ProQR®

DEVELOPING RNA-EDITING MEDICINES

for patients in need

Nasdaq: PRQR

Date: November 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.

ProQR Therapeutics

Short overview

Focus on Axiomer™

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

Novel mechanism of action, leading patent estate

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

Preclinically validated across multiple genes

Preclinical data demonstrate Axiomer™ is broadly validated across multiple genes

ADAR

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

Two pillars underlie strategy

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

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

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

Cash-runway into mid-2026

€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

2014

ProQR invents oligo mediated RNA Editing recruiting endogenous ADAR

2014-2018+

ProQR files key patents that protect ADAR mediated RNA editing broadly

2015-2021

ProQR optimizes the ADAR platform in stealth

2020-2023

Key ADAR patents get granted in EU and US

2021

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

2022

ProQR pivots to solely focus on ADAR editing

2022

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

2023-2024

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

2023

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

Late 2024 / Early 2025

ProQR will enter the clinic with ADAR mediated RNA editing

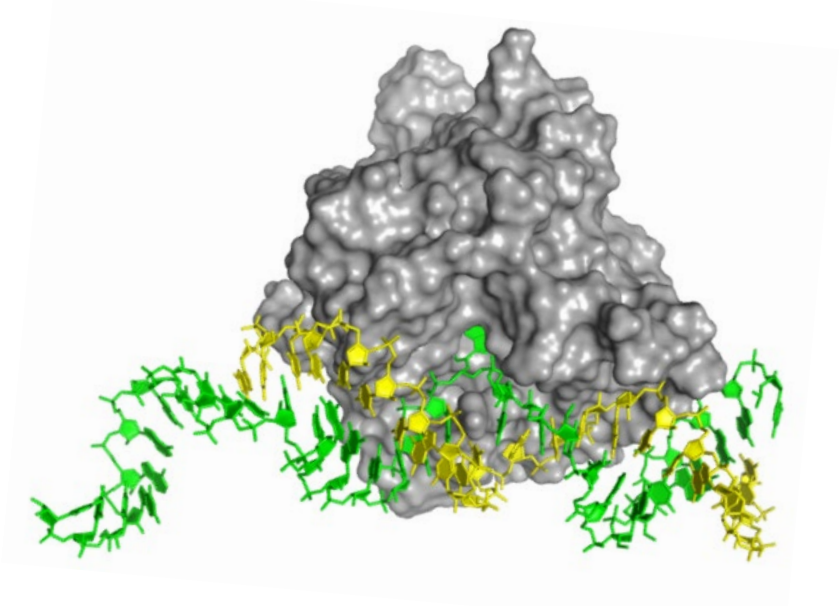
2024

- 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

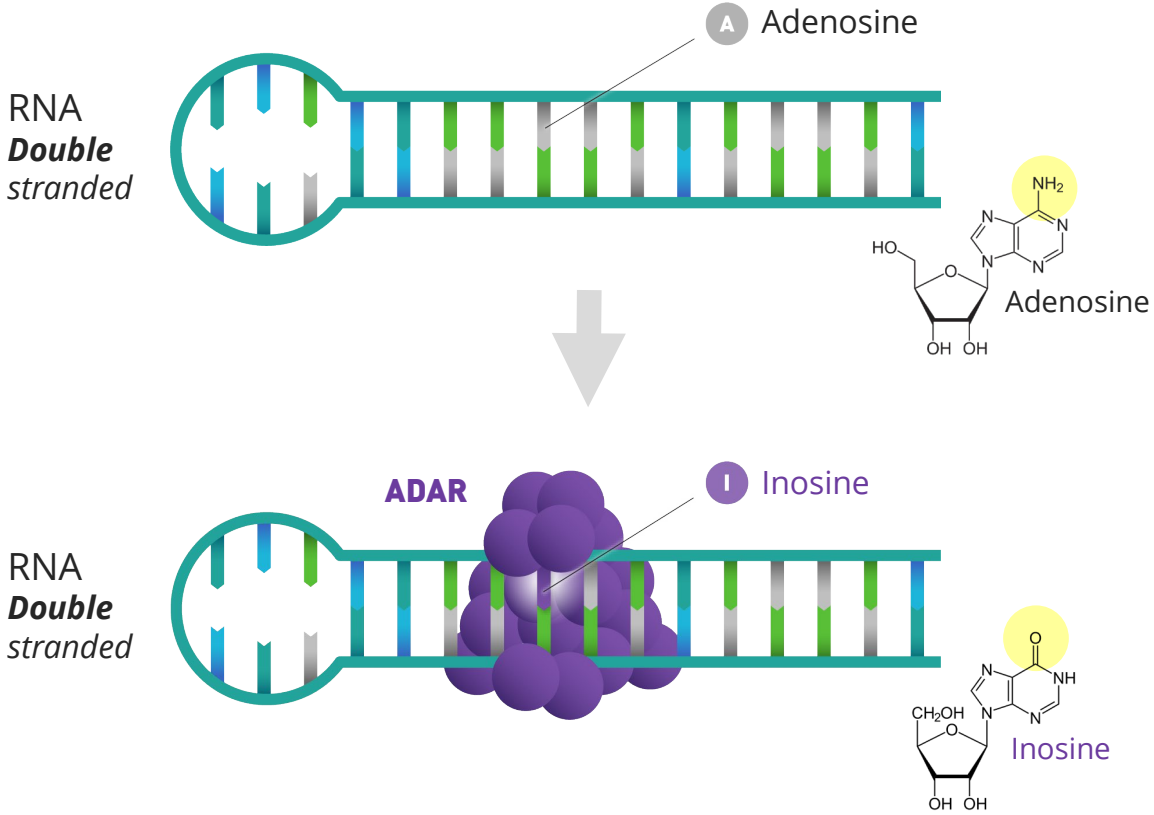
What is ADAR editing?

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)**

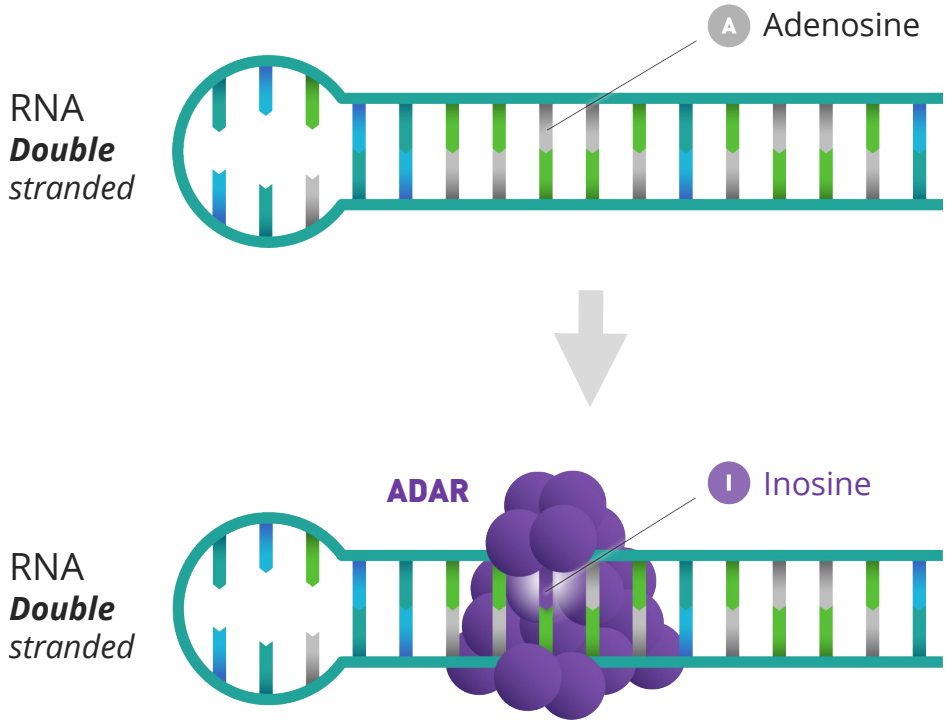
Natural ADAR editing *(A-to-I)*



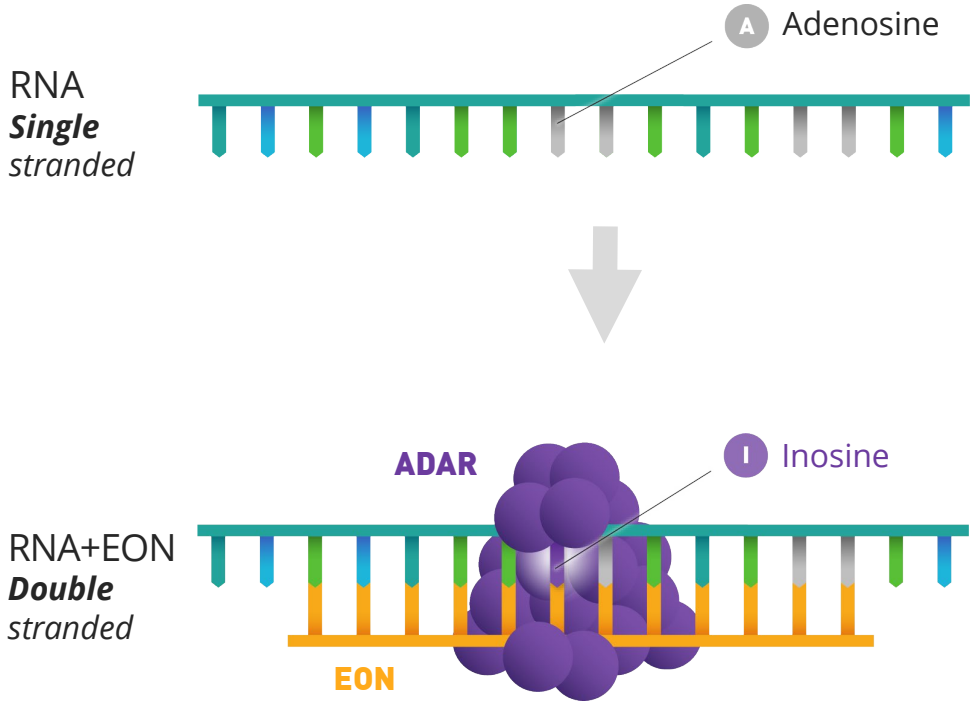
Axiomer™ EONs designed to unlock cellular machinery potential to treat diseases

By attracting ADARs and allowing highly specific editing

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



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



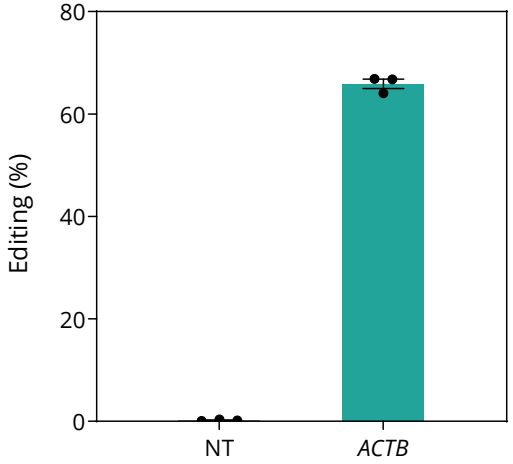
ADARs: Adenosine deaminases acting on RNA.

High intrinsic editing capability of Axiomer™ in the liver across models

Cell models

Up to 70% Editing of *ACTB* in primary human hepatocytes

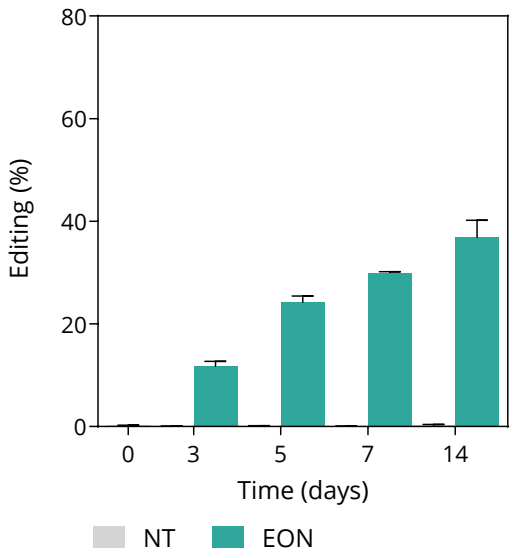
Gymnosis, 5µM, single dose, n=1 with triplicates, 72 hours, dPCR, mean, SD



Organoids

Up to 40% Editing of *ACTB* in human LMTs

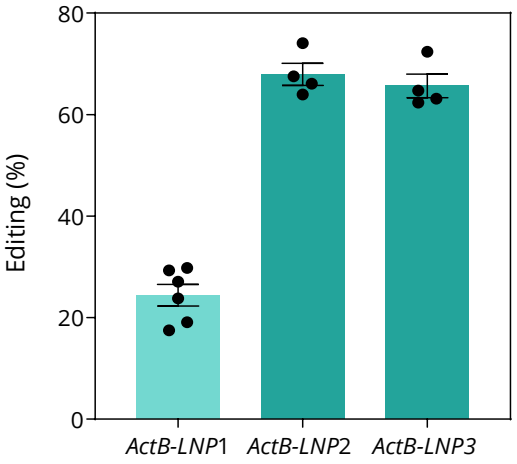
Gymnosis, 1µM, constant dose, 3 pools of 24 LMTs per condition, 14 days, dPCR, mean, SD



Mice *in vivo*

Up to 70% editing of ActB in liver

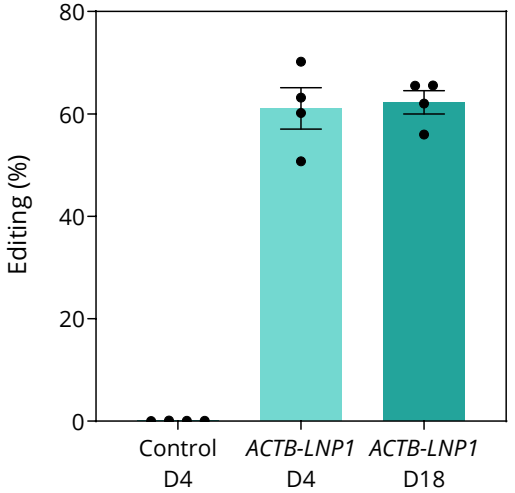
IV, 3mg/kg or 4mg/kg, N=4-6, LNP formulations, D7 data, dPCR, AVG±SEM



NHP *in vivo*

Up to 70% editing of *ACTB* in NHP

IV, 2mg/kg, 3 doses at D1, D8 and D15, LNP formulation, n=4, D4 and D18 data, dPCR, mean±SEM



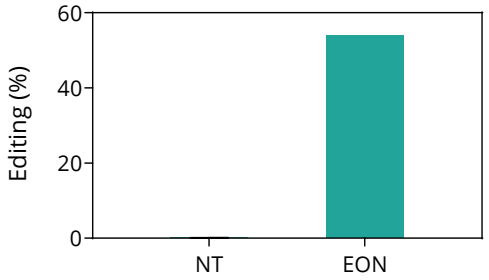
PHH: Primary Human Hepatocyte; LMT: Liver Micro Tissue; NHP: Non-human primate

Axiomer™ potential beyond liver

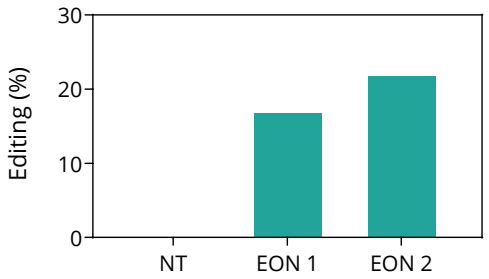
Strong editing in the nervous system across models

Cell models

More than 50% RNA editing of *ACTB* in human iPSC derived neurons

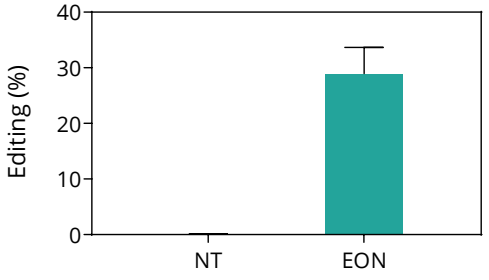


More than 20% RNA editing of APP in human iPSC derived neurons

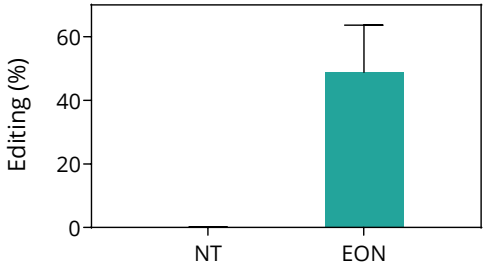


Organoids

Up to 35% RNA editing of *ACTB* in cerebral organoids

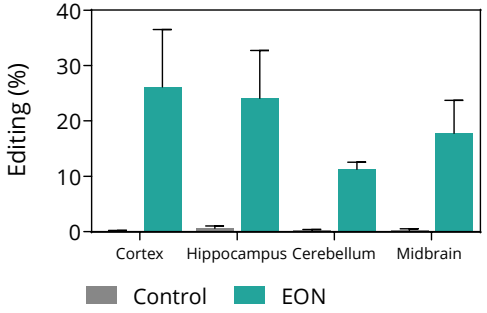


Up to 65% RNA editing of *APP* in cerebral organoids

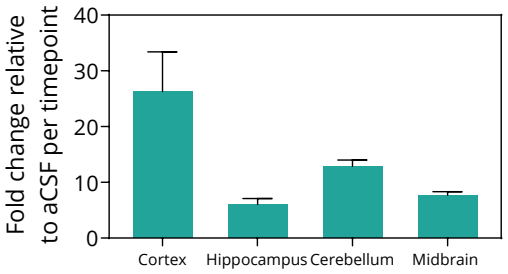


Mice *in vivo*

Up to 40% RNA editing in mice brain*

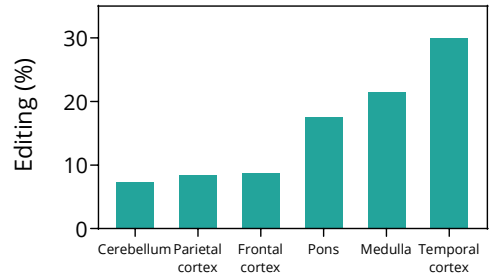


26-fold change in protein function in mice brain*

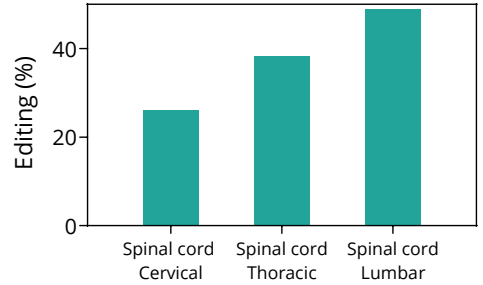


NHP *in vivo*

Up to 30% RNA editing in NHP brain*



Approx. 50% RNA editing in NHP spinal cord*



*Undisclosed target. Conditions of the *ACTB* iPSC derived neurons experiment: gymnosis, 2.5µM, single dose, n=1, 2 weeks, dPCR and conditions of the *APP* iPSC derived neurons experiment: gymnosis, 10µM, single dose, washout, n=1, 2 weeks, dPCR. Conditions of the *ACTB* cerebral organoids of 130 days: gymnosis, 10µM, single dose, washout, n=7, 6 days, ddPCR, mean, SD and *APP* cerebral organoids of 150 days: gymnosis, 5µM, single dose, washout, n=5, 2 weeks, ddPCR, mean, SD. Conditions of the mice *in vivo* experiment: intracerebroventricular (ICV), 250µg, single dose, N=6, 4 weeks, editing: ddPCR and protein function: western blot, mean, SD and SEM. Conditions of the non-human primate (NHP) *in vivo* experiment: intrathecal (IT), 12mg, single dose, n=3**, 7 days. ** Data of 2 NHPs not analyzable due to human error during injection procedure.

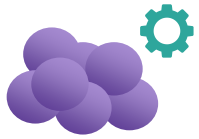
Axiomer™ aims to create a new class of medicines with broad therapeutic potential

Correction

Mutations correction

Thousands of G-to-A mutations, many of them described in literature

Protein modulation

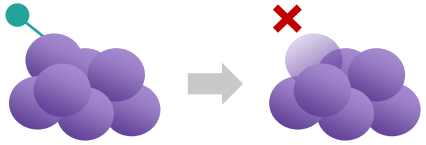


Alter protein function or include protective variants

Modified proteins achieving loss- or gain-of-functions that help addressing or preventing diseases

Disrupt >400 different types of post translational modifications

Regulate protein activity, change localization, folding, preventing immune escape or slowing down degradation



Change protein interactions

Changes localization, folding, protein function or prevents immune escape of glycosylated tumor antigens

BROAD THERAPEUTIC POTENTIAL

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

PTMs: Post-translational modifications.

Pipeline

ProQR development pipeline

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

[1]Approximately 100K people affected with Primary Sclerosing Cholangitis and Biliary Atresia in US and EU5. [2]Approximately 200 million people suffer from too high a level of cholesterol in US and EU5. *SLC10A1* is the gene that encodes for NTCP protein.
CVD: Cardiovascular Diseases, NASH: Nonalcoholic steatohepatitis, T2D: Type 2 Diabetes.
References: 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

AX-0810 for cholestatic diseases

RNA-editing therapy

for Primary Sclerosing Cholangitis and Congenital Biliary Atresia

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

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

- **Biliary Atresia** is projected to affect ~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 variants reduced bile acid uptake into liver in health population

- 95% of BA in liver is reuptaken from the bloodstream though the NTCP channel[1]
- Healthy population discovered with NTCP variants that reduces bile acid uptake into liver[2-4]
- Modulation of NTCP bile improved outcomes of cholestasis, reducing liver damage and inflammation in a mouse model[5,6]

REVIEW

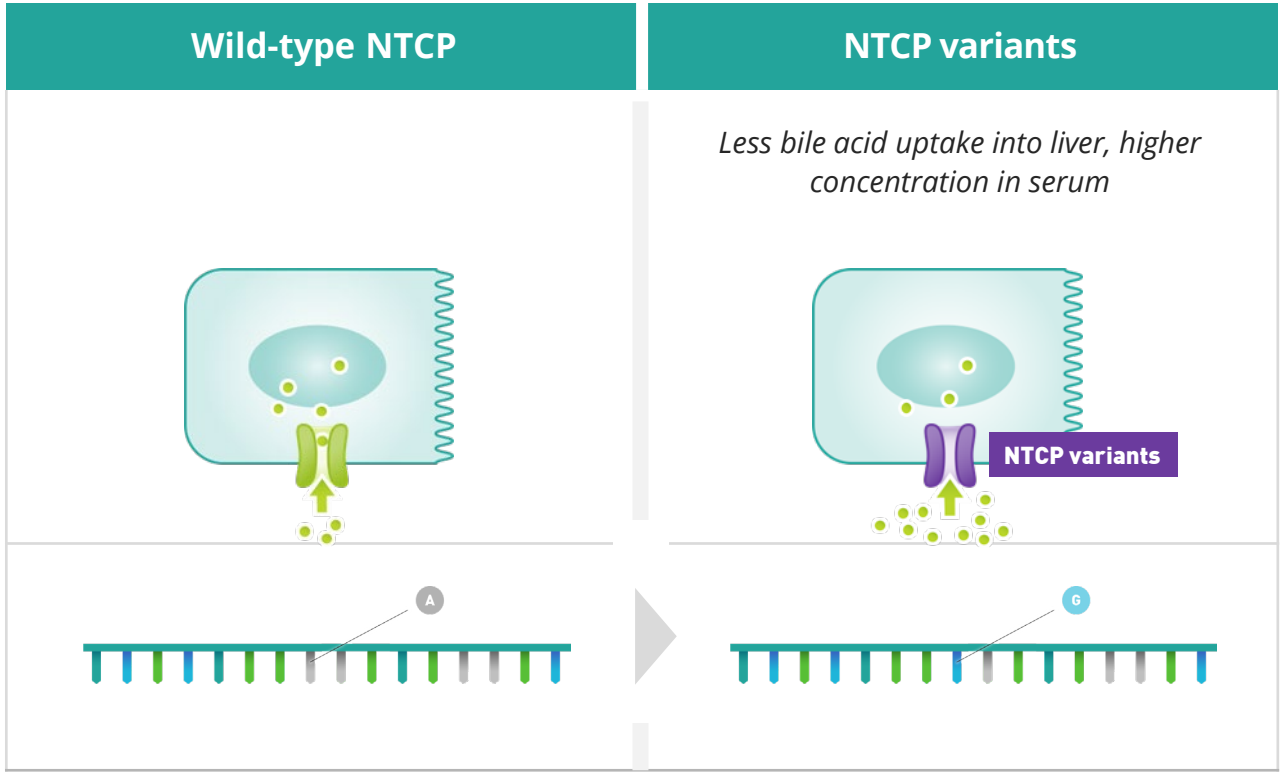
Sodium taurocholate co-transpo polypeptide deficiency

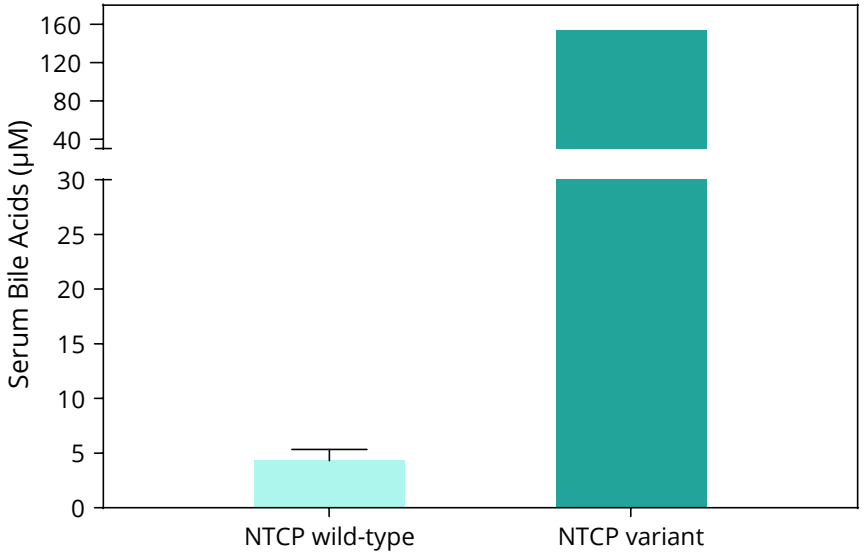
Ethnicity-dependent Polymorphism in Na$^+$-taurocholate Cotransporting Polypeptide (*SLC10A1*) Reveals a Domain Critical for Bile Acid Substrate Recognition*

Viruses and Bile Salts on Molecular Determinants on Sodium Polypeptide

[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 variants reduced bile acid uptake in liver

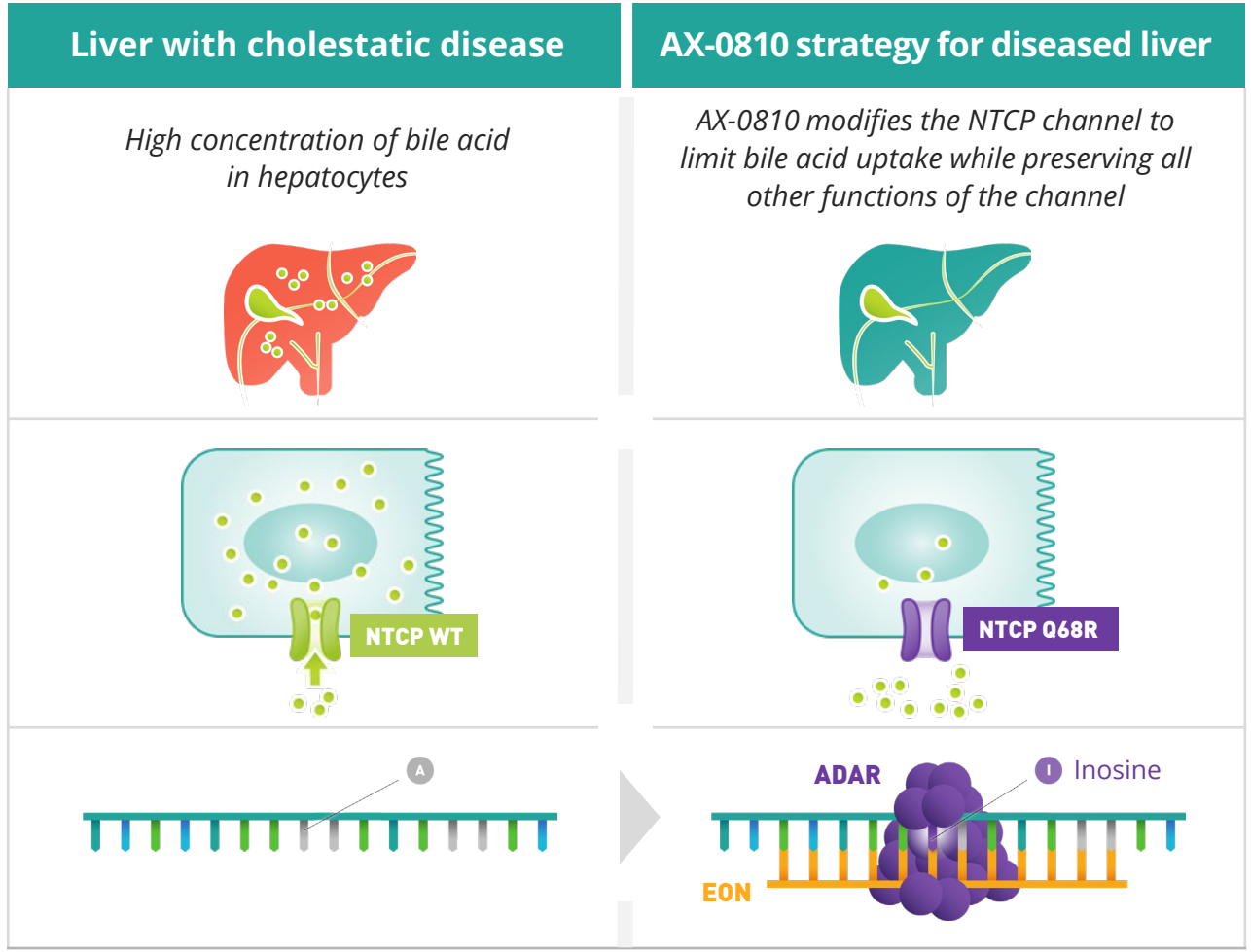




40-fold higher serum bile acid levels detected in healthy people living with the NTCP variant

References: Thakare R, et al . J Appl Toxicol. 2018 Oct;38(10):1336-1352; Shiffka SJ, et al. J Lipid Res. 2020 Nov;61(11):1524-1535; Mousa OY, et al. Hepatology. 2021 Jul;74(1):281-295; Haag M, et al. Anal Bioanal Chem. 2015 Sep;407(22):6815-25; Mao F, et al. J Biol Chem. 2019 Aug 2;294(31):11853-11862.

Q68R-NTCP as strategy for cholestatic disease

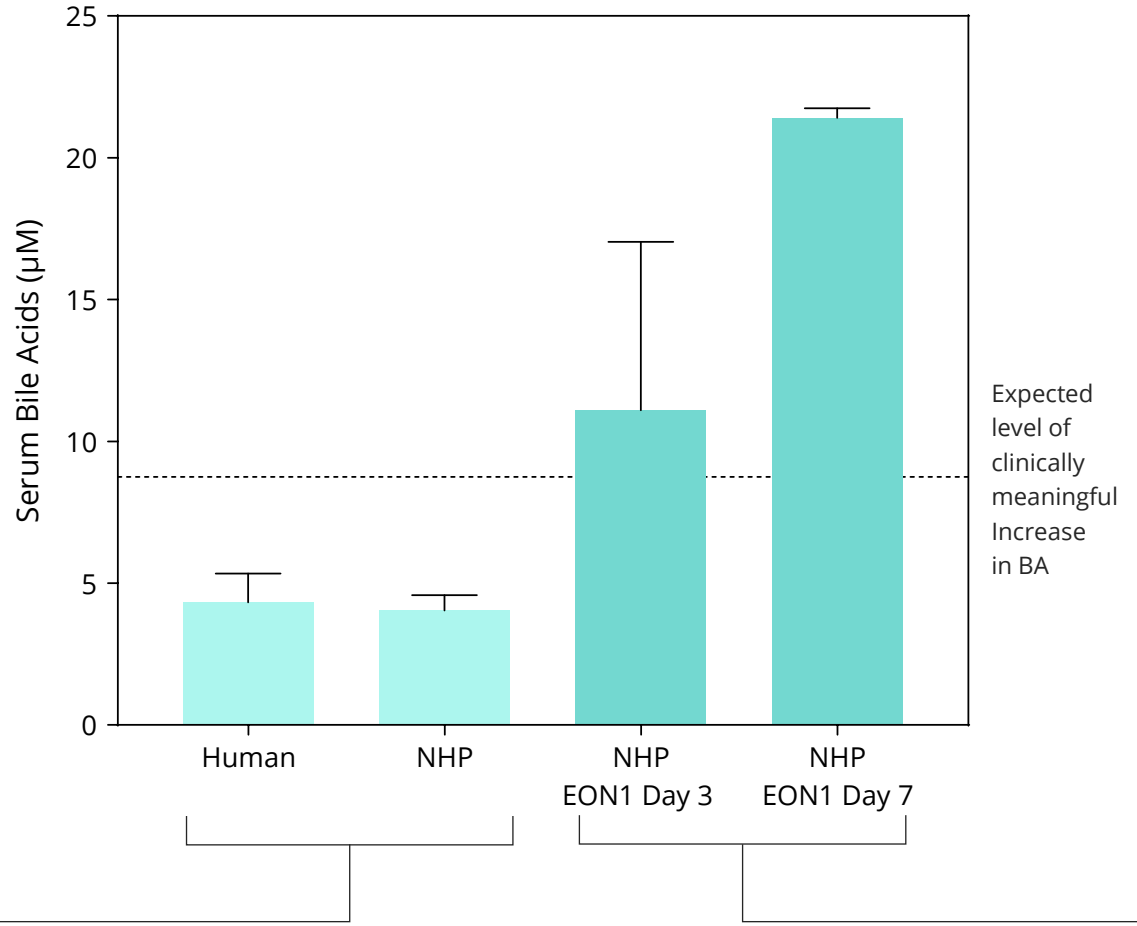


- The AX-0810 program introduces a variant in individuals with cholestatic disease to lower bile acid concentration in hepatocytes by a single A-to-I change
- The AX-0810 program is designed to be a disease modifying treatment
  - To alleviate symptoms in PSC and BA
  - To prevent or delay the development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

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

NHP is a predictive model for humans

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



NTCP editing oligonucleotides induce the desired change in biomarkers

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

References: Thakare R, et al . J Appl Toxicol. 2018 Oct;38(10):1336-1352; Shiffka SJ, et al. J Lipid Res. 2020 Nov;61(11):1524-1535; Mousa OY, et al. Hepatology. 2021 Jul;74(1):281-295; Haag M, et al. Anal Bioanal Chem. 2015 Sep;407(22):6815-25; Mao F, et al. J Biol Chem. 2019 Aug 2;294(31):11853-11862.

AX-0810 Target Engagement Clinical Study

To measure Bile Acid increase in healthy volunteers

Objectives

- Establish **target engagement** by **Bile Acids biomarkers**

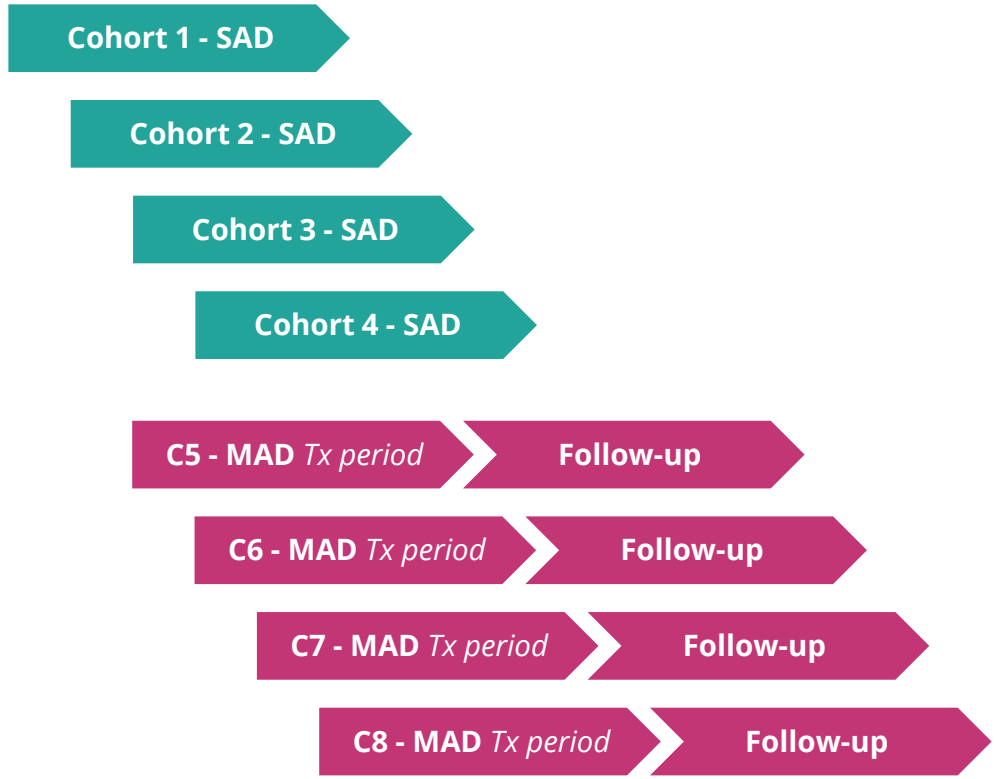
Endpoints will include

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

Entry into clinical trials in late 2024 / early 2025

- Further trial details to be announced in H2 2024

Preliminary study design



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

AX-1412 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.

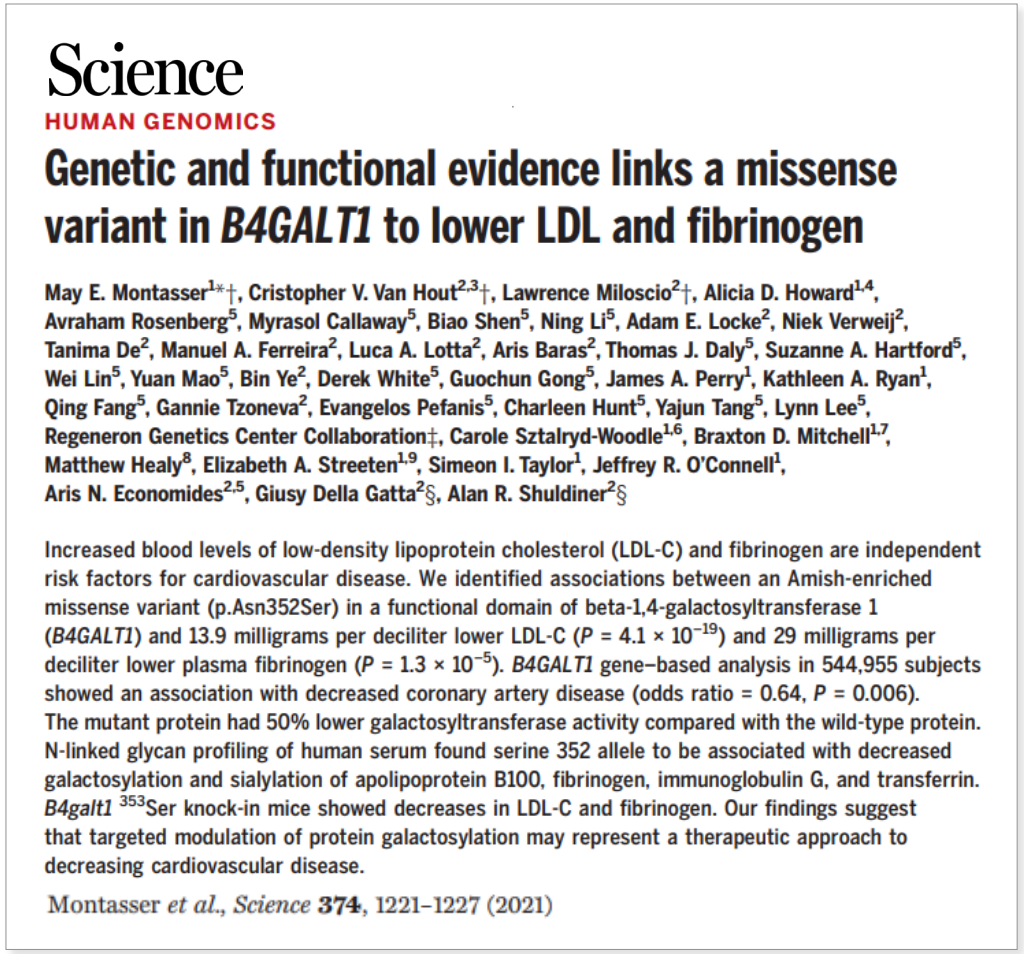
AX-1412 is designed to provide people with a protective genetic variant of B4GALT1 that is associated with **36%[1] reduction in the risk of cardiovascular disease**.

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 reduced CVD risk

- It is described that people who carry mutations like the p.Ans352Ser in the B4GALT1 gene, 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

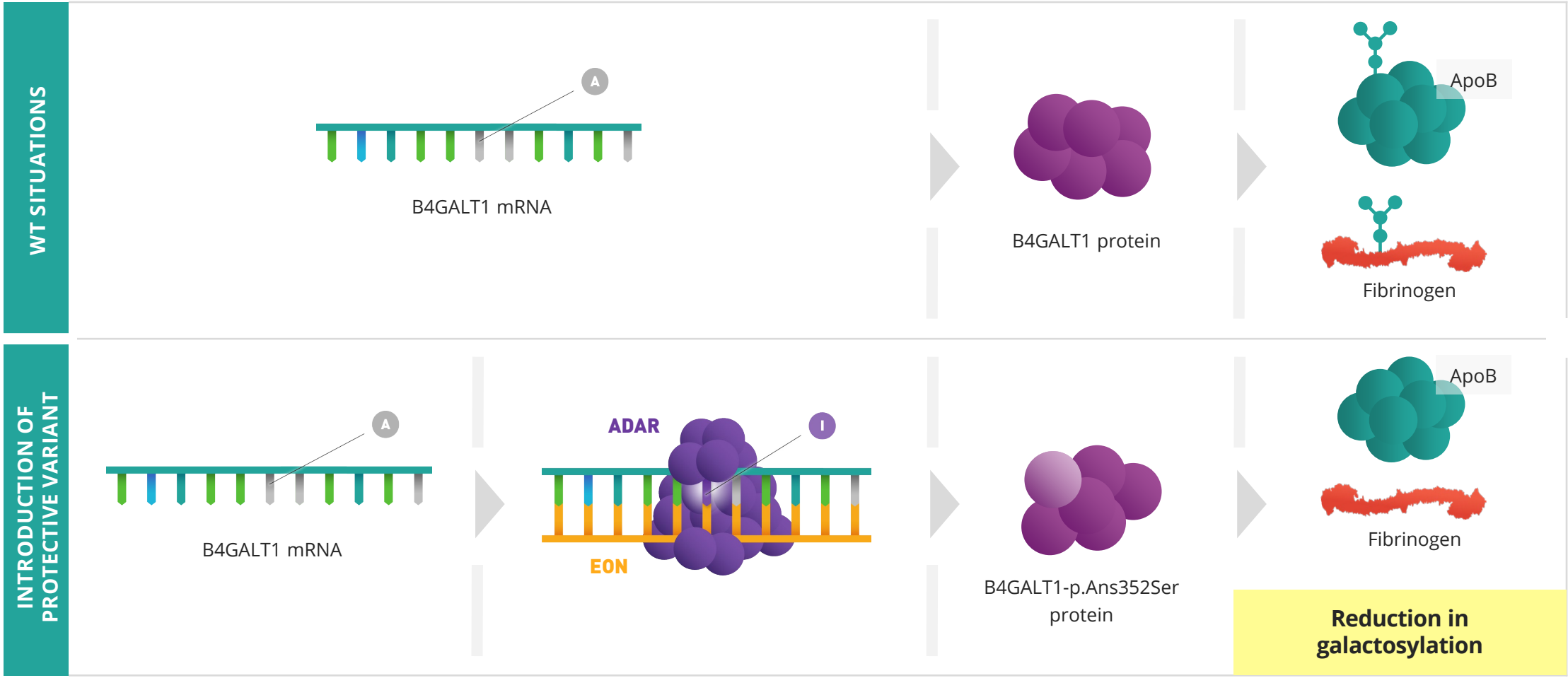
May E. Montasser[1*†], Cristopher V. Van Hout[2,3†], Lawrence Miloscio[2†], Alicia D. Howard[1,4], Avraham Rosenberg[5], Myrasol Callaway[5], Biao Shen[5], Ning Li[5], Adam E. Locke[2], Niek Verweij[2], Tanima De[2], Manuel A. Ferreira[2], Luca A. Lotta[2], Aris Baras[2], Thomas J. Daly[5], Suzanne A. 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* ^{353}Ser 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

B4GALT1 p.Ans352Ser variant reduced 2 cardiovascular risk factors



AX-1412 next steps

- Preclinical PoC data and translational data sets on AX-1412 will be announced in H2 2024
- AX-1412 is planned to subsequently enter the clinic around YE 2024 / early 2025
- The first in human trial will be a target engagement study measuring disease relevant biomarkers APO-B100 and Fibrinogen amongst others
  - As AX-1412 introduces a variant in a WT sequence, this trial can be conducted in healthy volunteers allowing for rapid and cost-efficient execution, proper sample sizing and dose range data without background disease noise.

AX-2402 for Rett Syndrome

Axiomer™ technology

targeting the transcription factor MECP2 and potential to correct nonsense variants

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.

[1]Krishnaraj R, et al. 2017. RettBASE: Rett syndrome database update; [2]RSRT 2023 conference; [3]Guy J, et al. 2007 Science 23;315(5815):1143-7.

ProQR strategy – develop own pipeline and selectively enter into partnerships

ProQR Pipeline

- Build **in-house pipeline** based on Axiomer™ RNA editing technology platform
- Initial focus on **liver and CNS originated diseases**

Partnerships

- Largely unencumbered platform, ProQR may **selectively enter partnerships**
- **Lilly partnership** with expansion announced December 2022 – total potential value of ~$3.9B

2024 and beyond outlook

Building momentum toward development

Pipeline

AX-0810 targeting NTCP for cholestatic diseases

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

AX-1412 targeting B4GALT1 for cardiovascular disease

- 2024 – report preclinical proof of concept data; announce clinical development candidate; report translational data; announce clinical trial design
- Late 2024/early 2025 – advance to clinic

New pipeline program announcement(s)
Potential in 2024 and beyond

IP

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

Partnerships

Eli Lilly

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

Rett Syndrome Research Trust

- Partnership announced January 2024

Potential new

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

Cash

Strong cash runway
€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

Well positioned

to advance Axiomer™

Science

- Deep understanding of basic science – ADAR, oligos
- Optimization of editing oligonucleotides (EONs) for therapeutic development

Axiomer™ has broad applicability

- Large number of potential therapeutic applications
- In vivo POC established in nervous system, liver

Advancing toward the clinic

- Extensive translational and developmental expertise with oligo modality
- AX-0810 and AX-1412 initial pipeline targets

Leading IP position

- Axiomer™ is protected by >10 published patent families
- Continuously investing in expanding IP estate

Strategic partnership

- Eli Lilly collaboration
- Rett Syndrome Research Trust
- Selectively form additional partnerships
- Optionality and multiple value creating opportunities

Experienced leadership

- Deep RNA, corporate finance, and business development expertise across Management Team, Supervisory Board, and Scientific Advisory Board

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

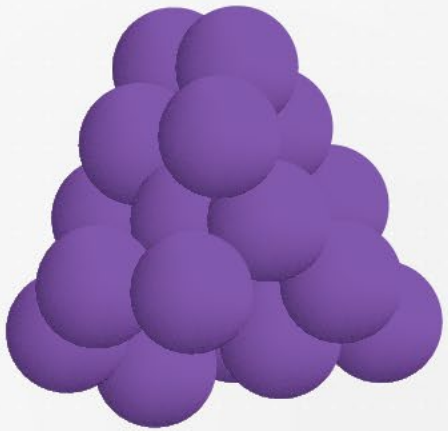
ProQR®

IT'S IN OUR RNA

Resource slides

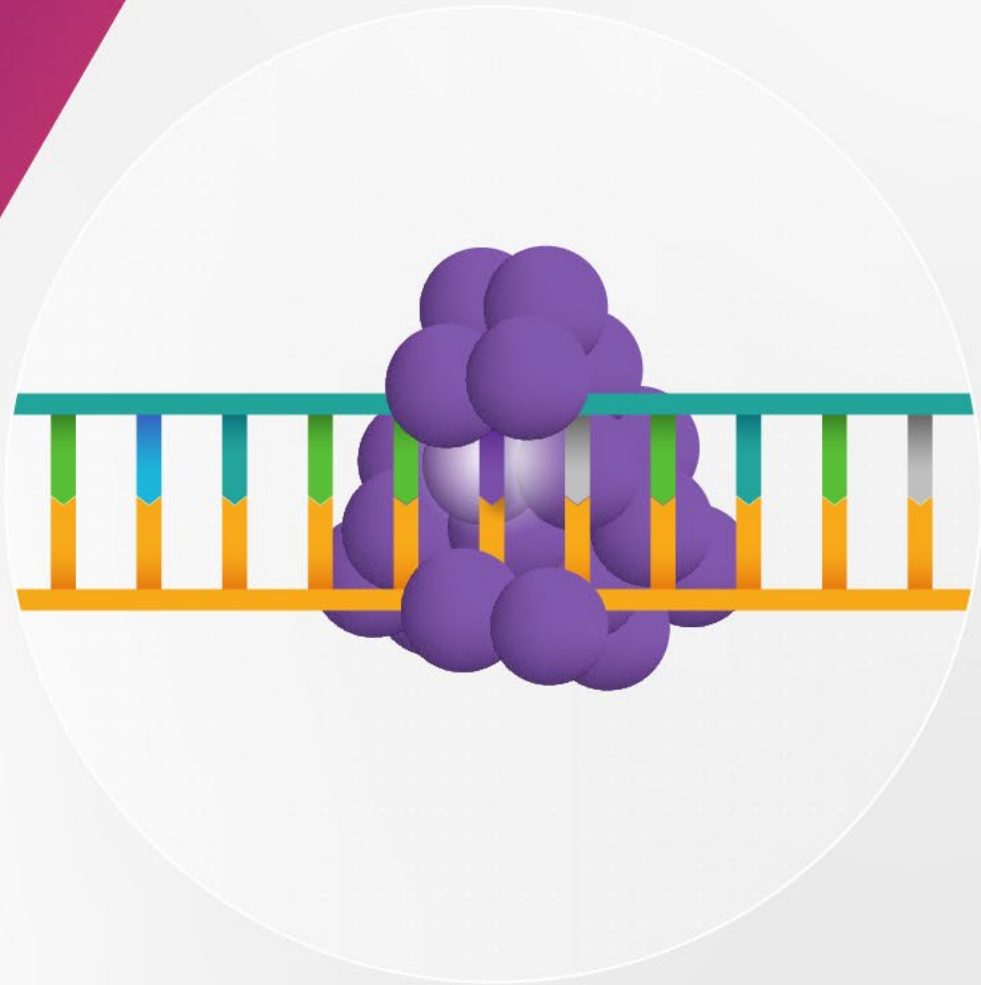
HOW DOES ADAR WORK?

Explained in 5 minutes



WHAT IS AXIOMER™?

Explained in 5 minutes



ProQR Leadership Team

Management Team

Daniel de Boer
Chief Executive Officer

Gerard Platenburg
Chief Scientific Officer

René Beukema
Chief Corporate Development Officer

Jurriaan Dekkers
Chief Financial Officer

Sheila Sponselee
VP, Head of People and Operations

Board of Directors

James Shannon, MD
Chair

Dinko Valerio

Alison Lawton

Martin Maier, PhD

Bart Filius

Theresa Heggie

Begoña Carreño

Board - Executive Directors

Daniel de Boer
Chief Executive Officer

Gerard Platenburg
Chief Scientific Officer

René Beukema
Chief Corporate Development Officer

Strategic Advisor

John Maraganore, PhD

In Memoriam

Henri Termeer
Honorary former board member

Scientific Advisory Board

James Shannon, MD
Chair

Phillip D. Zamore, PhD

Martin Maier, PhD

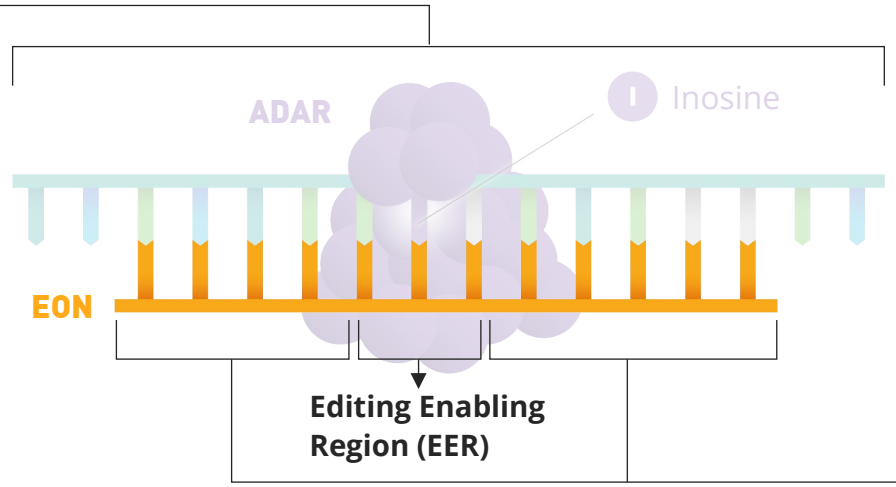
Peter A. Beal, PhD

Yi-Tao Yu, PhD

Driving the development of optimized EONs for therapeutic use

Sequence defines target
RNA binding and provides stability

EON total length
Between ~25 - 30 nt



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

Optimized sequence and chemistry define functionality

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

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

Leading IP supporting ADAR-mediated RNA editing platform technology

- Axiomer™ IP strategy commenced in 2014 with first patent application filings
- Currently 20 published patent families, comprising 30 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
1 (0004)	**17-12-2014**	**Targeted RNA Editing using endogenous ADARs**	Granted AU BR CA CN EP IL IN JP NZ US US ZA
2 (0013)	**22-06-2016**	**Short EONs with wobble and/or mismatch base pairs**	Granted AU IL JP KR US US US
3 (0014)	**01-09-2016**	**Chemically modified short EONs**	Granted AU CN EP JP KR NZ US US ZA
4 (0016)	19-01-2017	EONs + protecting SONs (heteroduplex formation)	Granted US
5 (0023)	18-05-2018	PS linkages / chiral linkages (*e.g.,* PS, PN)	Published
6 (0025)	28-01-2019	Editing of PTC in exon 61 USH2A	Published
7 (0026)	11-02-2019	Phosphonacetate linkages / UNA modifications	Published
8 (0029)	03-04-2019	MP linkages	Published
9 (0031)	24-04-2019	Editing inhibition	Published
10 (0032)	13-06-2019	Benner's base (dZ)	Published Granted ZA
11 (0035)	23-12-2019	Editing in exon 35 of ABCA4 for Stargardt disease	Published
12 (0039)	23-06-2020	Split EONs	Published
13 (0045)	14-02-2022	PCSK9 editing	Published
14 (0046)	15-07-2022	5'-GA-3' editing	Published
15 (0048)	15-07-2022	diF modification	Published
16 (0051)	21-10-2022	Heteroduplex oligonucleotide complexes	Published
17 (0052)	24-11-2022	HFE editing	Published
18 (0053)	09-12-2022	B4GALT1 editing	Published
19 (0054)	01-12-2022	ALDH2 editing	Published
20 (0055)	20-01-2023	AG1856 for RNA editing	Published

ProQR Axiomer™ IP

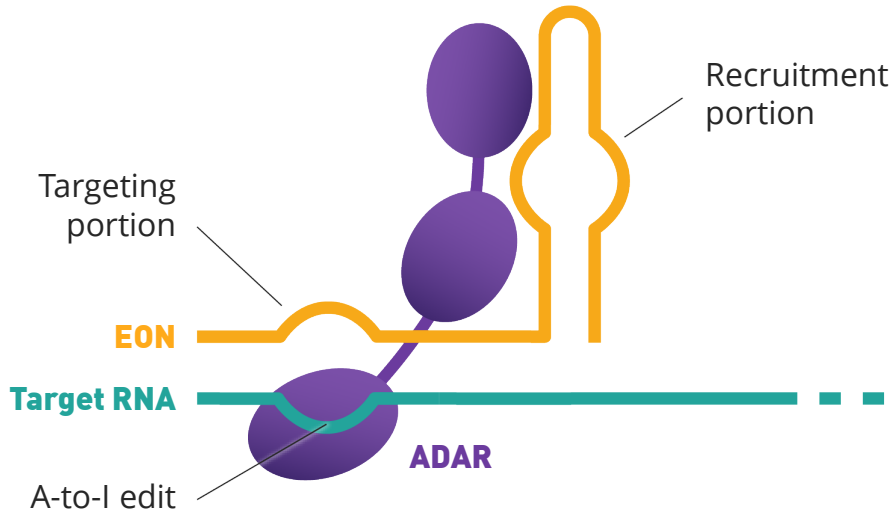
Broad coverage

- 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



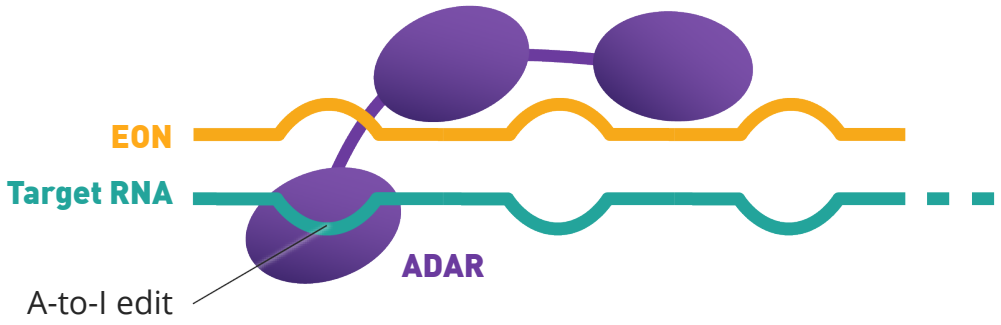
EP 3 234 134 B1 - **Granted; appeal pending**
US 10,676,737 - **Granted**
US 11,781,134 - **Granted**

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.



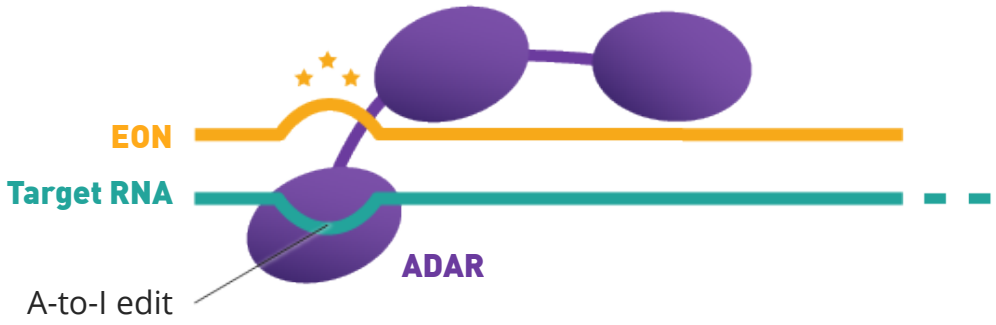
US 10,941,402 - Granted
US 11,851,656 - Granted
US 12,018,257 - Granted

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.

Well-defined development plan for AX-0810

PRECLINICAL STAGE	EARLY CLINICAL	LATE CLINICAL
Preclinical models available with strong translatability into the clinic	*Early insight on safety and target engagement using validated biomarkers*	*Clinical programs with disease specific endpoints for regulatory approval*
Translational models available • Organoids models • Animal models **Proof of mechanism measures in animal models** • Serum levels of ALP and γ-GT • Total bile acids in serum and liver • Hepatic inflammation and fibrosis	**Program with Phase 1 on healthy volunteers** **Validated biomarkers in cholestatic diseases** • Bile acids in serum, urine and feces • Liver enzymes • Serum cholesterol **Disease specific biomarkers in preparation for next trials** • ALP for PSC • Bilirubin for BA	**Primary Sclerosing Cholangitis** Co-primary endpoint for regulatory approval: • Reduction in ALP and • Histological liver evaluation **Biliary atresia** • Time to liver transplantation • Mean change in total serum bilirubin levels, liver enzymes, bile acid levels, blood platelets and serum albumin

γ-GT: γ-glutamyl transferase; ALP, Alkaline phosphatase; BA, biliary atresia; BDL, Bile duct ligation; LMT, Liver microtissues; NTCP, Na-taurocholate cotransporting polypeptide; PSC, Primary Sclerosing Cholangitis

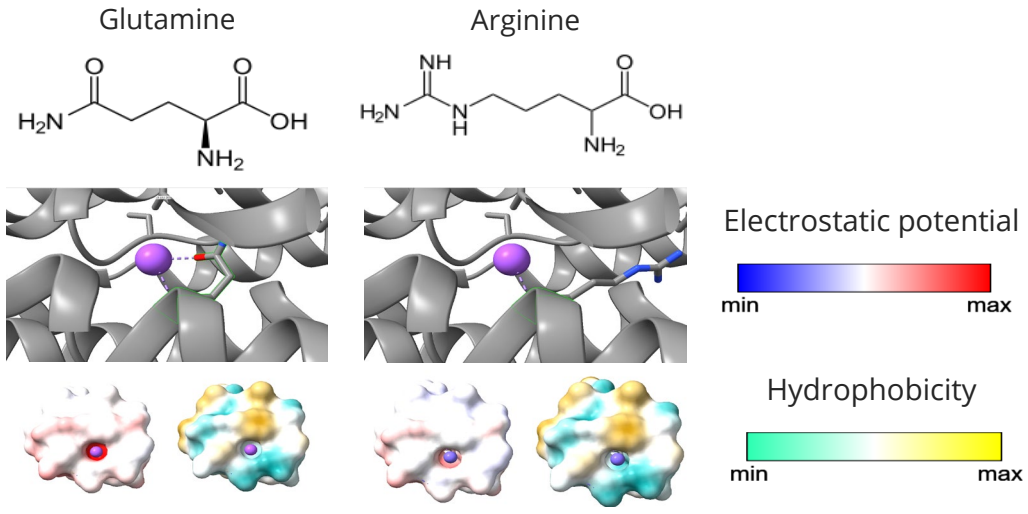
Well-defined development plan for AX-1412

PRECLINICAL STAGE	EARLY CLINICAL	LATE CLINICAL
Preclinical models available with strong translatability into the clinic	*Early insight on safety and target engagement using validated biomarkers*	*Clinical programs with disease specific endpoints for regulatory approval*
Organoids models for CVD • Blood-derived myeloid cells and THP-1 cells • Cell-laden microtissue spheroids **Animal models** • The Apoe−/− mouse model **Proof of mechanism measures in animal models** • Serum lipid levels • Atherosclerotic lesion area • C-reactive protein (CRP) and Interleukin 6 (IL-6) • Endothelial function	**Programs with Phase 1 on healthy individuals** • Reduce potential signal-to-noise ratio as CVD patients have many comorbidities **General CVD biomarkers** • non-HDL-C • Triglycerides • Apoliprotein B **Target specific biomarkers** • LDL-C • Fibrinogen	**Primary endpoints** 1. All-cause mortality and fatal CVD events or 2. Composite endpoints (incl. fatal and non-fatal CVD events) **Secondary endpoints** • Could consider using biomarkers as surrogate endpoints to reasonably predict treatment effects on outcome

Apoe: Apolipoprotein E, CVD: cardiovascular diseases, HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol, THP-1: human monocytic cell line

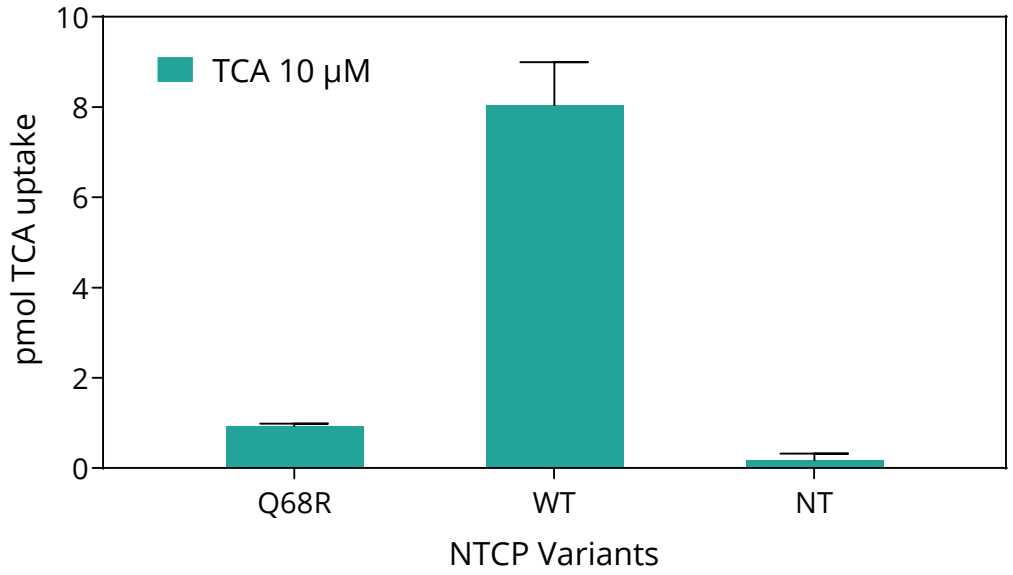
Introducing the Q68R variant led to modulation of bile acids reuptake

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



BAs uptake (TCA) *in vitro**

n=3, mean±SEM



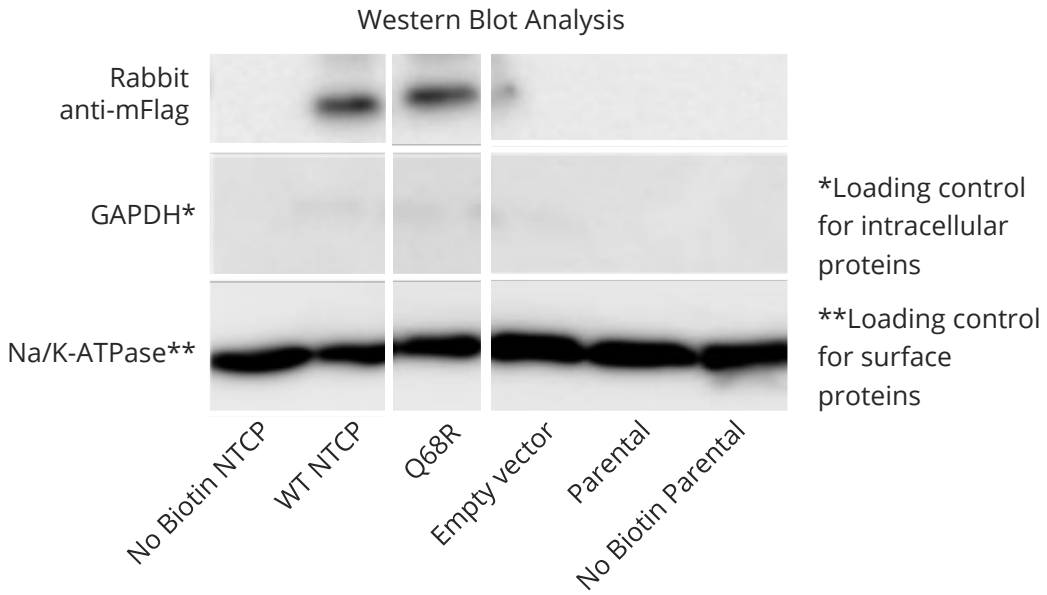
- The Q68R variant disrupts some hydrogen bonds and contacts in the Na^+ binding pocket.
- Clashes are inevitable since the Arg side chain is buried and likely to be found in one or another unfavorable rotamer state.

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

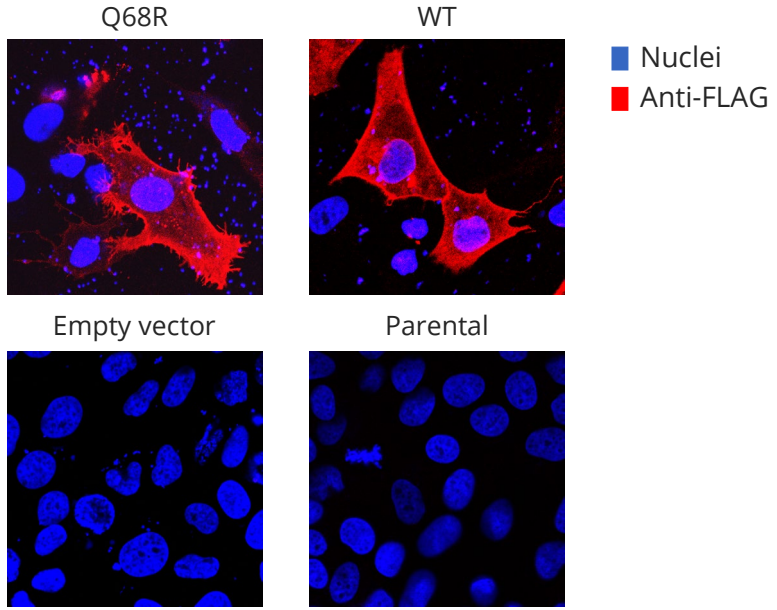
BAs: Bile acids, NTCP: Na-taurocholate cotransporting polypeptide, *transiently transfected U2OS cells. Reference: Cnubben, N. et al. (2024) ASGCT 27th Annual meeting abstracts, Molecular Therapy. Volume 32, Issue 4, 1 – 889 (Abstract 705, p. 355)

The Q68R variant solely affected NTCP bile acids reuptake function

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



NTCP protein localization in vitro*



- No significant differences in NTCP RNA and protein levels were detected. The plasma membrane location of the Q68R variant was also unaffected.
- The Q68R variant solely affects NTCP bile acid reuptake function making it an approach of interest for Axiomer EON therapeutic application.

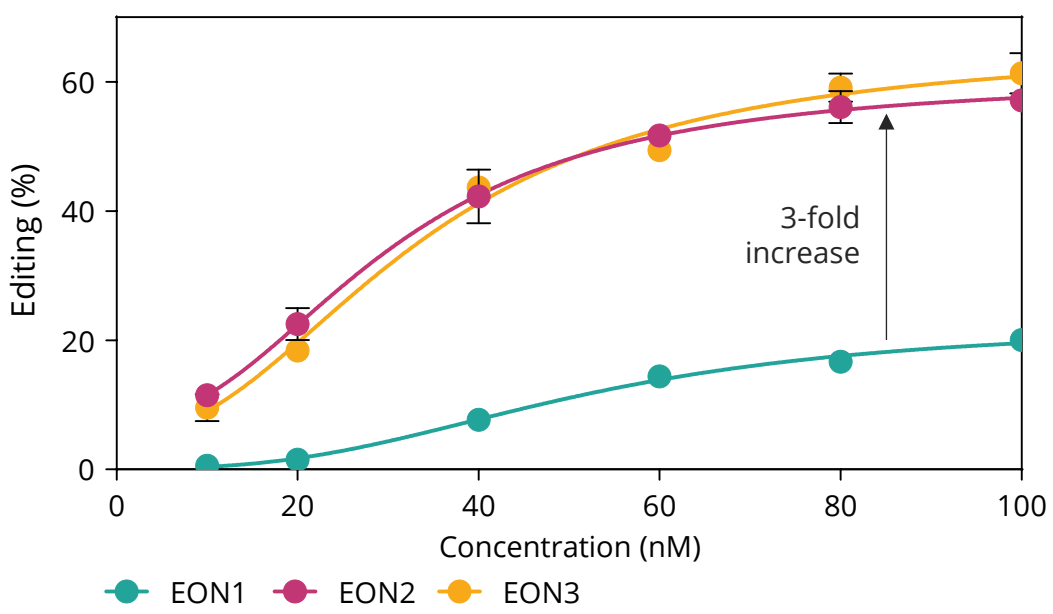
BAs: Bile acids, EON: editing oligonucleotide, NTCP: Na-taurocholate cotransporting polypeptide, *SLC10A1* is the gene that encodes for NTCP protein. Reference: Cnubben, N. et al. (2024) ASGCT 27th Annual meeting abstracts, Molecular Therapy. Volume 32, Issue 4, 1 – 889 (Abstract 705, p. 355)

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

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

EONs targeting NTCP RNA optimization in PHH

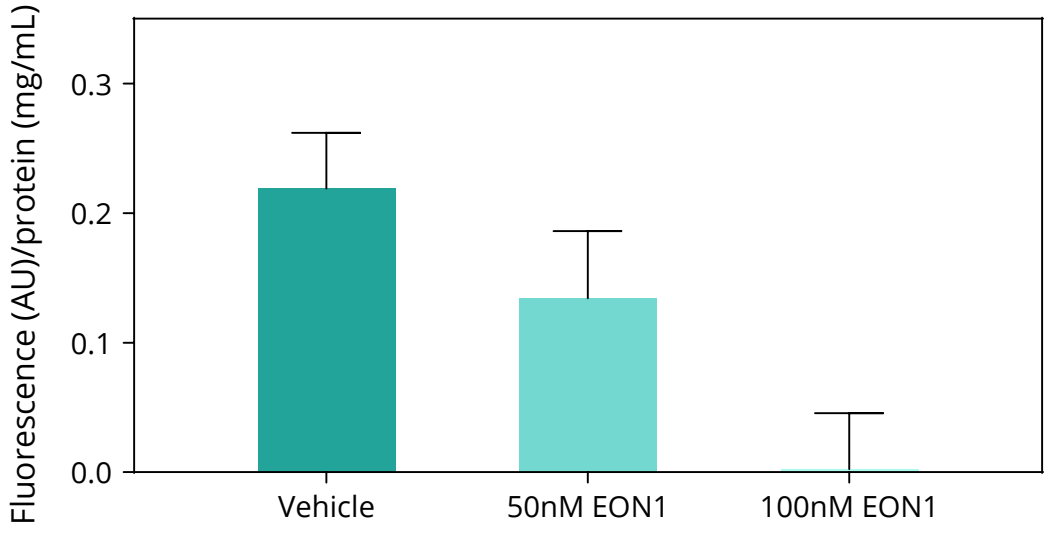
Transfection, n=3, 72 hours, dPCR, mean±SEM



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

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

n=3, 50-100nM, 72 hours, mean±SEM



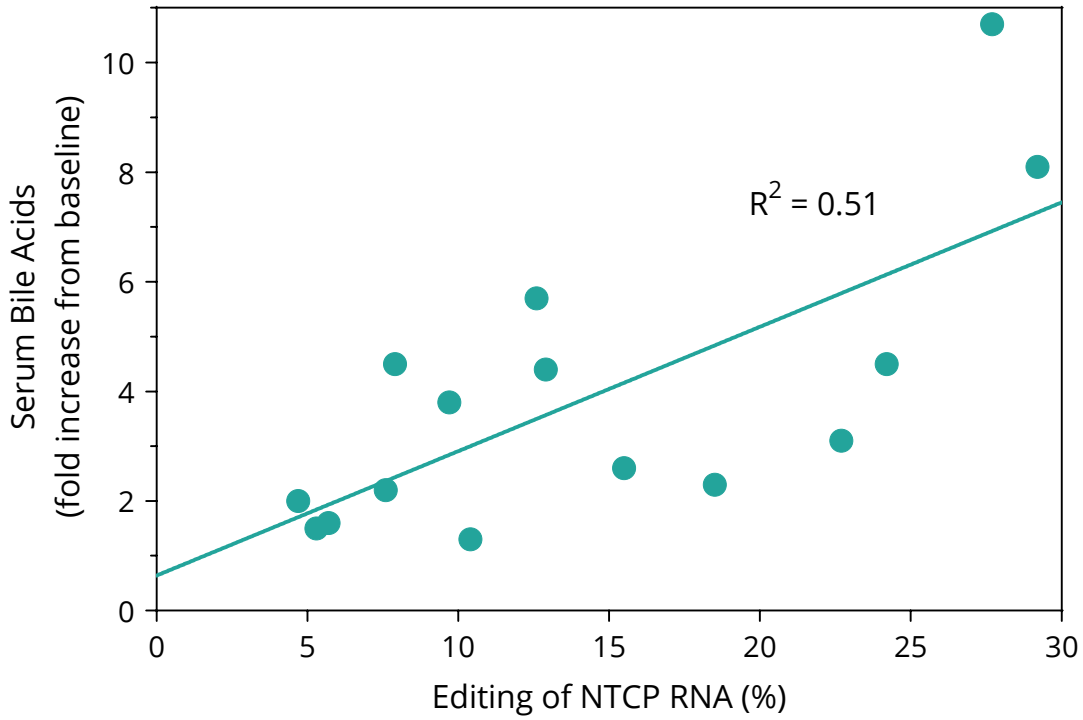
Early generation of EONs (EON1) induces a dose-response inhibition of BAs in vitro confirming its mediation by NTCP

BAs: Bile acids, NTCP: Na-taurocholate cotransporting polypeptide, BAs mentioned in this experiment are specifically Tauro-nor-THCA-24-DBD. *SLC10A1* is the gene that encodes for NTCP protein. Reference: Cnubben, N. et al. (2024) ASGCT 27th Annual meeting abstracts, Molecular Therapy. Volume 32, Issue 4, 1 – 889 (Abstract 705, p. 355)

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

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

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



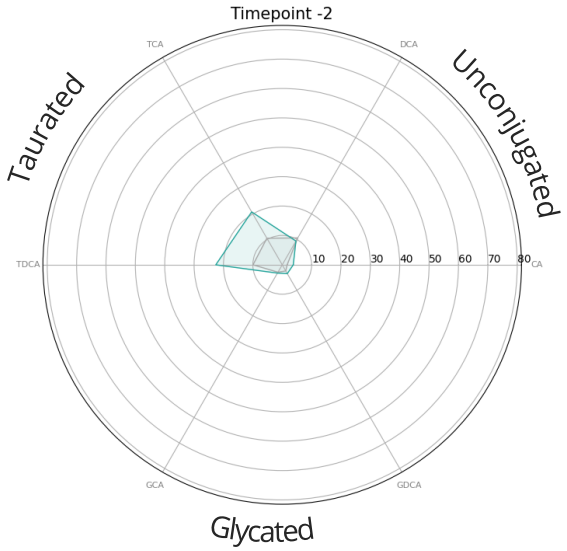
- NTCP target engagement with Axiomer EONs leads to the desired changes in biomarkers
- High correlation between serum bile acids and EON1 editing level in NHPs in vivo (linear regression R2 = 0.51)

BAs: Bile acids, EON1: early generation editing oligonucleotides targeting SCL10A1 (NTCP) mRNA, NTCP: Na-taurocholate cotransporting polypeptide, *SLC10A1* is the gene that encodes for NTCP protein. Reference: Cnubben, N. et al. (2024) ASGCT 27th Annual meeting abstracts, Molecular Therapy. Volume 32, Issue 4, 1 – 889 (Abstract 705, p. 355)

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

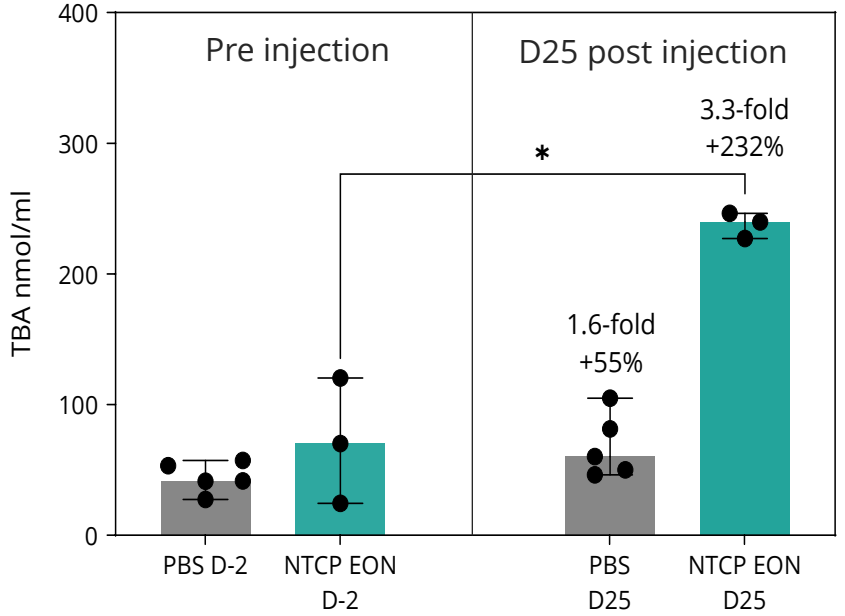
Confirming NTCP bile acids reuptake function modulation

Bile acid profile
Pre injection

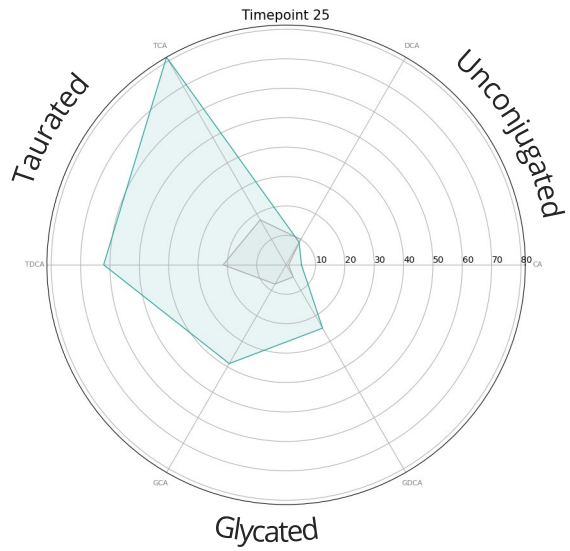


Change in serum bile acids with Axiomer EONs targeting NTCP RNA in humanized liver of mice *in vivo*

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

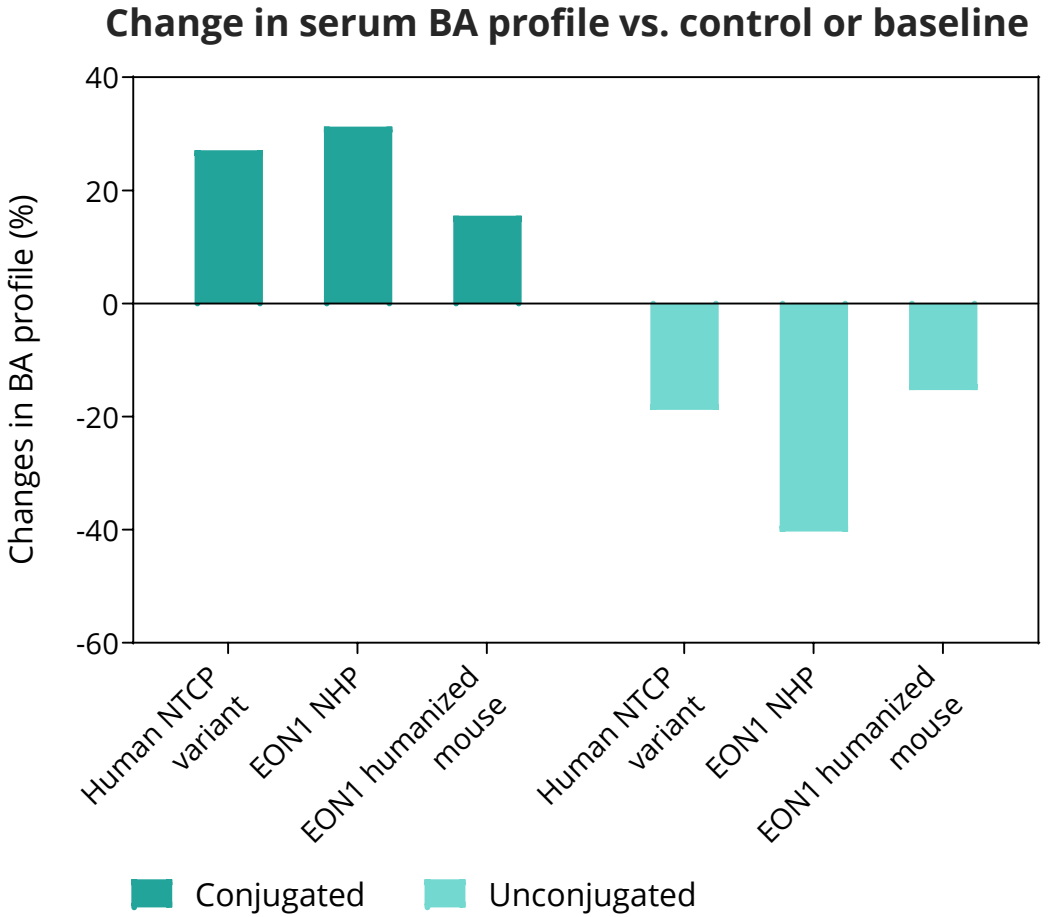


Bile acid profile
D25 post injection



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

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



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

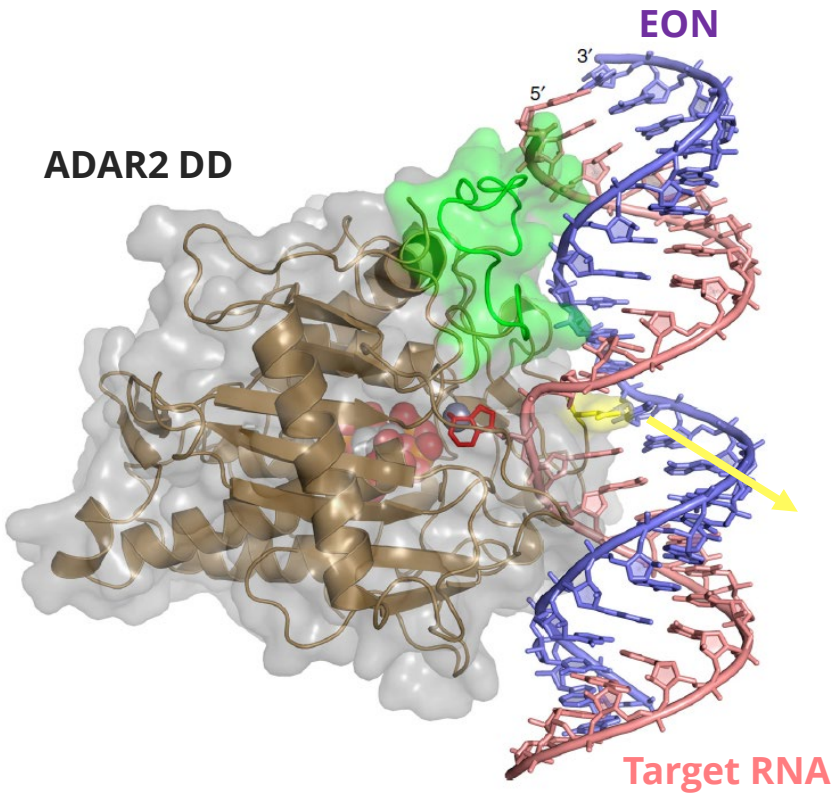
Modification in the Editing Enabling Region (EER)

Cytidine analogs as orphan base

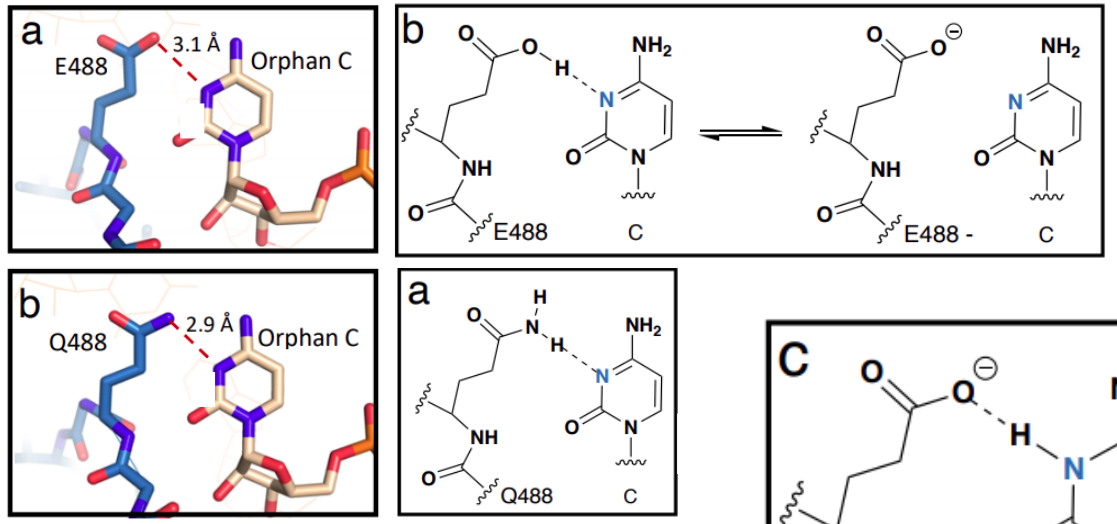
A single base modification of the EER increased ADAR activity

dZ base mimics E488Q mutation in ADAR2 causing hyperactivity

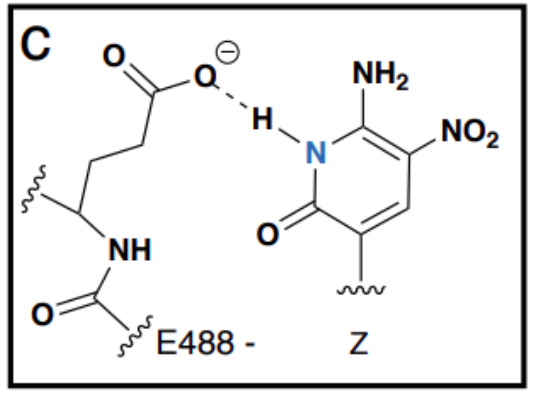
PROQR – UC DAVIS COLLABORATION



Protonation dependent hydrogen bond – pH dependency



Protonation independent hydrogen bond



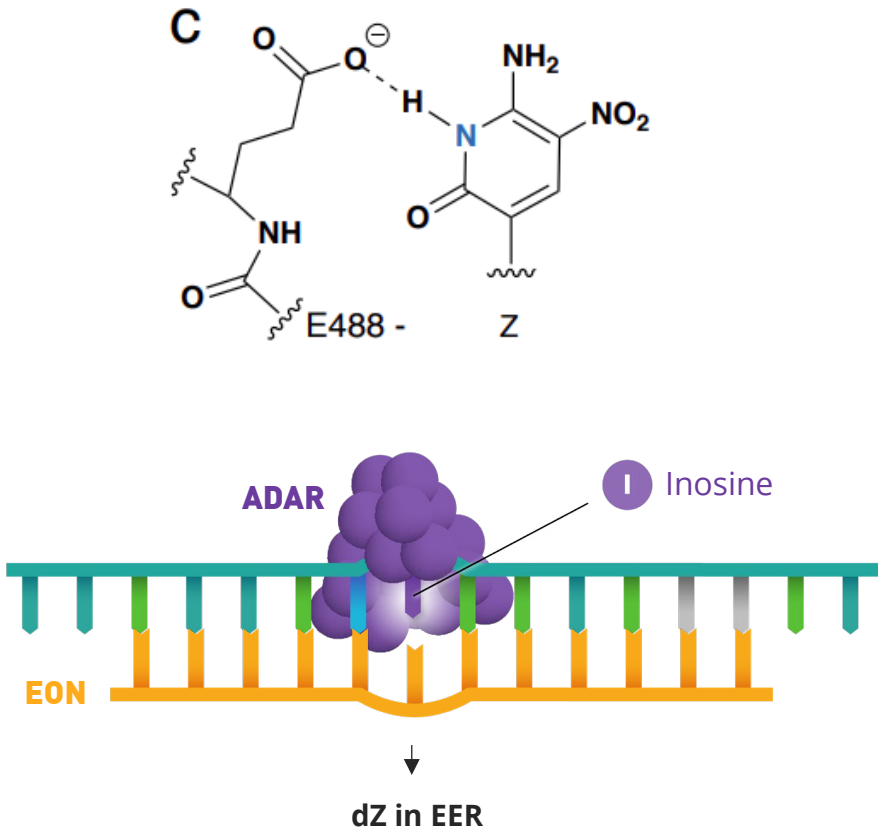
dZ base (dZ)

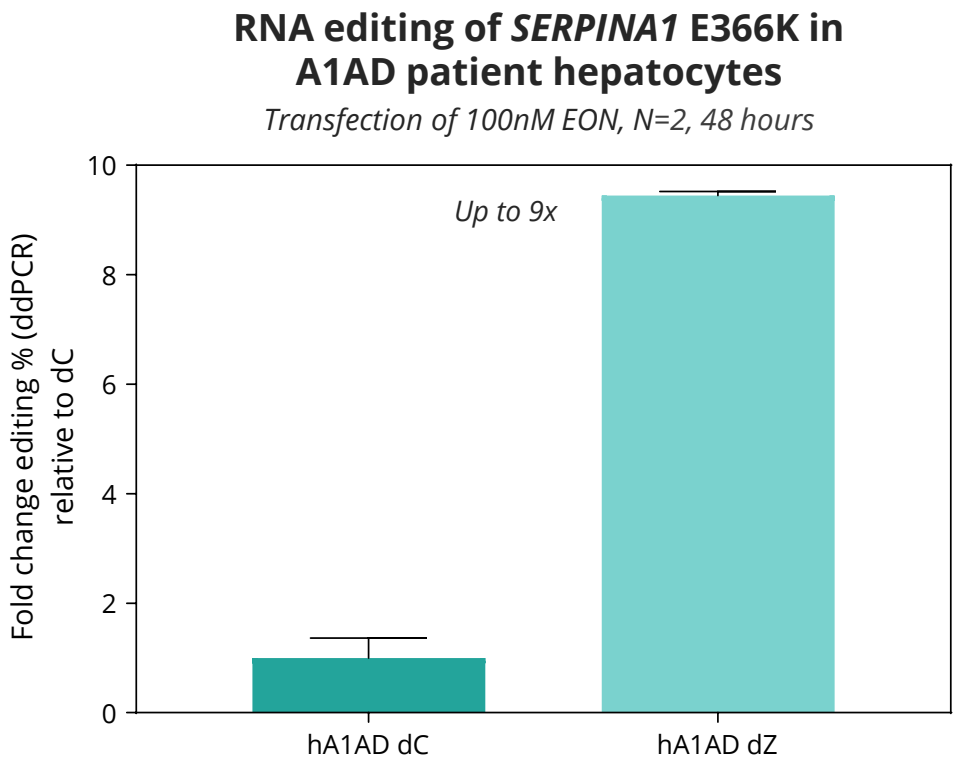
Matthews 2016, Nature Structural & Molecular Biology

Doherty et al., 2021, JACS, ProQR – UC Davis collaboration

dZ in the EER improved editing of SERPINA1 E366K in A1AD patient hepatocytes

PROQR – UC DAVIS COLLABORATION





Improved editing obtained for several systems

dZ improves editing in different cell types

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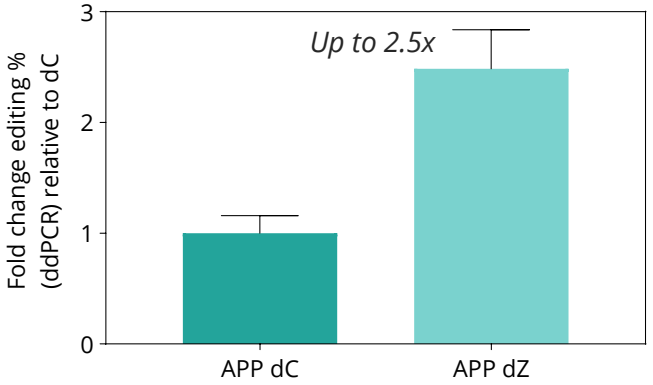
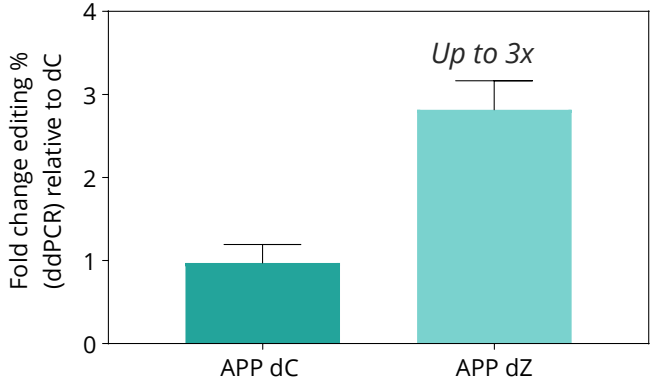
Editing of WT *APP* RNA in human ARPE-19

Transfection of 100nM EON, N=3, 48 hours



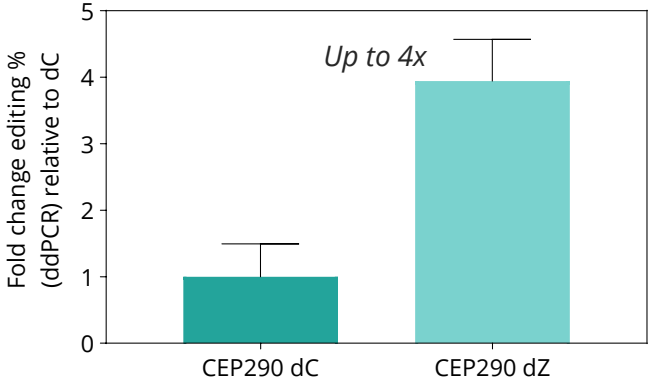
Editing of *APP* WT RNA in human retinal organoids

Gymnosis, 10μM single dose + 40μM CQ, N=6, 4 weeks



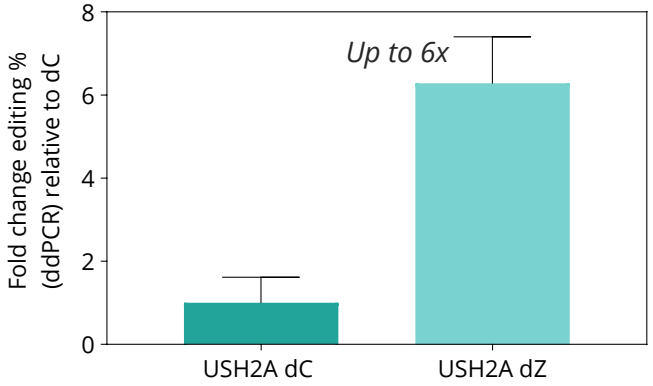
Editing of *CEP290* K1575X in human LCA retinal organoids

Gymnosis, 10μM single dose, N=8, 4 weeks



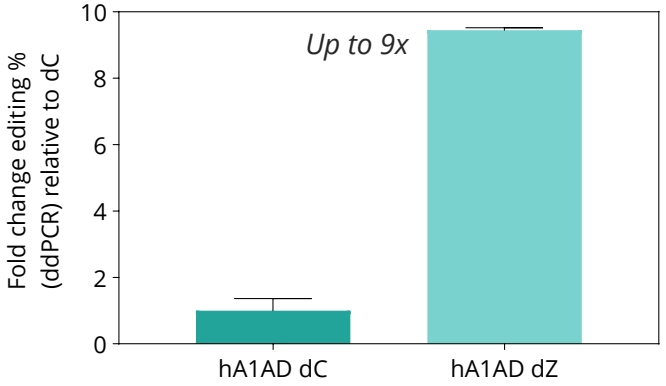
Editing of *USH2A* WT RNA in human retinal organoids

Gymnosis, 15μM single dose + 40μM CQ, N=4, 4 weeks



Editing of *SERPINA1* E366K in A1AD patient hepatocytes

Transfection of 100nM EON, N=2, 48 hours



ADAR knows few sequence constraints

With the exception of G upstream of target adenosine (5'-GA-3')

Location	**ADAR1/ADAR2 common**	**ADAR1 specific***	**ADAR2 specific***
	# sites (% WT/dHet sites)	# sites (% WT/dHet sites)	# sites (% WT/dHet sites)
Promoter/ 5' UTR	43 (57.33%)	13 (17.33%)	14 (18.66%)
Exon	225 (51.84%)	80 (18.43%)	92 (21.20%)
Intron	2239 (45.37%)	960 (19.45%)	1286 (26.06%)
3'UTR	142 (50.90%)	43 (15.41%)	66 (23.66%)
Intergenic	200 (65.36%)	47 (15.36%)	62 (20.26%)
Total	**2849 (47.25%)**	**1143 (18.96%)**	**1520 (25.21%)**

This has wide implications for the applicability of targeted RNA editing – guide RNAs with Watson-Crick complementarity are enough to recruit ADAR and induce targeted editing

Adapted from Eggington et al. Predicting sites of ADAR editing in double-stranded RNA. Nat Commun. 2011;2:319

A single base change opposite the 5’-G greatly enhances editing

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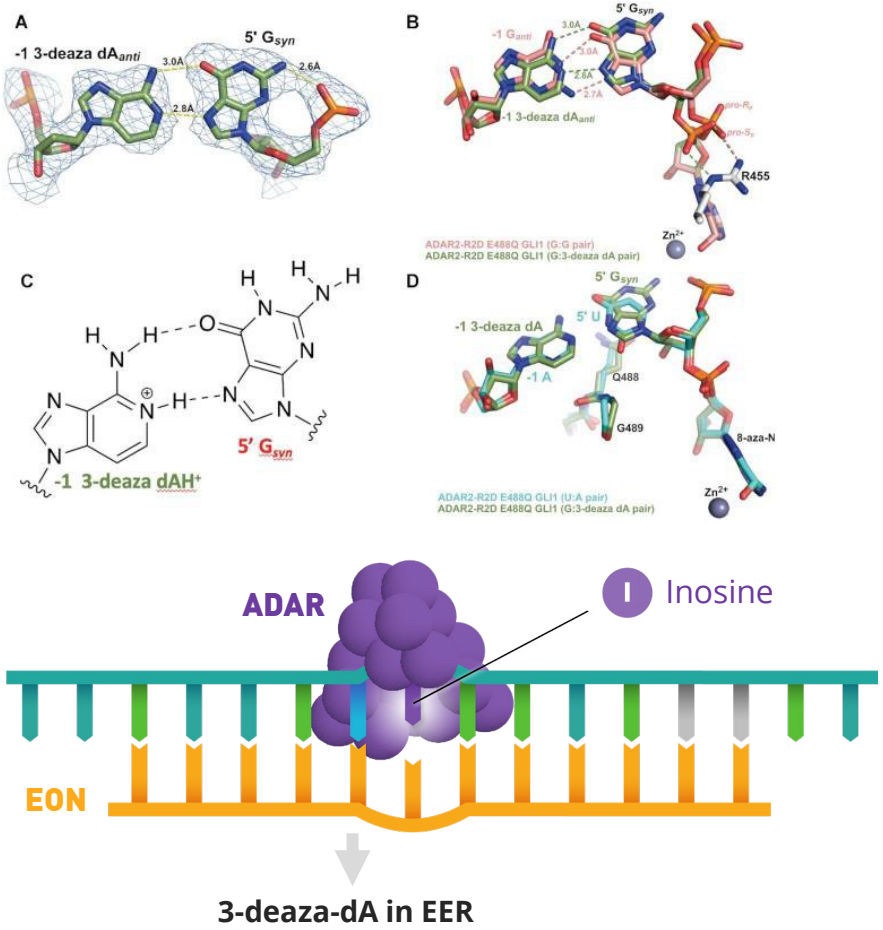
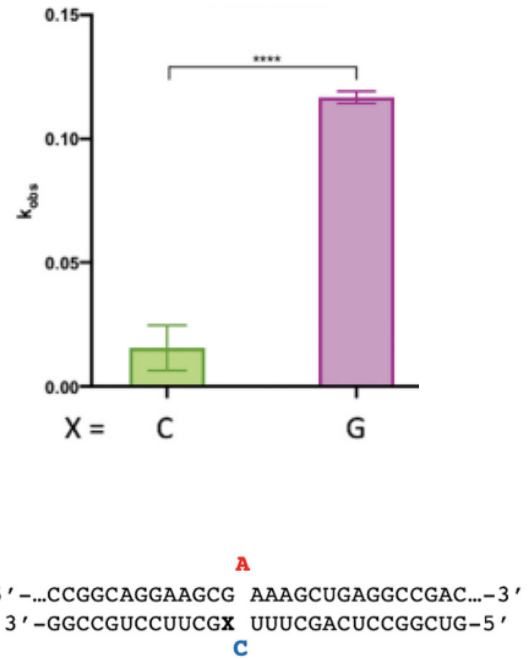


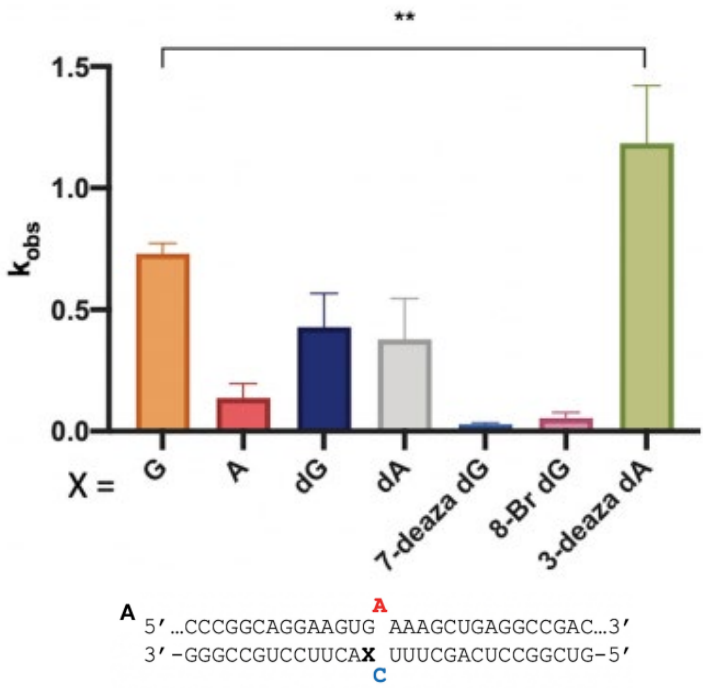
In vitro deamination kinetics for ADAR2 and duplex RNAs derived from WT _hMECP2_

100 nM ADAR2, 3 technical replicates, mean, SD



In vitro deamination kinetics for ADAR2 and duplex RNAs derived from _hMECP2_ R255X

100nM ADAR2, 3 technical replicates, mean, SD



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.

Adapted from Doherty EE, et al. Nucleic Acids Res. 2022;50(19):10857-10868.

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