Development of RNA Base Editing Technologies for Precision Medicines

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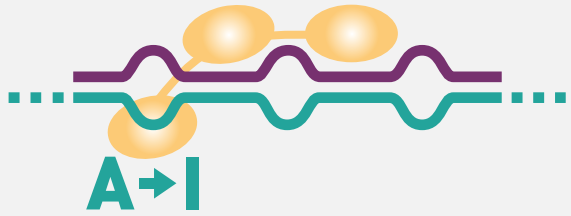
RNA toolbox – editing platform technologies

Axiomer® and Trident® in development by ProQR



Axiomer® A-to-I editing

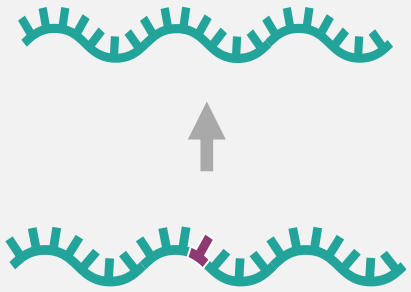
- Exploiting endogenous ADAR
- Recruited by synthetic Editing Oligonucleotide (EON)
- I is translated as a G, allowing to target G-to-A mutations
- Specific, potent, and stable by design
- >20,000 G-to-A mutations described in literature

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

Repairing G-to-A Mutations

Axiomer® has the potential to target broad range of diseases



Repairing G-to-A mutations

- More than 20,000 G-to-A mutations described in literature

Examples:

- IUDA in Hurler Syndrome
- SERPINA1 in A1AT

>20,000 G>A mutations

Ophthalmology

>1,100 targets

- Leber Congenital Amaurosis 4
- Usher syndrome
- Fuchs Endothelial Corneal Dystrophy
- Retinitis Pigmentosa type 3
- Stargardt Disease
- Primary Congenital Glaucoma

Skin

- Albinism
- Dystrophic Epidermolysis Bullosa
- Junctional Epidermolysis Bullosa
- Darier disease
- Epidermolysis Simplex

CNS

- Parkinson's Disease VIII
- Spinocerebellar Ataxia VII
- Alzheimer's Disease
- Huntington's Disease
- Pain disorders

Lung

- Cystic Fibrosis
- Primary ciliary dyskinesia
- Surfactant Metabolism Dysfunction
- ABCA3 deficiency
- Familial Pulmonary Fibrosis

Kidney

- Polycystic kidney disease

Oncology

- KRAS driven tumors
- P53 driven tumors

Blood / Cardiovascular system

- Beta thalassemia
- Alpha thalassemia
- Progeria

Liver

- Alpha-1 Antitrypsin Deficiency
- Hurler Syndrome
- Factor V Deficiency
- Transthyretin-related hereditary amyloidosis
- Wilson disease
- Hereditary Hemochromatosis
- Ornithine Transcarbamylase deficiency
- Hemophilia B
- Pompe Disease

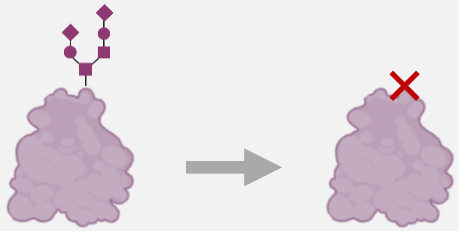
And many more...

Axiomer® - beyond mutation repair

Site-specific protein engineering & Post-Translational Modifications (PTMs)

Alter phosphorylation sites

Targeting of **phosphorylation** sites (activity switches) to regulate protein activity



Alter glycosylation sites

- Targeting of **glycosylation** sites changes localization, folding and protein function
- Prevent immune escape of **glycosylated** tumor antigens

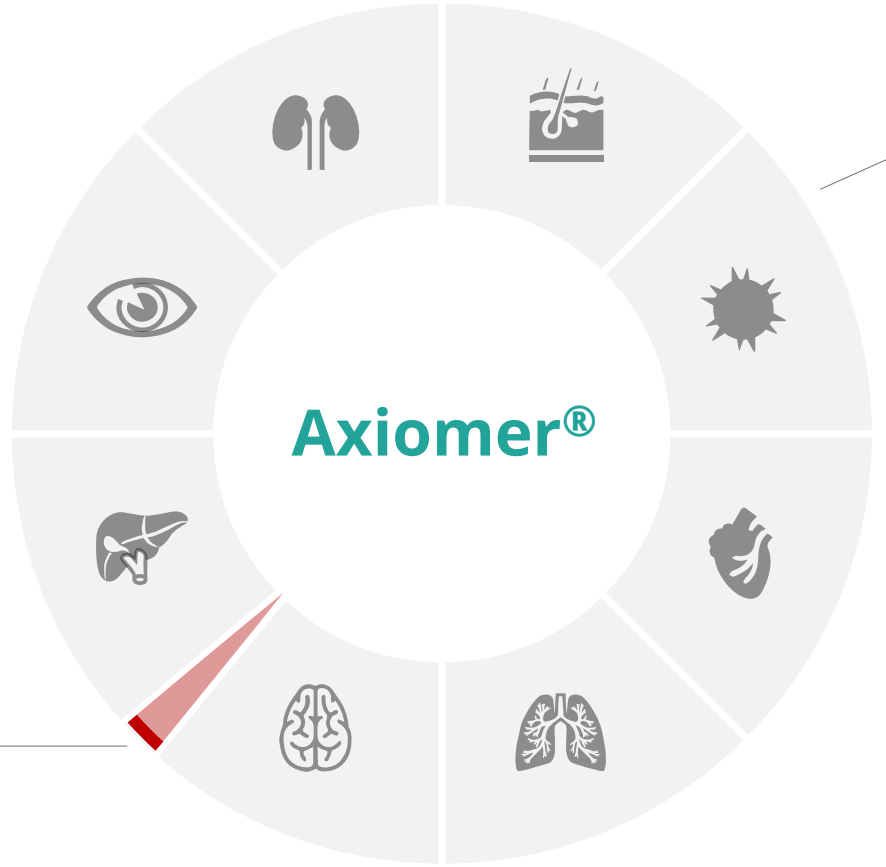
Alter ubiquitination sites

Changing a **ubiquitination** site slows down protein degradation (to treat haplo-insufficiencies)

Potential to edit more than 400 different types of PTMs

- Proteolytic cleavage
- Autocleavage
- Acetylation
- SUMOylation

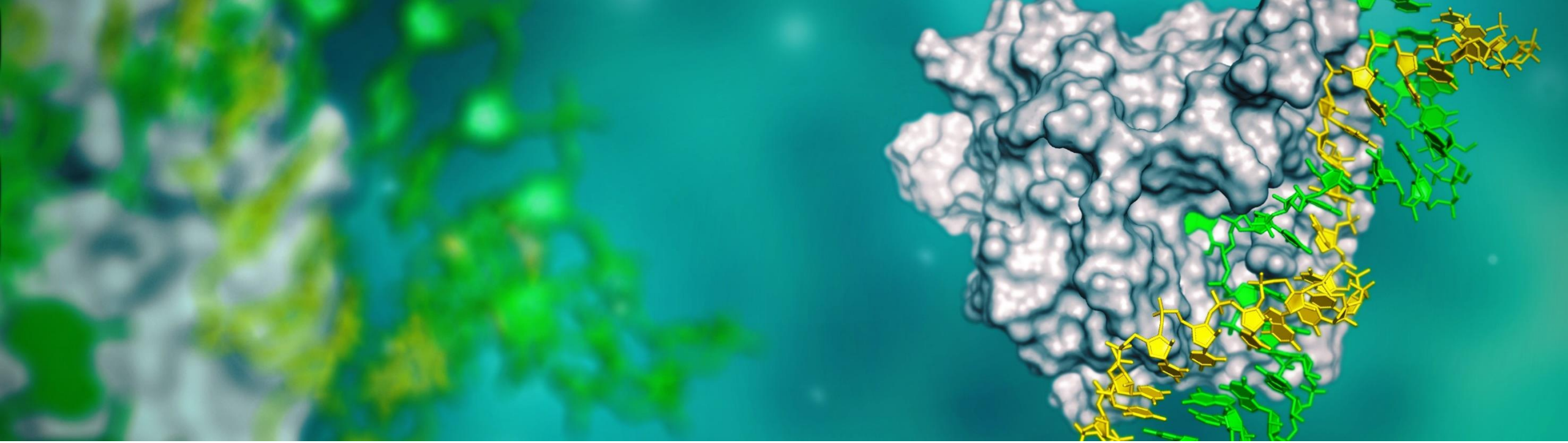
Axiomer® developed for partnership



ProQR will maintain all global exclusive rights to remainder of targets of Axiomer®

- strong potential for further value creation through additional partnerships

Up to 5 targets in liver and nervous system are licensed exclusively to Lilly

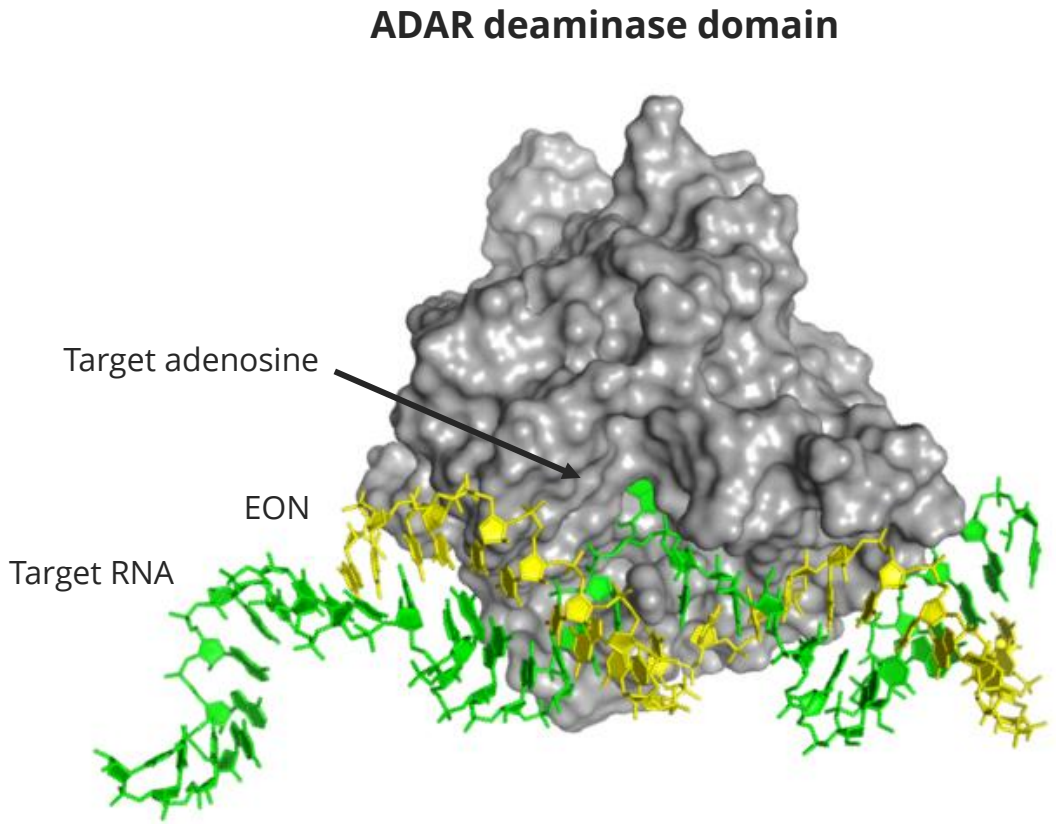


Axiomer®

A-to-I RNA Editing platform

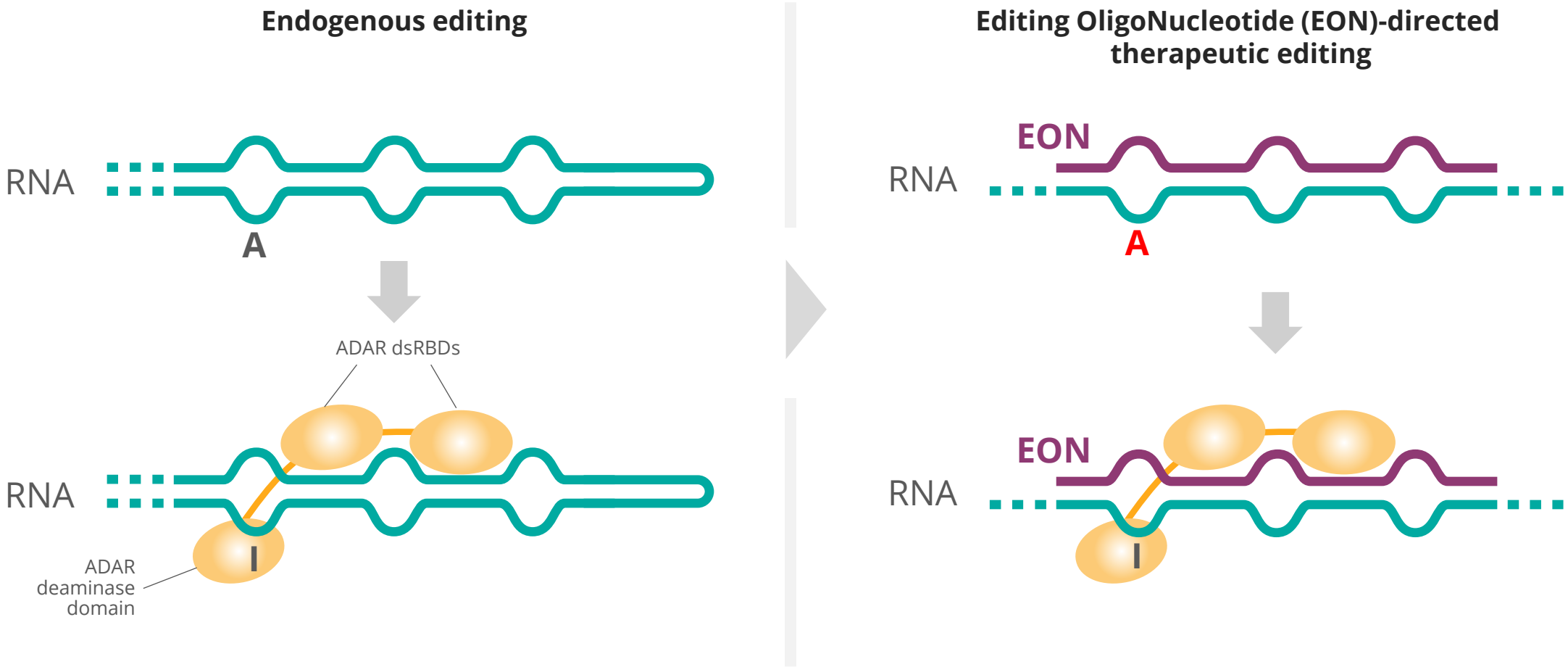
ADAR is the body's own system to edit RNA

- ADAR = **A**denosine **D**eaminase **A**cting on **R**NA
- ADAR is an RNA editing system that is present in all human cells
- In the human body, ADAR is responsible for editing RNA to, for example,
  - Create different isoforms of proteins
  - Change functionality of small RNA molecules
  - Regulate splicing



EONs designed to recruit endogenous ADAR

ADAR deaminates target A in EON-target RNA complex



dsRBDs, double-stranded RNA binding domain

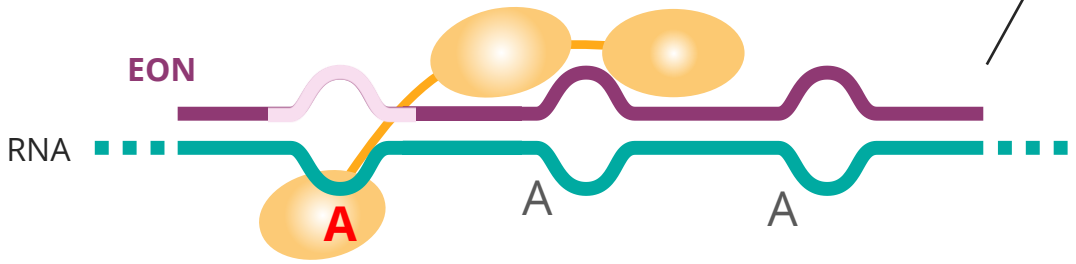
EONs designed for targeted RNA editing

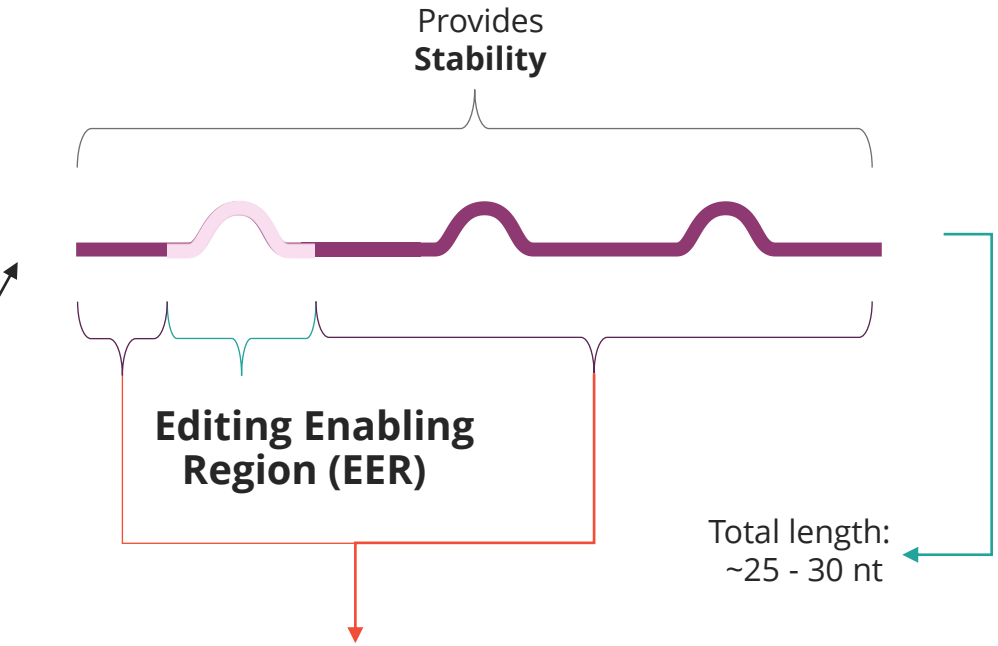
Functionality defined by sequence and chemistry

Sequence defines target RNA binding

EON chemical modifications define:

- Editing efficacy
- Editing specificity
- Stability (nuclease resistance)
- Cell and tissue uptake

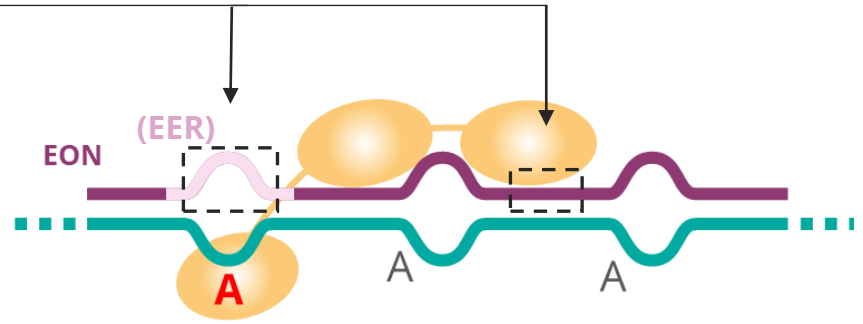




Optimizing EONs for therapeutic use

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

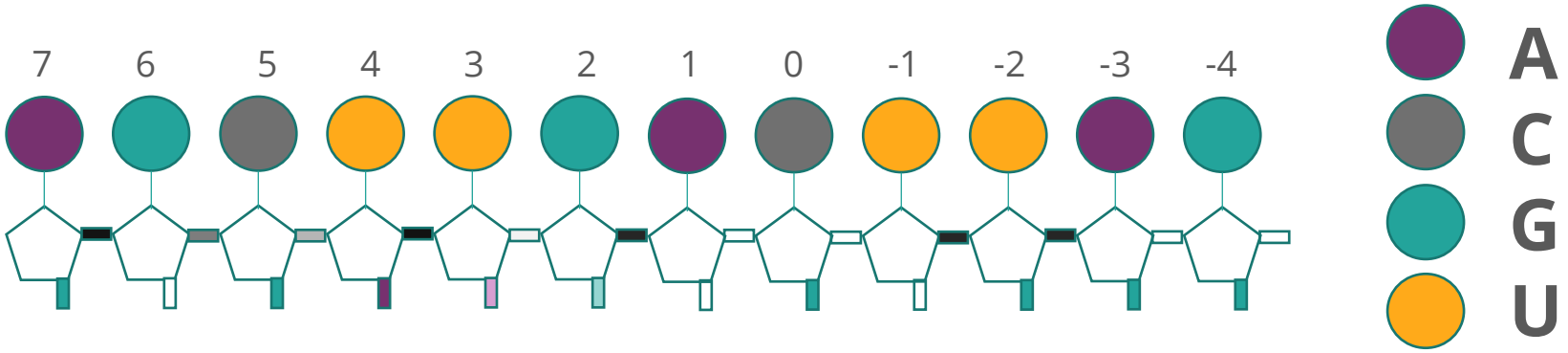
Validation phase

Cell-based assays

Further development

Focus on defining the ground rules

EON



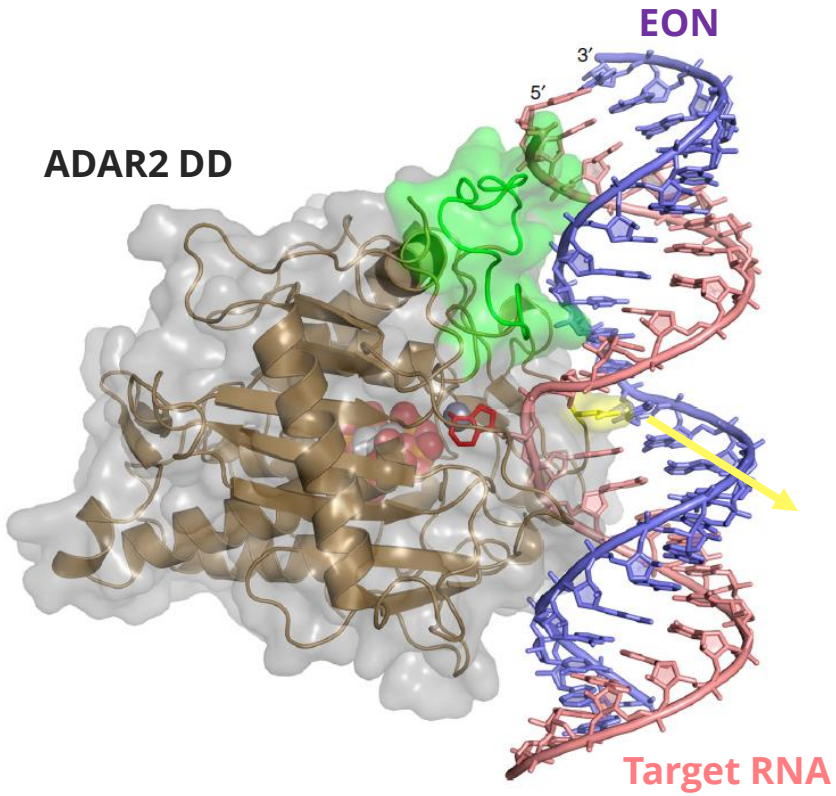
Aspect	Determined by	Modifications	Effects
Base	Target RNA	Mismatches and analogs	Improved PD
Ribose modification	ADAR structure	2’-H; 2’-OMe; 2’-MOE; 2’-F; 2’-NH2, LNA, TNA	Improved PK and PD
Linkage	ADAR structure	PO; PS; PN; MeP; UNA; PAc	Improved PK and PD

Single nucleotide modification

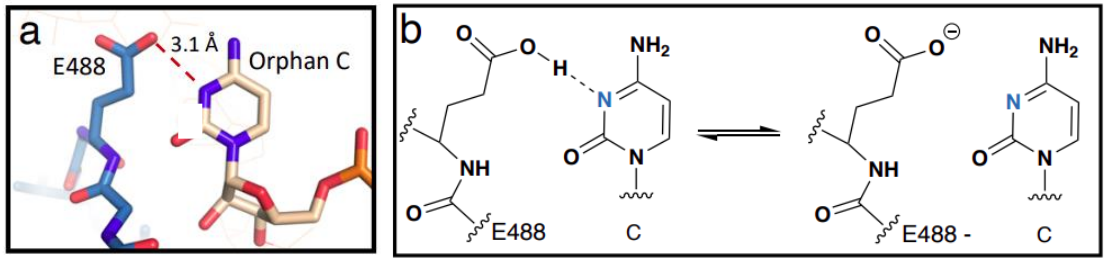
Within Editing Enabling Region (EER) increases EON efficacy

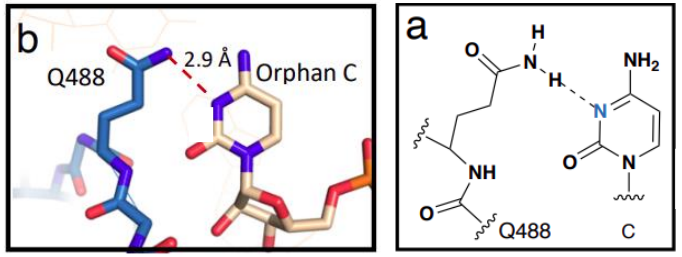
Modification improving EON efficacy identified

Mimicking E488Q mutation in ADAR2 causing hyperactivity

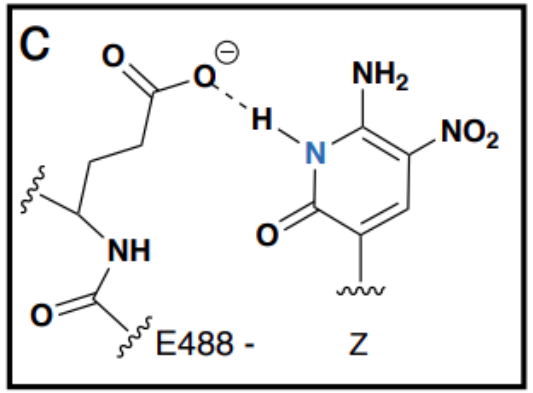


Protonation dependent hydrogen bond – pH dependency





Protonation independent hydrogen bond



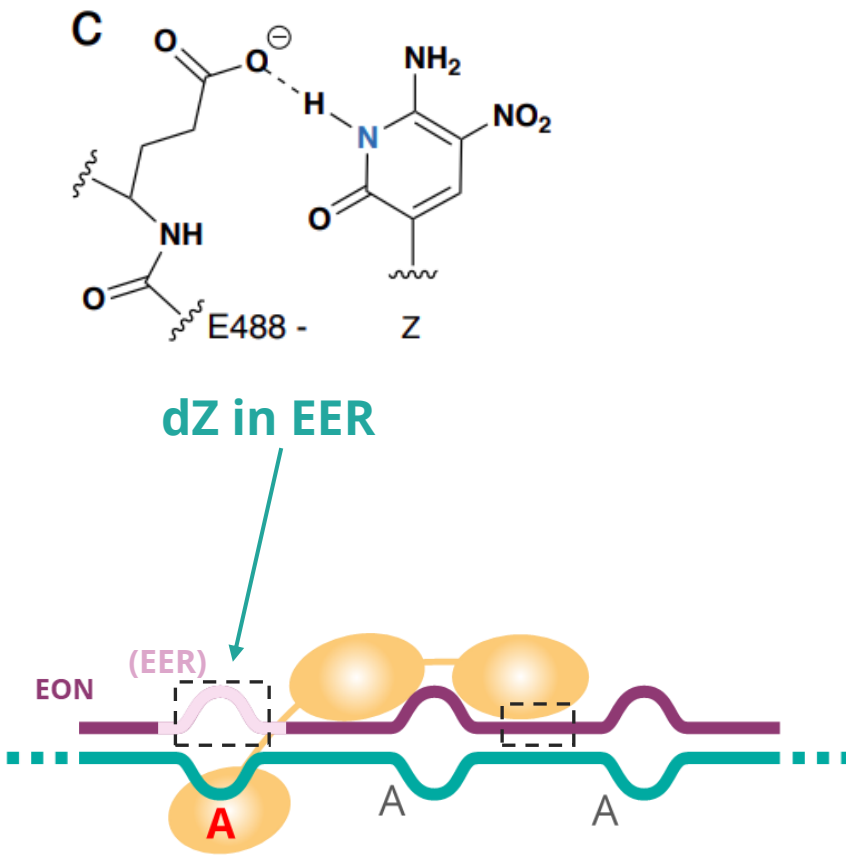
dZ base (dZ)

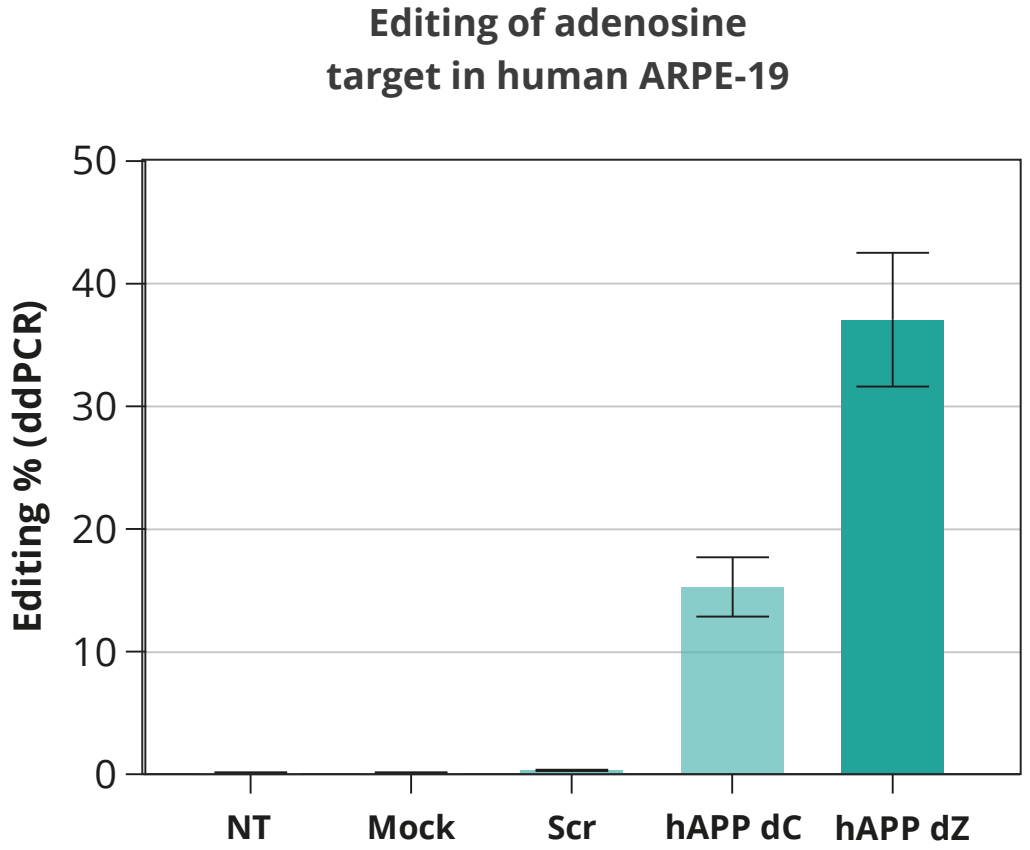
Metthews 2016, Nature Structural & Molecular Biology

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

dZ base (dZ) modification of the EER

dZ improves editing in human retinal pigment epithelial cells



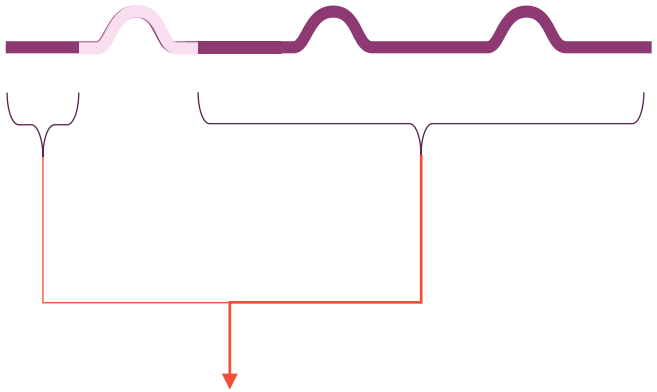


New chemical optimization

For EON ADAR-binding region (ABR) region

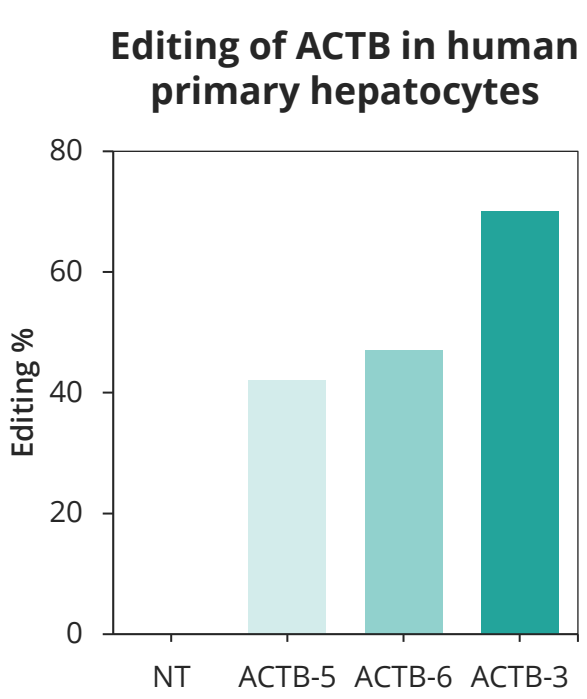
New chemical modification of the ABR

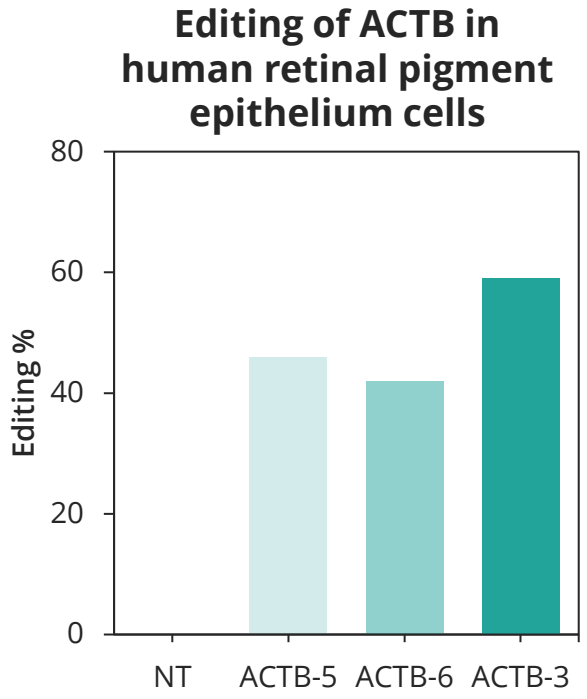
ABR modification greatly enhances editing



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

ADAR-binding region (ABR)

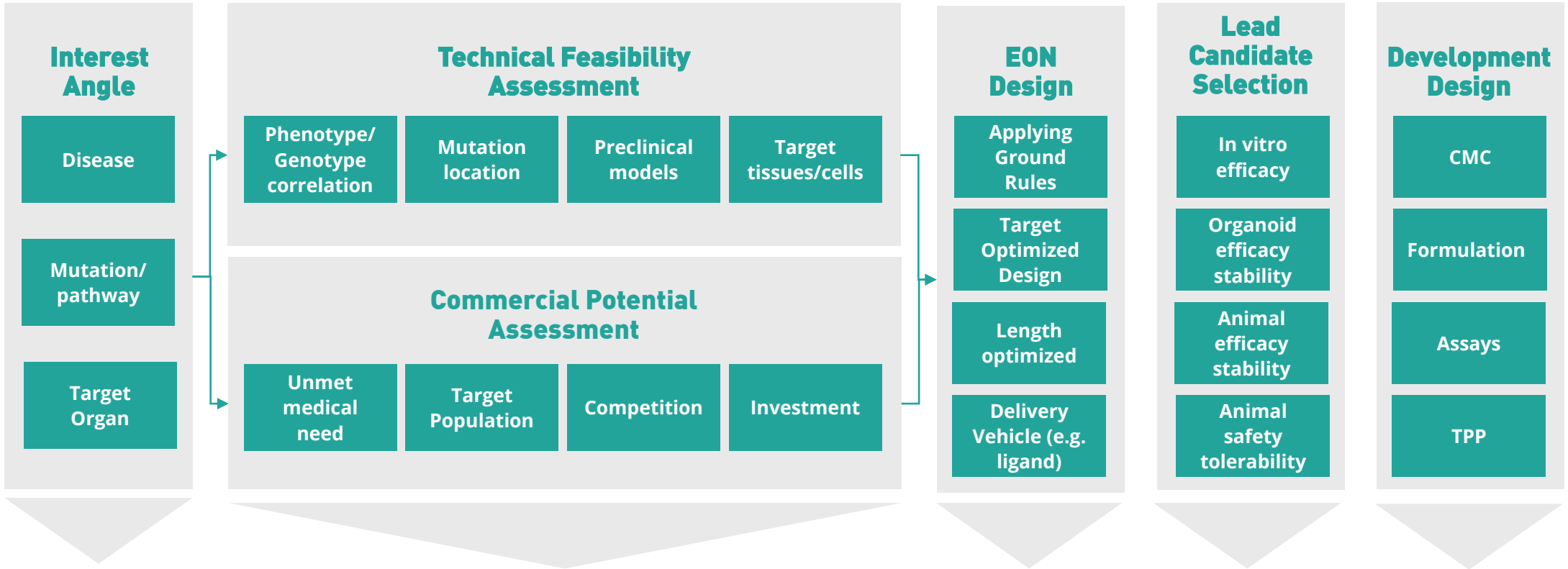




- Chemical optimization greatly increases EON editing in positions within ABR region
- SAR screen of 2nd backbone modification for best position within ABR region ongoing

Process: from target to lead candidate EON

How smart target ideas are transformed into products



Initial target screening generates candidate targets, between a handful to hundreds of targets

We focus on two dimensions: targets that can be technically addressed & which targets constitute a business case

Generation of drug candidates in a smart and efficient way

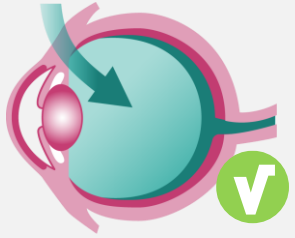
At this step we can prioritize between 1-3 lead candidates

First in human Trial

Axiomer® therapeutic applications

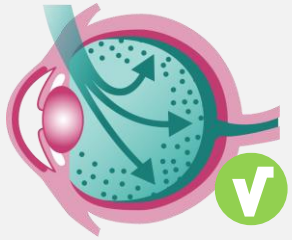
Inherited Retinal Diseases (IRD) indications

Targeting retinal diseases



Intravitreal delivery is routine procedure

- Long half-life in the eye allows for dosing once or twice yearly
- Chemical modification enables naked delivery



Broad distribution allows targeting of complete retina

- Oligonucleotides distribute broadly to all different cell types
- Allowing for targeting central and peripheral disease

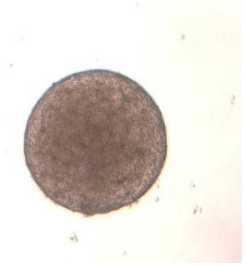
Optic cup model

- Sophisticated organoid model for retinal dystrophies
- Useful for:
  - PK/PD studies
  - Response to treatment
  - Time to onset of response

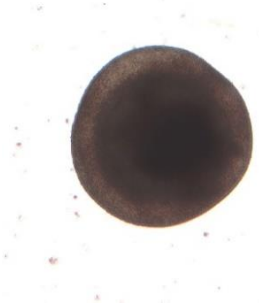
Human retinal organoids

Differentiation from induced pluripotent stem cells (iPSC)

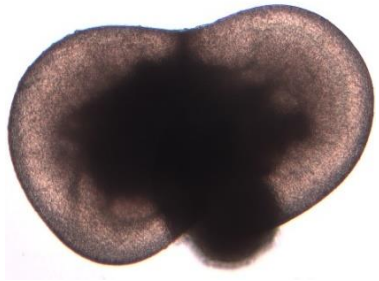
2 days



17 days

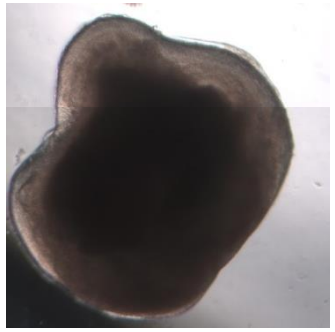


30 days

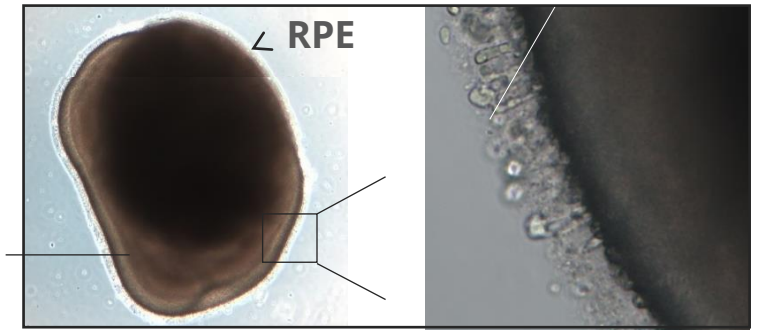


45 days

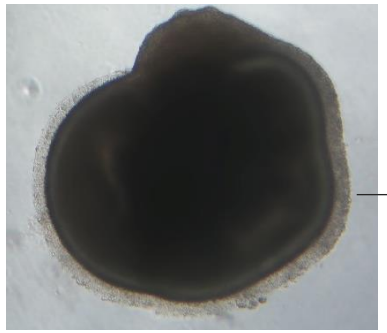
90 days



150 days



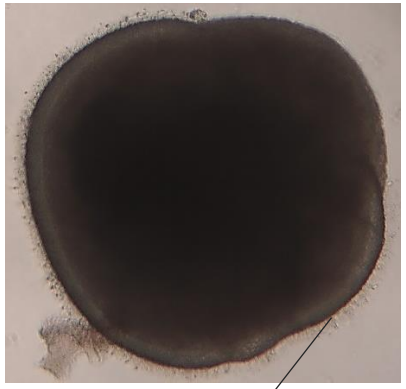
225 days



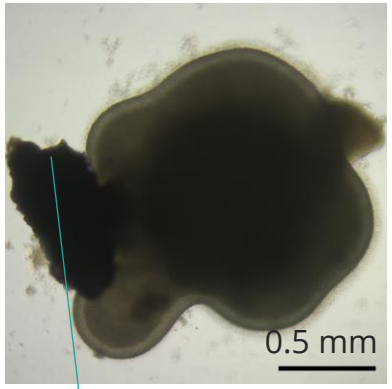
- Takes 150 days to generate organoids. After this they are ready treating with EONs
- Retinal organoids can be wild-type (volunteer derived) or mutant (patient derived)

Organoids fully recapitulate the human retina

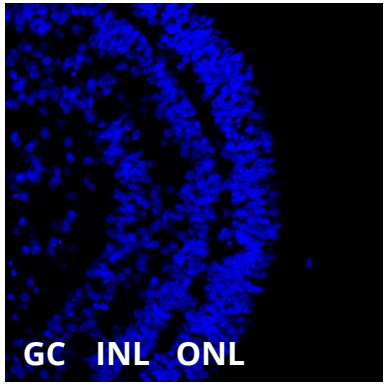
Reflected by cell layer organization and the presence of rods and cones



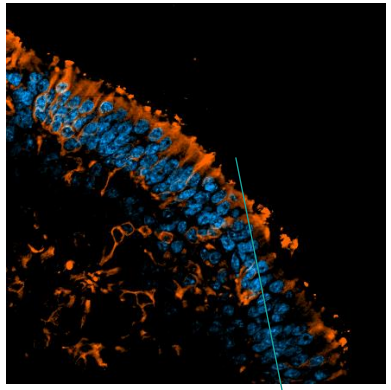
Photoreceptors



RPE

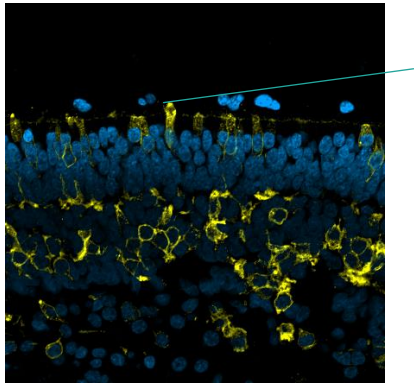


Rods



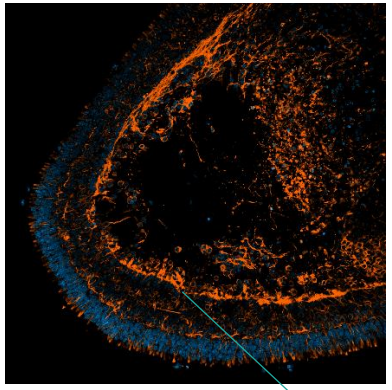
Rhodopsin

Cones



Opsin red/green

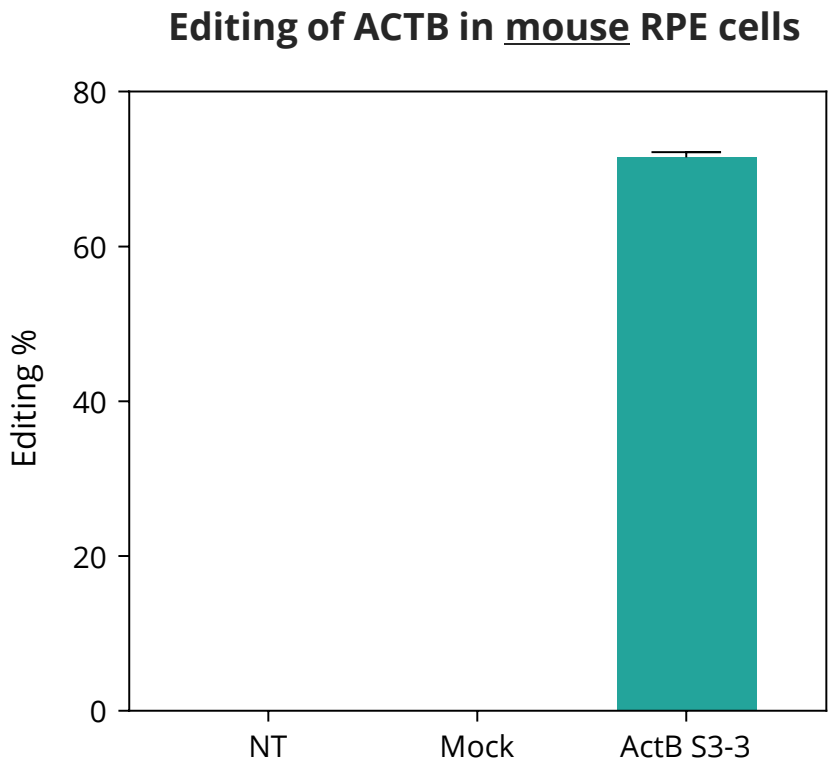
Ganglion Cells

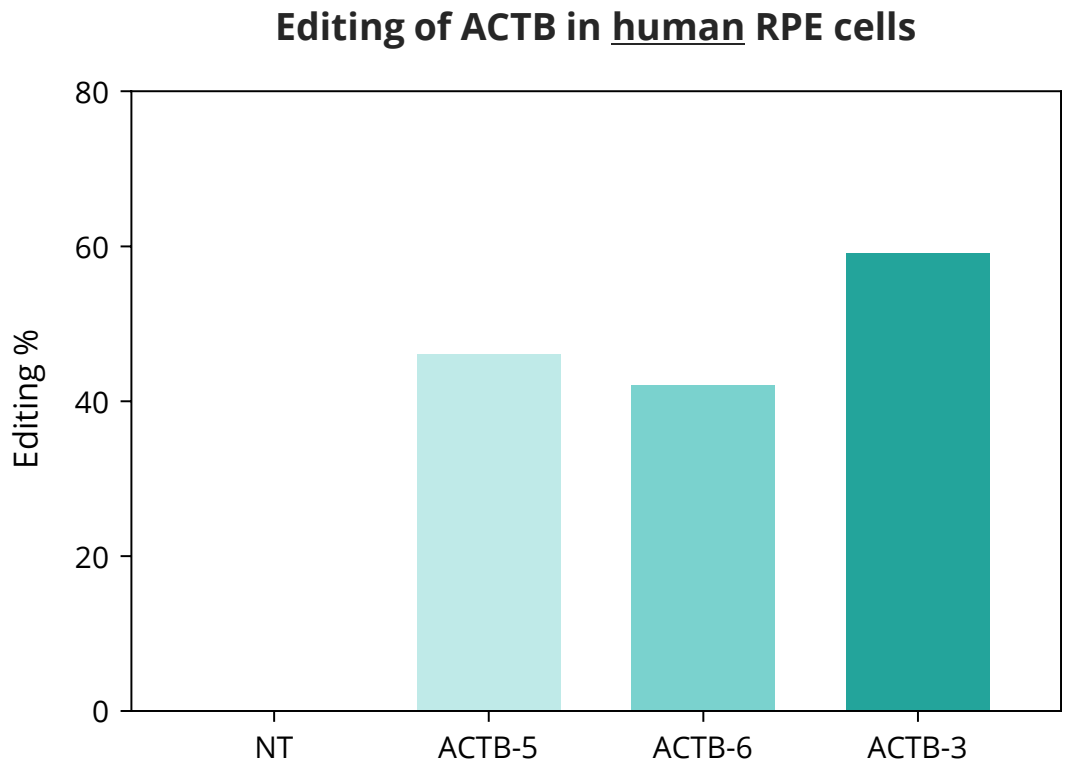


TuJ1

Efficient editing of ACTB in retinal cells

β-actin (ACTB) editing in different cells



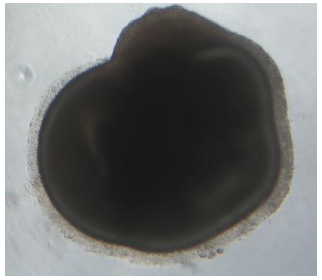


- Similar levels of editing of ACTB achieved in several models of retinal origin
- High confidence of translatability of the approach

Substantial A-to-I editing in retinal organoids

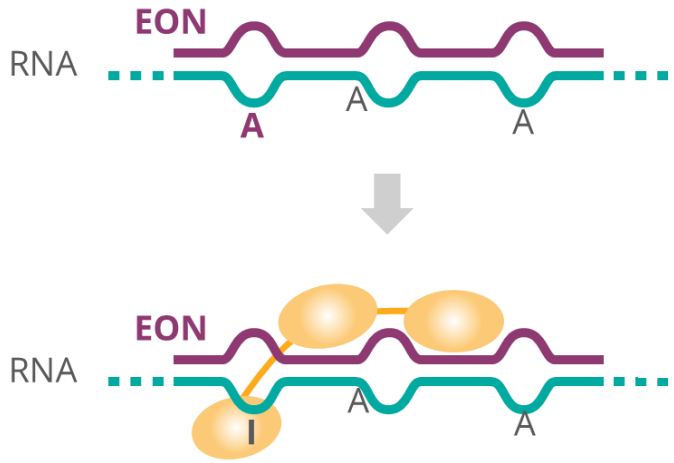
>40% editing was achieved in IPSC derived organoids

Retinal organoid

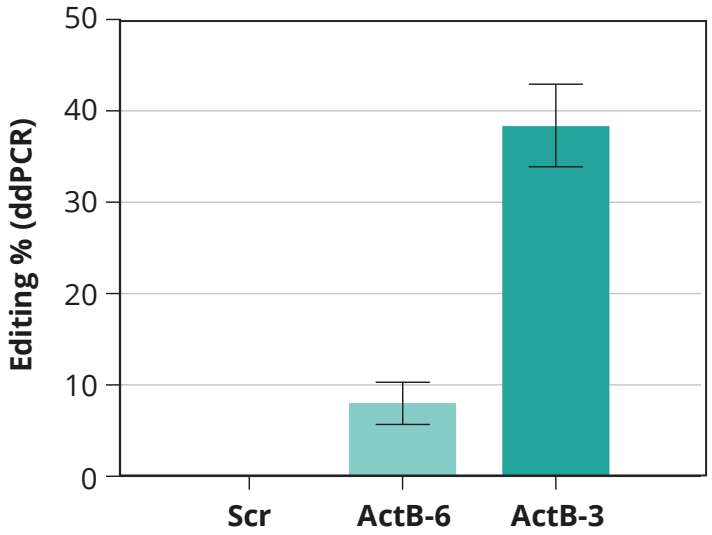


225 days

EON-directed therapeutic editing



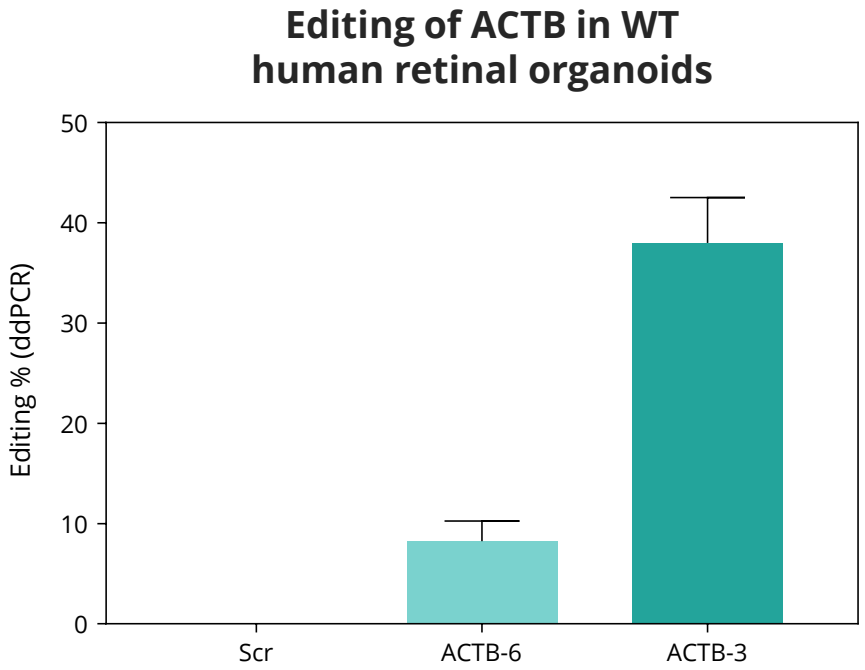
Editing of ACTB in human retinal organoids

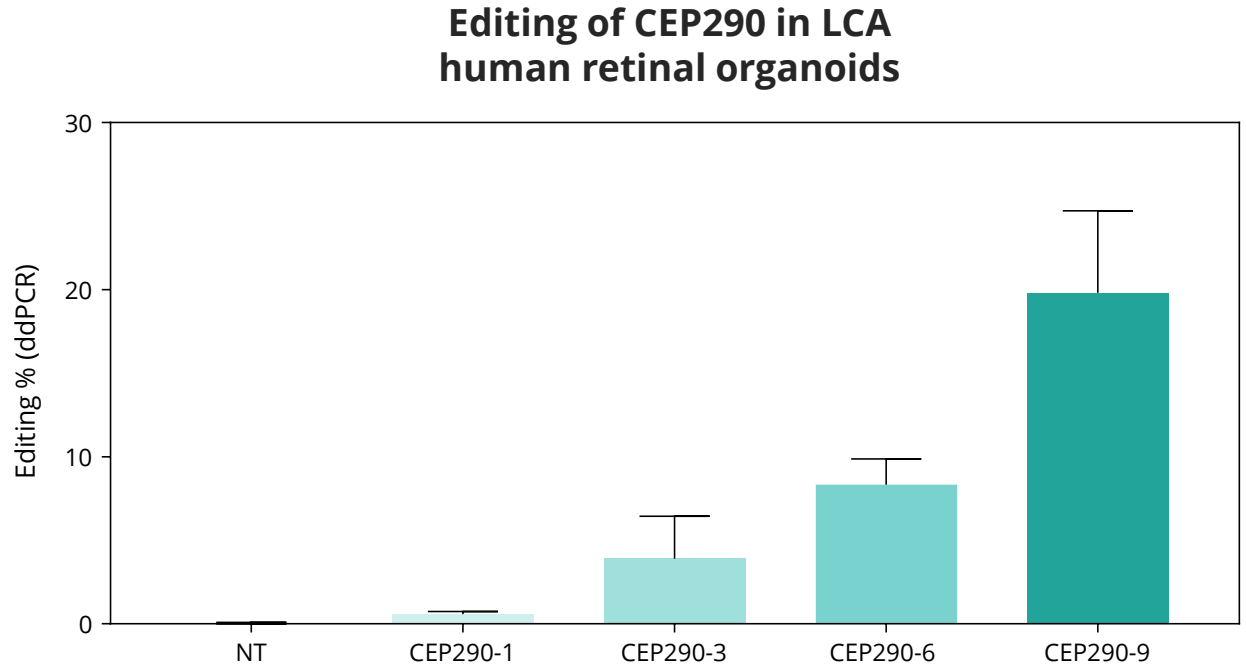


- Each chemical modification improves EON editing efficacy
- The highest editing efficacy increase is obtained for EONs with all modification combined
- Over 40% editing was observed after gymnosis

From model target to therapeutic IRD target

Tweaking basic EON design to meet a specific target's needs in organoids

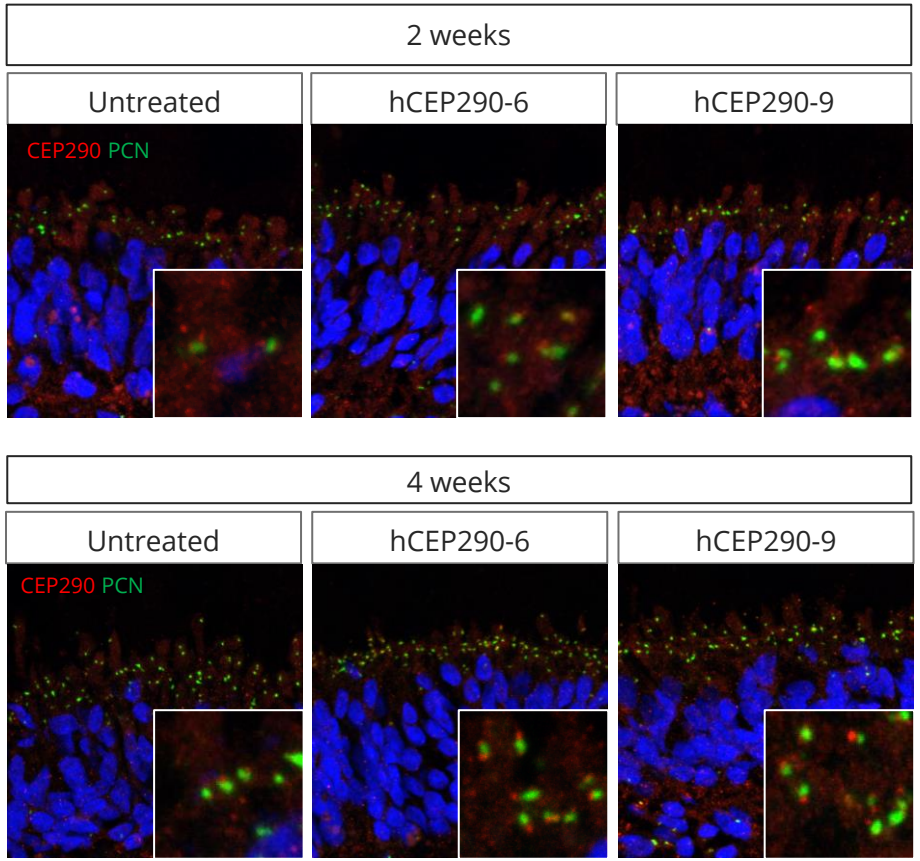


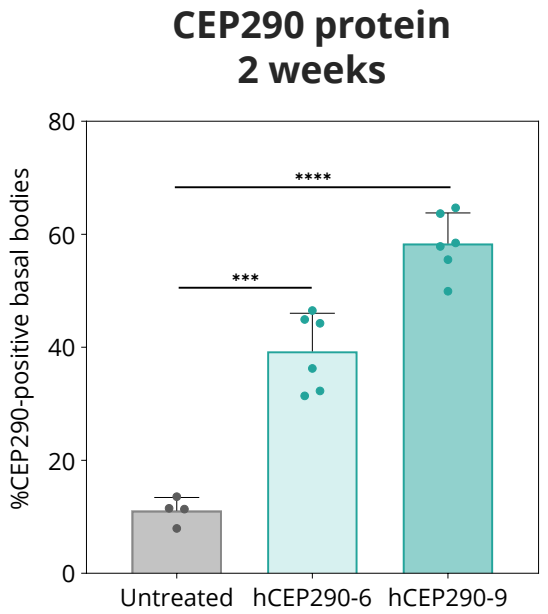


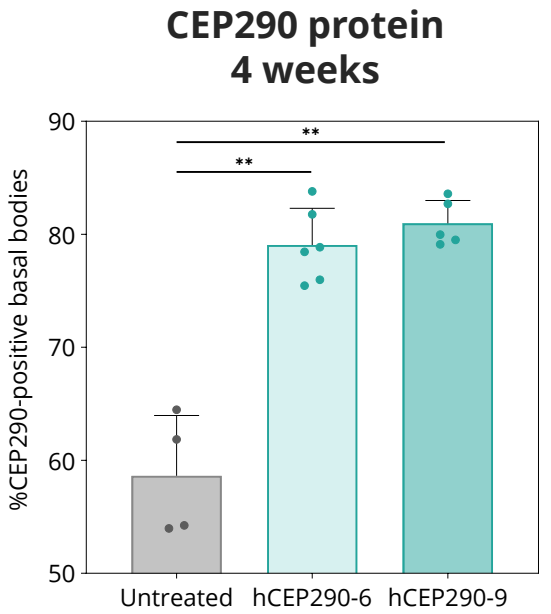
- Each chemical modification improves EON editing efficacy
- The highest editing efficacy increase is obtained for EONs with all modification combined
- Over 40% editing was observed after gymnosis for ACTB and over 20% editing observed after gymnosis for CEP290 (Work in progress)

Editing results in CEP290 protein expression

Quantification of CEP290 protein

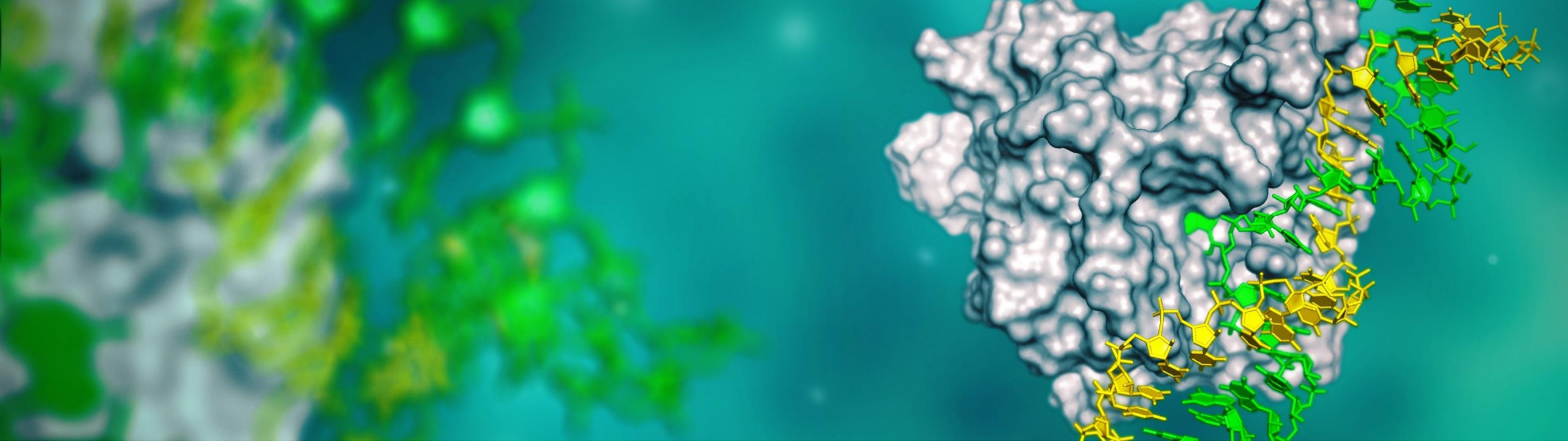






Significant increase in CEP290 protein levels and intensity was detected at the basal body of LCA07-3 organoids treated with hCEP290-6 and-9 after 2- and 4-weeks treatment

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



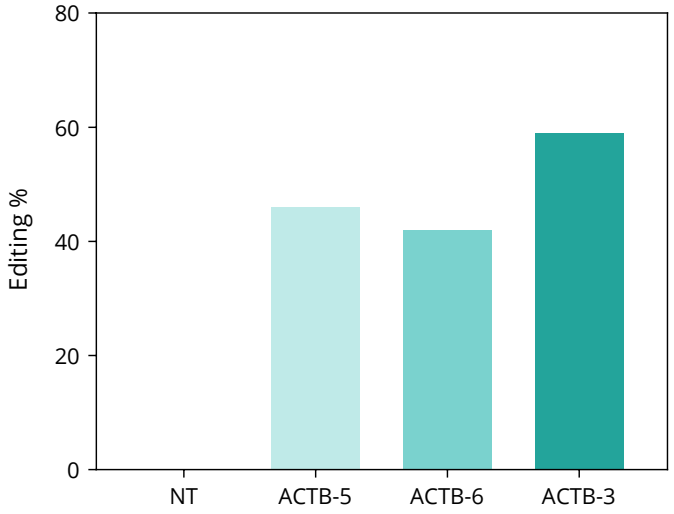
Axiomer®

Beyond IRDs

The liver as the next frontier

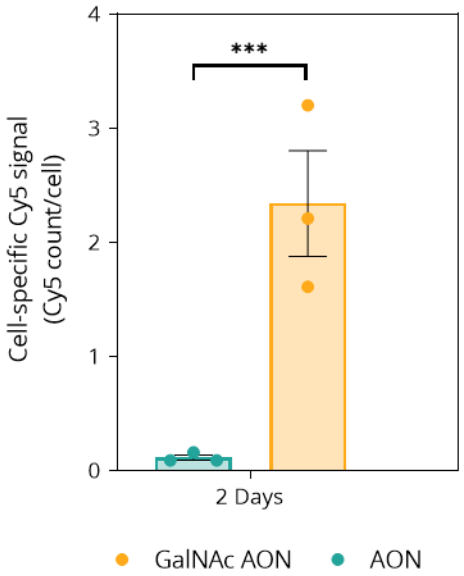
Targeted editing in liver is highly achievable

Editing of ACTB in human primary hepatocytes



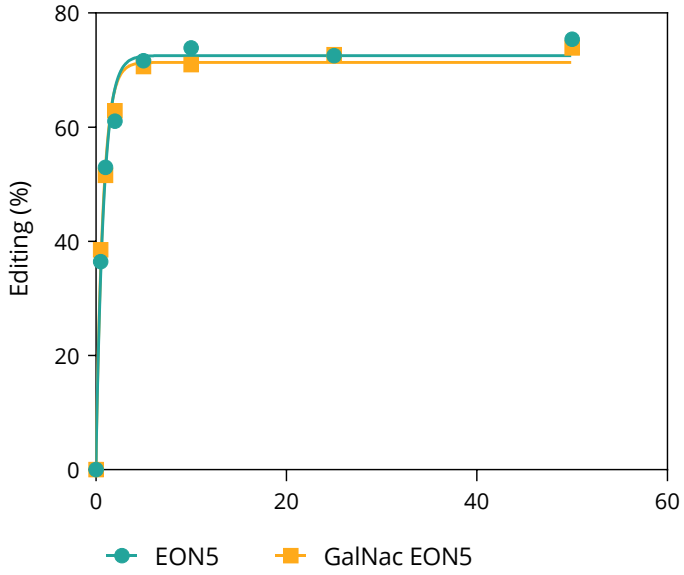
- Similar levels of editing of ACTB achieved in several models of liver origin
- High confidence of translatability of the approach

Targeting liver hepatocytes using GalNac conjugates



Selection of efficient GalNac conjugate targeting hepatocytes for liver targeting

A-to-I editing with GalNac conjugates in vitro



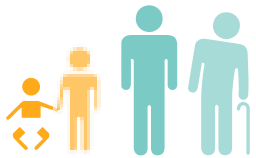
GalNAc appears not to interfere with ADAR binding or efficient RNA editing

Alpha-1-antitrypsin deficiency

First-in-class safe and unique approach restoring AAT protein function, targeting both liver and lung disease in A1AD patients

Liver & Lung disease

Inherited metabolic disease caused by a mutation in the SERPINA1 gene, primarily expressed in the liver. Mutated AAT accumulates in the liver and causes **liver cirrhosis**. Reduced AAT levels in the lung cause **respiratory failure**.

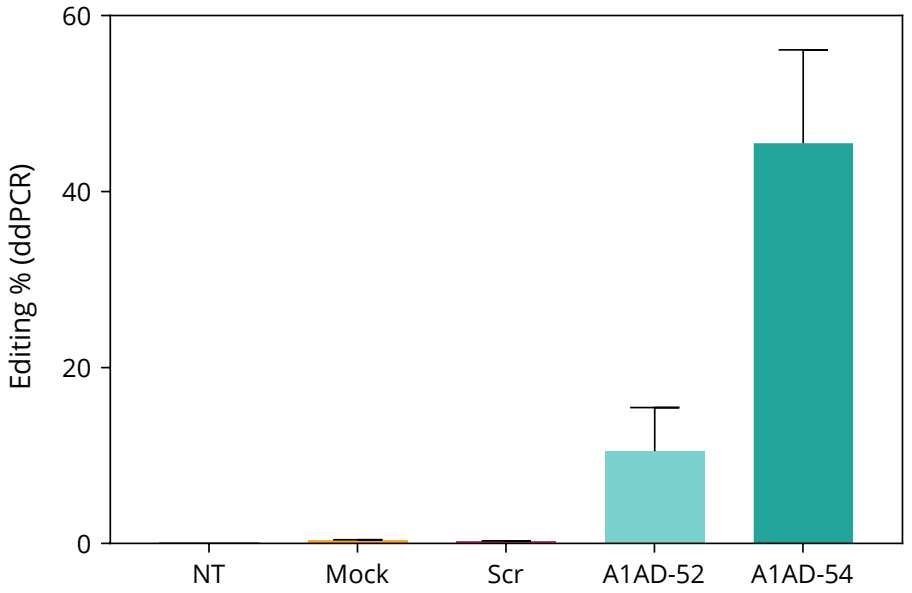


First symptoms at 20-50 years and more severe when patients have smoked.

Patients homozygous for **c.1096G>A (E366K)** in *SERPINA1* are at high risk for severe lung and liver disease.

There are **~130.000 patients** with this genotype in the Western world and more

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



Next steps Axiomer® platform

In house strategy

- Expand investments in Axiomer® platform, pipeline development and target selection activities
- Expect to present further non-clinical data updates throughout 2022
- Planning to announce internal development targets in H2 2022
  - Develop *in vivo* PoC in multiple programs with initial focus on Liver, CNS and ophthalmology
  - First IND expected in 18-24 months
  - Development of additional Therapeutic Areas in parallel

Partnership strategy

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

ProQR®

IT'S IN OUR RNA