A Versatile Platform for ADAR-mediated RNA Editing *in vivo* in Preclinical Models

Chikdu Shivalila, Prashant Monian, Genliang Lu, Bijay Bhattarai, David Boulay, Karley Bussow, Michael Byrne, Adam Bezigian, Arindom Chatterjee, David Chew, Jigar Desai, Frank Favaloro, Jack Godfrey, Andrew Hoss, Naoki Iwamoto, Tomomi Kawamoto, Jayakanthan Kumarasamy, Pachamuthu Kandasamy, Anthony Lamattina, Amber Lindsey, Fangjun Liu, Richard Looby, Jake Metterville, Ronelle Murphy, Tom Pu, Jeff Rossi, Stephany Standley, Snehlata Tripathi, Hailin Yang, Yuan Yin, Hui Yu, Cong Zhou, Luciano Apponi, Chandra Vargeese

Wave Life Sciences, Cambridge, MA,USA

Summary

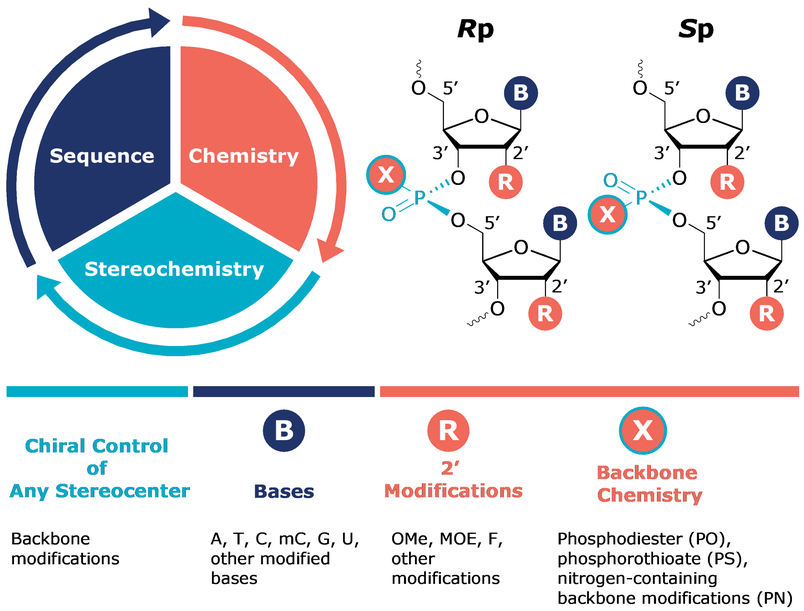
- With PRISM™, Wave can generate stereopure AIMers that promote RNA editing with endogenous ADAR enzymes in cellular and animal models
- We demonstrate that GalNAc-conjugated and unconjugated stereopure AIMers elicit robust ADAR-mediated RNA editing in primary hepatocytes as well as *in vivo* in NHPs and humanized mice
- Using our hADAR mouse model, we show that there are species-specific differences between ADAR enzymes, and that editing in this model replicates editing observed in NHPs
- We demonstrate that stereopure AIMers targeting distinct transcripts direct RNA editing throughout the CNS of hADAR mouse after a single ICV dose
- These preliminary preclinical findings provide an update on our advancing RNA-editing capabilities, which have potential to treat human genetic disease

Introduction

Application of PRISM to RNA editing

- PRISM, Wave's discovery and drug development platform, enables us to develop stereopure oligonucleotides—those in which the chiral configuration of backbone linkages (e.g., *R*p or *S*p) are precisely controlled at each position—to target genetically defined diseases (**Figure 1**).[1]
- ADAR (adenosine deaminase acting on RNA) enzymes catalyze adenine (A) to inosine (I) changes in coding and non-coding sequences throughout the transcriptome. Because inosine (I) is read as guanine (G) by the translational machinery, oligonucleotide-directed ADAR-mediated RNA editing has the potential to change myriad disease-causing mutations.[2]
- We have performed an extensive analysis to optimize our chemically modified editing oligonucleotides, called AIMers, to support RNA editing with endogenous ADAR enzymes.
- Herein, we present preliminary *in vivo* data in mouse and non-human primates (NHPs) showing that AIMers are effective when delivered as naked oligonucleotides or GalNAc-conjugates via systemic or intracerebroventricular (ICV) administration.

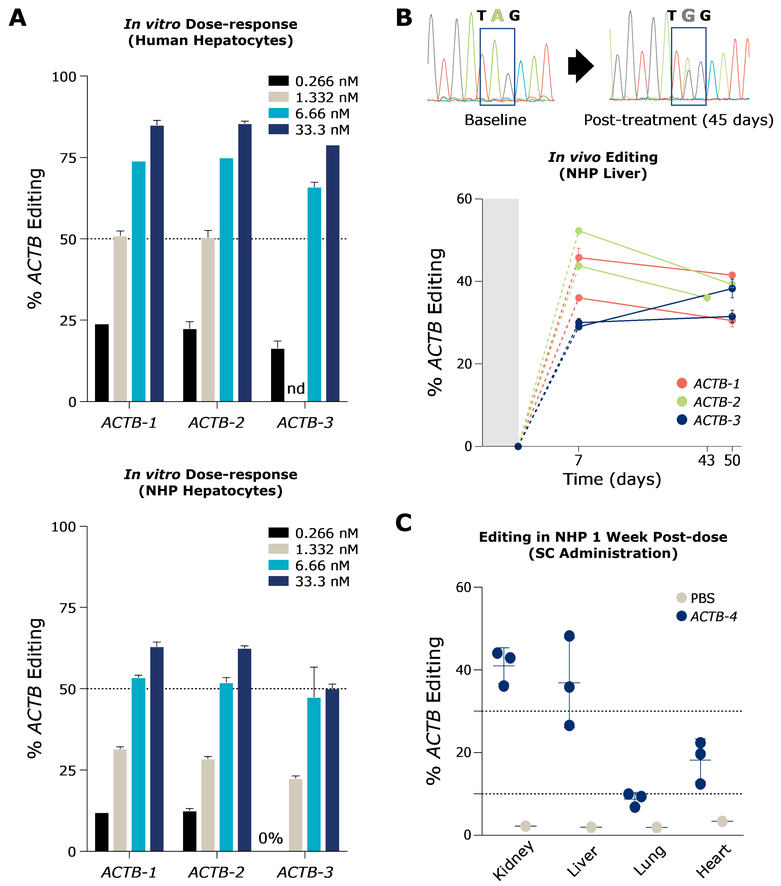
Figure 1. PRISM platform enables rational drug design



Chiral Control of Any Stereocenter	B Bases	R 2′ Modifications	X Backbone Chemistry
Backbone modifications	A, T, C, mC, G, U, other modified bases	OMe, MOE, F, other modifications	Phosphodiester (PO), phosphorothioate (PS), nitrogen-containing backbone modifications (PN)

Results

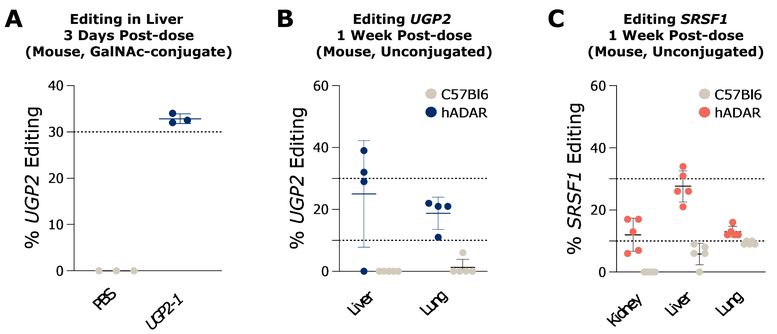
Figure 2. GalNAc-conjugated and unconjugated AIMers support efficient RNA editing



- We developed AIMers to edit an A in the 3′-UTR of *ACTB*.
- In primary hepatocytes (human and NHP), stereopure *N*-acetylgalactosamine (GalNAc)-conjugated AIMers led to efficient and dose-dependent *ACTB* editing (**Figure 2A**).
- We evaluated these GalNAc-conjugated AIMers *in vivo* in NHPs. At 7 days, we detected editing in all liver samples, with ~50% of the *ACTB* transcript edited with the most active AIMer. At 50 days, absolute editing percentage decreased slightly but ≥35% edited transcripts persisted for all AIMers tested. Representative Sanger sequencing traces for baseline and 45-day timepoints are shown (**Figure 2B**).
- We also evaluated an unconjugated AIMer *in vivo* in NHPs. At 1-week post-dosing, we detected >35% mean *ACTB* editing in liver in the absence of GalNAc. We also detected editing in kidney (41%), lung (8%), and heart (18%) (**Figure 2C**).

Primary hepatocytes (human or NHP) were treated with GalNAc-conjugated AIMer. Total RNA was harvested, reverse transcribed to generate cDNA, and the editing target site was amplified by PCR and quantified by Sanger sequencing. Percentage editing was quantified from Sanger sequencing using EditR.[3] 5 mg/kg GalNAc-conjugated AIMer was administered by subcutaneous (SC) injection to NHP once daily on days 1-5. Liver biopsies were collected on days 7 and 50. RNA was evaluated for editing as in panel A. 50 mg/kg unconjugated AIMer was administered by SC injection to NHP once on day 1. Tissue biopsies were collected on day 8.

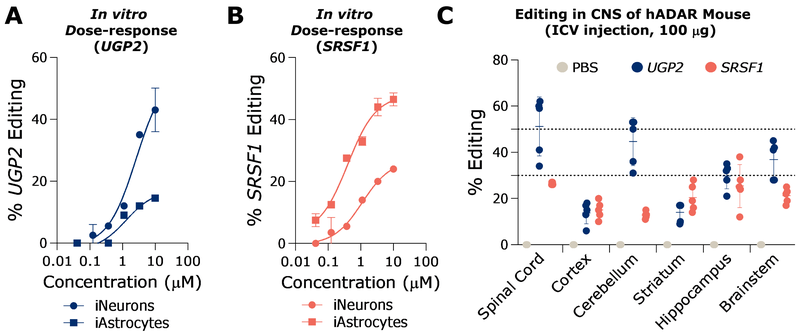
Figure 3. A humanized ADAR mouse model recapitulates findings in NHPs



- We developed a transgenic mouse model expressing human ADAR1 (hADAR) to test whether this model can support human ADAR-mediated editing. The expression of human ADAR1 in the liver and brain of hADAR mice is comparable to that in the respective human tissues. We developed stereopure AIMers (GalNAc-conjugated and unconjugated), which are designed to edit an A in the coding region and 3′-UTR of *UGP2* and *SRSF1*, respectively.
- At 1-week post-dose with GalNAc conjugate, we detected >30% *UGP2* editing in liver (**Figure 3A**).
- At 1-week post-dose with unconjugated AIMer, we detected ~25% *UGP2* and *SRSF1* editing in liver of hADAR mice in the absence of GalNAc. We also detected editing in lung (~15%) for both transcripts and in kidney for *SRSF1* (12%) (**Figure 3B,C**). No *UGP2* editing was detected in kidney (**Figure 3B**).
- In C57Bl/6 control mice, editing remained at background levels in all tissues evaluated, indicating that there are species-specific differences in ADAR enzymes, and our AIMers support editing by ADAR1 from human and NHP.

10 mg/kg GalNAc-conjugated AIMer was administered by SC injection to hADAR mice once daily on days 1-3. Liver biopsies were collected on day 8. RNA was evaluated for editing as in Figure 2. For the unconjugated AIMer, a single 100 mg/kg SC dose was administered on day 1. Tissue biopsies were collected on day 8.

Figure 4. AIMers direct editing throughout CNS of hADAR mouse



- To assess editing in CNS, we generated AIMers against multiple transcripts.
- In cultured iNeurons and iAstrocytes, unconjugated stereopure AIMers promote efficient and dose-dependent editing of *UGP2* and *SRSF1* (**Figure 4A,B**).
- In hADAR mice, the same AIMers elicit substantial transcript editing in multiple regions of the CNS 1-week after a single 100 μg ICV dose (**Figure 4C**). Dotted lines demarcate 30% and 50% editing levels.
- Editing of both *UGP2* and *SRSF1 in vivo* supports general applicability of these AIMers in CNS (**Figure 4C**).

Human iCell neurons (iNeurons) or iCell Astrocytes (iAstrocytes) were treated with increasing concentrations of unconjugated AIMers under gymnotic conditions. Percentage editing was calculated as described in Figure 2. 100 μg unconjugated AIMer was administered by ICV injection to hADAR mice once on day 1. Tissue biopsies were collected on day 8. RNA was evaluated for editing as in Figure 2.

References: 1. Iwamoto, N. et al., 2017. Control of phosphorothioate stereochemistry substantially increases the efficacy of antisense oligonucleotides. *Nat. Biotech.* 35, 845-851; 2. Chen, G., Katrekar, D. & Mali, P. 2019. RNA-guided adenosine deaminases: Advances and challenges for therapeutic RNA editing. *Biochemistry* 58, 1947-1957; 3. Kleusner, M.G., et al., 2018. EditR: A method to quantify base editing from Sanger sequencing. *CRISPR J.* 1, 239-250. **Acknowledgments:** Authors are grateful to Eric Smith and Amy Donner for graphical and editorial support, respectively, and Wave Life Sciences for funding. Authors are also grateful to Paloma Giangrande for critical feedback on the content.