Paratek's small molecule splice modulator

By Kai-Iye Lou, Staff Writer

U.S. researchers have shown that derivatives of tetracycline, a common antibiotic, can increase levels of SMN protein, whose deficiency is the root cause of spinal muscular atrophy.¹ The findings are the first fruits of a deal between **Paratek Pharmaceuticals Inc.** and **Families of Spinal Muscular Atrophy** to develop a therapeutic for the indication. The biotech's next step is to find a derivative that can actually penetrate the blood brain barrier.

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

The body does have another gene—*survival of motor neuron 2 centromeric* (*SMN2*)—that codes for functional SMN protein. However, most *SMN2* mRNA transcripts naturally lose an exon during splicing

and end up encoding a truncated, unstable version of the SMN protein. As a result, *SMN2* is unable to compensate for the loss of *SMN1* (see **Figure 1, "Regulation of SMN protein production"**).

Paratek, which has been developing new tetracycline derivatives to treat infection, entered the SMA space in 2002 after a 2001 paper by a group at **The Ohio State University** showed that aclarubicin modulated *SMN2* splicing and increased formation of stable, full-length SMN protein.²

Aclarubicin was too toxic for clinical development, but Paratek researchers noted that the small molecule chemotherapeutic was structurally similar to the tetracyclines, which are regularly used as broad-spectrum antibiotics and acne treatments. The company set about screening its library of tetracyclines and hit upon compounds that could modulate *SMN2* splicing without toxic effects.

In 2003, Paratek and Families of Spinal Muscular Atrophy (FSMA), a private foundation that funds research on the disease, partnered to evaluate compounds from the company's tetracycline library to treat SMA. In 2006, the two signed a new deal to develop one of the company's tetracycline derivatives—PTK-SMA1—to treat the disease.

Now, Paratek and collaborators at **Cold Spring Harbor Laboratory** and **Rosalind Franklin University of Medicine and Science** have published in *Science Translational Medicine* that, compared with vehicle, PTK-SMA1 increased the concentration of SMN protein in liver cells in two mouse models of SMA.

"In this paper we demonstrate a tetracycline derivative that has a direct effect on correcting mRNA splicing of *SMN2*," said Paul Higgins, a corresponding author on the paper and director of inflammation drug

Figure 1. Regulation of SMN protein production.

Spinal muscular atrophy is predominantly caused by a mutation or deletion in the *survival of motor neuron 1 telomeric* (*SMN1*) gene, resulting in insufficient expression of functional SMN protein and thus progressive loss of spinal α -motor neurons.

[a] In normal subjects, splicing leads to the inclusion of exon 7 in the majority of mRNAs generated from *SMN1*. This results in the translation of stable, full-length SMN protein. An unstable, truncated form of the protein is generated from the mRNA of another gene, *survival of motor neuron 2 centromeric* (*SMN2*), due to the exclusion of the exon caused by a single nucleotide substitution within the gene. A small percentage of *SMN2* mRNA retains exon 7 and codes for full-length SMN protein.

[b] Because *SMN1* is mutated or deleted in spinal muscular atrophy patients, *SMN1* mRNA fails to code for a functional SMN protein or is absent altogether. As a result, the majority of SMN protein in these patients is of the truncated, unstable form derived from *SMN2* mRNAs.

[c] In *Science Translational Medicine*, researchers showed that treatment with the tetracycline derivative PTK-SMA1 promotes the inclusion of exon 7 into *SMN2* mRNA during the splicing step. This results in a higher percentage of *SMN2* mRNAs that code for the stable, full-length SMN protein. The mechanism of splice modulation and molecular target of PTK-SMA1 are not yet known.

discovery at Paratek. “The effect of this compound on splicing appears not to affect the constitutive splicing of normal genes, at least for the genes we have tested, indicating that its effects on SMN2 are specific.”

An assay showed that PTK-SMA1 works by promoting the inclusion of exon 7 into SMN2 mRNA. The assay also showed that PTK-SMA1 had minimal splice-modulating activities on five other genes, including SMN1.

“It is very encouraging to see a small molecule compound that directly influences specific splicing decisions, rather than having a more global effect on many genes,” said Meg Winberg, director of discovery research at the **Spinal Muscular Atrophy Foundation**. “Although other compounds like HDAC [histone deacetylase] inhibitors and phenylbutyrate have also been reported to modulate SMN2 splicing, such compounds appear to work more upstream on global factors that affect the transcription or splicing of many genes. This could be an issue because the more genes you modulate, the more likely you are to get off-target effects.”

The increased specificity also has the potential to boost efficacy.

“Many of the therapeutics that have been proposed up to now do actually stimulate the production of normal SMN protein from the backup SMN2 gene, but their effects have been modest, in part because they have acted through fairly nonspecific mechanisms of changing gene expression,” said Winberg. “Since a key aspect of SMN2 is that it tends to be spliced incorrectly, compounds that specifically target splicing, and specifically target SMN2, represent an exciting mechanism by which to achieve a more robust effect.”

Gordon Lutz, an associate professor in the Department of Biochemistry and Molecular Biology at **Drexel University College of Medicine**, thinks the work with PTK-SMA1 represents a positive step toward the development of a small molecule treatment for SMA. However, he added that oligomer-based treatment modalities also have been making headway in this disease space.

In June, Lutz’s group showed that intracerebroventricular delivery of splice-modulating oligomers that correct SMN2 splicing increased SMN expression throughout the CNS and partially ameliorated disease pathology in a mouse model of SMA.^{3,4}

Breaching the BBB

Despite the safety and efficacy benefits that could come from PTK-SMA1’s ability to directly influence SMN2 splicing, the compound does not cross the blood brain barrier (BBB). Thus, Paratek is now looking for derivatives that retain the SMN2-specific, splice-modulating effect but also get into the CNS.

“In the paper, we reported *in vivo* activity in the liver because the compound is unable to penetrate the blood brain barrier when injected systemically,” Higgins told *SciBX*. “However, we have performed subsequent experiments, injecting drug directly into the brain, which have shown that the drug also increases SMN protein expression. Therefore, we believe that a modified tetracycline will have the ability to alter splicing mechanisms in the appropriate target tissues relevant to SMA. We have already identified some tetracyclines that are active in increasing full-length SMN and are bioavailable to the brain, and [we] are currently planning experiments in mice to test for efficacy.”

If that doesn’t work, said Jill Jarecki, research director at FSMA, a secondary option would be to deliver a splice-modulating tetracycline derivative to the CNS via a pump.

Efficacy also will need to be addressed. Both Winberg and Arthur Burghes noted that PTK-SMA1’s potency likely falls into the micromolar range and would need to be improved. Burghes was senior author on the 2001 aclarubicin study and is a professor in the Department of Molecular and Cellular Biochemistry at Ohio State.

“Right now, splice-modulating oligomers appear to be the most potent and specific compounds to turn up SMN expression in the CNS, but the technology to develop oligomers that cross the blood brain barrier is not yet available,” Lutz told *SciBX*. “At this time, continuous intrathecal infusion by osmotic pumps would be the most feasible option for delivering splice-modulating oligomers to the CNS, as this technology is already being used to deliver other medications.”

Indeed, Paratek’s small molecule could have some competition from the oligonucleotides space. Last week, **Isis Pharmaceuticals Inc.** announced that it has added ISIS-SMNRx to its drug development pipeline. The antisense therapeutic is designed to treat SMA by modulating SMN2 splicing to increase SMN protein levels.

“We are about a year or more away from dosing patients, but we would use a delivery device similar to what we will be using with our other neurodegenerative disease drug, ISIS-SOD1rx,” wrote Amy Blackley, assistant director of corporate communications at Isis. “This is a small pump that would administer the drug directly into the CSF [cerebrospinal fluid],” she added.

Earlier this year, FSMA said Paratek received a grant from the NIH’s **National Institute of Neurological Disorders and Stroke** to continue their work. They hope to submit an IND within four to five years. FSMA has provided Paratek and its collaborators with about \$2 million in research funding since 2006.

Paratek has a pending patent covering PTK-SMA1 and its use in SMA. The program is available for licensing.

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