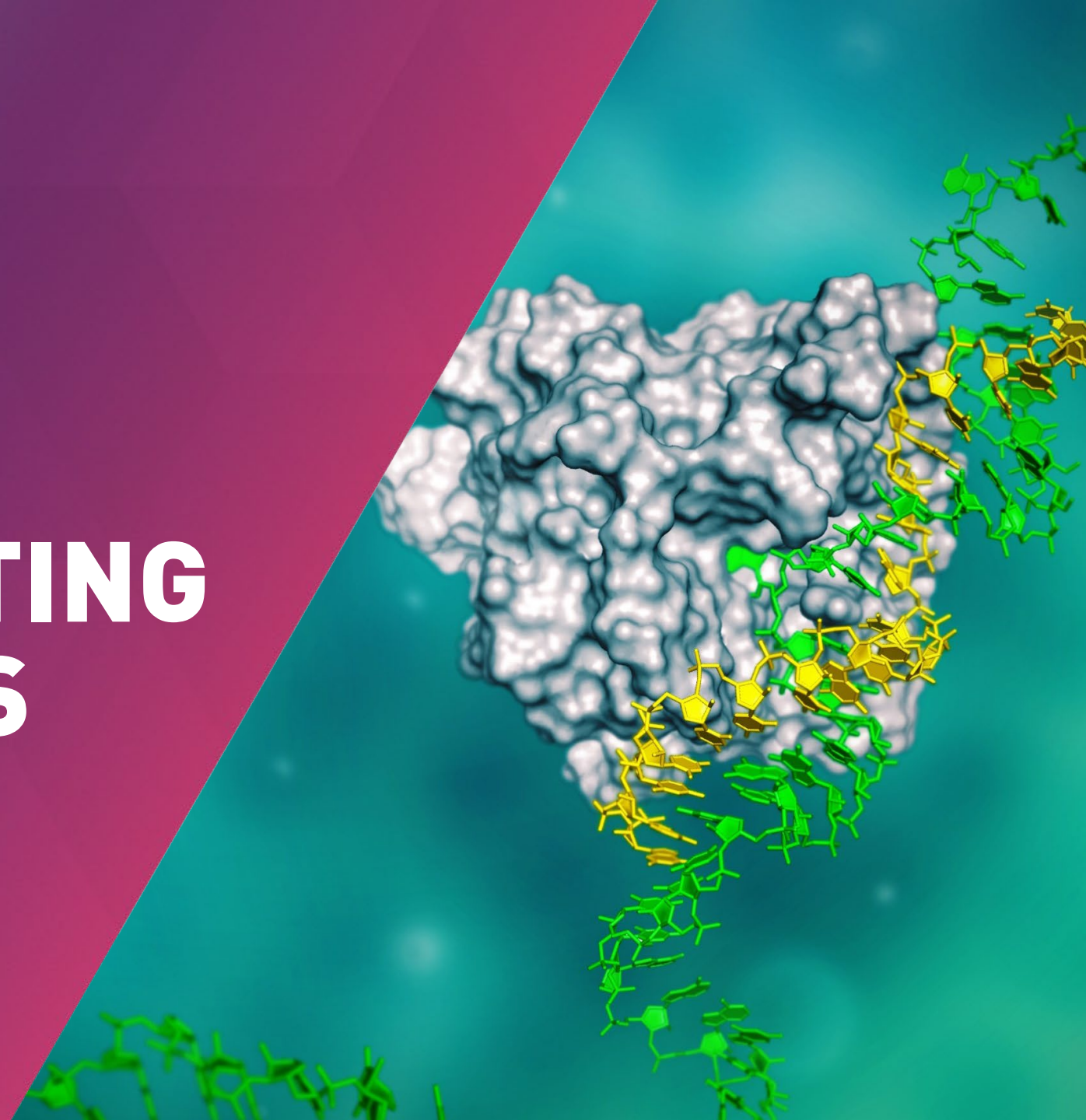
ProQR®

DEVELOPING RNA BASE EDITING TECHNOLOGIES

For precision medicines



Gerard Platenburg, Chief Scientific Officer

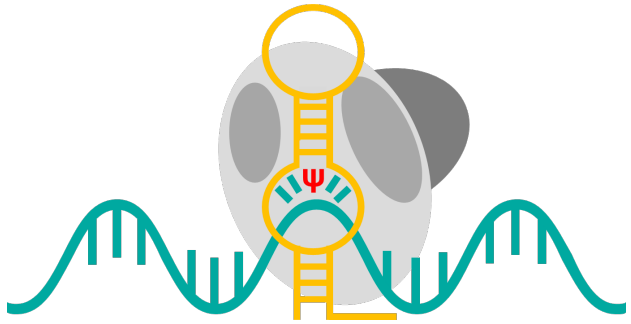
November 18th , 2022

RNA toolbox – editing platform technologies

Axiomer® and Trident® in development by ProQR

Axiomer® A-to-I editing

- Exploiting endogenous ADAR
- Recruited by synthetic Editing Oligonucleotide (EON)
- I is translated as a G, allowing to target G-to-A mutations
- Specific, potent, and stable by design
- Thousands of G-to-A mutations described in literature



Trident® U-to-Ψ editing

- Exploiting endogenous pseudouridylation machinery
- Recruited by single stranded pseudouridylation EON (psEON)
- Specifically target PTC mutations (~11% of all known disease-causing mutations)
- Broad applicability in RNA and protein engineering

Axiomer® Targeted RNA editing

Our journey with Axiomer® since 2014

2014

2022
and beyond

The state of the art before 2014

- Artificial editors (ADAR without dsRNA binding domains)
- Non-modified guide RNAs
- *In vitro* proof-of-concept

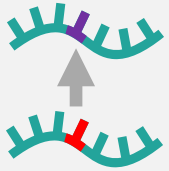
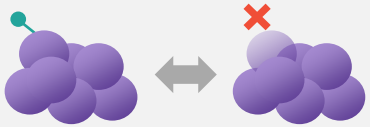
Axiomer® *(2014)*

- Natural and endogenously expressed ADARs
- Completely modified synthetic oligonucleotides (EONs)
- Designed for *in vivo*/therapeutic use
- IP filling

Axiomer® constant optimization to further therapeutic potential

- Proof of concept studies
- Optimized platform to potentialize
  - Therapeutic uses
  - Efficacy and safety
  - Delivery and cellular uptake
  - Limit off target effect

Axiomer® technology potential

Correction	Modulation / Regulation		Modification	
				
Correcting G-to-A mutations Thousands of G-to-A mutations, many of them described in literature	**Disrupt >400 different types of PTMs** Regulate protein activity, change localization, folding, preventing immune escape or slowing down degradation	**Change protein-protein interactions** Changes localization, folding, protein function or prevent immune escape of glycosylated tumor antigens	**Alter or create de novo protein function** Modified or de novo proteins achieving loss- or gain-of-functions that helps addressing diseases	**Include protective variants** At the RNA level, to develop new functions that help prevent disease

Broad therapeutic potential

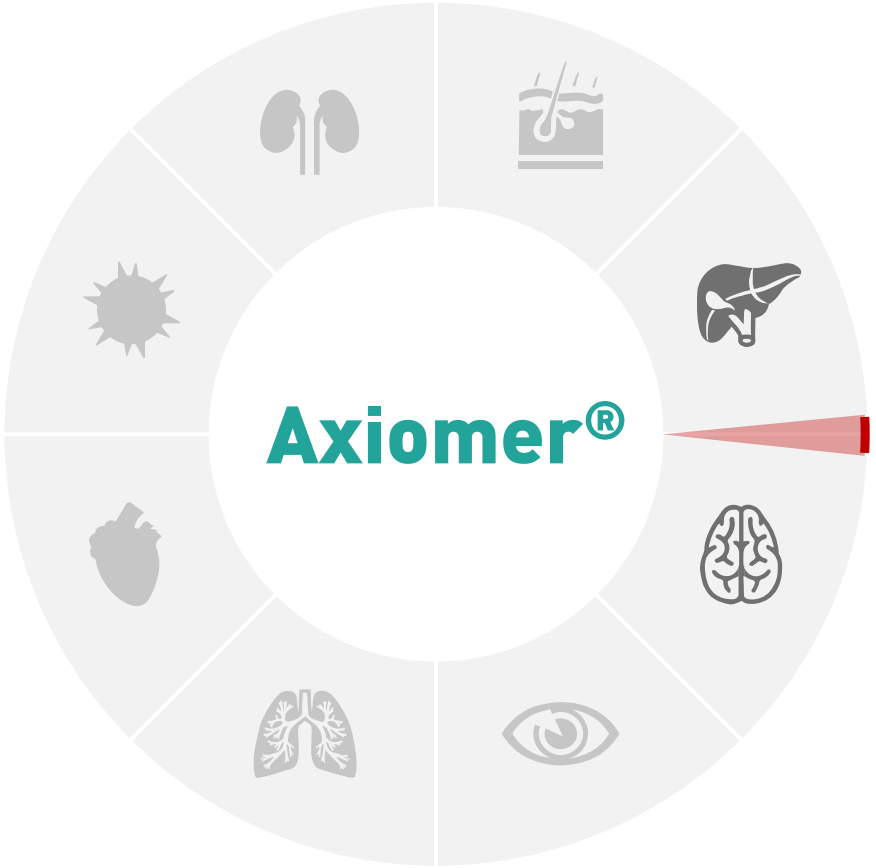
- ✓ Common diseases
- ✓ Rare diseases
- ✓ Target a wide variety of organs
- ✓ Treat so-far undruggable targets

Axiomer® Strategy

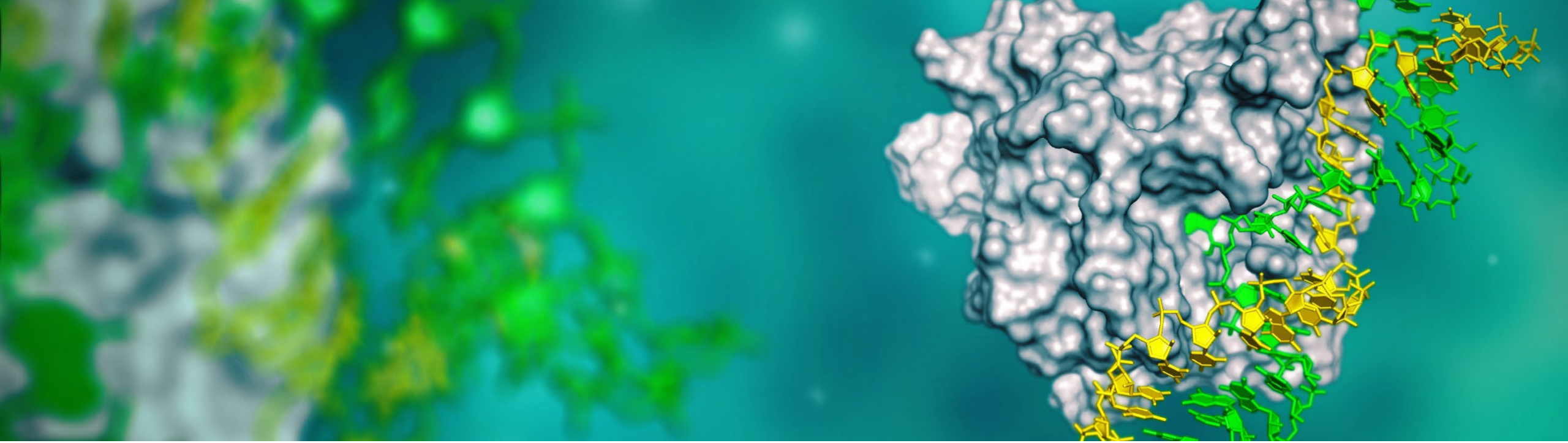
ProQR will develop its own pipeline and selectively enter partnerships

Diversified value creation strategy

- ProQR to build **in-house pipeline** based on Axiomer® RNA editing technology platform.
  - Initial focus on **liver** and **CNS** applications
- Largely unencumbered platform, **great potential for additional Axiomer® partnerships**



Partnership with Eli Lilly on up to 5 targets in liver and nervous system



Axiomer®

EONs optimized to precisely edit with endogenous ADAR

ADAR mediated A-to-I editing

The most prevalent editing event in human tissues

ADAR *(Adenosine Deaminase Acting on RNA)*
ADAR is an RNA editing system that is present in all human cells and performs A-to-I editing

Advantages

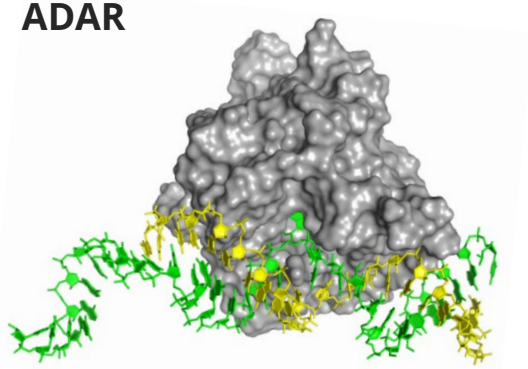
No sequence dependence

- 16 million A-to-I sites in the human transcriptome
- Extent of editing similar in most human tissues making therapeutic editing feasible in all disease areas

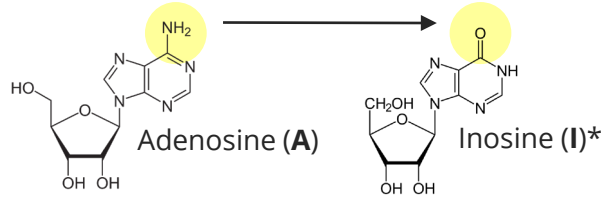
Biological roles of A-to-I editing

- Recoding during the maturation of neurons
- Self vs. non-self discrimination
- Regulating genome stability
- Changing RNA processing (e.g., Splicing, miRNAs)
- And many more!

ADAR

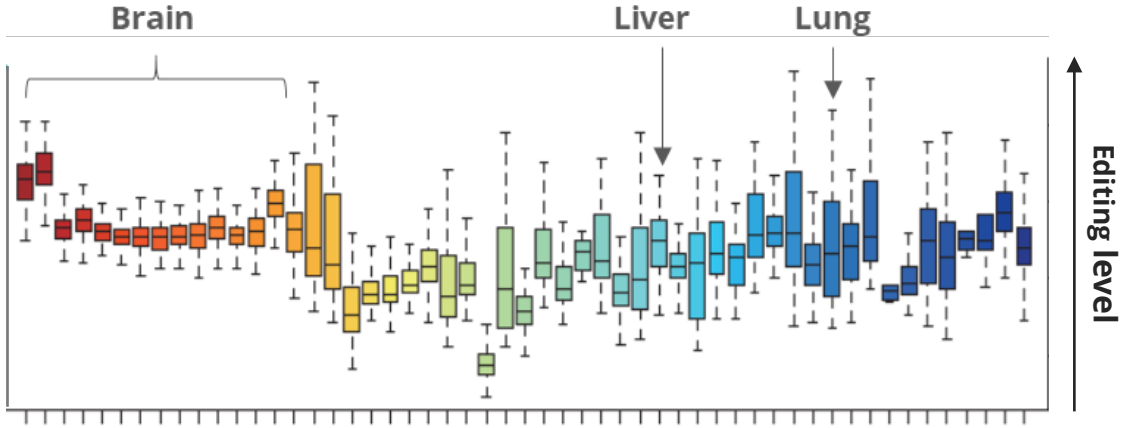


A-to-I editing



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

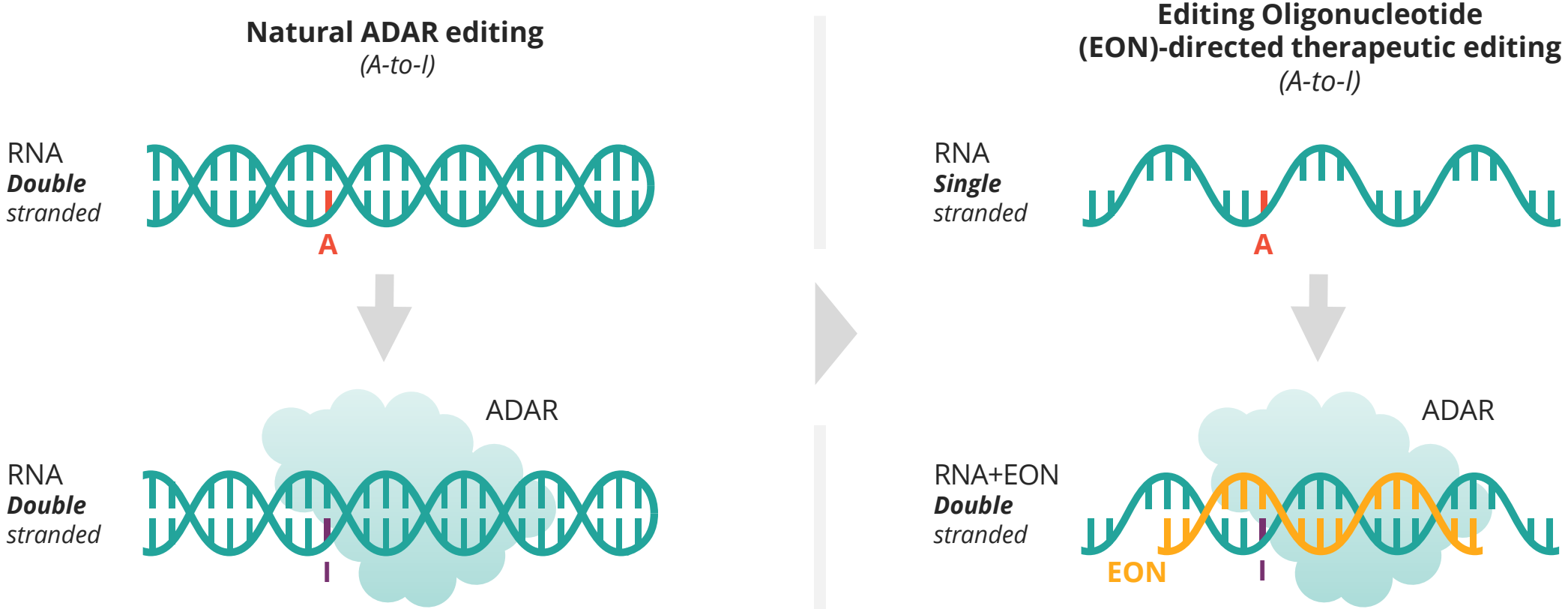
ADAR mediated A-to-I editing in human tissues



Adapted from Tan et al. 2017 Nature 550:249-254

EONs designed to recruit endogenous ADAR

ADAR deaminates target A in EON-target RNA complex



dsRBDs, double-stranded RNA binding domain

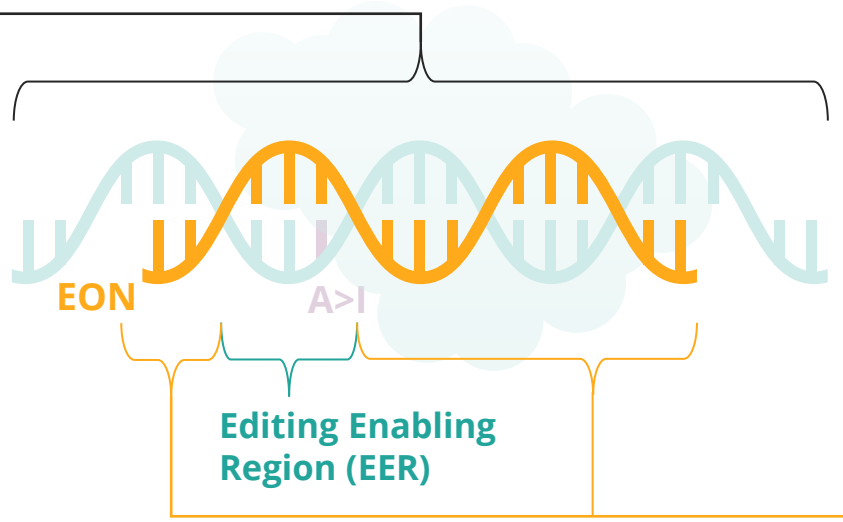
EONs optimization for therapeutic use

To increase editing efficacy and specificity

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

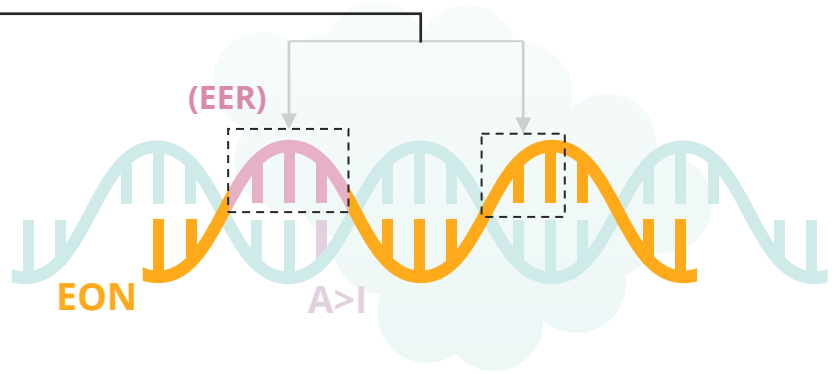
Optimized sequence and chemistry define functionality

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

Optimizing EONs for therapeutic use

Separate screening for potency, stability and bioavailability

Challenge: Replace defined regions in EONs with new chemical modifications, without compromising ADAR binding and activity



EON design

Selection of new modifications based on structural analysis and modelling: Fit with ADAR binding

High-throughput screening

Efficacy optimization: Biochemical editing assay

Stability optimization: Biochemical stability assays

Validation phase

Cell-based assays

Further development

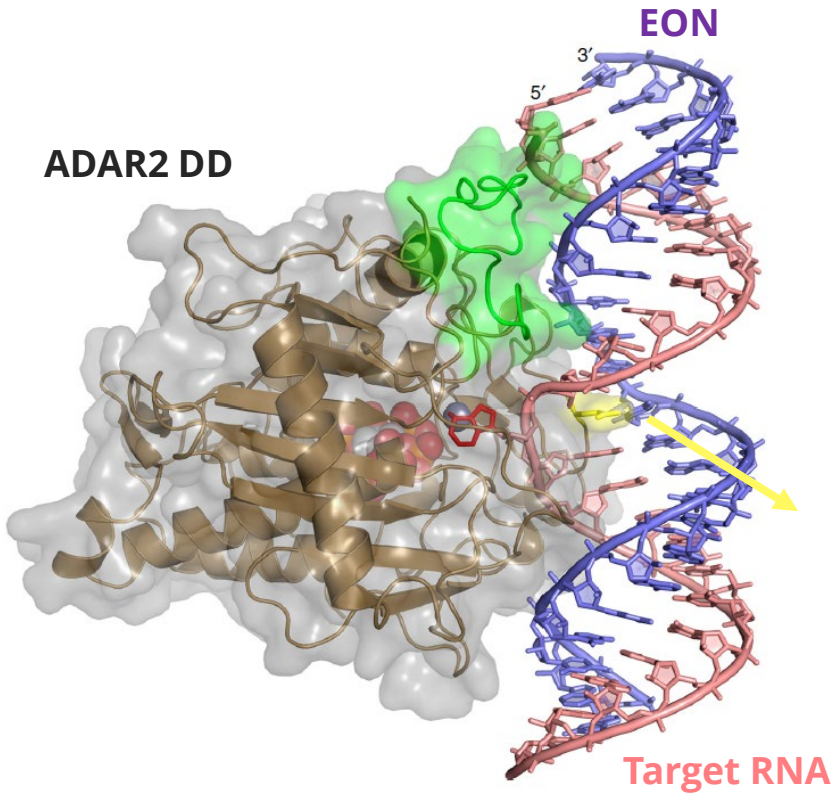
Modification in the Editing Enabling Region (EER)

Cytidine analogs as orphan base

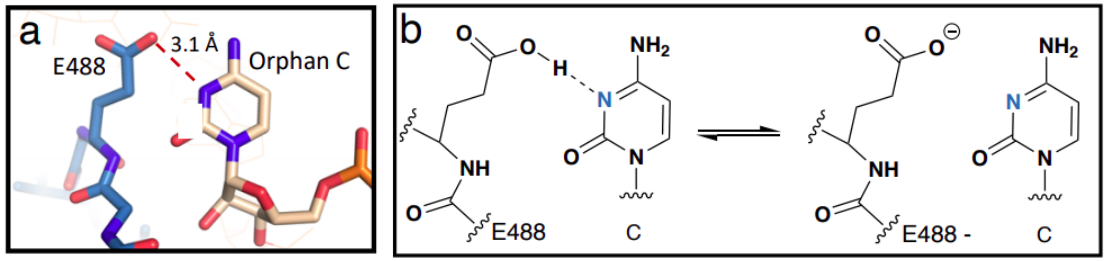
A single base modification of the EER increases ADAR activity

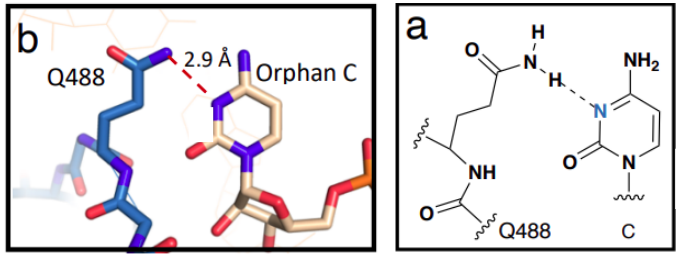
dZ base mimics E488Q mutation in ADAR2 causing hyperactivity

ProQR – UC DAVIS Collaboration

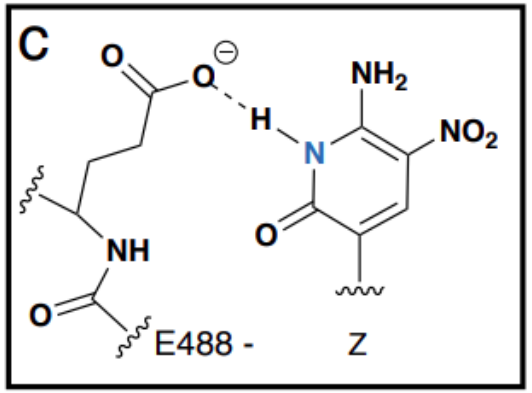


Protonation dependent hydrogen bond – pH dependency





Protonation independent hydrogen bond



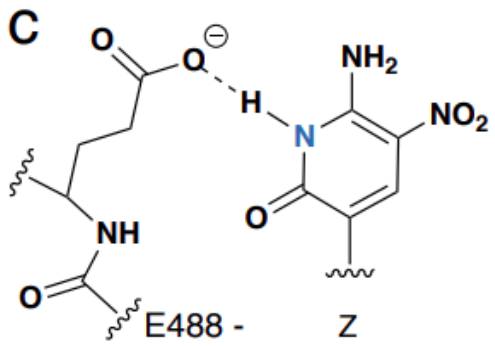
dZ base (dZ)

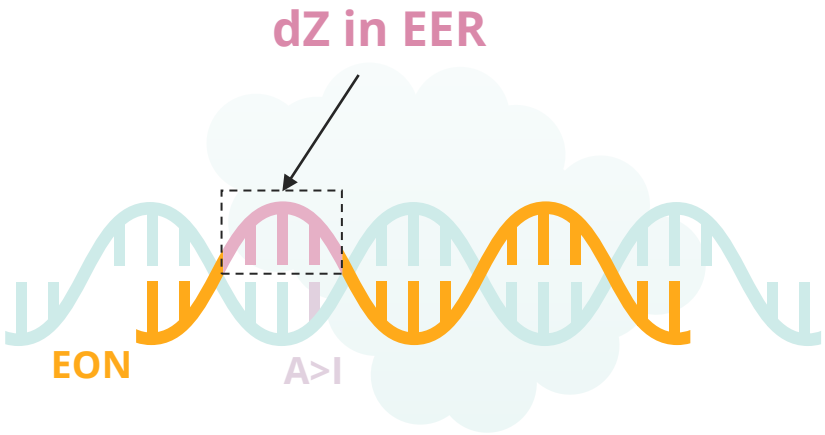
Metthews 2016, Nature Structural & Molecular Biology

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

dZ improves editing in human retinal pigment epithelial cells

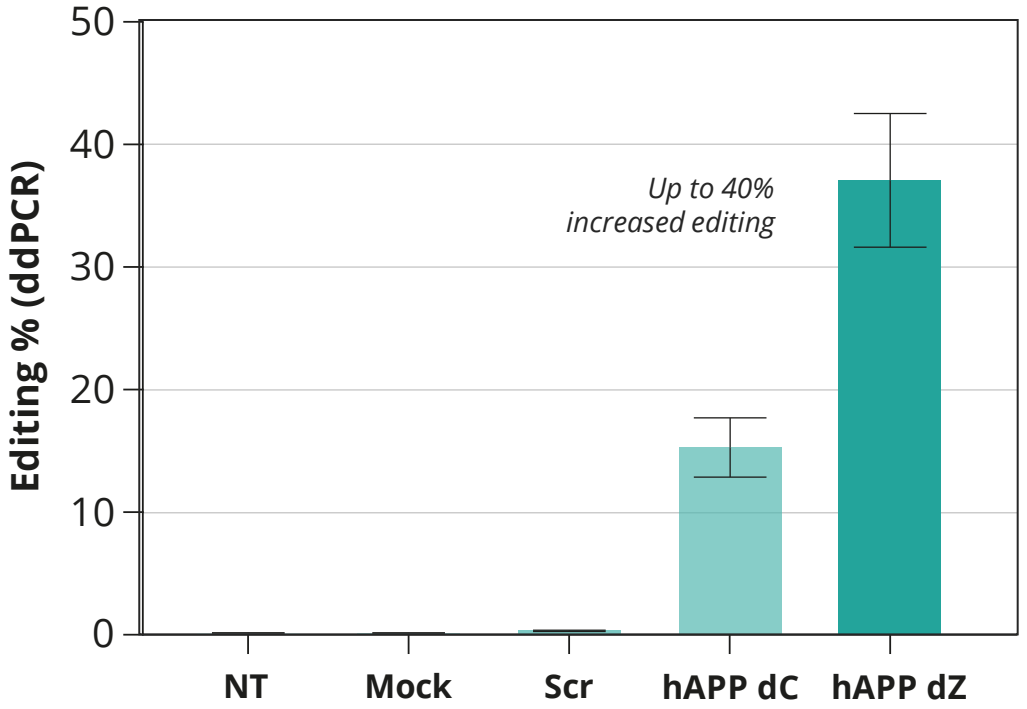
ProQR – UC DAVIS Collaboration





Editing of adenosine target in human ARPE-19

(Transfection, 100nM, single dose, N=3, 48 hours)



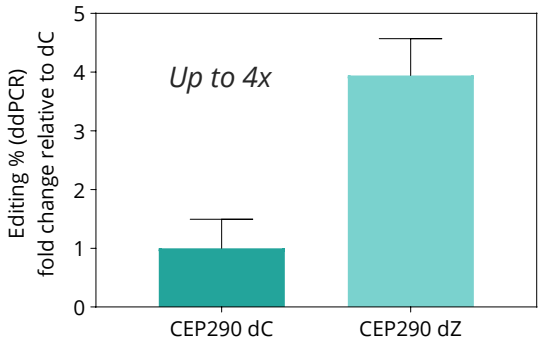
Improved editing obtained for several targets

dZ improves editing in different cell types

ProQR – UC DAVIS Collaboration

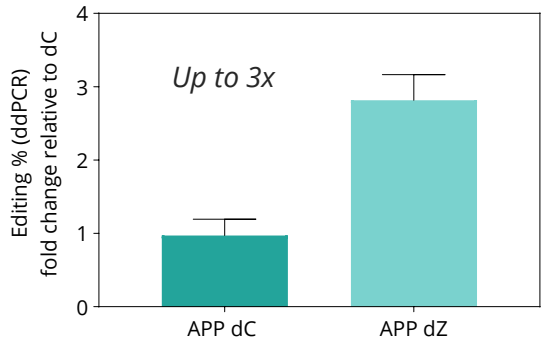
Editing of *hCEP290* K1575X in human LCA retinal organoids

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



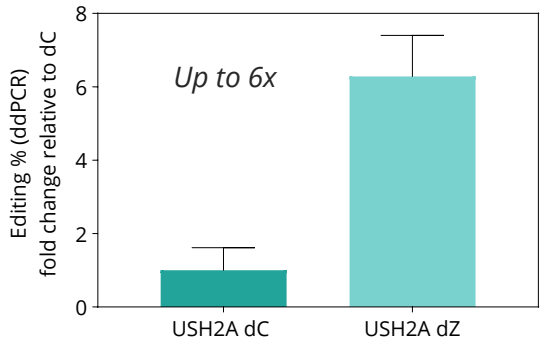
Editing of *APP* WT RNA in human retinal organoids

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



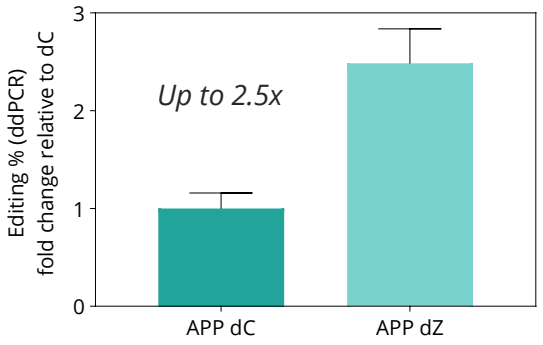
Editing of *USH2A* WT RNA in human retinal organoids

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



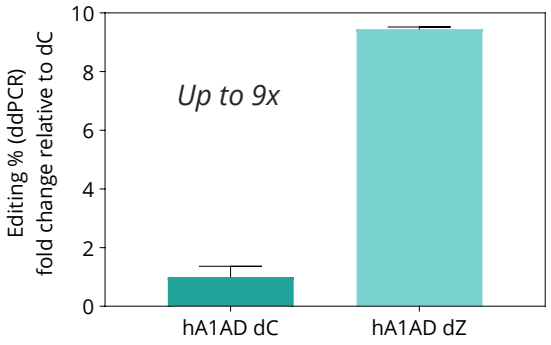
Editing of WT *APP* RNA in human ARPE-19

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



Editing of *SERPINA1* E366K in A1AD patient hepatocytes

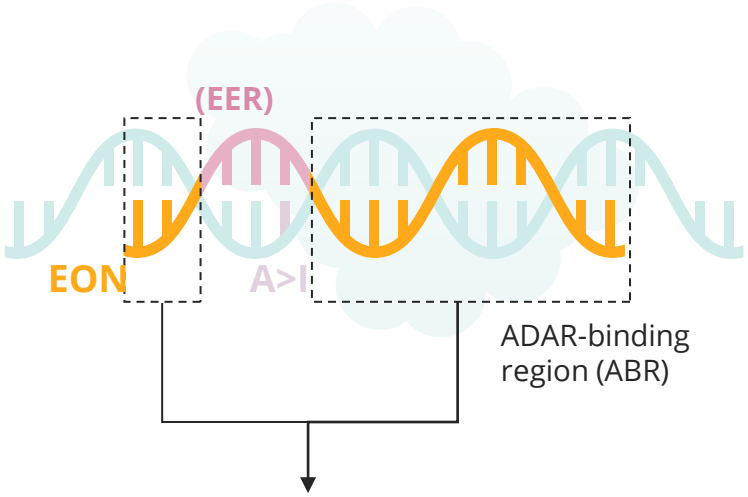
Transfection of 100nM EON, n=2, 48 hours



Modification in the ADAR-binding region (ABR)

New EONs chemical optimization

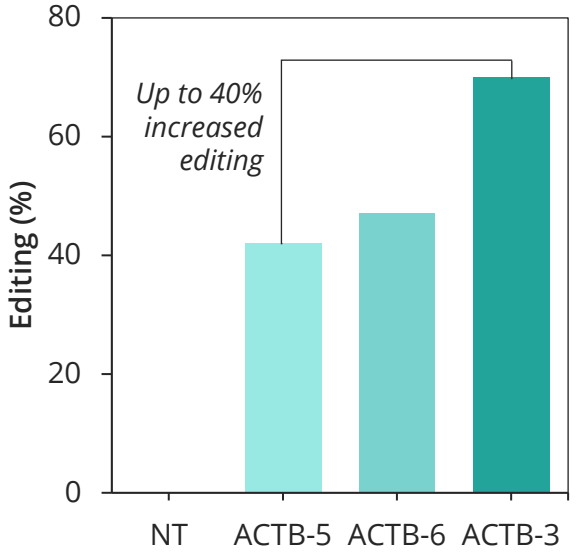
ADAR-binding region (ABR) modification greatly enhances editing



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

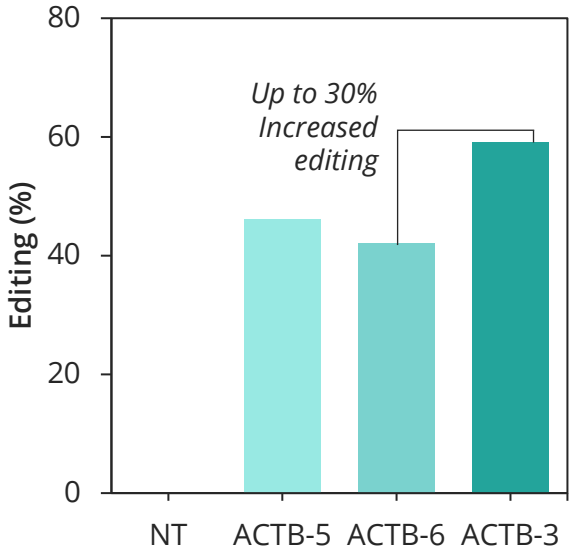
Editing of *ACTB* in human primary hepatocytes

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



Editing of *ACTB* in human retinal pigment epithelium cells

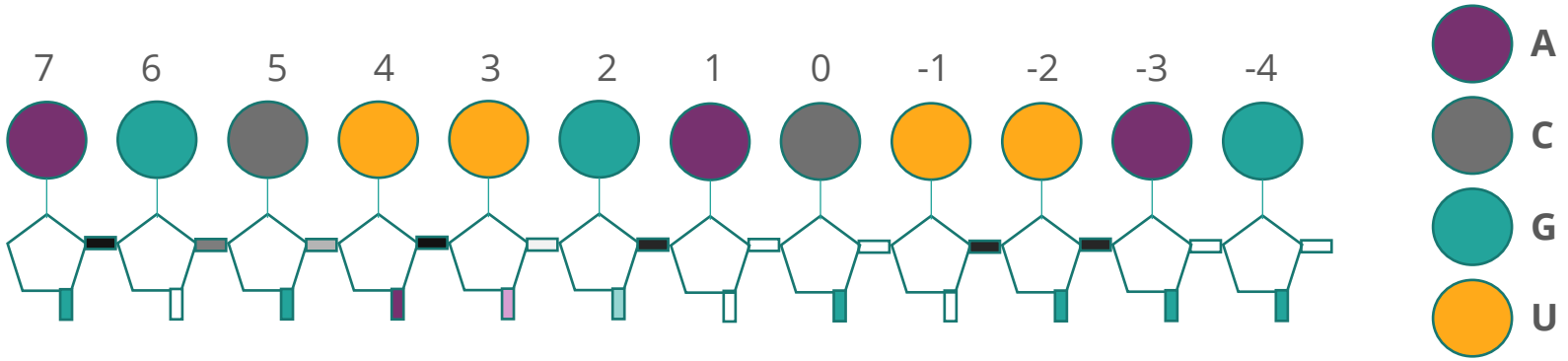
(Transfection, 100nM, single dose, N=1, 48 hours, dPCR)



- 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® platform over time

Optimization is yielding stability improvements and efficacy increase in cells and in vivo

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 liver
as a promising area of development

The CNS
The CNS as the next frontier

Model targets

PoC therapeutic targets
Tool targets used for optimization

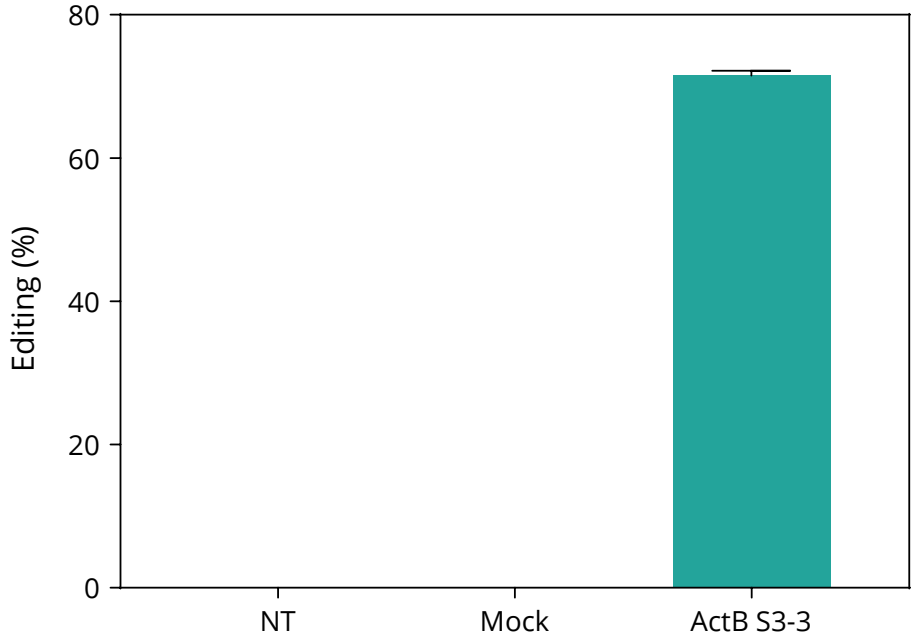
Pipeline targets

The retina as early proof-of-concept

Efficient editing of ACTB in mouse and human retinal cells

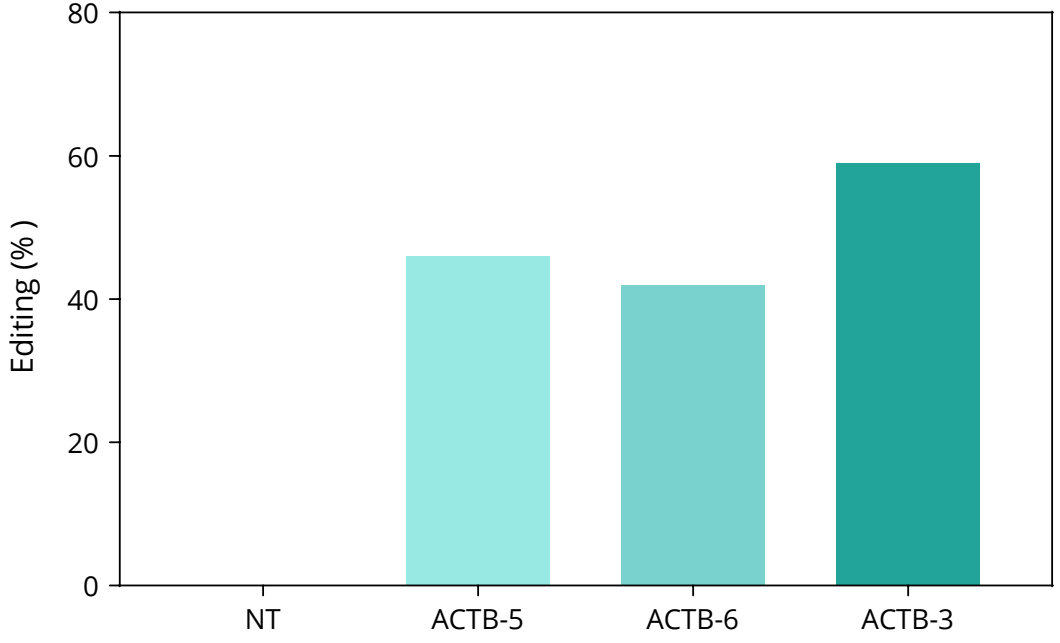
Editing of *ActB* in mouse RPE cells

(Transfection, 100nM, single dose, N=2, 24 hours, Sanger sequencing)



Editing of *ACTB* in human RPE cells

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



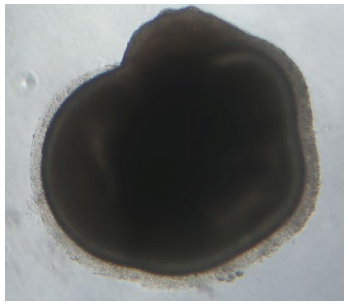
- Similar levels of editing of *ACTB* achieved in mouse and human models of retinal origin
- High confidence of translatability of the approach

The retina as early proof-of-concept

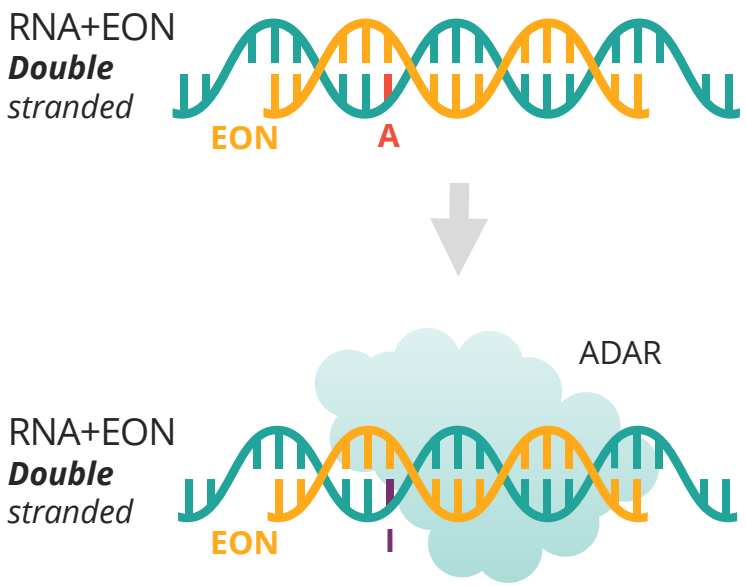
Efficiency confirmed in human retinal organoids with >40% editing achieved

Retinal organoid

225 days

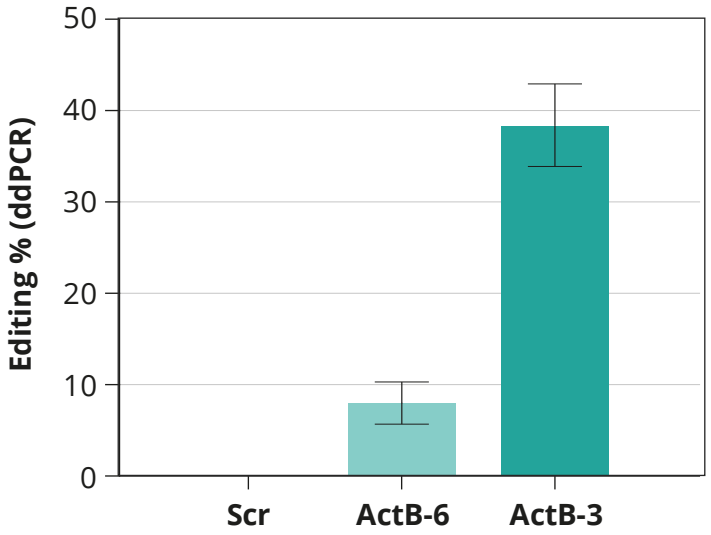


EON-directed therapeutic editing



Editing of *ACTB* in iPSC-derived human retinal organoids

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



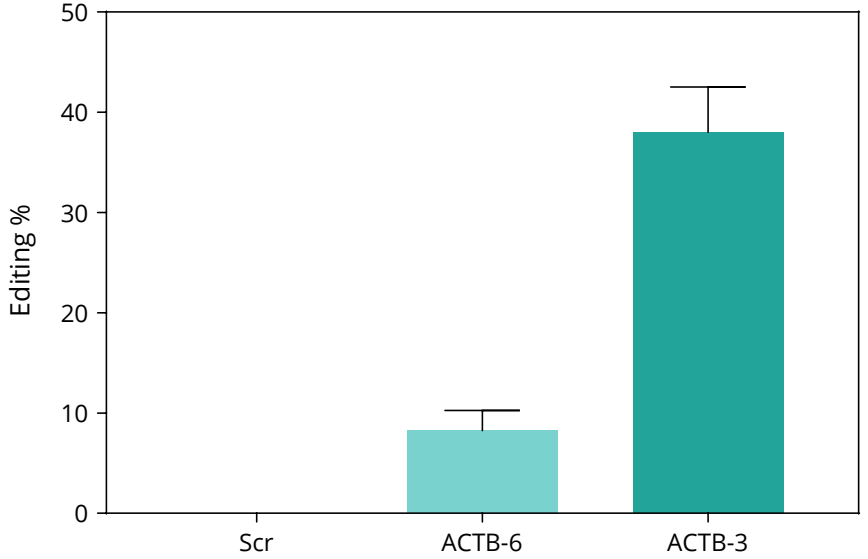
- Chemical iterations improve EON editing efficiency
- The highest editing efficacy increase is obtained for EONs with multiples modification combined
- Over 40% editing was observed after gymnosis

From model target to PoC therapeutic targets

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

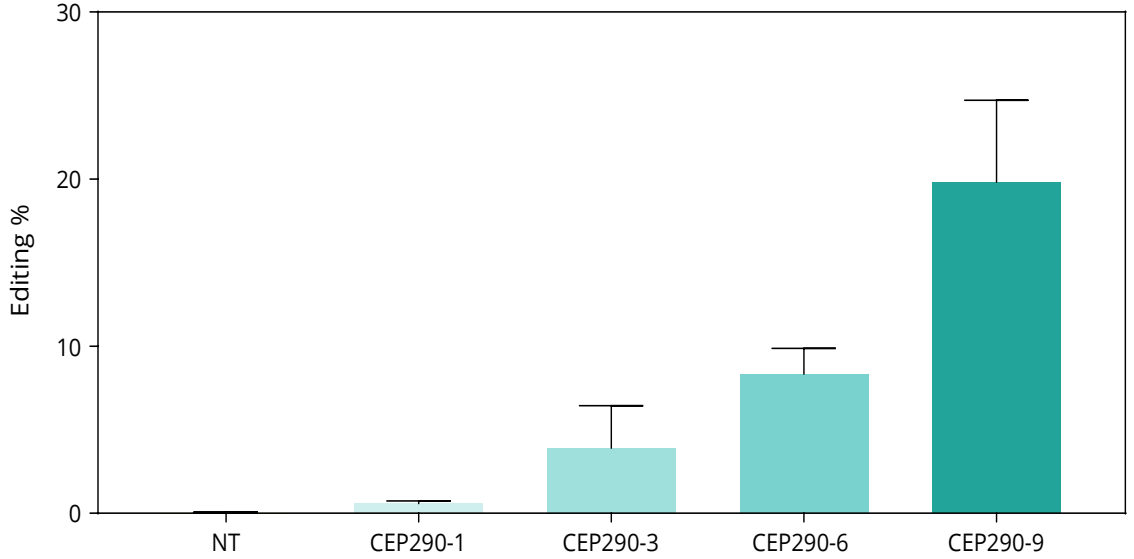
Editing of *ACTB* in human retinal organoids

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



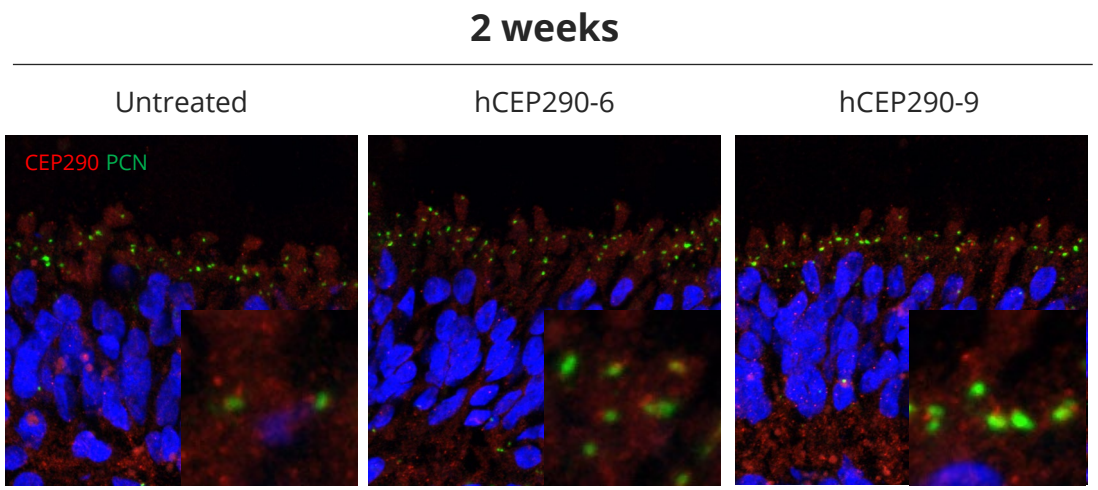
Editing of *CEP290* in human LCA-specific retinal organoids

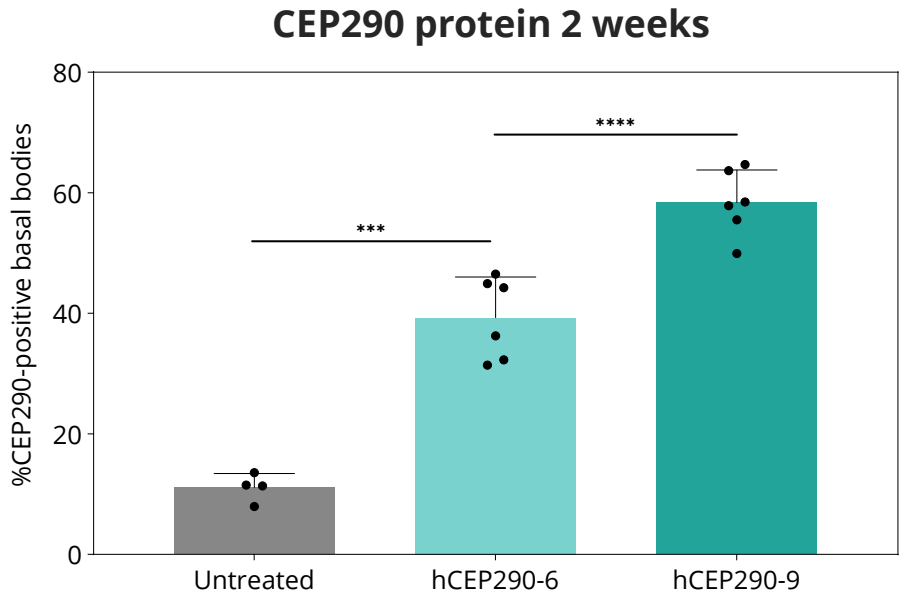
(Gymnosis, 10uM, single dose, N=8, 2 weeks, ddPCR)



- 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

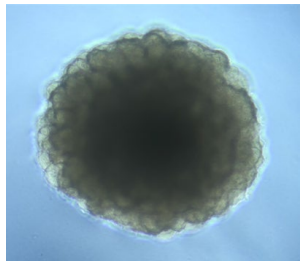
Significant increase in CEP290 protein levels and intensity was detected at the basal body of LCA07-3 organoids treated with hCEP290-6 and-9 after 2-weeks treatment

The CNS as the next frontier

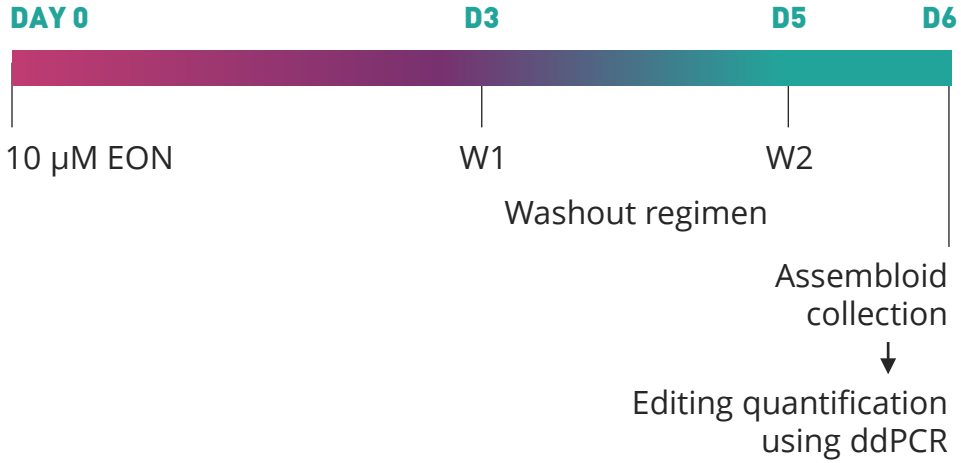
>30% editing was achieved in IPSC derived brain organoids

Human brain organoid

130 days

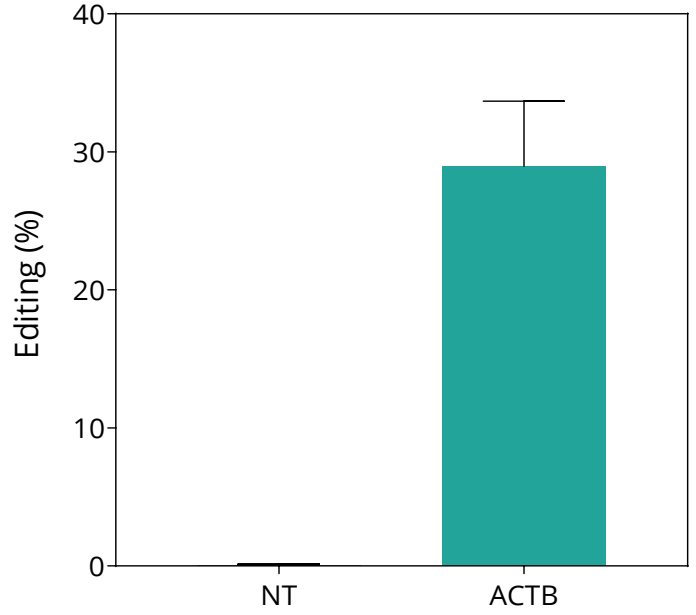


Gymnotic uptake



Editing of *ACTB* in iPSC derived brain organoids

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

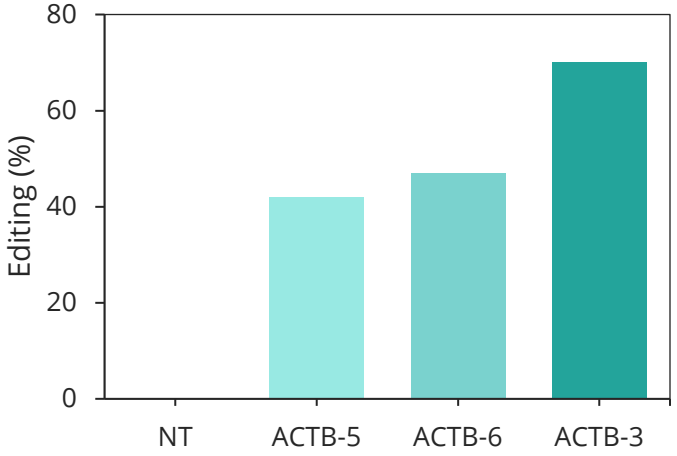


The liver as a promising area of development

High potential of EONs editing in the liver

Editing of *ACTB* in human primary hepatocytes

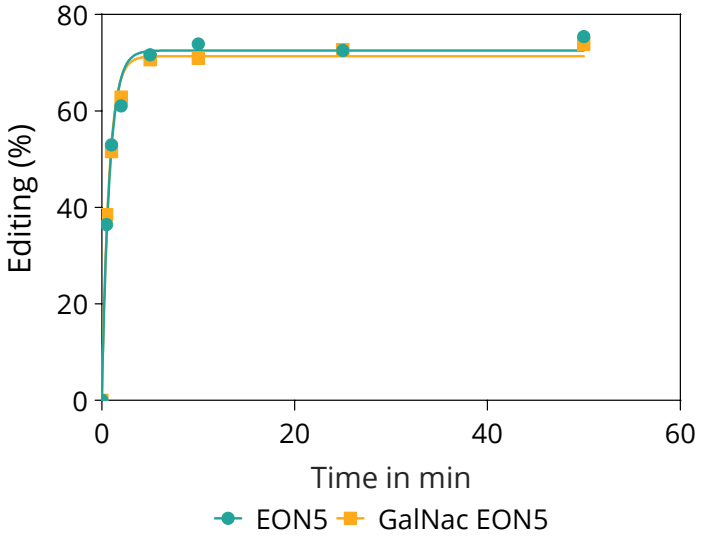
Gymnosis, 10uM, single dose, N=1, 48 hours, dPCR



- 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*

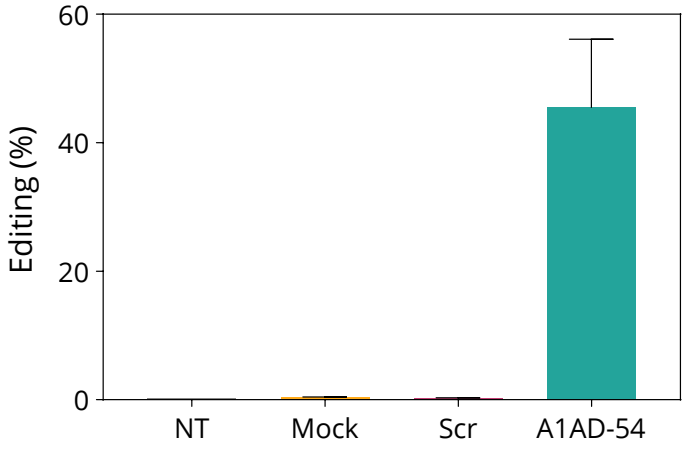
N=1, BEA assay



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



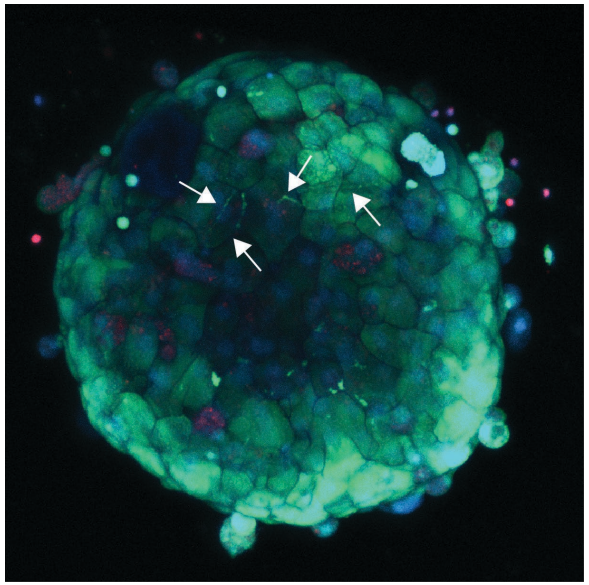
>50% Editing of *SERPINA1* E366K in human A1AD patient hepatocytes

Editing in InSphero Human Liver microtissues (LMTs)

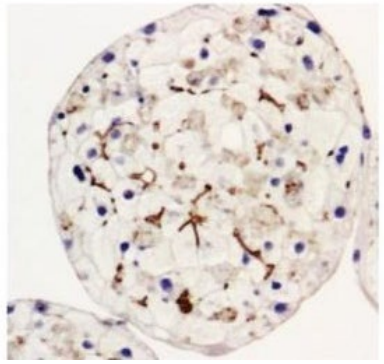
Primary hepatocytes, Kupffer cells and liver endothelial cells in 3D spheroid

Live image of LMT

Stained with 5-CFDA (green), PI (red) and Hoechst (blue)



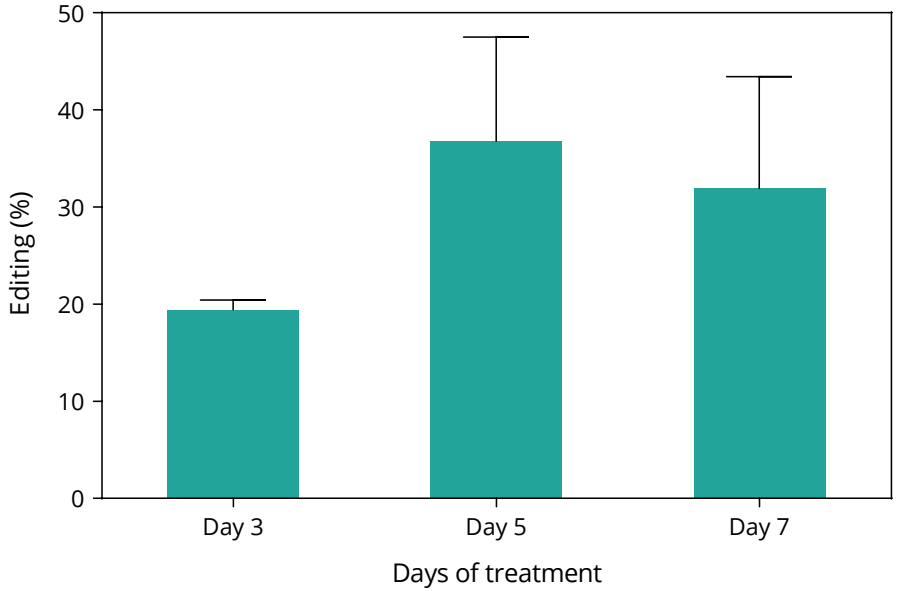
BSEP Bile Canaliculi
(InSphero data)



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)

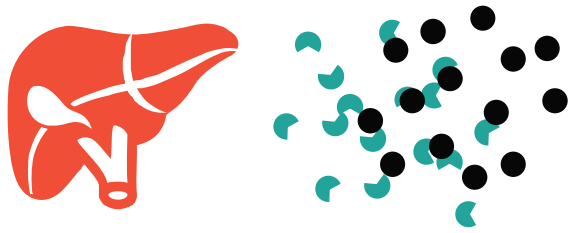


Treatment of LMTs with 5µM EON for 7 days results in up to 40% of edited *ACTB*.

Liver targeted editing of *PSCK9*

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

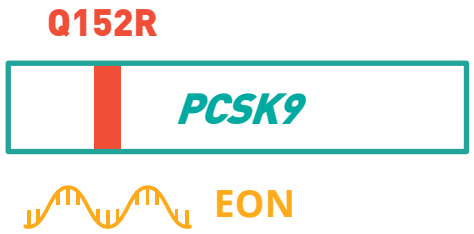
FEH patients



↑ PCSK9 ● ↑ LDL

Axiomer® edit

↓ PCSK9 ● ↓ LDL



Disruption of PCSK9 autocleavage site reduces protein in bloodstream

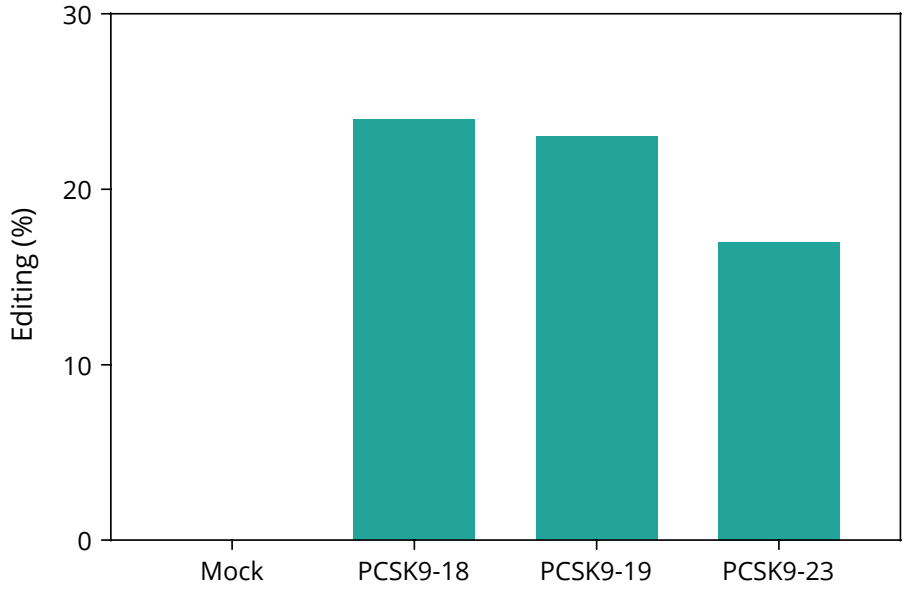
- Less PCSK9 leads to increase of LDL-R on cells, decrease of 'bad' LDL in bloodstream
- Loss-of-function *PCSK9* variant Q152H is associated with low plasma LDL cholesterol in a French-Canadian family and with impaired processing and secretion in cell culture

PCSK9 mRNA editing leads to reduced PCSK9 protein levels

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

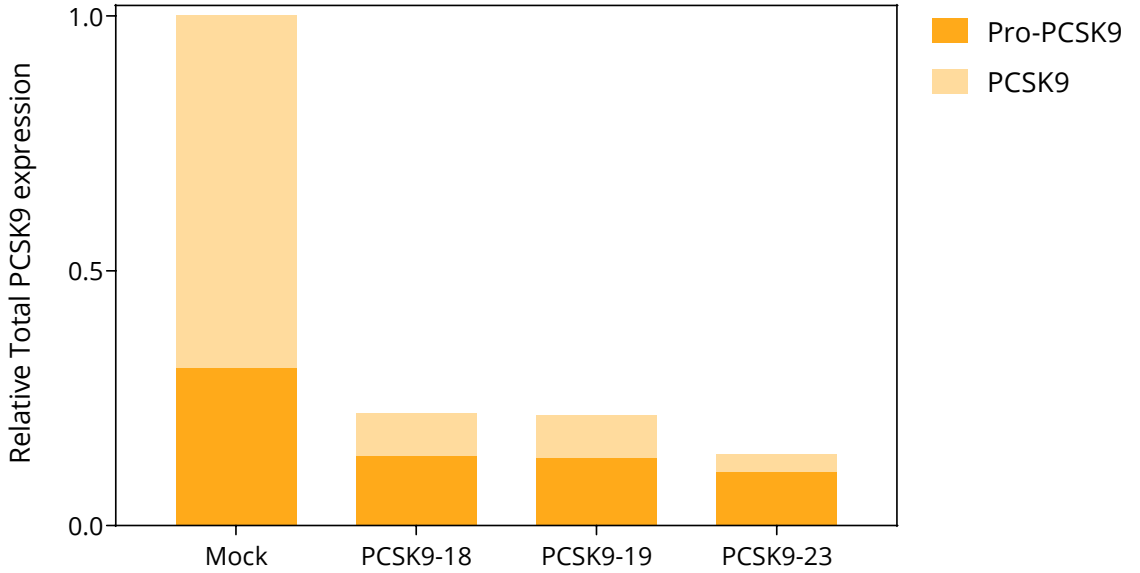
Editing of *PCSK9* in HeLa cells

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% 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

Axiomer® to date

- Successful primary optimization of the platform
- PoC studies showing RNA editing at potential therapeutic levels in multiple targets

In house strategy

- Continue platform optimization and *in vivo* PoC in multiple programs with initial focus on Liver and CNS
- Axiomer® platform, pipeline development and target selection activities
- Planning to announce pipeline development targets in early 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

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