

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

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Disclosures

• I am an employee of ProQR Therapeutics

Axiomer[™] EONs unlock cellular machinery potential to treat diseases

By attracting ADARs and allowing highly specific editing



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)



Creating a new class of medicines with broad therapeutic potential



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.



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

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



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

NTCP modulation validated in vivo and clinic

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



Bulevirtide (Hepcludex) is a daily SC injected NTCP inhibitor approved for Hepatitis D. NTCP channel is a known transporter for bile acids and hepatitis virus from bloodstream to the liver. 1. Slijepcevic D, et al. Hepatology. 2018 Sep;68(3):1057-1069; 2. Wedemeyer H, et al. N Engl J Med. 2023 Jul 6;389(1):22-32; 3. Wedemeyer H, J Hepatol. 2024 Oct;81(4):621-629.; 4. Dietz-Fricke C, JHEP Rep. 2023 Mar 15;5(4):100686.

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

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Healthy population discovered with NTCP variants that reduces bile acids uptake into liver¹⁻⁴

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			THE JOURNAL OF BREACHER CHEMISTRY 0 2004 by The American Society for Biochemistry and Molecular Biology, Inc.	Vol. 279, No. 8, Issue of February 20, pp. 7213–7222, 2004 Printed in U.S.A.	
			Ethnicity-dependent Polymorphism in Na ⁺ -taurocholate Cotransporting Polypeptide (<i>SLC10A1</i>) Reveals a Domain Critical for Bile Acid Substrate Recognition*		
and the factor			Received for publication, June 2, 2003, and in revised form. December 1, 2003 Published, JBC Papers in Press, December 2, 2003, DOI 10.1074/jbc.M305782200		dD
1			Richard H. Ho‡§1, Brenda F. Leake‡, Richard L. Roberts, Wooin Lee‡, and Richard B. Kim‡**		on Me
LSEVIER			From the Division of Clinical Pharmacology, Departments of Medicine and Pharmacology, Vanderbill University Medical Center, Nashville, Tennesses 3723-8962, Med Ebitssion of Pediatric Hematology Olnoclogy, Department of Pediatrics, Vanderbill University Medical Center, Nashville, Tennesses 37232-8510, the Uppartment of Pethology, Vanderbill University Medical Center, Nashville, Tennesses 37232-8510, and the Master of Science in Clinical Investigation Program, Vanderbilt University School of Medicine, Nashville, Tennesses 3732		Polyp
EVIEW Social and tauting Social and tauting Social and tauting AL Schneider*.1 * Swiss Pediatric Liver Cere Cenevo, Genero, Sentere * Department of Pediatri * Laboratory for humons * a Department of Pediatri * Available online 29 Oct Kerver0005 Failure to thrive; Hypercholanemia	A service of the serv	holate co-transpo eficiency I. Köhler ^{1,1} , B. Röthlisber Witin of Pediatric Speciative, Department pittel of Array, Switzerland itesting and generatic conselling, Zurich aptitol of Array, Switzerland 2021 Material Introduction: Little in Schötzer Introduction: Little Schötzer Int	The key transporter responsible for hepatic uptake of bulk acids from portal circulation is Na*taurocholate cotransporter is thought to be critical for the maintenance of enterohepatic recirculation of bile acids and hepato- phisms in this transporter would be predicted to have an important impact on bile acid homeostasisfiver func- tion. However, little is known regarding genetic heteror geneticy in NTCP, In this study, we demonstrate the pres- transporter is thought to be critical for the maintenance of the transport of the transport of the transporter of the transport of the transport function were identified. Cell surface bioinglation experiments indicated that he al- st of transport function were identified. Cell surface bioinglation experiments indicated that he al- st of transport function were identified. Cell surface bioinglation experiments indicated that the al- ter of transport function were identified. Cell surface bioinglation experiments indicated that the al- ter of transport function were identified. Cell surface bioinglation experiments indicated that the al- ter of the transport activity of T668C (lle ²² \rightarrow Thr), a vari- ant seen only in African Americans, was due at least in spression patterns were observed when the variant alterse were expressed in HepG2 cells, and plasma mem- brane expression was assessed using immunofluores- transport of a region in the transporter critical index points for bile acid substrate recognition. Accord- ing any the part of a region in the transportant poly- nophiles with for bile acid substrate recognition in the transports in the index points in NTCP exist and that the likelihood ob sub- ing carries of such polymorphisms is dependent on the acid substrate extrements in the accord- ing points in NTCP exist and that the likelihood ob sub-	Bile acids, synthesized from the enzymatic catabolism of cholesterol, are the major solutes in bile, essential for the maintenance of bile flow and biliary lipid secretion (1). In addition, an important mechanism for cholesterol homeostasis occurs through its elimination in the form of bile acids. Indeed de noos synthesis of bile acids from cholesterol is thought to account for nearly half of the daily elimination of cholesterol from the body (1). In the gastruintestinal tract, bile acids also modulate the release of pancreautic secretions and gastruintes- tinal peptides and activate enzymes required for the absorption of lipid-soluble virtamins (2, 3). Furthermore, their detergent properties assist solubilization of cholesterol in the small intestine and are returned to the liver via the portal circulation and resecreted into bile, thus forming an enterohegatic circuit (4). The efficient enterohegatic recirculation of bile acids is the aniantained by polarized expression of bile acids the bile bay for any transporters in the intestine and liver (4). Moreover, taurine or glycine conjugates of bile acids the bile bay polarized conjugated bile acids that bile acids that bile bay for any polarized bile acids and the bay and the source of a polarized bile acids and the bay and the source of conjugated bile acids that bile acids that bile acids that bile protective conjugates of bile acids that bile acids that bile protective conjugates of bile acids that bile bay and the protective conjugates of a source bile acids that bile acid protection for greater than 80% of the bayatic tuptake of conjugated bile acids that bile acids that bile acids that bile protective conjugates of a coupled to a source bile state that to be appressive that bile acids that bile acid protective the bayatic tuptake of the discussive bile acids that bile acid protective transporter whose function is coupled to a source and market to the savelater and mean-rane of the bayative (14) and shares 175 manion acid sequence identity with rat NK (14)	nnsportin o function eraction b ons of NTCP blocks: DP blocks: DP blocks: DP blocks: discourse ivity or th ivity o
	Abbreviation: BA, Bile acid; BM, Body Mans Inder; VTCP, Na-ta obspretive; SL-OMA, Solar Genter 1041; TBA, Iota bile acid; Compositive; SL-OMA, Solar Genter 1041; TBA, Iota bile acid. Compositive; SL-OMA, Solar Genter; 1041; TBA, Iota bile acid. Com		Grants GM54724 and GM31304, by the NIGMS, National Institutes of Health Pharmacogenetics: Research Network and Database (U01GM61374) under Grant U01 HL60982, and by an NCI, National Institutes of Health-funded vanderhilt Cinical Oncology Beenarch De- minents, data analysis, and data presentation were performed in part through the use of the Yanderhilt University Medical Center Cell Im- aging Core Resource (supported by National Institutes of Health Grants CA88465, DK20305, and DK58404. The cents of publication of this article were defrayed in part by the payment of page charges. This article must therefore be herefore paraled "activement" in accordance with 18 U.S.C. Section 1754 solely to indicate this fact.	nucleotides specific to Ntep, the expressed Na ⁻¹ -dependent tau- rocholate transport activity was reduced by 95%. This finding suggests a potentially central role for Ntep in the hepatic up- take of bile acids. Accordingly, the extent of its expression or function would be predicted to significantly affect enterohe- patic circulation of bile acids and directly affect cellular signal- ing pathways importantly involved in cholesterol homeostasis and hepatocyte function. One potential source of altered NTCP function may be ge-	with huma with huma ulture syst ism of vir ugs. Human 1 n that is pr

Viruses and Bile Salts olecular Determinants on Sodium

jie Ren," Zhiyi Jing," Jianhua Sul," Wenhui Li" ces, Peking Union Medical College and Chinese Academy of Medical Sciences

ag polypeptide (NTCP) is responsible for the majority of sodior as a cellular receptor for viral entry of hepatitis B virus etween NTCP and the pre-S1 domain of HBV large envelope CP are independent or if they interfere with each other. Here aurocholate uptake by the receptor; conversely, some bile NTCP residues critical for bile salts binding severely impair artant for sodium binding also inhibit viral infection. The (SNP) found in about 9% of the East Asian population, e ability to support HBV or HDV infection in cell culture. BV and HDV entry overlap with that for bile saits uptake by anction of NTCP, and bile acids and their derivatives hold

(HDV), are important human pathogens. Available ther-(112) 73, are important frames partogens. Avalance incr-available for HDV infection. A liver bile acids transporter anintaining homeostasis of bile acids serves as a funcig lipopeptide that originates from the first 47 amino ssport. Some bile salts dose dependently inhibit HBV critical for HBV and HDV entry overlap with that for diated HBV and HDV infection in relation to NTCP's inter ray and rary successing in reaction to react

lls complemented with human or treeshrew NTCP. Refew amino acids of crab-eating monkey (amino acids the minute actual of control and a statistic function of the statistic of rts converted these NTCPs to functional receptors for HDV, respectively. Thus, HepG2 cells complemented in NTCP provide a valuable and convenient in vitro cell tem for increasing our understanding of the mechaal entry and for the development of novel antiviral

NTCP (SLC10A1) is a multiple-transmembrane pro edominantly expressed at the basolateral membrane

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

Human genetics validates NTCP modulation as strategy for cholestatic disease



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

BA, Biliary atresia; PSC, Primary Sclerosing Cholangitis

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

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



- 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.

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



BAs uptake (TCA) in vitro*

 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 Q68R variant solely affects bile acids re-uptake function

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



Western Blot Analysis

• No significant differences in NTCP RNA and protein levels were detected. The plasma membrane location of the Q68R variant was also unaffected.

NTCP protein localization in vitro*



NucleiAnti-FLAG



Parental

WT

• 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 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 R² = 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 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, change in plasma BA profile confirms NTCP specific modulation
- High confidence on NTCP EON treatment to positively impact BA toxic load in the liver

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

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

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

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

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



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

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

Integrated single/multiple ascending dose study design



Treatment

AX-0810 GalNAc conjugated editing oligonucleotide

Objectives

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

Trial design

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

 DMC safety reviews before proceeding to next dose and dose escalation

Key endpoints

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

Top-line data in Q4 2025

Summary & next steps AX-0810 for cholestatic diseases





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

- ✓ 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

Thank you!



Prof. Peter Beal, and his group

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



Prof. Stan van de Graaf, and his team

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

ProQR R&D Department

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