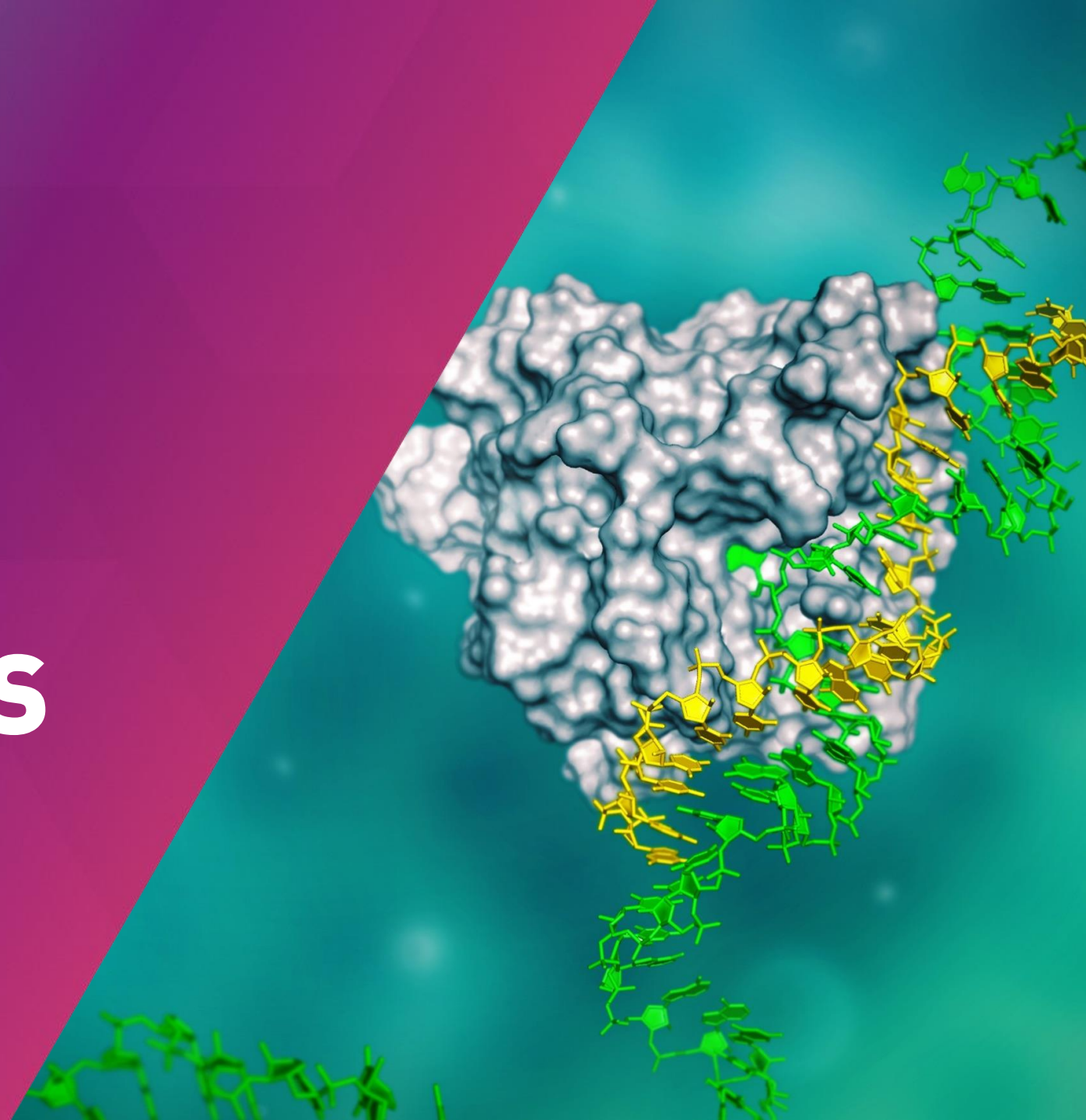
ProQR®

HARNESSING CHEMICAL MODIFICATIONS

to improve ADAR potency and unlock Axiomer's broad applicability

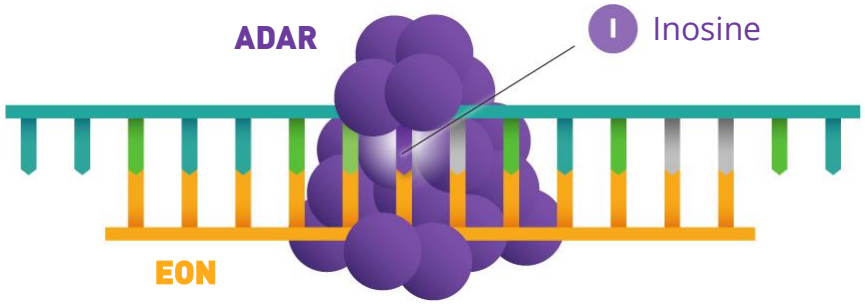
Lenka van Sint Fiet, PhD, *Sr. Director Technology*

RNA Editing Summit – July 12, 2023



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

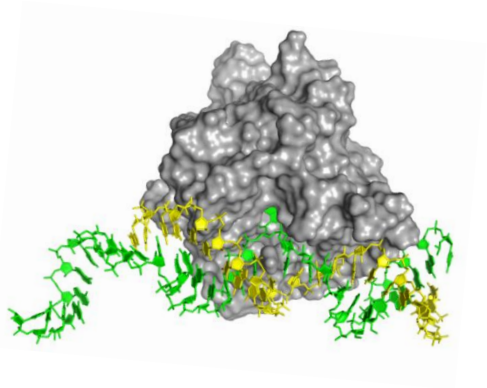
Trident® U-to-Ψ editing

- Exploiting endogenous pseudouridylation machinery
- psEON adopts a hairpin structure with a guiding sequence ultimately recruiting the machinery
- Specifically target PTC mutations (~11% of all known disease-causing mutations)
- Broad applicability to various diseases caused by PTCs

Axiomer® EONs unlock cellular machinery potential to treat diseases

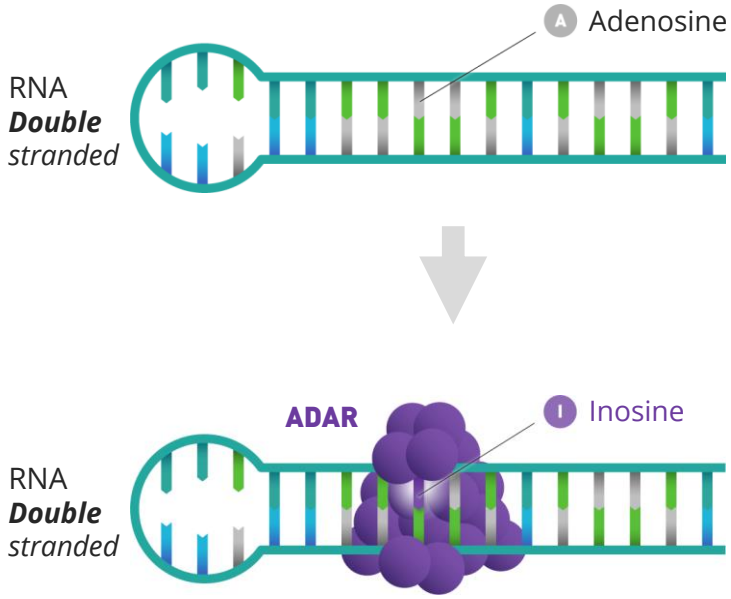
By attracting ADARs and allowing highly specific editing

ADAR *(Adenosine Deaminase Acting on RNA)*

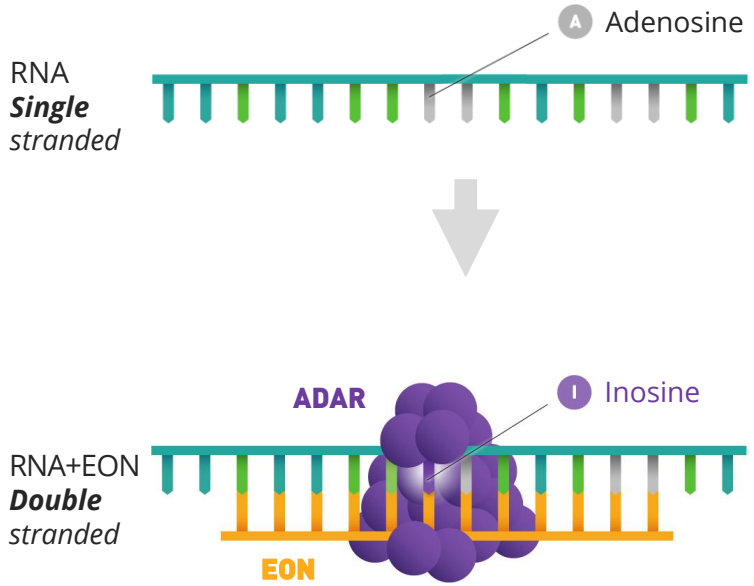


Enzyme that performs specific form of natural RNA editing, called **A-to-I editing.** During A-to-I editing an **A nucleotide (adenosine)** is changed into an **I nucleotide (inosine)**

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



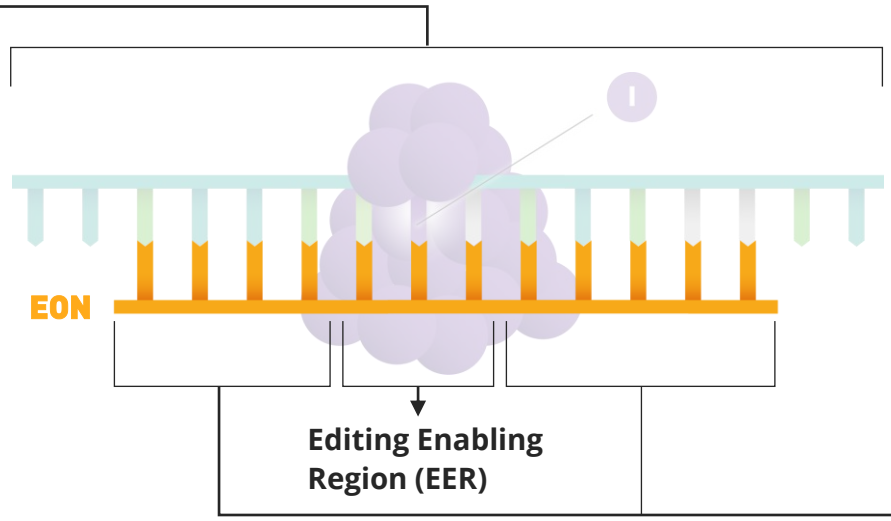
Editing Oligonucleotide (EON)-directed therapeutic editing *(A-to-I)*



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

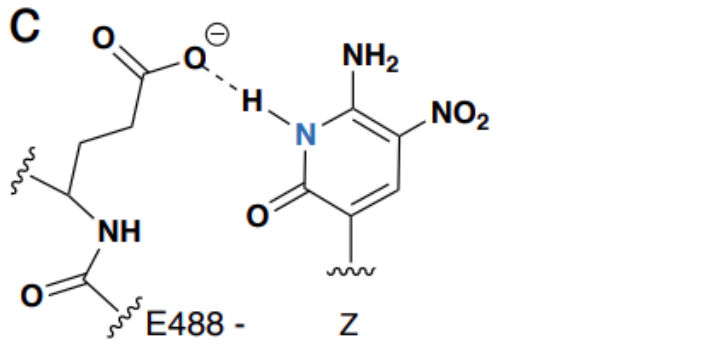
Optimized sequence and chemistry define functionality

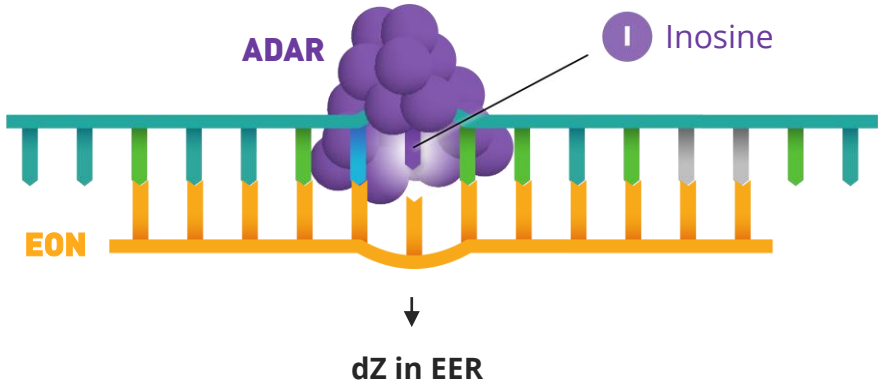
- Increase editing efficacy
- Bring metabolic stability
- Prevent off-target ('bystander') editing
- Ensure bioavailability (cell and tissue uptake)
- Offer safety and tolerability at therapeutic doses

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

dZ in the EER improves editing of *SERPINA1* E366K in A1AD patient hepatocytes

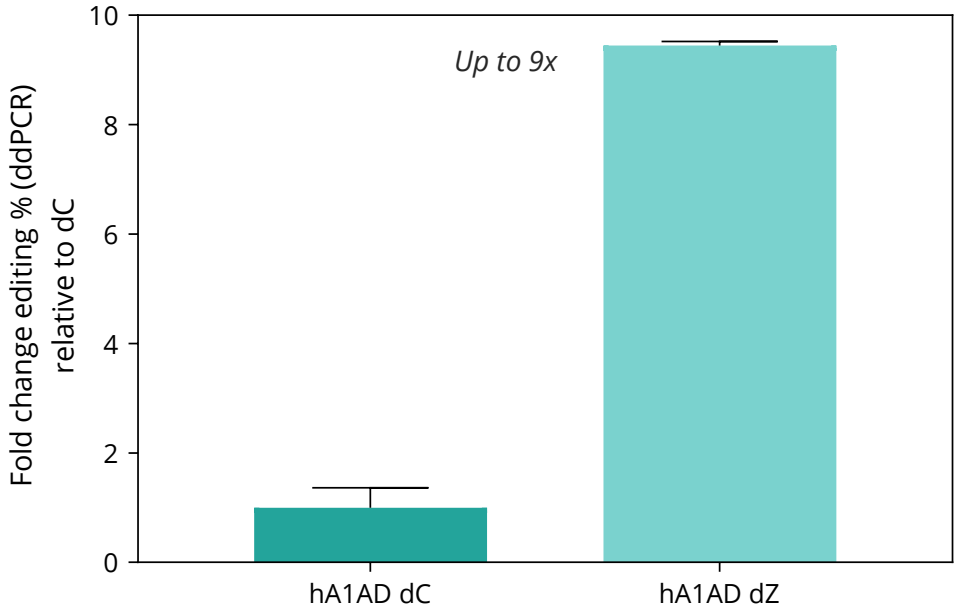
PROQR – UC DAVIS COLLABORATION





RNA editing of *SERPINA1* E366K in A1AD patient hepatocytes

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



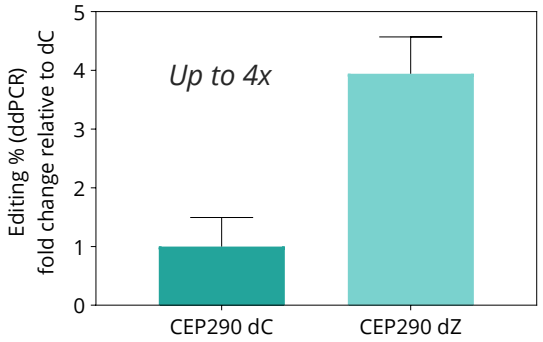
Improved editing obtained for several targets

dZ modification on EER improves editing in different cell types

ProQR – UC DAVIS Collaboration

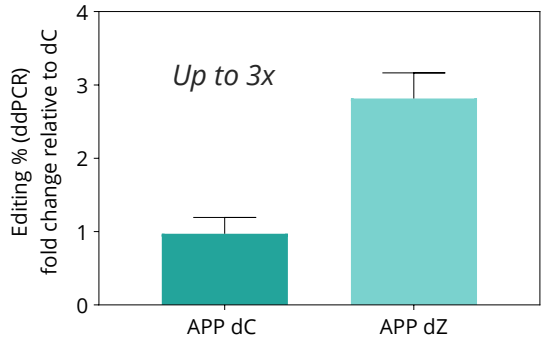
Editing of hCEP290 K1575X in human LCA retinal organoids

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



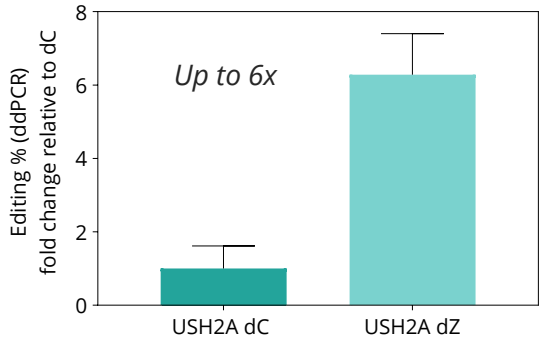
Editing of APP WT RNA in human retinal organoids

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



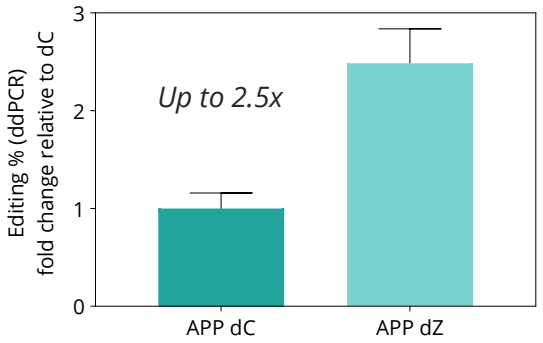
Editing of USH2A WT RNA in human retinal organoids

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



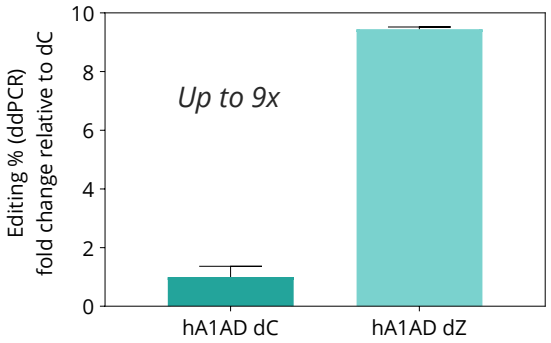
Editing of WT APP RNA in human ARPE-19

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



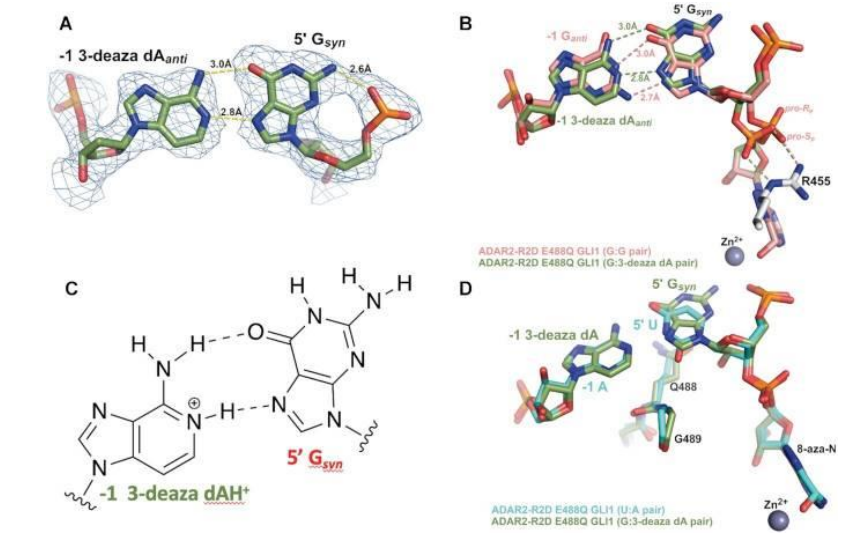
Editing of SERPINA1 E366K in A1AD patient hepatocytes

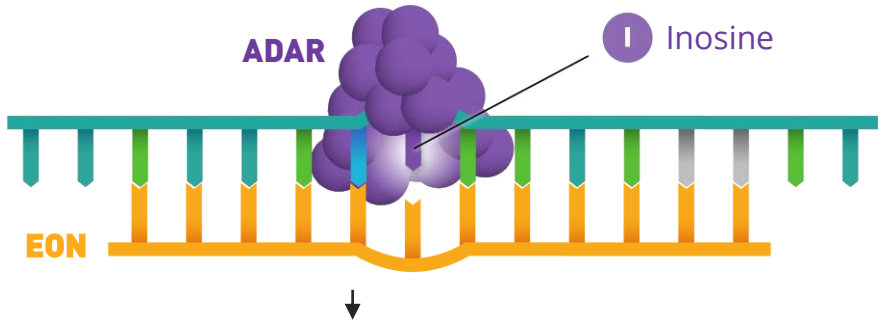
Transfection of 100nM EON, n=2, 48 hours



A single base change opposite the target 5'G greatly enhances editing

PROQR – UC DAVIS COLLABORATION

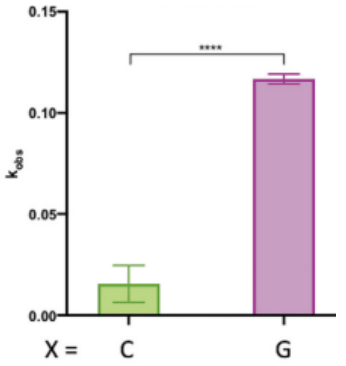


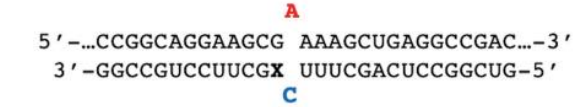


3-deaza-dA in EER

In vitro deamination kinetics for ADAR2 and duplex RNAs derived from WT *hMECP2*

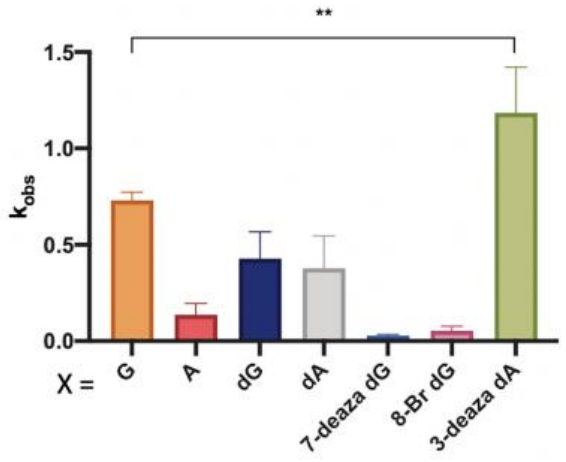
100 nM ADAR2, 3 technical replicates, mean, SD

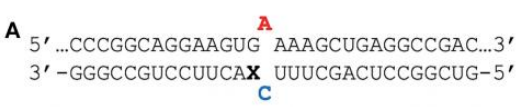




In vitro deamination kinetics for ADAR2 and duplex RNAs derived from *hMECP2* R255X

100nM ADAR2, 3 technical replicates, mean, SD

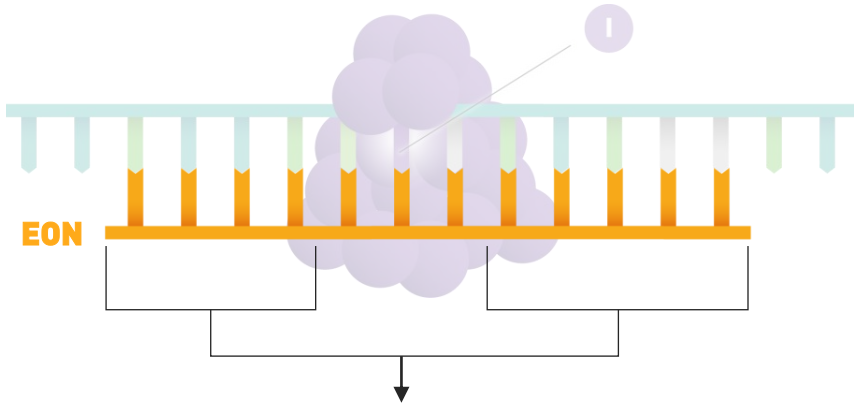




Adapted from Doherty EE, et al. Nucleic Acids Res. 2022;50(19):10857-10868.

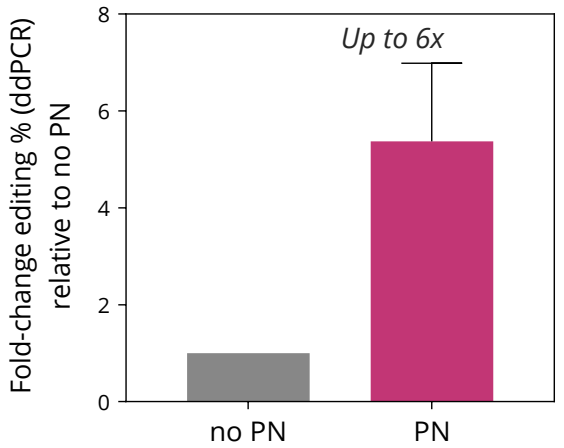
Statistical significance between groups was determined using one-way ANOVA with Tukey's multiple comparisons test or an unpaired t-test with Welch's correction; **P < 0.01; ***P < 0.001; ****P < 0.0001.

ADAR-binding region (ABR) modification greatly enhances editing

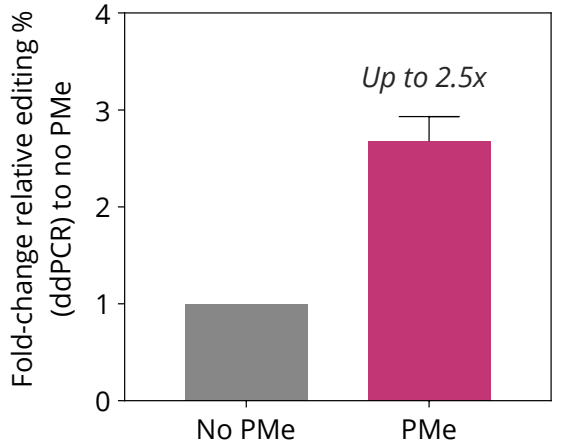


ADAR-binding region (ABR)
Backbone modifications enable ADAR binding, and **improve** stability

RNA editing of *hACTB* in Weri-rb1 cells
Gymnosis, 5µM single dose, N=3, 5 days, ddPCR, mean, SEM



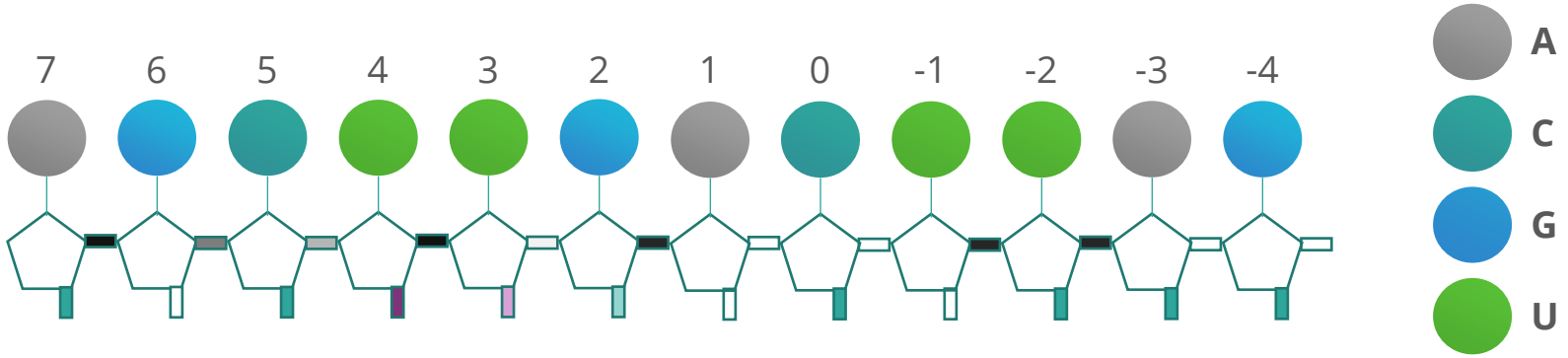
RNA editing of *hAPP* in HepG2 cells
Gymnosis, 5µM single dose, N=2, 5 days, ddPCR, mean, SEM



PN and PMe linkages greatly increase EON editing efficiency in positions within ABR region

Accelerating program advancement with focus on 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 10 published patent families

ADAR: Adenosine deaminase acting on RNA, EON: Editing oligonucleotide, PD: pharmacodynamic, PK: pharmacokinetic

Axiomer® RNA editing platform has broad potential

Increased editing efficiency

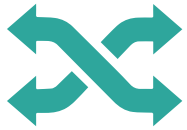
EER and ABR modifications greatly enhance editing

Consistent RNA editing

in all models evaluated in nervous system and liver, including NHP *in vivo*

Validation of Axiomer's potential for therapeutic targets

With positive effect on protein expression



Broad applicability

With proof of concept in mutation correction and multiple forms of protein modulation

Establishing a strong platform in multiple organs, targets and models

Nervous system

Targeting CNS and PNS

Liver

Targeting liver-originated diseases

Cell models

Organoids

Mice *in vivo*

NHP *in vivo*

Model target

PoC therapeutic targets

Tool targets used for optimization

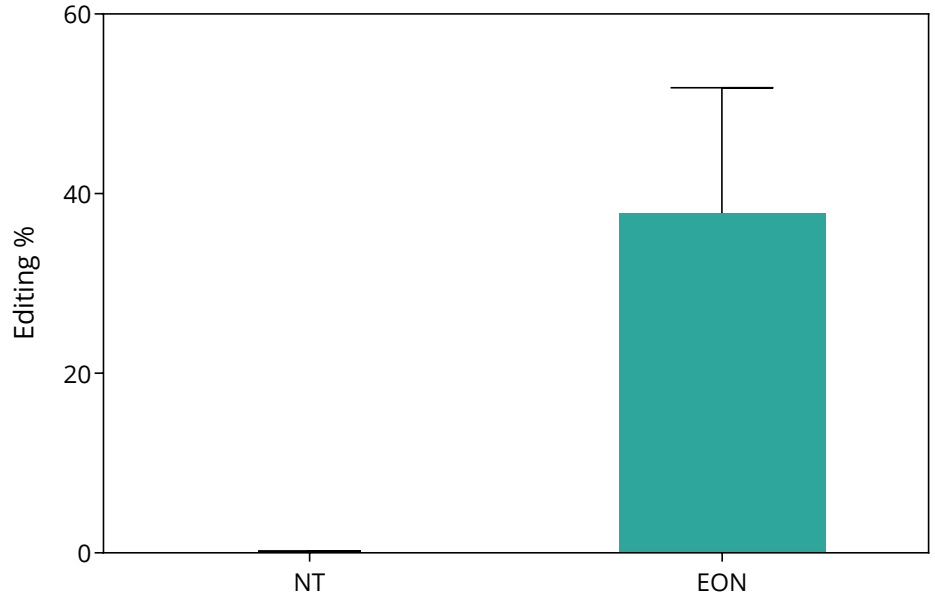
Pipeline targets

CNS: Central nervous system, NHP: Non-human primate, PNS: peripheral nervous system

More than 50% RNA editing achieved in human iPSC derived neurons

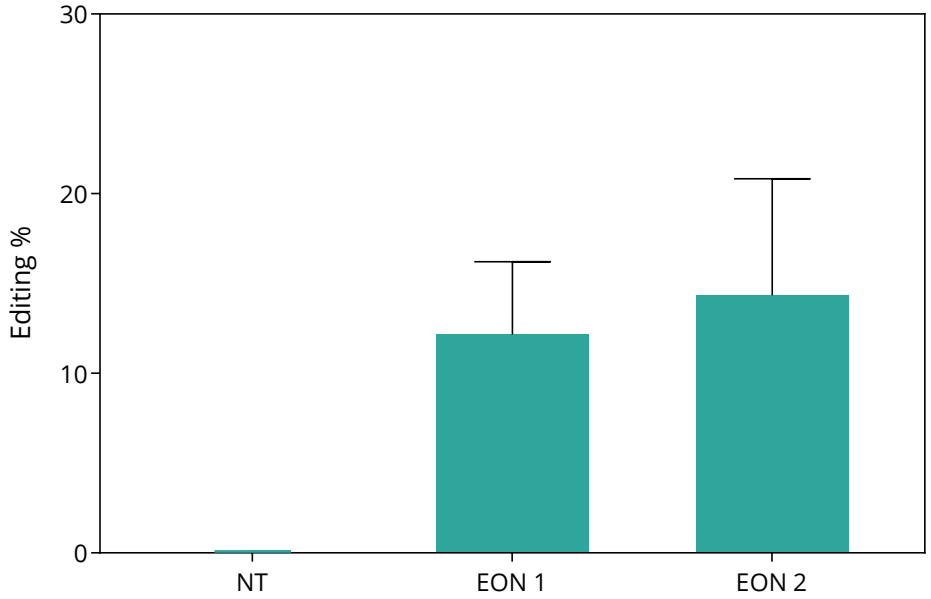
RNA editing of *ACTB*

Gymnosis, 2.5µM, single dose, n=3-4, 2 weeks, dPCR



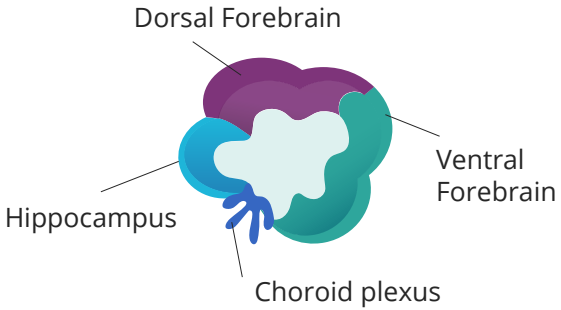
RNA editing of *APP*

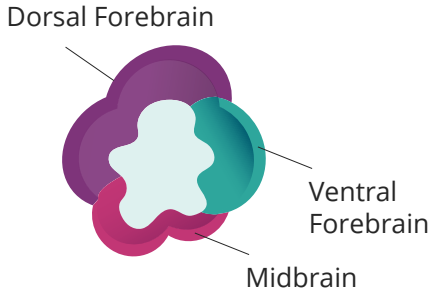
Gymnosis, 10µM, single dose, washout, n=3, 2 weeks, dPCR



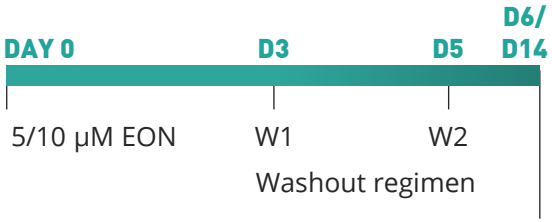
In iPSC-derived neurons of 4-6 weeks maturation, more than 20% RNA editing of APP and 50% RNA editing of ACTB was achieved after 2 weeks

Up to 65% RNA editing achieved in iPSC derived cerebral organoids





Gymnotic uptake

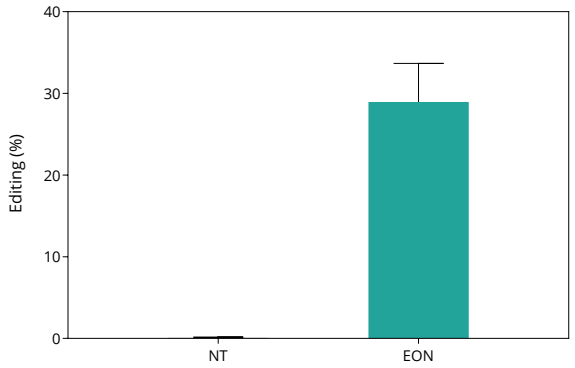


Human cerebral organoids

130-150 days

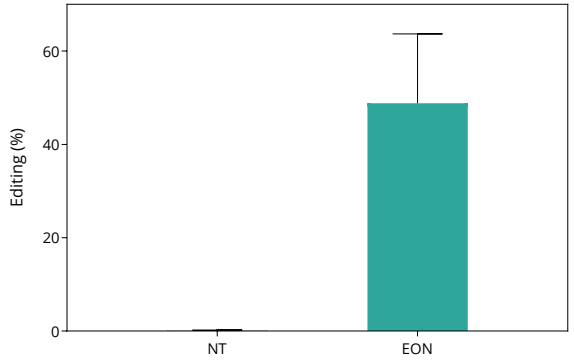
RNA editing of *ACTB*

Gymnosis, 10µM, single dose, washout, n=7, 6 days, ddPCR, mean, SD



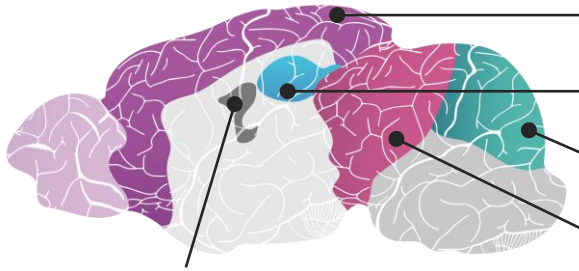
RNA editing of *APP*

Gymnosis, 5µM, single dose, washout, n=5, 2 weeks, ddPCR, mean, SD



RNA editing leads to protein function recovery in brain tissues of interest *in vivo*

Mouse brain



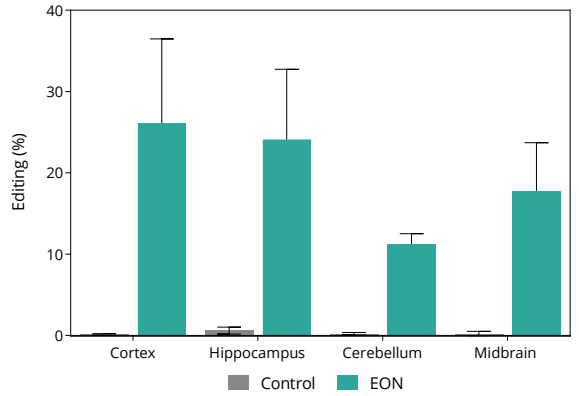
ICV Injection
(lateral ventricle)

RNA editing (%)	Protein function (Fold change to aCSF)
15-40%	Cortex **26**
15-39%	Hippocampus **6**
11-14%	Cerebellum **13**
14-25%	Midbrain **8**

Up to 40% editing *in vivo* leading to 26-fold change in protein function recovery at 4 weeks with a single dose

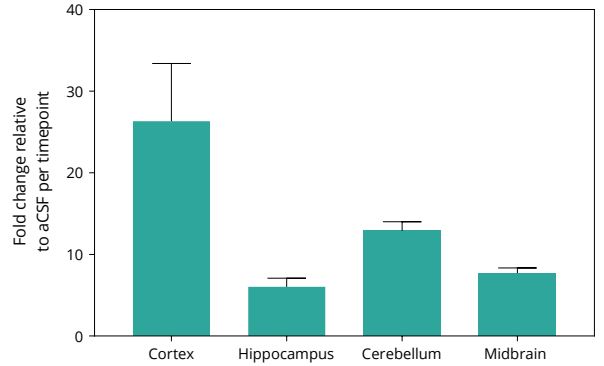
RNA editing in mice brain*

ICV, 250µg, single dose, n=6, 4 weeks, ddPCR, mean, SD



Protein function in mice brain*

ICV, 250µg, single dose, n=6, 4 weeks, western blot, mean, SEM

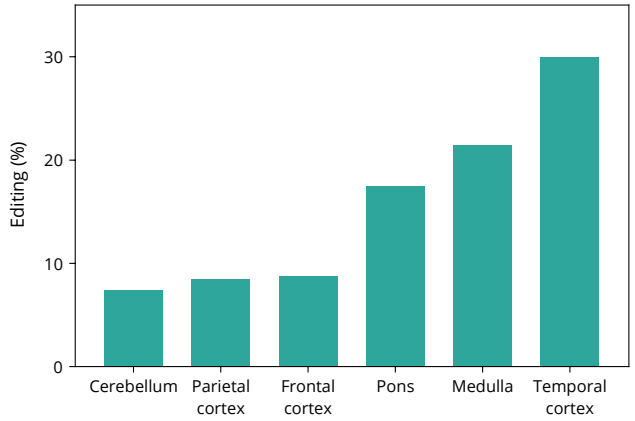


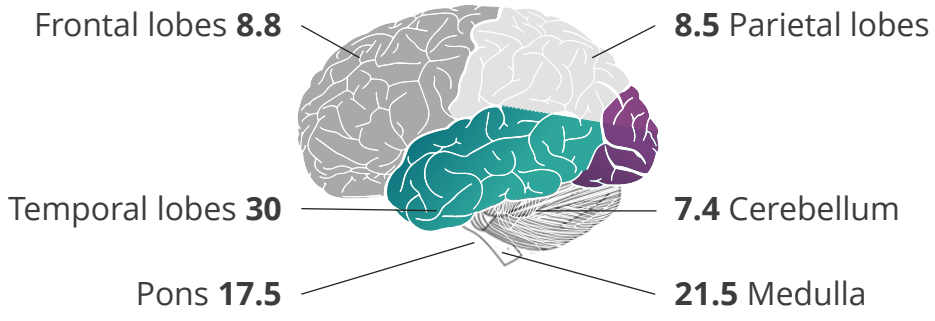
*Undisclosed target. ICV: intracerebroventricular, aCSF: artificial cerebrospinal fluid. Mouse brain (sagittal) from Allen Mouse Brain Atlas

Up to 30% RNA editing reported in brain and approx. 50% in spinal cord in NHP *in vivo*

RNA editing *in vivo* in NHP brain*

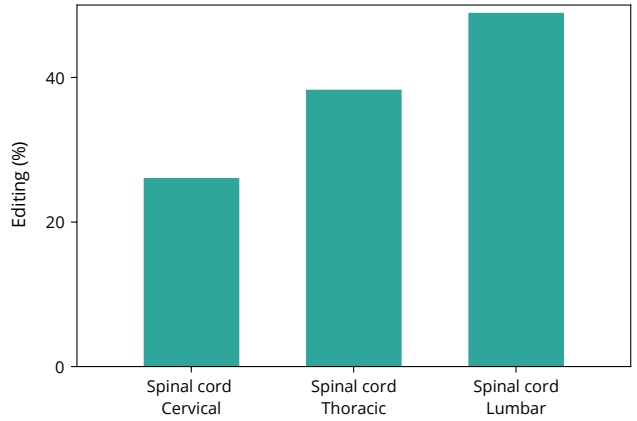
*IT administration, 12mg, single dose, n=3**, 7 days, ddPCR*

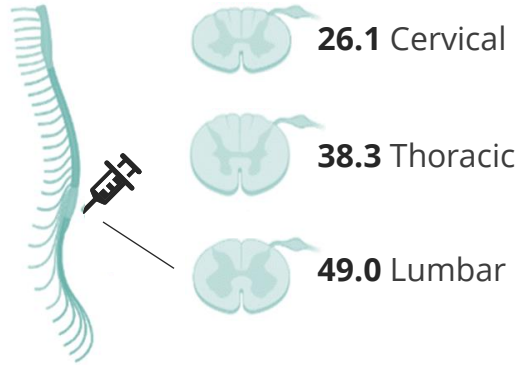




RNA editing *in vivo* in NHP spinal cord*

*IT administration, 12mg, single dose, n=3**, 7 days, ddPCR*



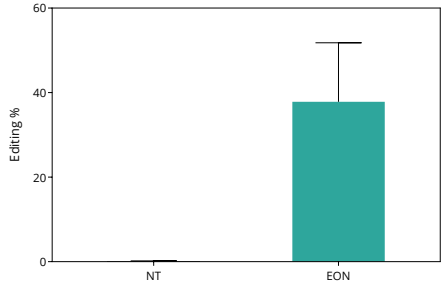


*Undisclosed target. **Data of 2 NHPs not analyzable due to human error during injection procedure. IT: intrathecal, NHP: non-human primate

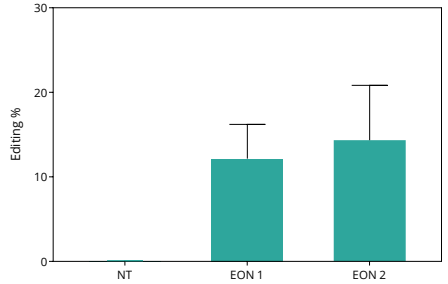
Consistent editing reported – including *in vivo* NHP – in the nervous system

Cell models

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

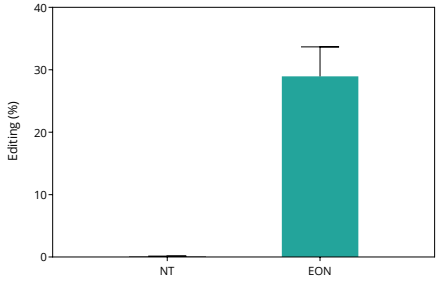


More than 20% RNA editing of *APP* in human iPSC derived neurons

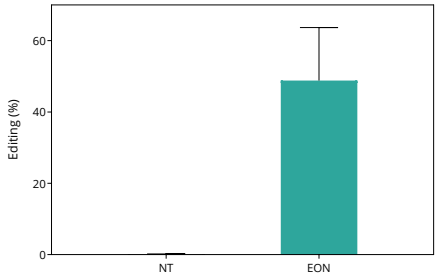


Cerebral organoids

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

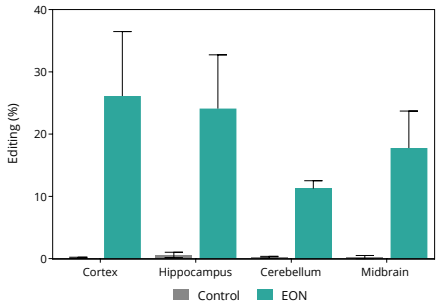


Up to 65% RNA editing of *APP* in cerebral organoids

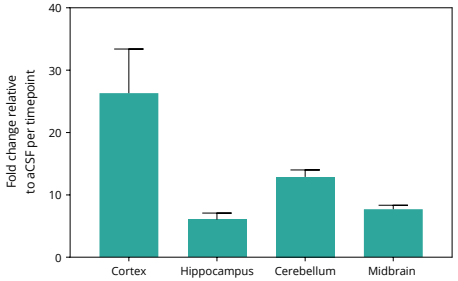


Mice *in vivo*

Up to 40% RNA editing in mice brain*

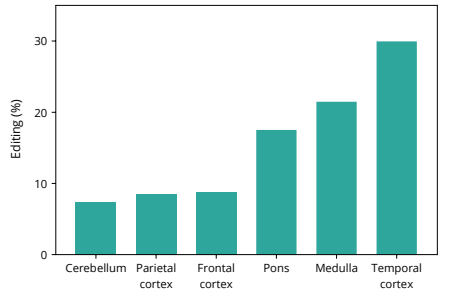


26-fold change in protein function in mice brain*

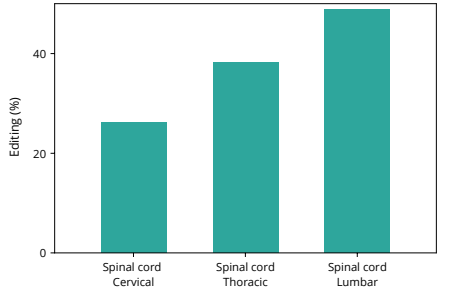


NHP *in vivo*

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=3-4, 2 weeks, dPCR and conditions of the *APP* iPSC derived neurons experiment: gymnosis, 10µM, single dose, washout, n=3, 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.

Establishing a strong platform in multiple organs, targets and models

Nervous system

Targeting CNS and PNS

Liver

Targeting liver originated diseases

Cell models

Organoids

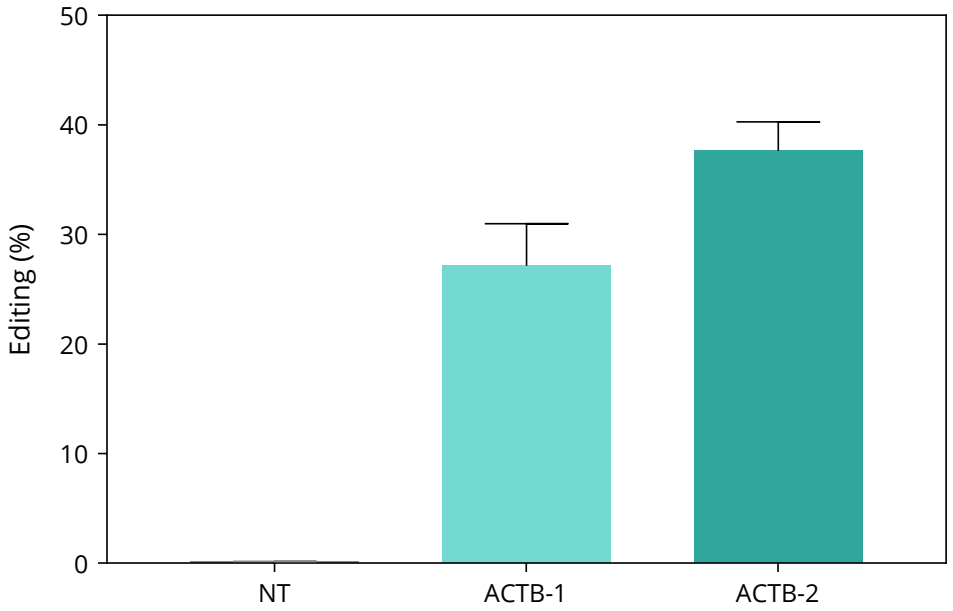
Mice *in vivo*

CNS: Central nervous system, PNS: peripheral nervous system

More than 50% RNA editing in human primary hepatocytes

RNA editing of *ACTB* in human primary hepatocytes

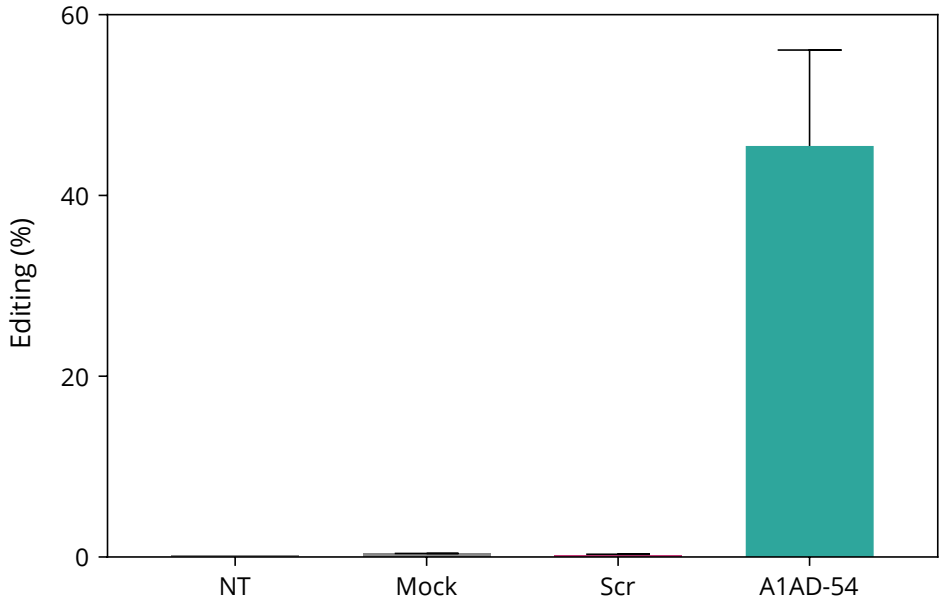
Gymnosis, 1 µM, single dose, n=6, 72 hours, SEM, dPCR



Similar levels of RNA editing of *ACTB* achieved in several models of liver origin (not presented here)

RNA editing of *SERPINA1* E366K in human A1AD patient hepatocytes

Transfection, 100 nM, single dose, n=2, 47 hours, dPCR, mean, SD



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

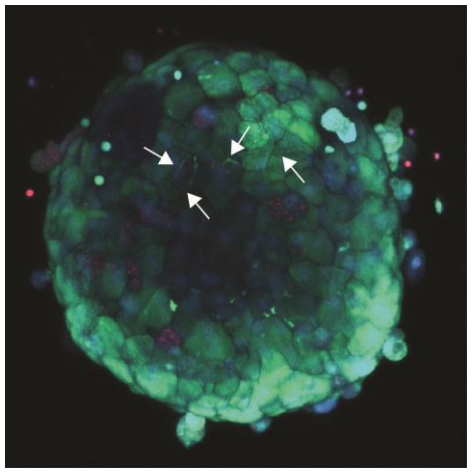
A1AD: Alpha-1 antitrypsin deficiency.

Editing in InSphero Human Liver microtissues (LMTs)

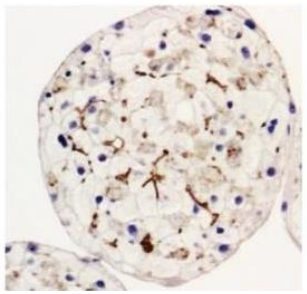
Primary hepatocytes, Kupffer cells and liver endothelial cells in 3D spheroid

Live imaging of LMT

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



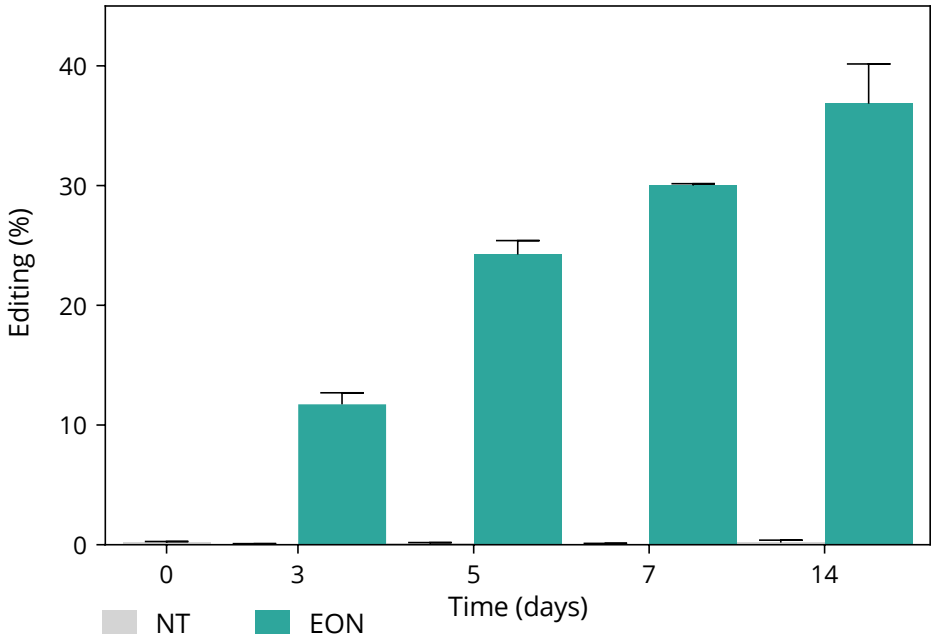
BSEP Bile Canaliculi (InSphero data)



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



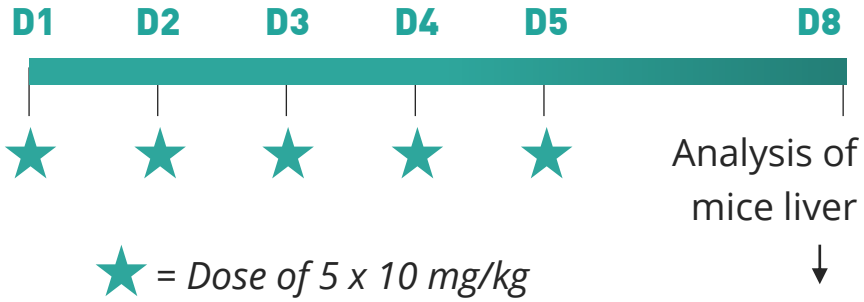
Treatment of LMTs with 1µM EON for 14 days results in up to 40% RNA editing of *ACTB*

BSEP: Bile salt export pump, LMTs: Liver Microtissues constituted of primary hepatocytes, Kupffer cells and liver endothelial cells in 3D spheroid.

Up to 50% RNA editing of *ActB* in liver of mice

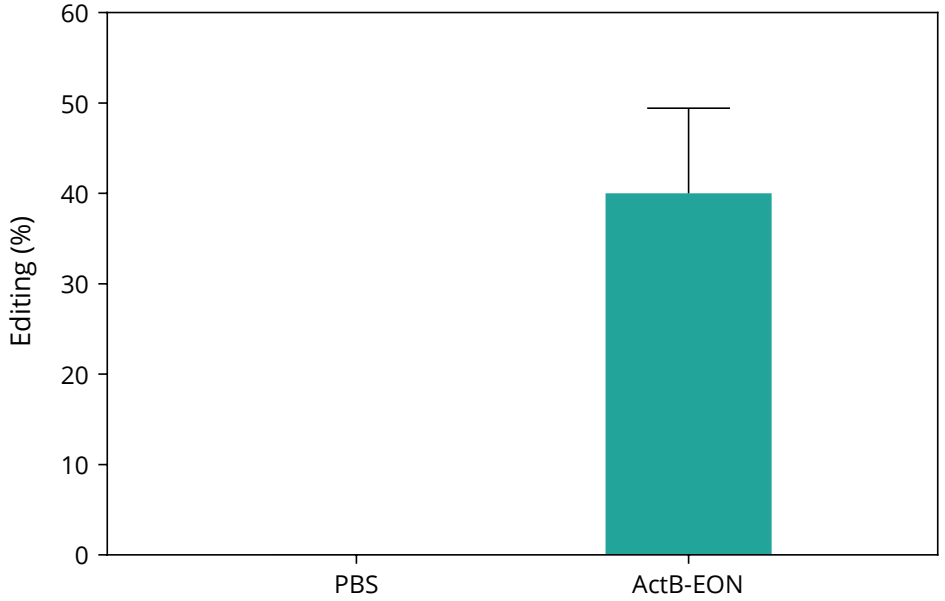
Mice treatment

In vivo



RNA editing of *ActB* in liver of mice

SC, 5 daily doses of 10 mg/kg, N=4, 1 week, ddPCR, mean, SD

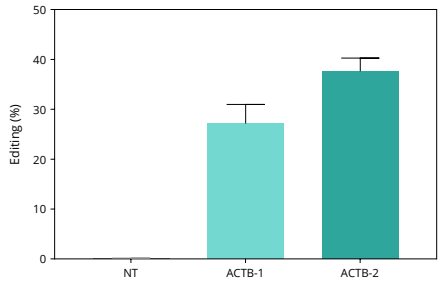


High *in vivo RNA* editing of *ActB* in the liver of mice reaching up to 50%

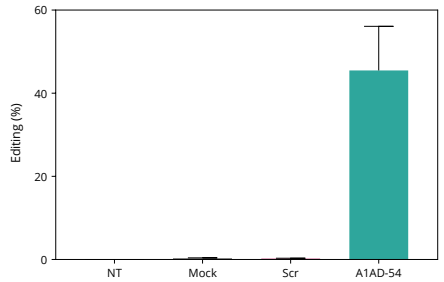
Advancing Axiomer® development across different models and targets in the liver

Cell models

Up to 40% RNA editing of *ACTB* in human primary hepatocytes



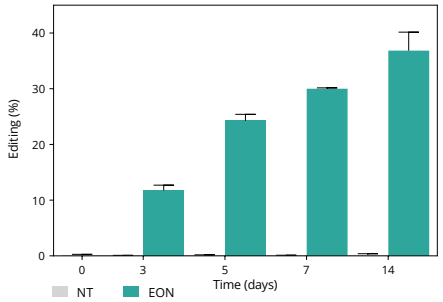
>50% RNA editing of *SERPINA1* E366K in human A1AD patient hepatocytes



Liver organoids

Up to 40% RNA editing of *ACTB* in human LMTs

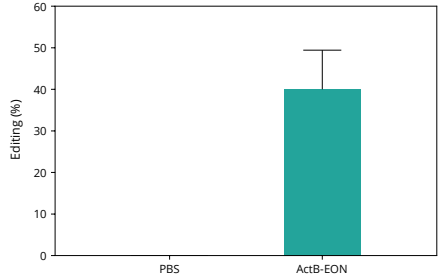
Gymnosis, 1µM, constant dose, 3 pools of 24 LMTs per condition, 14 days, dPCR, mean, SD



Mice *in vivo*

Up to 50% RNA editing of *ActB* in liver of mice

SC, 5 daily doses of 10 mg/kg, N=4, 1 week, ddPCR, mean, SD



Conditions of *ACTB* editing experiment in human primary hepatocytes experiment: gymnosis, 10µM, single dose, N=6, 48 hours, dPCR; Conditions of the of *SERPINA1* editing experiment in human A1AD patient hepatocytes experiment: transfection, 100 nM, single dose, N=2, 47 hours, dPCR, mean, SD. LMTs: human liver microtissues.

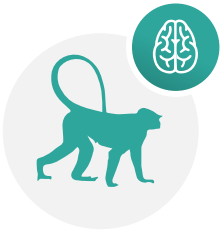
Axiomer® PoC in the nervous system and liver across multiple models including *in vivo*

Consistent RNA editing reported

in all models in nervous system and liver

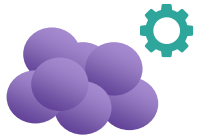
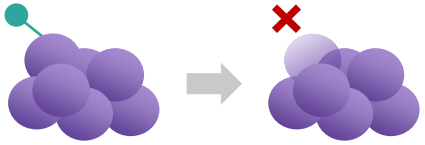
Up to 40% editing reported in the nervous system of mice *in vivo*

Up to 50% editing reported in the liver of mice *in vivo*



Approx. 50% editing reported in the nervous system of NHP *in vivo*

Axiomer® creating a new class of medicines with broad therapeutic potential

Correction	Protein modulation		
			
Mutations correction Thousands of G-to-A mutations, many of them described in literature	**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 PTMs** 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

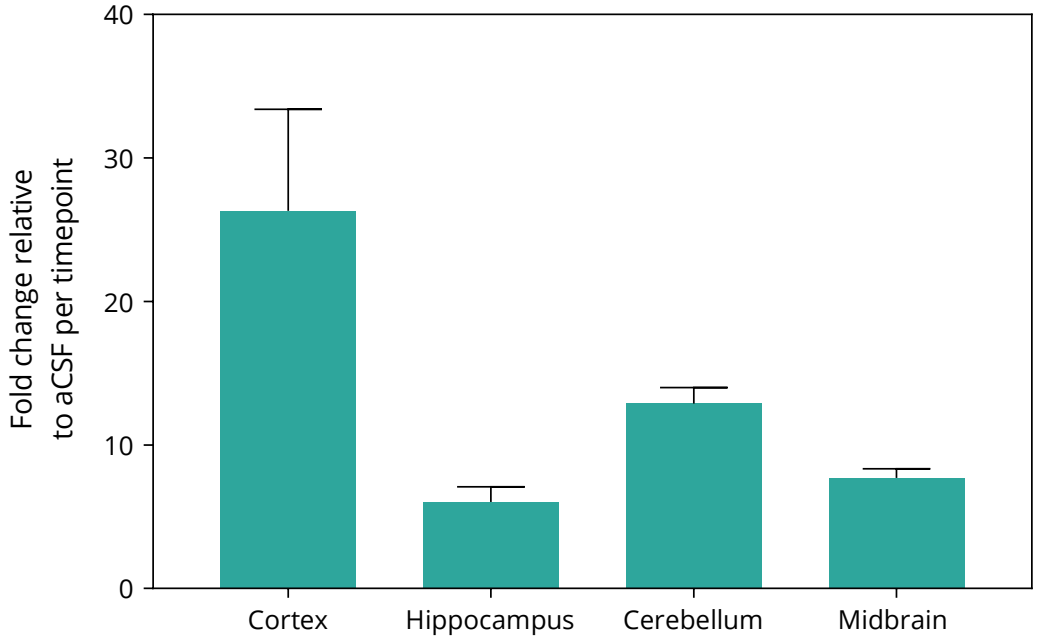
- Common diseases
- Rare diseases
- Target a wide variety of organs
- Treat so-far undruggable targets

PTMs: Post-translational modifications.

Mutation correction with Axiomer® leads to protein recovery

Protein function in mice

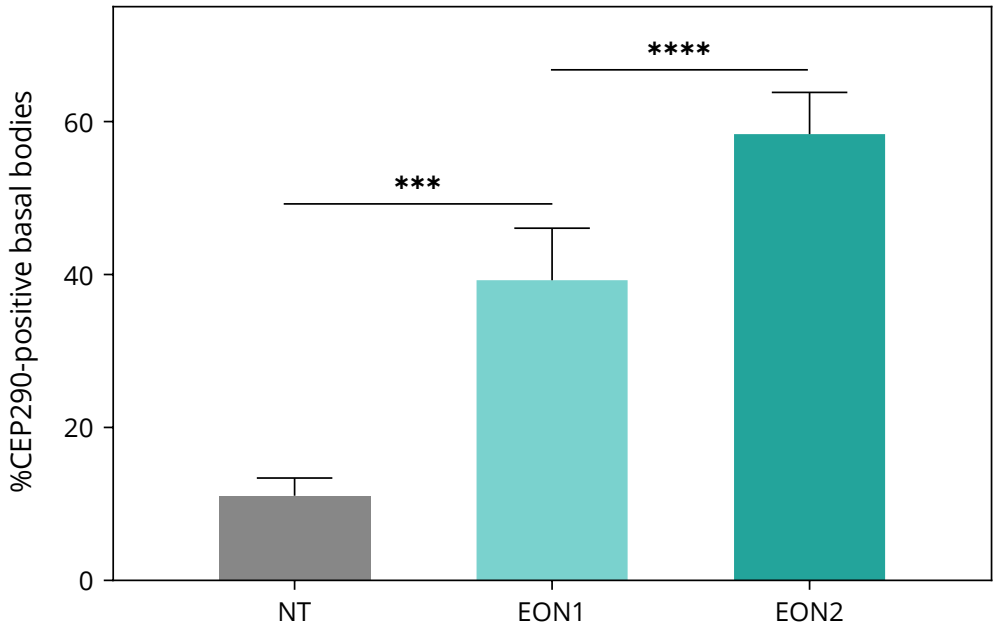
ICV, 250µg, single dose, N=6, 4 weeks, western blot, mean, SEM



In the brain, Axiomer® EONs lead to 26-fold increase in protein function in the cortex after editing

CEP290 protein recovery in organoids

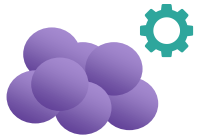
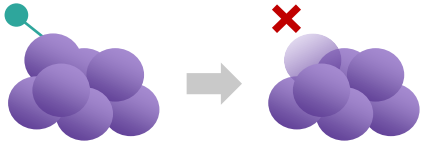
Gymnosis, 10µM, single dose, N=8, 2 weeks, IF, mean, SD



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

ICV: Intracerebroventricular injection, IMF: Immune Fluorescence; SD: standard deviation, SEM: Standard error of the mean, WT: wild type. Statistical significance was determined using Brown-Forsythe and Welch ANOVA test.

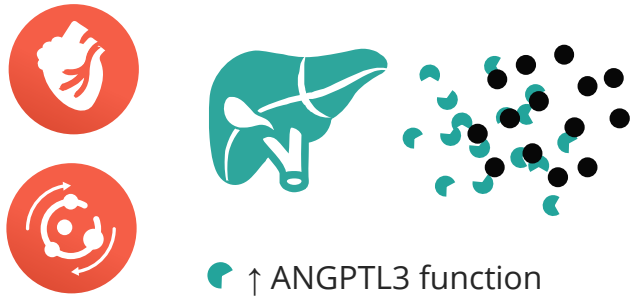
Axiomer® creating a new class of medicines with broad therapeutic potential

Correction	Protein modulation		
			
Mutations correction Thousands of G-to-A mutations, many of them described in literature	**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 PTMs** 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
Mutation correction leading to protein recovery	Variant resulting in a dominant negative effect	Reduction of protein phosphorylation altering protein function	Variant impacting protein interaction with sugar

Changing a protein binding site with Axiomer® leads to an increased LPL activity

Generation of an ANGPLT3 variant to activate lipoprotein lipases

Individuals with increased CVD risk



↑ ANGPTL3 function

↑ LDL + triglycerides

Axiomer® edit

↓ ANGPTL3 function

↓ LDL + triglycerides

Wildtype ANGPTL3

AAAGACTTTGTCCAT**AAG**ACGAAGGGCCAAATTAAT

-K--D--F--V--H--**K**--T--K--G--Q--I--N-

Edited ANGPTL3

AAAGACTTTGTCCAT**GAG**ACGAAGGGCCAAATTAAT

-K--D--F--V--H--**E**--T--K--G--Q--I--N-

= Heparin-binding motif

ANGPTL3 is an angiopoietin-like factor that inhibits lipoprotein lipases (LPL)

- Increase triglyceride, cholesterol, and non-esterified fatty acids in plasma leading to an increased risk of CVD

Reported variant changing protein binding site of ANGPTL3

- Significantly decreased triglycerides, LDL-cholesterol, and total cholesterol
- Significantly decreased odds ratio for coronary artery disease

Heparin binding was shown to be essential for proper ANGPTL3 function

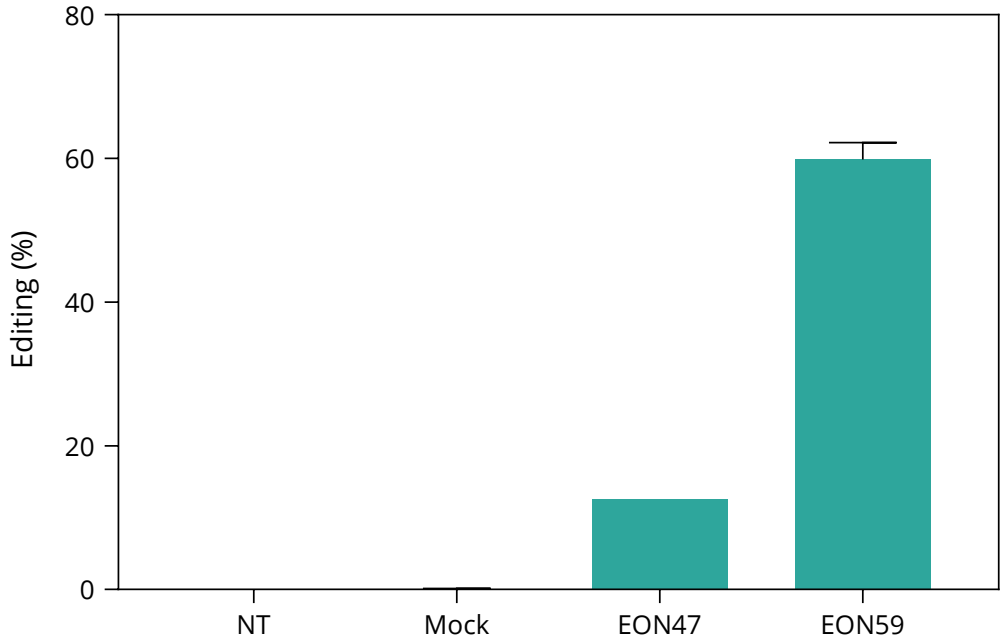
- Disruption of the heparin binding site is highly likely to abrogate LPL inhibition, ultimately leading to lipid lowering in the serum

CVD; cardiovascular disease. LDL: low density lipoprotein, LOF: Loss of function. References: Ono M et al. J Biol Chem. 2003 Oct 24;278(43):41804-9; Romeo S et al. J Clin Invest. 2009 Jan;119(1):70-9; Dewey FE et al. N Engl J Med. 2017 Jul 20;377(3):211-221.

ANGPTL3 variant disrupting essential protein binding site

More than 60% RNA editing of *ANGPTL3* in primary human hepatocytes derived spheroids

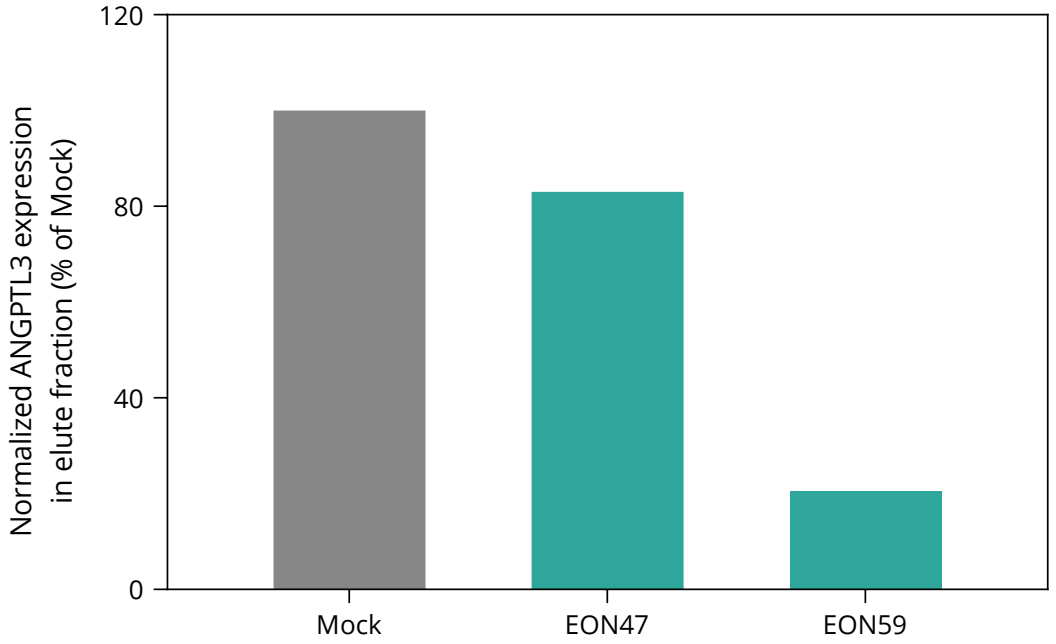
Gymnosis, 1µM, single dose, N=1 or 2, 5 days, dPCR, mean, SD



More than 60% RNA editing of *ANGPTL3* in primary human hepatocytes derived spheroids

Up to 80% decrease in heparin binding in Huh-7 cells

Gymnosis, 1µM, single dose, N=1, 72 hours, western blot



Up to 80% decrease in heparin binding in Huh-7 cells

Axiomer® RNA-editing platform technology

Versatile

- Ability to target multiple organs and a wide range of diseases with numerous applications
- Potential to include protective variants
- Designed to target a variety of RNA species (mRNA, miRNA, lncRNA)

Safety

- No permanent changes
- No irreversible DNA damages and less risk of permanent side effects

High specificity

- Highly targeted therapeutic with potential to minimize off-target effects and reduce the risk of adverse reactions

Transient

- Provide a long-lasting therapeutic effect that does not require frequent dosing
- Potential to target diseases for which permanent changes would be deleterious

No viral vector

- No risk of immunogenicity or capacity limitation due to the vector
- Efficient development and faster production increase the chance to reach market

Endogenous ADARs

- Leverage body's potential to treat disease
- Less risk of off-target effect vs. exogenous ADARs

ADAR: Adenosine deaminase acting on RNA, mRNA: messenger RNA, miRNA: microRNA, lcRNA: long non-coding RNA

ProQR®

IT'S IN OUR RNA