Drexel's splice rack

By Lauren Martz, Staff Writer

Previous studies of antisense oligonucleotides geared at fixing aberrant *SMN2* splicing to treat spinal muscular atrophy have shown either increases in the key disease-related protein or phenotypic improvements in the disease,¹ but never both. Now, a team at **Drexel University College of Medicine** thinks it has the first data conclusively showing that correcting *SMN2* splicing with antisense directly leads to better outcomes in mice.²

Spinal muscular atrophy is an autosomal recessive neuromuscular disorder that is caused by a mutation or deletion in *survival of motor neuron 1 telomeric (SMN1; SMN)*. The result is reduced expression of SMN protein, which leads to progressive loss of spinal α -motor neurons and muscular atrophy that can cause infant death.

SMN2 is almost identical to *SMN1*, differing in only a single nucleotide base. Whereas *SMN1* encodes the full-length SMN protein, *SMN2* predominantly encodes a truncated version with exon 7 spliced out and only produces very low levels of functional SMN. Thus, one proposed strategy for treating spinal muscular atrophy involves antisense oligonucleotides that prevent the alternative splicing in *SMN2* transcription.³

In a paper published in the *Journal of Neuroscience*, the Drexel group built on previous *in vitro* work with an antisense oligonucleotide. Now, using mice, the team showed that the oligonucleotide not only increased expression of full-length SMN, but also improved motor deficits caused by spinal muscular atrophy.

In *SMN1*^{-/-} mice expressing a homolog of the human *SMN2* gene, intracerebroventricular delivery of the oligonucleotide on postnatal days 1, 3, 5, 7 and 10 prevented the gene's aberrant splicing and restored SMN protein levels in brains and spinal cords to 50% of those in wild-type mice. In untreated transgenic mice, SMN protein levels were about 15% of those in healthy controls.

The oligonucleotide also increased body weight and improved righting response, a measure of improvements in motor defects, compared with a scrambled antisense oligonucleotide control.

The team was led by Gordon Lutz, associate professor in the Department of Pharmacology and Physiology at Drexel.

"The two questions we were addressing with this work were: did the powerful stimulatory effects of the oligonucleotide on full-length SMN expression shown *in vitro* translate into animals, and did it ameliorate spinal muscular atrophy pathology," Lutz told *SciBX*. "I believe our paper provides compelling affirmative evidence in both cases, and therefore that this oligonucleotide should be further explored and developed with the ultimate goal of clinical trials in patients with spinal muscular atrophy."

Meg Winberg, director of discovery research at the **Spinal Muscular Atrophy Foundation**, noted that several labs are working on antisense molecules for the disease. Many, she said, are addressing "what is the optimal nucleotide sequence and length, plus backbone chemistry of an oligonucleotide to promote exon 7 inclusion. This study addresses a different question, namely, strategies for administering the oligonucleotides within a living organism and promoting its distribution to the cells that need it most."

The foundation funded the study that was published in the *Journal of Neuroscience*.

Advancing SMA treatment

With data in hand showing that an *SMN2* oligonucleotide produced both molecular and phenotypic changes, the next hurdles relate to timing and delivery.

"One might argue that oligonucleotide delivery beginning on postnatal day 1 will be before neuromuscular deficits have had time to develop, because the neuromuscular junction in mice completes development postnatally," said Rebecca Pruss, CSO at **Trophos S.A.**

Indeed, Pruss said it is possible the antisense oligonucleotide was actually preventing the onset of spinal muscular atrophy rather than reversing the disease. "It would be interesting to show that the phenotype could be reversed or even stabilized when administered after defects are seen in a less severe model of spinal muscular atrophy—however, these studies are not feasible with the currently available models of spinal muscular atrophy that display a severe phenotype beginning around birth," she said.

Trophos' olesoxime (TRO19622), a small molecule with cholesterol-like structures that interacts with the mitochondrial permeability transition pore (MPTP), is in Phase IIa testing to treat the disease.

Pruss also noted that although the study showed positive results up to 12 days after birth, only one dose and one administration strategy were tested, suggesting that the best approach has yet to be identified. "While functional improvements were noted, no histological or electrophysical correlates were reported," she added.

Pruss also said that direct delivery of the antisense oligonucleotide into the brain may not produce the best results. "Delivery was restricted to the central nervous system, but decreased SMN in muscle may also play a role in spinal muscular atrophy," she said. "Both systemic and central nervous system delivery may be necessary to provide maximum benefit."

Lutz said the inability of the oligonucleotides to cross the blood brain barrier is a double-edged sword. "Although the inability of oligonucleotides to readily cross the blood brain barrier is certainly in some cases viewed as a drawback," he said, "if you do deliver the oligonucleotides directly into the cerebral spinal fluid, they are not likely going anywhere else, which prevents off-target systemic effects."

The Drexel team does not own the antisense molecule described in the *Journal of Neuroscience* article—it was patented by the **University of Massachusetts Medical School**—and thus does not plan to move forward with it.

Ravindra Singh, associate professor in the Department of Biomedical Sciences in the College of Veterinary Medicine at **Iowa State University**, was previously at the University of Massachusetts Medical School and part of the team that developed the antisense molecule.

Singh told *SciBX* that his current team at Iowa State has generated a series of new compounds that may be as effective, or more effective, than the previously developed antisense compound in spinal muscular atrophy. Iowa State has filed patents on the newer compounds and is interested in industrial collaborations.

Broad indications

Drexel's work has implications beyond a single molecule or spinal muscular atrophy, according to Philip Caldwell, associate director of the university's Office of Research Technology Commercialization.

The paper, "while so important for developing a therapeutic for spinal muscular atrophy, also sheds light on the use of the compound class more generally," he noted.

Indeed, the university has patent applications covering other splice-modulating antisense oligonucleotides for neurological indications, including amyloid lateral sclerosis (ALS) and epilepsy.

"Our data demonstrate excellent diffusional distribution and cellular uptake as evidenced by the pronounced upregulation of SMN expression and partial amelioration of pathology. This suggests that other compounds of the same class that alter pre-mRNA splicing can be used as therapeutics for other neurological conditions as well," said Lutz.

He said his group has developed other splice-modulating oligonucleotides directed against the AMPA glutamate receptor (GRIA; GLUR) that potentially could be used to treat ALS and other neurological disorders such as traumatic brain injury or chronic pain.

"We have also developed oligonucleotides that modulate splicing of the serotonin (5-HT_{2C}) receptor that is applicable to a range of neurological disorders," Lutz said.

Compared with classic antisense compounds or other RNA-based oligomers such as small interfering RNA that cleave the mRNA

transcript, Lutz said, the oligomers that modulate pre-mRNA splicing are designed with extreme target specificity and are unlikely to modulate splicing of off-target pre-mRNA.

"This class of compounds has the capacity to be inherently specific and safe," he told *SciBX*. "As our appreciation for the scope and importance of alternative splicing in disease is growing exponentially, pre-mRNA splice-modulating oligomers are poised to show extraordinary growth as drug candidates of choice by pharmaceutical companies."

According to Christian Lorson of the **University of Missouri**, the benefits of such oligonucleotides include their ability to target the desired nervous system cell populations—a hurdle for most CNS disorders—and the specificity that oligonucleotides of a sufficient length provide.

"Modulating pre-mRNA expression holds tremendous promise, and diseases such as spinal muscular atrophy, Duchenne muscular dystrophy and ALS are poised to benefit from related applications," said Lorson, who is associate professor of veterinary pathology and of molecular microbiology and immunology at

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**—Christian Lorson,
University of Missouri**

the university.

Caldwell told *SciBX* that the oligonucleotides covered by Drexel's patent applications are available for licensing.

Martz, L. *SciBX* 2(26); doi:10.1038/scibx.2009.1030
Published online July 9, 2009

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