AXIOMER™, AN RNA EDITING TECHNOLOGY

Building confidence towards clinical development

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Deaminet, January 18, 2024

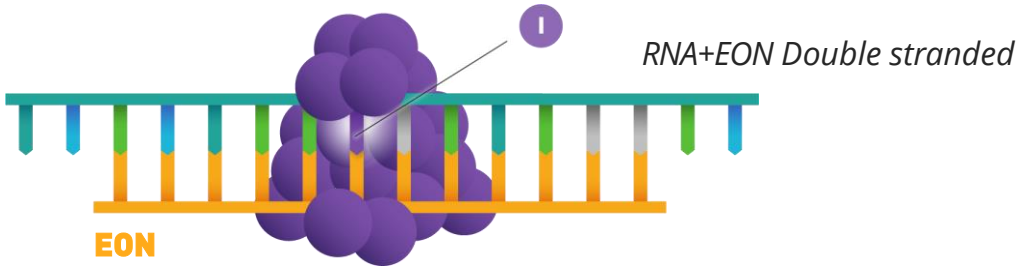
Forward-looking statements

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 preclinical model data, our pipeline targets, 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

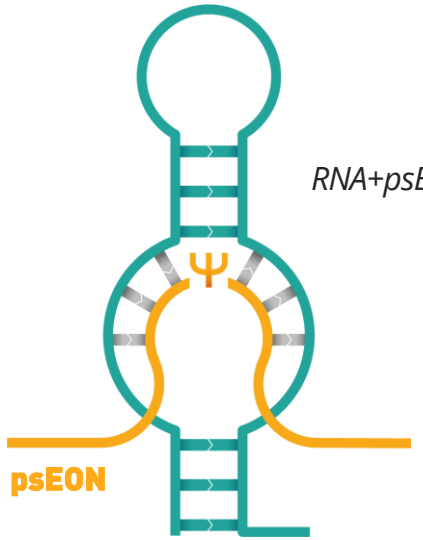
Axiomer™ A-to-I editing



RNA+EON Double stranded

- 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
- Broad applicability to various diseases

Trident U-to-Ψ editing



RNA+psEON Double stranded

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

Axiomer™ technology

Leading the science to expand the potential of RNA editing

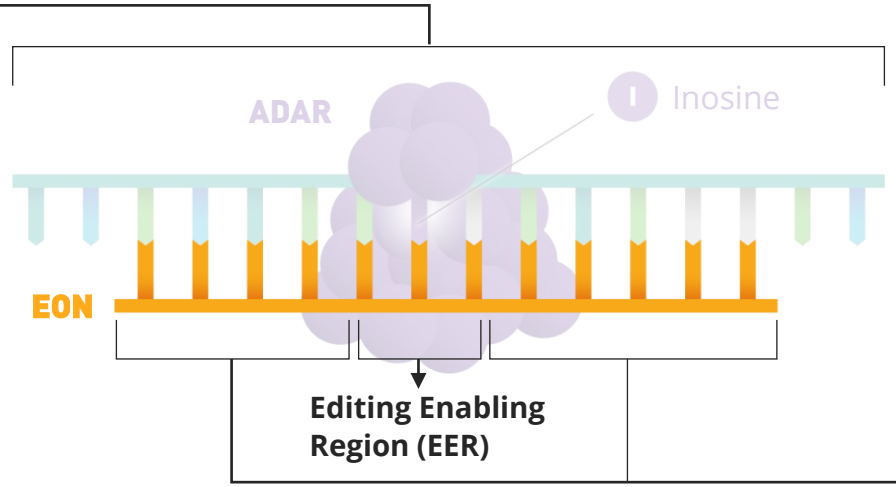
- **Increased knowledge of ADAR biology**
- **Selection of models for prediction of potential in human tissue**
- **EON sequence optimization**
- **Broad applicability**
- **Robust editing capability**
- **Safety profile**

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

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

Establishing a platform with strong translatability across models

Liver

Targeting liver-originated diseases

Nervous system

Targeting CNS and PNS

Cell models

Organoids

Mice *in vivo*

NHP *in vivo*

Model target

PoC therapeutic targets

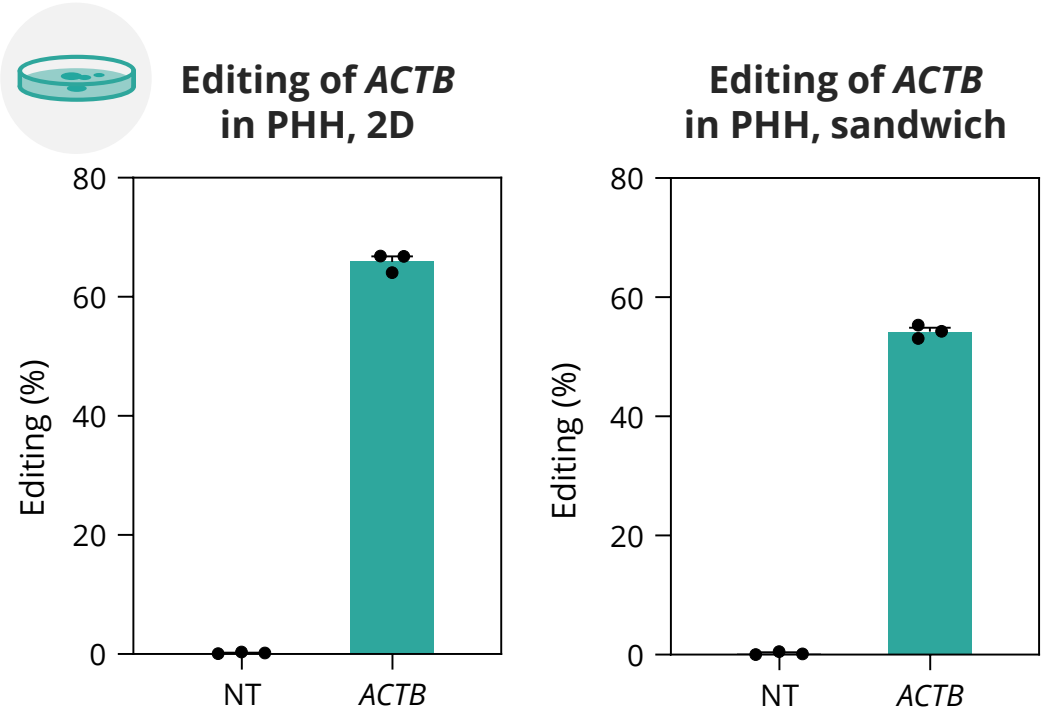
Tool targets used for optimization

Pipeline targets

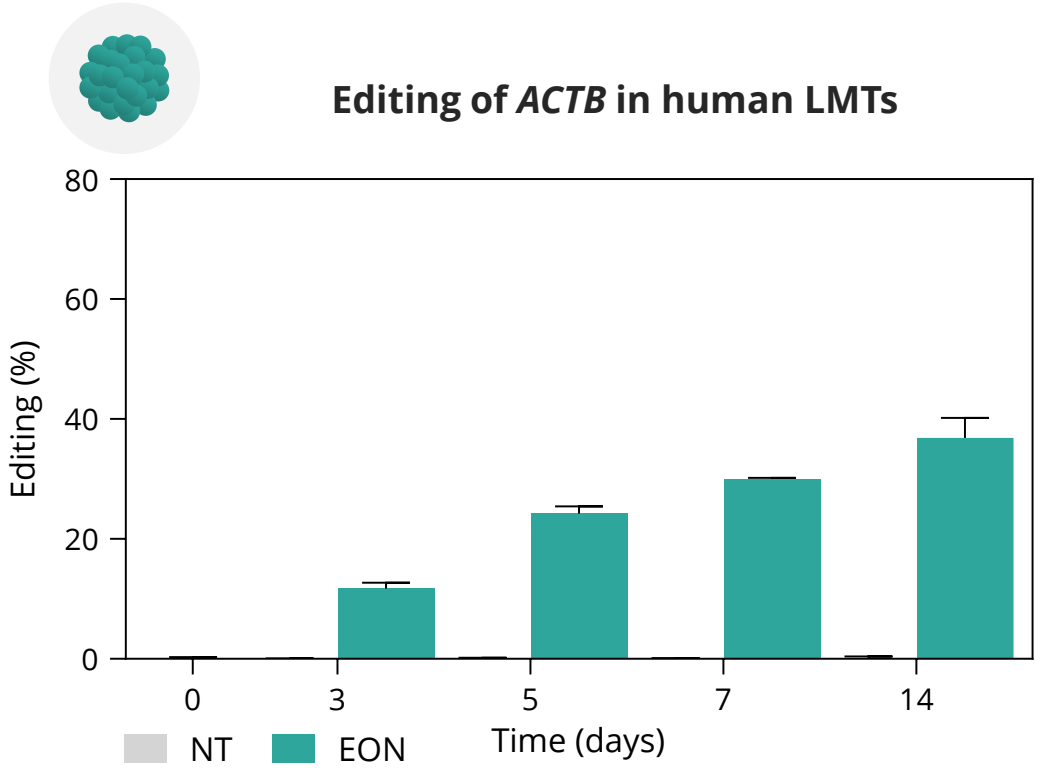
CNS: Central nervous system, NHP: Non-human primate, PNS: peripheral nervous system

Exploring intrinsic editing capacity *in vitro*

Cultured as 2D Primary Human Hepatocytes or Liver Micro Tissues



Editing of ACTB in Primary Human Hepatocytes cultured in 2D format reached 70% and 55% in PHH cultured in sandwich format



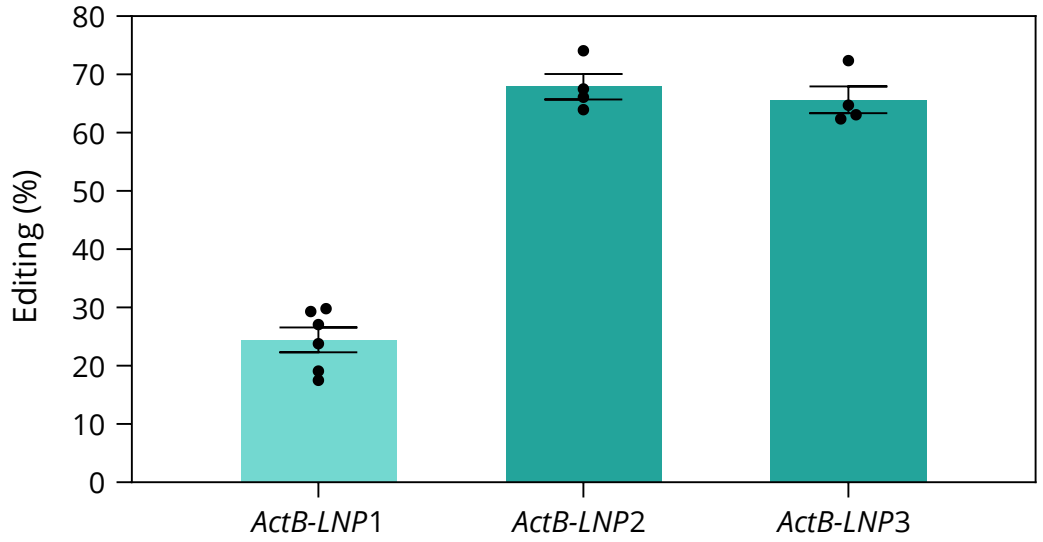
Treatment of Liver Micro Tissues with 1µM EON for 14 days resulted in up to 40% RNA editing of *ACTB*

PHH: Primary Human Hepatocyte; LMT: Liver Micro Tissue. Editing of ACTB in PHH, 2D Gymnosis, 5µM, single dose, n=1 with triplicates, 72 hours, dPCR, mean, SD; Editing of ACTB in PHH, sandwich Gymnosis, 5µM, single dose, n=1 with triplicates, 72 hours, dPCR, mean, SD; Editing of ACTB in human LMTs Gymnosis, 1µM, constant dose, 3 pools of 24 LMTs per condition, 14 days, dPCR, mean, SD

Exploring intrinsic editing capability of Axiomer™ EONs in liver *in vivo*

Editing of *ActB* in mice *in vivo*

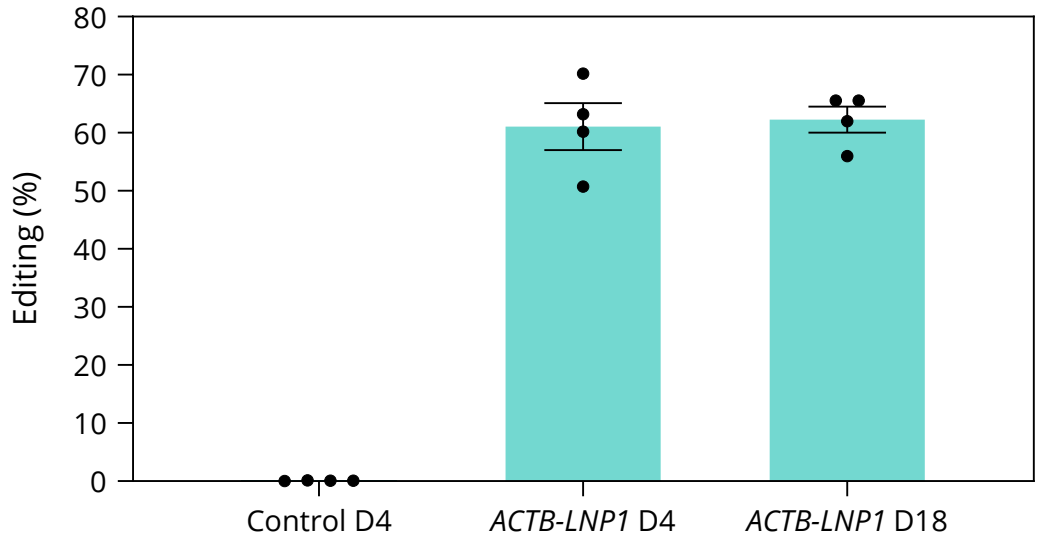
IV, 3mg/kg or 4mg/kg, LNP formulations, N=4-6, D7 data, dPCR, AVG±SEM



- Strong editing efficiency with ActB EON
- Up to **74% editing** reported in the liver of mice at D7
- High intrinsic editing capability of Axiomer EONs in the liver

Editing of *ACTB* in NHP *in vivo*

IV, 2mg/kg, 3 doses at D1, D8 and D15, LNP formulation, n=4, D4 and D18 data, dPCR, mean±SEM



- Editing efficiency **up to 70%** reported in NHP in vivo
- An **average of 61% and 62% editing** efficiency was observed at D4 and D18 respectively
- Robust and consistent editing level reported in mice and NHP *in vivo*

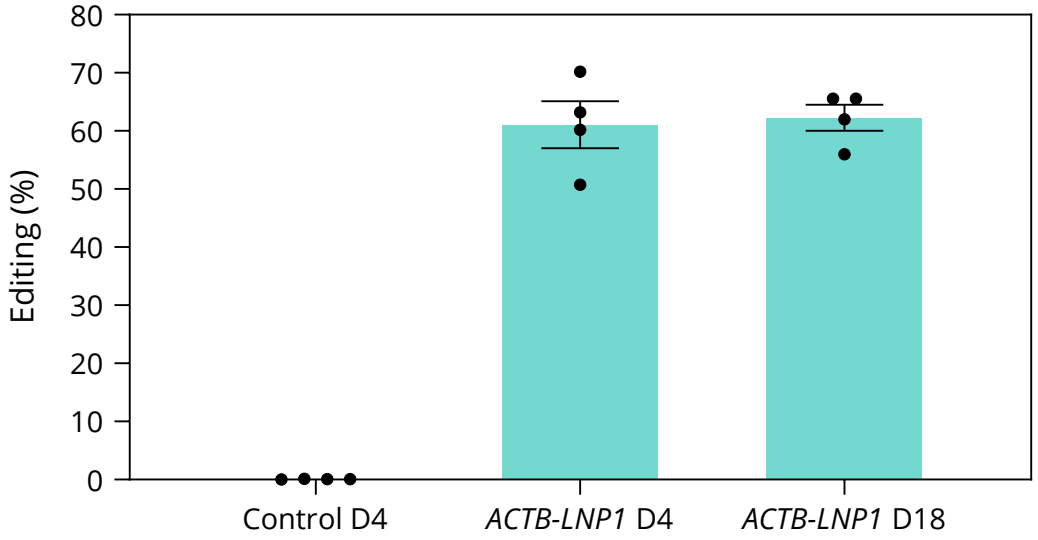
AVG: average; EON: Editing Oligonucleotide; IV: Intravenous; LNP: Lipid Nanoparticle; NHP: Non-human primate; SEM: standard error of the mean

Editing capabilities confirmed across RNA analysis methods

dPCR and RNAseq showing consistent results

Editing of *ACTB* in NHP *in vivo - dPCR*

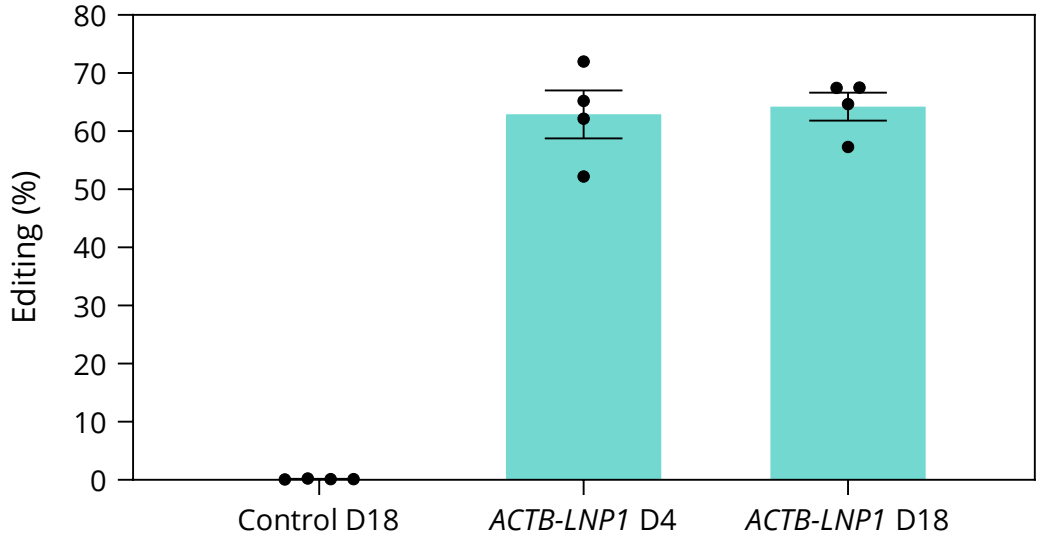
IV, 2mg/kg, 3 doses at D1, D8 and D15, LNP formulation, n=4, D4 and D18 data, dPCR, mean±SEM



- Editing efficiency **up to 70%** reported in NHP in vivo
- An **average of 61% and 62% editing** efficiency was observed at D4 and D18 respectively

Editing of *ACTB* in NHP *in vivo – RNAseq*

IV, 2mg/kg, 3 doses at D1, D8 and D15, LNP formulation, n=4, D4 and D18 data, RNAseq, mean±SEM



- Editing efficiency **up to 72%** reported in NHP in vivo
- An **average of 63% and 64% editing** efficiency was observed at D4 and D18 respectively

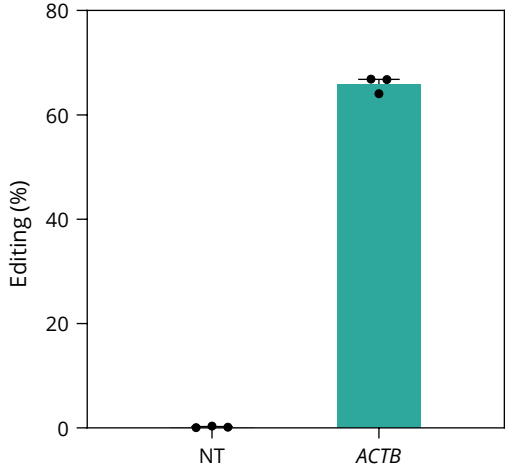
EON: Editing Oligonucleotide; NHP: Non-human primate

High intrinsic editing capability of Axiomer™ in the liver across models

Cell models

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

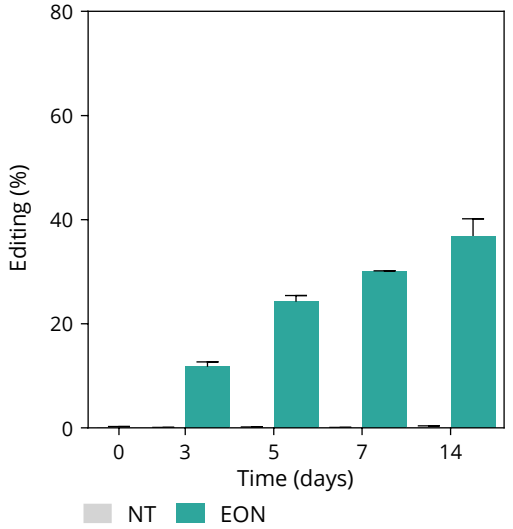
Gymnosis, 5µM, single dose, n=1 with triplicates, 72 hours, dPCR, mean, SD



Organoids

Up to 40% Editing of *ACTB* in human LMTs

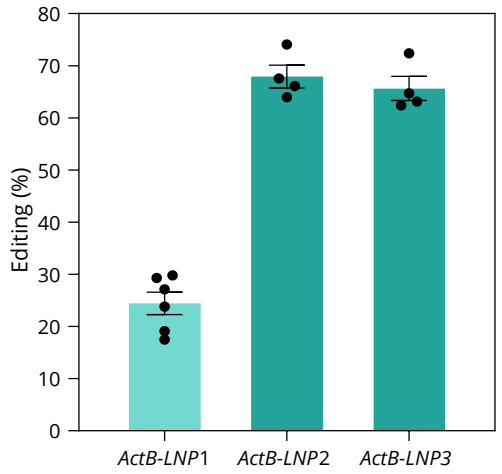
Gymnosis, 1µM, constant dose, 3 pools of 24 LMTs per condition, 14 days, dPCR, mean, SD



Mice *in vivo*

Up to 70% editing of *ActB* in liver

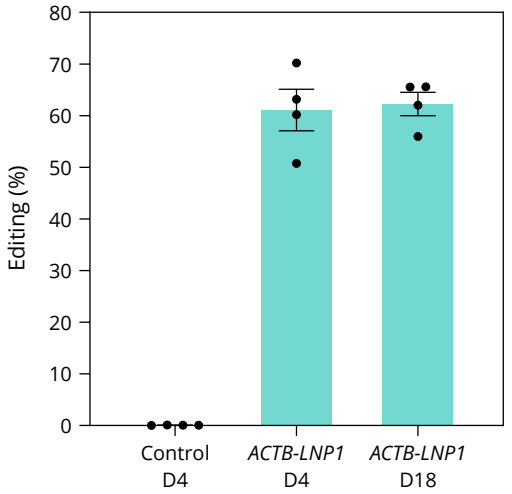
IV, 3mg/kg or 4mg/kg, N=4-6, LNP formulations, D7 data, dPCR, AVG±SEM



NHP *in vivo*

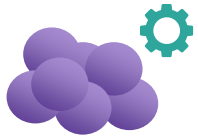
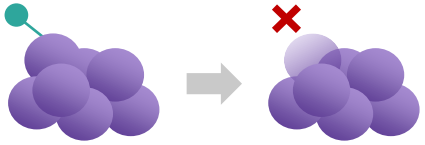
Up to 70% editing of *ACTB* in NHP

IV, 2mg/kg, 3 doses at D1, D8 and D15, LNP formulation, n=4, D4 and D18 data, dPCR, mean±SEM



PHH: Primary Human Hepatocyte; LMT: Liver Micro Tissue; NHP: Non-human primate

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

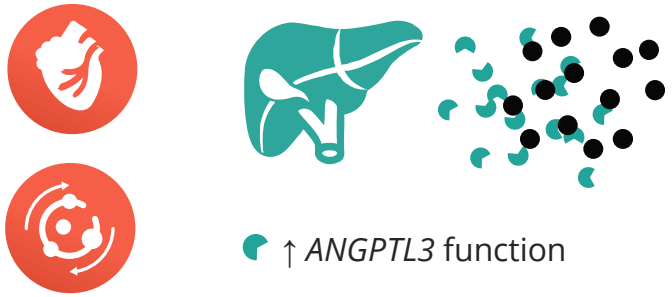
BROAD THERAPEUTIC POTENTIAL

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

PTMs: Post-translational modifications.

Axiomer™ can generate an ANGPTL3 variant reported to have positive impact on CVD risk

Individuals with increased CVD risk



Axiomer™ edit

***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 K63E variant of *ANGPTL3*

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

CVD; cardiovascular disease. LDL: low density lipoprotein.; LPL: Lipoprotein lipase. 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.

Changing a ligand binding site in *ANGPLT3* with Axiomer™ leads to LPL activation

The K63E variant result in significant change in local electrostatic properties

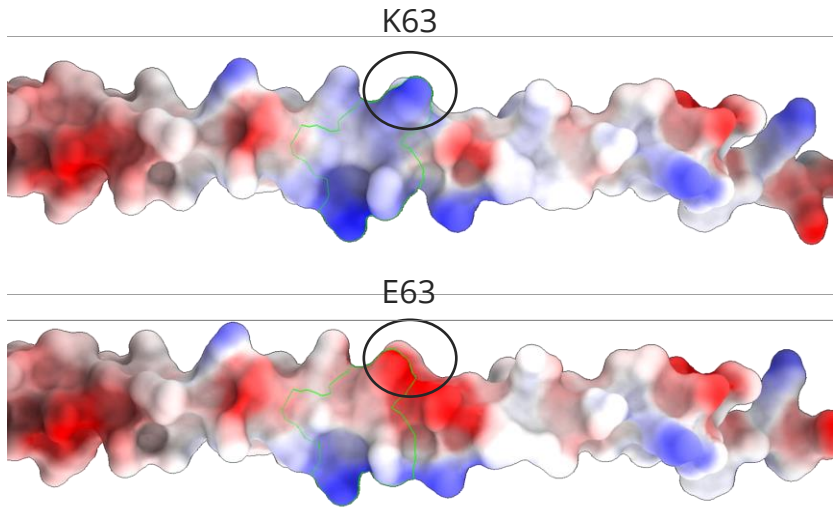
Wildtype *ANGPTL3*

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

Edited *ANGPTL3*

AAAGACTTTGTCCATGAGACGAAGGGCCAAATTAAT
-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)

- At position 63, lysine (K) has a long, flexible side chain, which is replaced by the shorter, negatively charged side chain of glutamic acid (E).

Heparin binding was shown to be essential for proper _ANGPTL3_ function

- Disruption of the heparin binding site by introducing negative charge is highly likely to increase LPL activity, ultimately leading to lipid lowering in the serum

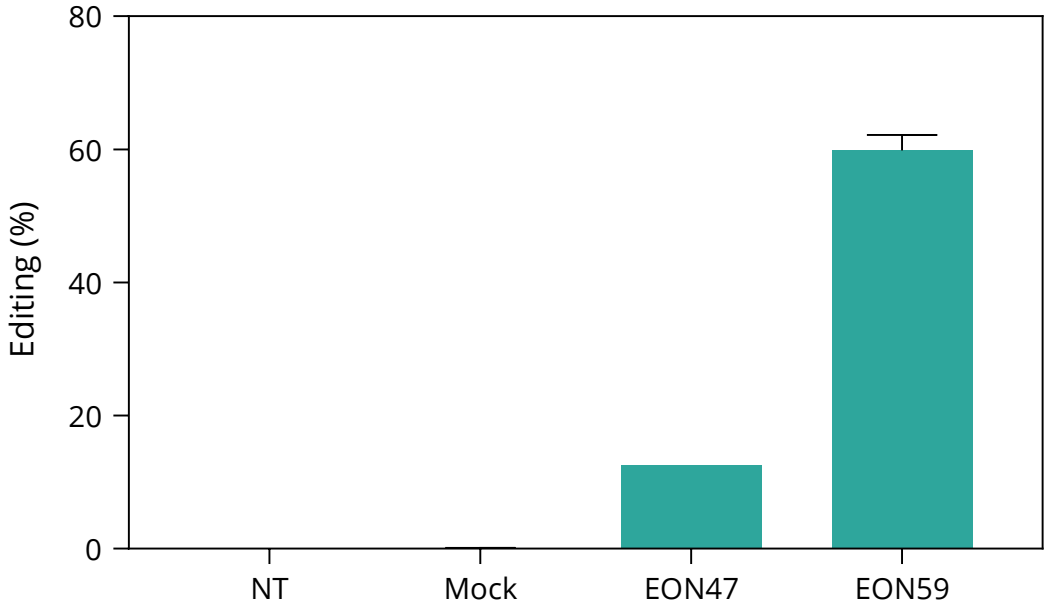
LPL: Lipoprotein lipase.

ANGPTL3 variant disrupting essential protein binding site

The K63E variant result in significant change in local electrostatic properties

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

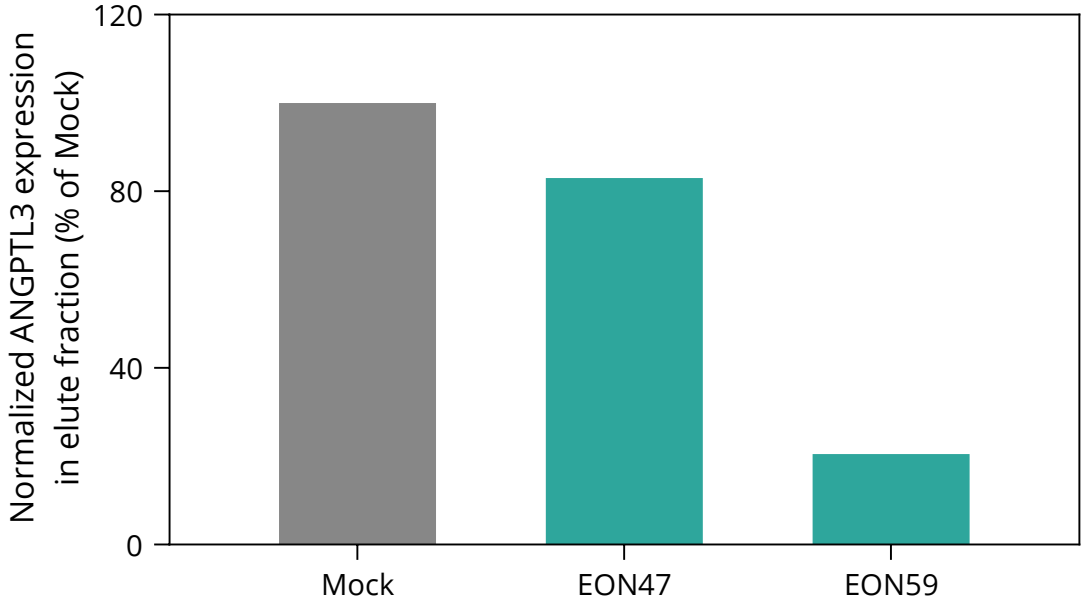
Gymnosis, 1µM, single dose, N=1 or 2, 5 days, dPCR, mean, SD



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



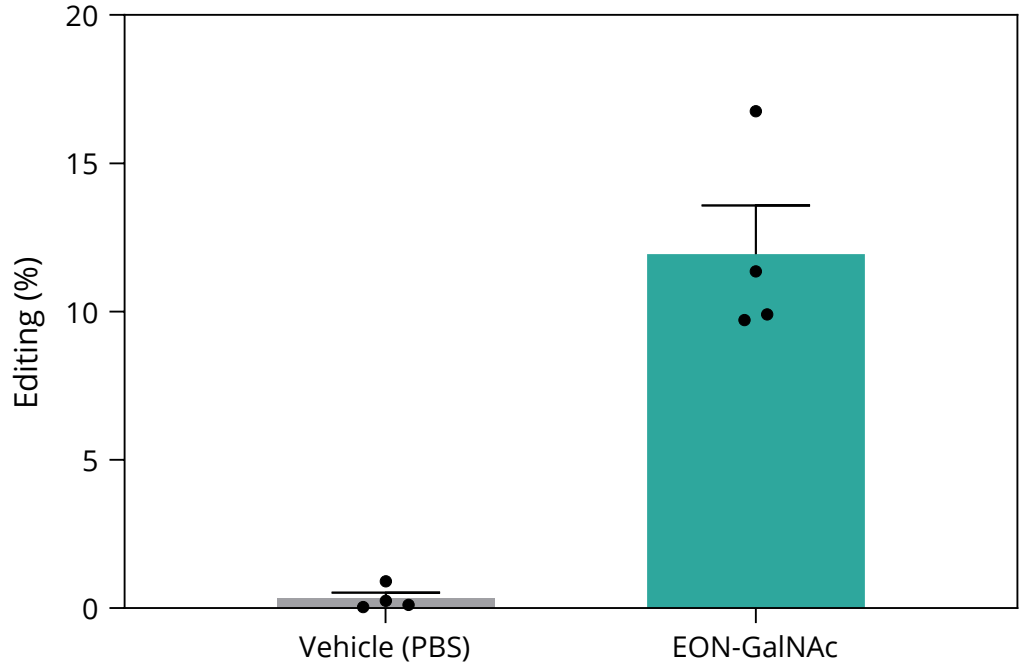
Up to 80% decrease in heparin binding in Huh-7 cells

SD: Standard deviation..

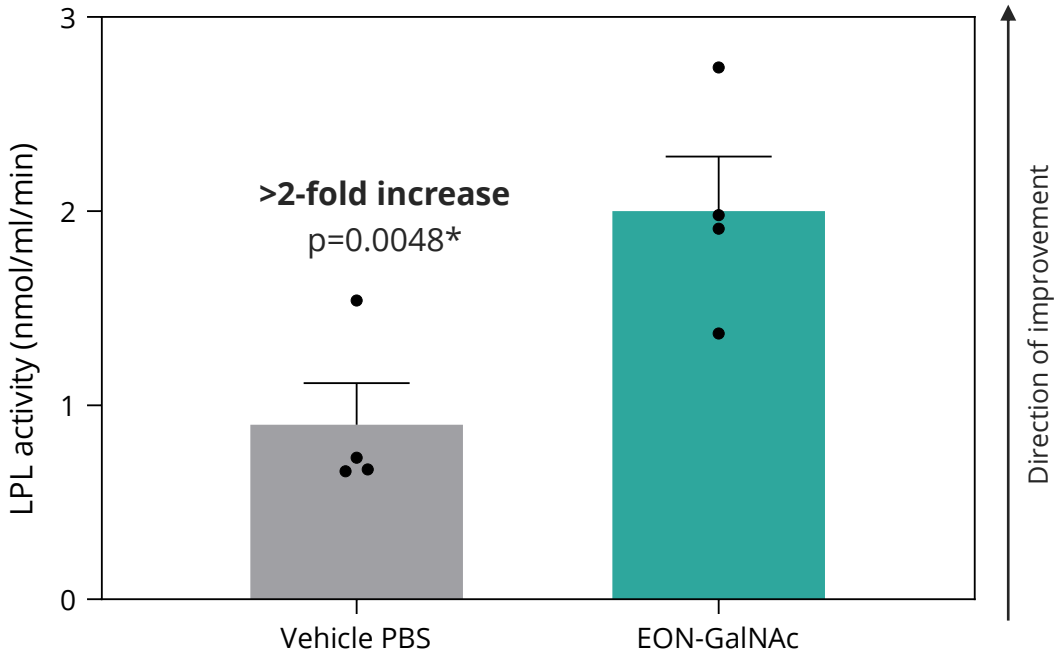
EON treatment led to an increase in LPL activity in liver of WT mice

Editing of *ANGPTL3* in mice *in vivo*

SC, 50mg/kg at D0, D2 and D4, N=4, D7 data, dPCR, AVG±SEM



Approx. 2-fold increase in LPL activity at D7



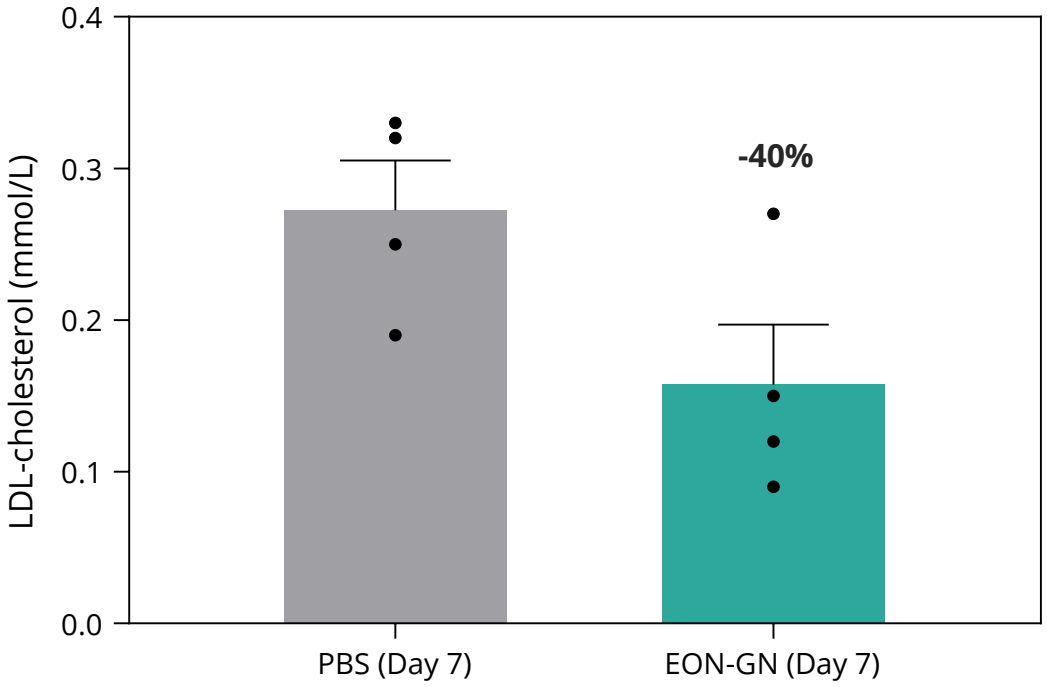
17% editing of *ANGPTL3* reported in this pilot study and approx. **2-fold increase in LPL activity** in WT mice *in vivo*

AVG: average; EON: Editing Oligonucleotide;; LPL: Lipoprotein lipase; SEM: standard error of the mean; WT: Wild Type. *Adjusted p-values from one-way ANOVA with Dunnett.

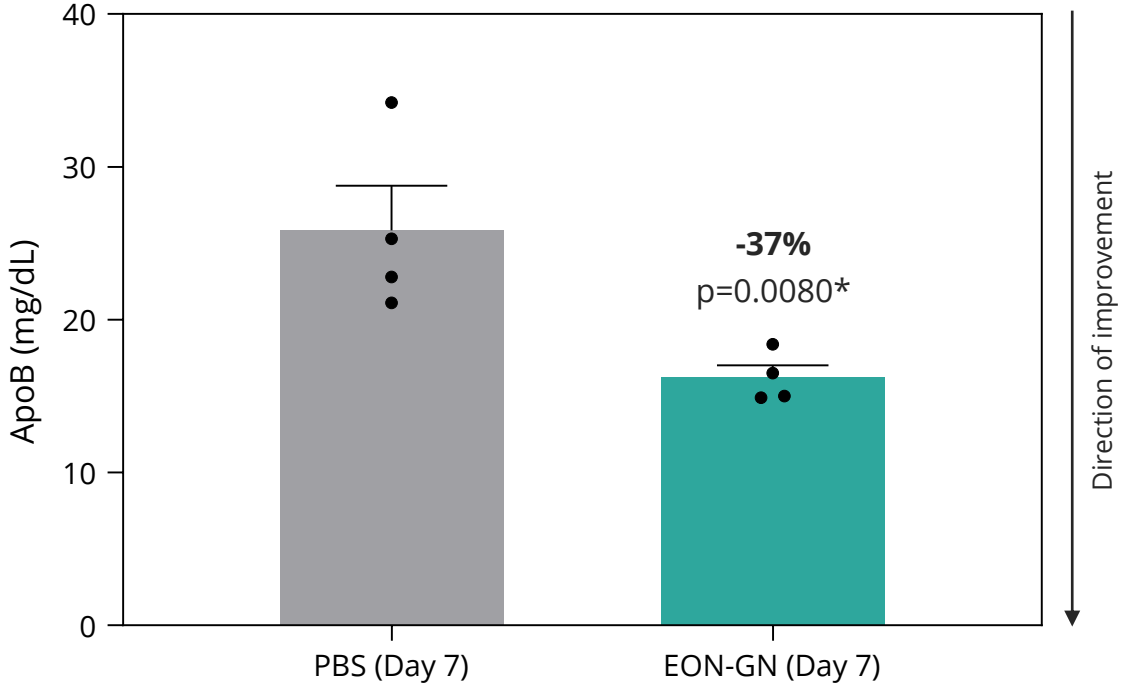
Positive impact on biomarkers observed

EON leading to a decrease in LDL-c and ApoB in a pilot study

Reported reduction of plasma LDL-c at D7



Reported reduction of plasma ApoB at D7



~40% and ~37% reduction in plasma LDL-c and ApoB, respectively, in WT mouse *in vivo*

ApoB: Apolipoprotein B; LDL-c:: Low-density lipoprotein cholesterol; WT: Wild type. *Adjusted p-values from one-way ANOVA with Dunnett.

Nonclinical safety assessment

No safety concerns upon unconjugated and GalNAc conjugated EONs

In vitro hepatotoxicity

- No clear hepatotoxic effects at the tested concentration
- Robust *in vitro* stability in nuclease assay (88%)

In vivo mice toxicity

- Multiple high dose (SC, 9x, 100 mg/kg) well tolerated with no signs of discomfort or changes in body-weights
- No relevant change in hematology parameters
- No relevant changes in clinical chemistry (ALT, AST and ALP within normal range) and histopathology

In vivo NHP toxicity

- Clinically and locally well tolerated following SC and IT dosing
- No relevant change in hematology & clinical chemistry parameters
- Typical ASO-class profile behavior & no red flags for EONs

Overall, both EONs (unconjugated and GalNAc conjugated) show a similar safety profile compared to other single-stranded RNA oligonucleotides

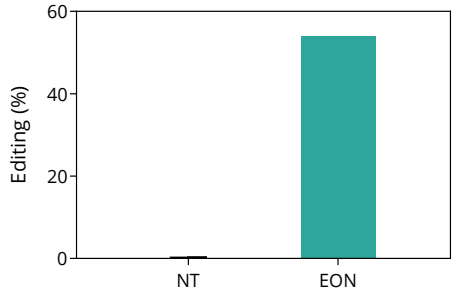
ALP: alkaline phosphatase; ALT: Alanine transaminase; ASO: Antisense oligonucleotide; AST: Aspartate Transferase ; EON: Editing oligonucleotide; IT: Intrathecal; SC: Subcutaneous;

Axiomer™ potential beyond liver

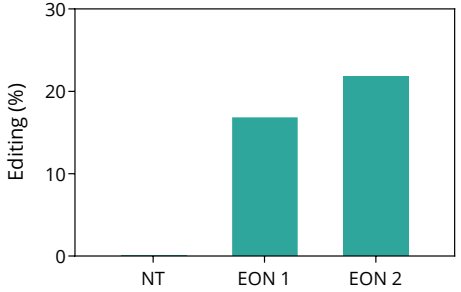
Strong editing in the nervous system across models

Cell models

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

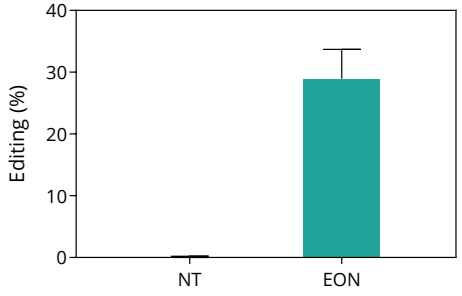


More than 20% RNA editing of *APP* in human iPSC derived neurons

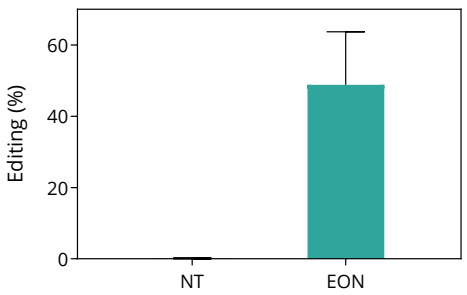


Organoids

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

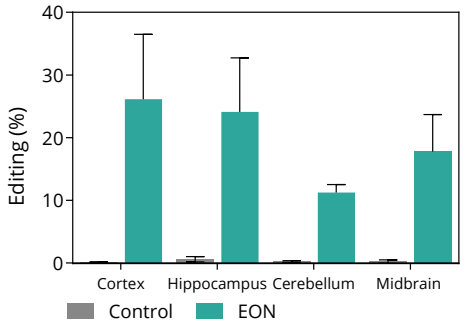


Up to 65% RNA editing of *APP* in cerebral organoids

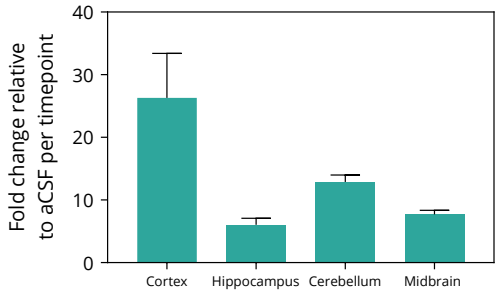


Mice *in vivo*

Up to 40% RNA editing in mice brain*

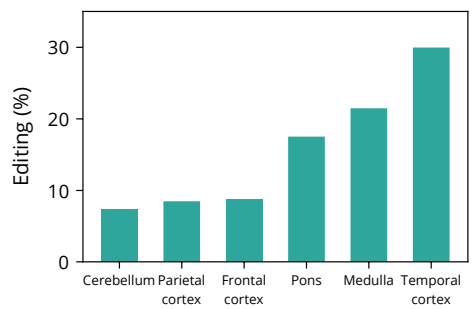


26-fold change in protein function in mice brain*

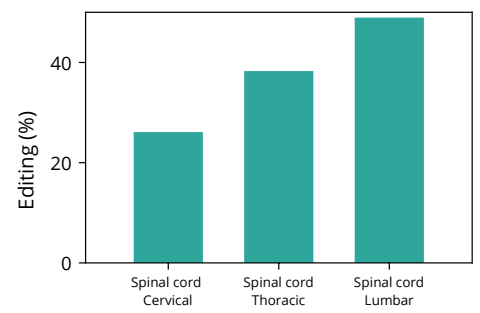


NHP *in vivo*

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=1, 2 weeks, dPCR and conditions of the *APP* iPSC derived neurons experiment: gymnosis, 10µM, single dose, washout, n=1, 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.

Summary

Building confidence towards clinical development

ProQR®

Axiomer™

Increased knowledge of ADAR biology

- Modification of the orphan base to optimize ADAR activity
- Collaborative work to address 5'GA context

EON sequence optimization

- Improvement in editing with linkage modifications
- Structure–activity relationship (SAR) assessment

Robust editing capability

- High intrinsic editing capability
- Editing reported in liver and CNS

Selection of models for prediction of potential in human tissue

- Strong and consistent editing reported in various models: *in vitro*, *mouse in vivo*, NHP *in vivo*
- Functionality reported in liver and CNS

Broad applicability

- Capacity to modulate protein functions beyond correction of G-to-A mutations
- Leading future direction for EON access to various cells and organs

Safety profile

- Favorable early safety profile
- Clinically and locally well tolerated *in vivo*, with no unexpected findings

ProQR®

IT'S IN OUR RNA