RNA Editing via Endogenous ADARs Using Stereopure Oligonucleotides

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Summary

- PRISM™ is Wave Life Sciences' proprietary discovery and drug development platform that enables us to generate stereopure oligonucleotides in which the chiral configuration of each internucleotide linkage in backbone-modified oligonucleotides is precisely controlled. PRISM combines our unique ability to construct stereopure oligonucleotides with a deep understanding of how the interplay among oligonucleotide sequence, chemistry, and backbone stereochemistry impacts key pharmacologic properties.
- With PRISM, Wave can generate stereopure antisense oligonucleotides (ASOs) that promote RNA editing with endogenous adenosine deaminase acting on RNA (ADAR) enzymes in cellular models.
- Here, we demonstrate that optimized stereopure editing ASOs elicit more potent ADAR-mediated RNA editing than stereorandom editing ASOs *in vitro* in luciferase assays.
- We also show that stereopure editing ASOs induce more potent RNA editing with endogenous ADAR than stereorandom editing ASOs under gymnotic conditions (i.e., in the absence of transfection reagents) in multiple primary human cell lines.
- Similarly, a *N*-acetylgalactosamine (GalNAc)-conjugated stereopure ASO elicits efficient RNA editing with endogenous ADAR in primary human hepatocytes.
- Finally, we demonstrate that our stereopure editing ASOs can elicit ADAR-mediated editing across several distinct RNA transcripts in primary human hepatocytes, indicating that our technology is applicable across multiple sequences.

Background

Stereopure Oligonucleotides

- Natural oligonucleotides make poor drugs because they are easily degraded in the body. Chemical modifications are necessary to improve their stability and pharmacokinetic properties.[1]
- Phosphorothioate (PS) modification is one of the most common backbone modifications used in oligonucleotide synthesis to improve stability, biodistribution, and cellular uptake of nucleic acid therapeutics.[2]
- A consequence of introducing PS modifications is that it also creates a chiral center at each modified phosphorous (**Figure 1A**), creating stereoisomers designated as either "*R*p" or "*S*p".[2]
- In traditionally synthesized PS-modified oligonucleotides, the chiral configuration at each PS modification is random (either *R*p or *S*p) (**Figure 1B**). Hence, each PS linkage doubles the number of stereoisomers in the product (2^n, where n=the number of PS modifications).[3]
- At Wave, we design and synthesize oligonucleotides by controlling the orientation of each chiral linkage (**Figure 1B**) to confer improved pharmacological properties.[4] We call these stereopure oligonucleotides.

Figure 1. Phosphorothioate (PS) modifications introduce chiral centers

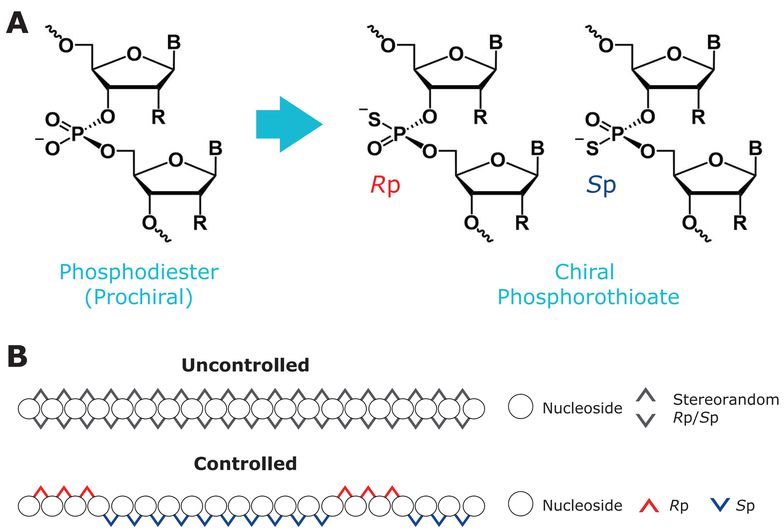
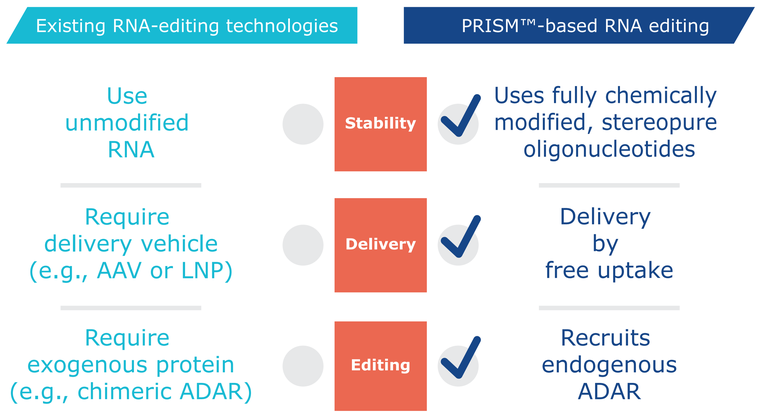


Figure 2. Advantages of Wave's approach to RNA editing with ADAR



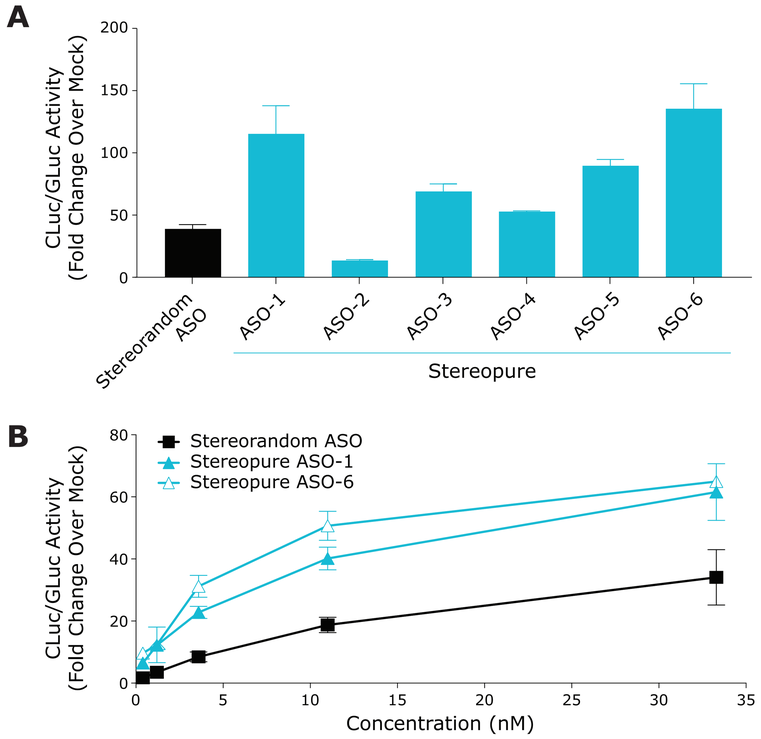
- With PRISM, Wave can generate stereopure ASOs that promote RNA editing with endogenous ADAR enzymes under gymnotic conditions in cellular models.
- Stereopure editing ASOs generated with PRISM have the potential to be a best-in-class RNA-editing modality (**Figure 2**).

Objective

- To determine the effects of optimized stereopure ASOs on ADAR-mediated RNA editing *in vitro*.

Results

Figure 3. *In vitro* RNA-editing activity with stereopure ASOs

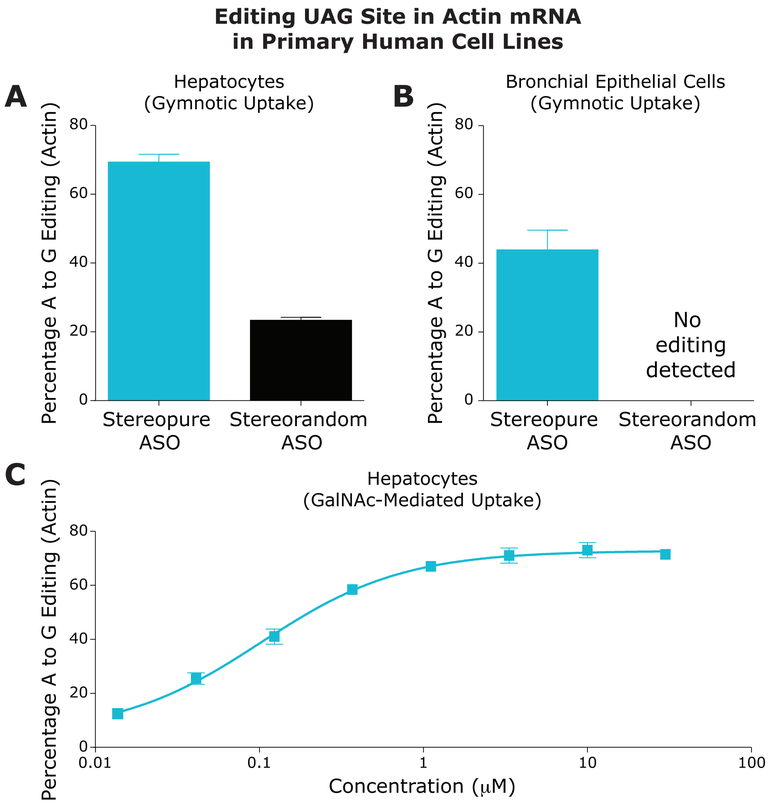


- Stereopure ASOs elicit a range of RNA-editing activity in luciferase assays (**Figure 3A**).
- Optimized stereopure ASOs elicit dose-dependent RNA editing that was more potent than a stereorandom ASO in luciferase assays (**Figure 3B**).

Data presented are mean ± SD. Stereorandom ASO and Stereopure ASOs 1-6 have the same sequence and 2'-ribose modifications. Each stereopure ASO has a unique backbone chirality.

293T cells were co-transfected with a human ADAR expression plasmid and a dual luciferase reporter plasmid expressing *Cypridina* (CLuc) and *Gaussia* (GLuc) luciferases under the same promoter. The CLuc reporter contains a stop codon and can only become active with ADAR-mediated A>I editing. After 24 hours, cells were reverse transfected with 0-35 nM ASO targeting CLuc and luciferase activity was quantified 48-96 hours later. Editing is measured as a ratio between luminescence from CLuc and GLuc, and normalized to a mock-treated sample.

Figure 4. Stereopure ASOs achieve more potent RNA editing with endogenous ADAR than stereorandom ASOs *in vitro*

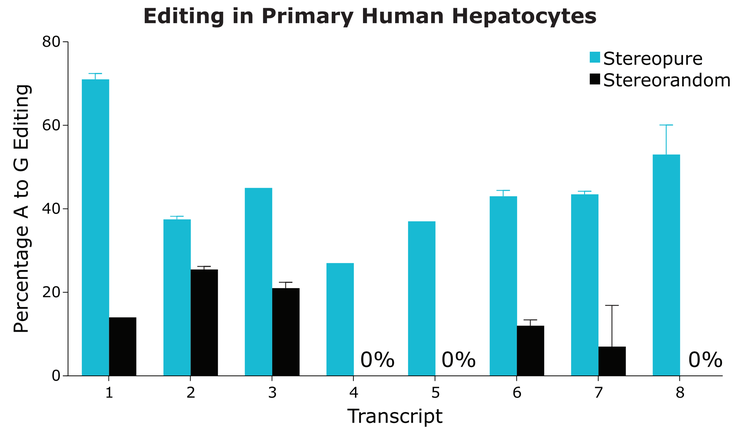


- Stereopure ASOs elicit more potent RNA editing than a stereorandom ASO in primary human hepatocytes (**Figure 4A**) and bronchial epithelial cells (**Figure 4B**).
- A GalNAc-conjugated stereopure ASO induced dose-dependent RNA editing with an EC_{50} of ~100 nM in primary human hepatocytes (**Figure 4C**).

Data presented are mean ± SD. Stereorandom ASO and stereopure ASOs have the same sequence. Each stereopure ASO has a unique backbone chirality.

ASOs targeting a UAG site in *β-actin (ACTB)* mRNA were delivered to primary human cells under gymnotic conditions. After 48 hours, total RNA was harvested, reverse transcribed to generate cDNA, and the editing target site was amplified by PCR. The PCR product was sent for Sanger sequencing, and editing efficiency was calculated using the EditR program.[5] RNA editing with a GalNAc-conjugated stereopure ASO targeting a UAG site in *β-actin (ACTB)* mRNA was evaluated as described above.

Figure 5. Stereopure ASOs achieve greater editing than stereorandom ASOs across multiple RNA transcripts *in vitro*



- Stereopure ASOs achieve greater ADAR-mediated editing compared to stereorandom ASOs across several distinct RNA transcripts in primary human hepatocytes (**Figure 5**), which validates that our technology is applicable across multiple sequences.

Data presented are mean ± SD. For each transcript, stereorandom and stereopure ASOs have the same sequence but differ in backbone chirality.

ASOs targeting several distinct RNA transcripts were transfected into primary hepatocytes. RNA harvesting, cDNA synthesis, Sanger sequencing, and quantification of editing efficiency was completed as described in Figure 4.

References: 1. Wan, W. and Seth, P. (2016). The Medicinal Chemistry of Therapeutic Oligonucleotides. *J. Med. Chem.* 59(21):9645-9667; 2. Eckstein, F. (2014). Phosphorothioates, essential components of therapeutic oligonucleotides. *Nucleic Acid Ther.* 24(6):374-387; 3. Eckstein, F. (2000). Phosphorothioate oligodeoxynucleotides: What is their origin and what is unique about them? *Antisense Nucleic Acid Drug Dev.* 10(2):117-121. 4. Iwamoto, N., et al., (2017). Control of phosphorothioate stereochemistry substantially increases the efficacy of antisense oligonucleotides. *Nat. Biotechnol.* 35(9):845-851; 5. Kluesner MG., et al., (2018) EditR: A method to quantify base editing via Sanger sequencing. *The CRISPR Journal.* 1(3):239-250. **Acknowledgments:** Editorial support was provided by Allison Lord, Amy Donner, and Gene Petrella. Artistic support was provided by Eric Smith. **Disclosures:** All authors are employees of Wave Life Sciences.