Gene therapy successes point to better therapies

Despite some data concerns, two treatments for a rare pediatric killer could usher in a new wave of innovative medicines for neurological conditions.

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The drug Zolgensma was recently in the news for all the wrong reasons.

In August, the US Food and Drug Administration (FDA) gave drug manufacturer AveXis, Inc, a subsidiary of Novartis AG, a major slap on the wrist for violations related to the approval of Zolgensma, a new treatment for spinal muscular atrophy (SMA). The agency said the company had failed to promptly report to the proper regulatory authorities issues of data manipulation in some product testing. Ominous newspaper headlines followed.

It didn't help that the drug is extremely expensive—a record-setting $2.1 million for a single dose. In part, the need for only limited doses drove the price up; pharmaceutical companies typically develop drugs that patients need to take for a lifetime. Even so, the price tag sparked debates anew about drug affordability.

Adding to Zolgensma's woes: In October, Novartis and AveXis halted a study of the drug's use in adults, citing safety concerns observed in monkey studies when the therapy was administered directly into the spinal fluid.

But some of the regulatory concerns, at least, seem to be overblown. According to the FDA's inspection report, the initial data discrepancies were limited to a handful of mouse experiments, and importantly, the human clinical results look sound. In an August 28 webinar hosted by Cure SMA, senior FDA official Peter Marks said the agency continues to "remain confident in the safety and efficacy of Zolgensma," terming the miscues an "isolated incident." And despite the October setback, the original aim for the drug—administered to kids via infusion into

The drug milasen, tailor-made by Timothy Yu (*Left*) for a young girl named Mila Makovec (*Center*; *Mother on Right*) who has Batten disease, was based on the drug Spinraza, which is helping pave the way for future gene therapies. Image credit: Boston Children's Hospital.

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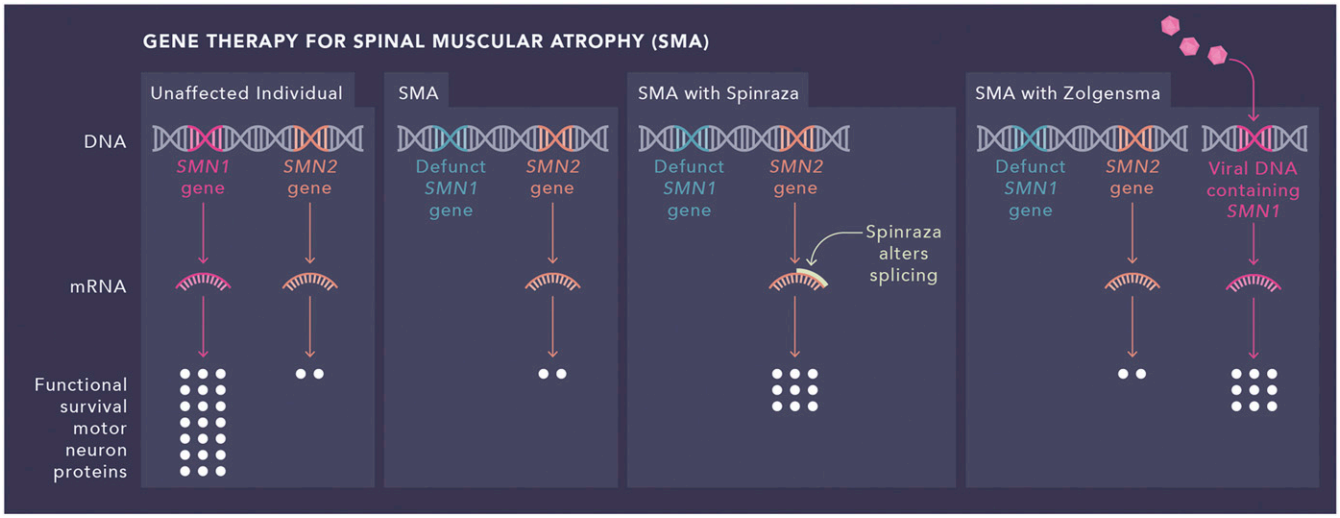

Spinraza and Zolgensma treat spinal muscular atrophy via different mechanisms of action. Image credit: Lucy Reading (artist).

the bloodstream—was not affected and continues to find success.

In the eyes of the public and politicians, the reputation of Zolgensma—the first gene replacement therapy to hit the market for a neurological disease—may be tarnished, at least in the near term. But the flurry of media coverage may also have obscured what the drug development community believes to be the more enduring story of Zolgensma: its impact not only on patients but on the entire field of gene therapy.

Zolgensma's approval in May capped off a landmark run for the biotech industry, coming just 2.5 years after the arrival of Spinraza (jointly developed by Biogen and Ionis Pharmaceuticals), the first antisense drug to substantially alter the course of a life-threatening disease. Thanks to these two cutting-edge medicines, a diagnosis of SMA is no longer a death sentence. Researchers and pharmaceutical companies alike now look to Zolgensma and Spinraza for inspiration as they pursue the next generation of therapies that target the root causes of other devastating neuromuscular diseases—Huntington's, amyotrophic lateral sclerosis (ALS), and muscular dystrophy chief among them.

"This is the beginning of a coming revolution in the treatment of severe neurological diseases," says Michael Ehlers, the former head of research and development at Biogen. "And we look forward to a time when we look back at this period and say, 'SMA paved the way for an entire set of severe neurological diseases that now have treatments.'"

The regulatory process for Zolgensma fell short. And drug prices will continue to prompt outrage among patients and policy makers. But the science behind these drugs suggests that both could prove to be therapeutic trailblazers.

Historic Killer

First described in 1891, SMA has long been the most common genetic killer of infants and toddlers, affecting around 1 in 10,000 newborns and responsible for as many as 1,000 pediatric deaths annually in the United States alone. The disease is caused by having two faulty versions of *SMN1*, a gene needed for the health of specialized nerve cells that control muscle movement. Without this protein, crucial motor neurons progressively die off. Eating, moving, and breathing become difficult. Whole-body paralysis eventually leads to early death.

How long this process takes depends on a quirk of the human genome. Most people have one or more copies of a nearly identical backup gene, *SMN2*, which differs from *SMN1* by just a single DNA nucleotide. That change alters how the resulting RNA transcript is processed, leading neurons to produce a defective, truncated protein. The gene-splicing machinery is sloppy, though, and around 5 to 10% of the time a full-length—and fully functioning—protein gets made.

Spinraza works to boost that percentage. The therapy is an 18-letter string of DNA that binds part of the coding strand of the *SMN2* gene transcript—also known as the "sense" strand, hence the name antisense oligonucleotide therapy—where it gets in the way of regulatory proteins involved in RNA splicing.

For people with SMA, the amount of working protein, and the severity of their disease, used to depend on how many *SMN2* copies occurred naturally in their genome: one or two, and babies would normally die before their second birthday; three or more, and patients might live into adulthood but with physical and respiratory disabilities. With Spinraza, patients with only a few copies are now blowing right past those historical expectations. If treated early enough, they are sitting, walking, and even developing fine motor skills.

Zolgensma has a different mechanism of action but has produced equally dramatic results. The drug uses a genetically engineered virus to deliver a functional copy of *SMN1* back into the motor neurons. The therapy thus aims to fix the underlying defect responsible for SMA in the first place, rather than trying to make the most of an imperfect back-up pathway.

The growing number of youngsters who, thanks to Spinraza or Zolgensma, are now meeting their motor

milestones demonstrates the huge promise of gene therapy—an umbrella term that encompasses antisense therapies, virus-mediated gene replacement strategies, and other gene-directed medicines. And the science that underpins those therapies offers a roadmap for drug development more broadly.

"SMA is the poster child of the gene therapy renaissance in neurological conditions," says James Sleigh, a neurogeneticist at University College London.

The path culminating in both strategies started with animal model success in the lab. To study SMA in mice, researchers first knocked out the mouse counterpart of the *SMN1* gene and then inserted two or more copies of human *SMN2*. The motor impairments in some early mouse models were so severe that many researchers feared the animals would be too sick for proper drug testing.

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>
> —Christian Lorson

But the faithful replication of the human condition on an accelerated timeline proved to be a blessing for the field, notes Arthur Burghes, a molecular geneticist at The Ohio State University in Columbus who developed some of the first and most commonly studied mouse models of the disease (1, 2). It meant that "if you have molecules that work, you can really see a big effect," he says.

Early mouse studies of Spinraza (3) and Zolgensma (4) conducted about a decade ago showed those kinds of effects—and they set the bar for the entire field. As Burghes points out, few gene therapies now enter human testing without similarly impressive preclinical data.

Yet, as the FDA-identified violations make clear, the rollout had problems. Later mouse studies of Zolgensma conducted in AveXis labs included instances of time stamp inconsistencies, failure to run certain quality control experiments, and other cases of noncompliance to regulatory standards. Still, these appear to be the result of process failures, not nefarious manipulation—in part a result of the drug's research roots.

Purely Academic?

Zolgensma grew out of academic work performed by The Ohio State University's Brian Kaspar and his postdoc Kevin Foust, both of whom eventually left the university and its affiliated Center for Gene Therapy at Nationwide Children's Hospital, also in Columbus, to direct research efforts at their fledgling startup. The University of North Carolina's Jude Samulski, who has founded several gene therapy–focused companies, attributes the missteps to a lack of industry experience. "It's a byproduct of academics doing drug development with insufficient resources to do it correctly," Samulski says. "None of it is excusable, but none of it shocks me." (Kaspar, who served as chief scientific officer, was dismissed from AveXis in August. Through a lawyer, Kaspar has denied any wrongdoing. AveXis declined to make Foust available for an interview.)

A look at the science and its impact reveals a much more promising tale. Take the viral vector, for example. Researchers had struggled for years to identify a virus capable of ferrying genetic material across the stalwart blood-brain barrier. And without one, most early gene therapy trials for neurological disorders relied on direct injection of vectors through burr holes drilled into the skull.

Then came the discovery—first made by Kaspar's group and an independent team in France (5)—of adeno-associated virus serotype 9 (AAV9). In 2009, The Ohio State University researchers (6) showed that AAV9 could traverse the anatomical gateway of mouse brains to enter tissues throughout the central nervous system (CNS), presumably because the vector engages with different cell-entry receptors than other related viruses. A year later, they demonstrated the potential for therapeutic benefit in a mouse model of SMA (4).

"It's an amazingly efficacious vector," says Christian Lorson from the University of Missouri in Columbia, who now uses AAV9 in his own gene therapy research. "To get something that you can dose at really high levels peripherally, and then get it into the CNS was what was really missing." Now, many other brain-targeted gene-corrective therapies in development are also delivered via AAV9. These include experimental treatments for rare pediatric diseases such as Sanfilippo syndrome, Batten disease, and more.

Before Zolgensma, many researchers also feared unwanted immune responses to viral vectors, including various types of AAV, as had been shown in preclinical studies involving dogs affected by Duchenne muscular dystrophy (7). This raised the specter of patients rejecting the therapy, a safety concern that prompted most clinicians to favor low doses for human trials. (For diseases that required the gene therapy to reach muscle cells, early studies also took advantage of inflatable tourniquets to sequester the vector inside treated arms or legs, thereby minimizing the risk of systemic toxicity.) But then came clear indications that the vector was innocuous.

Although most investigators at the time were playing it safe and keeping their viral dose low, neurologist Jerry Mendell from Nationwide Children's Hospital felt strongly that a high dose of Zolgensma was needed to adequately rescue motor neuron function in the sickest of SMA-affected babies. Kaspar had generated safety data in mice (4) and monkeys (8) to back him up. And the severity of the disease seemed to support taking a higher risk for the potential therapeutic reward.

Mendell ultimately won federal regulators over to his side and showed in 2017 that infusions of high-dose Zolgensma carried a reasonable safety profile (9)—producing what Mendell describes as "unprecedented" results in youngsters otherwise fated for death. Ordinarily, those children would rarely if ever achieve head control or gain the ability to stand independently, walk without support, or speak. Patients who got high-dose Zolgensma were doing all those

things, whereas low doses had far less of an impact on motor function. "It's rather dramatic," Mendell says.

For drug development, "it was equivalent to breaking the sound barrier," Samulski says. "After that everyone knew you could go to high doses." In muscular dystrophy, for example, three companies—Solid Biosciences, Inc, Sarepta Therapeutics, and Pfizer Inc (advancing a therapy initially developed by Samulski)—are all now evaluating AAV-based gene therapies delivered intravenously at viral titers of two to three times the approved dose for Zolgensma.

"I'm not sure they would have done that if they hadn't had the SMA example to guide them," says Dominic Wells, a neuromuscular scientist at the Royal Veterinary College in London, United Kingdom, adding that some of the early patient responses to these high-dose gene therapies have been "stunning."

Design Decisions

Spinraza set many scientific benchmarks of its own. From the architecture of the drug molecule to the way it's administered, experts cite a slew of design features they say are worth emulating in other antisense therapies for neuromuscular disease.

For instance, Toshifumi Yokota, a muscular dystrophy researcher at the University of Alberta in Edmonton, Canada, credits the originators of Spinraza with picking the best site to target within the *SMN2* gene and optimizing the nucleotide sequence for maximal splicing activity. "That was an important key to the success of their therapy," he says.

And although some antisense drugs are given via the bloodstream, Spinraza is injected into fluid surrounding the spinal cord, and the neurons defective in SMA are quickly bathed in the therapeutic molecules—even though the drug's developers initially had different delivery plans.

Researchers had formulated Spinraza with a chemistry designed for potency and stability in the body's peripheral tissues, not for distribution throughout the CNS, and initial mouse experiments involved injecting the antisense therapy directly into the blood (10). However, after those studies demonstrated changes in *SMN2* splicing only in the liver and kidney—but not in the brain or spinal cord where the motor neurons are found—researchers changed course.

They began administering the therapy directly into the cerebrospinal fluid via lumbar punctures, first in mice (3), then in monkeys (11), and eventually in patients (12). That delivery mechanism performed well: "The compound with this chemistry that was optimized for completely different purposes actually was found to distribute broadly in the brain," notes Paul Burke, a biotech consultant who formerly led oligonucleotide therapeutics units at Pfizer and Merck & Co, Inc. The intrathecal delivery strategy also proved effective with few serious side effects—although some children did experience headaches, back pain, and spinal fluid leakage problems as a result of the procedure.

Confirmation for this approach came from research into antisense therapies for ALS that Ionis has pursued over the past decade in tandem to its SMA program. Working with neurologist Timothy Miller from the Washington University School of Medicine in St. Louis, MO, the company developed antisense molecules that prevent the production of SOD1, a protein responsible for a particular hereditary form of ALS. As Miller reported in May at the American Academy of Neurology's annual meeting, study subjects in a small, early-phase trial who received the latest version of the therapy—called tofersen—via spinal tap showed a 37% reduction in SOD1 protein levels compared with those who received the placebo, with better scores on functional tests to boot.

Experimental antisense therapies in clinical testing now for Huntington's, Alzheimer's, and another form of ALS are all administered the same way. "There's a lot of excitement around intrathecal delivery," Miller says.

Clinical development of Spinraza also showed the benefit of treating patients as early as possible, especially among those with the most severe forms of the disease. In a study of infants that had already developed symptoms of SMA, the therapy proved a lifesaver—but around half the children still required respiratory intervention within a year of initiating treatment, and only 41% achieved a motor-milestone response (13). By comparison, babies diagnosed genetically and treated before symptom onset were far less likely to need breathing assistance, and a majority could even walk unassisted, according to data presented at the Cure SMA conference in July.

The bottom line, says Sleigh: "The timing of a therapy is very important."

Roche Holding AG took that lesson to heart with its trials for the oral medicine risdiplam. Like Spinraza, it helps the *SMN2* gene produce more functional SMN protein. After first testing the drug in children and young adults, the company launched a pivotal trial in babies aged 1–7 months old with early signs of disease, and most recently opened a study for newborns under 6 weeks old who had a genetic diagnosis of SMA but no discernible health problems. Interim results from the studies involving symptomatic patients have looked promising. Roche is expected to file for marketing approval before the end of the year.

Therapeutic Template

The parallels between Zolgensma or Spinraza and subsequent gene therapies only go so far, though. In the case of antisense drugs, for example, several of the clinical-stage candidates now in testing for diseases such as ALS, Huntington's, and Alzheimer's involve destroying target RNA rather than modulation of splicing.

That kind of drug action requires changing the structural and chemical design of the antisense molecule—modifications that, at least in mouse models, can lead to toxicity problems, according to unpublished data from Jonathan Watts, a medicinal chemist at the University of Massachusetts Medical School in Worcester. As a result, "you have to be a little more creative" when devising safe and effective molecules intended for target destruction, says Watts, who is developing antisense therapeutics for ALS and frontotemporal dementia.

In the laboratory, that kind of creativity often requires a lot of trial and error—as was the case with tofersen. "It was a lot of screening," says Alex McCampbell, senior director of neuromuscular research at Biogen. As reported last year, McCampbell and his collaborators synthesized more than 2,000 different molecules and then vetted the library of candidate antisense therapies in cell lines to find the most potent one (14). It's thus hard to draw a straight line from Spinraza to tofersen, McCampbell cautions. There were many experimental zigs and zags along the way.

Not so with milasen, a bespoke antisense oligonucleotide that clinicians at Boston Children's Hospital in Massachusetts tailor-made for a 6-year-old named Mila Makovec with Batten disease. DNA sequencing revealed that Mila's rare brain disorder was caused by a mutation in a gene called *CLN7* that led to a shortened, useless protein. The genetic defect reminded neurogeneticist Timothy Yu at Boston Children's of the splicing fault in *SMN2* that gets remedied by Spinraza. And so, using the SMA therapy as a template, Yu quickly adapted the chemistry to fix the splicing of *CLN7* transcripts, tested the custom antisense drug in cell culture, and then gave it to young Mila.

It seemed to work, as recently detailed in the *New England Journal of Medicine* (15). Although Mila has continued to lose brain volume since treatment began, her symptoms have dramatically improved. Episodes of sudden, abnormal electrical activity used to strike Mila's brain up to 30 times a day and last for minutes. Now, after several doses of her eponymous therapy, Mila seizes two to six times daily, and only for a few seconds each time, according to her mother Julia Vitarello.

The development of milasen, in turn, had knock-on effects, notes Frank Bennett, senior vice president of research at Ionis. It established a "precedent for how to get a drug to a single patient," he says. Yu's team has designed another custom antisense drug for a toddler with ataxia telangiectasia, a rare genetic disorder affecting movement, brain development, and the immune system. Meanwhile, clinicians at Columbia University's ALS clinic in New York have already started administering a one-off antisense therapy to Jaci Hermstad, a 25-year-old from Iowa who petitioned to receive an experimental Ionis drug for a rare form of the disease that killed her twin sister.

Earlier this year, Ionis worked with neurologist Neil Shneider and with regulatory authorities to allow compassionate-use access to the therapy. Ionis had not yet fully vetted the drug for safety in animals, let alone tested it in any people, before the first spinal infusion of jacifusen was administered on June 11. Jaci has since received several more doses of the drug and, according to her mother Lori, has begun to show some improvements in her physical and occupational therapy sessions.

Spinraza, says Bennett, "laid a foundation that was augmented by other drugs." And therein may lie the greatest value of the pioneering SMA therapies: Now that safety profiles have been established and regulators have outlined the types of data they want from drug sponsors, the process of moving forward with another gene therapy product should be much more streamlined, says James Valentine, an attorney at the law firm Hyman, Phelps & McNamara, PC, in Washington, DC, which specializes in pharma and biotech regulatory issues. "It's always most difficult to be first," he says.

This first may have been marred by Zolgensma's data problems and safety concerns in animal studies, leaving behind a complex legacy for that drug. But in the end, if the promise of these drugs and their development are borne out in other therapies, the enduring legacy will be lives saved.

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