Stereochemical Control of Antisense Oligonucleotides Enhances Target Efficacy

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Acknowledgements & Disclosures

- All Wave Life Sciences employees
- Prof. Gregory Verdine, co-founder & Director Wave Life Sciences
- Prof. Takeshi Wada, co-founder Wave Life Sciences
- Prof. Matthew Wood, Department of Physiology, Anatomy and Genetics, University of Oxford

- Chandra Vargeese is an employee of Wave Life Sciences

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Forward looking statements

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Architects of transformation

Wave Life Sciences is a clinical-stage, genetic medicines company unlocking the potential of a proprietary chemistry platform that enables the precise design, optimization and production of stereopure nucleic acid therapies.

Wave's chemistry platform is built on a foundation of two core capabilities

PRECISION

Ability to design nucleic acid compounds that have **one defined and consistent profile**

SCALE

Platform potential across **multiple modalities and tissues**

Internal expertise and capacity for **large-scale GMP manufacturing**

Wave has reinvented the design, synthesis and manufacture of nucleic acid therapies to potentially optimize potency, durability and safety

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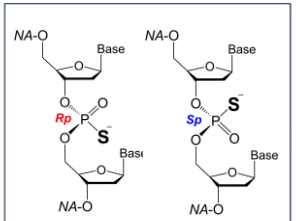
History of oligonucleotide therapeutics

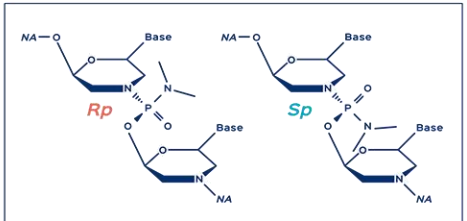
Backbone modifications
- Introduce chiral centers
- Generate mixtures

Sugar modifications

Drug approvals (FDA)



Chiral Phosphorothioate



Chiral Phosphorodiamidite Morpholino (PMO)

Mixtures of 2^n molecules (n=No. of chiral centers) ~500,000 different molecules per dose

1975

Stec WJ, et al. J Am Chem Soc. 1989

Fomiversen

Pegaptanib

2000

Oka N, Wada T, Saigo K. JACS. 2002

Mipomerse

Nusinersen

Eteplirsen

Patisiran

2018

Stereopure ASOs enter clinic

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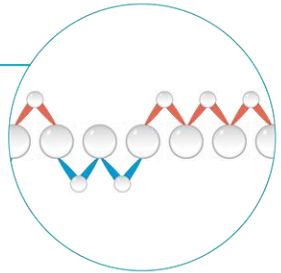
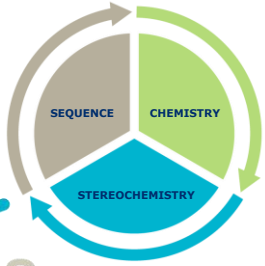
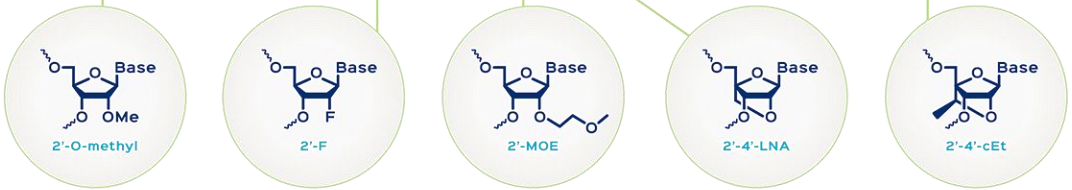
Wave Stereopure ASOs



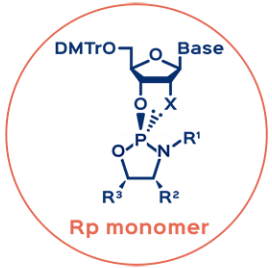


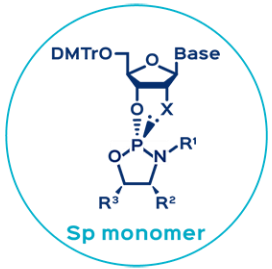


Advances in stereopure oligonucleotide synthesis and manufacturing

Versatility in Chemistry

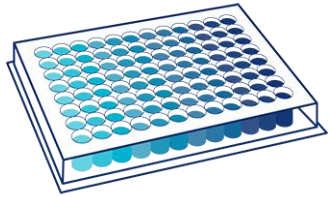
- Improved synthetic capabilities
- Custom building blocks
  - Tunable 'R' groups
  - Various 2'-modifications





Versatility in Scale

Candidate Optimization and Selection

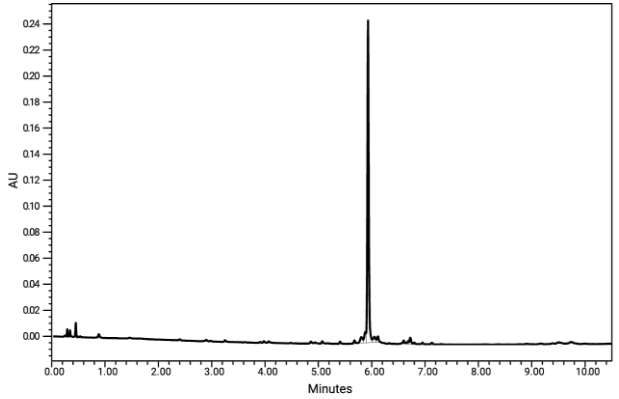


High-throughput scale

GMP Quality

Manufacturing scale

High Crude Purity



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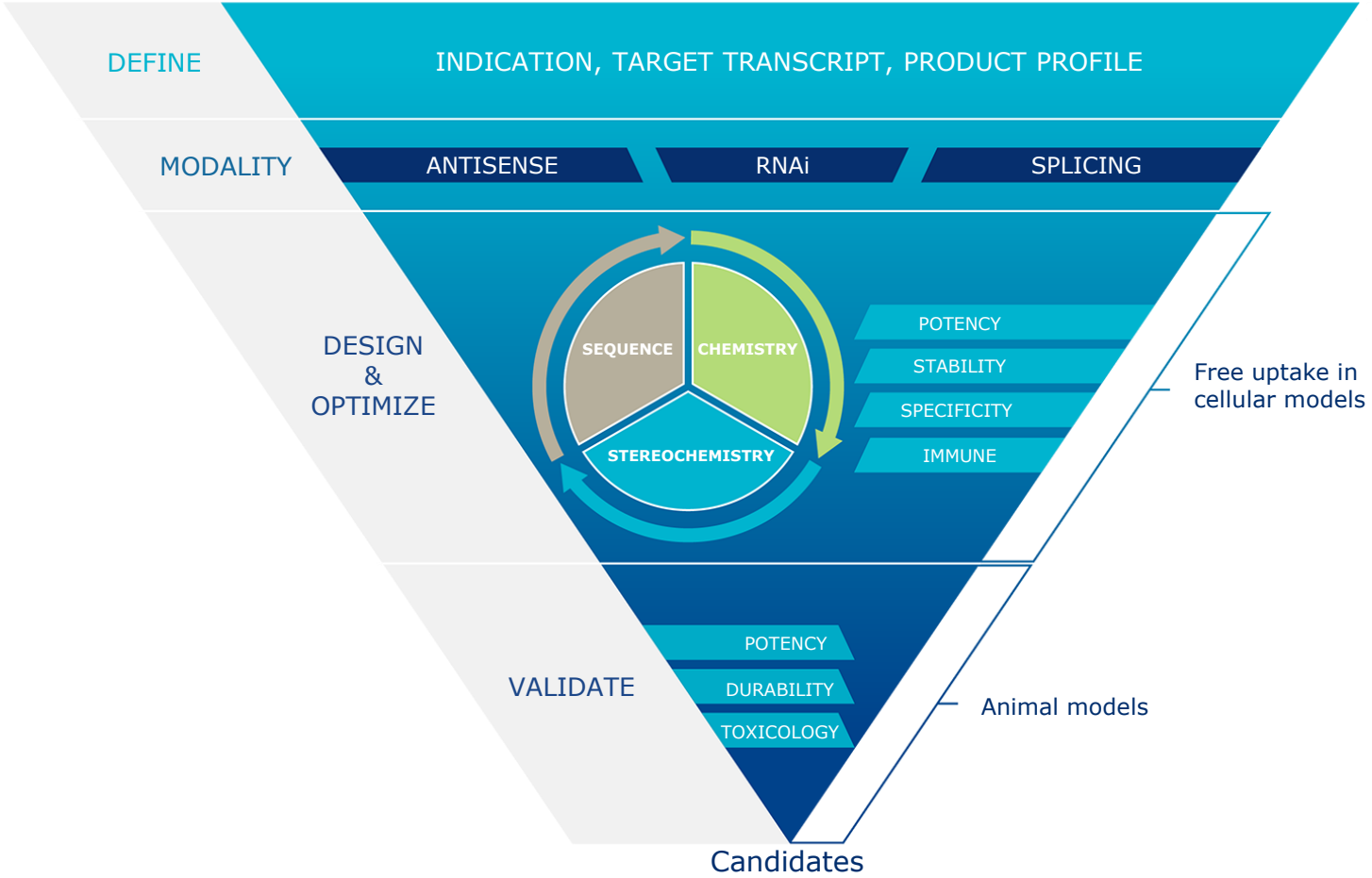
Wave's chemistry platform



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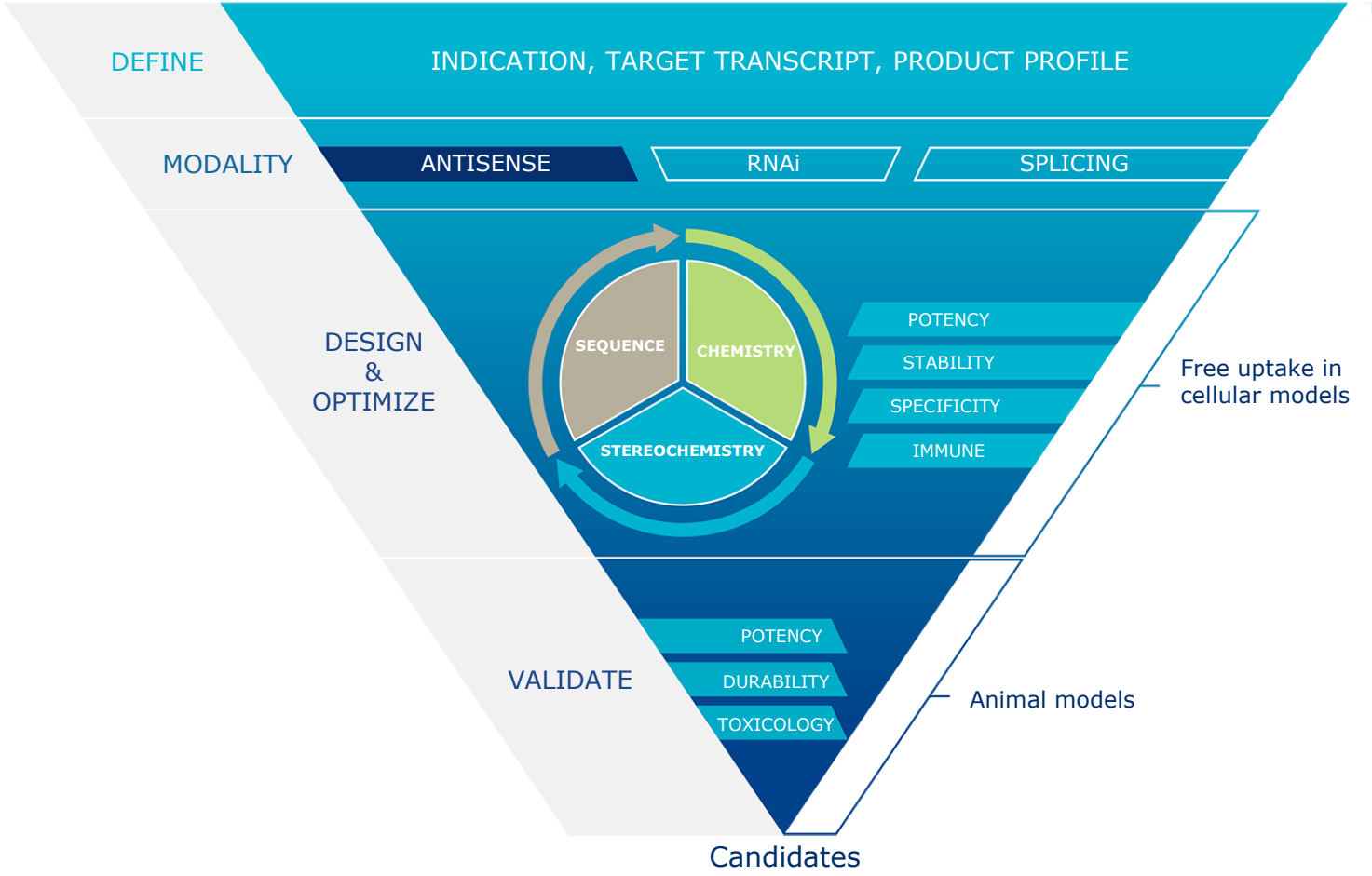
Wave's chemistry platform: Antisense



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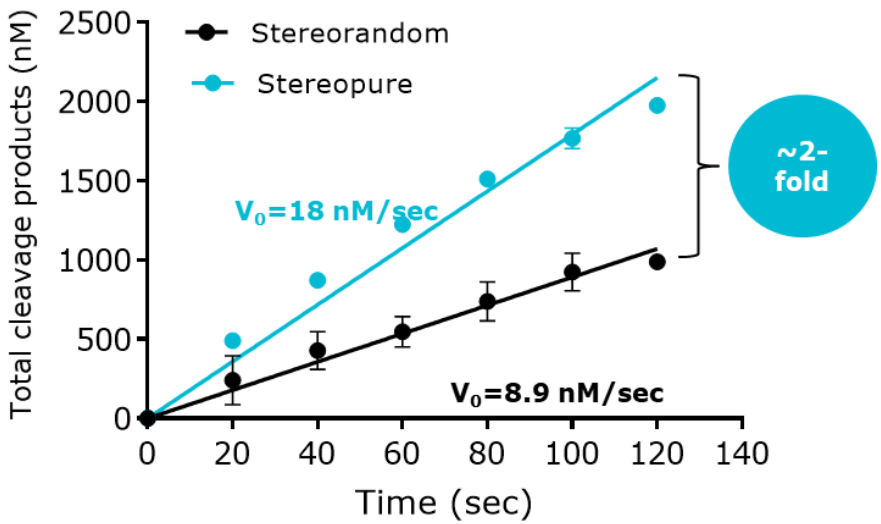
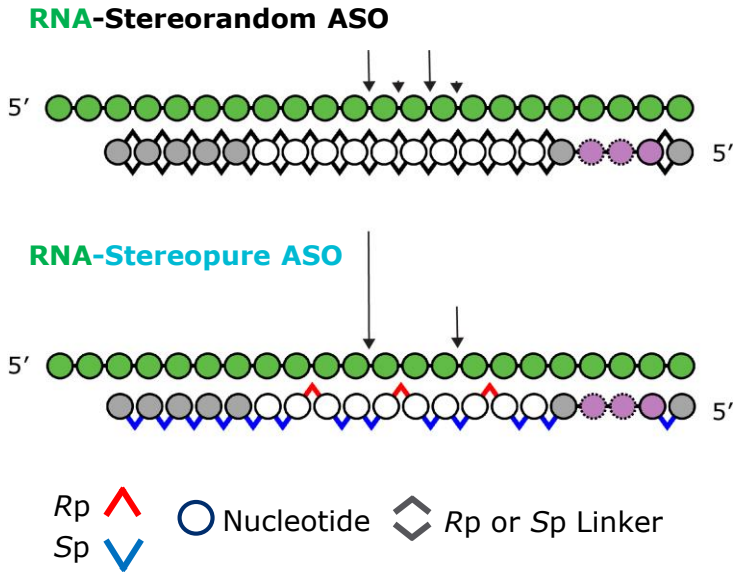
Precision RNase H-mediated RNA degradation

Initial Velocity (V_0)



Cleavage Activity

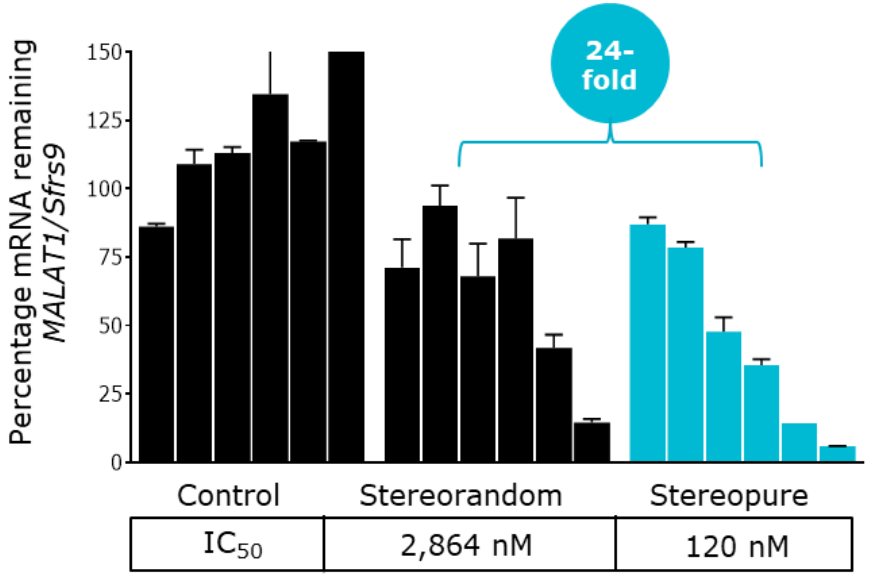


In RNase H1 assay, ASOs were pre-annealed to surrogate *MALAT1* mRNA (1:1, Cf=5 μM). RNase H (250:1, E:S) was added and quenched with EDTA at the indicated times. Products were quantified, and V_0 was calculated from the best-fit line (n=3 per time point).

Potency of stereopure oligonucleotides under *in vitro* free-uptake conditions translates *in vivo*

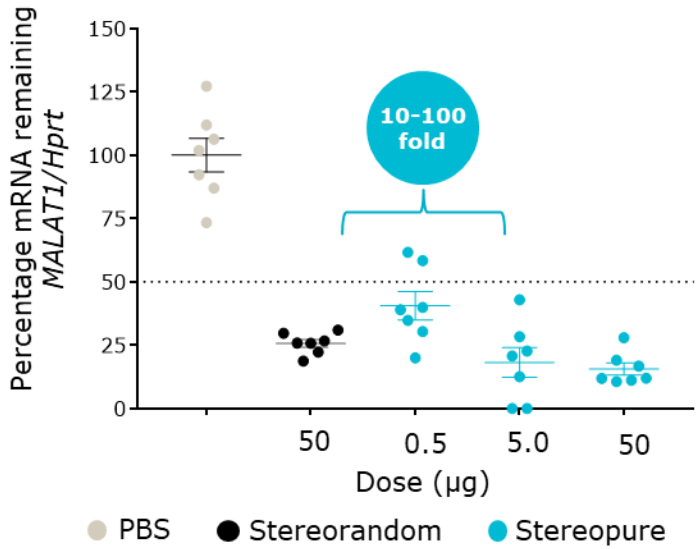
In Vitro

MALAT1 Knockown in iCell Neurons



In Vivo

MALAT1 Knockdown in Posterior Mouse Eye at 1 Week

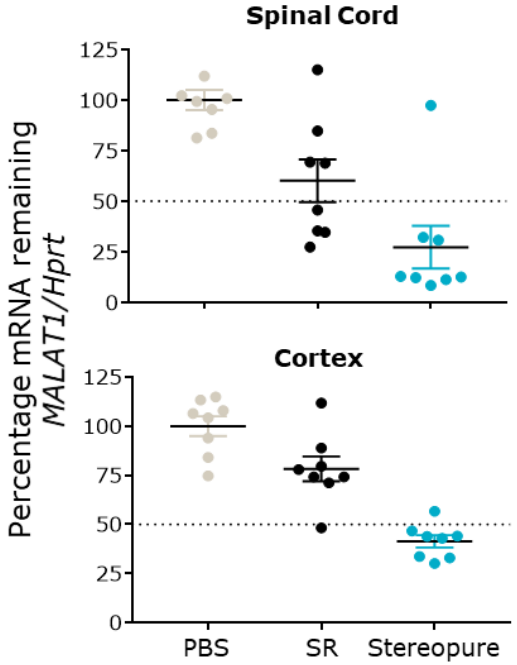


In vitro: In iCell neurons, 10, 30, 100, 300, 1,000 or 3,000 nM ASO was added to iCell neurons under free-uptake conditions. 4-days post-treatment, RNA was harvested and processed. *MALAT1* mRNA expression was determined by qPCR (n=2 per concentration). *In vivo*: Mice received a single IVT injection. 1 week post-injection, tissues were frozen and processed for RNA. *MALAT1* mRNA expression was determined by qPCR (n=7).

Stereopure oligonucleotides enhance potency across tissues *in vivo*

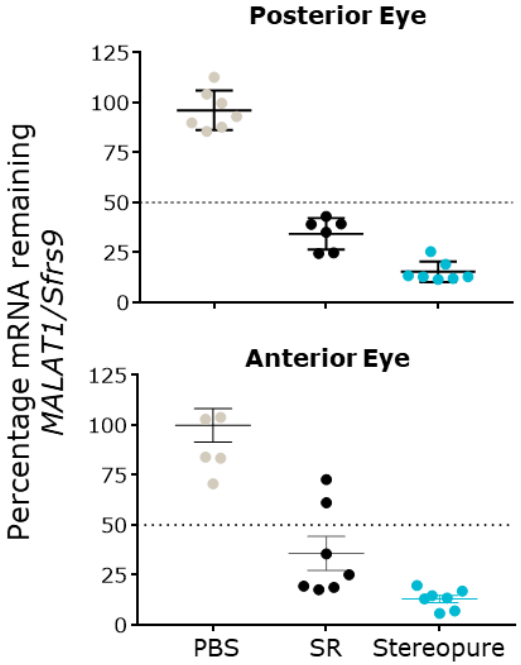
CNS

Single ICV injection in Mice (50 µg)



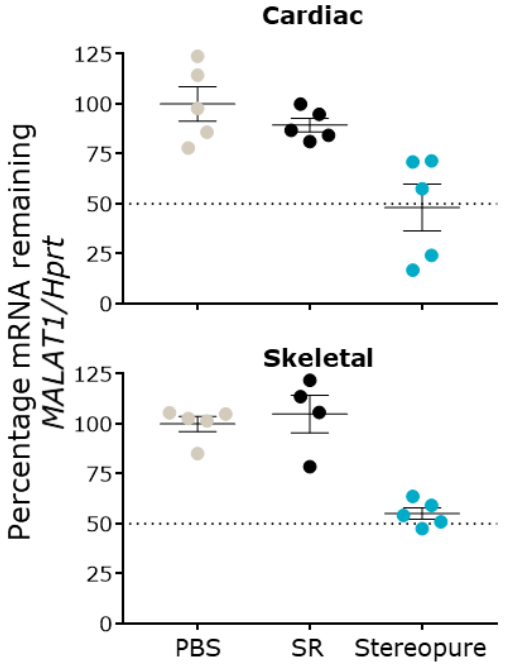
Eye

Single IVT injection in Mice (50 µg)



Muscle

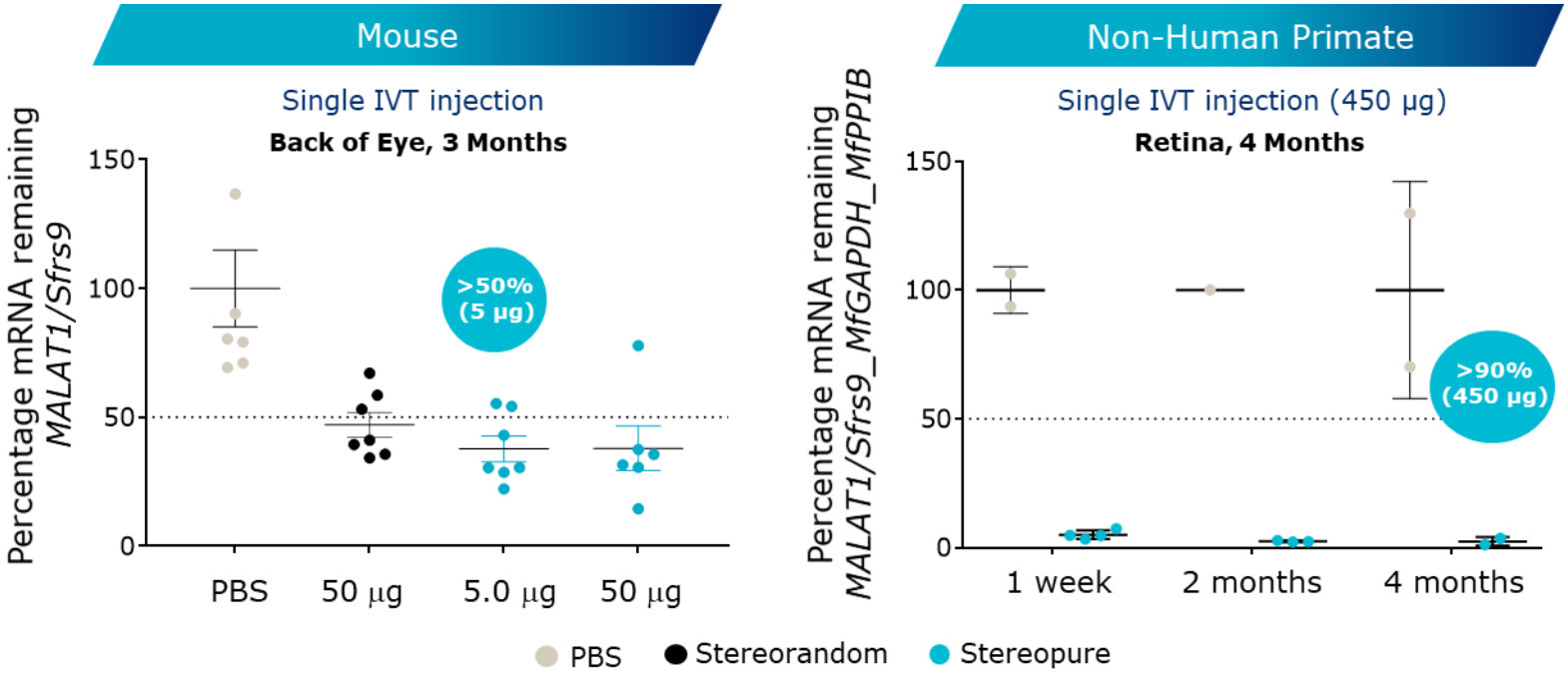
Single SC injection in Mice (25 mg/kg)



Tissues were harvested 1 week post-dose and processed for RNA. mRNAs were quantified by qPCR. Plots show relative fold change or percentage mRNA remaining with respect to control mRNA. Each symbol represents one animal. SR = Stereorandom

Stereopure oligonucleotides induce potent and durable activity in the eye

(OTS poster #030)



9-fold greater eye volume

Tissues were harvested at the indicated time points, post-dose and processed for RNA. mRNAs were quantified by qPCR. Plots show percentage mRNA remaining with respect to control mRNA. Each symbol represents one animal.

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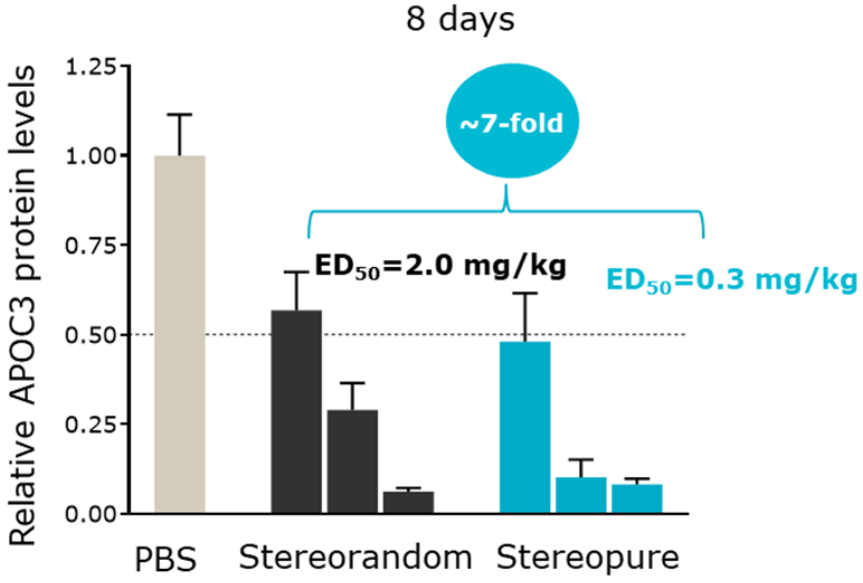
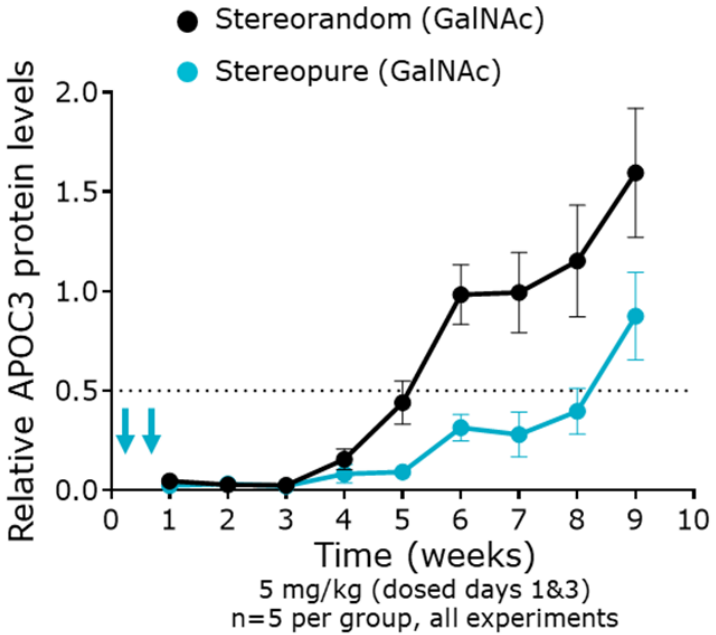
Stereopurity improves potency and durability of GalNAc-conjugated oligonucleotides

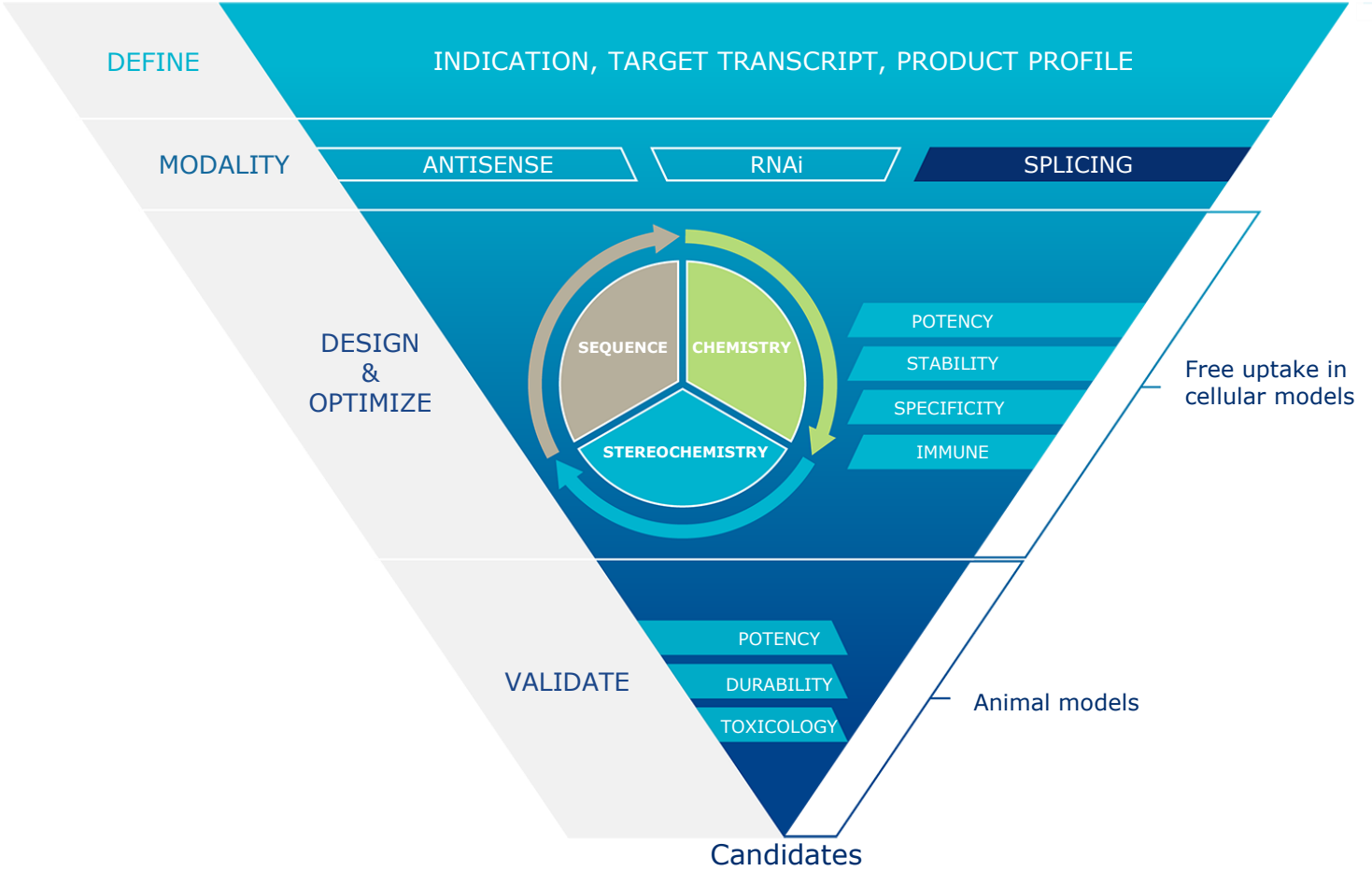
Stereopure ASO yields potency comparable to state-of-the-art GalNAc-dsRNAi



Stereopure ASO yields durable effect in transgenic mice



Wave's chemistry platform: Splicing



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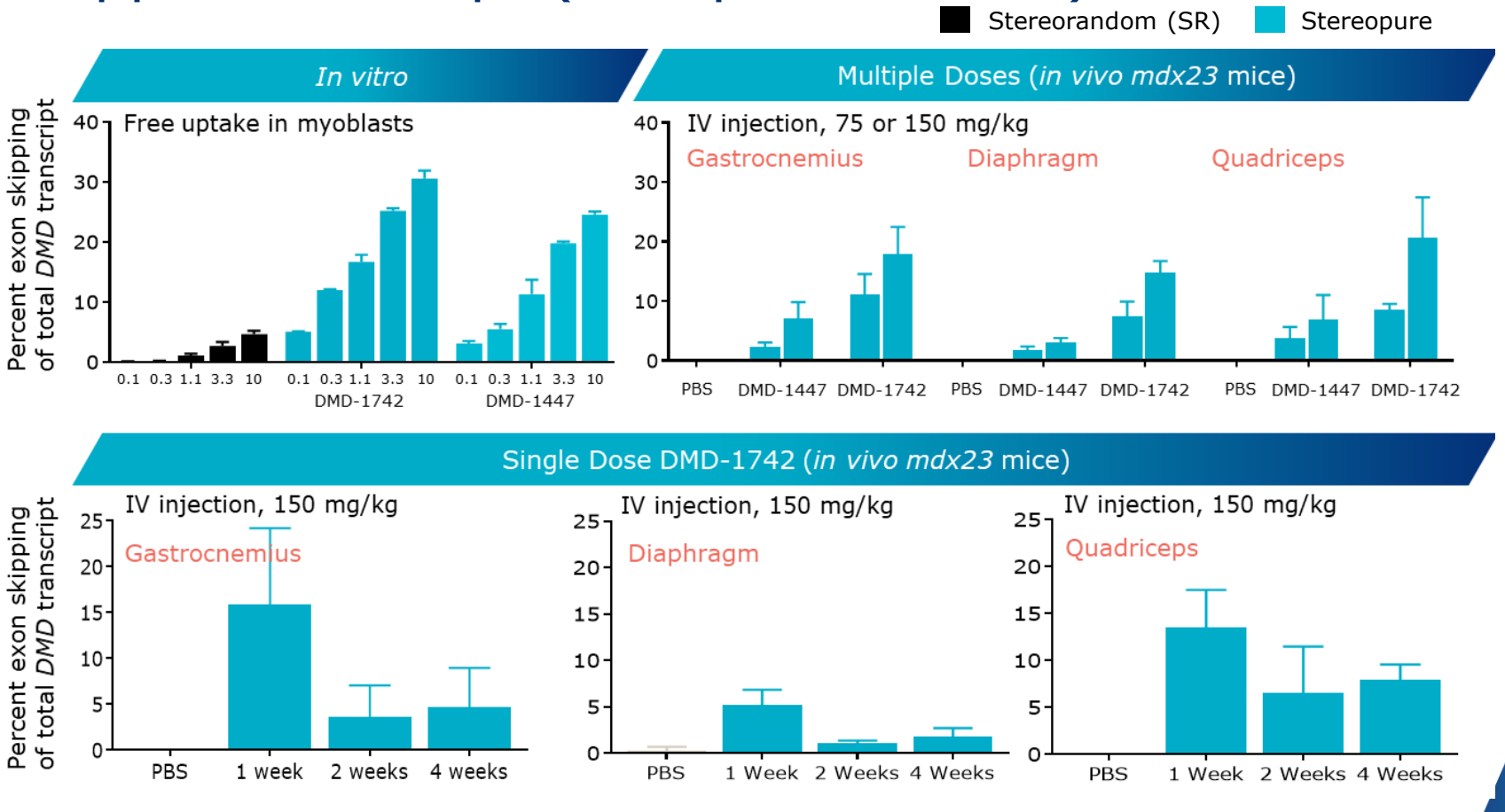
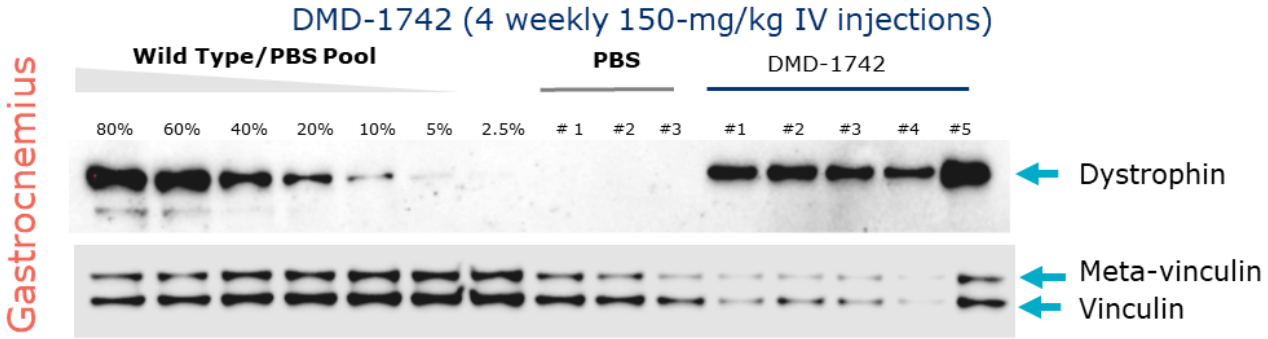
Stereopure oligonucleotides induce exon 23 skipped transcript (OTS poster #119)



Stereopure oligonucleotide induces dystrophin protein restoration and reduces elevated serum enzymes

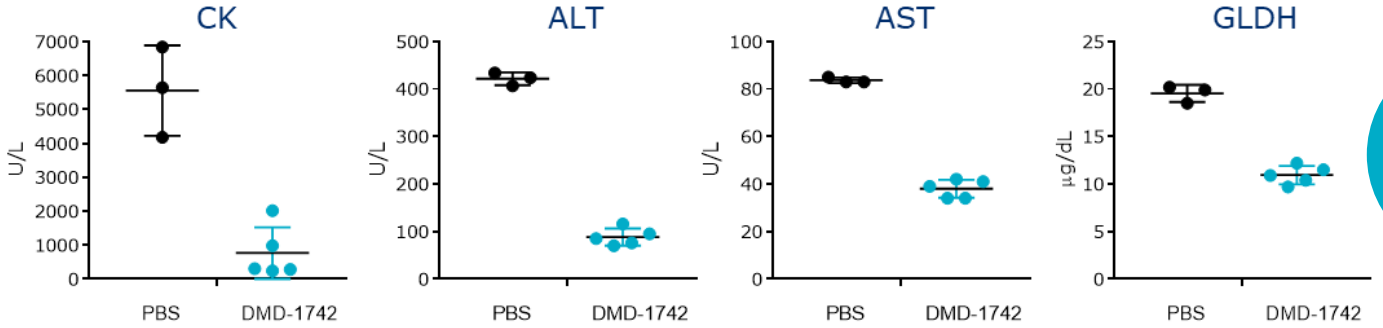
Multiple Doses (*in vivo mdx23* mice)

Dystrophin Protein Restoration



70-90% of natural dystrophin restoration

Serum Enzyme Levels



87% reduction in creatine kinase levels

ALT=alanine aminotransferase; AST=aspartate aminotransferase; CK=creatine kinase; GLDH=glutamate dehydrogenase.
Serum and plasma clinical chemistry were measured with an Olympus AU640 (Olympus America) and the manufacturer's reagents and procedures.

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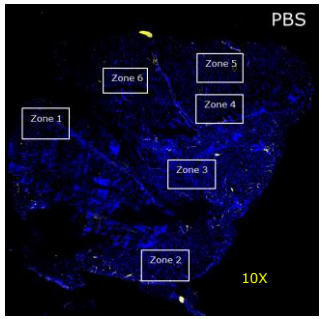
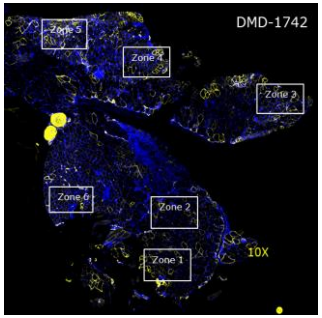
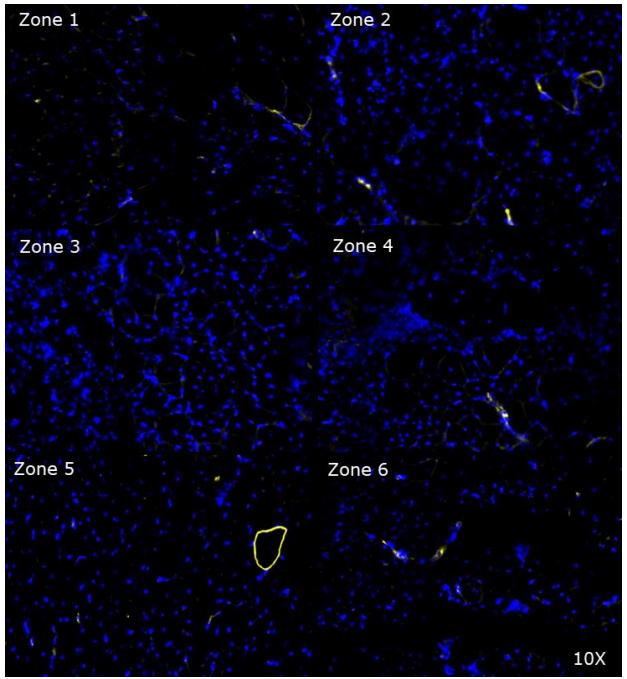
Stereopure surrogate restores dystrophin in muscle fibers after single dose

Immunohistochemistry of dystrophin in gastrocnemius in *mdx23* mice at 4 weeks

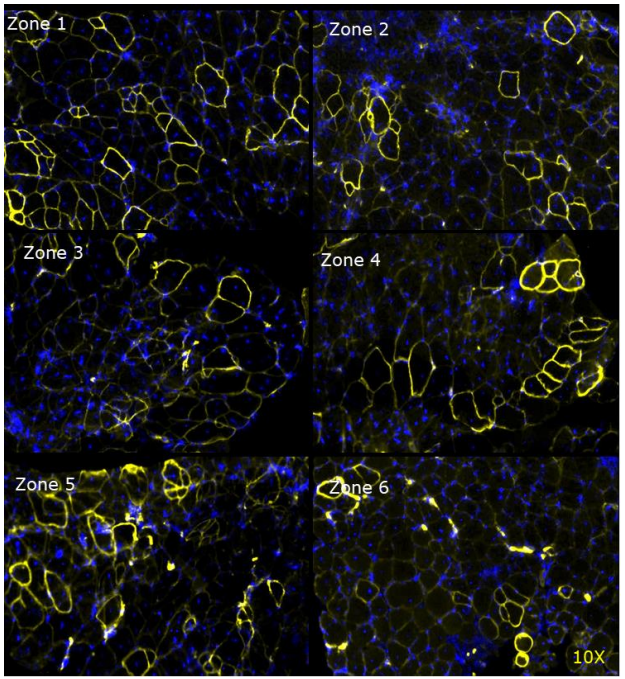




PBS



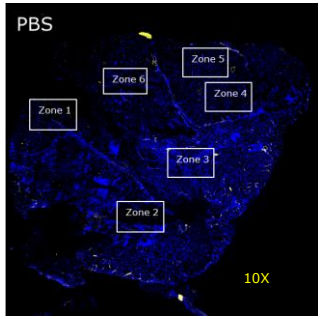
DMD-1742

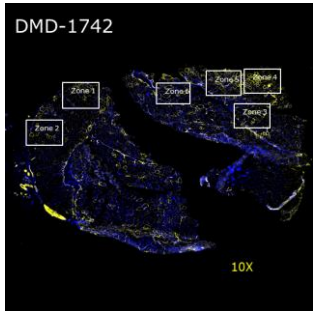


Experimental conditions: *mdx23* mice received a single IV injection of PBS or DMD-1742 (150 mg/kg).
Immunohistochemistry: Blue: Nuclei, Hoechest; Yellow: Rabbit anti-Dystrophin(#ab15277) 1:400 diluent, 555/Cy3, Yellow is a fake color for Cy3.
10X magnification.

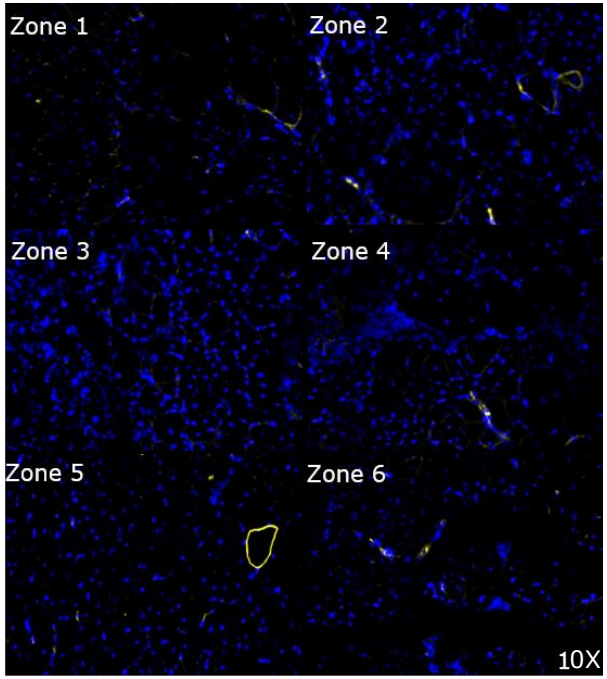
Stereopure surrogate restores dystrophin in muscle fibers after multiple doses

Immunohistochemistry of dystrophin in gastrocnemius in *mdx23* mice at 4 weeks

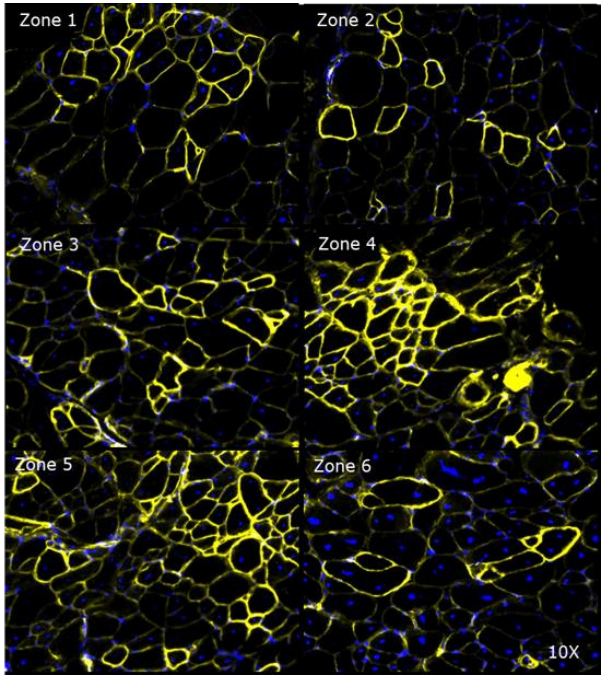




PBS



DMD-1742



Experimental conditions: *mdx23* mice received 4 weekly IV injections of PBS or DMD-1742 (150 mg/kg).
Immunohistochemistry: Blue: Nuclei, Hoechest; Yellow: Rabbit anti-Dystrophin(#ab15277) 1:400 diluent, 555/Cy3, Yellow is a fake color for Cy3.
10X magnification.

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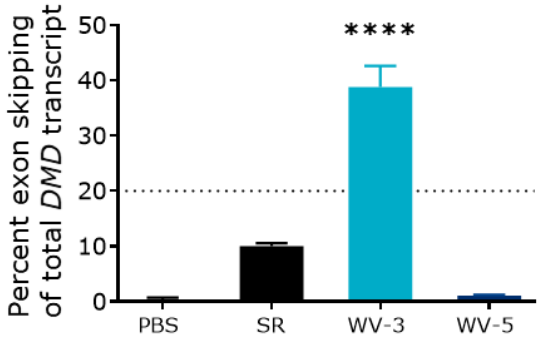
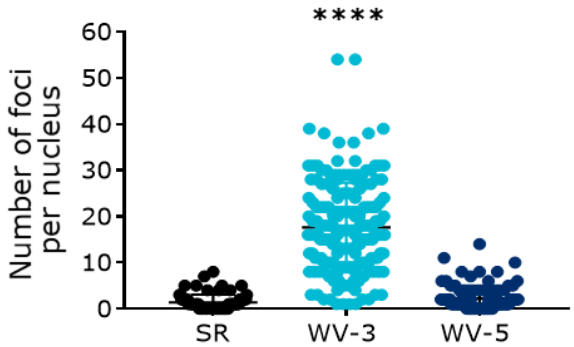
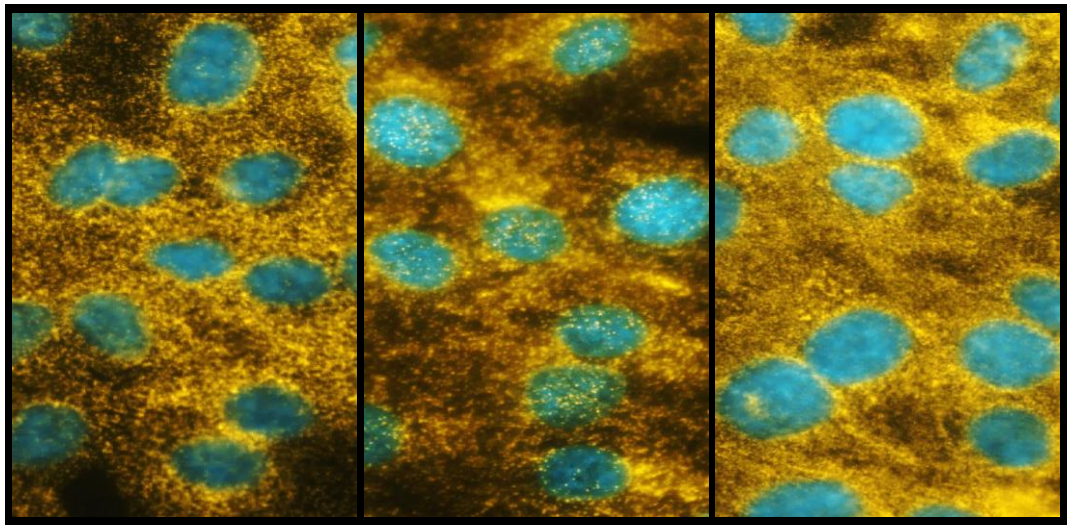
Stereopure oligonucleotide traffics to nuclei in myoblasts





Stereopure ASO enters the nuclei of cultured myoblasts and promotes efficient exon 51 skipping

Stereorandom (SR) Stereopure Stereopure (opposite)



SR WV-3 WV-5

Cultured myoblasts were treated with 10 μM of the indicated ASO under free-uptake conditions. ASO was detected with ViewRNA; nuclei are stained with DAPI. Exon skipping efficiency was quantified by Taqman assay. Nuclear ASO was quantified with ImageJ software (https://imagej.nih.gov/ij/).

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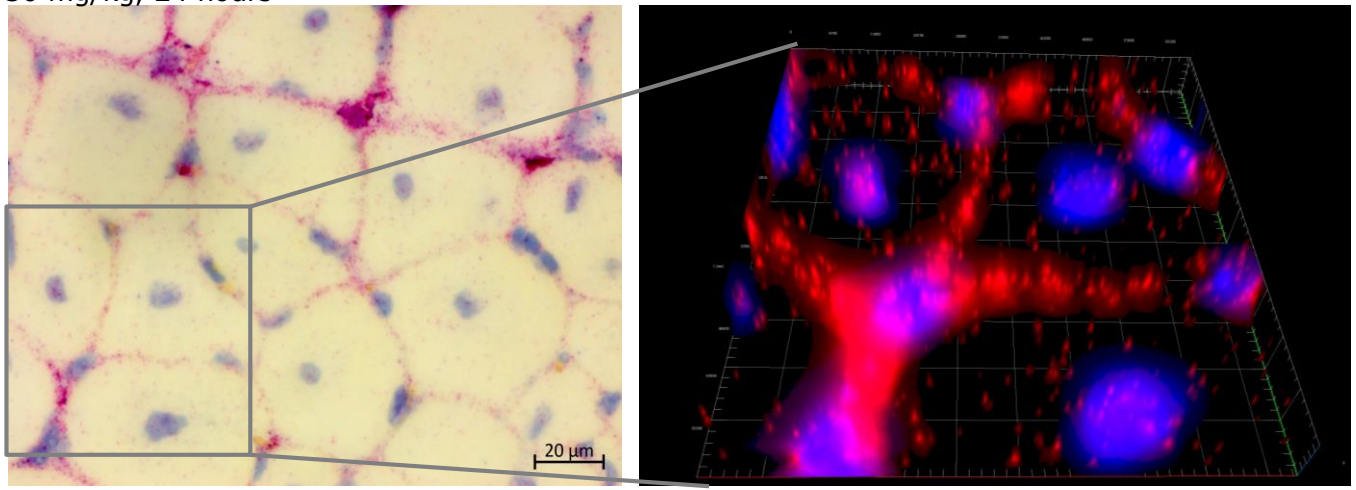
Stereopure oligonucleotides access myofiber nuclei in mice

Stereopure ASO targeting exon 53 rapidly enters myofibers in *mdx23* mice

30 mg/kg, 24 hours



Bright-field view
Nucleus: Hematoxylin (blue)
ASO: ViewRNA (red)

Fluorescence-field view (z stack)
Nucleus: Hoechst33342 (blue)
ASO: Fast Red (pink)

Mdx23 mice were treated with a single 30 mg/kg dose of optimized, stereopure ASO (IV). Tissues were collected 24-hours post-dose. ASO was detected using ViewRNA, and nuclei were stained with Hoechst33342 or hematoxylin.

Summary

- We have developed a scalable process for generating stereopure ASOs
- Compared with stereorandom, stereopure ASOs are:
  - Taken up more readily by cells under gymnotic conditions in multiple cell lines
  - More potent in multiple tissues
  - More durable *in vivo*
- Optimized, stereopure ASOs exhibit improvements in multiple properties:
  - Precision and activity of RNase H
  - Potency correlation between *in vitro* and *in vivo*
  - Exon skipping efficiency
  - Rapid and broad tissue distribution
  - Nuclear uptake

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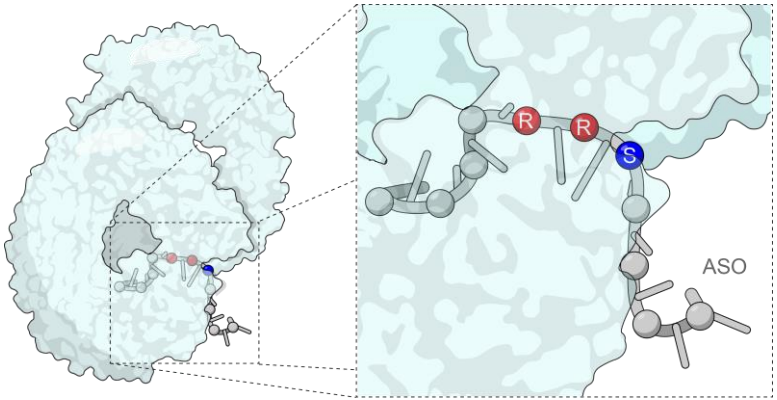
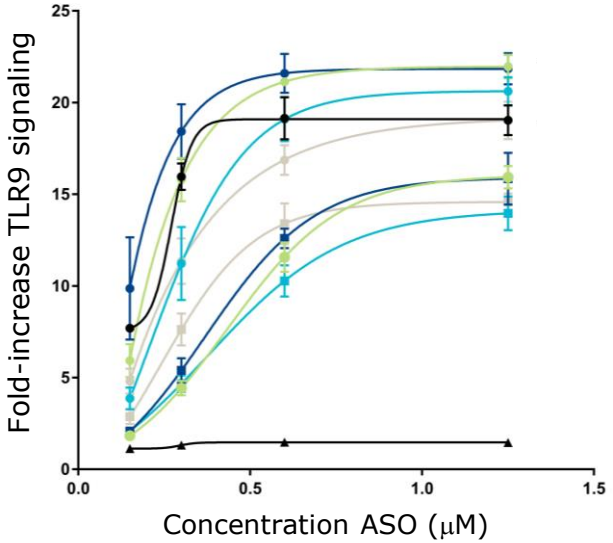
Future: Improving nucleic acid therapeutics through greater understanding of protein-nucleic acid interactions

Understanding innate immune receptor and broader DNA/RNA-protein interactions

TLR9 bound to stereopure, CpG-containing oligonucleotide



Stereochemistry of CpG-containing oligonucleotides impacts TLR9 activity



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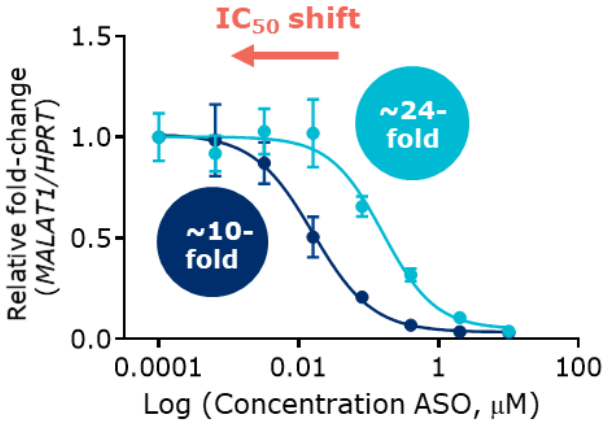
Future: More potent and durable CNS targeting with new chemistries

In vitro potency

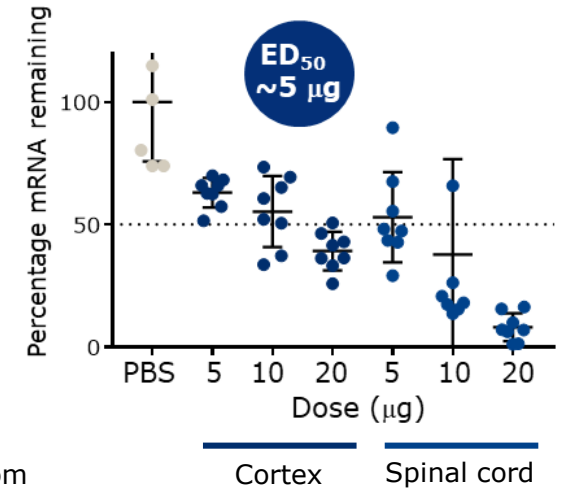
MALAT1 Knockdown in Human iCell Neurons
Under Free-Uptake Conditions



	Stereopure	Stereopure	Stereorandom
IC_{50}	15.9 nM	150 nM	2,900 nM

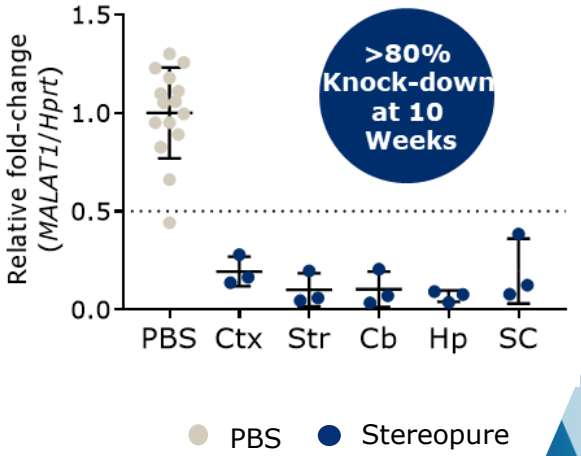
In vivo potency

MALAT1 Knockdown in Mice
1 week after single ICV injection



In vivo durability

MALAT1 Knockdown in Mice
10 weeks after single 100 μg injection



PBS = phosphate buffered saline; Ctx = cortex; Str = striatum; Cb = cerebellum; Hp = hippocampus; SC = spinal cord.