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Cellular Free Uptake of Optimized, Stereopure Antisense Oligonucleotides Predicts Exon-Skipping Efficiency and Dystrophin Protein Restoration in Mice

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Summary

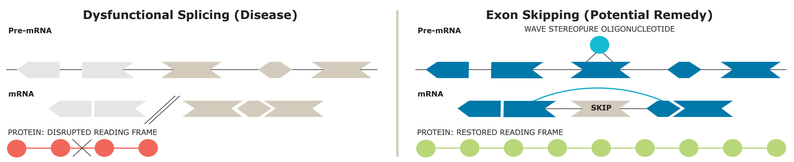
- WVE-210201 is an investigational stereopure oligonucleotide that targets exon 51 in the *dystrophin* (*DMD*) gene to induce efficient production of *DMD* transcript and dystrophin protein restoration. WVE-210201 is being studied in a Phase 1 clinical trial in Duchenne muscular dystrophy (DMD).
- We designed optimized, stereopure oligonucleotides that target exon 23 in the *DMD* gene and yielded potent *DMD* exon skipping in both *in vitro* (free uptake in myoblasts) and *in vivo* (*mdx23* mouse) models.
- In *mdx23* mice, exon skipping and dystrophin protein restoration were observed at 7 days and for at least 28 days following a single intravenous (IV) dose of a stereopure oligonucleotide; 4 weekly IV doses restored dystrophin protein levels to 70%–90% of normal in multiple muscle tissues. Creatine kinase levels were reduced by 87% compared with untreated mice.
- Transcript levels observed with free uptake of olignonucleotides in cultured myoblasts were predictive of transcript and dystrophin protein levels in the *mdx23* mouse model.
- Efficient exon skipping and resulting dystrophin protein restoration are expected to result in therapeutic benefits for patients.
- Results of *in vivo* experiments with exon 23 stereopure oligonucleotides support WVE-210201 as a potentially viable therapeutic approach to DMD.

Background

Exon Skipping Therapeutic Approach to DMD

- DMD is a fatal X-linked neuromuscular disorder in which mutations in the *DMD* gene result in absent or defective dystrophin protein.[1]
- Normal dystrophin protein is part of a protein complex called the dystrophin-glycoprotein complex, which provides structural stability to skeletal muscle and protects the muscle from injury during contraction and relaxation.[2,3]
- Oligonucleotide therapeutics that promote exon skipping may enable production of functional dystrophin (**Figure 1**); partial restoration of dystrophin is expected to result in therapeutic benefits for patients.[4]

Figure 1. Exon-skipping strategy to restore dystrophin expression

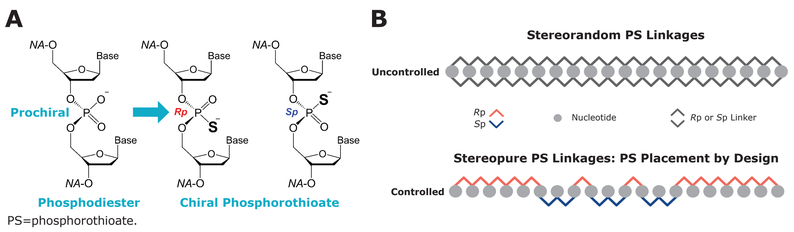


mRNA=messenger RNA.

Stereopure Oligonucleotides

- Phosphorothioate (PS) modification is one of the most common backbone modifications used to improve the stability, biodistribution, and cellular uptake of oligonucleotides.
- PS modification creates a chiral center at every modified phosphate, and during traditional chemical synthesis, "*Sp*" or "*Rp*" diastereoisomers are randomly incorporated, yielding uncontrolled, complex mixtures of stereoisomers (**Figure 2A**).
- Wave has established a targeted approach to designing stereopure nucleic acid compounds based on the interplay among sequence composition, chemical design, and backbone stereochemistry.
- Wave's nucleic acid compounds are different because they are designed to be stereopure, defined as precisely controlled chirality of each PS inter-nucleotide backbone linkage during chemical synthesis (**Figure 2B**).

Figure 2. Wave's chemistry platform generates optimized stereopure oligonucleotides

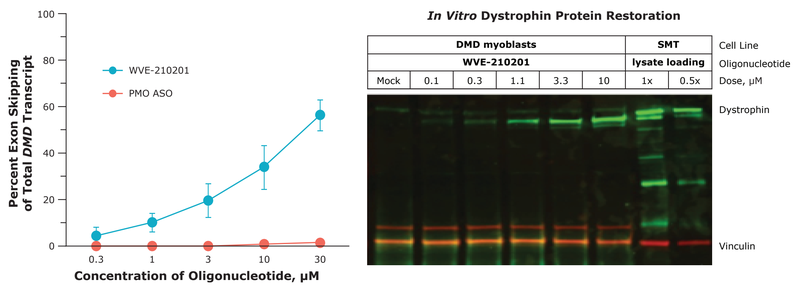


PS=phosphorothioate.

WVE-210201

- WVE-210201 is an investigational, stereopure oligonucleotide currently being evaluated in a Phase 1 clinical trial as a potential treatment for DMD; it is designed to promote skipping of exon 51 and production of a truncated dystrophin protein.
- WVE-210201 induced more efficient exon 51 skipping than an optimized phosphorodiamidate morpholino antisense oligonucleotide (PMO ASO) in Δ48–50 patient-derived myoblasts (**Figure 3,** left panel).
- WVE-210201 dose-dependently restored dystrophin protein expression (**Figure 3,** right panel).

Figure 3. WVE-210201 induced dose-dependent exon skipping and dystrophin restoration in patient-derived myoblasts in culture under free-uptake conditions



PMO ASO=phosphorodiamidate morpholino antisense oligonucleotide; SMT=human skeletal muscle lysate.

Objective

- To determine the effects of optimized stereopure oligonucleotides targeting *DMD* exon 23 on exon skipping and dystrophin protein restoration in the *mdx23* mouse model.

Methods

In Vitro Skipping and Dystrophin Protein Expression

- Cultured DMD patient-derived myoblasts or murine *mdx23*-derived myoblasts were treated with oligonucleotide under free-uptake conditions in differentiation media.
- After 4 days of oligonucleotide treatment, RNA was extracted with Trizol and skipping efficiency was evaluated by TaqMan® assays using custom primer-probe sets specific for the exon junction of skipped *DMD* transcripts or total *DMD* transcripts, normalized to an internal control (SFSR9) for human myoblasts or to gene-block standard curves for murine myoblasts.
- After 6 days of oligonucleotide treatment, total protein lysate was analyzed by western blot. Dystrophin signal was normalized to vinculin loading control and compared to healthy human skeletal muscle lysate.

In Vivo Exon 23 Skipping, Dystrophin Protein Expression, and Serum Enzyme Profiles in *mdx23* Mice

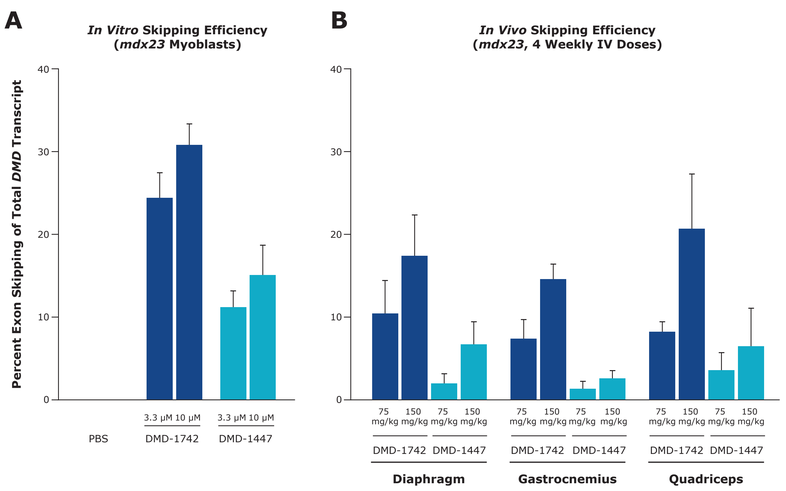
- Treatment of *mdx23* mice was conducted as follows:
  - Single IV dose 150 mg/kg of oligonucleotide, necropsies at 1, 3, 7, 14, and 28 days postdose
  - Four weekly IV doses of 75 mg/kg or 150 mg/kg of oligonucleotide, necropsy at 4 days after the last dose
  - Tissues were fresh frozen for RNA skipping analysis (as above) or western blot analysis (as above) or microscopy. Quantification of *in vivo* dystrophin was performed according to a standard curve of CD1 wild-type mouse gastrocnemius lysate titrated into untreated *mdx23* gastrocnemius lysate. Immunofluorescent microscopy was performed with a polyclonal rabbit anti-dystrophin and DAPI nuclear stain.
  - Serum and plasma clinical chemistry were measured with an Olympus AU640 (Olympus America) and the manufacturer's reagents and procedures, at Charles River Laboratory.

Results

Potency of Stereopure Oligonucleotides *In Vitro* and *In Vivo*

- Stereopure oligonucleotides targeting murine exon 23 (DMD-1742 and DMD-1447) were designed and synthesized to determine *in vivo* exon skipping and dystrophin protein restoration in the *mdx23* mouse model of DMD.
- Stereopure oligonucleotides targeting exon 23 induced dose-dependent exon 23 skipping *in vitro* in *mdx23*-derived myoblasts (**Figure 4A**) and in target muscle tissues *in vivo* in *mdx23* mice, including gastrocnemius, diaphragm, and quadriceps following 4 weekly IV doses (**Figure 4B**).
- Stereopure oligonucleotides induced dose-dependent increases in exon 23 transcript, and the pattern was similar between *in vitro* and *in vivo* models.

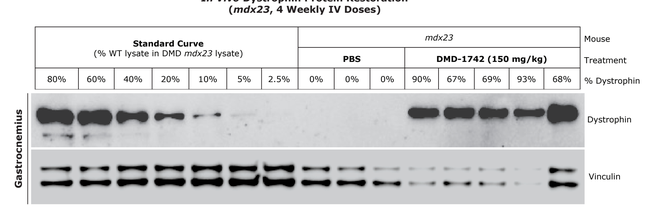
Figure 4. Potency of stereopure oligonucleotides targeting exon 23 *in vitro* and *in vivo*



IV=intravenous; PBS=phosphate-buffered saline.
Exon 23 skipping was analyzed by TaqMan in (A) *mdx23*-derived myoblasts dosed with stereopure oligonucleotides under free-uptake conditions and in (B) *mdx23* mice treated with 4 weekly IV doses, sacrificed at 4 days after the last dose.

- Stereopure oligonucleotides targeting exon 23 induced dystrophin protein restoration in target muscle tissues, including gastrocnemius (**Figure 5**), diaphragm, quadriceps, and heart (data not shown) following 4 weekly IV doses.

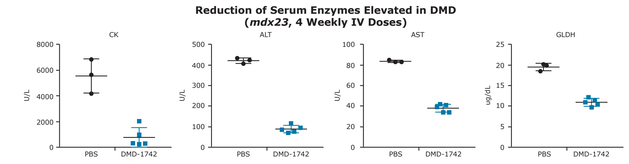
Figure 5. Efficient exon 23 skipping yielded substantial dystrophin restoration in *mdx23* mice



IV=intravenous; PBS=phosphate-buffered saline; WT=wild type.
Dystrophin quantification by western blot of the gastrocnemius muscle of mice treated with 4 weekly IV doses, sacrificed at 4 days after the last dose.

- Stereopure oligonucleotides targeting exon 23 reduced serum enzymes elevated in DMD. Creatine kinase (CK) levels were reduced by 87%.

Figure 6. Reduction of Serum Enzymes Elevated in DMD in *mdx23* mice

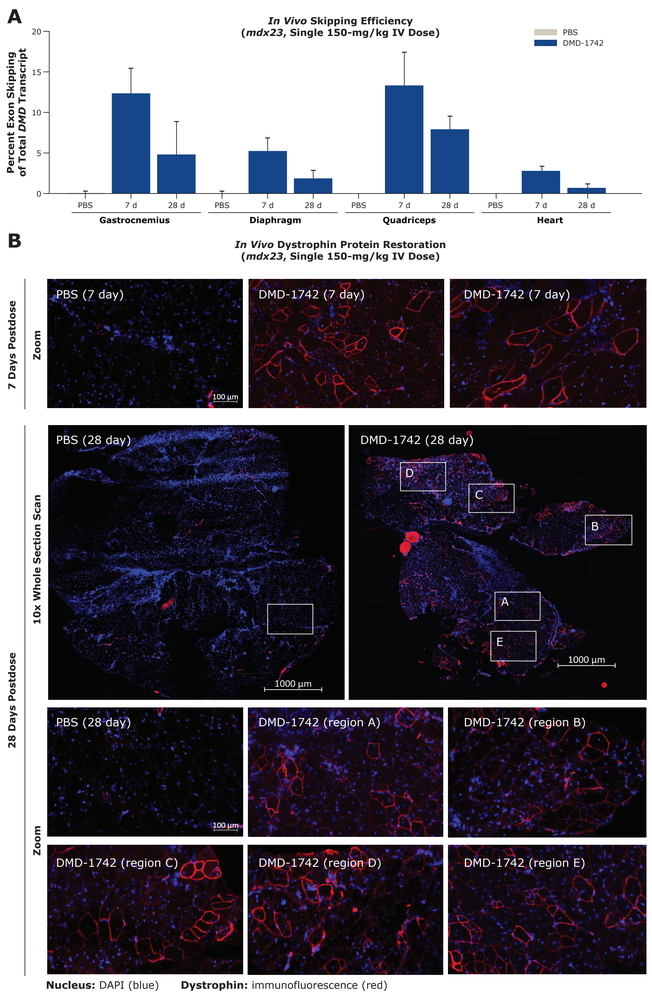


ALT=alanine aminotransferase; AST=aspartate aminotransferase; CK=creatine kinase; GLDH=glutamate dehydrogenase

Potency and Durability of Stereopure Oligonucleotides *In Vivo*

- Single IV doses of stereopure oligonucleotides induced efficient exon 23 transcript in diaphragm, gastrocnemius, quadriceps, and heart that was observed for at least 28 days after administration (**Figure 7A**).
- Single IV doses of stereopure oligonucleotides resulted in myofiber membrane localization of restored dystrophin for at least 28 days postdose in gastrocnemius tissue, as would be expected for functionally restored dystrophin (**Figure 7B**).

Figure 7. Durability of effect of stereopure oligonucleotide targeting exon 23 *in vivo* following single IV dose



IV=intravenous; PBS=phosphate-buffered saline.
(A) Exon skipping in indicated tissues analyzed by TaqMan.
(B) Microscopy of dystrophin expression and membrane localization in gastrocnemius cross-section.
Blue=nuclei (DAPI stain); red=dystrophin (rabbit anti-dystrophin); 10× magnification.

References: 1. Mah JK. *Neuropsychiatr Dis Treat.* 2016;12:1795-1807. 2. Kole R, Krieg AM. *Adv Drug Deliv Rev.* 2015;87:104-107. 3. Wallace GQ, McNally EM. *Annu Rev Physiol.* 2009;71:37-57. 4. Verhaart IE, et al. *Mol Ther Nucleic Acids.* 2014;3:e148. **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.

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

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