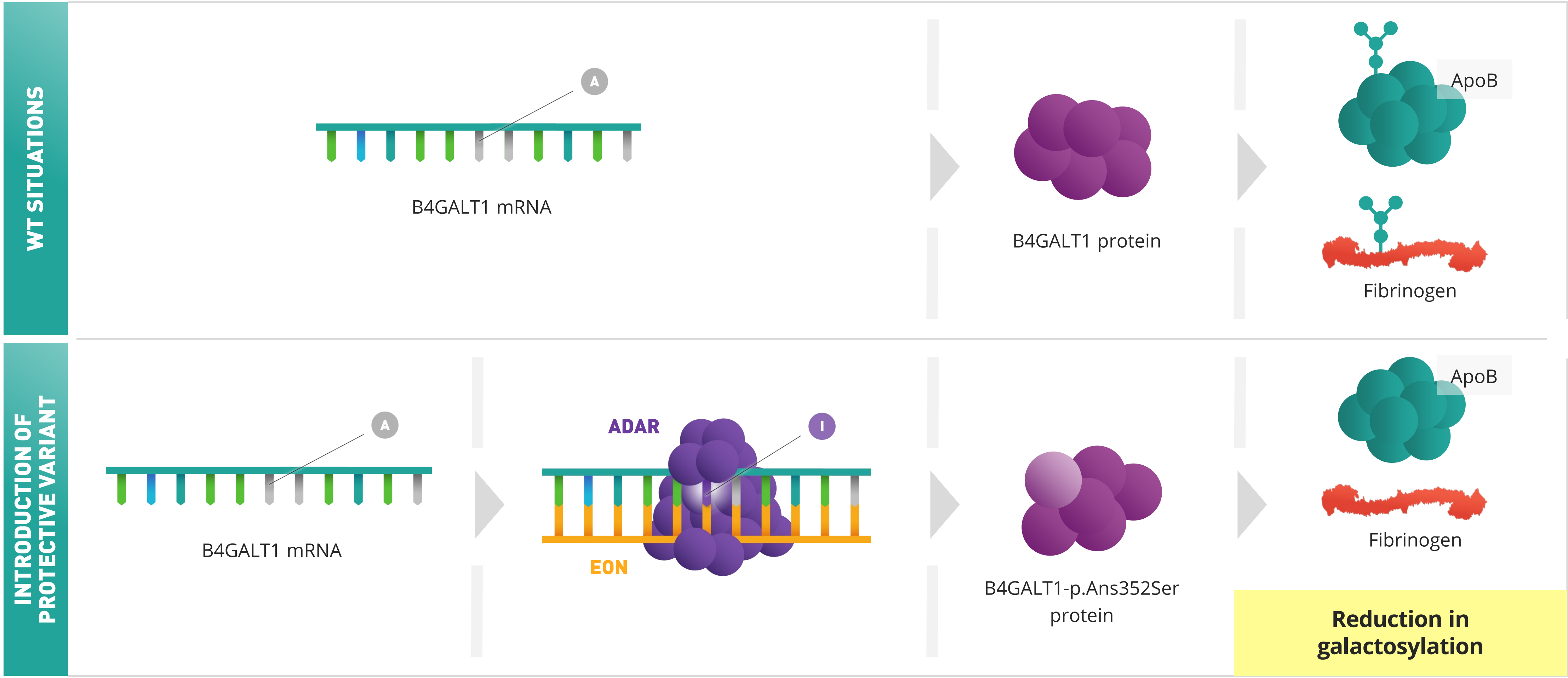
Physiological lipoprotein glycosylation[1]



- Glycosylation stabilizes the folding and conformation of apolipoproteins (e.g., ApoB, ApoE), ensuring proper assembly and secretion of lipoproteins such as LDL and HDL.
- Glycosylation of receptors like LDLR is critical for their membrane localization and ligand binding, enabling efficient lipoprotein clearance from the bloodstream.
- Aberrant glycosylation can lead to dysfunctional lipoproteins, a key driver of atherosclerosis.

[1]Pirillo A, et al. Cardiovasc Res. 2021 Mar 21;117(4):1033-1045.

B4GALT1 p.Asn352Ser variant to reduce galactosylation of CVD risk factors

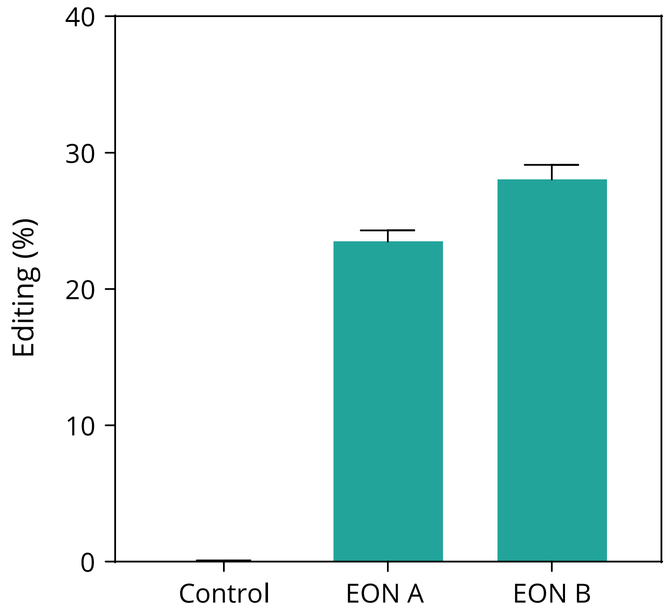


EON-mediated editing of B4GALT1 leads to reduced glycosylation activity

In line with natural population

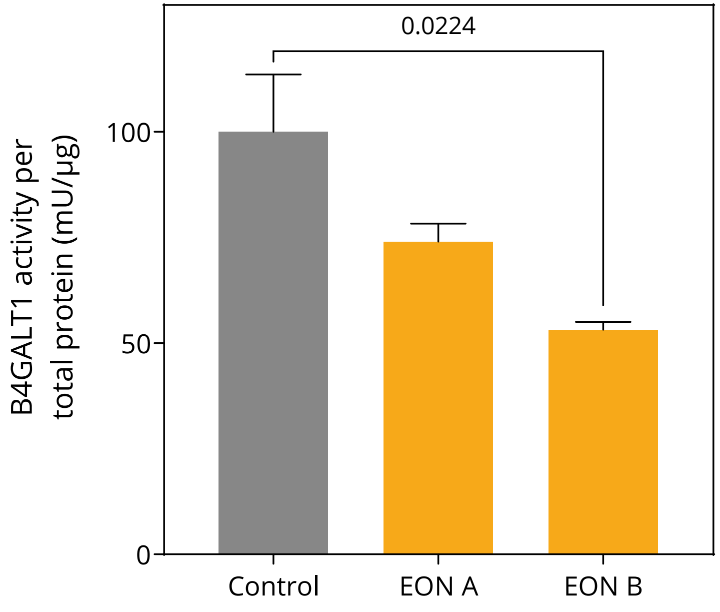
EON mediated editing of B4GALT1 in PHH

5µM transfection, dPCR, mean, SD, n=3



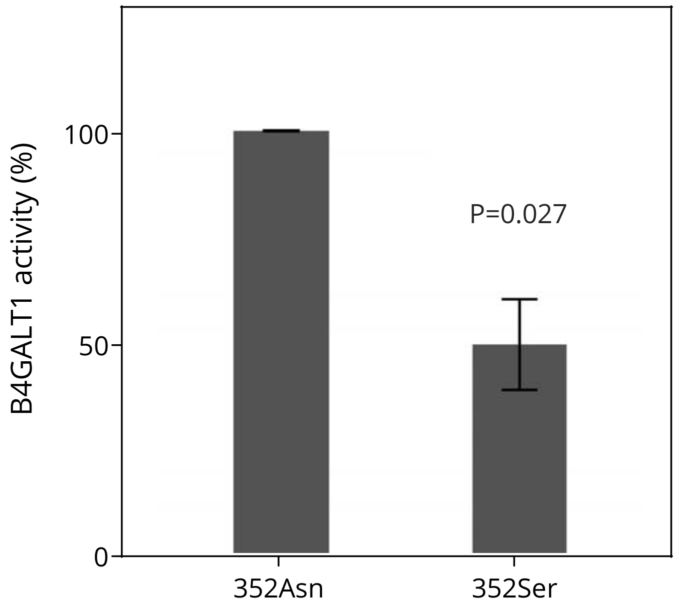
B4GALT1 activity following editing

PMH, cell lysate, transfection 5µM, 4 days, mean, SD, ANOVA



B4GALT1 activity reported in Montasser et al.[1]

COS-7 cells, cell lysate, transfection, n=4, mean, SEM

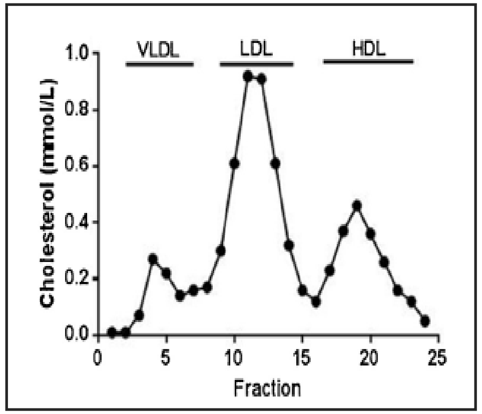


[1]Montasser ME, et al. Science. 2021 Dec 3;374(6572):1221-1227. Percentage of 352Asn B4GALT1 galactosylation activity of 352Asn B4GALT1 and 352Ser B4GALT1 immunoprecipitated proteins

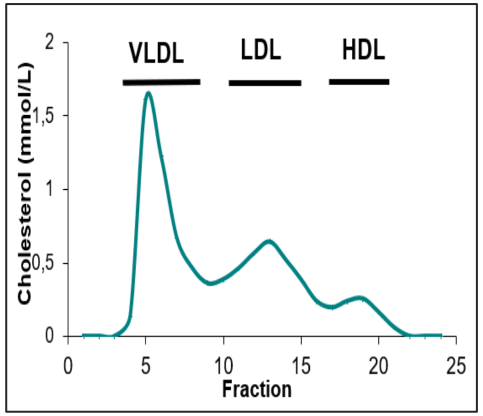
E3L.CETP mice disease model is industry standard for assessing CVD therapeutics

- CETP facilitates the transfer of cholesteryl esters from HDL to VLDL and LDL, a key process in human lipid metabolism that is absent in most rodent models.
- These mice, fed a high-fat high-cholesterol diet (HFCD), exhibit a biphasic dyslipidemic response, closely mimicking plasma lipid changes in humans
- The presence of CETP in this model makes it uniquely suited to study dyslipidemia and cholesterol metabolism, especially in relation to B4GALT1, which is involved in glycosylation processes affecting lipid metabolism.
- In humans, most circulating lipids are confined to VLDL/LDL particles

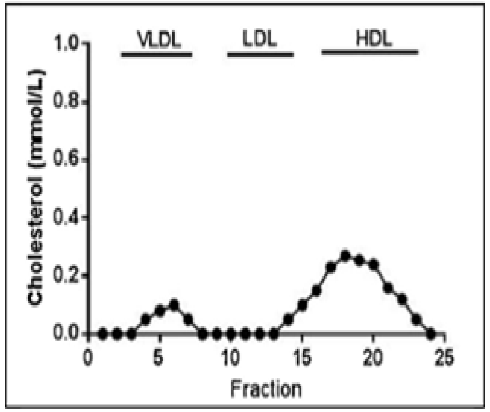
Human



E3L.CETP mice disease model



Wildtype healthy mice

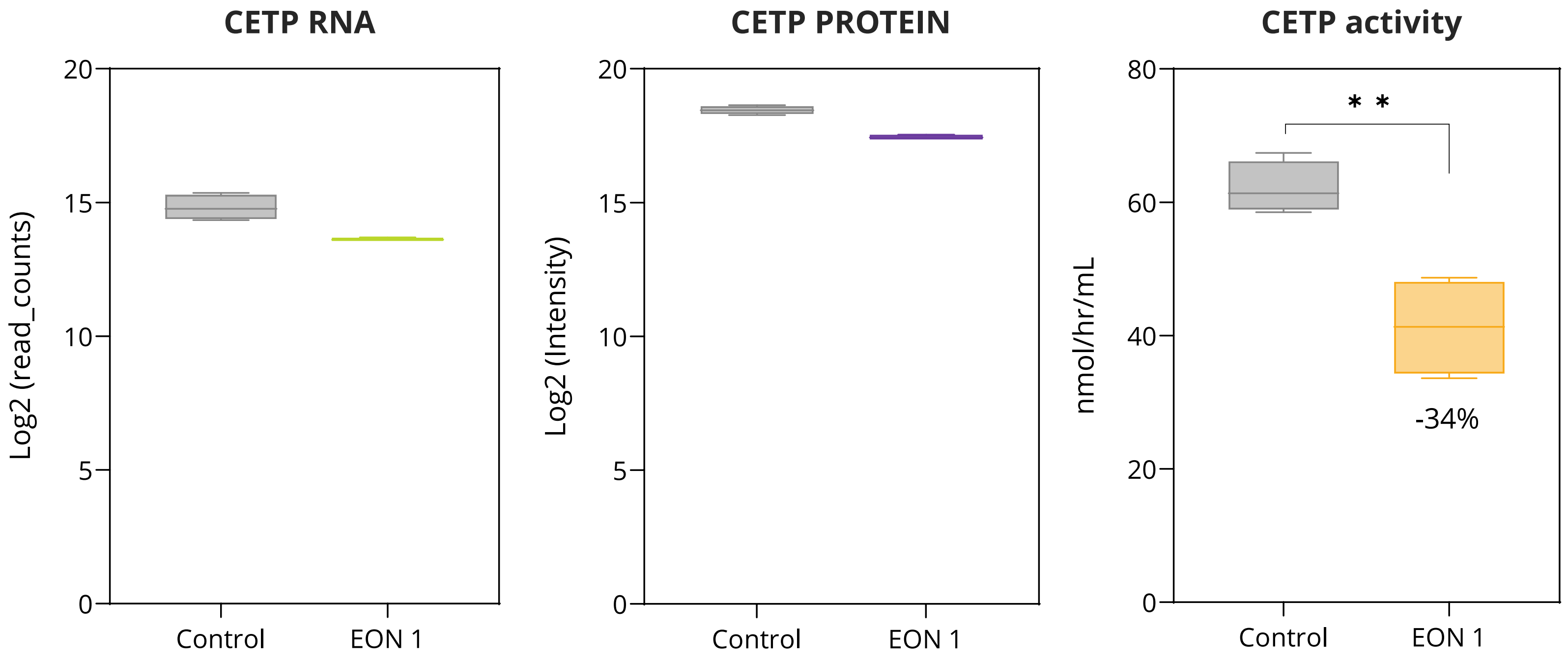


B4GALT1 editing impacts activity of key proteins involved in lipid metabolism

Minimal changes in transcript and protein levels associated with decrease in CETP activity in vivo

CETP RNA, protein and activity following EON B treatment

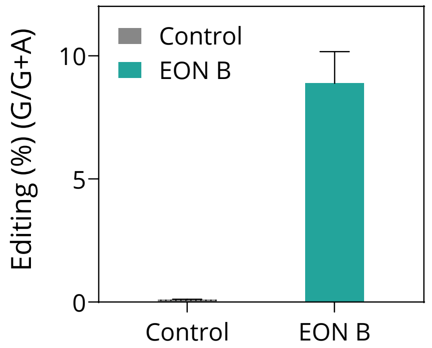
E3L.CETP mice, LNP formulation, 2mg/kg, Q1W, D31, RNAseq, Roar, mean, max-min n=3, T-test



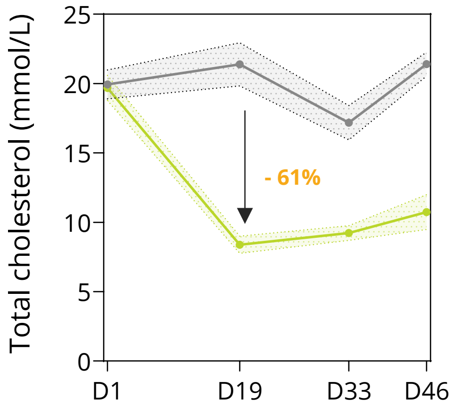
Reduced CETP activity in the absence of changes at the transcriptomic or proteomic levels highlights the impact of EON on glycosylation rather than on expression levels

EON-mediated editing of B4GALT1 leads to meaningful effect on key biomakers in E3L.CETP Mice

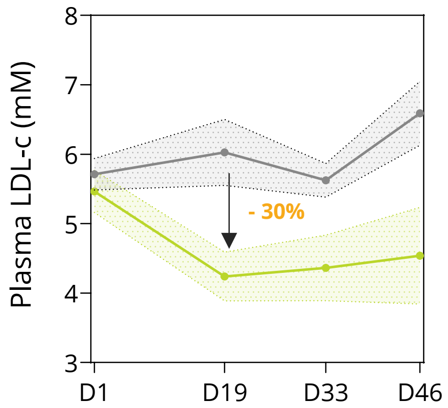
B4GALT1 editing and biomarkers in E3L.CETP mice *(N=10, 2mg/kg, LNP formulation, IV Q1W, D46, ddPCR)*



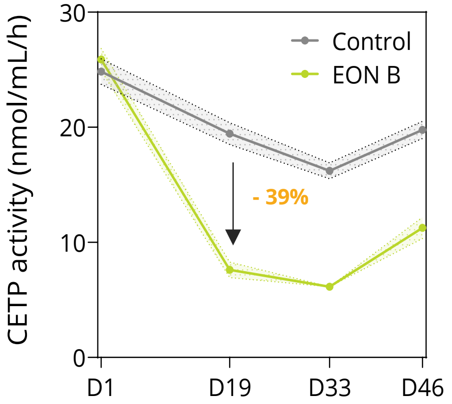
Total Cholesterol



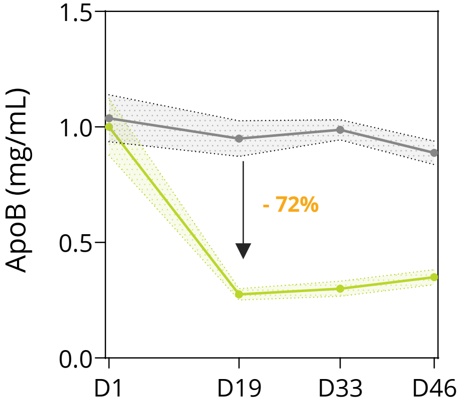
Plasma LDL-c



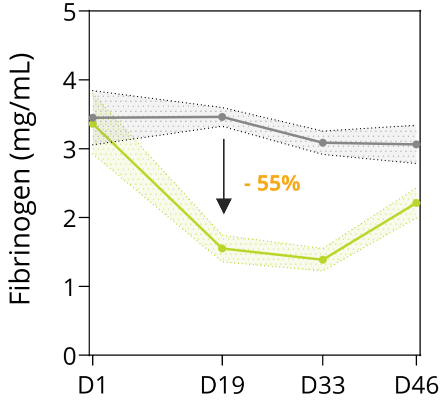
CETP activity



ApoB



Fibrinogen

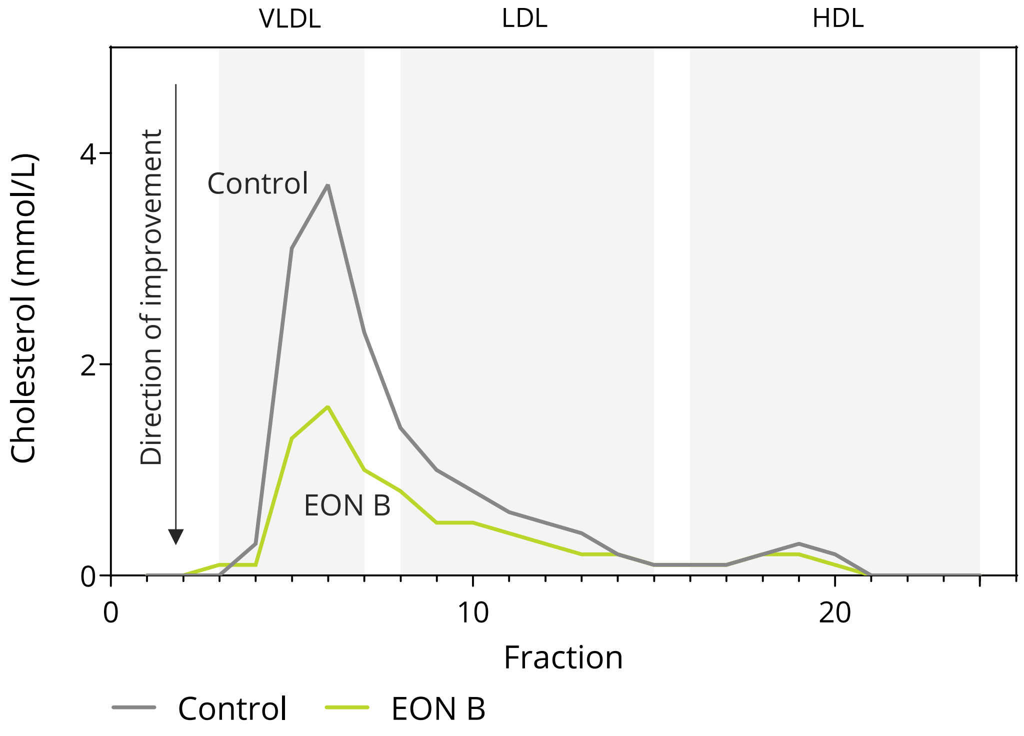


Following treatment with EON B, a marked reduction in total cholesterol, ApoB, and LDL-c by observed already at Day 19 confirms our approach to address cardiovascular diseases

B4GALT1 EON leads to a positive shift in lipoprotein profiles

Specifically targeting atherogenic lipoproteins

Impact on lipoprotein profile following editing of B4GALT1 in E3L.CETP mice
(N=10, 2mg/kg, LNP formulation, IV Q1W, D46)



- Treatment with EON B significantly decreases VLDL and LDL cholesterol compared to control
- These lipoproteins are associated with increased cardiovascular risk due to their role in atherosclerotic plaque formation
- HDL cholesterol which supports reverse cholesterol transport and is associated with reduced cardiovascular risk, remains unchanged

Summary & next steps

AX-1412 for CVD

EON-mediated RNA editing of B4GALT1 leads to the required reduction in galactosylation

reflecting the human genetics observed effect

LNP-delivered EON editing B4GALT1 leads to editing and meaningful changes

in biomarker effect on LDLC, CEPT, cholesterol and fibrinogen in an industry-standard in vivo disease model

Further optimization of a GalNAc delivered EON ongoing

to achieve a TPP desirable for CVD

Expected to provide an update on the optimization efforts in mid 2025

AX-2911 Program

Targeting PNPLA3 to address unmet medical needs in MASH

Presenter: Gerard Platenburg

AX-2911 RNA-editing therapy to address Metabolic dysfunction associated steatohepatitis (MASH)

MASH and subsequent stages of liver disease **are very prevalent and still on the rise worldwide**. MASH individuals have a high unmet medical needs due to the **progressive** nature of the disease (cirrhosis and hepatocellular carcinoma) and **limited therapeutic options** available[1]

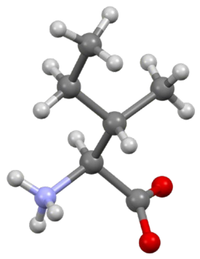
PNPLA3 (patatin-like phospholipase domain-containing 3) I148M is a variant **commonly reported** in the MASH population worldwide (20-60% of the patients) and is known as **associated risk factor**.[2,3] Approximately 8 million individuals in US and EU are homozygous for the 148M variant.

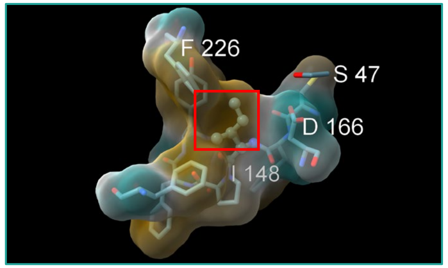
Axiomer EONs have the potential to change the Methionine into a Valine bringing the **PNPLA3 protein back to a WT-like functional conformation**.

[1]Sandireddy R, et al. Front Cell Dev Biol. 2024 Jul 16;12:1433857; [2]Romeo S, et al. Nat Genet. 2008 Dec;40(12):1461-5; [3]Salari N, et al. BMC Endocr Disord. 2021 Jun 19;21(1):125.

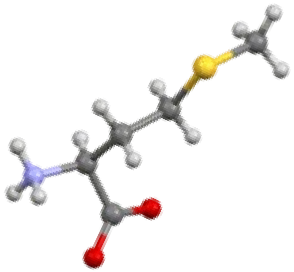
Axiomer™ creates a PNPLA3 protein with WT-like functionality

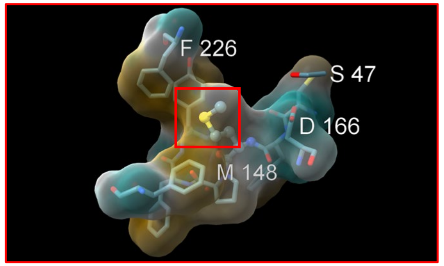
PNPLA3 148I (WT)
Isoleucine (ATC)



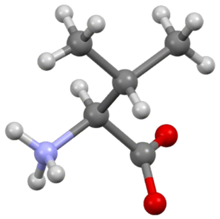


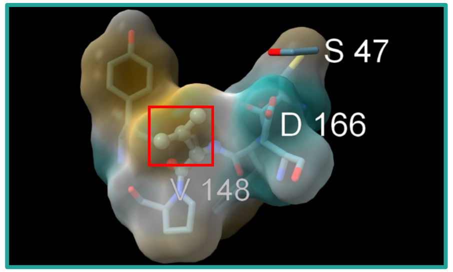
PNPLA3 148M (Mutant)
*Methionine (**A**TG)*





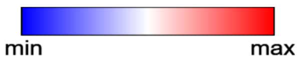
PNPLA3 148V
(Axiomer *de-novo* variant)
*Valine (**G**TG)*





F226 not included here

Electrostatic potential



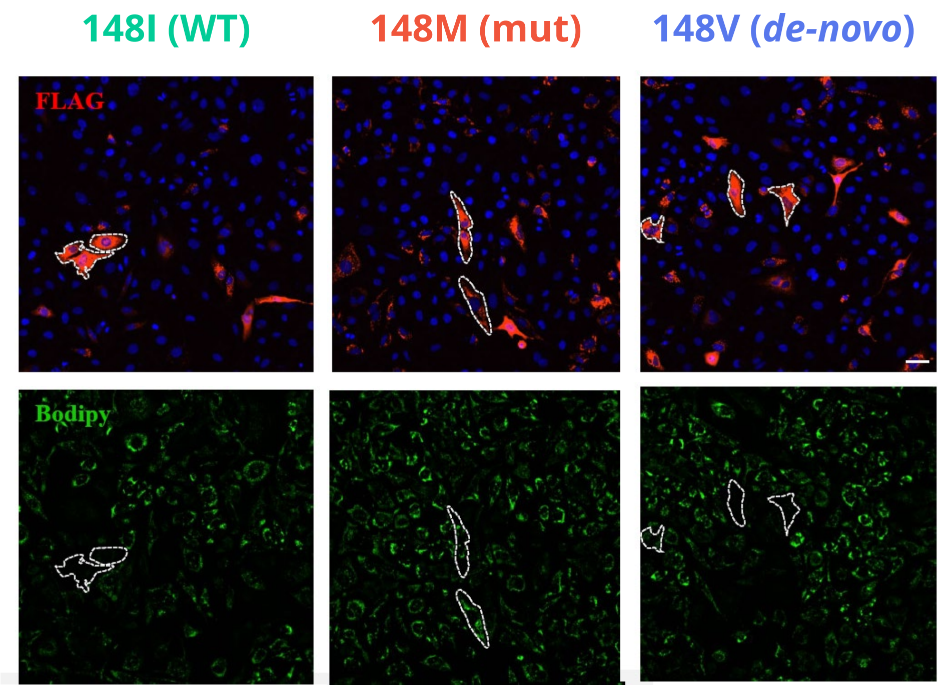
Hydrophobicity

***In silico* analysis of variants**

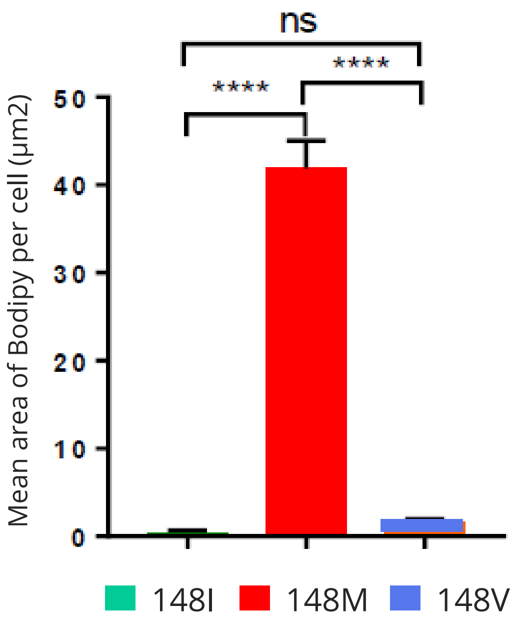
- 148M shows a non-conservative substitution with predicted **functional consequences, with change in binding cavity volume limiting access of substrate to the active site**
- Equivalent potential between Isoleucine (WT) or Valine (Axiomer correction) at location 148 in 3D models
- 148I and 148V predict **no functional consequences** for PNPLA3, with valine expected to behave like isoleucine

PNPLA3 148V variant has WT-like lipid metabolizing properties

148I and 148V reports equivalence in lipid droplet sizes



Hoechst (nuclei), Bodipy (Lipids) and M2 anti-flag (PNPLA3)



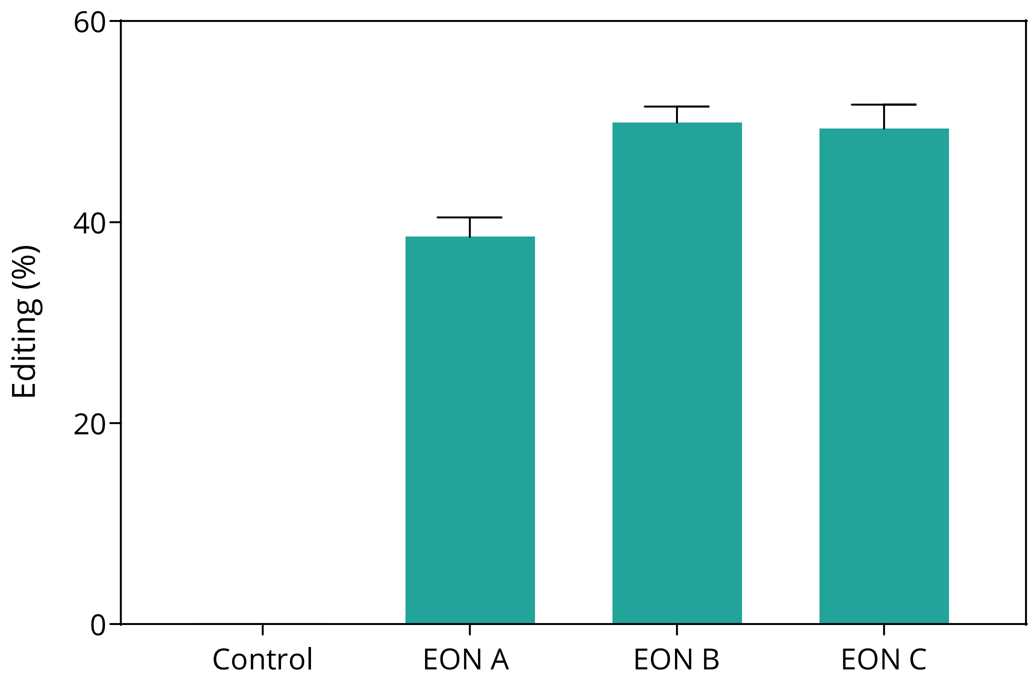
- The wild-type 148I shows smaller lipid droplets, reflecting normal lipid metabolism
- The 148M variant induces significantly larger lipid droplets, consistent with its pathogenic role in lipid metabolism disorders
- The corrected variant 148V results in wild-type like droplet sizes, suggesting a corrective effect on lipid accumulation, similar to 148I

Treatment conditions: HeLa cells, plasmid, transfection, 250uM linoleic acids, 24h, cell lipase activity by IF One-way ANOVA, ****, P<0.0001; Mean, SEM.

EON mediated PNPLA3 editing leads to over 50% RNA editing and change in lipid droplet

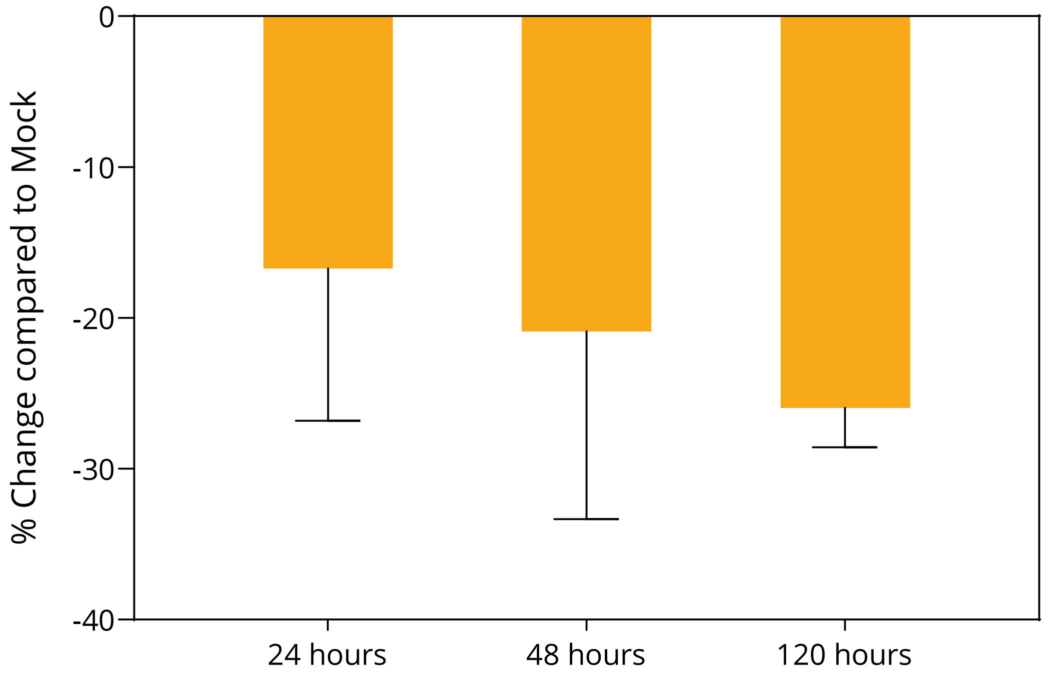
Editing of PNPLA3 in PHH

100nM EON, transfection, 72h, dPCR, mean, SEM, n=3



Change in intracellular lipid droplets post PNPLA3 148V EON treatment in HepG2

Bodipy/DAPI stainings, 5µM EON, transfection, exposure to linoleic acid, mean, SEM, n=2



Summary & next steps

AX-2911 for MASH

Final optimization of AX-2911 EONs ongoing for clinical candidate selection in 2025

Expected 3-6 monthly dosing interval subcutaneous GalNAc-delivered

Development activities to start in 2025

Start clinical trial in 2026

Closing summary

Daniel A. de Boer

ProQR development pipeline

	TARGET	DISCOVERY	NON-CLINICAL	CLINICAL	NEXT MILESTONE	ESTIMATED POPULATION
DEVELOPMENT PIPELINE						
AX-0810 *for Cholestatic diseases*	NTCP				CTA filing in Q2 2025	~100K patients
AX-2402 *for Rett syndrome*	MECP2 R270X				Candidate selection in 2025	~5K patients
AX-1412 *for Cardiovascular disease*	B4GALT1				Scientific update in mid 2025	~200M patients
AX-2911 *for MASH*	PNPLA3				Candidate selection in 2025	~8M patients
DISCOVERY PIPELINE						
AX-1005 *for CVD*	Undisclosed					~200M patients
AX-0601 *for obesity and T2D*	Undisclosed					~650M patients
AX-9115 *for rare metabolic condition*	Undisclosed					
AX-2403 *for Rett syndrome*	MECP2 R168X					~6K patients
AX-2404 *for Rett syndrome*	MECP2 R255X					~5K patients
AX-2405 *for Rett syndrome*	MECP2 R294X					~6K patients
AX-2406 *for Rett syndrome*	MECP2 R133H					
AX-3875 *for rare metabolic & CNS disorder*	Undisclosed					
AX-4070 *for rare CNS disorder*	Undisclosed					
PARTNERED PIPELINE						
10 targets (option to expand to 15)	Undisclosed	*Progress undisclosed*				Lilly

[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: Trivedi PJ, et al. Clin Gastroenterol Hepatol. 2022 Aug;20(8):1687-1700.e4; Schreiber RA, et al. J Clin Med. 2022 Feb 14;11(4):999; Tsao CW, et al. Circulation. 2022;145(8):e153–e639. World Health Organization, World Gastroenterology Organization

Catalyst overview

4 trial readouts expected in 2025-2026, cash runway into mid-2027

AX-0810 for Cholestatic disease

- CTA submission Q2 2025
- Top-line data Q4 2025

AX-2402 for Rett Syndrome

- Clinical candidate selection in 2025
- Anticipated trial start and top-line data in 2026

AX-1412 for Cardiovascular disease

- Non-clinical data update in mid 2025

AX-2911 for MASH

- Clinical candidate selection in 2025
- Anticipated trial start and top-line data in 2026

Partnerships

- Opportunity to earn up to $3.75B in milestones in the Lilly partnership
- Opportunity to receive a $50 M opt-in fee from Lilly for expansion to 15 targets
- Opportunity for other strategic partnerships

Well positioned

to advance Axiomer™

Clinical trial results across 4 trials in 2025 and 2026 expected

- Clinical PoC data of NTCP trial in 2025
- Up to 4 clinical trials with data readouts in 2025/2026

Rich discovery pipeline with potential for broad pipeline expansion

- Large number of potential therapeutic applications in discovery pipeline
- Broad applicability beyond current discovery pipeline

Leading IP position

- Axiomer™ is protected by >20 published patent families
- Continuously investing in expanding IP estate

Validating Strategic Partnerships

- Eli Lilly collaboration valued up to $3.9B, with opportunity for near-term milestones
- Rett Syndrome Research Trust cofinancing of AX-2402 program
- Selectively form additional partnerships

Strong balance sheet

- €89.4 million cash and cash equivalents as of end of Q3, plus $82.1 million gross proceeds from October financing
- Cash runway to mid-2027, excluding potential for additional BD-related upside

ProQR®

IT'S IN OUR RNA