



NTCP MODULATION IN BILIARY ATRESIA VIA LIVER-TARGETED RNA EDITING

*Understanding the biology, target engagement,
and path to clinical translation*

ProQR Therapeutics | September 30, 2026



Forward looking statements

This presentation has been prepared by ProQR Therapeutics N.V. ("we", "us" or "our") and contains forward-looking statements. All statements other than statements of historical fact are forward-looking statements, which are often indicated by terms such as "continue," "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 business, technology, strategy, preclinical and clinical model data; our initial pipeline targets and the upcoming strategic priorities and milestones related thereto; the continued advancement of our lead development pipeline programs, including ongoing and planned clinical trials; expectations regarding the ongoing Phase 1 clinical trial of AX-0810 in NTCP for cholestatic diseases, including our ability to complete the Phase 1 clinical trial for AX-0810, biliary atresia as our primary indication for AX-0810 and/or AX-0811, and the anticipated timing of additional target engagement data readout for Phase 1 clinical trial in 2026 and the initiation of Phase 2 trial for AX-0810 and/or AX-0811 in mid-2027, subject to regulatory interactions, with first interim analysis expected by mid-2028; expectations regarding the safety and therapeutic benefits of AX-0810 and AX-0811, including the planned dosing levels and their efficacy; our ability to collaborate with investigators to execute and recruit for an investigator-initiated trial of AX-0810 and/or AX-0811 in China in pediatric participants with biliary atresia and to generate meaningful data therefrom, including the anticipated timing of initial data readout in the first half of 2027; risks and uncertainties associated with conducting clinical trials in China, including evolving regulatory requirements; our pipeline targets, including the planned Phase 1 clinical trial of AX-0811 in NTCP for cholestatic diseases; our ability to recruit for and complete a Phase 1 clinical trial for AX-0811, including the anticipated initial data readout by early January 2027; the anticipated first-in-human study of AX-0422 targeting IDUA for Hurler syndrome, with a CTA filing expected in early 2027 and anticipated initial clinical data readout in the first half of 2027; the anticipated investigator-initiated study in China of AX-2911 targeting PNPLA3 for MASH in the first half 2027; our expectations regarding clinical updates across multiple programs in 2026 and 2027; the therapeutic potential and development timeline regarding AX-0810, AX-0811, AX-0422, AX-2911 and AX-2402; the continued development and advancement of our Axiomer platform; the therapeutic potential of our Axiomer RNA editing oligonucleotides and product candidates; the timing, progress and results of our preclinical studies and other development and pipeline activities, including the release of data related thereto; our patent estate, including our anticipated strength and our continued investment in it, as well as the timing of our clinical development; our AI strategy and expectations regarding AI's ability to accelerate Axiomer discovery; our partnership with Ginkgo and the anticipated benefits thereof; our collaboration with Lilly and the intended benefits thereof; our ability to selectively form new

partnerships and enter into future collaborations; our financial position and cash-runway; and the potential of our technologies and product candidates. 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 expressed or implied by 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 most recent annual report filed on Form 20-F. These risks and uncertainties include, among others, the cost, timing and results of preclinical studies and clinical trials 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 chains and logistics in the global market, economic sanctions and international tariffs; the likelihood of our preclinical and clinical programs being initiated and executed on timelines provided and our reliance on our contract research organizations and predictability of timely enrollment of subjects and patients to advance our clinical trials and maintain their own operations; our reliance on contract manufacturers to supply materials for research and development and the risk of supply interruption from a contract manufacturer; the potential for future data to alter initial and preliminary results of early-stage clinical trials; the unpredictability of the duration and results of the regulatory review of applications or clearances that are necessary to initiate and continue to advance and progress our clinical programs; 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; and risks related to macroeconomic conditions and market volatility resulting from global economic developments, geopolitical events and conflicts, inflationary pressures, fluctuating interest rates, tariffs and potential for significant changes in U.S. policies and regulatory environment. 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.

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Agenda

Welcome & agenda

Sarah Kiely

Opening remarks

Daniel A. de Boer

NTCP modulation in biliary atresia and AX-0810 FIH

Golnar Karimian

Clinical perspective on the role of bile acids in cholestasis

Prof. Gideon Hirschfield

Clinical development and next steps

Cristina Lopez Lopez

Q&A

*Daniel A. de Boer,
Cristina Lopez Lopez,
Golnar Karimian,
Prof. Gideon Hirschfield,
Gerard Platenburg,
Dennis Hom*

Speakers



Sarah Kiely

*VP Investor Relations
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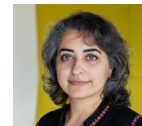
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Founder & CEO



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Chief Medical Officer



Golnar Karimian, MD, PhD

Lead Liver Development



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Gerard Platenburg

Chief Scientific Officer



Dennis Hom

Chief Financial Officer

Advancing multi-asset Axiomer™ clinical pipeline

	TARGET	AXIOMER APPLICATION	DISCOVERY	NON-CLINICAL	CLINICAL	ANTICIPATED MILESTONES	ESTIMATED POPULATION
DEVELOPMENT PIPELINE							
AX-0810 <i>for Cholestatic diseases</i>	NTCP	Modulate				Phase 1 full results YE 2026	~100K patients
AX-0811 <i>for Cholestatic diseases</i>	NTCP	Modulate				Target engagement data in early January 2027	
AX-0422 <i>for Hurler Syndrome</i>	IDUA	Correct				CTA filing early 2027; Initial patient data in H1 2027	~500-1000 patients
AX-2911 <i>for MASH</i>	PNPLA3	Correct				FIH H1 2027	~8M patients
AX-2402 <i>for Rett syndrome</i>	MECP2 R270X	Correct					 ~5K
PARTNERED PIPELINE 10 undisclosed targets (option to expand to 15)			Progress undisclosed				



FIRST CLINICAL VALIDATION OF AXIOMER RNA EDITING

Initial AX-0810 target engagement data
Biliary atresia selected as initial indication for NTCP



HIGH IMPACT PARTNERSHIP

Ongoing execution across
\$3.9B collaboration with Eli Lilly



ADVANCE AXIOMER PLATFORM

ProQR's AI-enabled discovery;
autonomous HTS with Ginkgo Bioworks



RUNWAY THROUGH MID 2028

€117.1M cash and cash equivalents as of end Q2 2026;
multiple upcoming clinical catalysts

Biliary atresia is a severe pediatric disease

with no approved therapies



DIAGNOSIS

Pediatric: in the first weeks of life



POPULATION

1 in 8,000 to 18,000 live births



SYMPTOMS

Jaundice, poor weight gain, pale stool, dark urine



PROGRESSION

Rapid progression to cirrhosis and portal hypertension early in life



STANDARD OF CARE

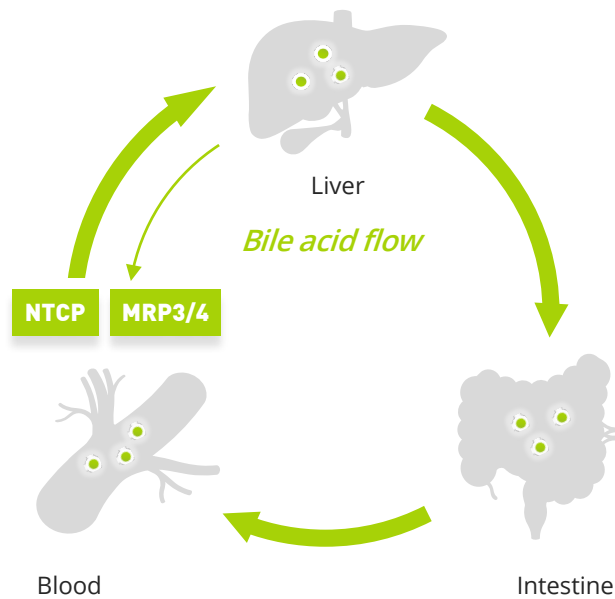
Kasai portoenterostomy as first-line therapy

Adike A, et al. Expert Rev Gastroenterol Hepatol. 2018 Oct;12(10):1025-1032; Verkade HJ, et al. J Hepatol. 2016 Sep;65(3):631-42; Sundaram SS, et al. Liver Transpl. 2017 Jan;23(1):96-109; Raghu VK, et al. Liver Transpl. 2021 May;27(5):711-718; NORD, 2019, Japanese Biliary Atresia Society. Japanese Biliary Atresia Registry (JBAR). <https://jbas.net/en/national-registration/>.

NTCP RNA editing redirects toxic bile acids away from the liver

HEALTHY LIVER

BAs homeostasis



Bile acids (BAs) homeostasis and recycling in the enterohepatic cycle

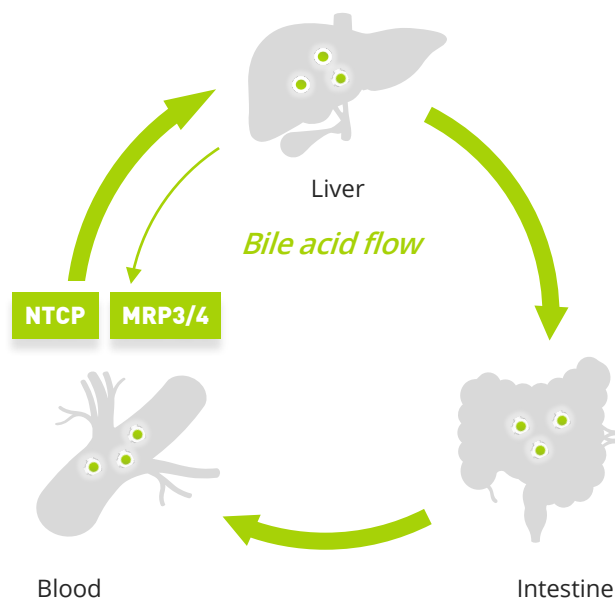
BILIARY ATRESIA

NTCP MODULATION *with RNA editing*

NTCP RNA editing redirects toxic bile acids away from the liver

HEALTHY LIVER

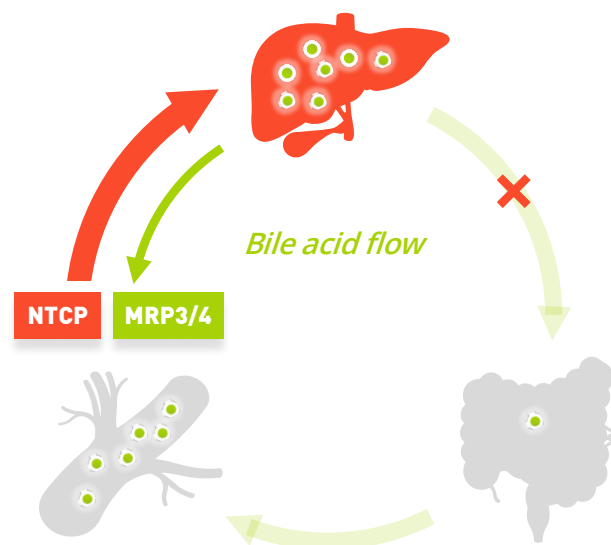
BAs homeostasis



Bile acids (BAs) homeostasis and recycling in the enterohepatic cycle

BILIARY ATRESIA

Toxic BAs lead to intrahepatic cholestasis



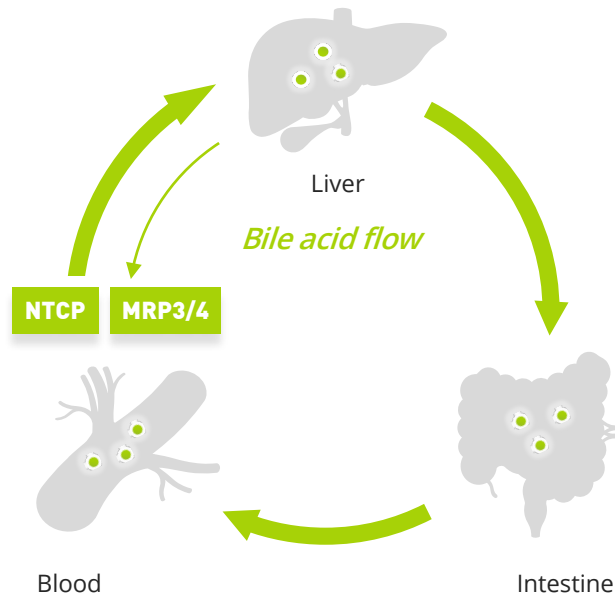
Deterioration of liver health biomarkers (increase in ALT, AST, GGT, bilirubin, INR), fibrosis biomarkers and negative clinical outcomes

NTCP MODULATION *with RNA editing*

NTCP RNA editing redirects toxic bile acids away from the liver

HEALTHY LIVER

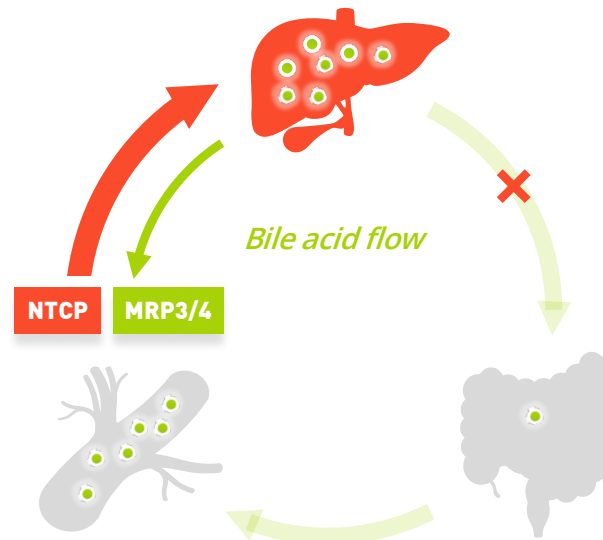
BAs homeostasis



Bile acids (BAs) homeostasis and recycling in the enterohepatic cycle

BILIARY ATRESIA

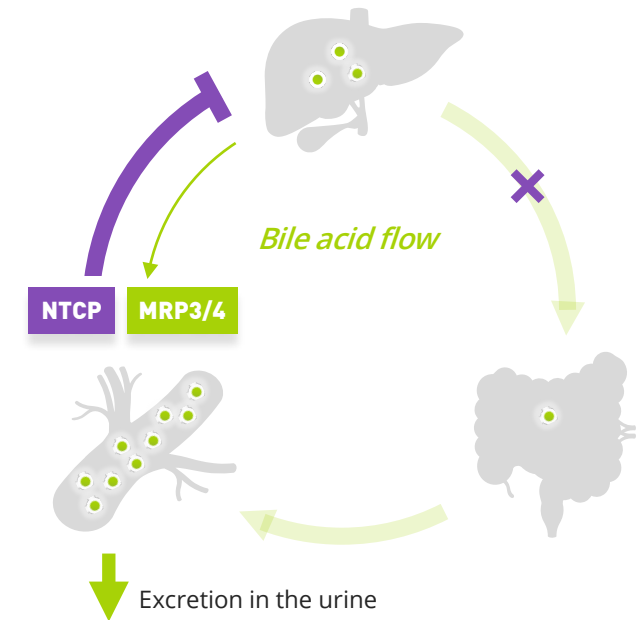
Toxic BAs lead to intrahepatic cholestasis



Deterioration of liver health biomarkers (increase in ALT, AST, GGT, bilirubin, INR), fibrosis biomarkers and negative clinical outcomes

NTCP MODULATION with RNA editing

Decrease in toxic BAs in liver



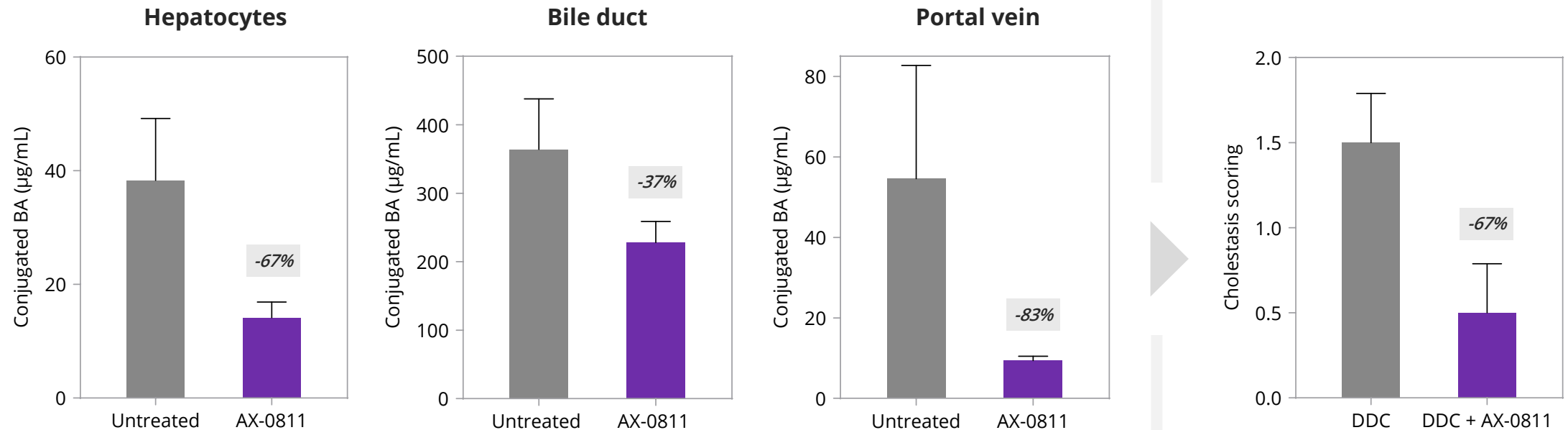
Improvement in liver health biomarkers
Positive clinical outcomes

NTCP modulation decreases toxic bile acids in the liver and improves cholestasis in vivo



REDUCED BILE ACID UPTAKE THAT DRIVES LIVER TOXICITY

IMPROVED CHOLESTASIS



AX-0811 led to 67% decrease in hepatocytes bile acid levels

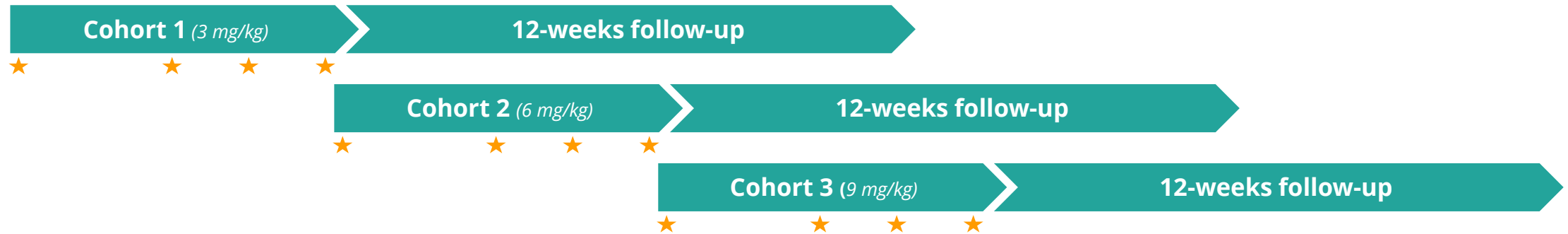
Lower intrahepatic load reduced downstream bile acid levels

AX-0811 improved cholestasis by ~67% in cholestatic animals

Conjugated bile acid (BA) reported is relative quantification of taurocholic acid (TCA) compared to internal standard, the primary substrate for NTCP-mediated hepatic uptake in mice, and is consistently detected above the quantification threshold, enabling reliable spatial measurements across the porto-central axis. Treatment conditions: left 3 graphs, humanized mice treated with SC administration of AX-0811 at 30 mg/kg, 10 doses, n=2, Maldi MS Imaging, Mean, SEM; Right, Humanized mice treated with SC administration of AX-0811, 30 mg/kg, 12 doses, n=4, D31, Mean, SEM

AX-0810 FIH Phase 1 study

Multiple ascending dose (MAD) N=33 healthy volunteers (24 on treatment, 9 on placebo)



DMC safety reviews before proceeding to next dose and dose escalation is sequential during the dosing phase; Cohort 3 ongoing

Objectives

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

Key biomarkers of target engagement

- TUDCA challenge
- Bile acids profile
- Change in bile acid levels

Cohort 3 and 12-week follow-up full results by YE 2026

DMC, Data Monitoring Committee; MAD, Multiple Ascending Dose; PK, Pharmacokinetics; NTCP, sodium taurocholate co-transporting polypeptide; TUDCA, Tauroursodeoxycholic acid; AX-0810 CTA approved in Europe (Oct 2025)

Baseline characteristics AX-0810 Phase 1

Characteristic	Placebo (Cohort 1 + 2, n=6)	3 mg/kg (Cohort 1, n=8)	6 mg/kg (Cohort 2, n=8)
Age, years (mean ± SD)	40.8 ± 8.98	40.9 ± 9.36	40.1 ± 11.4
Age, years (min-max)	27 – 50	26 – 55	27 – 54
Male, n (%)	5 (83%)	8 (100%)	8 (100%)
Female, n (%)	1 (17%)	0	0
Weight, kg (mean ± SD)	80.1 ± 10.4	80.6 ± 10.1	78.5 ± 10.2
Weight, kg (min-max)	66 – 97	67.1 – 98.1	67.9 – 95.7
BMI, kg/m ² (mean ± SD)	24.1 ± 2.46	25.1 ± 1.91	24.4 ± 2.48
BMI, kg/m ² (min-max)	22.3 – 29	22.8 – 28.4	20.1 – 27.4
TBA, µmol/L (mean ± SD)	2.2 ± 1.7	3.2 ± 1.3	1.9 ± 1.0
TBA, µmol/L (min-max)	1.1 - 5.5	1.9 – 5.9	0.8 - 3.8

The two cohorts are well balanced with respect to age, body weight, and BMI

AX-0810 demonstrated favorable profile

Category	3 mg/kg AX-0810 + placebo (N=11), blinded, n (%)	6 mg/kg AX-0810 + placebo (N=11), blinded, n (%)	Overall (N=22) n (%)
Number of all AEs	35	16	51
Participants with ≥1 AE	7 (63.6)	6 (54.5)	13 (59.1)
AEs by relationship			
AEs related to study drug	1 (9.1)	3 (27.3)	4 (18.2)
AEs unrelated to study drug	6 (54.5)	3 (27.3)	9 (40.9)
AEs by severity			
Mild	5 (45.5)	6 (54.5)	11 (50.0)
Moderate	2 (18.2)	0	2 (9.1)
Severe	0	0	0
SAEs	0	0	0
AEs leading to study drug withdrawal	0	0	0
AEs leading to early termination	0	0	0

No safety or tolerability issues to date

- No discontinuations
- No SAE's reported

NTCP modulation mechanism as expected

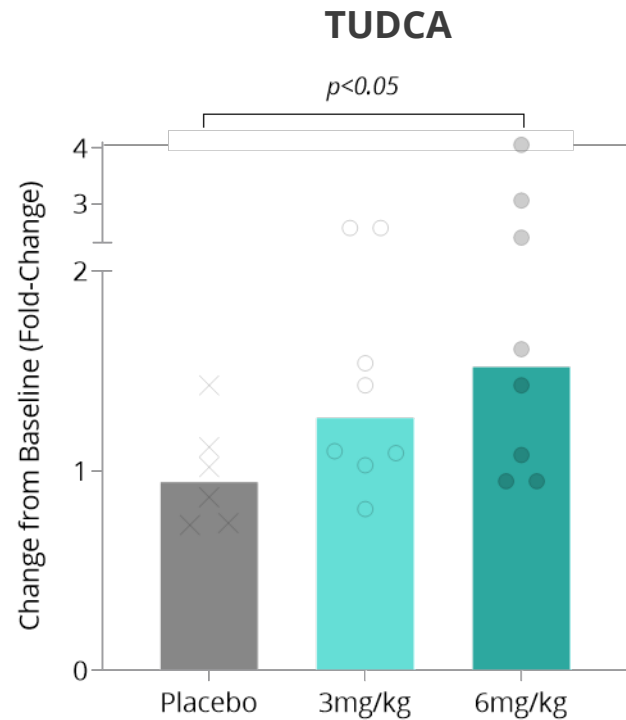
- No clinically significant changes in liver enzymes
- No pruritus
- No changes in hormone or vitamin D levels
- No changes in bilirubin (no OATP off-target)

PK in line with expected profile

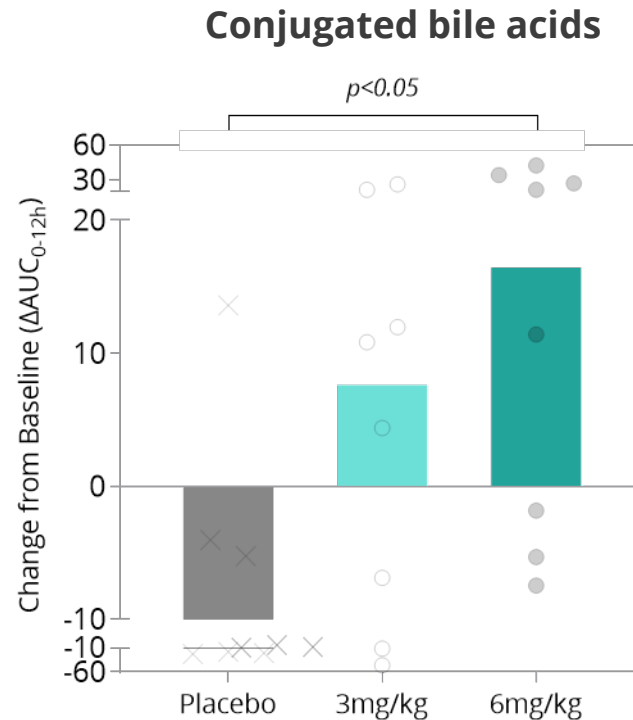
- AX-0810 has a half-life in human of 8 weeks

AEs, Adverse events; SAEs, Serious adverse events. Values represent n (%) of participants unless otherwise stated. Data shown through the cutoff date (May 26, 2026 for AEs summaries, and June 1, 2026 for Laboratory data); covers 30 days on treatment and additional post-treatment follow-up. PK half-life based on available data (3mg/kg)

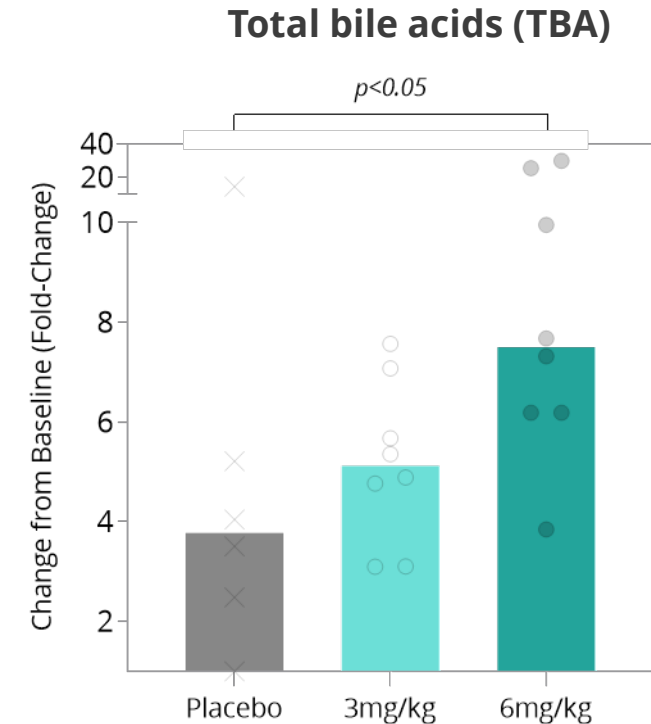
All 3 biomarkers confirmed target engagement



Increased circulating TUDCA confirmed
NTCP specificity



Selective increase in conjugated bile
acids showed specific NTCP modulation

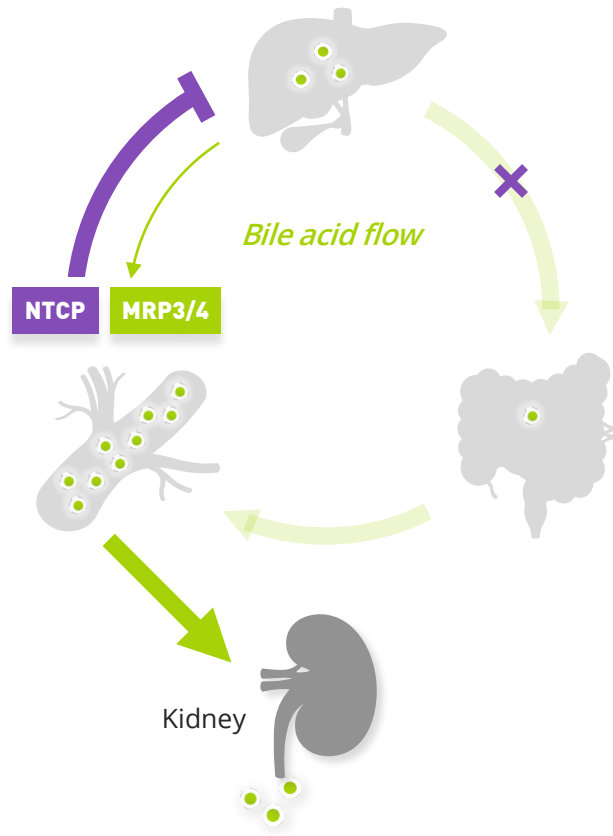


Up to 8-fold increase in TBA in serum
showed bile acids uptake modulation

Figures: median + individual values. Total Bile Acids (TBA) and TUDCA: C_{max} week 5 / baseline. Conjugated bile acids: ΔAUC_{0-12h} (μmol/L x h) (week 5 – baseline). Post-hoc statistical analyses. N=22 (n=6 Placebo, crosses; n=8 Cohort 1 | 3mg/kg, circles; n=8 Cohort 2 | 6mg/kg, dots); 4 doses AX-0810; GalNAC conjugation; Subcutaneous

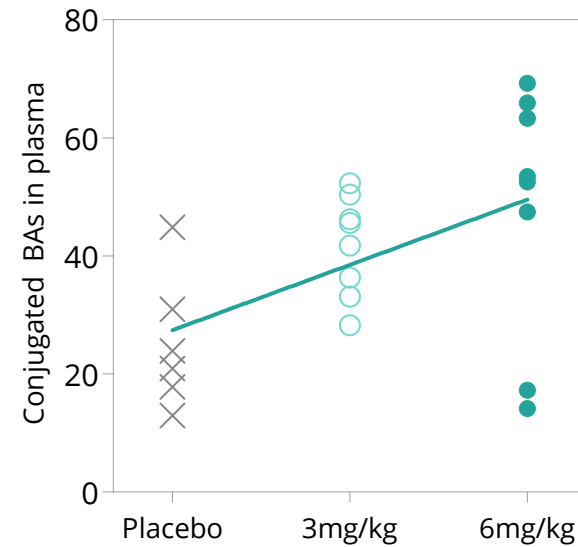
AX-0810 increases renal clearance of conjugated bile acids

NTCP MODULATION with RNA editing



Plasma conjugated BAs

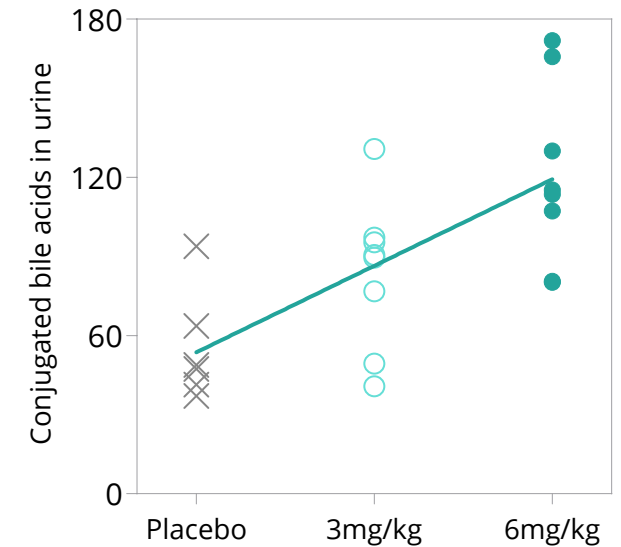
$r = 0.53, p = 0.012$



Decreased
liver exposure

Urinary conjugated BAs

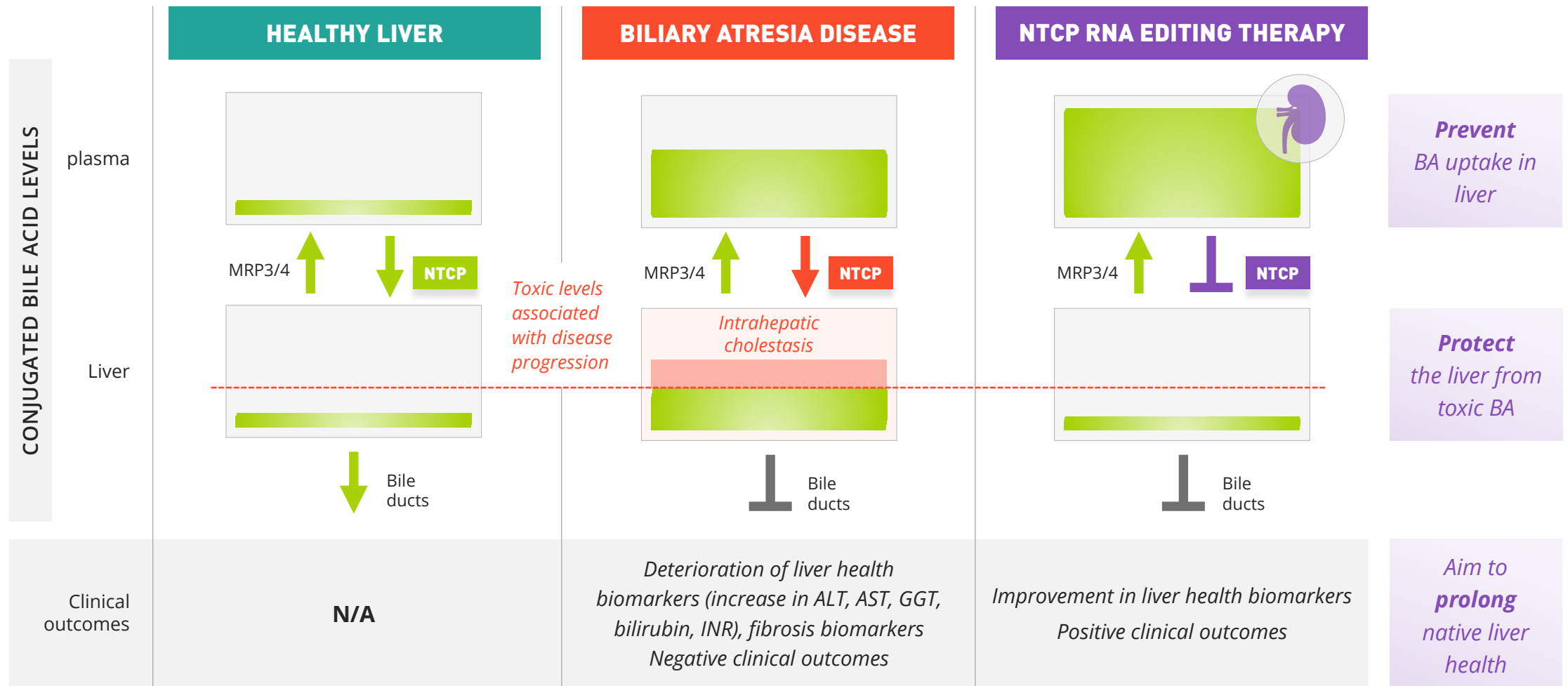
$r = 0.68, p = 0.0004$



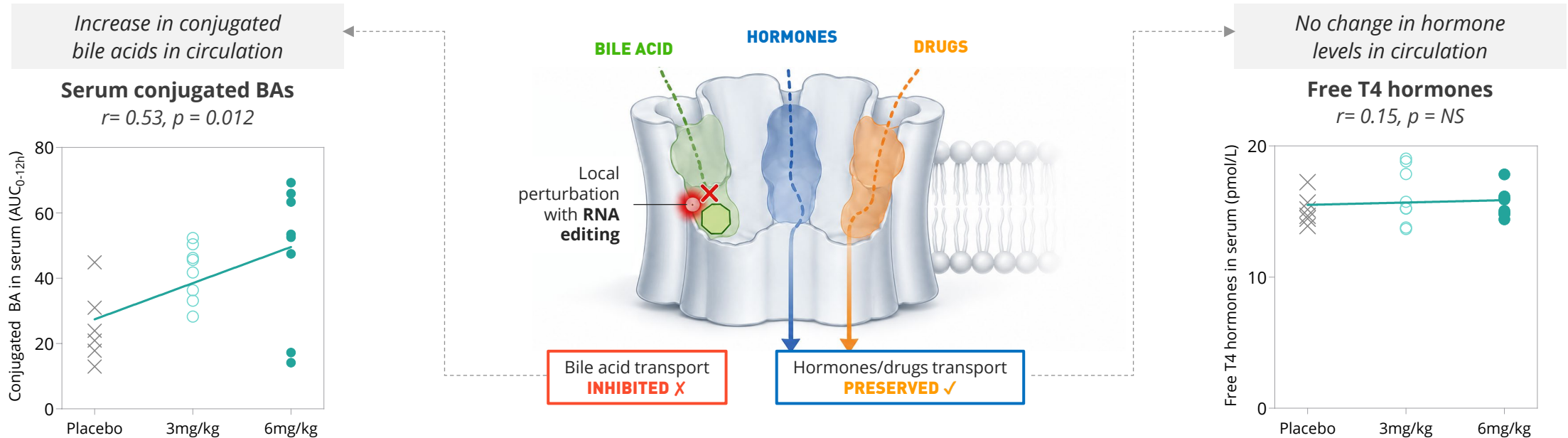
Increased
renal clearance

In serum, values represent each individual AUC (in serum from 0-12h) on Week 5 ($\mu\text{mol/L} \times \text{h}$). In urine, represented total amount cleared 0-24h on week5 ($\text{nmol/L} \times \text{h}$). Pearson correlation calculated and significance indicated. Post-hoc statistical analyses. N=22 (n=6 Placebo, crosses; n=8 Cohort 1 | 3mg/kg, circles; n=8 Cohort 2 | 6mg/kg, dots); 4 doses AX-0810; GalNAc conjugation; Subcutaneous

NTCP RNA editing to improve clinical outcomes in cholestasis



NTCP RNA modulation is highly selective



RNA editing is designed to modulate NTCP bile acid reuptake and maintain its other functions

- NTCP protein transports bile acids and hormones through distinct binding sub-pockets
- AX-0810 only edits the sub-pocket that transports bile acid, leaving the sub-pocket that transports hormones functional

AX-0810 Phase 1 clinical data demonstrates that hormonal levels are unchanged Important to maintain NTCP's other functions

- Mitigates thyroid dysfunction, growth and neurodevelopmental concerns associated with full NTCP inhibition
- Maintains normal drug metabolism
- Enables safer, longer-term therapy for chronic cholestasis patients

NS, not significant. References: Ruggiero MJ, et al. J Biol Chem. 2021 Jan-Jun;296:100047; Park JH, et al. Nature. 2022 Jun 30;606(7916):1027-1031; Ho RH, et al. J Biol Chem. 2004 Feb 20;279(8):7213-7222.

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

Importance of bile acids and enterohepatic circulation in cholestatic diseases

Prof. Gideon Hirschfield

**THE AUTOIMMUNE & RARE
LIVER DISEASE PROGRAMME**

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

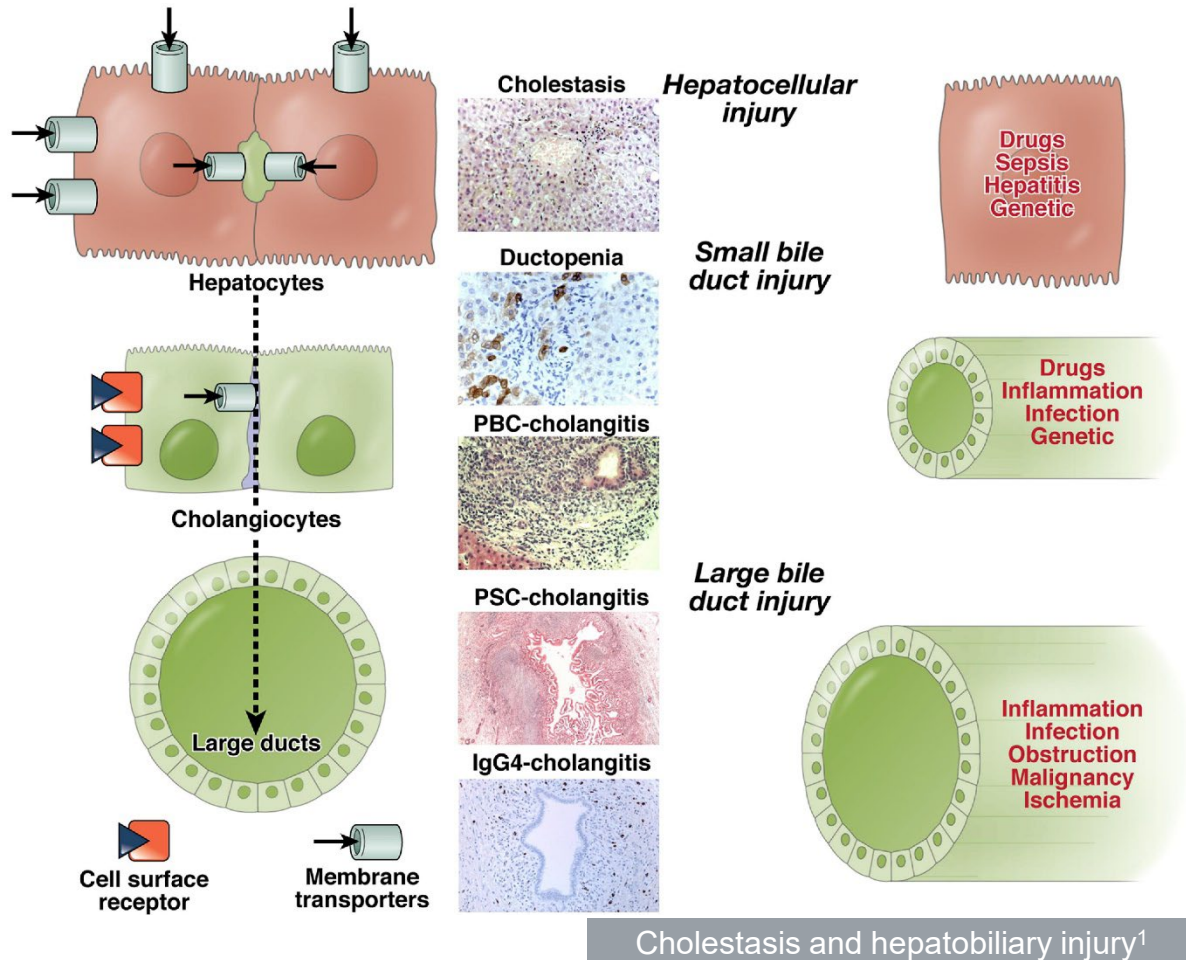
Care

Teaching

Research

Causes of Cholestasis

Aetiologies of cholestatic liver disease are related to the site of hepatobiliary insult¹



Causes of Cholestasis ²	
Intrahepatic Cholestasis	Extrahepatic Biliary Tract Diseases
Biliary Intrahepatic PBC PSC Malignancy Granulomatous Post-transplant ductopenia PFIC3 Alagille Syndrome Biliary atresia	Biliary atresia Choledocholithiasis Bile duct tumors Ampullary tumors Pancreatic carcinoma Mirizzi's syndrome AIDS cholangiopathy Parasites PSC
Hepatocellular ICP Drug injury PFIC1, PFIC2, PFIC4+ Hepatitis (viral and alcoholic)	

AIDS, acquired immunodeficiency syndrome; ICP, Intrahepatic cholestasis of pregnancy; PBC, primary biliary cirrhosis; PFIC, Progressive familial intrahepatic cholestasis; PSC, primary sclerosing cholangitis.

1. Hirschfield GM, et al. Gastroenterol. 2010;139(5):1481-1496. 2. Adapted from Sadiku E, et al. Albanian Med J. 2015;4:72-82.

2. Slide courtesy of Richard Thompson

ProQR Therapeutics – Educational Event September 2026

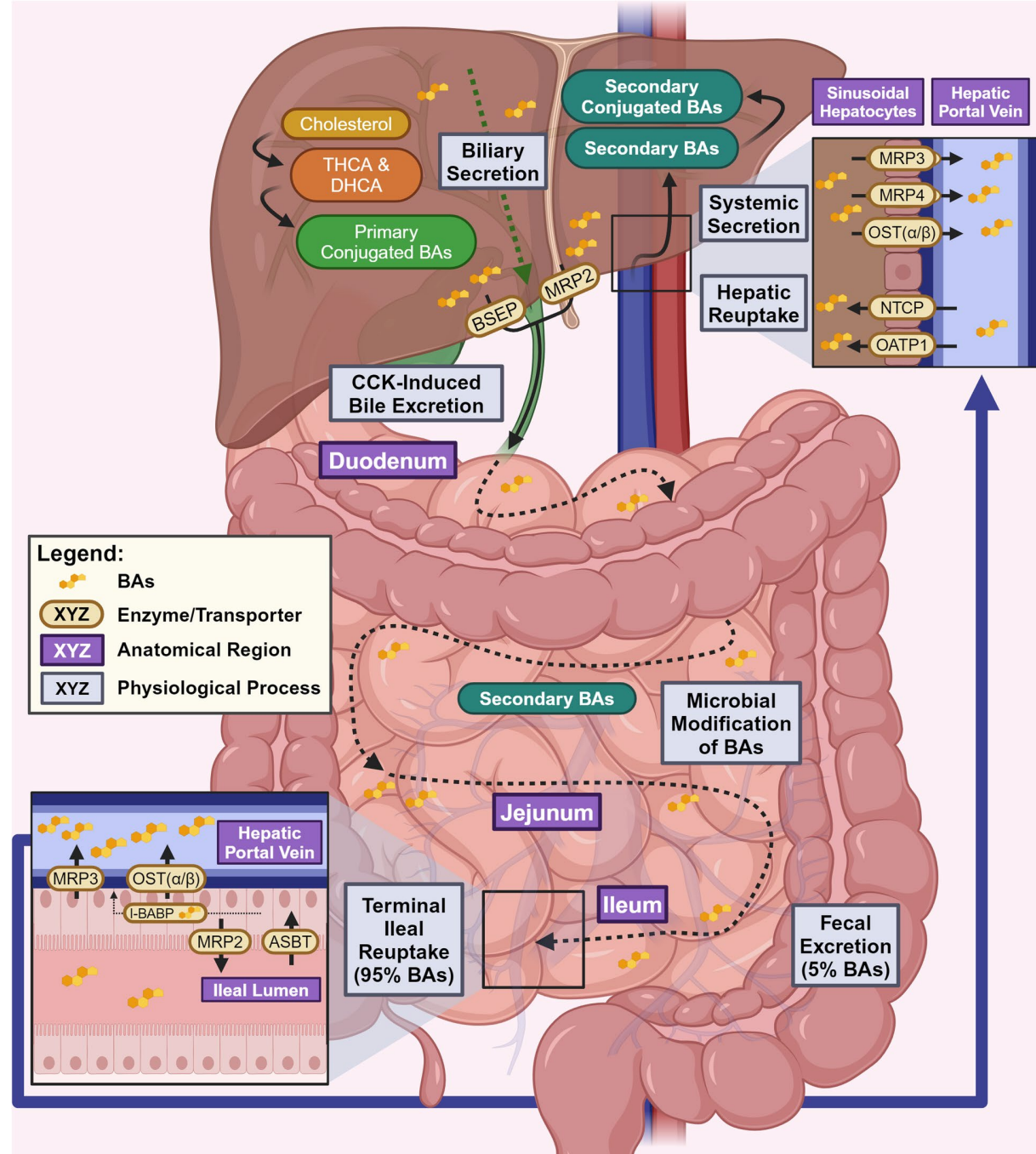


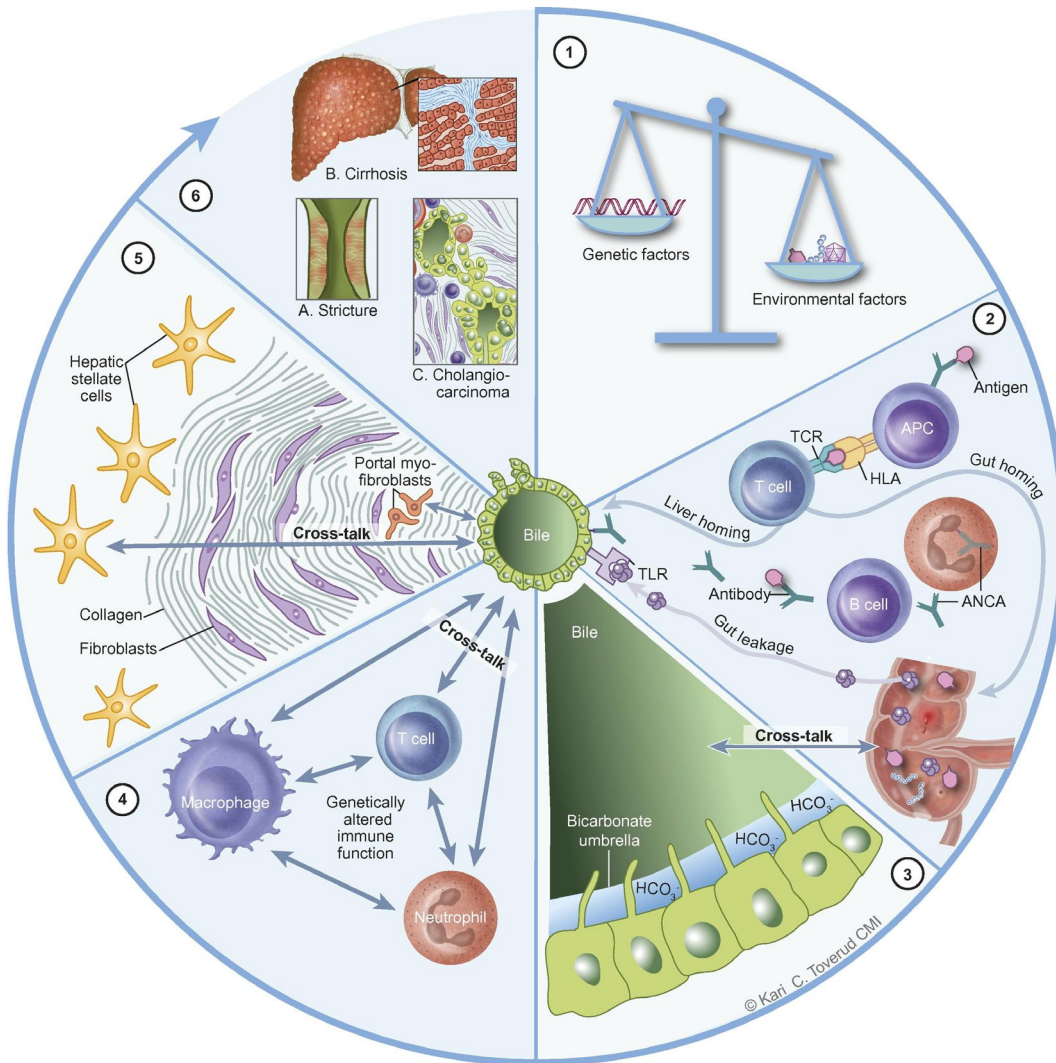
Table 1. Functions of Bile Acids*

Process	Site	Mechanism
Whole Organism		
Cholesterol elimination	Hepatocyte	Conversion to bile acids
		Stimulation of biliary cholesterol secretion
	Biliary tract	Solubilization of cholesterol in mixed micelles
	Intestine	Fecal excretion of cholesterol and bile acids
Hepatobiliary		
Stimulation of bile flow	Hepatocyte	Canalicular secretion of osmotically active membrane-impermeable molecules
	Cholangiocyte	Potentiation of secretin-stimulated bicarbonate secretion
Stimulation of biliary PC secretion	Hepatocyte	Detachment of PC from canalicular membrane
	Biliary tract	Solubilization of PC in mixed micelles
Feedback inhibition of bile acid and cholesterol biosynthesis	Hepatocyte	Repression of cholesterol 7 α -hydroxylase and HMG-CoA reductase
Intestinal		
Enhancement of lipid absorption	Small intestine	Formation of mixed micelles that promote diffusion through the unstirred layer
Cleaning of absorptive surface†	Small intestine	Surface activity of the bile acid anions
Stimulation of intestinal motility†	Large intestine	Neural arc

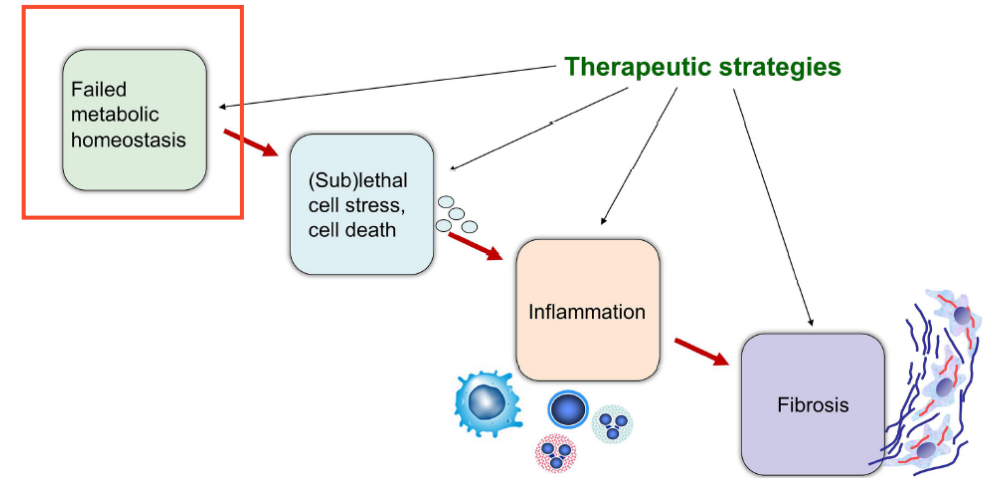
*PC indicates phosphatidylcholine; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A.

†Postulated, not an established function.

Multiple molecular mechanisms involved in cholestatic diseases



Toxic bile acid accumulation



Article

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

<https://doi.org/10.1038/s41586-019-1785-z>
Received: 24 October 2018
Accepted: 17 September 2019
Published online: 27 November 2019

Saiyu Hang^{1,2}, Donggi Paik^{1,2}, Lina Yao², Eunha Kim¹, Trinath Jamma², Jingping Lu⁴, Soyoung Ha¹, Brandon N. Nelson⁵, Samantha P. Kelly⁶, Lin Wu⁶, Ye Zheng⁷, Randy S. Longman⁸, Fraydoon Rastinejad⁴, A. Sloan Devlin², Michael R. Krout⁵, Michael A. Fischbach^{2*}, Dan R. Littman^{5,10*} & Jun R. Huh^{1,9*}

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

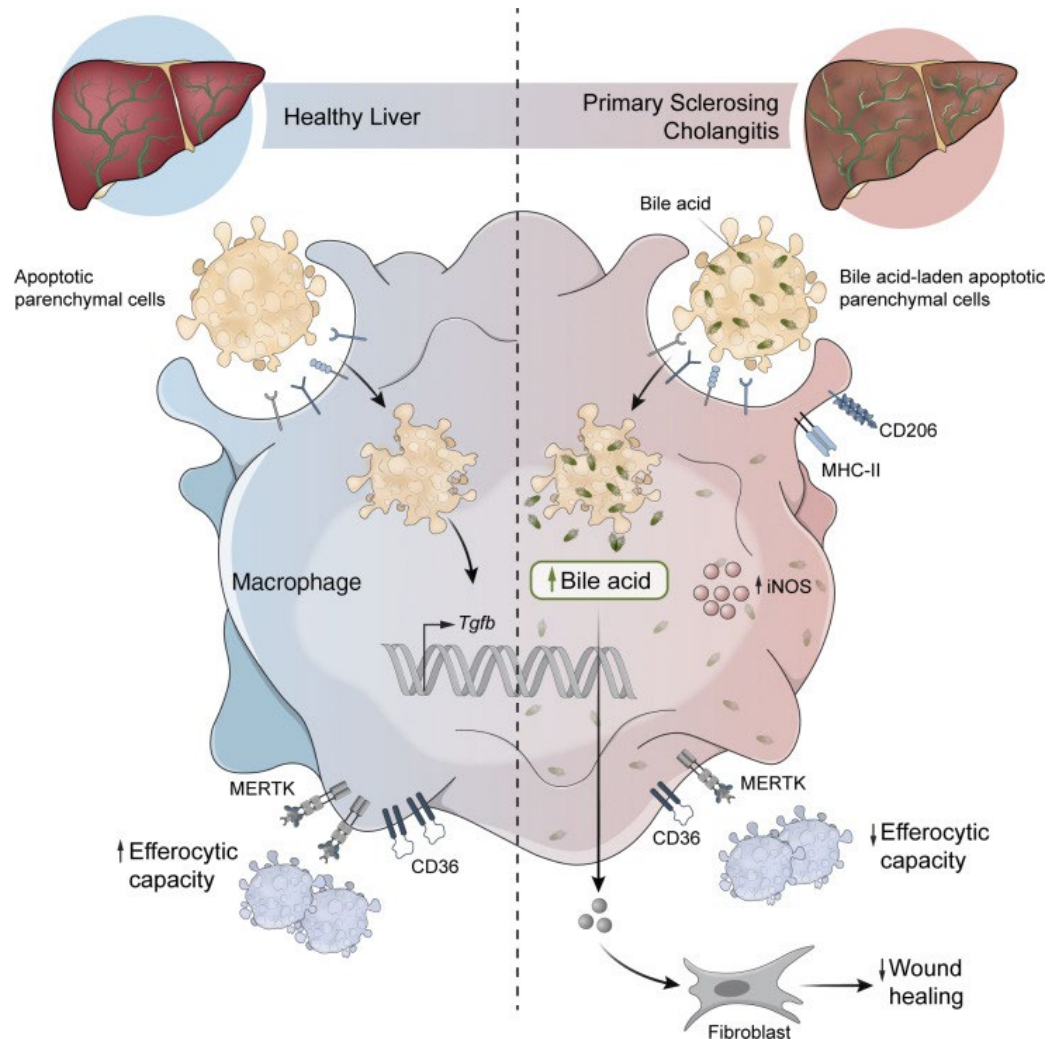
<https://doi.org/10.1038/s41586-022-04480-z>
Received: 4 December 2020
Accepted: 27 January 2022
Published online: 16 March 2022

 Check for updates

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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 differentiation¹. 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.

Bile acid retention drives pro-inflammatory program in macrophages



Content of the phagocytosed dying cells, specifically bile acid-laden hepatocytes, drives a pro-inflammatory program in the corresponding efferocytic macrophages, potentially contributing to chronic hepatic inflammation.

Bile acid physiology and potential toxicity

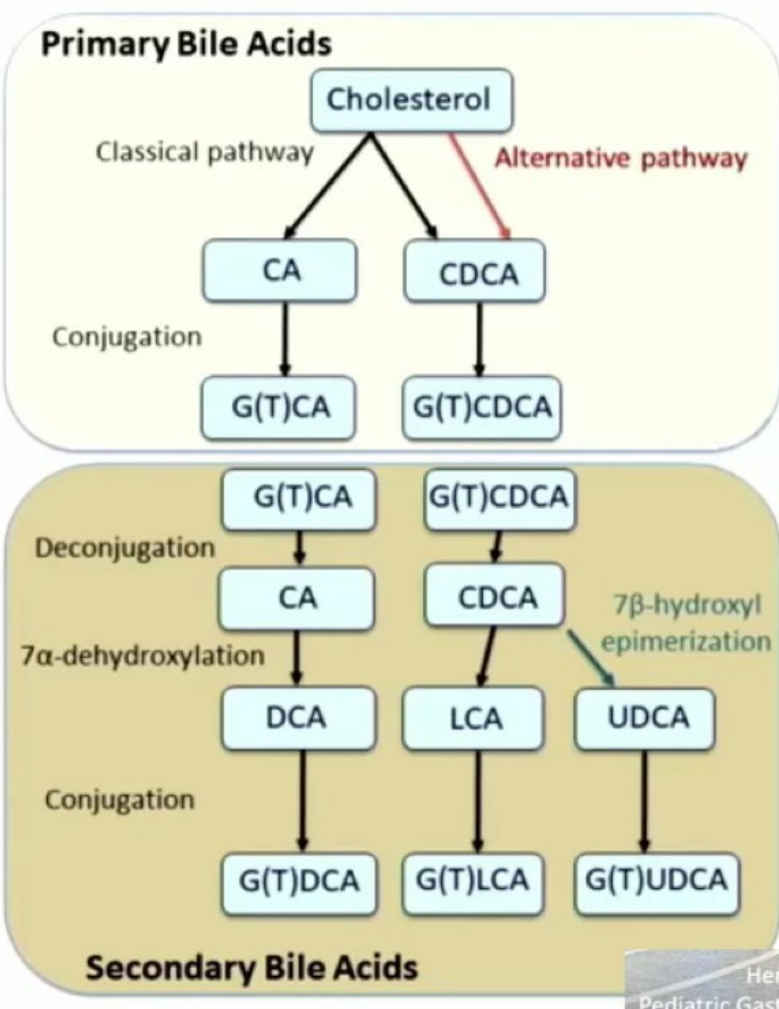
Bile acids are cytotoxic when their concentrations increase to abnormally high levels, either intracellularly or extracellularly

Bile acid cytotoxicity is strongly affected by structure: the greater the hydrophobicity, the greater the cytotoxicity. Hydrophobicity is defined operationally by the extent to which bile acids bind to hydrophobic surfaces, and this can be determined by measuring the retention time during liquid chromatography using a hydrophobic adsorbent

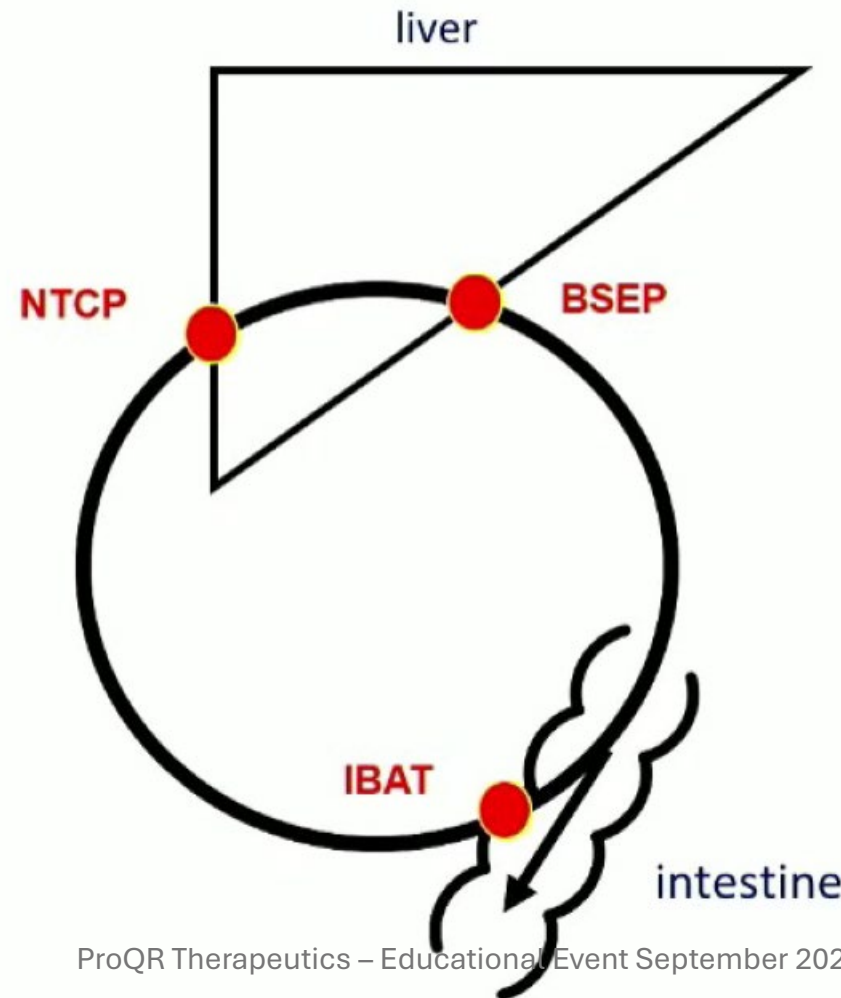
The natural **dihydroxy bile acids CDCA and DCA** bind tightly to the adsorbent, have a long retention time, are hydrophobic by this definition, and are **highly cytotoxic**.

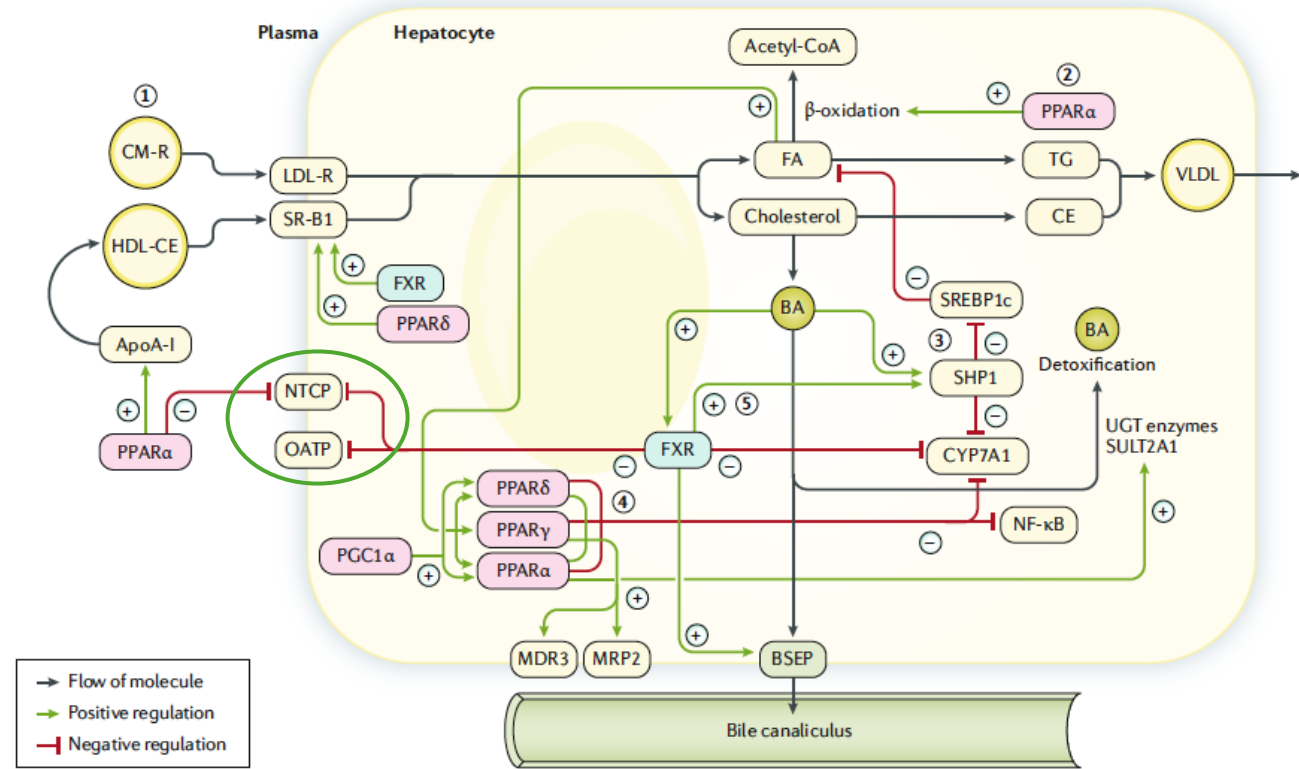
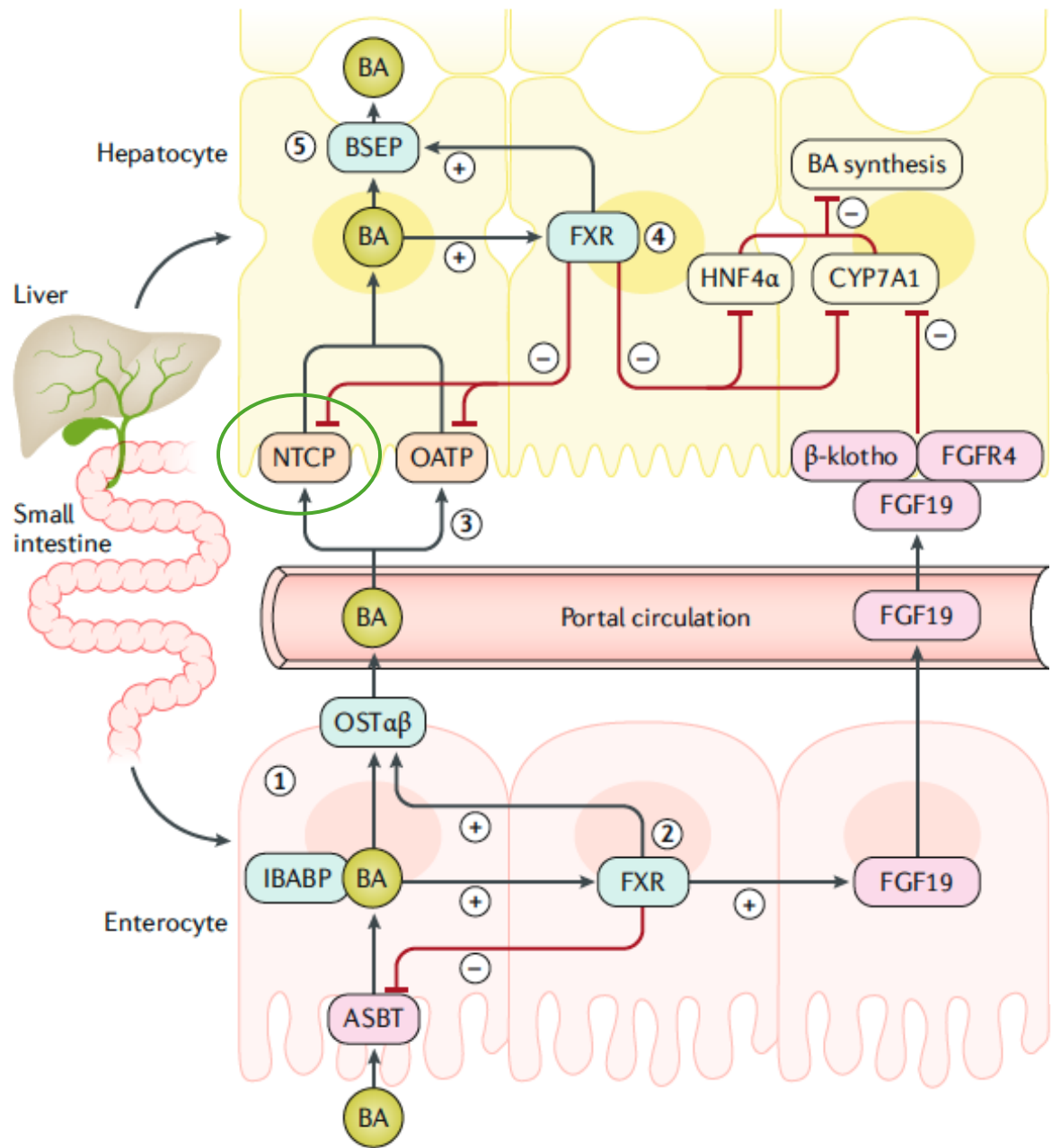
Ursodeoxycholic acid, although a dihydroxy bile acid, does not bind to the adsorbent, has a short retention time, is hydrophilic, and **devoid of cytotoxic properties** in most model systems.

Cholic acid is intermediate, being noncytotoxic at low concentrations, but cytotoxic at very high concentrations.



The three major protein transporters for conjugated bile acids





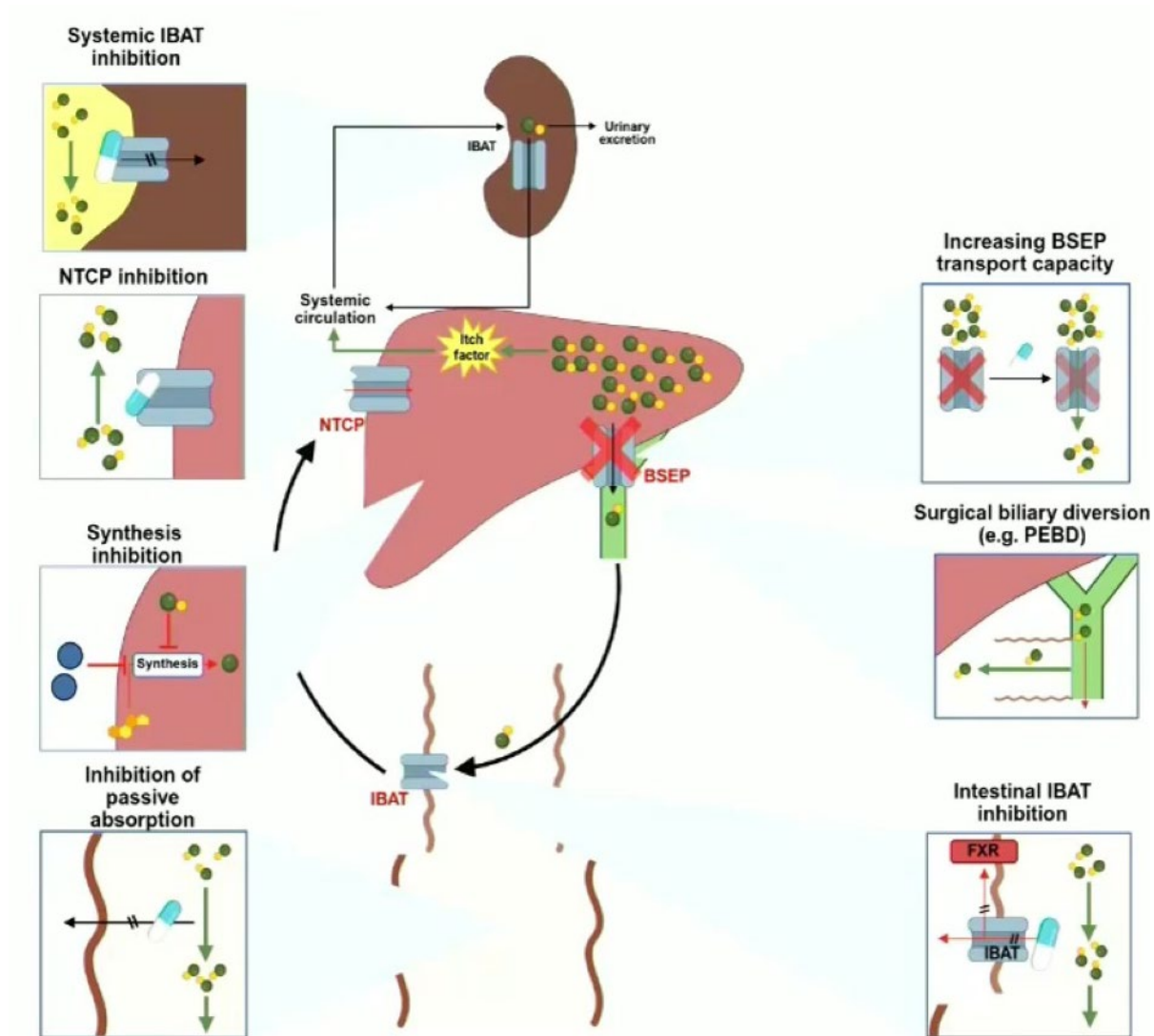
NTCP deficiency leads to conjugated hypercholanemia without a clear clinical phenotype

Table 1. Routine Clinical Chemistry and Bile Salt Profiles

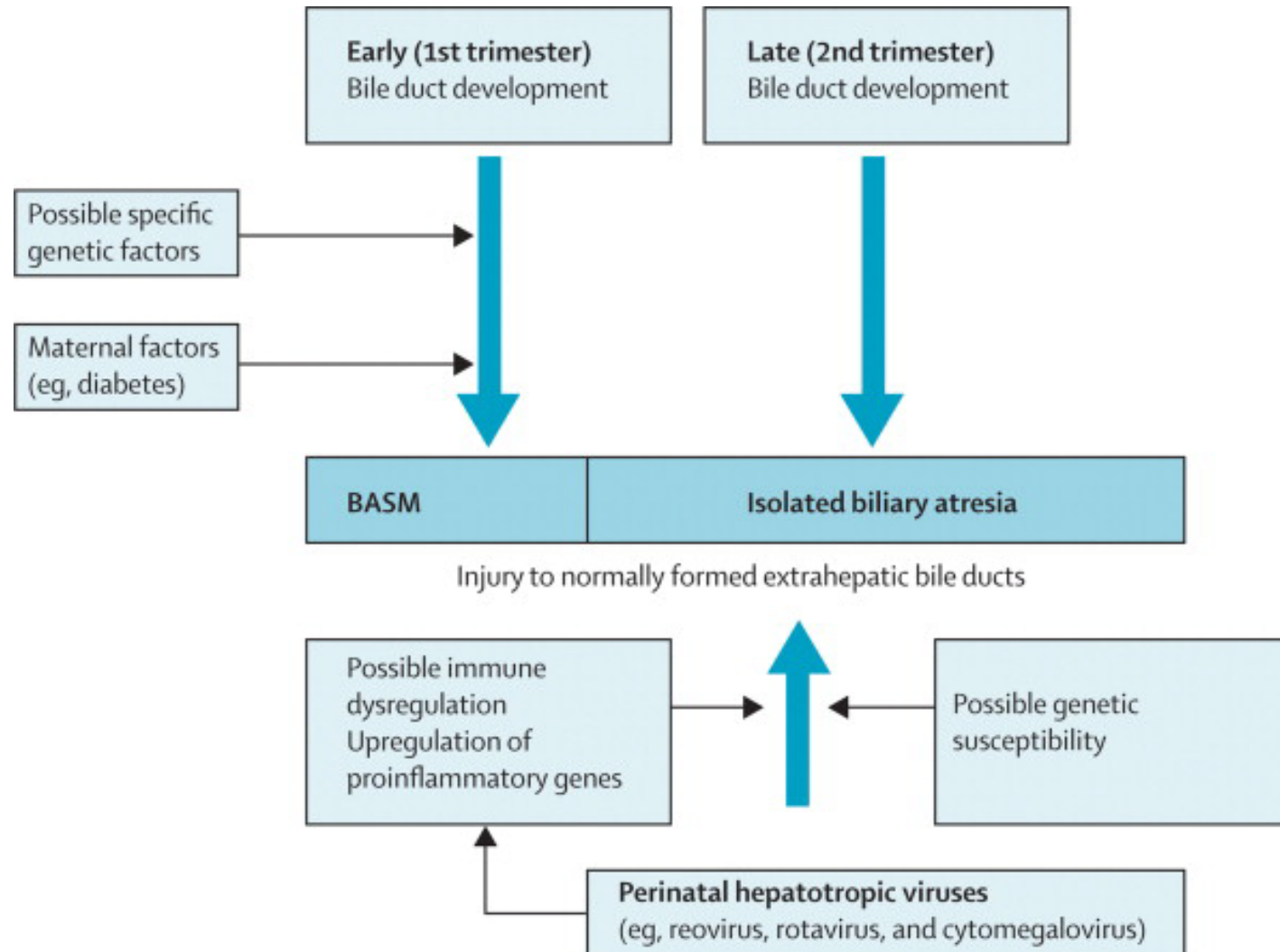
			Age (years)							
Ref.			0.8	1.3	2.4	2.8	2.9*	3.3	4.8 [†]	5.3 [†]
Total bile salts	μM	0.8-16.3		445	1531		1009 (2.1)	1225 (2.4)	533 (4.5)	494 (6.7)
Routine Clinical Chemistry										
Test	Unit	Ref.								
ASAT	(U/l 37°C)	0-40	46	37	37	34	40	33	34	38
ALAT	(U/l 37°C)	0-45	36	17	19	15	22	17	22	47
Bili tot	μM	0-17		13	13	13	13	14	8	11
Bili dir	μM	0-7					11	4	5	5
ALP	(U/l 37°C)	60-325	112	88	89	72	105	75	80	122
γGT	(U/l 37°C)	0-56	18	14	14	11	14	11	11	14
APTT	sec	22-30		25					25	27
PT	sec	9.7-11.6		13.0					13.0	12.6

- BA retention in systemic circulation
- No pruritus despite high serum BA
- Low intrahepatic BA levels
- Low BA levels in small intestine

Targeting intrahepatic cholestasis



Biliary Atresia: destructive, inflammatory process that affects intra- and extrahepatic bile ducts, leading to fibrosis and obliteration of the biliary tract with the development of biliary cirrhosis

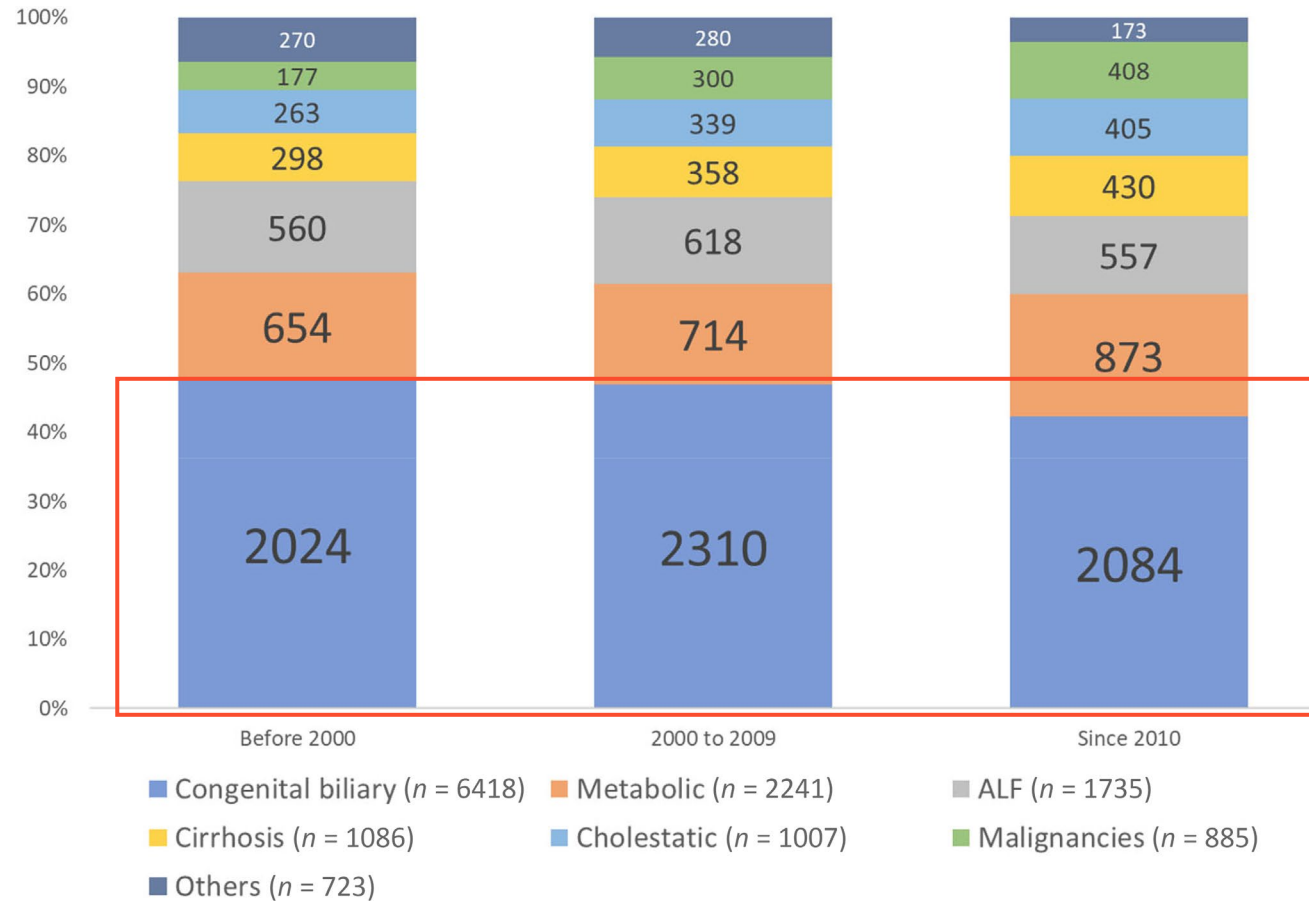


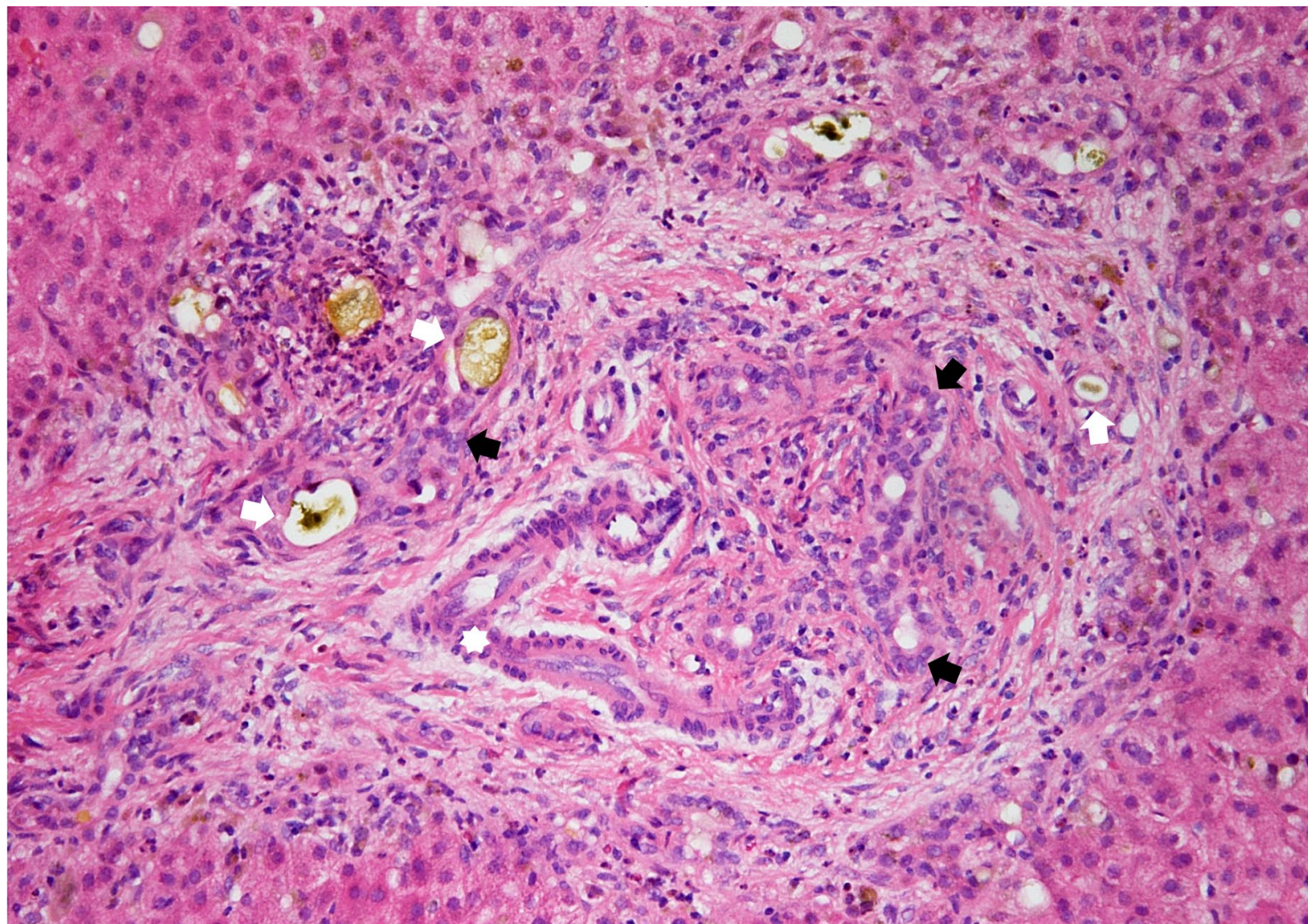
High unmet medical needs in cholestatic diseases: Biliary Atresia

DIAGNOSIS	Pediatric: first weeks of life
POPULATION*	≈ 1 in 10–19,000 in Europe and North America
SYMPTOMS	Jaundice, poor weight gain, pale stool, dark urine
PROGRESSION	Rapid progression to cirrhosis and portal hypertension early in life
STANDARD OF CARE	Kasai procedure as first-line therapy UDCA SoC for infants and children post-Kasai
SIGNIFICANT UNMET NEED	No approved pharmacological treatments 60-80% require liver transplant despite Kasai

Adike A, et al. Expert Rev Gastroenterol Hepatol. 2018 Oct;12(10):1025-1032; Verkade HJ, et al. J Hepatol. 2016 Sep;65(3):631-42; Sundaram SS, et al. Liver Transpl. 2017 Jan;23(1):96-109; Raghu VK, et al. Liver Transpl. 2021 May;27(5):711-718; NORD, 2019. Japanese Biliary Atresia Society. Japanese Biliary Atresia Registry (JBAR). <https://jbas.net/en/national-registration/>.

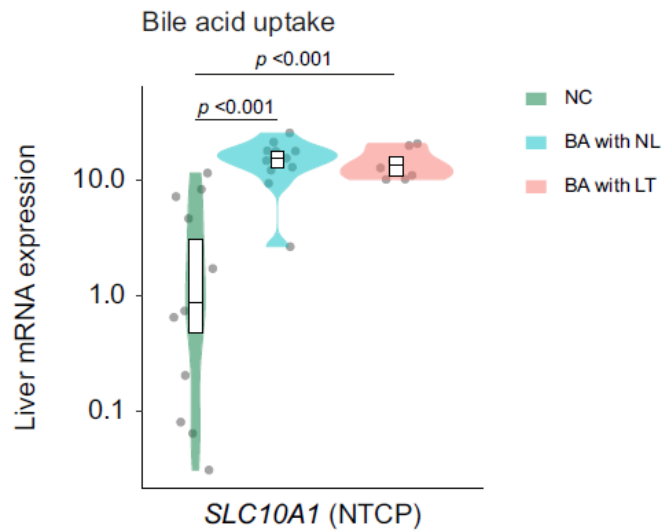
Biliary Atresia is responsible for ~45% of all pediatric liver transplantations



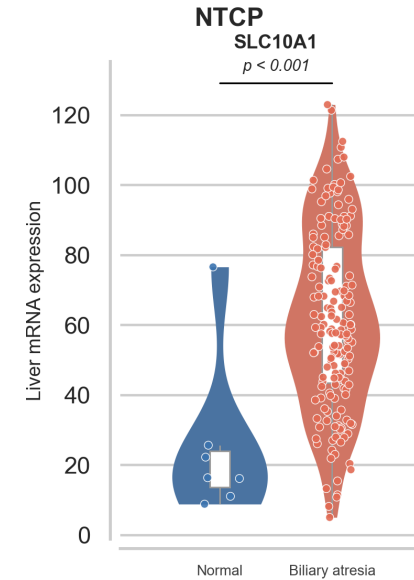


Histologic findings include expansion of the portal tract with stromal edema, prominent fibrosis, bile duct proliferation (black arrows), and bile plugs (white arrows). White star delineates a branching artery.

***SLC10A1* (NTCP: Sodium-taurocholate cotransporting polypeptide) mRNA expression is upregulated in Biliary Atresia**



Nyholm I, et al. J Hepatol. 2025 Aug;83(2):440-452

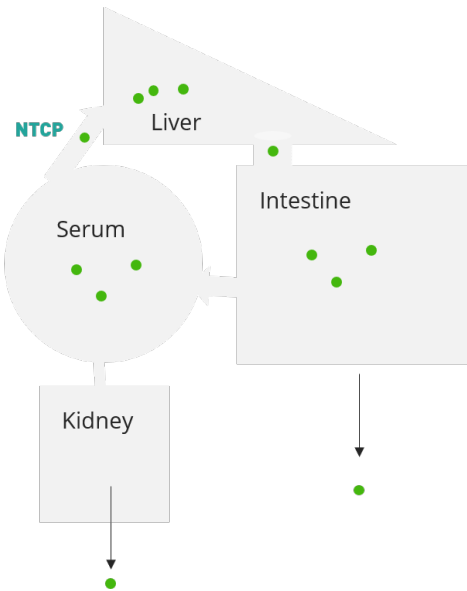


Luo Z, et al. Gastroenterology. 2019 Oct;157(4):1138-1152.e14

Accumulation of bile acids in the liver is the major factor in pathophysiology of disease

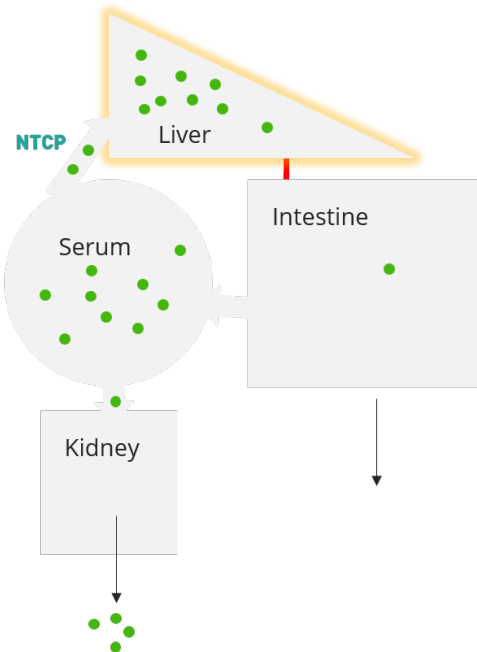
IN HEALTHY INDIVIDUALS - BILE ACIDS HOMEOSTASIS IN THE ENTEROHEPATIC CYCLE

Bile acid levels in each compartment are tightly regulated



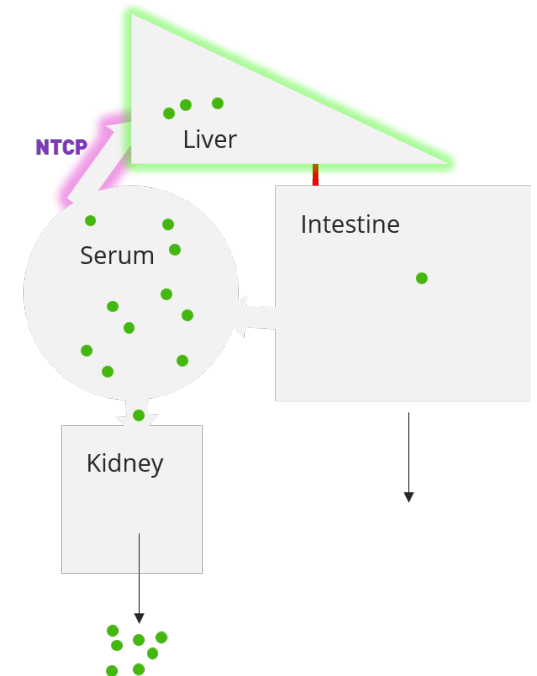
IN BILIARY ATRESIA PATIENTS - CONSISTENT INCREASE IN LIVER AND SERUM

Accumulation of bile acids in the liver, spill over in the circulation / Associated with decrease in native liver survival, portal hypertension and varices development



NTCP MODULATION LEADS TO OPPOSITE PATTERN BETWEEN LIVER AND PLASMA

Increase in serum bile acids is associated with a decrease liver toxic bile acid load



Disease vs NTCP modulation: plasma bile acids increase leading to opposite effect

	PLASMA BILE ACIDS	LIVER BILE ACIDS	EXPECTED OUTCOME
DISEASE STATE <i>untreated biliary atresia</i>	 HIGH (Nyholm 2025)	 HIGH toxic load → fibrosis (Nyholm 2025)	 POOR High rate of transplant/death (Harpavat 2022)
NTCP MODULATION <i>AX-0810 / AX-0811 editing therapy</i>	 HIGH Demonstrating target engagement in healthy subject	 LOW ↓~67% in animal model	 GOOD Decrease in liver BA load with potential for decrease in negative outcomes (Harpavat 2022)



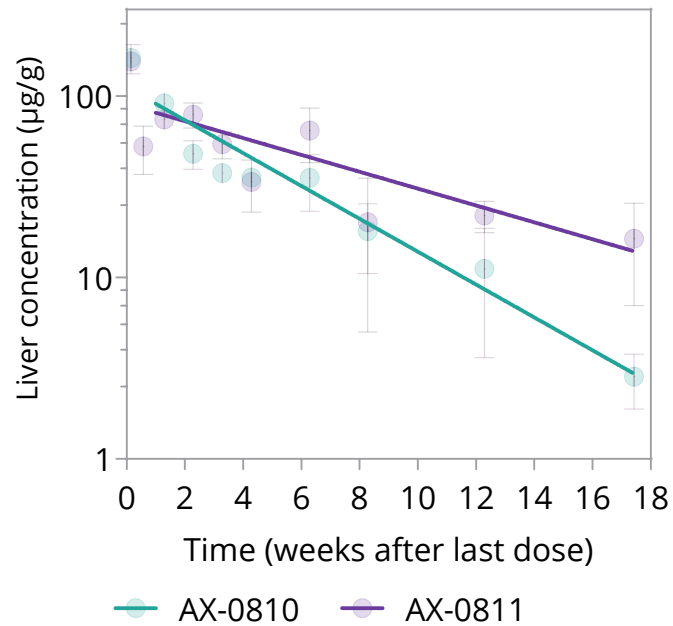
From target engagement to therapeutic effect

Clinical development and next steps

AX-0811 projected to achieve up to 65% editing



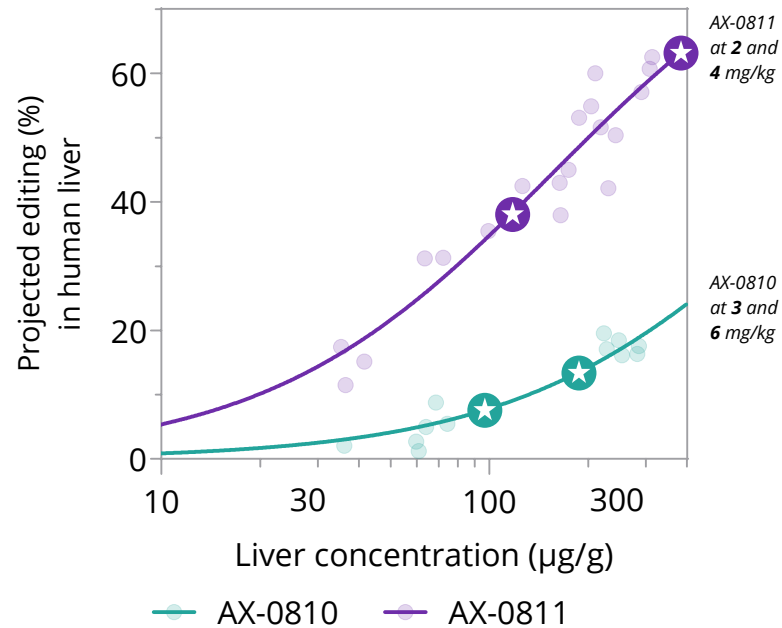
AX-0810 and AX-0811
liver concentration in NHP



AX-0811 has 1.6-fold longer half life



AX-0810 and AX-0811 projected
editing in human liver based
on liver concentration



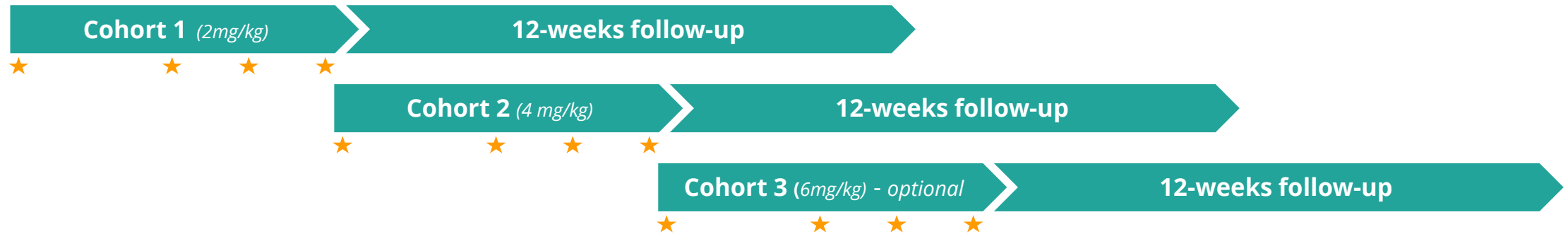
AX-0811 has >4x greater potency *in vivo*

- Based on the AX-0810 human PK correlation, AX-0811 PK modeling predicts **40 and 65% editing in human** at doses of **2 and 4 mg/kg** respectively
- AX-0811 is projected to have
 - 3+ month half-life
 - lower dose levels
 - less frequent dosing

Treatment conditions: left, NHP treated with SC administration of AX-0810 2mg/kg at D1, D3 and D5 or AX-0811 2 mg/kg at D1, D4 and D7. n=4 per treatment - sparse sampling; right, humanized liver mice treated with SC administration of AX-0810 at 50 mg/kg, (1, 4 and 13 injections) and 30 mg/kg (10 injections), n=18, HPLC, and AX-0811 at 3.75 - 30 mg/kg (8 injections), n=19, hECLIA. PK half-life based on available data (3mg/kg)

AX-0811 FIH Phase 1 study

Multiple ascending dose (MAD) N=33 (*healthy volunteers: 24 on treatment, 9 on placebo*)



DMC safety reviews before proceeding to next dose and dose escalation is sequential during the dosing phase

Treatment

AX-0811 GalNAc conjugated editing oligonucleotide

Objectives

- Assess safety, tolerability, and PK of AX-0811
- Confirm target engagement as measured by biomarkers

Key biomarkers of target engagement

- TUDCA challenge
- Bile acids profile
- Change in bile acid levels

CTA enabling activities ongoing

- ✓ CTA filing in mid-2026
- Target engagement data expected from Cohorts 1 and 2 by early January 2027

CTA, Clinical Trial Application; DMC, Data Monitoring Committee; FIH, First in Human; MAD, Multiple Ascending Dose; PK, Pharmacokinetics; TUDCA, Tauroursodeoxycholic acid

Clear biomarker strategy to assess AX-0811 target engagement

AX-0811 expected to exceed AX-0810 in all biomarkers

Greater potency and improved durability at lower dose levels

1

TUDCA

*High specificity to NTCP
and controlled conditions*

2

BILE ACIDS PROFILE

*Translation into the
disease population*

3

TOTAL BILE ACIDS

*Supportive target
engagement*

Translate into disease modification in Biliary Atresia



IIT TRIAL

- *Children 5–11 years with biliary atresia post-Kasai with native liver*
- *Active disease enrichment (e.g., abnormal liver enzymes, presence of liver fibrosis, and abnormal bile acids level)*



PHASE 2 TRIAL

- *BA patients post-Kasai procedure with native liver*
- *Excluding patients with decompensated liver disease or prior liver transplantation*

Target engagement Bile Acid biomarkers

- TUDCA
- Conjugated Bile Acids
- Total Bile Acids

Liver health biomarkers

- Liver injury enzymes
ALT, AST, GGT
- Liver function and fibrosis biomarkers bilirubin, albumin and INR, ProC3, ELF
- Liver stiffness ultrasound elastography

Clinical outcomes

- Liver-related clinical events
- Native liver survival
- Death

Summary of learnings from this event

Framework for evaluating AX-0811 is expected to exceed AX-0810 across all biomarkers in equivalent cohorts, listed in order of importance

- TUDCA – most sensitive PD biomarker
- Conjugated bile acids increase – most informative for translation to disease
 - Urine conjugated BA to directionally align with plasma
- Total bile acids increase - supportive target engagement

Clear strategy to translate from NTCP target engagement into liver health in biliary atresia

- Biological rational for NTCP editing in biliary atresia is very strong
- Robust PoC in disease model demonstrating a 67% reduction in cholestasis
- Clinical data confirms elimination of the disease-causing toxic conjugated bile acids from the system
- NTCP editing is expected to translate into improvements in liver health for biliary atresia, as measured by liver injury enzymes, and liver function, fibrosis and stiffness biomarkers.

A development pipeline *with rich catalyst calendar*



NTCP / CHOLESTATIC DISEASE



IDUA / MPS1



PNPLA3 / MASH

*AX-0810 Ph 1
Cohort 3 and
12-week follow-
up full results
YE 2026*

*AX-0811 target
engagement
HV data
Cohorts 1 & 2
expected by
early Jan 2027*

*IIT initial data
in biliary
atresia patients
H1 2027*

*Ph 2
development
expected start
mid 2027, with
IA mid 2028*

*AX-0422 for
MPS I Hurler
syndrome
initial human
data H1 2027*

*AX-2911 for
PNPLA3 IIT
H1 2027*

Adike A, et al. Expert Rev Gastroenterol Hepatol. 2018 Oct;12(10):1025-1032; Verkade HJ, et al. J Hepatol. 2016 Sep;65(3):631-42; Sundaram SS, et al. Liver Transpl. 2017 Jan;23(1):96-109; Raghu VK, et al. Liver Transpl. 2021 May;27(5):711-718; NORD, 2019, Japanese Biliary Atresia Society. Japanese Biliary Atresia Registry (JBAR). <https://jbas.net/en/national-registration/>.



**IT'S IN
OUR RNA**