Selectively splicing SMN2

By Kai-Jye Lou, Senior Writer

A team from **PTC Therapeutics Inc.**, **Roche** and the **Spinal Muscular Atrophy Foundation** has generated selective, orally available, blood brain barrier-penetrant small molecule modulators of *SMN2* splicing that are highly effective in mouse models of spinal muscular atrophy.¹

The lead *SMN2* (*survival of motor neuron 2 centromeric*) splice modulator discovered under the collaboration, RG7800, completed a Phase I trial in healthy volunteers this year. The partners are planning a trial to evaluate the candidate in patients with spinal muscular atrophy (SMA) but declined to disclose details.

SMA is an autosomal recessive neuromuscular disorder characterized by the progressive loss of spinal α -motor neurons, leading to muscle atrophy and death. It is predominantly caused by a mutation or deletion in the *SMN1* (*survival of motor neuron 1 telomeric*) gene that leads to decreased expression of functional SMN protein.

A homologous gene—*SMN2*—also codes for functional SMN, but most *SMN2* RNA transcripts lose exon 7 during splicing. These truncated transcripts encode an unstable, rapidly degraded version of SMN that cannot compensate for the loss of *SMN1*. Promoting the inclusion of the lost exon results in a full-length *SMN2* mRNA transcript that encodes stable SMN (see Figure 1, “Modulating *SMN2* splicing”).

PTC entered the SMA space in 2006 by partnering with the SMA Foundation to discover small molecule *SMN2* splice modulators. In 2011, Roche licensed exclusive worldwide rights to PTC’s then-preclinical SMA program in exchange for \$30 million up front, up to \$460 million in milestones and potential double-digit royalties.²

Preclinical data published by the partners last month in *Science* has now provided the first detailed look into the program. In the study, the researchers first screened PTC’s compound library with a cell-based reporter assay designed to detect compounds that can shift splicing activity toward the production of full-length *SMN2* mRNA. PTC developed the assay in-house. It uses a human cell line harboring a construct composed of exons 6–8 from the *SMN2* gene.

The group then validated screening hits in a series of assays designed to remove false positives and nonspecific compounds and then carried out medicinal chemistry optimization.

In patient-derived fibroblasts, the top three splice modulators resulting from these studies—SMN-C1, SMN-C2 and SMN-C3—increased levels of full-length *SMN2* mRNA with nanomolar potency.

Transcriptional profiling of SMN-C1 and SMN-C3 showed that they altered the expression of very few genes across a panel of 11,714. Splice-junction profiling showed that SMN-C1 and SMN-C3 were highly specific for modulating *SMN2* splicing.

“The molecules described in this study are the first orally available small molecules to modify alternative splicing of *SMN2* with remarkable selectivity,” said Sergey Paushkin, director of research at the SMA Foundation. “The field of RNA splicing has been questioning whether or not it is possible in principle to selectively modify alternative splicing of a particular pre-mRNA with a small molecule.”

Oral dosing of SMN-C2 and SMN-C3 in mouse models of mild and severe SMA showed that the compounds increased Smn protein levels in both brain and muscle tissues compared with vehicle. The molecules also efficiently crossed the blood brain barrier (BBB). In the severe SMA mouse model, both compounds normalized motor behavior and increased weight and survival compared with vehicle.

Proof is in the process

Other research groups, including a team from **Paratek Pharmaceuticals Inc.**, have reported the development of small molecule splice modulators,^{3,4} but RG7800, a successor to the compounds described in the *Science* paper, is the only one that has advanced into clinical trials. Low selectivity and lack of BBB penetration are two issues that have slowed the development of such compounds.

Earlier small molecule splice modulators such as chlorhexidine and flunarizine have low selectivity. Chlorhexidine is used in over-the-counter disinfectants, and flunarizine is a generic calcium channel blocker used to prevent migraines.

Paratek’s tetracycline derivative, PTK-SMA1, does appear to modulate *SMN2* splicing in a specific manner but does not cross the BBB.⁵ The biotech’s SMA program is still in preclinical development.

PTC CEO Stuart Peltz told *SciBX* that the complementary expertise brought to the project by each of the three parties was critical to discovery of a clinical candidate. He said PTC developed the cell-based reporter assay system used to screen for *SMN2* splice modulators, as well as many of the validation and selectivity assays.

Paushkin added, “SMA Foundation scientists were engaged in all aspects of this project, from designing experiments and evaluating scientific data to preparing the manuscript. The foundation also provided a large portfolio of drug discovery and research assets that were previously developed by the foundation’s academic and biotech partners.”

The foundation also provided \$13 million in grant support for PTC’s SMA program before Roche became a partner.

Anirvan Ghosh, global head of neuroscience discovery at Roche’s Pharma Research and Early Development (pRED) unit, said the

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Figure 1. Modulating SMN2 splicing. Spinal muscular atrophy (SMA) is caused by insufficient expression of functional and stable survival of motor neuron (SMN) protein, leading to progressive loss of spinal α -motor neurons. *Survival of motor neuron 1 telomeric (SMN1)* and *survival of motor neuron 2 centromeric (SMN2)* both encode SMN. Most patients who have SMA carry loss-of-function mutations in *SMN1* but retain normal copies of *SMN2*.

By default, most mRNA transcripts generated from *SMN2* lack exon 7. The resulting truncated transcript encodes an unstable SMN protein. Several companies working on therapies for SMA are developing splice modulator compounds designed to promote the inclusion of exon 7 into the *SMN2* mRNA transcript and thus increase expression of the stable, full-length SMN.

One strategy is to use compounds such as antisense oligonucleotides (ASOs) that mask intronic splicing silencer (ISS) regulatory sequences (red line) found near exon 7. These sequences inhibit inclusion of the exon into the final mRNA transcript during pre-mRNA splicing.

Another strategy is to use small molecule splice modulators that promote the inclusion of exon 7 into the *SMN2* mRNA transcript. Naryshkin *et al.* have described CNS-penetrant, orally available small molecules that can promote exon 7 in a potent and selective manner, but the exact mechanism of splice modulation and molecular target is currently unknown.

pharma brought in its medicinal chemistry and bioinformatics expertise, which enabled the selection and optimization of the right compounds. “Our collaboration program has benefited tremendously from combining research expertise from the three organizations,” he said.

Isis Pharmaceuticals Inc.’s antisense oligonucleotide ISIS-SMNRx is the most advanced *SMN2* splice modulator in development for SMA. The biotech started a Phase III trial last month. The compound promotes the inclusion of exon 7 into the final *SMN2* mRNA transcript by binding to an intronic splicing silencer regulatory sequence on *SMN2* pre-mRNA. The compound is not BBB penetrant and is being delivered to CNS tissues via intrathecal injections.

In 2012, Isis granted **Biogen Idec Inc.** an exclusive, worldwide option to develop and commercialize ISIS-SMNRx for \$29 million up front and up to \$45 million in development milestones prior to exercise of the option. The option expires at the completion of a clinical trial that would support a regulatory filing. If Biogen exercises the option, Isis would be eligible for an additional \$225 million in a license fee and regulatory milestones plus double-digit royalties.

Isis declined requests for comment.

Ghosh said there is evidence to suggest that correcting neuromuscular pathology in SMA would require increasing SMN protein expression in

both muscle and CNS tissues.^{6,7} In a 2011 study published in *Nature*, delivery of ISIS-SMNRx to both the CNS and periphery in a mouse model of severe SMA yielded greater gains in survival than CNS or peripheral delivery alone. Peripheral delivery of ISIS-SMNRx increased SMN protein expression across multiple disease-relevant tissues, including cardiac muscle and skeletal muscle. Cardiomyopathies and arrhythmias have been linked to SMA.⁸

“Our oral small molecule reaches the tissues relevant to SMA. With oligonucleotide therapeutics, there are still questions on whether they can be delivered in an effective manner to all disease-relevant tissues,” said Peltz.

Roche and PTC have cofiled multiple patent applications since their 2011 collaboration covering small molecule splice modulators for treating SMA, including RG7800. Peltz added that the proof-of-concept data generated under the *SMN2* program suggest PTC’s alternative splicing technology could be used to discover small molecules that modulate the splicing of traditionally difficult targets, such as those involved in protein-protein interactions.

The SMA deal is Roche and PTC’s second partnership. In 2009, the two agreed to use PTC’s Gene Expression Modulation by Small-Molecules (GEMS) technology to develop oral small molecules against undisclosed CNS targets. Under the deal, PTC received \$12 million up

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—Anirvan Ghosh, Roche

front plus research funding from Roche. PTC is eligible for up to \$239 million in milestones per target plus double-digit royalties.

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COMPANIES AND INSTITUTIONS MENTIONED

Biogen Idec Inc. (NASDAQ:BIIB), Weston, Mass.
Isis Pharmaceuticals Inc. (NASDAQ:ISIS), Carlsbad, Calif.
Paratek Pharmaceuticals Inc., Boston, Mass.
PTC Therapeutics Inc. (NASDAQ:PTCT), South Plainfield, N.J.
Roche (SIX:ROG; OTCQX:RHHBY), Basel, Switzerland
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