THERAPEUTIC POTENTIAL OF AN RNA EDITING PLATFORM USING EDITING OLIGONUCLEOTIDES

OPT Congress

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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 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

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

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

Validated across multiple genes

Preclinical data demonstrate Axiomer® is broadly validated across multiple genes

ADAR

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

Two pillars underly strategy

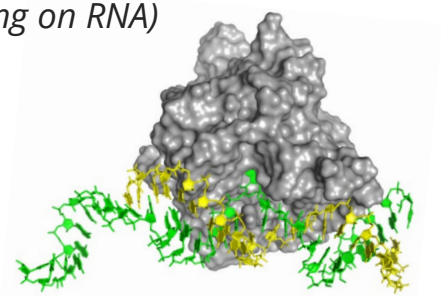
- ProQR developing wholly owned pipeline: Initial targets to be disclosed in early 2023
- Selectively enter into partnerships: initial partnership with Lilly in Sept 2021, expansion announced Dec 2022

Ophthalmology partner

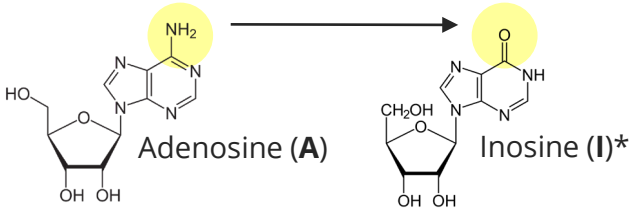
Seeking strategic partner for ophthalmology assets

What is ADAR editing?

ADAR *(Adenosine Deaminase Acting on RNA)*



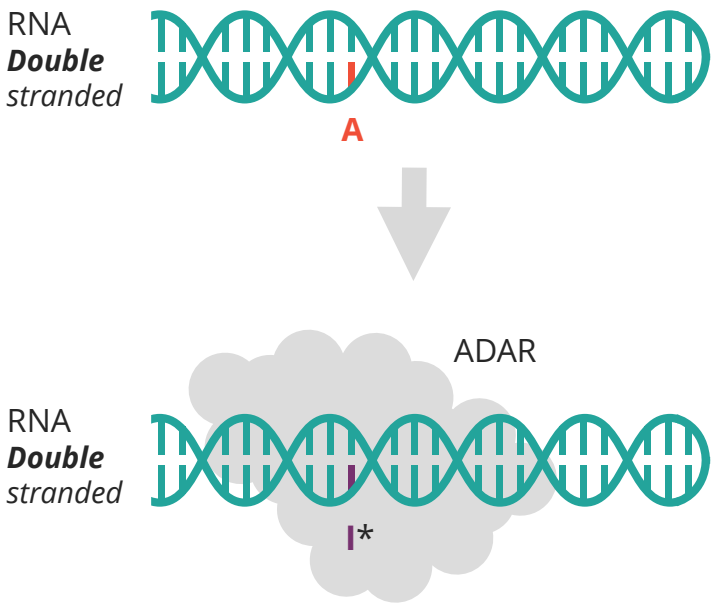
Enzyme that performs specific form of natural RNA editing, called **A-to-I editing.** During A-to-I editing an **A nucleotide (adenosine)** is changed into an **I nucleotide (inosine)**



*Inosine will be read as Guanosine (**G**)

Natural ADAR editing

(A-to-I)



A = Adenosine **I** = Inosine *Will be read as **G** (Guanosine)

- ADAR normally binds to **double stranded structures** in RNA to perform A-to-I editing
- Later, during the translation process, the 'I' in the RNA is read as a 'G' (guanosine) by the cell

What is Axiomer®?

How Axiomer® works

- Uses short strands of synthetic RNA, called **EONs** (Editing Oligonucleotides)

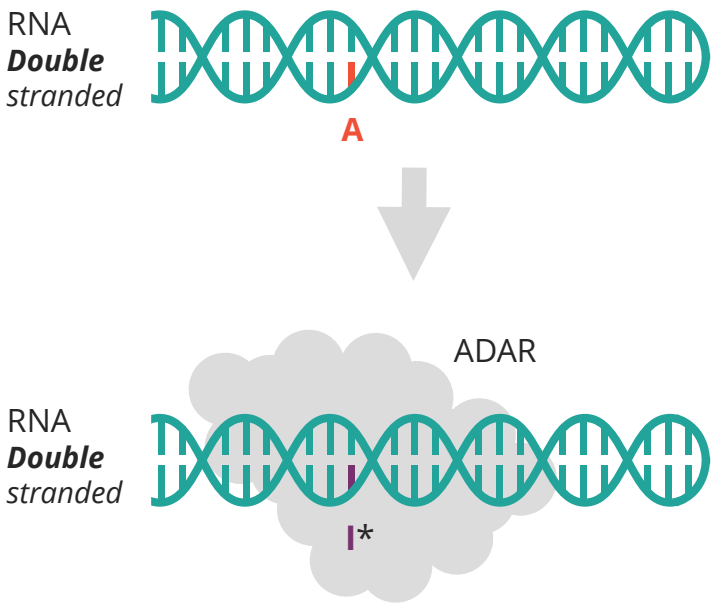
- EONs bind to the target (**single stranded**) RNA and mimics double stranded structure that attracts ADAR
- EONs attract ADAR to specific location in RNA to make A-to-I edit

Results

- RNA with disease-causing mutation is corrected back to normal RNA
- Function of protein is changed to help prevent or treat disease

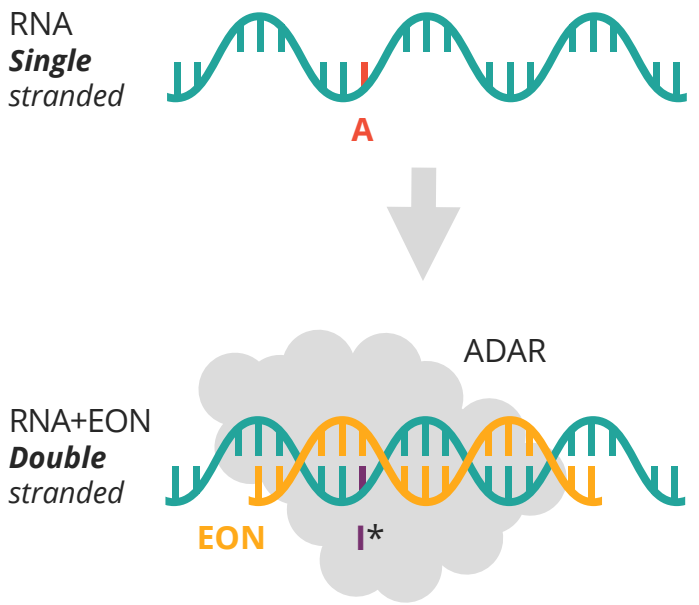
Natural ADAR editing

(A-to-I)



EON-directed therapeutic editing

(A-to-I)

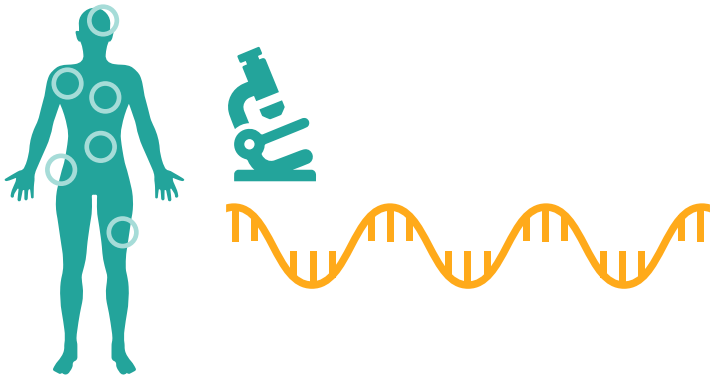


A = Adenosine **I** = Inosine *Will be read as **G** (Guanosine)

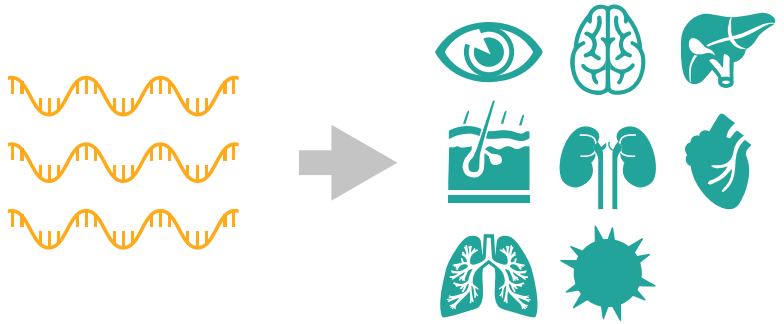
How does Axiomer® work?

Step by step

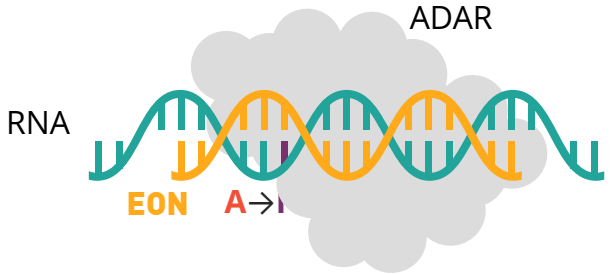
1 We identify where an A-to-I edit could treat disease, and design an EON



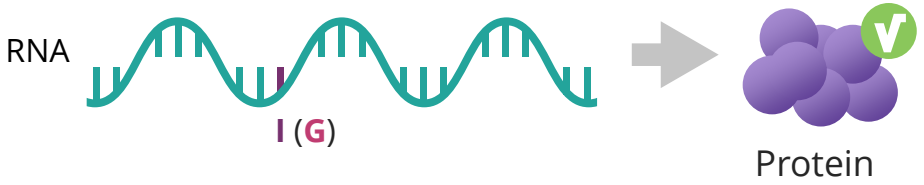
2 The EON is periodically delivered to the targeted organ or tissue



3 The EON binds to the target RNA and attracts ADAR to make an A-to-I edit



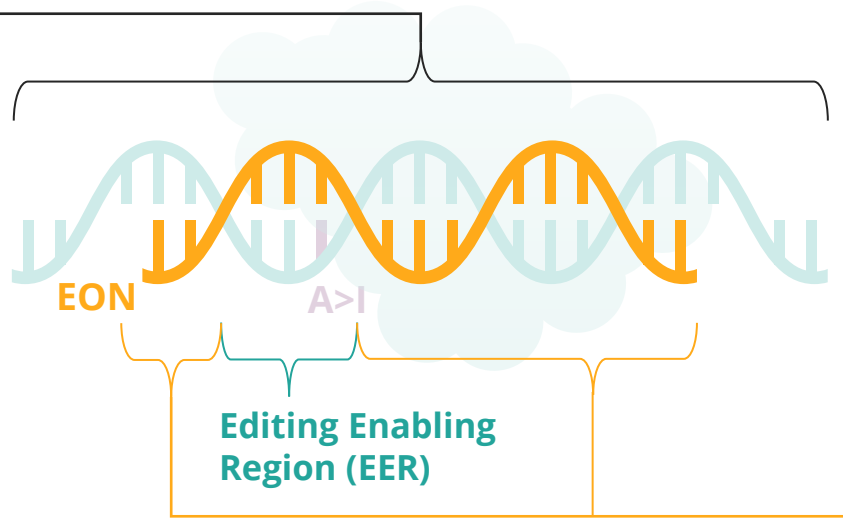
4 During translation, the 'I' is read as a 'G', resulting in a corrected or altered protein



ProQR expertise 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

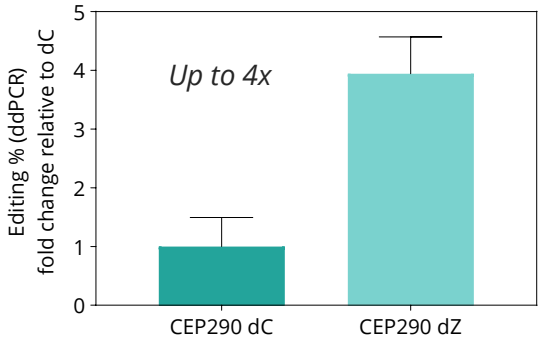
Improved editing obtained for several targets

dZ modification on EER improves editing in different cell types

ProQR – UC DAVIS Collaboration

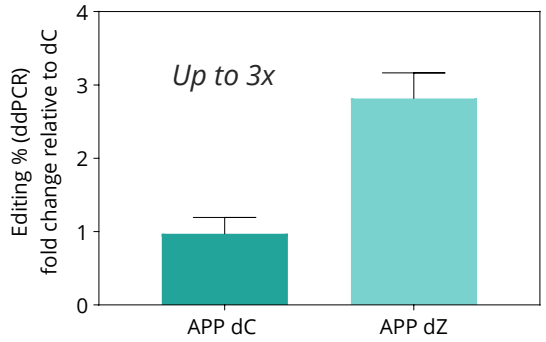
Editing of hCEP290 K1575X in human LCA retinal organoids

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



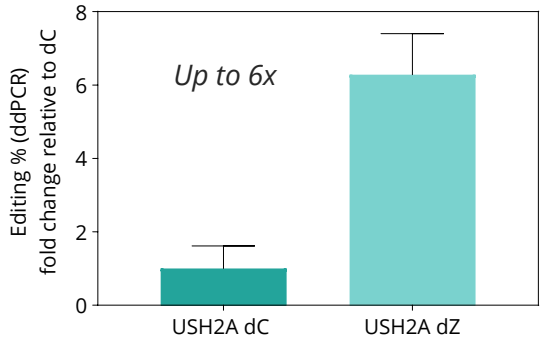
Editing of APP WT RNA in human retinal organoids

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



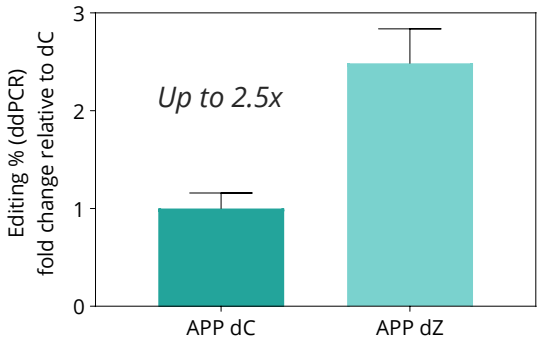
Editing of USH2A WT RNA in human retinal organoids

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



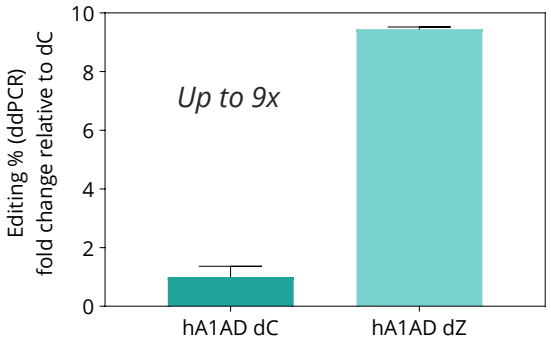
Editing of WT APP RNA in human ARPE-19

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

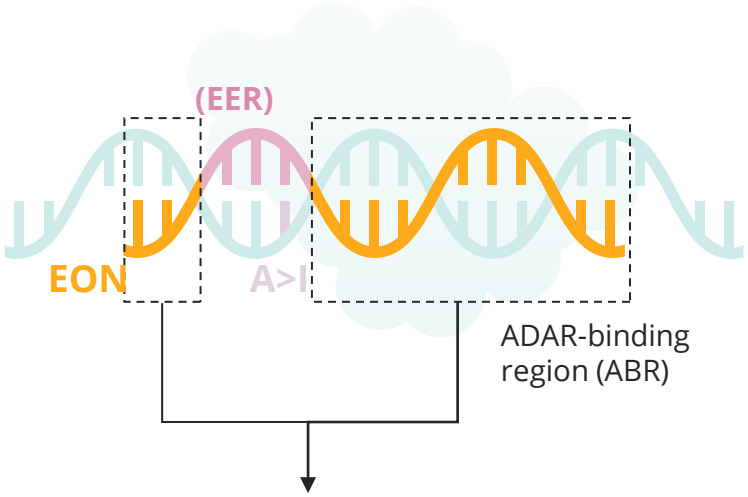


Editing of SERPINA1 E366K in A1AD patient hepatocytes

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



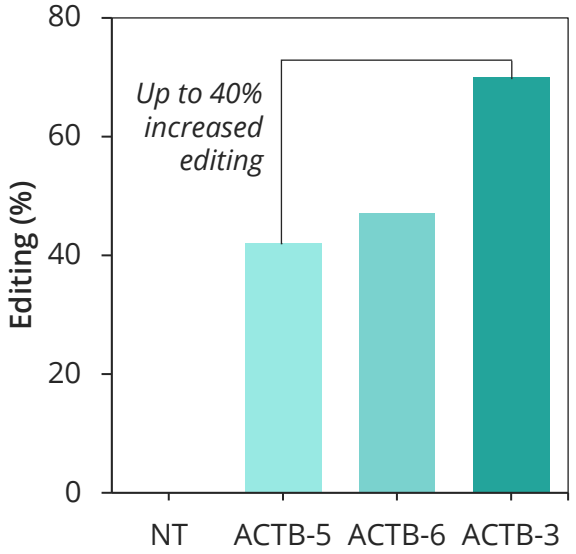
ADAR-binding region (ABR) modification greatly enhances editing



Backbone modifications enable ADAR binding, and **improve** stability

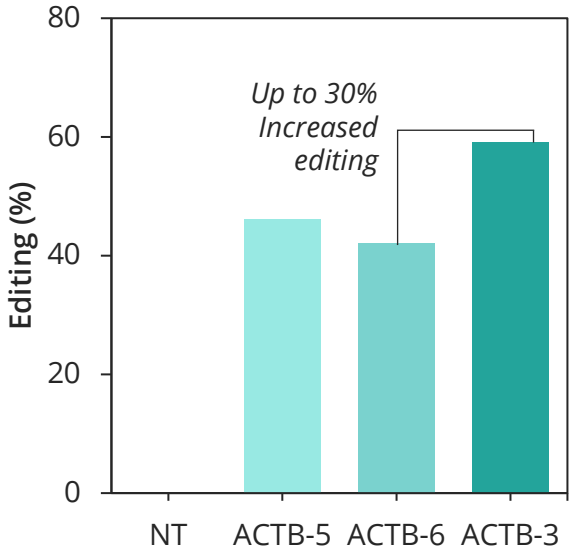
Editing of ACTB in human primary hepatocytes

(Gymnosis, 10uM, single dose, N=1, 48 hours, dPCR)



Editing of ACTB in human retinal pigment epithelium cells

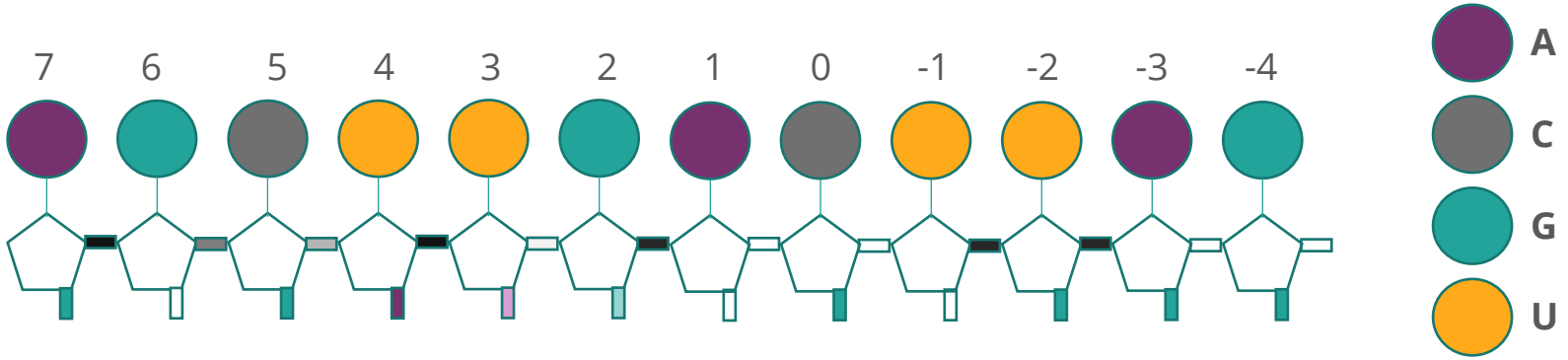
(Transfection, 100nM, single dose, N=1, 48 hours, dPCR)



- Chemical optimization greatly increases EON editing in positions within ABR region
- SAR screen of 2nd backbone modification for best position within ABR region ongoing

Focus on the EON design principles

EON

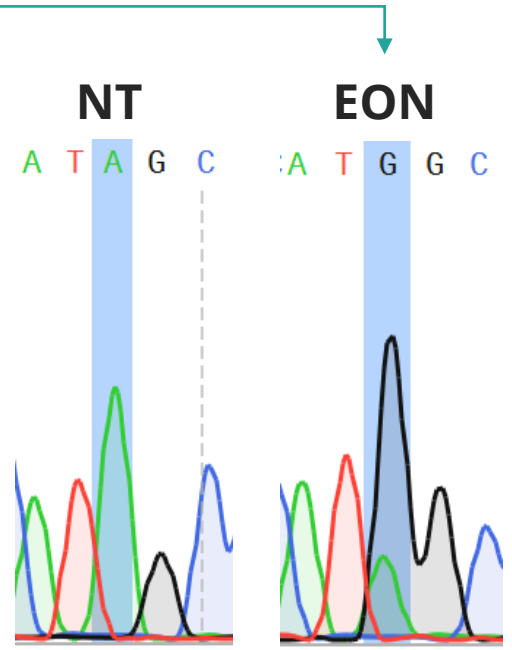


Aspect	Determined by	Modifications	Effects
Base	Target RNA	Mismatches and analogs	Improved PD
Ribose modification	ADAR structure	2′-H; 2′-OMe; 2′-MOE; 2′-F; 2′-NH2, LNA, TNA, diF, 2′-FANA	Improved PK and PD
Linkage	ADAR structure	PO; PS; PN; MeP; UNA; PAc	Improved PK and PD

This work led to a portfolio of 13 foundational platform patents

Axiomer® is broadly validated across multiple genes

Functional aim of editing	Target RNA	Editing up to*
Reverse G-to-A mutation	*GFP*	85 %
Reverse G-to-A mutation	*mIdua*	60 %
(None; WT target)	*mUsh2a*	80 %
Reverse G-to-A mutation	*hUSH2A*	50 %
Inactivate protease site	*hAPP*	50 %
Inactivate kinase site	*hEPHB3*	60 %
Inactivate kinase site	*hEPHA7*	60 %
Reverse G-to-A mutation	*hSERPINA1*	70 %



GFP reporter in Hepa1-6 cells

*ProQR data on file

Optimization of Axiomer® in multiple models, targets and organs

Opening the pathway for new class of medicines targeting diverse types of diseases

The retina
as early proof of concept

The CNS
The CNS as the next frontier

The liver
as a promising area of development

Model targets

PoC therapeutic targets
Tool targets used for optimization

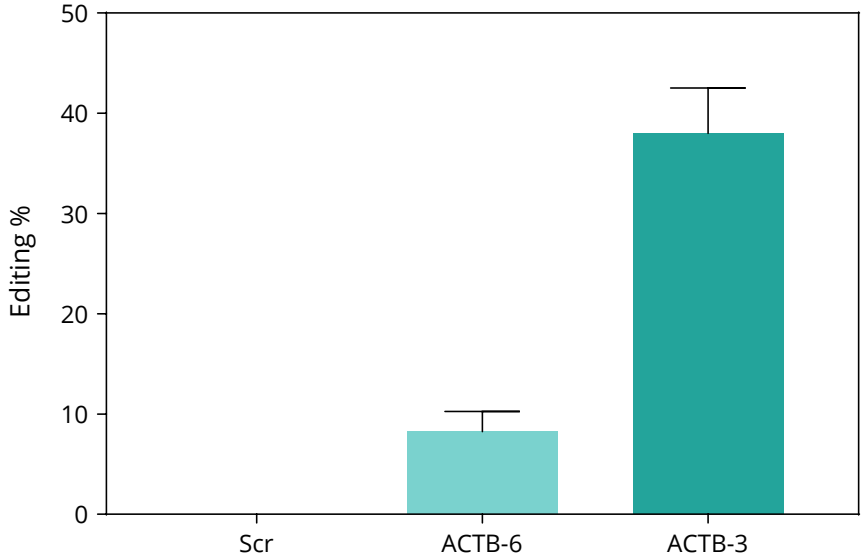
Pipeline targets

The retina as early proof-of-concept

Approx. 20% editing was observed after gymnosis for CEP290, a tool targets used for optimization

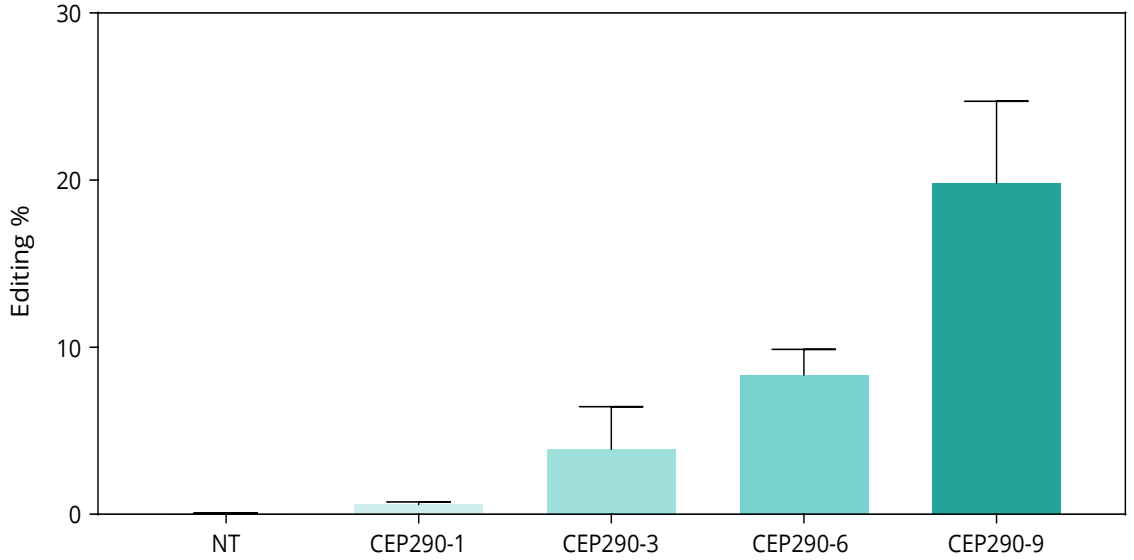
Editing of ACTB in human retinal organoids

(Gymnosis, 20 uM, single dose, N=6, 7 days, ddPCR)



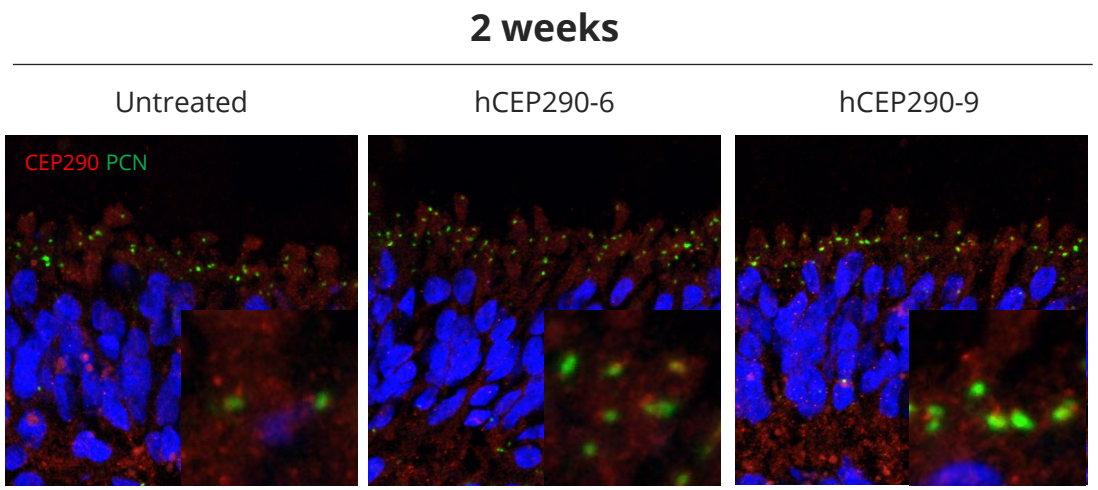
Editing of CEP290 in LCA human retinal organoids

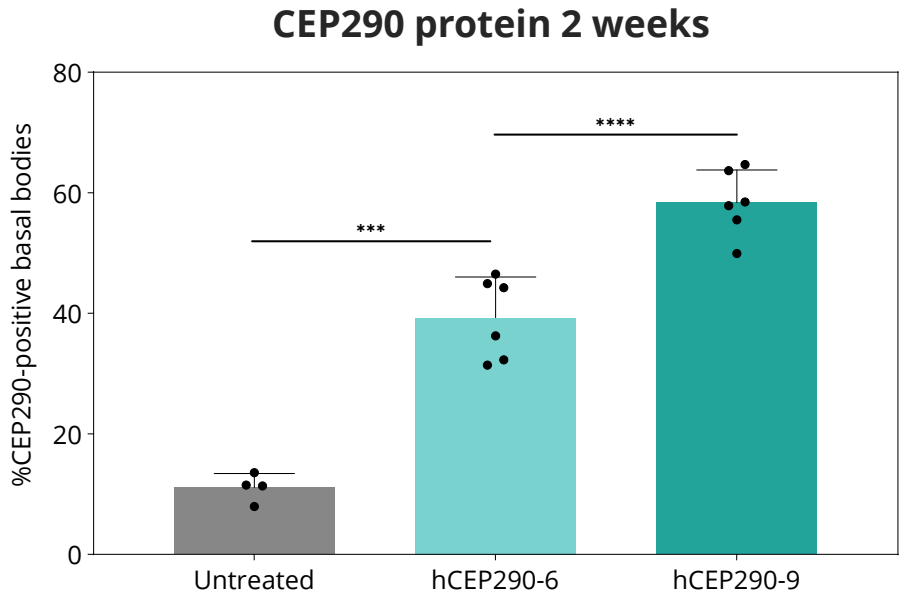
(Gymnosis, 10uM, single dose, N=8, 2 weeks, ddPCR)



- Each chemical modification improves EON editing efficacy
- The highest editing efficacy increase is obtained for EONs with all modification combined
- Over 40% editing was observed after gymnosis for ACTB and over 20% editing observed after gymnosis for CEP290

Editing results in significant increase in CEP290 protein levels and intensity at the basal body



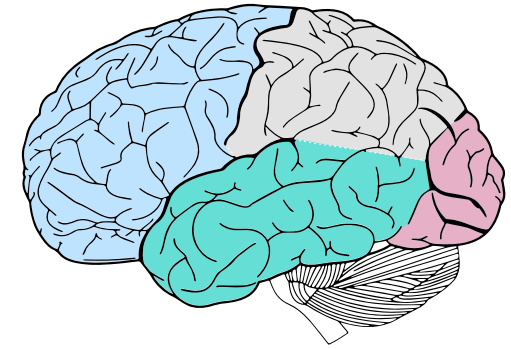


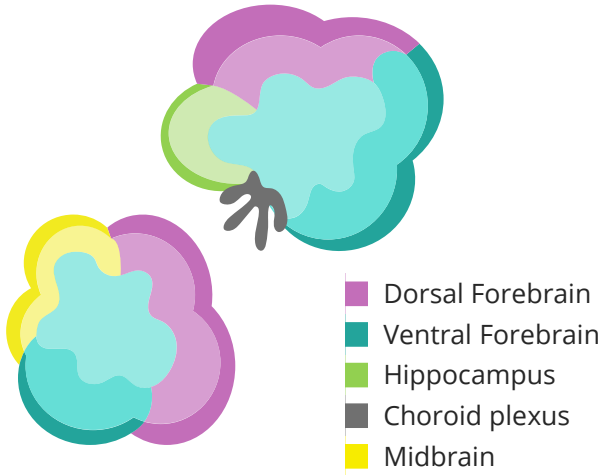
Mean ±SEM. Statistical significance was determined using Brown-Forsythe and Welch ANOVA test

Significant increase in CEP290 protein levels and intensity was detected at the basal body of LCA07-3 organoids treated with hCEP290-6 and-9 after 2-weeks treatment

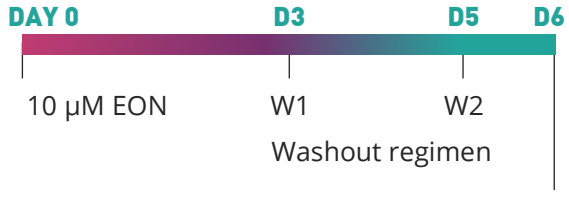
The CNS as the next frontier

>30% editing was achieved in cerebral brain organoids



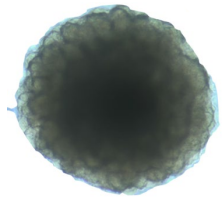


Gymnotic uptake



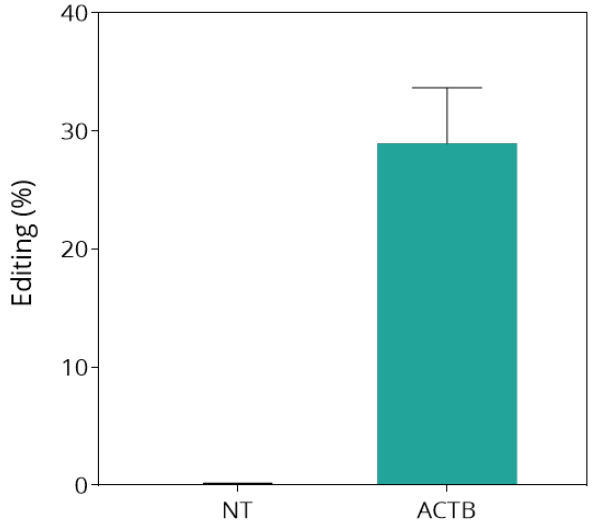
Human brain organoid

10 days



Editing of ACTB in IPSC derived brain organoids

(Gymnosis, 10uM, single dose, n=7, 6 days, ddPCR)

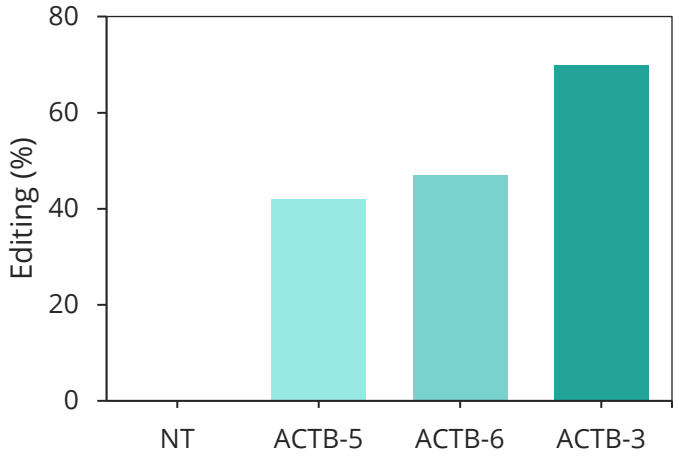


The liver as a promising area of development

High potential of EONs editing in the liver

Editing of ACTB in human primary hepatocytes

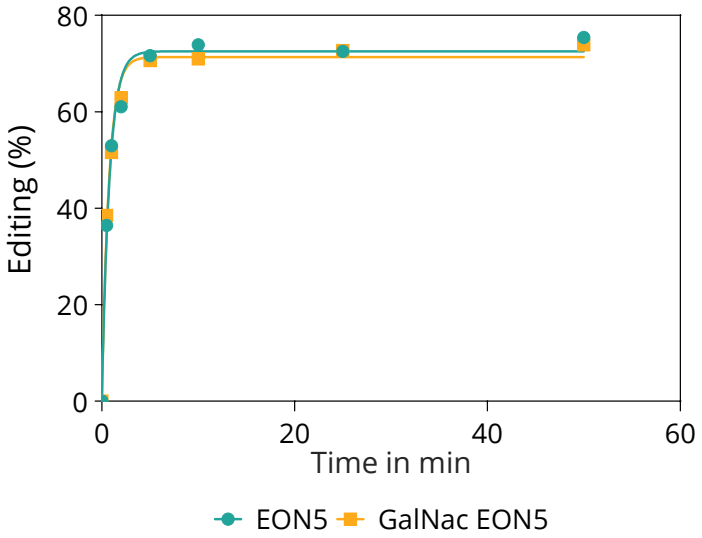
Gymnosis, 10uM, single dose, N=1, 48 hours, dPCR



- Similar levels of editing of ACTB achieved in several models of liver origin
- High confidence of translatability of the approach

GalNac does not interfere A-to-I editing *in vitro*

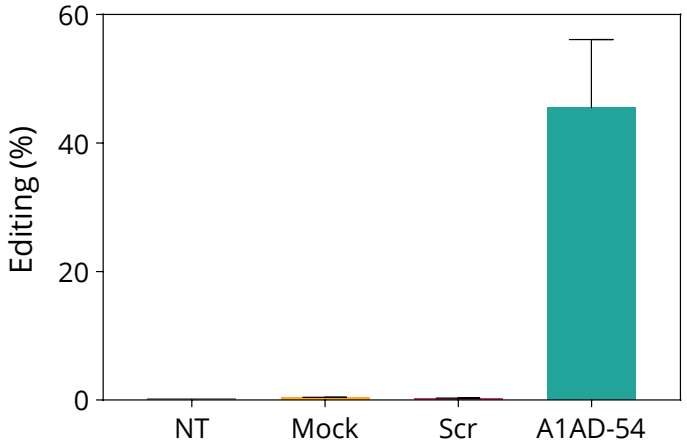
N=1, BEA assay



GalNAc appears not to interfere with ADAR binding or efficient RNA editing

Editing of SERPINA1 E366K in human A1AD patient hepatocytes

Transfection, 100 nM, single dose, N=2, 47 hours, dPCR



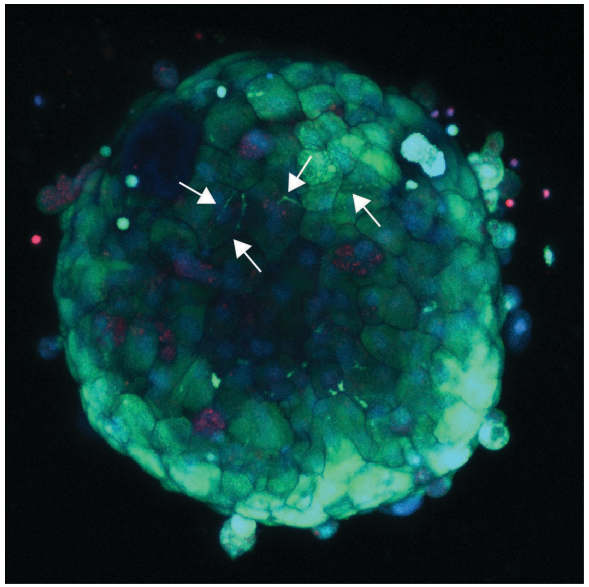
>50% Editing of SERPINA1 E366K in human A1AD patient hepatocytes

Editing in Human Liver microtissues (LMTs)

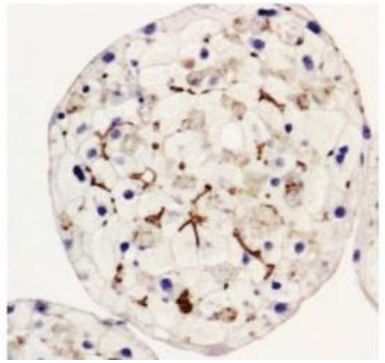
Primary hepatocytes, Kupffer cells and liver endothelial cells in 3D spheroid

Live image of Day 7 LMT

Stained with 5-CFDA (green), PI (red) and Hoescht (nuclei; blue)



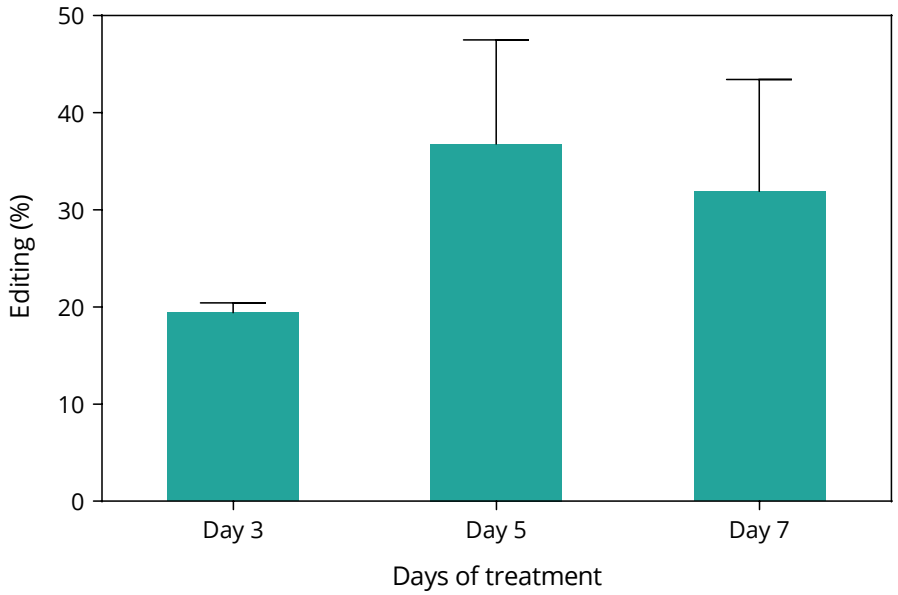
BSEP Bile Canaliculi
(InSphero data)



Presence of bile channels in LMTs by day 7
Fluorescent dye 5-CFDA secreted from healthy cells into bile channels (canaliculi)

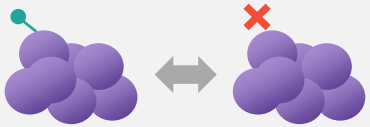
Editing of ACTB in human LMTs

(Gymnosis, 5µM, single dose, 3 pools of 6 LMTs per condition, 7 days, dPCR)



Treatment of LMTs with 5µM EON for 7 days results in up to 40% of edited ACTB.

Axiomer® technology potential

Correction	Modulation / Regulation		Modification	
				
Repairing G-to-A mutations Thousands of G-to-A mutations described in literature	**Edit >400 different types of PTMs** *(Post-translational modifications)* To regulate protein activity, changes localization, folding, prevent immune escape and slows down degradation	**Change protein-protein interactions** Changes localization, folding and protein function and prevent immune escape of glycosylated tumor antigens	**Create new proteins** New proteins achieving loss or gain of functions that helps address disease	**Include protective variants** At the RNA level, to develop new functions that help prevent disease

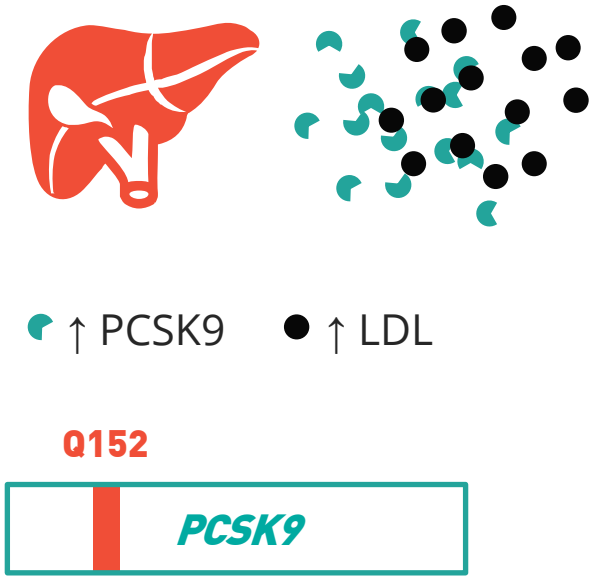
Broad therapeutic potential

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

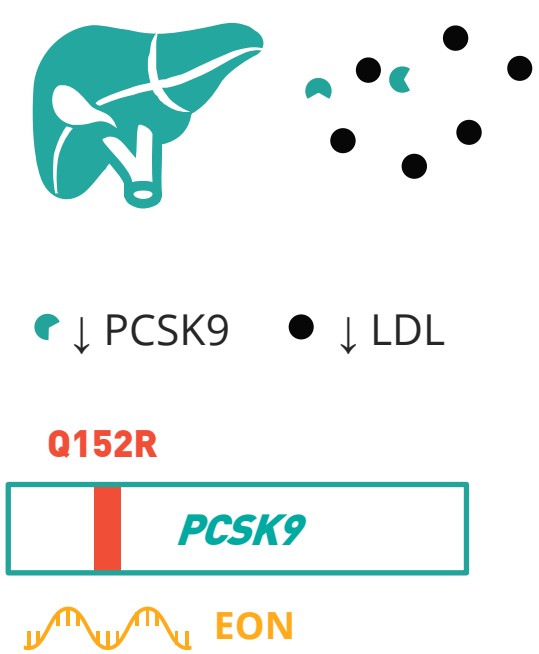
Liver targeted editing of *PSCK9*

De novo generation of a loss-of-function variant to lower PCSK9

FEH patients



Axiomer® edit



Disruption of PCSK9 autocleavage site reduces protein in bloodstream

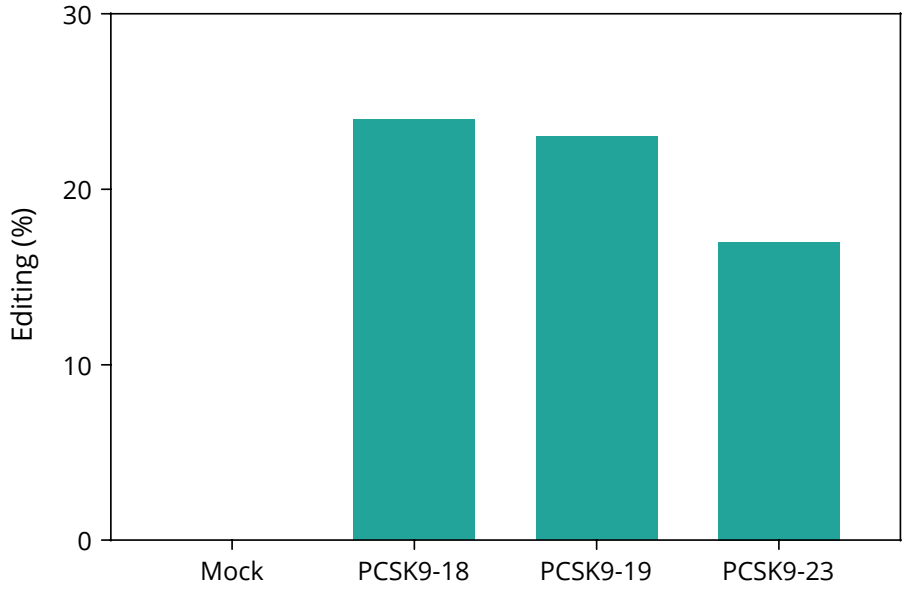
- Less PCSK9 leads to increase of LDL-R on cells, decrease of 'bad' LDL in bloodstream
- Loss-of-function *PCSK9* variant Q152H is associated with low plasma LDL cholesterol in a French-Canadian family and with impaired processing and secretion in cell culture

PCSK9 mRNA editing reduces PCSK9 protein levels

Editing of PCSK9 mRNA results in a loss-of-function phenotype

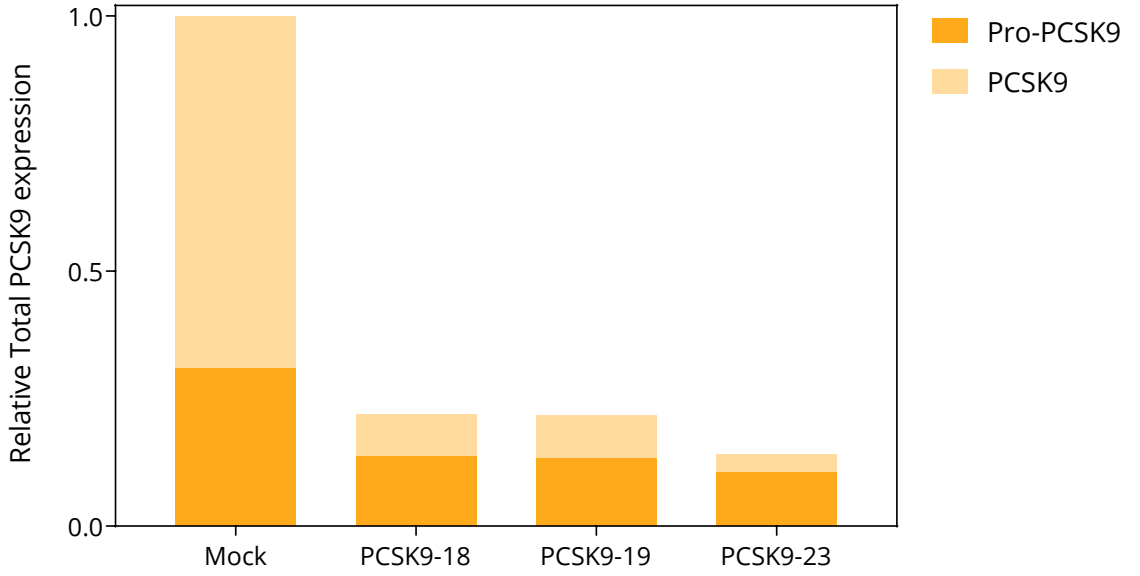
Editing of PCSK9 in HeLa cells

Transfection, 100nM, single dose, N=2, 48 hours, dPCR



PCSK9 protein expression in HeLa cells

Transfection, 100nM, single dose, N=2, 48 hours, western blot



- Up to 25% precent A-to-I editing of PCSK9 mRNA detected using ddPCR assays
- EONs treated HeLa cells produce lower levels and more uncleaved PCSK9 protein
- Up to 80% reduction of total PCSK9 protein measured in treated samples
- Shift in the ratio cleaved to uncleaved PCSK9 observed; 70%:30% to 25%:75%

Next steps Axiomer® platform

In house strategy

- Expand investments in Axiomer® platform and pipeline development and target selection activities
- Initial focus on Liver originated diseases and CNS
- Planning to announce internal development update during the R&D day scheduled March 29, 2023

Partnership strategy

- Continue to execute on the partnership with Lilly
- Potential for additional partnerships, building on industry leading IP estate and strong development capabilities

RNA editing expert advisory board

Scientific Advisory Board

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IT'S IN OUR RNA