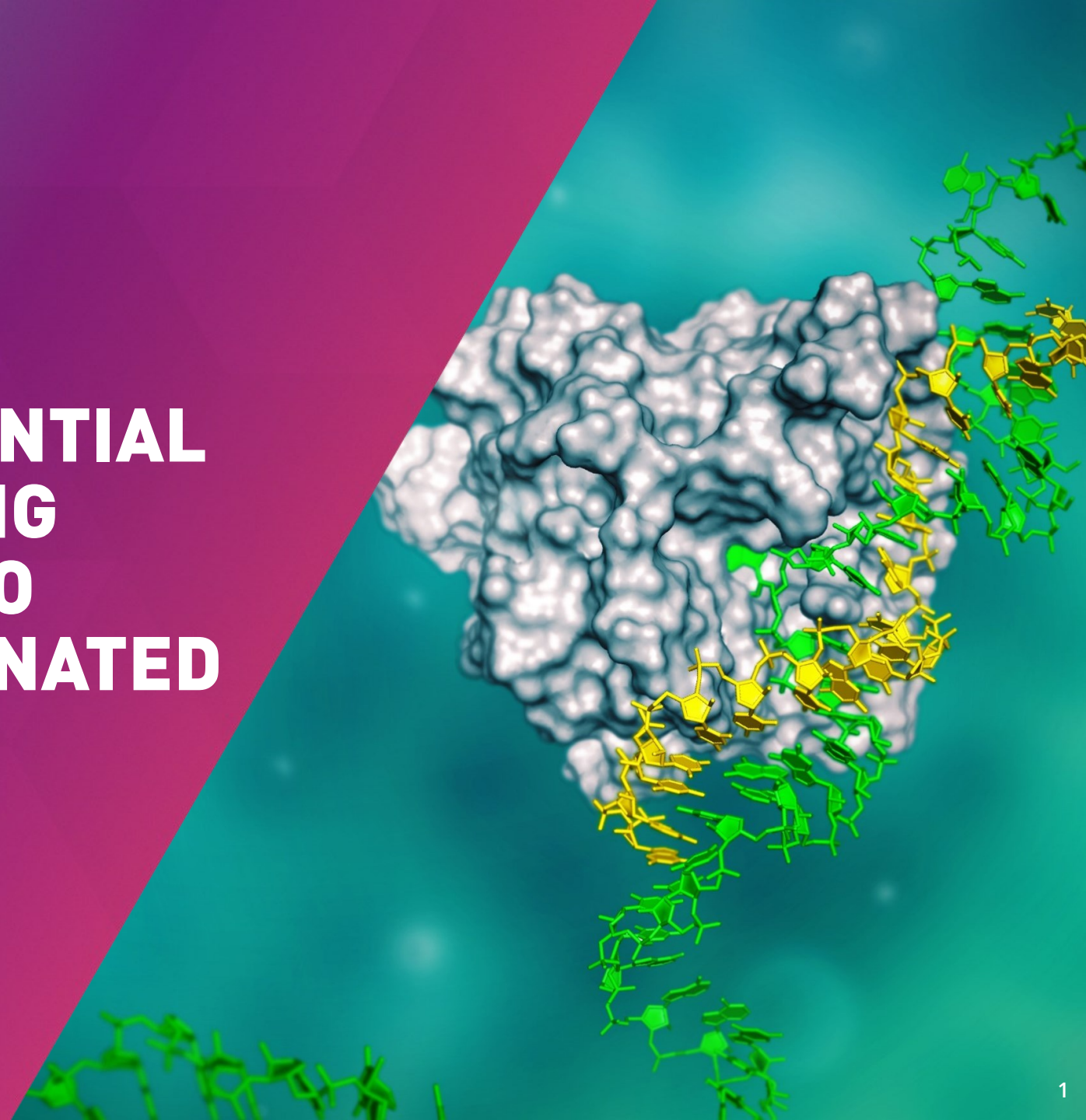
# UNLOCKING THE POTENTIAL OF INNOVATIVE EDITING OLIGONUCLEOTIDES TO ADDRESS LIVER ORIGINATED DISORDERS

*Gerard Platenburg, CSO and co-founder*
*ProQR Therapeutics*

RNA Editing Gordon Research Conference
Thursday, March 23

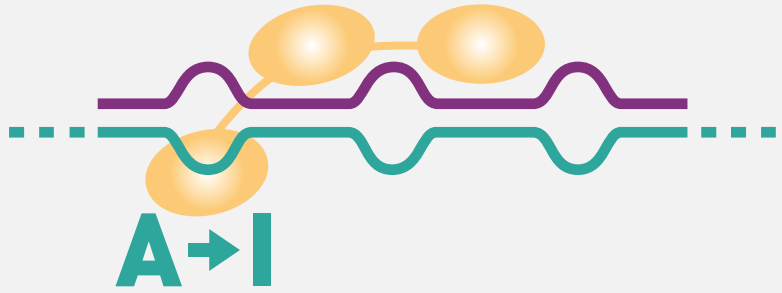


# Forward looking statements

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.

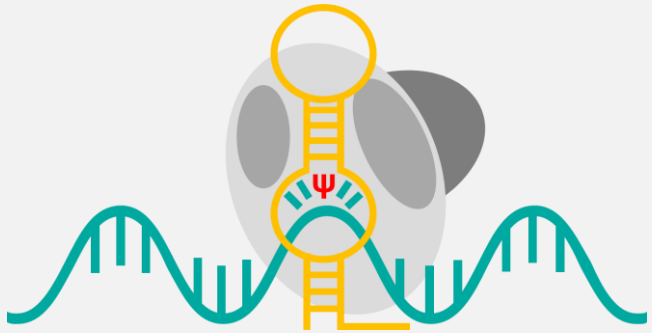
# RNA toolbox – editing platform technologies

*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

# 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)

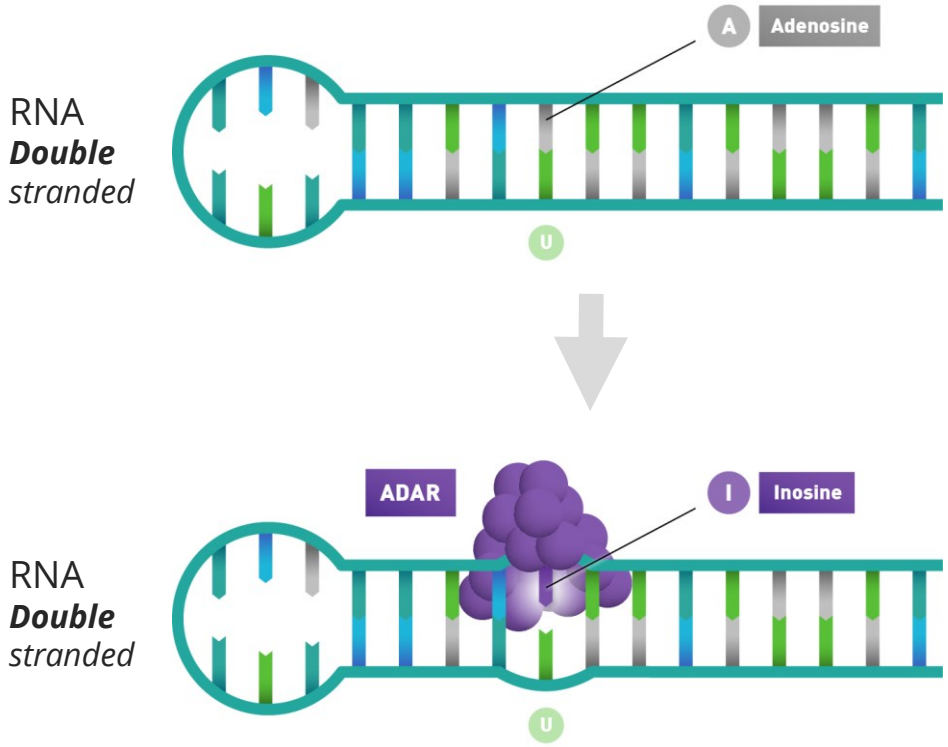
### Two pillars underly strategy

- 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

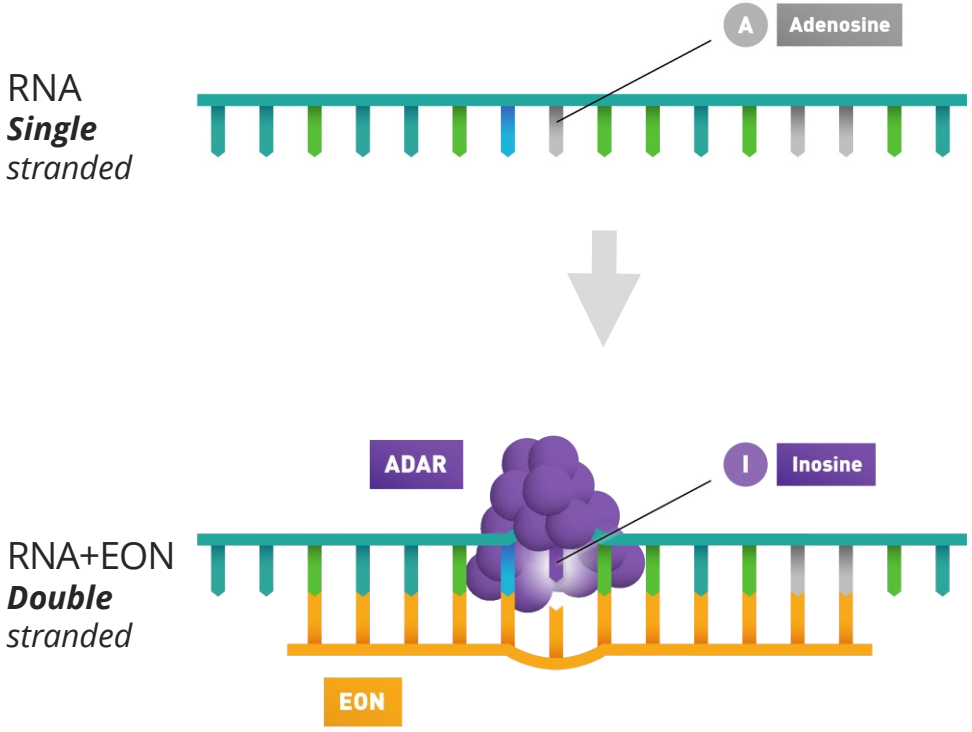
# Axiomer® EONs unlock cellular machinery potential to treat diseases

*By attracting ADAR and allowing highly specific editing*

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




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

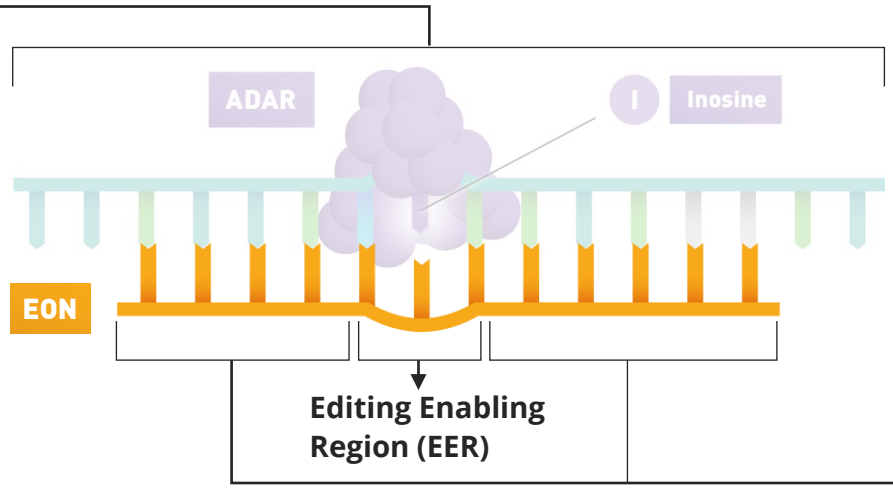



dsRBDs, double-stranded RNA binding domain

# Driving the development of optimized EONs for therapeutic use

**Sequence defines** target
RNA binding and provides stability

**EON total length**
Between ~25 - 30 nt



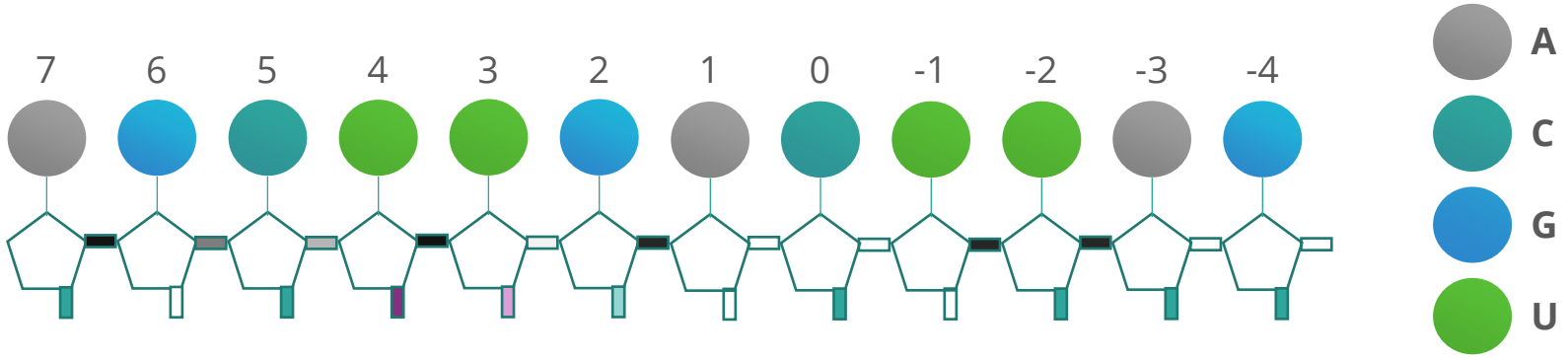

**ADAR-binding region (ABR)**
Backbone modifications enable ADAR binding, and disable off-target editing

## 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

# Accelerating programs advancement with focus on design principles

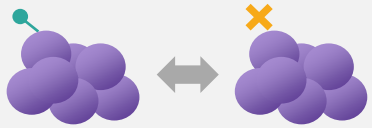
EON




| 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**

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

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

# Establishing a strong platform in the liver in multiple targets and models

**Liver**

Targeting liver originated diseases

**GalNAc**

Optimizing liver delivery

**Cell models**

**Liver organoids**

**Mice *in vivo***

**Model target**

**PoC therapeutic targets**

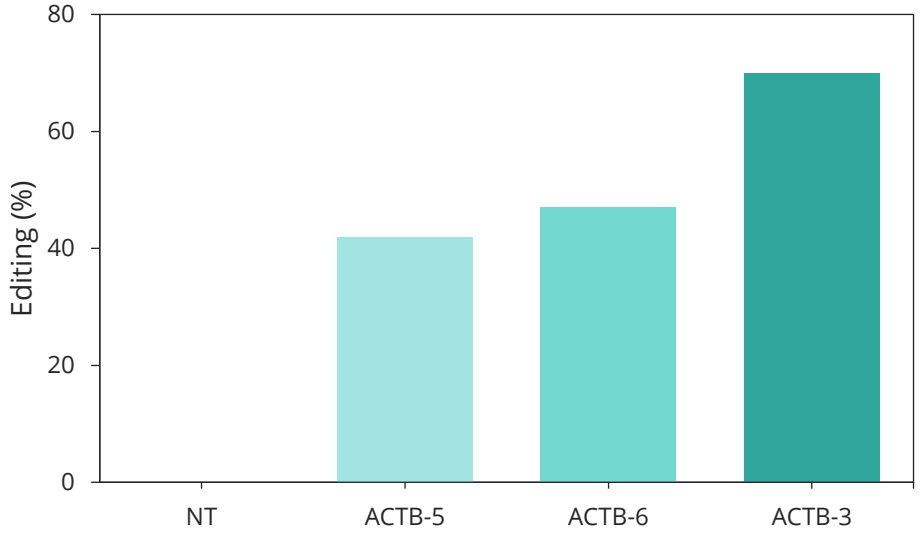
Tool targets used for optimization

**Pipeline targets**

# Up to 70% editing in human primary hepatocytes

**Editing of *ACTB* in human primary hepatocytes**

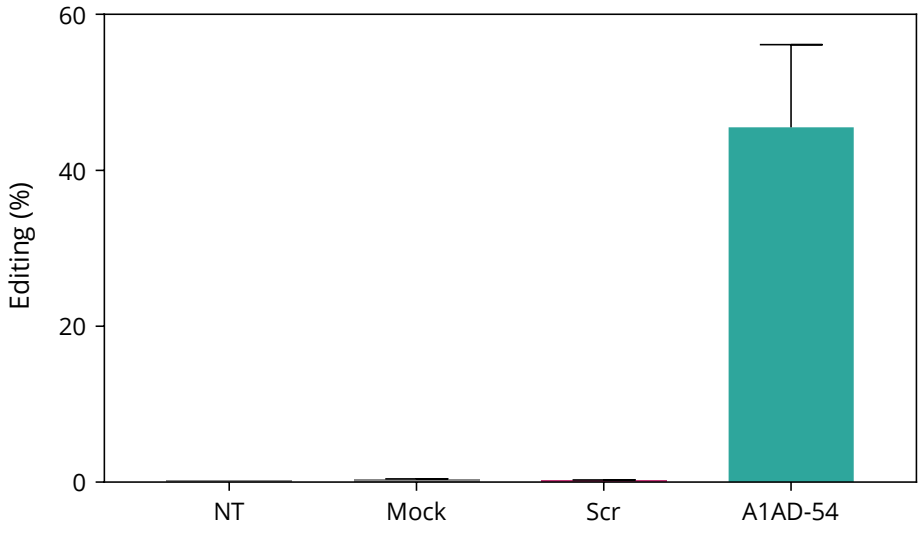
*Gymnosis, 10µM, single dose, N=1, 48 hours, dPCR*




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

**Editing of *SERPINA1* E366K in human A1AD patient hepatocytes**

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



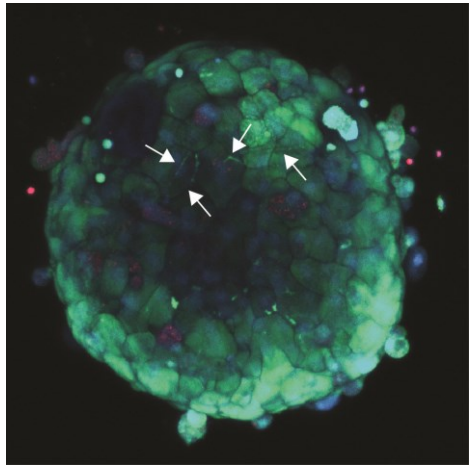

>50% Editing of *SERPINA1* E366K in human A1AD patient hepatocytes

# Editing in InSphero Human Liver microtissues (LMTs)

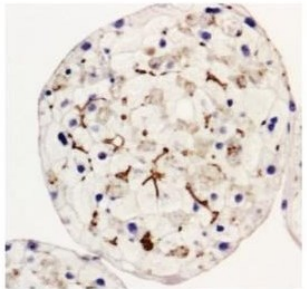
*Primary hepatocytes, Kupffer cells and liver endothelial cells in 3D spheroid*

### Live imaging of LMT

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



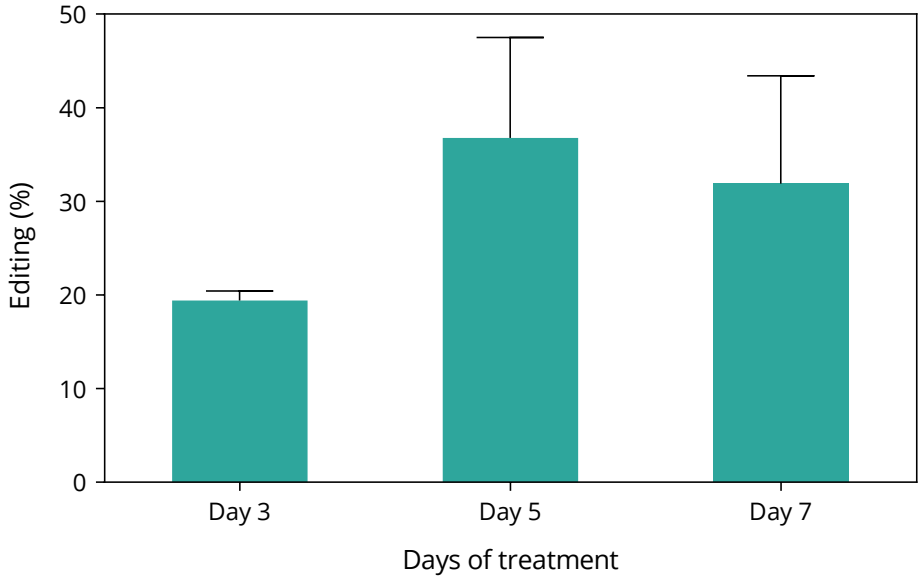
**BSEP** Bile Canaliculi (InSphero data)



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, 5µM, single dose, 3 pools of 6 LMTs per condition, 7 days, dPCR, mean, SD*



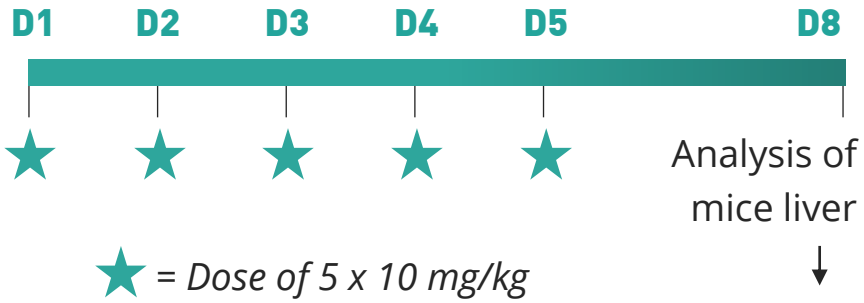

Treatment of LMTs with 5µM EON for 7 days results in up to ~50% of edited *ACTB*

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

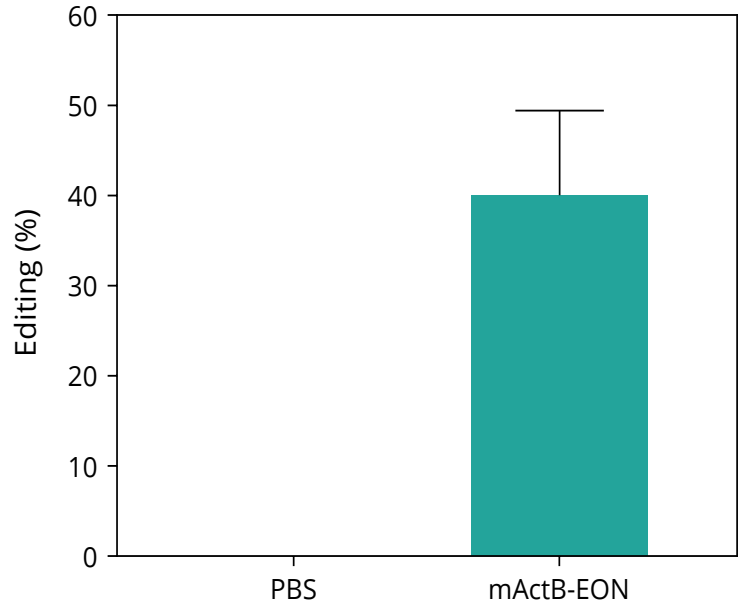
**Mice treatment**

*In vivo*




**RNA editing of *ActB* in liver of mice**

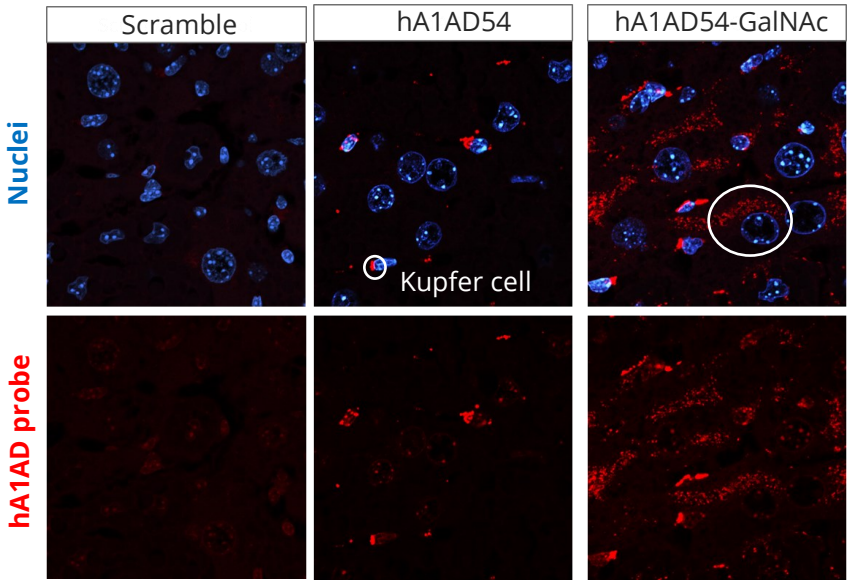
*SC, 5 daily doses of 10 mg/kg, N=4, 1 week, ddPCR, mean, SD*



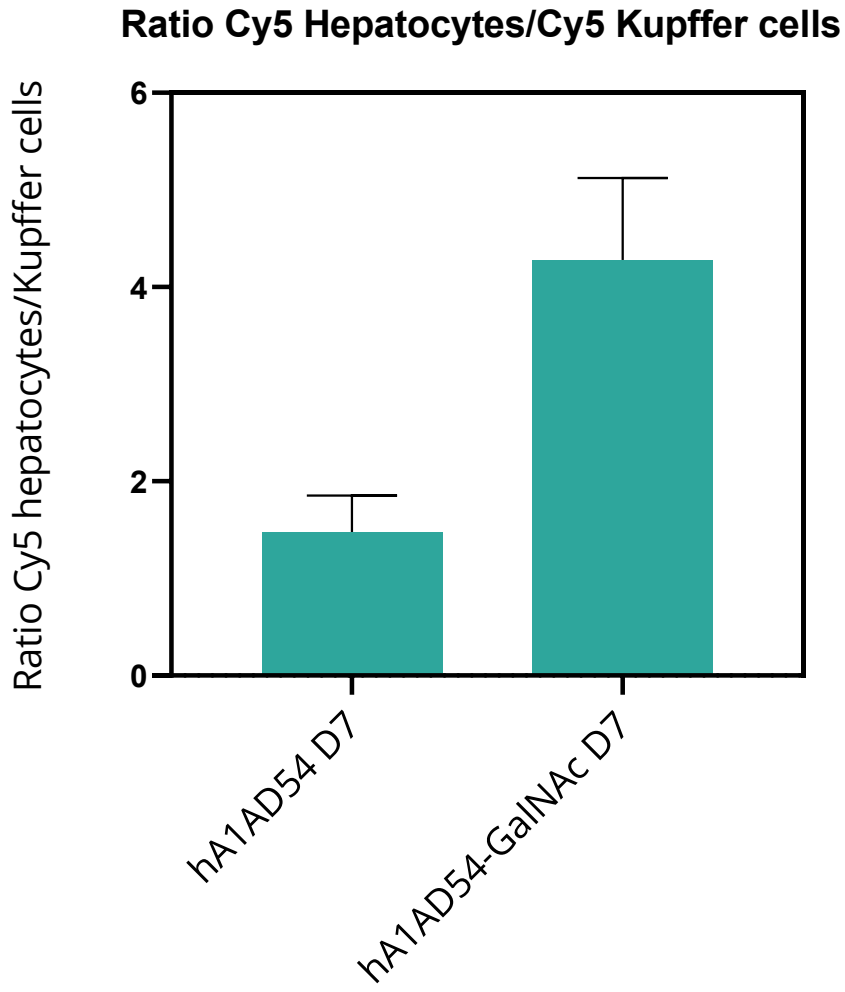
# Hepatocytes uptake of EONs explored by FISH *in vivo;* unconjugated vs GalNAc conjugate

**FFPE liver tissue sections of EON-treated NSG-PiZ mice *in vivo***

*SC, 3x 10 mg/kg on Day 0, 2 and 4, N=4, 7 days, FISH*



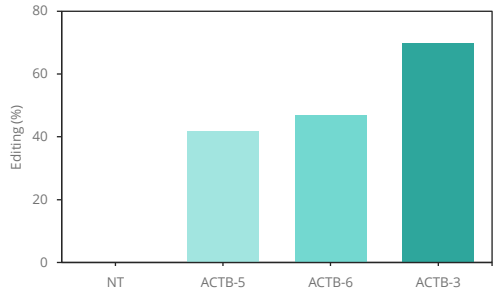

FISH signal detected in Kupffer cells
After 7 days, hA1AD54-GN-derived FISH signal mainly localized in hepatocytes



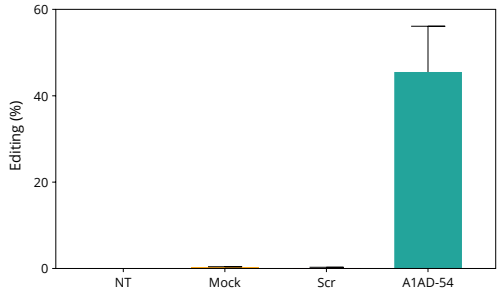
# Axiomer advancement in the liver, with consistent editing across models

## Cell models

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



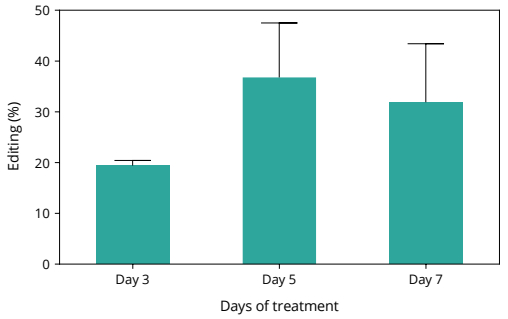

**>50% editing of *SERPINA1* E366K in human A1AD patient hepatocytes**




## Liver organoids

**Up to ~50% editing of *ACTB* in human LMTs**

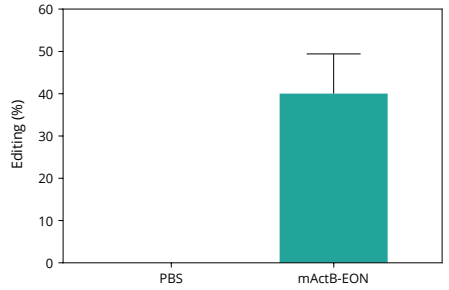
*Gymnosis, 5µM, single dose, 3 pools of 6 LMTs per condition, 7 days, dPCR, mean, SD*




## 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*

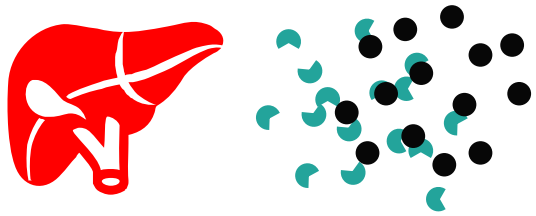



Conditions of *ACTB* editing experiment in human primary hepatocytes experiment: gymnosis, 10uM, single dose, N=1, 48 hours, dPCR; Conditions of the *of SERPINA1 editing experiment* in human A1AD patient hepatocytes experiment: transfection, 100 nM, single dose, N=2, 47 hours, dPCR, mean, SD. LMTs: Liver microtissues

# Liver targeted editing of *PCSK9* involved in hypercholesterolemia

## *Generation of a loss-of-function variant to lower PCSK9*

**FEH patients**



↑ PCSK9 ● ↑ LDL


**Axiomer® ® edit**

↓ PCSK9 ● ↓ LDL

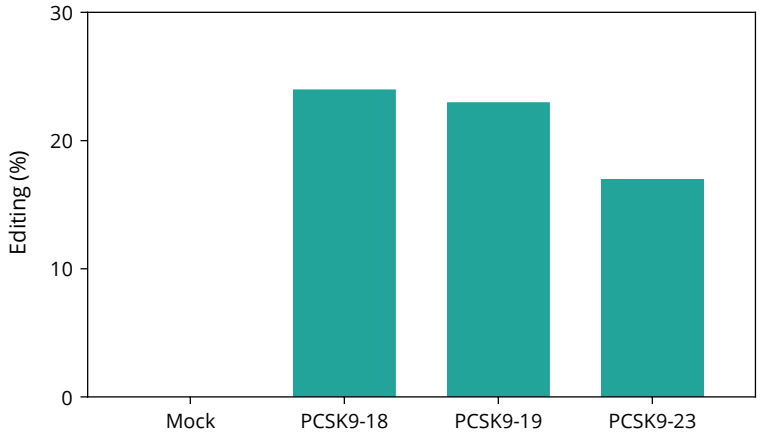

**Disruption of PCSK9 autocleavage site reduces protein in bloodstream**

- 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

# Editing of PCSK9 mRNA results in a loss-of-function phenotype and reduces protein levels

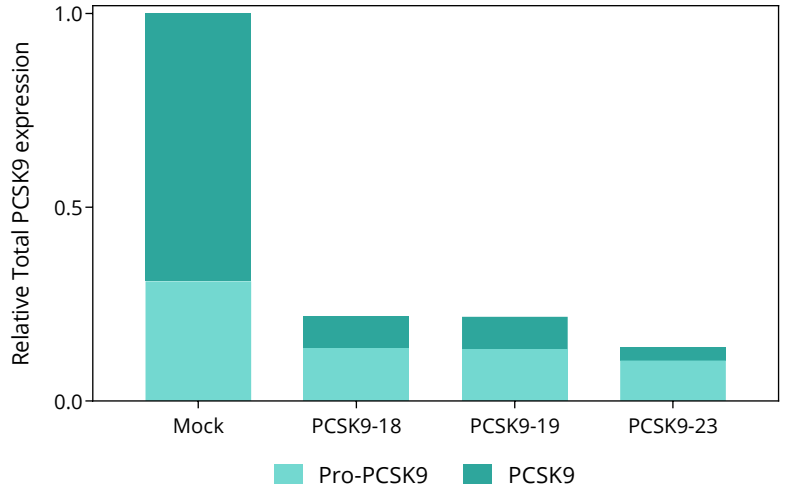
### Editing of *PCSK9* in HeLa cells

*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%

# PoC data of Axiomer in the liver, including in *in vivo* model

- Consistent RNA editing reported in all liver models assessed
- Up to 50% editing reported in the liver of mice *in vivo*
- Increased hepatocyte uptake *in vivo* with GalNAc conjugate
- *In vitro* proof of concept of a new modality generating a loss-of-function variant

# 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

Art Levin
*PhD*

Peter A. Beal
*PhD*

Phillip D. Zamore
*PhD*

Yi-Tao Yu
*PhD*

Martin Maier
*PhD*

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