FROM CHEMICAL DESIGN OPTIMIZATION TO CLINICAL APPLICATION

Advancing ADAR RNA Editing in Liver and CNS

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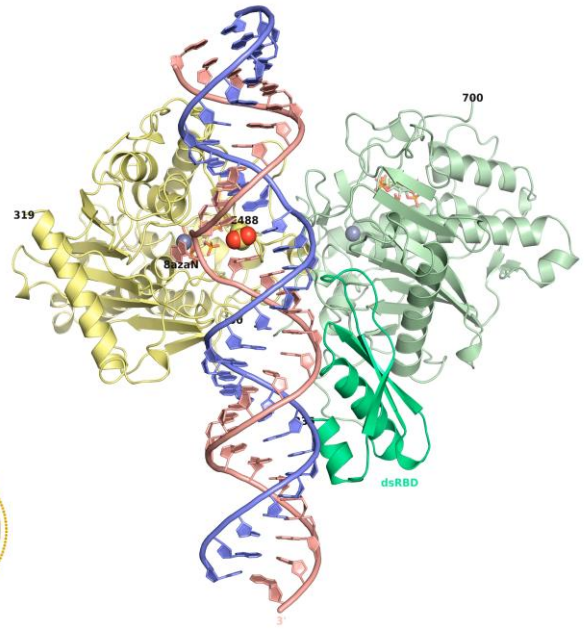
Disclosure

- I am an employee of ProQR Therapeutics

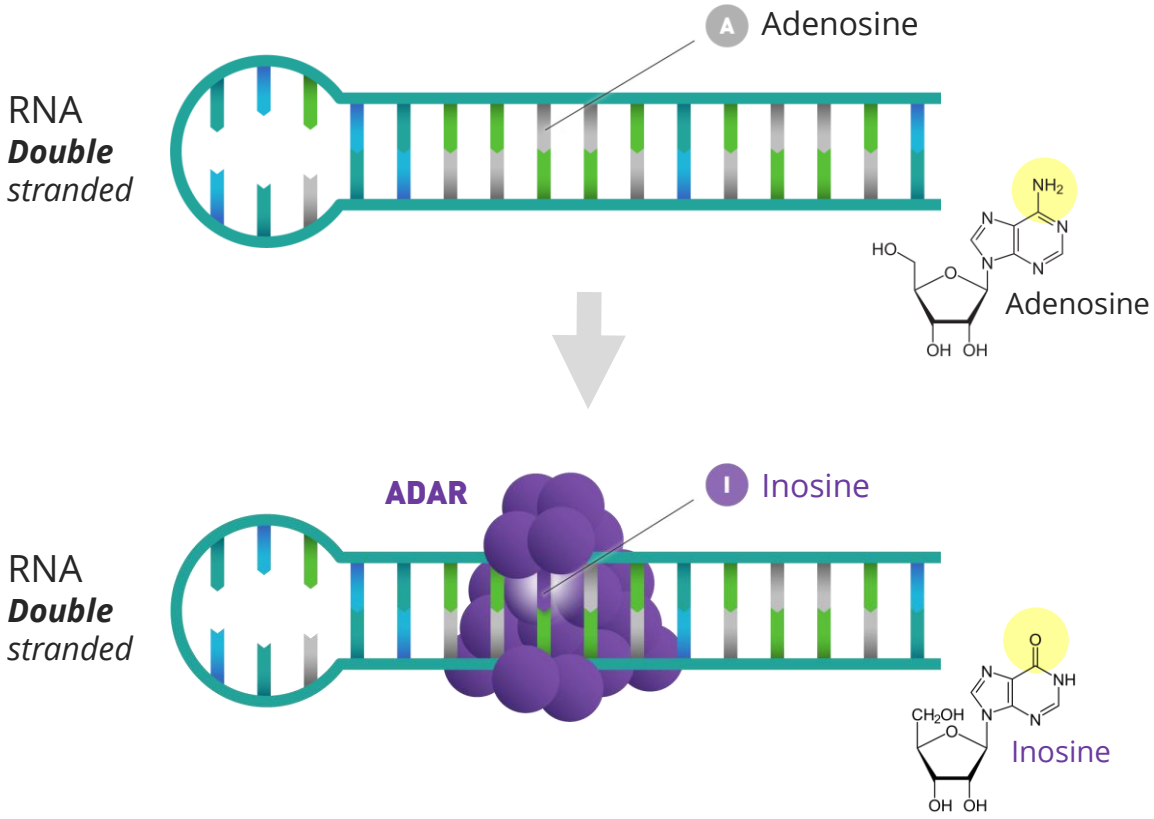
What is ADAR editing?

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)**

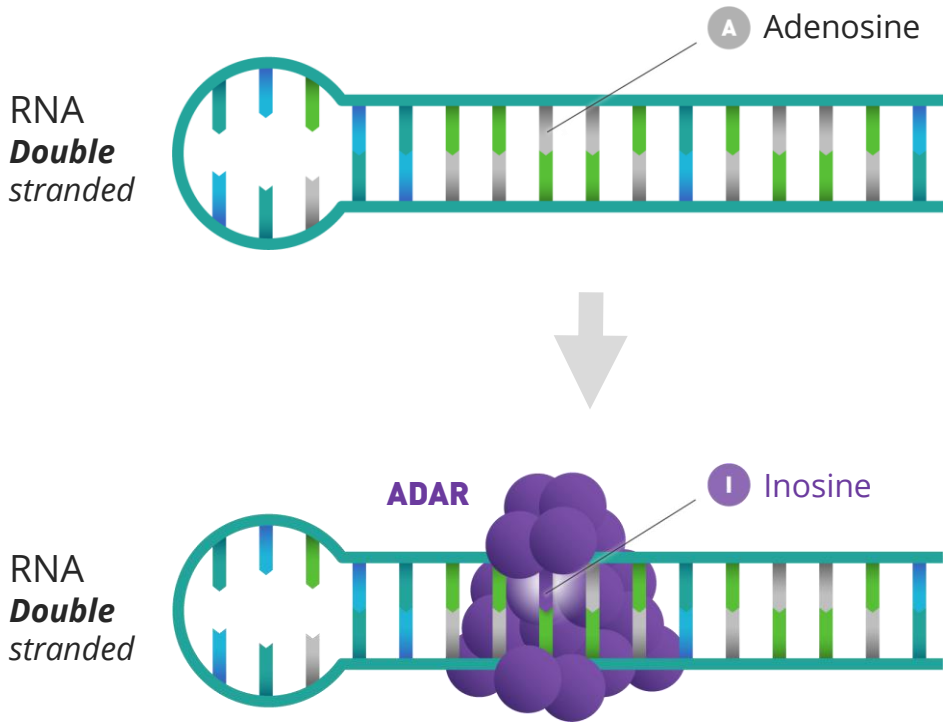
Natural ADAR editing *(A-to-I)*



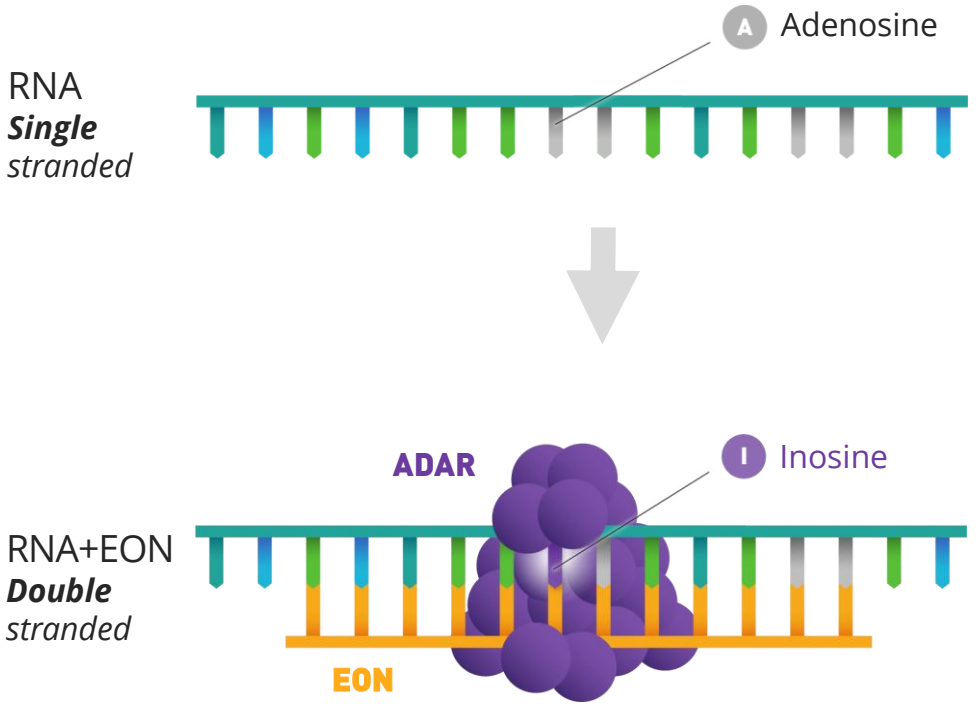
Axiomer EONs unlock cellular machinery potential to treat diseases

By attracting ADARs and allowing highly specific editing

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



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



ADARs: Adenosine deaminases acting on RNA.

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 leading research to optimize editing oligonucleotides for therapeutic use

Modification of the orphan base

Zd in the Editing Enabling Region (EER) maximizes ADAR activity

Translation of findings to NTCP-targeted therapeutic program: AX-0810 to address **Cholestatic Diseases**

Modification of the base opposite to 5'G

3 and 7-deaza-dA in EER to increase editing activity in 5'G unfavorable context

- Therapeutic relevance for Rett syndrome Axiomer program: AX-2402 to address **Rett syndrome**

Modification of the orphan base

Zd in the Editing Enabling Region (EER) maximizes ADAR activity

A single base modification of the EER increases ADAR activity

Zd base mimics E488Q mutation in ADAR2 causing hyperactivity

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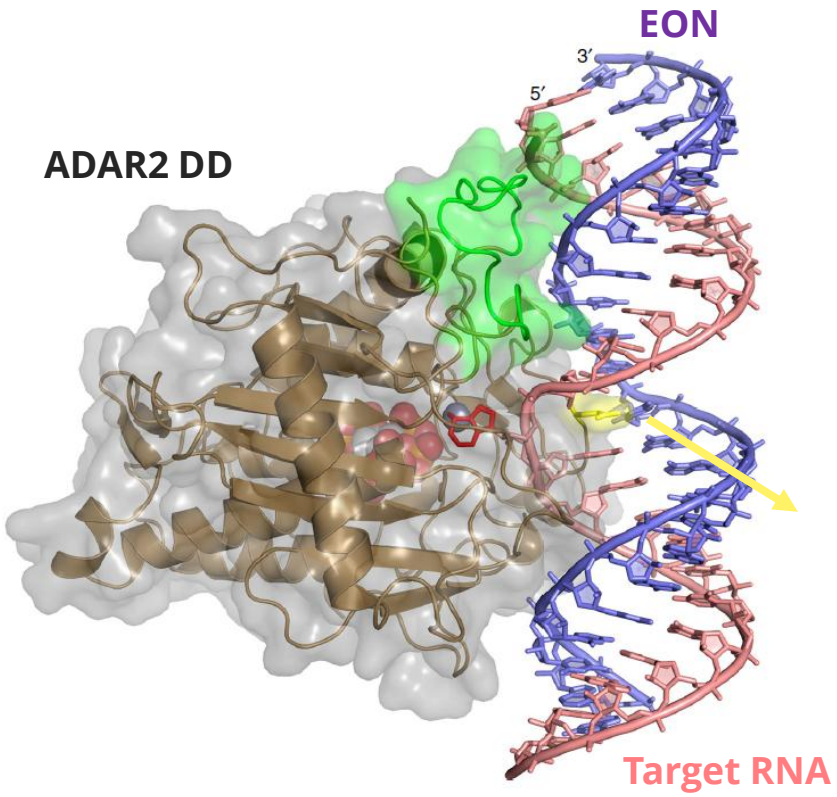
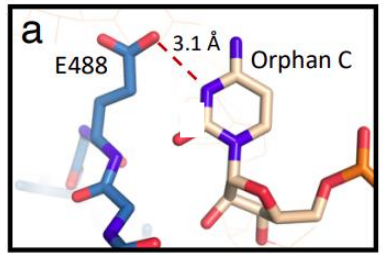
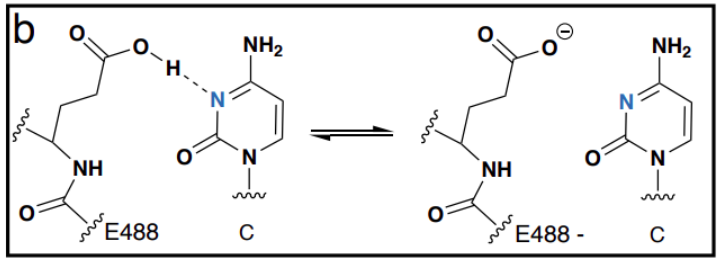
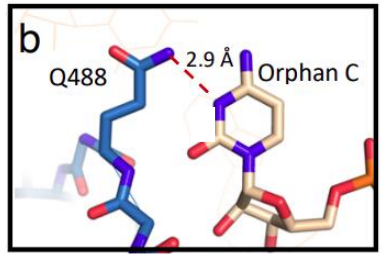
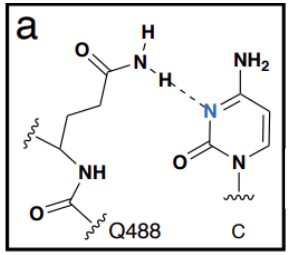


Protonation dependent hydrogen bond – pH dependency

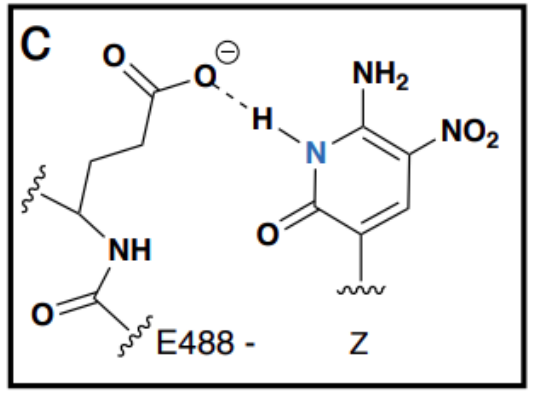








Protonation independent hydrogen bond

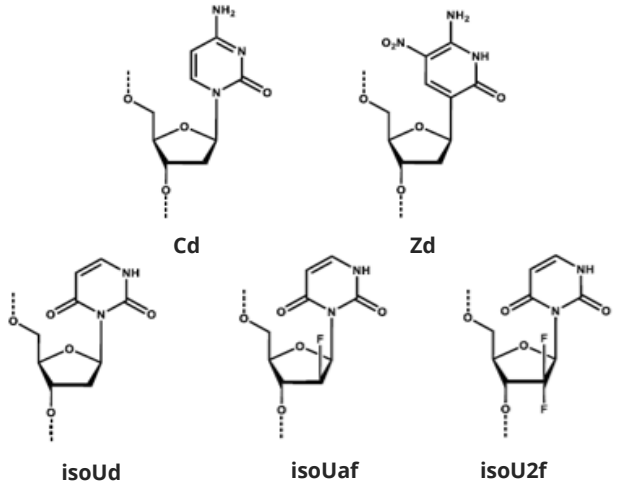


Zd base (dZ)

Metthews 2016, Nature Structural & Molecular Biology

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

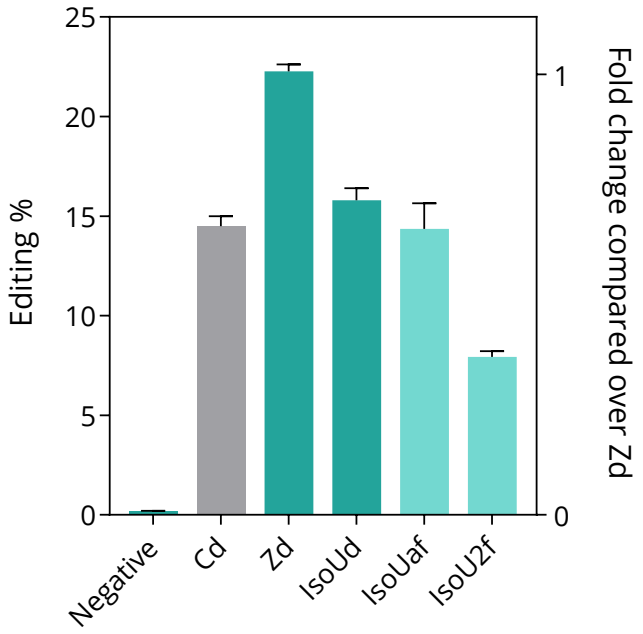
Zd in the Editing Enabling Region (EER) maximizes ADAR activity



With the same N3 H-bond mechanism **none of the tested base-modifications outperformed the editing efficiency of Zd**, indicating that the N3 hydrogen bonding ability of Zd is not the only key chemical component responsible for the high editing efficiency seen for Zd

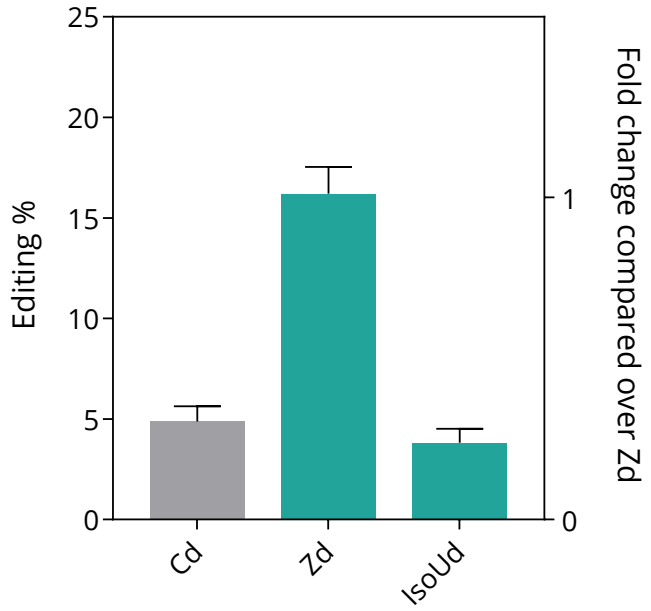
RNA editing of *ACTB* in PHH

Gymnosis, N=3-5, 0.1 µM, dPCR, mean, SEM



RNA editing of *Angptl3* in liver of C57BL/6JRj mice

SC, 1 µmol/kg, 3x dose (D0, D2, D4), N=4, Necropsy D7, dPCR analysis, mean, SEM



Addressing unmet need in cholestatic diseases through NTCP modulation

Cholestatic diseases have high unmet medical need, especially **Primary Sclerosing Cholangitis** affecting adults (~80,000 patients) and Congenital **Biliary Atresia** affecting pediatrics early in life (~20,000 patients). Both conditions have no approved therapies and may require liver transplantation.[1,2]

Patients **accumulate bile acids** in liver leading to fibrosis and ultimately liver failure.

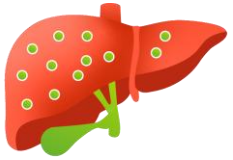
Learnings from human genetics and literature demonstrate that **modulation of the NTCP channel** responsible for majority of bile acids re-uptake in liver cells could lead to **hepato-protective effects**.

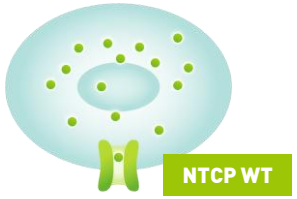
NTCP, sodium taurocholate co-transporting polypeptide. References: [1]Trivedi PJ, et al. Clin Gastroenterol Hepatol. 2022 Aug;20(8):1687-1700.e4; [2]Schreiber RA, et al. J Clin Med. 2022 Feb 14;11(4):999

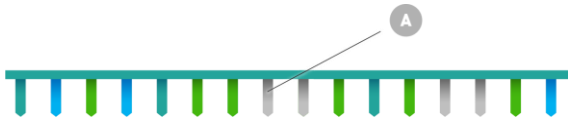
AX-0810: first-in-class RNA editing therapy targeting NTCP for cholestatic diseases

LIVER WITH CHOLESTATIC DISEASE

High concentration of bile acids in hepatocytes

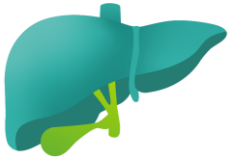


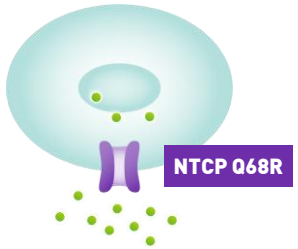


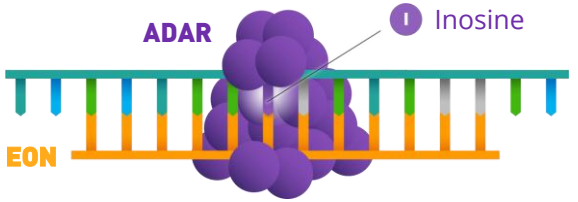


AX-0810 STRATEGY FOR DISEASED LIVER

AX-0810 modifies the NTCP channel to limit bile acids uptake while preserving all other functions of the channel







- AX-0810 makes an A-to-I edit that mimics a variant to enable lower bile acids concentration in hepatocytes
- AX-0810 is designed to be a disease-modifying treatment

Therapeutic goals

- Reduce inflammation and fibrosis from bile acids toxicity
- Alleviate symptoms in PSC and BA
- Prevent or delay cirrhosis, organ failure, and transplant

ADAR, Adenosine Deaminase Acting on RNA; BA, Biliary atresia; EON, Editing Oligonucleotide; NTCP, sodium taurocholate co-transporting polypeptide; PSC, Primary Sclerosing Cholangitis; WT, Wild Type.

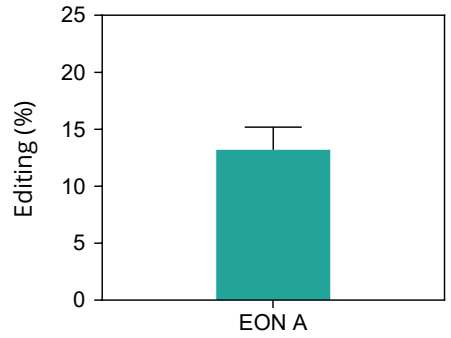
EON mediated editing demonstrates consistent editing of NTCP and impact on biomarker *in vivo*

MICE *in vivo*

EDITING EFFICIENCY

NTCP RNA Editing in Humanized Mice

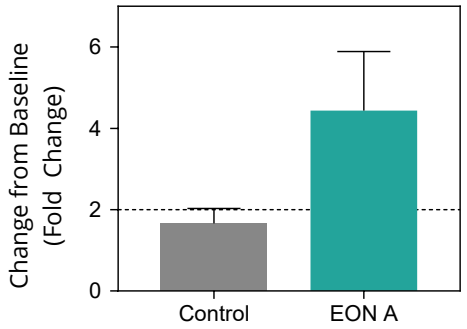
(N=4, 20 mg/kg, 6 doses, GalNAc conjugation, SC, D25, ddPCR)



PLASMA TOTAL BILE ACIDS

Plasma TBA in Humanized Mice

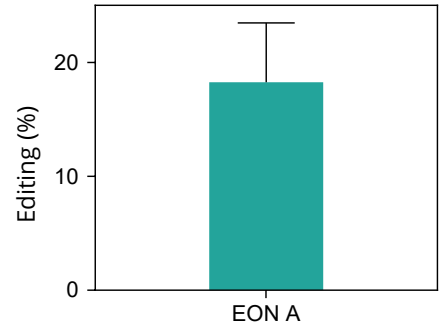
(N=4, 20 mg/kg, 6 doses, GalNAc conjugation, SC, D25)



NHP *in vivo*

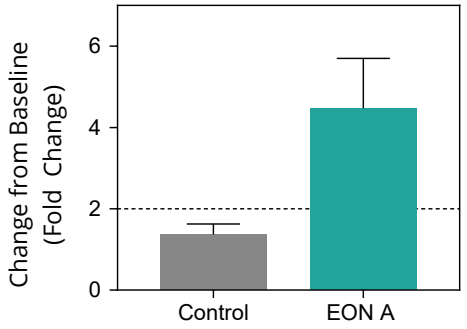
NTCP RNA Editing in NHP

(N=1, 1-4 mg/kg, 4 doses, LNP formulation, IV, up to D46, ddPCR)



Plasma TBA in NHP

(N=1, 1-4 mg/kg, 4 doses, LNP formulation IV, up to D39)

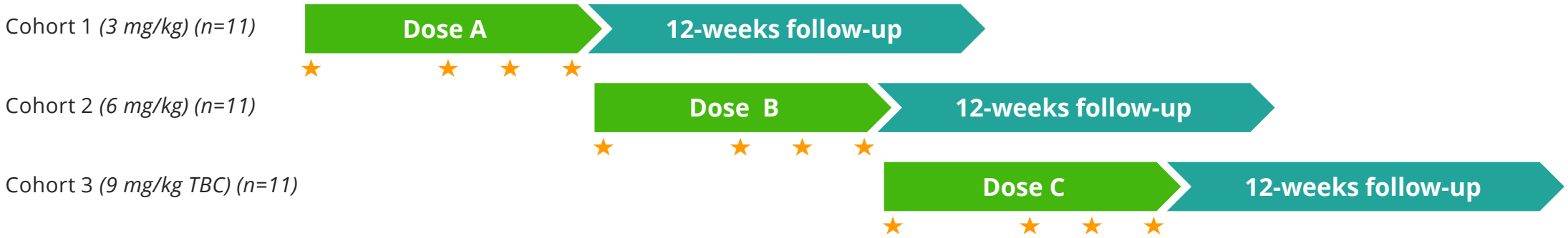


- EON A results in consistent editing data in humanized mouse model and NHP *in vivo* with approx. 15% editing reaching expected NTCP modulation.
- Reaching >2-fold changes in biomarkers - expected impact on plasma bile acids levels following NTCP EON treatment

CTA approved for first-in-human (FIH) trial

Safety, tolerability, PK, and biomarker-based target engagement of AX-0810 in healthy volunteers

Multiple ascending dose (MAD) N=33 (24 on treatment, 9 on placebo)



DMC safety reviews before proceeding to next dose and dose escalation is sequential during the dosing phase

Treatment

AX-0810 GalNAc conjugated editing oligonucleotide

Objectives

- Assess safety, tolerability, and PK of AX-0810
- Confirm target engagement as measured by biomarkers

Key endpoints

- Change in bile acids levels
- Bile acids profile
- TUDCA challenge
- Liver biomarkers

CTA approved and open

- Cohort 1 safety and tolerability towards year end
- Target engagement data on all cohorts in H1 2026

CTA, Clinical Trial Application; DMC, Data Monitoring Committee; MAD, Multiple Ascending Dose; PK, Pharmacokinetics; TUDCA, Tauroursodeoxycholic acid; AX-0810 CTA has been approved in Europe.

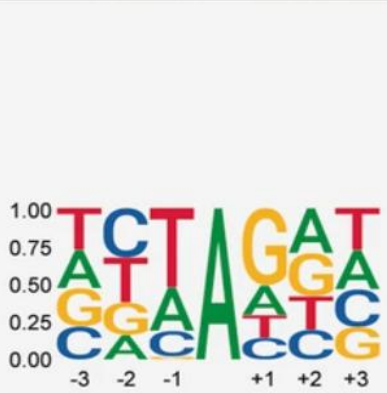
Modification of the base opposite to 5’G

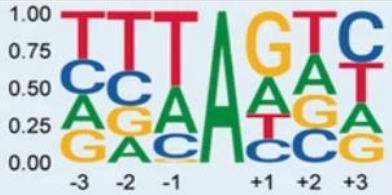
3- and 7-deaza-dA in EER to increase editing activity in 5’G unfavorable context

ADAR knows few sequence constraints

With the exception of G upstream of target adenosine (5'-GA-3')

Location	ADAR1/ADAR2 common	ADAR1 specific*	ADAR2 specific*
	# sites (% WT/dHet sites)	# sites (% WT/dHet sites)	# sites (% WT/dHet sites)
Promoter/ 5' UTR	43 (57.33%)	13 (17.33%)	14 (18.66%)
Exon	225 (51.84%)	80 (18.43%)	92 (21.20%)
Intron	2239 (45.37%)	960 (19.45%)	1286 (26.06%)
3'UTR	142 (50.90%)	43 (15.41%)	66 (23.66%)
Intergenic	200 (65.36%)	47 (15.36%)	62 (20.26%)
Total	**2849 (47.25%)**	**1143 (18.96%)**	**1520 (25.21%)**





This has wide implications for the applicability of targeted RNA editing – guide RNAs with Watson-Crick complementarity are enough to recruit ADAR and induce targeted editing

Adapted from Eggington et al. Predicting sites of ADAR editing in double-stranded RNA. Nat Commun. 2011;2:319

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

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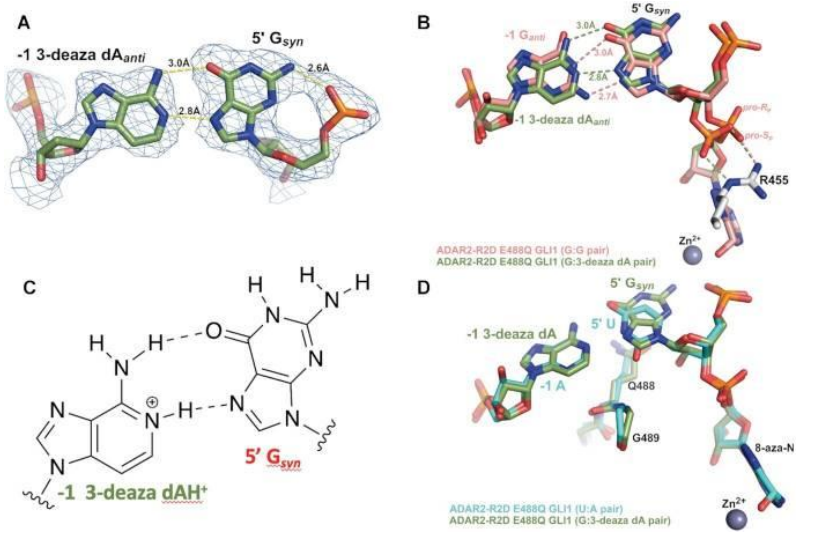
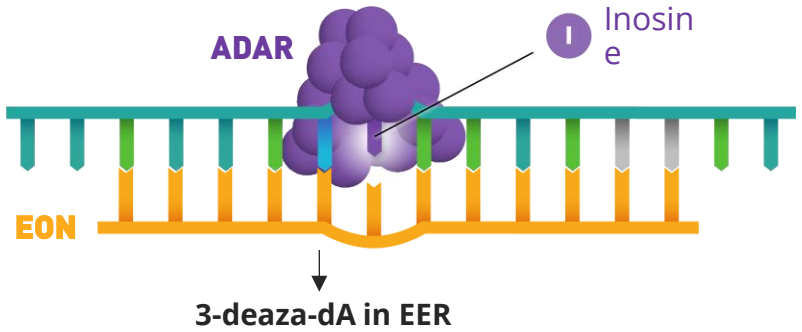
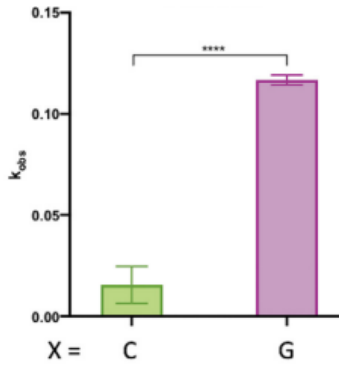
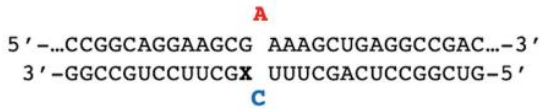




In vitro deamination kinetics for ADAR2 and duplex RNAs derived from WT _hMECP2_

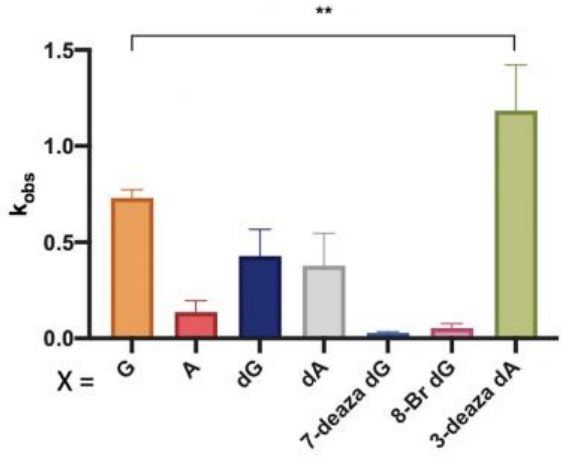
100 nM ADAR2, 3 technical replicates, mean, SD

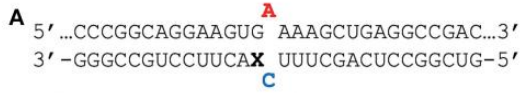




In vitro deamination kinetics for ADAR2 and duplex RNAs derived from _hMECP2_ R255X

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.*

Effect of other purine analogs on editing at 5’ GA site

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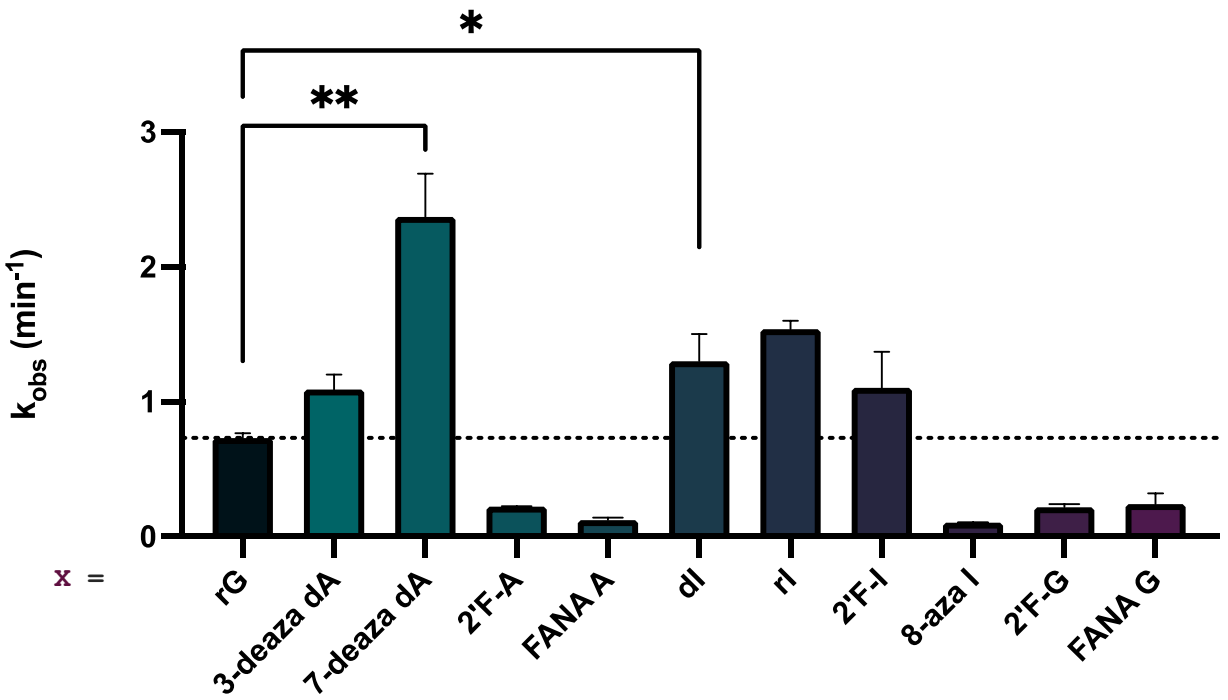
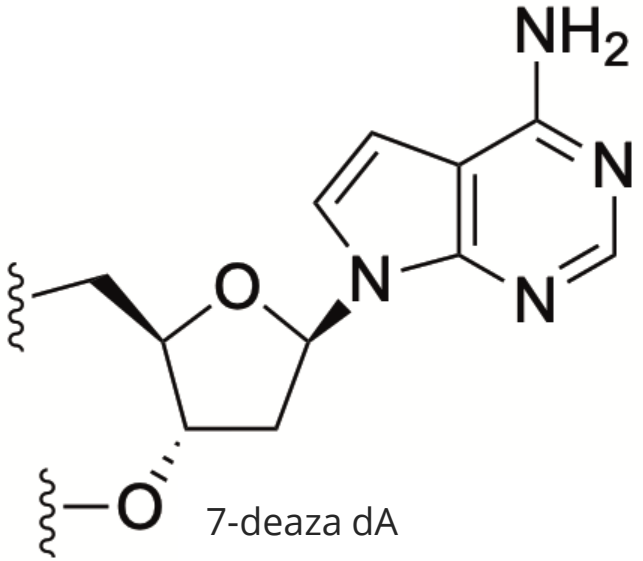
***In vitro* deamination kinetics for ADAR2 and duplex RNAs derived from *hMECP2* R255X**

*10nM hybrid, 100nM ADAR2, 3 technical replicates, mean, SD. Two-tailed Welch’s t test, *p<0.05, **p<0.01*





7-deaza dA

Adapted from: Manjunath, A. et al. Biomolecules 2024, 14, 10, 1229.

AX-2402 RNA editing therapy targeting *MECP2* for Rett Syndrome

RETT SYNDROME RESEARCH TRUST

Rett Syndrome is a **devastating and progressive neurodevelopmental disorder** caused by variants in the transcription factor Methyl CpG binding protein 2 (*MECP2*). There is a **high unmet need for a disease modifying therapy**.

Nonsense variants lead to **severe phenotypes.** They represent more than one third **of Rett Syndrome** cases and are projected to affect **20,000 individuals** in US and EU.[1,2]

Rett Syndrome is **not a neurodegenerative disorder** and restoring levels of the MeCP2 protein has shown to **reverse symptoms** in mice.[3]

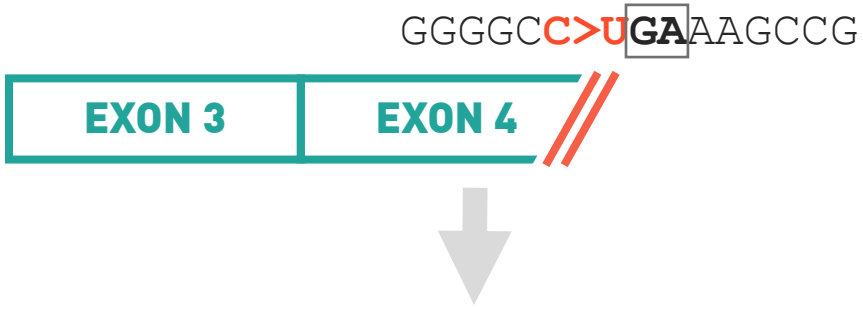
Axiomer has the potential to **restore the precise level of MeCP2 protein regulatory function,** which is lacking in Rett Syndrome, and become a disease modifying therapy.

Rett Syndrome Research Trust partnership includes $9.2 M in funding; collaboration established in January 2024, expanded in December 2024.

[1]Krishnaraj R, et al. Hum Mutat. 2017 Aug;38(8):922-93; [2]RSRT 2023 conference; [3]Guy J, et al. Science. 2007 Feb 23;315(5815):1143-7.

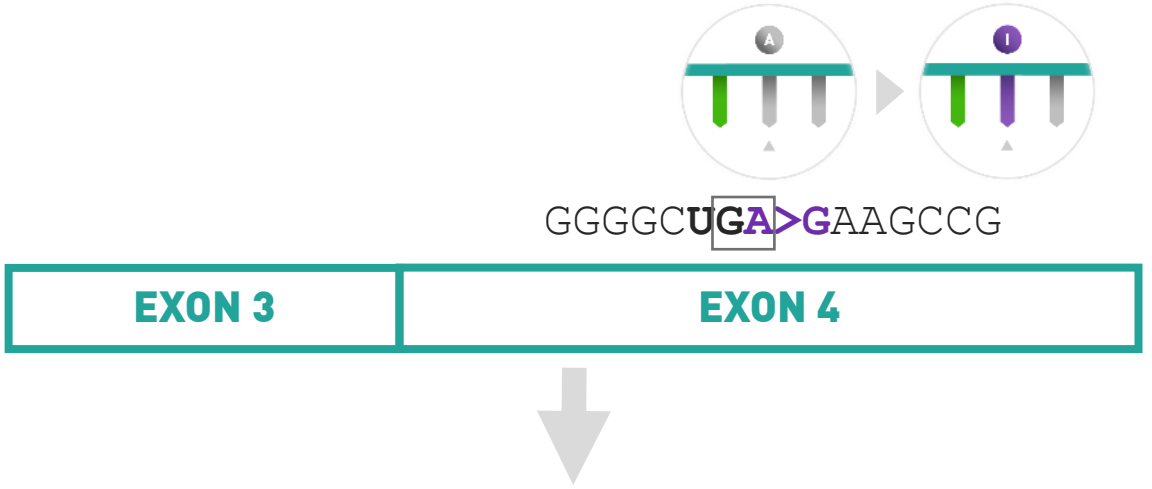
Axiomer™ has the potential to restore physiological levels of functional MECP2

AX-2402 correcting MECP2 R270X into WT-like R270W



RETT syndrome

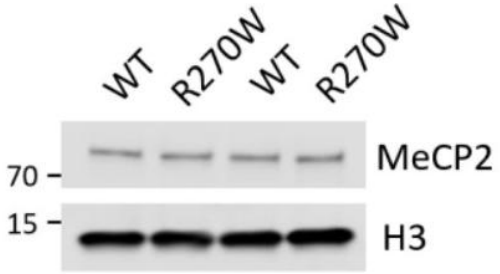
Postnatal microcephaly, stereotypic hand movements, ataxia, abnormal breathing, and growth retardation, social withdrawal, loss of speech, seizures

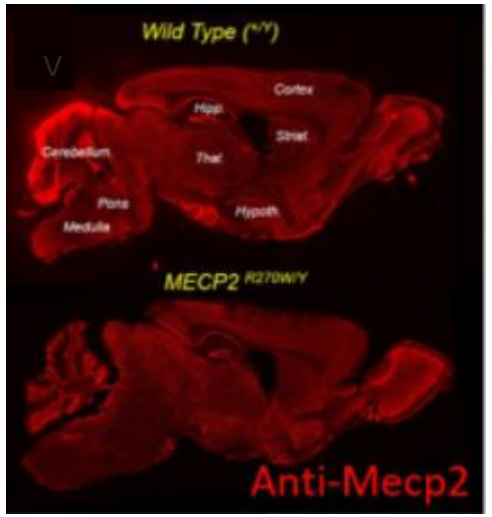


WT like phenotype

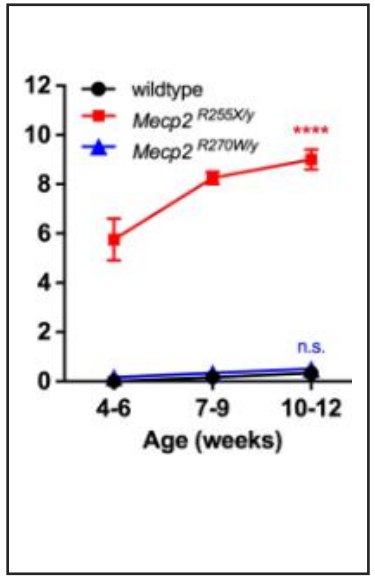
- MeCP2 protein restoration/recovery
- MeCP2 R270W (Arg > Trp) mouse model indistinguishable from wild type mice

R270W variant demonstrates wild-type like profile *in vivo*

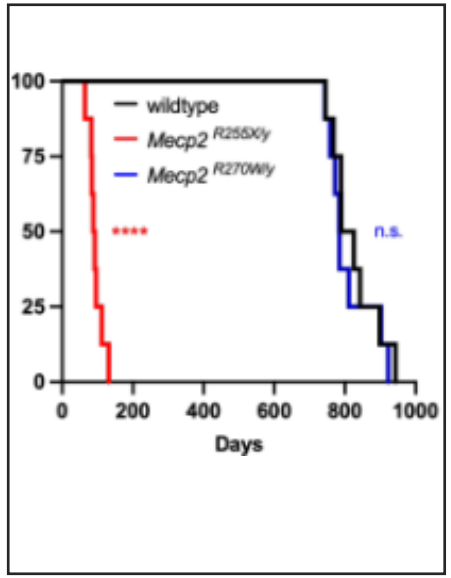




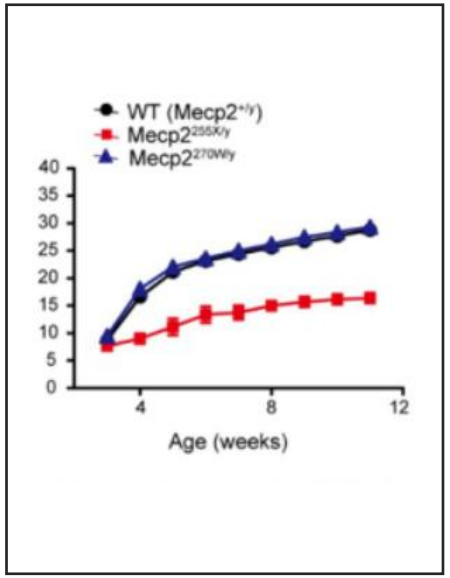
Severity score (0-12)



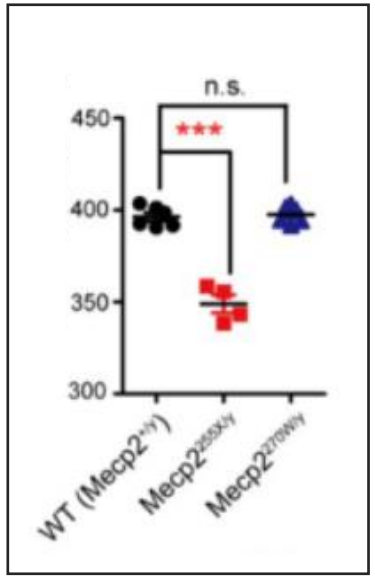
Probability of survival



Body weight (g)



Brain weight (mg)



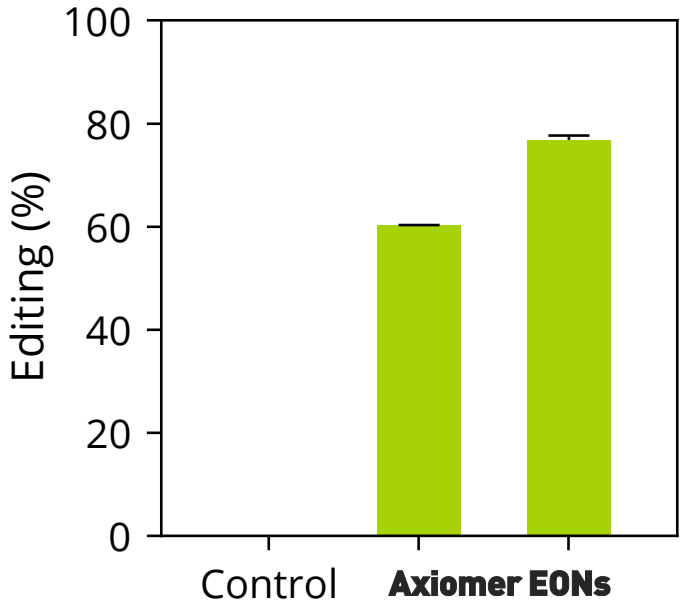
AX-2402 can restore physiological levels of functional MeCP2 potentially reverting Rett syndrome into a WT like phenotype[1]

[1]Colvin, S. (2023) thesis. Massachusetts Institute of Technology. Figures adapted from: Colvin, S. (2023) thesis. Massachusetts Institute of Technology

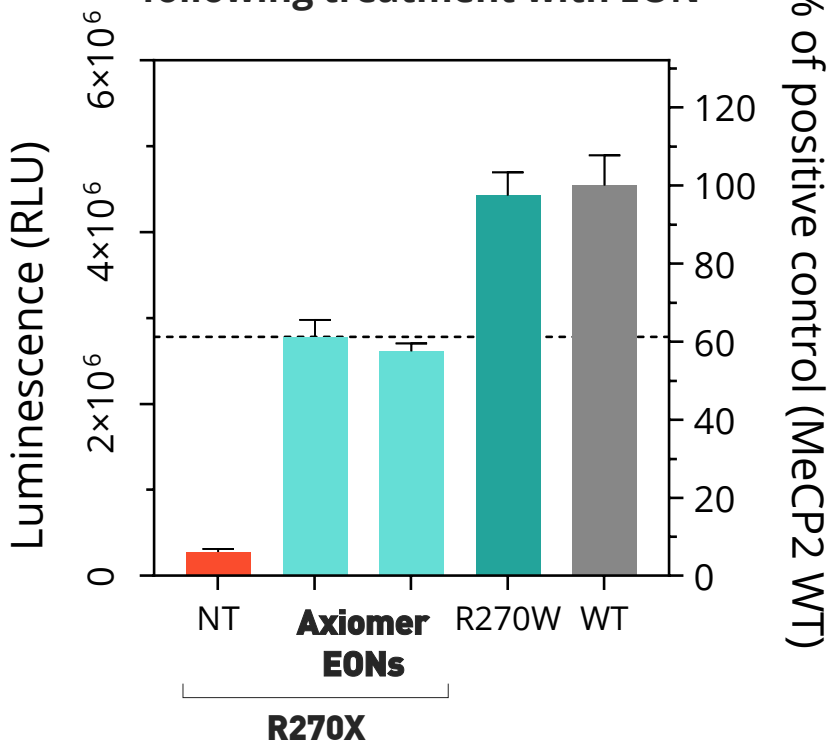
EON mediated editing in patient cells restores MeCP2 protein expression

Up to 60% of WT protein levels

RNA editing of the R270X MECP2 in patient fibroblasts[1]



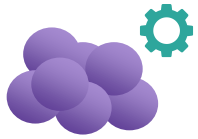
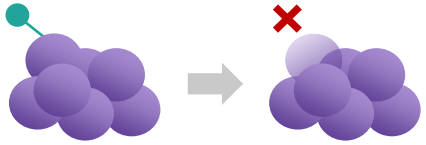
MeCP2 protein reporter activity following treatment with EON[2]



- Up to 80 % editing of R270X *MECP2* in patient fibroblasts
- EON treatment increases MeCP2 protein levels up to 60% of the WT level
- *In vitro* validation of Axiomer as a potential therapeutic approach for Rett

Treatment conditions: 1. Transfection, 25nM, N=2, 48h; 2. Plasmid reverse transfection (MECP2 R270X, MECP2 R270W or MECP2 WT) 24h , 100ng/ml, turbofect, EON forward TF, 100nM, 48h, RNAiMax, N=4; data represented are mean ± SEM.

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

Axiomer RNA editing science translating toward therapeutic applications

ADAR-mediated RNA editing as a versatile therapeutic approach

- Harnessing advanced knowledge of ADAR and oligonucleotide science
- Pioneering the optimization of editing oligonucleotides (EONs) to achieve best-in-class therapeutic solutions

Optimization of editing oligonucleotides (EONs) through chemical modifications to enhance editing efficiency, specificity, and cellular uptake.

- Demonstrating proven success in correcting genetic mutations and enabling diverse protein modulation strategies
- Platform with potential to address diverse conditions rooted in human genetics

Translation of optimized EON strategies into therapeutically relevant applications addressing high unmet medical needs in the liver and CNS.

- Driving innovation in the ADAR RNA editing science with Axiomer EONs since 2014
- Dominant IP position to drive ADAR-mediated RNA editing platform innovation

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