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ADAR-Mediated RNA editing of a premature stop codon enables functional correction of MECP2 in Rett syndrome

Marko Potman[1], Kelvin E Lau[1], Maurits van den Berg[1], Christopher Langeveld[1],Jeroen van de Giessen[1],Bart Snieder[1], Wawah Zhu[1], Macha de Drue[1], Tushar Deshpande[1], Seda Yilmaz-Elis[1], Gerard Platenburg[1] | [1]ProQR Therapeutics N.V. Leiden, the Netherlands

Introduction

The premature stop codon p.R270X, a common pathogenic variant in Rett syndrome

Rett syndrome (RTT) is a severe neurodevelopmental disorder affecting ~350,000 people worldwide, mainly females, most often caused by mutations in the X-linked *MECP2* gene[1]. ~900 *MECP2* mutations have been identified thus far, with disease severity influenced by mutation type, location, and X-chromosome inactivation[2]. The p.R270X variant (c.808T) is among the most frequent RTT-associated nonsense mutations[3].

Guide RNA editing oligonucleotides (Axiomer™), or "EONs" are designed to bind a single stranded RNA with high specificity and recruit endogenously expressed ADARs to change an Adenosine (A) to an Inosine (I). The Inosine (I) is then translated as a Guanosine (G). EONs can be utilized to recode the MECP2 premature stop codon to tryptophan (p.270W), restoring MeCP2 protein function *in vitro* and *in vivo*[4] (**Fig. 1**). This work evaluates leading EON candidates in patient-derived induced pluripotent stem cells (iPSC) forebrain neurons and a humanized mouse model.

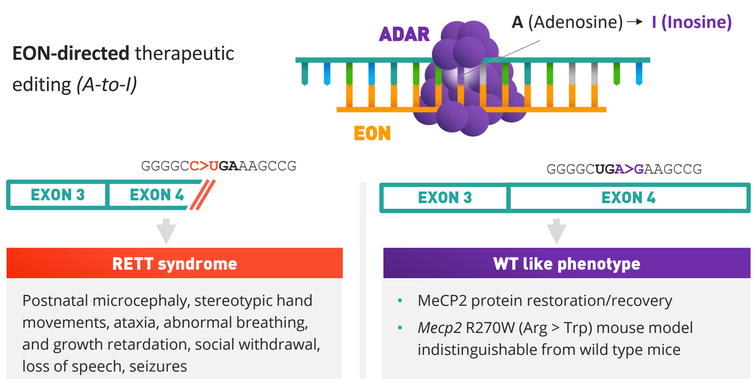

Figure 1. Schematic illustration of the Axiomer oligonucleotide-guided RNA–mediated ADAR editing mechanism for the treatment of R270X premature termination codon (PTC)–dependent Rett syndrome (RTT).

Mecp2 tryptophan variant (p.R270W) mouse model is indistinguishable from wild-type controls

In 2023, a transgenic mouse model showed that correction to the tryptophan variant (p.R270W) of Mecp2 renders mice indistinguishable from wild-type controls.[4] (**Fig. 2**)

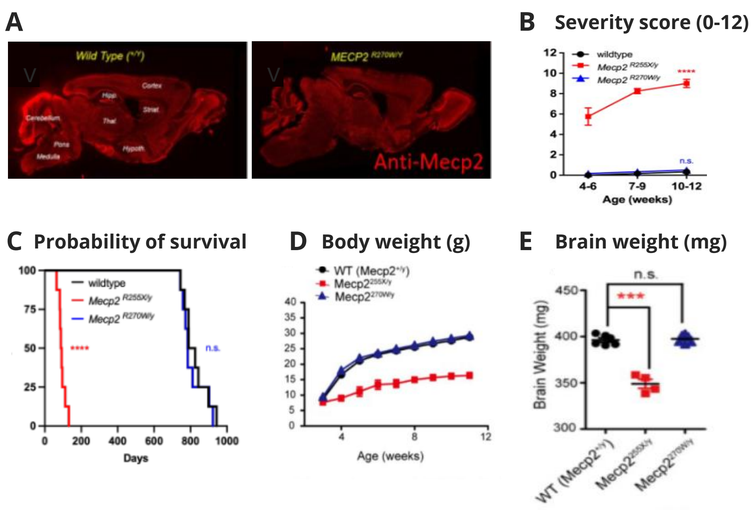

Figure 2. Comparison of WT, R255X, and R270W Mecp2 mouse models: (**A**) protein expression by immunostaining; phenotypes shown as (**B**) severity score (0–12), (**C**) survival probability (%), (**D**) body weight (g), and (**E**) brain weight (mg). *Adapted from Colvin, S. (2023) thesis.*

Methods

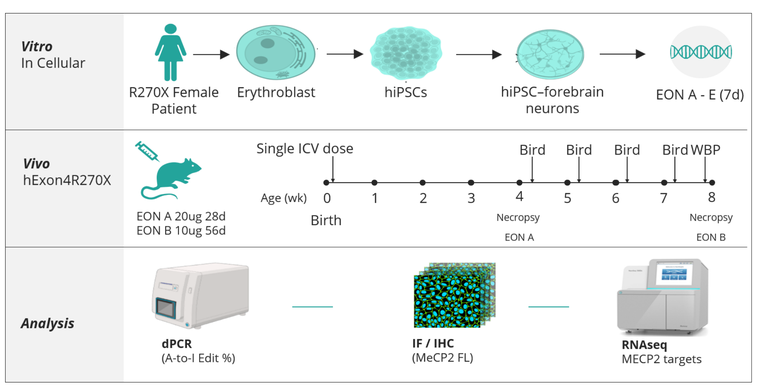

Figure 3. Methods to evaluate leading Axiomer™ EON candidates for restoring functional MeCP2 expression in patient-derived iPSC forebrain neurons and an exon 4 humanized mouse model.

Objectives

- **To assess Axiomer EON editing efficiency *in vitro,* capacity to restore full length MeCP2 protein expression and effect on gene expression.**
- **To assess Axiomer capacity to restore full length protein expression and function *in vivo***

Results

Editing efficiency correlates with increased protein restoration *in vitro*

To assess Axiomer potential in Rett syndrome, patient-derived hiPSC forebrain neurons carrying the MECP2 mutation (p.R270X, c.808T) were transfected with multiple EON iterations or left untreated [UT]. EON-induced editing ranged from 30% - 90% (dPCR). (**Fig. 4**)

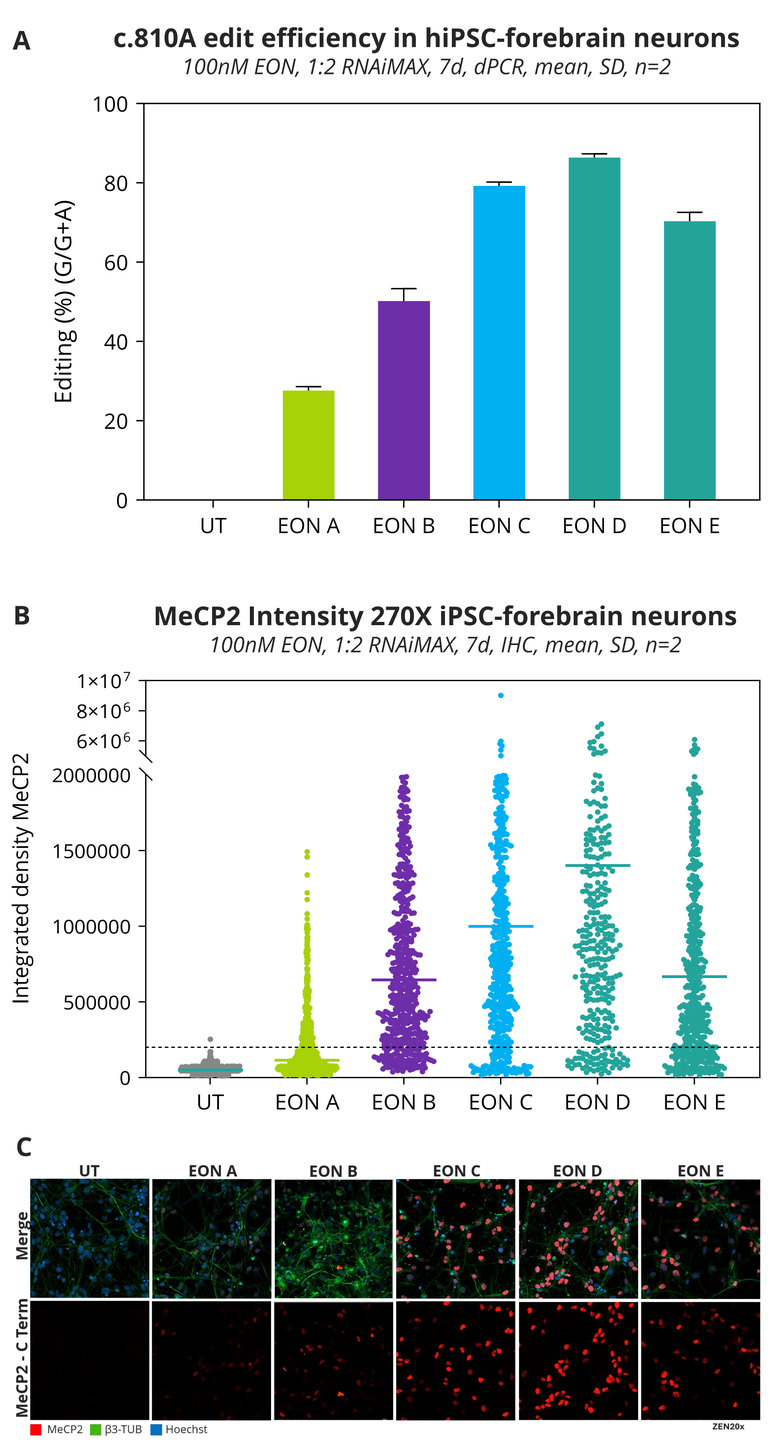

Figure 4. Assisted uptake of EONs (EONs A-E; early lead and follow-up candidates, shown in green, purple, blue and teal respectively) in forebrain neurons results in efficient full-length protein restoration, consistent with *MECP2* RNA c.810A editing efficiency (**A)** c.810A RNA editing after EON treatment in *MECP2* R270X hiPSC neurons 100 nM; 7-day washout; untreated [UT], N=2, mean ± SD; dPCR). (**B)** Integrated immunofluorescence as a semi-quantitative measure of full-length MeCP2 restoration (ZEN ZEISS). (**C)** Representative immunostaining of MeCP2 and βIII-tubulin in hiPSC-derived forebrain neurons (ZEN ZEISS).

MeCP2 downstream target gene expression rescue in EON C treated hiPSC-forebrain neurons *in vitro*

To assess the effect of *MECP2* c.810A>G editing on downstream MeCP2 targets, patient-derived hiPSC neurons carrying the *MECP2* R270X mutation were transfected with EON C. >60% A-to-I editing was achieved with EON C (data not shown). Genes suppressed in Rett (SET A) are restored by EON C (Inc. key synaptic genes (BDNF, GRIN2B). Genes elevated in Rett are normalized by (consistent with rebalancing neuronal and glial signaling) (**Fig. 5**).

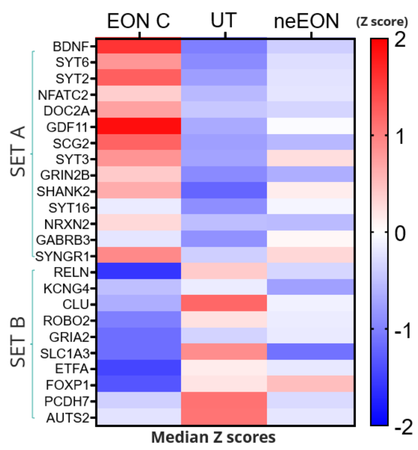

Figure 5. RNA-seq–derived events across EON, neEON, Mock, and UT conditions. (100 nM; 7-day washout) Normalized enrichment scores highlighting RTT-relevant, editing-affected genes. (Not shown: GSEA (MSigDB, https://www.gsea-msigdb.org) analysis identified 25 significantly enriched pathways associated with Rett syndrome, neurodevelopment, maturation and synaptic signaling

EON mediated RNA editing restores MeCP2 protein expression and nuclear localization *in vivo*

A single 20 µg intracerebroventricular (ICV) dose of EON A resulted in editing in the humanized *MECP2* exon 4 (p.R270X) mouse model and subsequent nuclear-localized full-length MeCP2 protein restoration as shown by IHC MeCP2 C-terminus immunoreactivity at day 28. (**Fig. 6**)

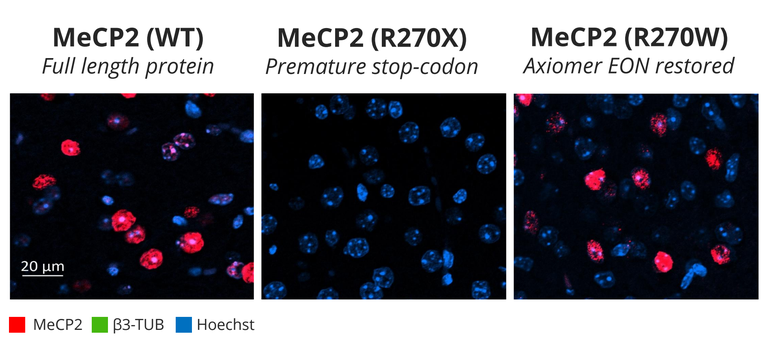

Figure 6. Representative immunohistochemistry staining of MeCP2 C-terminus. Tissue: right brain - sectioned coronally (ZEN ZEISS).

EON treatment delays disease severity, as assessed by the Bird score

A single 10 µg neonatal intracerebroventricular administration of EON B in a severe RTT model produced a statistically significant improvement in hindlimb clasping score at week 7. The composite Bird score demonstrated sustained statistically significant improvement through weeks 6 and 7. (**Fig. 7**)

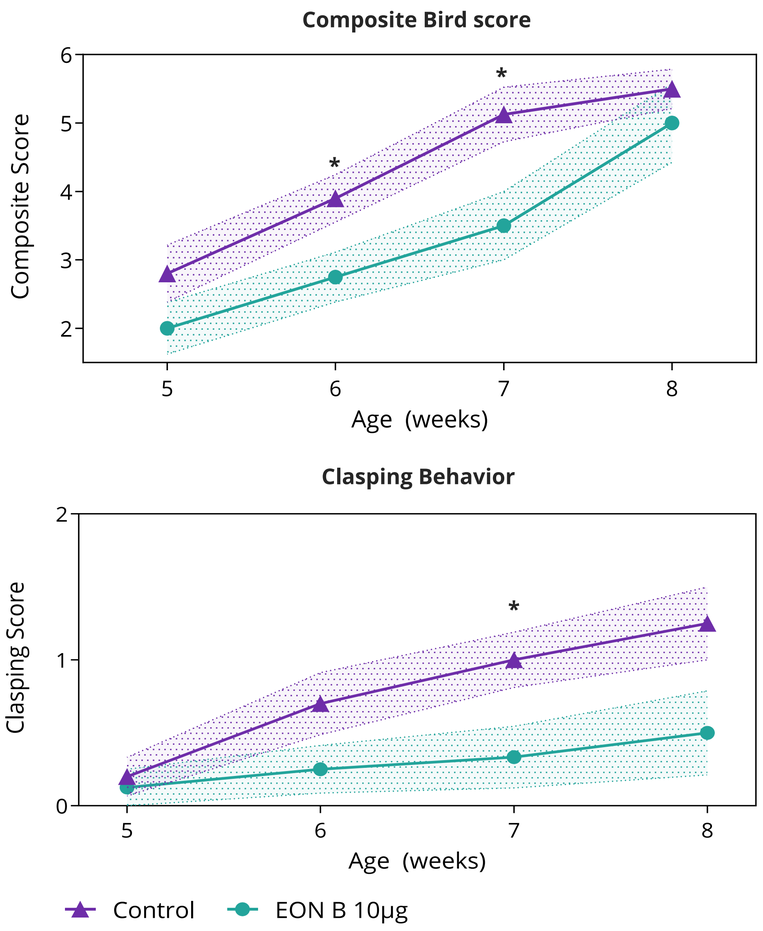

Figure 7. Neonatal male hExon4 R270X mice (N=9) received a single 10 µg ICV dose of EON B or control (vehicle) at postnatal day 1 and were monitored from weeks 5 to 8. (Left) Hindlimb clasping score. (Right) Composite Bird score (mobility, gait, tremor, breathing, condition, hindlimb clasping). Y-axis indicates severity. Data are mean ± SEM, mixed-effects model (repeated measures) + Tukey's posthoc test (* p<0.05)

Conclusions

- **Axiomer RNA editing demonstrated robust editing efficiency, restoration of full-length protein and gene expression rescue in human forebrain neurons *in vitro*, with translation to an RTT mouse model *in vivo* including functional improvement over control**
- **Follow-up experiments on optimized oligonucleotides will be conducted to assess potential for increased potency, durability and therapeutic window**

Literature

1. Amir, R.E. et al. (1999) Rett syndrome is caused by mutations in X-linked MECP2, encoding methyl-CpG-binding protein 2. Nature Genetics, 23(2), 185–188. https://doi.org/10.1038/13810
2. Townend, G.S. et al. (2018) MECP2 variation in Rett syndrome: an overview of genetic and phenotypic data coverage in existing databases. Human Mutation, 39(7), 914–924. https://doi.org/10.1002/humu.23542
3. Krishnaraj, R., Ho, G. and Christodoulou, J. (2017) RettBASE: Rett syndrome database update. Human Mutation, 38(8), 922–931. https://doi.org/10.1002/humu.2326
4. Colvin, S. (2023) Modeling Autism Spectrum Disorder: Fragile X syndrome and Rett syndrome. Department of Biology, MIT.