030

# Optimized, Stereopure Antisense Oligonucleotides Achieve Broad Tissue Distribution and Excellent Exposure, Enabling Potent and Durable Knockdown of Nuclear *Malat1* in Mice and Nonhuman Primates

Michael Byrne, Luciano Apponi, J. C. Dodart,* Naoki Iwamoto, Tomomi Kawamoto, Fangjun Liu, Yuanjing Liu, Kenneth Longo, Richard Looby, Lauren Norwood, Erin Purcell-Estabrook, Anee Shah, Chikdu Shivalila, Kris Taborn, Vinod Vathipadiekal, Hailin Yang, Yuan Yin, Zhong Zhong, Chandra Vargeese

Wave Life Sciences, Cambridge, MA, USA

## Summary

- Stereorandom antisense oligonucleotides (ASOs) contain large numbers of potentially inactive isomers as well as those that may have unintended biological activities.
- Controlling chirality to generate a single stereopure ASO provides enhanced potency *in vitro* that translates *in vivo* in animal models.
- Wave has developed stereopure ASOs targeting *MALAT1*, a nuclear-enriched, ubiquitously expressed, long noncoding RNA.
- *In vitro*, under free-uptake gymnotic conditions, a *MALAT1*-targeting stereopure ASO had a 24-fold lower half-maximal inhibitory concentration ($IC_{50}$) than a stereorandom ASO with the same sequence and other chemical modifications.
- The potent *MALAT1* knockdown observed *in vitro* under free-uptake conditions translates to *in vivo* animal models.
- Stereopure ASOs are amenable to multiple routes of administration, distribute across multiple tissue types, and penetrate multiple cell types within complex tissues.
- Stereopure ASOs exhibit enhanced potency *in vivo* in mice across multiple tissues.

## Nucleic Acid Therapeutics

### Conventional Oligonucleotide Synthesis

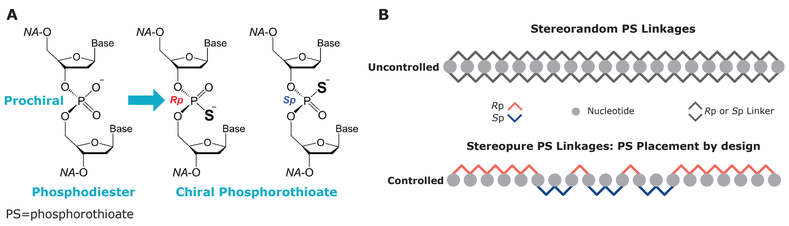
- Nucleic acid therapeutics, including oligonucleotides, are innovative drugs comprising chemically modified, short-length RNA or DNA strands.[1]
- Phosphorothioate (PS) modification is one of the most common backbone modifications used in oligonucleotide synthesis to improve stability, biodistribution, and cellular uptake of nucleic acid therapeutics.[2]
- However, PS modification creates a chiral center at every modified phosphate, and during traditional synthesis, "*Sp*" or "*Rp*" diastereoisomers are randomly incorporated.[3]
- A conventional, fully PS-modified oligonucleotide (20 nucleotides in length, 19 PS modifications) is a mixture of more than 500,000 stereoisomers ($2^n$), each having the same nucleotide sequence but differing in the stereochemistry of its backbone, resulting in heterogeneous and uncontrolled pharmacologic properties.[3]

## Stereopure ASOs

### Wave's Foundational Chemistry

- We can design and synthesize optimized, stereopure ASOs with backbone stereochemistry defined at every PS linkage (**Figure 1**).

Figure 1. PS Modification Creates Chiral Centers




- We explore the relationships among sequence, chemical modification, and backbone stereochemistry to generate optimized, stereopure ASOs with desirable pharmacologic profiles.

## Objective

- Using *MALAT1* as a surrogate target, the hypothesis was tested that controlling the chirality of PS linkages in the backbone to generate stereopure compounds will provide a benefit in potency, distribution, and duration of effect in multiple tissues.
  - *MALAT1* is a single exon gene that is transcribed as a long noncoding RNA; it is specifically enriched in cellular nuclei.

## Methods

- To compare stereopure ASOs with stereorandom ASOs, iCell® Neurons were cultured at 400,000 cells/well.
  - Fresh media with the indicated concentration of ASO was added 48 hours postculture, allowing free uptake of ASO (n=2 per concentration).
  - 4 days after treatment, cells were lysed, RNA was extracted, and *MALAT1* expression was determined using quantitative polymerase chain reaction (qPCR).

### Ocular Delivery

- To assess ocular delivery of ASO, mice were given bilateral intravitreal (IVT) injections of stereopure ASO (0.5, 5, or 50 µg), stereorandom ASO (50 µg), or phosphate-buffered saline (PBS) on day 1.
  - Whole eyes were harvested and flash frozen (at 1 week or 3 months postdose) and were bisected to separate posterior (choroid, sclera, retina, lens) and anterior (iris, cornea) regions.
  - Frozen samples were lysed and processed for RNA, which was quantified using qPCR.
  - Pharmacokinetic (PK) data were generated from ASO-specific hybridization assays in tissue lysates.
- To assess ocular delivery in a primate model, NHPs received bilateral IVT injections of stereopure ASO (45, 150, or 450 µg) or PBS on day 1.
  - Eyes and RNA were processed as described for the mouse model.
  - For histologic evaluation, whole eyes were enucleated, placed in Davidson's fixative for 24 hours, transferred to 70% ethanol, then formalin fixed and paraffin embedded.
  - 5-µm micro sections were evaluated using ViewRNA™ assay for *MALAT1* ASO.

### Central Nervous System Delivery

- To assess central nervous system (CNS) delivery of ASO, mice received a single 100-µg intracerebroventricular (ICV) injection of stereopure ASO or PBS on day 1.
  - At days 2, 7, 28, and 70 postinjection, spinal cord (lumbar) and brain (cortex) were harvested and flash frozen.
  - Frozen samples were lysed, and RNA levels were quantified using qPCR.

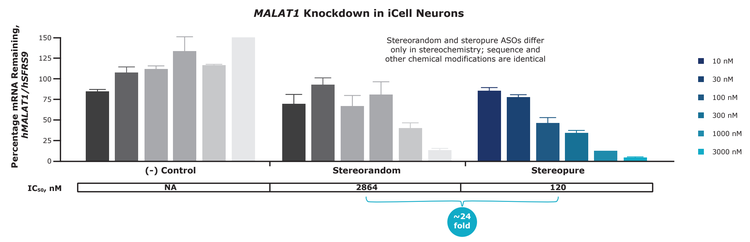
### Systemic Delivery

- To assess systemic delivery of ASO, mice received a single 25-mg/kg subcutaneous (SC) injection of stereopure ASO or stereorandom ASO, or PBS.
  - Muscles were harvested 1 week postdose, dissected, and flash frozen.
  - Frozen samples were lysed and RNA levels were quantified using qPCR.

## Results

- An optimized, stereopure *MALAT1*-targeting ASO decreased $IC_{50}$ 24-fold compared with a stereorandom ASO in cultured iCell Neurons (**Figure 2**).

Figure 2. Effect of an Optimized, Stereopure *MALAT1*-targeting ASO Compared With a Stereorandom ASO in Cultured iCell Neurons

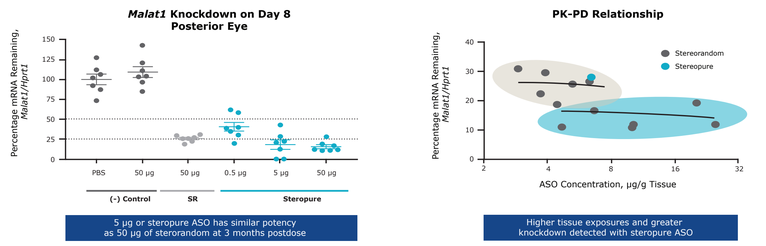



ASO=antisense oligonucleotide; $IC_{50}$=half-maximal inhibitory concentration; NA=not applicable

### Ocular Delivery: Intravitreal Injection

- PK analysis demonstrated that an optimized, stereopure ASO had greater tissue exposure at 7 days post-IVT injection than a stereorandom ASO.
- The PK-pharmacodynamic relationship revealed that the stereopure ASO accumulated to a greater extent in posterior eye and had enhanced potency compared with stereorandom ASO (**Figure 3**).

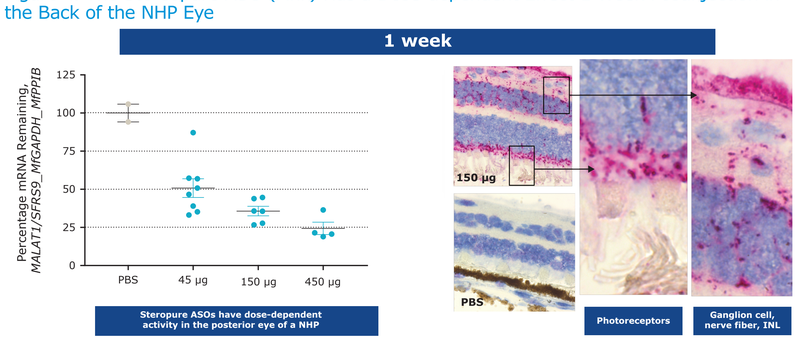
Figure 3. An Optimized, Stereopure ASO Achieves Greater Tissue Exposure and *Malat1* Knockdown 1 Week Postinjection in the Posterior of the Mouse Eye Than the Stereorandom ASO




Each data point represents an individual eye
ASO=antisense oligonucleotide; PBS=phosphate-buffered saline; PK=pharmacokinetic; PD=pharmacodynamic; SR=stereorandom

- Stereopure ASO has dose-dependent activity in the posterior eye of a NHP (**Figure 4**).
  - ASO reaches multiple cell types in the posterior eye, including photoreceptors, ganglion cells, and the inner nuclear layer (**Figure 4**)

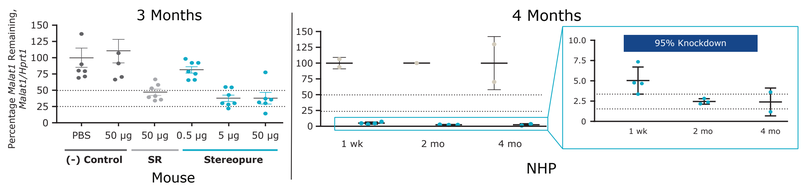
Figure 4. The Stereopure ASO (Pink) Has a Dose-dependent Effect 1 Week Postinjection in the Back of the NHP Eye

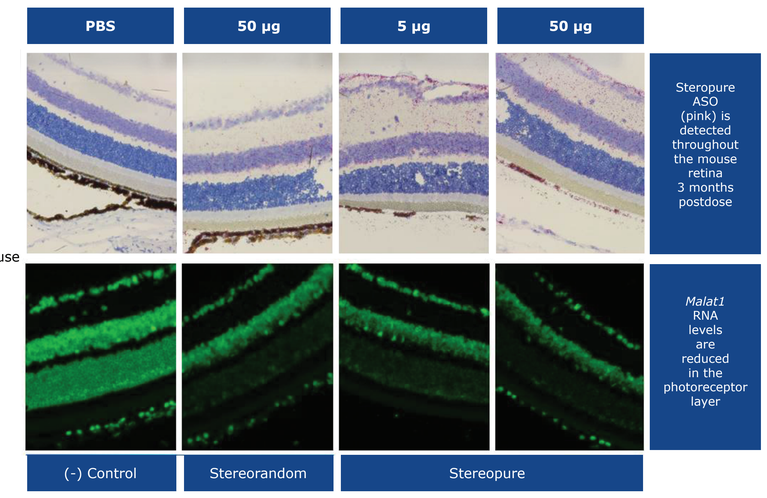



In left panel, each data point represents an individual eye
ASO=antisense oligonucleotide; INL=inner nuclear layer; NHP=nonhuman primate; PBS=phosphate-buffered saline

- A single IVT injection of 5 µg stereopure ASO in mouse results in:
  - Greater *Malat1* knockdown than 50 µg of stereorandom ASO through 3 months in the posterior of the mouse eye (**Figure 5**)
  - Enhanced distribution of ASO throughout the retina, including photoreceptors
  - Reduced *Malat1* RNA throughout the retina, including photoreceptors

Figure 5. A Single IVT Injection of Stereopure ASO Leads to Durable *MALAT1* Knockdown in Mouse and NHP Eyes



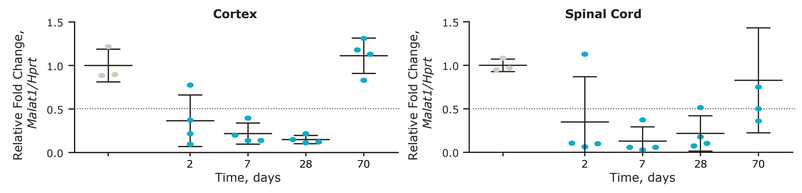

Each data point represents an individual eye
ASO=antisense oligonucleotide; IVT=intravitreal; NHP=nonhuman primate; PBS=phosphate-buffered saline; SR=stereorandom

- A 10-fold lower dose of stereopure ASO is as effective as a stereorandom ASO.
- A single IVT injection of 450 µg stereopure ASO in NHP results in:
  - >95% knockdown of *MALAT1* that persists in retina for at least 4 months (**Figure 5**)
  - Distribution of ASO throughout the eye

### CNS Delivery: ICV and IT Injections

- In the spinal cord and cortex, a single 100-µg dose of stereopure ASO leads to >50% knockdown of *MALAT1* by day 2 (**Figure 6**).
  - This effect is durable, lasting for at least 1 month.

Figure 6. *Malat1* Knockdown Persists in the Mouse CNS for 1 Month Following a Single 100-µg Intracerebroventricular Injection

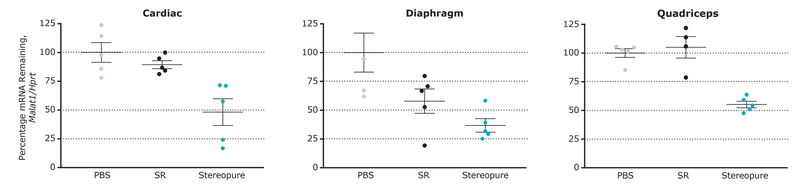



Each data point represents an individual animal
CNS=central nervous system

### Systemic Delivery: SC Injection

- After a single SC injection, stereopure ASO leads to more potent *Malat1* knockdown (>50%) than stereorandom ASO at 1 week in skeletal muscle, heart, and diaphragm (**Figure 7**).

Figure 7. Stereopure ASO Leads to More Potent *Malat1* Knockdown in Muscle than Stereorandom ASO After a Single 25-mg/kg SC Injection




Each data point represents an individual animal
ASO=antisense oligonucleotide; PBS=phosphate-buffered saline; SC=subcutaneous; SR=stereorandom

*Currently at United Neuroscience, Dublin, Ireland

**References:** 1. Evers MM, et al. *Adv Drug Deliv Rev.* 2015;87:90-103. 2. Eckstein F. *Antisense Nucleic Acid Drug Dev.* 2000;10:117-121. 3. Iwamoto N, et al. *Nat Biotechnol.* 2017;35:845-851. **Acknowledgments:** Editorial support was provided by ICON plc (North Wales, PA) and funded by Wave Life Sciences Ltd. **Disclosures:** All authors are employees of Wave Life Sciences Ltd, except J. C. Dodart who is currently an employee of United Neuroscience, Dublin, Ireland.

Presented at Oligonucleotide Therapeutics Society; September 30–October 3, 2018; Seattle, WA

Supported by Wave Life Sciences Ltd, Cambridge, MA, USA.