Life-saving SMA drug is a triumph

Adrian Krainer speaks of a moment in his scientific career when something unexpected placed everything he'd previously done in sharp relief. "It was 1999, and I was attending a workshop at the National Institute of Neurological Diseases and Stroke (NINDS)," says the CSHL Professor and recent inductee into the National Academy of Arts and Sciences. "The meeting was about spinal muscular atrophy, an illness I knew almost nothing about."

SMA, he would learn, is a motor-neuron disease, "not unlike Lou Gehrig's disease (ALS), except that SMA tends to affect babies and young children. It's the leading genetic cause of infant mortality."

"You have progressive loss of motor neurons. As these neurons degenerate, the muscles they activate weaken and atrophy. This impairs one's ability to move—in young children, the ability to sit up, eat, even breathe." Infants with the most serious form of SMA, called type 1, often don't survive to their 2nd birthday.

By 1999 Krainer was already widely respected for research he describes as "a relentless pursuit of RNA splicing." Splicing is an activity that takes place continuously in the nucleus of cells: the editing of RNA messages copied from the DNA of genes. Each edited message serves as a template for the manufacture of a specific protein, one of the many thousands our lives depend upon.

At the NIH workshop, Krainer instantly understood that his research might help identify the molecular basis of the splicing problem in SMA, and ways to fix it. In patients, a gene called SMN1 is either missing or doesn't function. The gene encodes a protein called "survival of motor neuron" (SMN) that motor neurons need to flourish. Humans have a nearly identical backup gene called SMN2, but due to a mutation, its RNA copy does not splice properly. As little as 10 percent of the critical SMN protein is made in patients with severe SMA, not nearly enough to support normal neuromuscular development.

Krainer began to investigate the splicing error in SMN2. Today, 17 years later, a drug based on a molecule his lab designed and tested in mice has had impressive successes in late-stage clinical trials, dramatically improving the lives of babies and children with SMA. Biogen and Ionis Pharmaceuticals, commercial developers of the drug, called nusinersen, have filed for approval with the FDA.

This is the story behind the story of nusinersen. It's about how basic research produced the Nobel Prize-winning discovery of RNA splicing, and later, knowledge about its complex machinery, which then provided a basis for efforts to invent a therapy for SMA. Scientists at Cold Spring Harbor Laboratory play prominent roles throughout, illuminating how basic research brings benefits to society. The story line takes us back to the declaration of the War on Cancer by President Nixon in 1970, which spawned research leading to the discovery of RNA splicing.

Discoveries 'neither planned nor expected'

Almost simultaneously in 1977, a number of teams that included Richard Roberts [above, left] and Louise Chow at CSHL and another at MIT led by CSHL alumnus Phillip Sharp [above, right], discovered RNA splicing. James Watson called it "a once-in-a-lifetime event that completely transformed all of biology." Roberts and Sharp won a Nobel Prize for the work in 1993.

Yet its full impact did not register until after the human genome was sequenced 25 years later. Scientists then learned that human beings have about 21,000 genes, a number shockingly small considered against the vast number of distinct proteins these genes encode. This diversity is possible because the RNA copies of most human genes are spliced in different ways to generate different proteins, something called alternative splicing.

> "The pursuit of fundamental knowledge makes possible discoveries and breakthroughs that can't be anticipated..."
>
> *Bruce Stillman*

Splicing thus helps explain the evolution of biological complexity and one of the means by which organisms adapt. The other great implication of Roberts' and Sharp's discovery pertains to illness. Not just SMA but a vast number of human maladies, from muscular dystrophy to breast cancer, sometimes can be caused by errors in the splicing process.

CSHL figures prominently in the story of splicing's discovery and the development of the first drug therapy to correct a splicing error, for two key reasons, says President Bruce Stillman: "Our bedrock commitment to basic research, and our strategy of investing in talented young scientists and then giving them room to run."

Nusinersen was invented at CSHL because the Laboratory had long been committed to basic splicing research. "Importantly," Stillman stresses, "that outcome was neither planned nor expected, much as the discovery of splicing itself had not been years earlier. The pursuit of fundamental knowledge makes possible discoveries and breakthroughs that can't be anticipated, and it is often these that lead to spectacular practical advances like new life-saving drugs."

Rich Roberts, who since leaving CSHL in 1992 as Assistant Director has been Chief Scientific Officer at New England Biolabs, explained that "all this early splicing research was going on at Cold Spring Harbor because a decision had been made to study tumor viruses—and *that* was because we were going to study cancer."

Jim Watson, after taking the helm at CSHL in 1968, responded to Nixon's War on Cancer with a commitment to studying viruses, because viruses were genetically simple and some were known to cause cancer. They offered a way of studying how genes are expressed and how the proteins they encode can change a normal cell into a cancer cell. Among Watson's early decisions was to hire dozens of the

Adrian Krainer embarked on splicing research as the first CSHL Fellow.

smartest young scientists he could find. Among them were Sharp and Roberts, who arrived in 1971 and '72, respectively.

Sharp and Roberts were given the freedom to follow where their research took them. Neither had the faintest notion they would discover RNA splicing. Roberts proposed to use a newly discovered class of proteins called restriction enzymes to cut the gigantic DNA molecule into small bits that could then be sequenced, if slowly, using a manual method. Working separately, Sharp had already used the first such enzymes to map and sequence parts of a viral genome.

Both men applied their skills to a basic mystery that molecular biology was then tackling: When an activated gene's "message" is copied into RNA—that first step toward making a protein—how does the RNA message actually form? Their conclusion was that genes were "split." They were not copied directly into the form of a protein-encoding RNA. Rather, a preliminary, raw RNA copy of the gene was edited, or spliced. [illustration, facing page]

A young talent hand-picked to explore

"Splicing was discovered when I was a freshman in college," Adrian Krainer remembers. "It opened the door to a whole series of questions that motivated me. How does this splicing process happen? What is the machinery? We had no idea."

Most newly minted Ph.D.s in biology go on to serve as postdoctoral researchers in other labs and are obliged to shift their research focus. An innovative program at CSHL enabled Krainer to continue splicing research he had begun at Harvard under his Ph.D. advisor, Tom Maniatis. In 1986 he was named a CSH Fellow, the first in a distinguished line that includes Nobel laureate Carol Greider. He was hand-picked by Rich Roberts, who remembered being impressed with a talk Krainer had given at the 1984 CSHL meeting on RNA Processing.

Krainer, with Roberts as a mentor, was able in the late 1980s to pursue "frontier" questions. He would use an experimental system in which cells are broken open and their contents sifted to understand the components required for splicing. At Harvard, he had devised such a "cell-free system" to study splicing in a test tube. At CSHL he could add and subtract various "fractions" from the cell nucleus, where the splicing reaction occurs, to isolate the individual components needed to make splicing happen.

Krainer recalls: "My first real breakthrough at CSHL was to take one of these fractions [a cell extract that spliced RNA] and purify a single protein out of it, which is now called SRSF1." It has proven to be one of the most important of the 200-odd proteins now known to be involved in splicing. In July 1990 he published two key papers: one characterizing SRSF1 as a factor that binds to RNA and that must be present if splicing is to occur; the other reporting that its concentration influences *alternative* splicing, the phenomenon that accounts for the ability of a single gene to encode different proteins.

For a full explanation of RNA splicing watch our cartoon: http://bit.ly/RNAsplicing To see how the SMA drug works: https://youtu.be/YLIuIVwg_y4

Krainer and postdoc Akiya Mayeda soon made a second major discovery: They identified the function of another regulatory splicing factor, an RNA-binding protein with the unwieldy name hnRNPA1. Curiously, it had an antagonistic effect on SRSF1 when the splicing machinery was faced with choosing between two competing splice-sites. The site ultimately chosen for the cut depended on which of the two proteins was more prevalent. They later understood that SRSF1 acts as a splicing activator, and hnRNPA1 as a splicing repressor.

Krainer's team applied what they'd learned in cell-free systems to the much more complex environment of living cells. In dozens of papers written over a decade, they took apart and reassembled various parts of the splicing machinery and factors that encouraged and impeded it. They were fleshing out the complex workings and regulation of the phenomenon that Sharp and Roberts discovered in 1977.

When Krainer attended the NIH workshop on SMA in 1999, he was working on a problem called exon skipping in the messenger RNA of a gene called BRCA1. Various mutations in BRCA1 are associated with heightened ovarian and breast cancer risk. Krainer was studying a rare BRCA1 mutation in which the change of a single DNA letter caused the gene's RNA copy to splice incorrectly.

"The NIH workshop was a watershed moment for me because in SMA the splicing error in SMN2 is so obviously similar to the error we were studying in BRCA1," Krainer

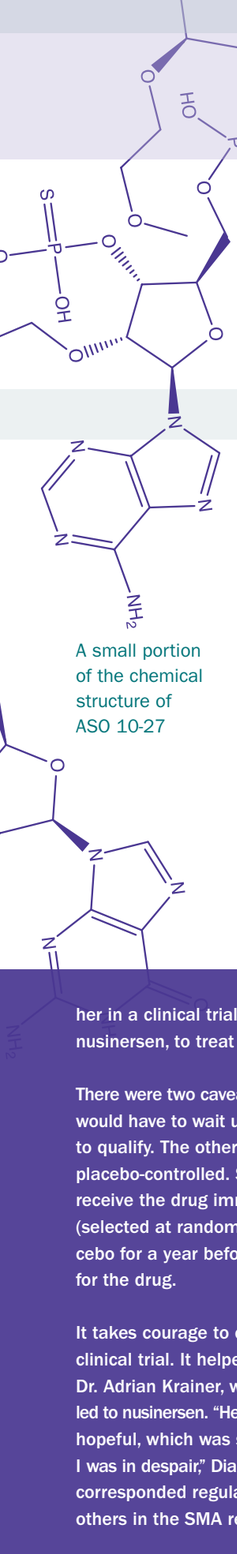
"She's my little fighter"

"You feel like the rug is ripped out from under you with this disease."

That's how Dianne Larson, the mother of an SMA-affected child, describes the experience of finding out. The problem in most cases, including that of her daughter, Emma, is that when the illness begins, "there are no signs whatsoever."

She learned to sit up and to crawl, like other toddlers her age. At Emma's 12-month pediatrician checkup, her mom remembers: "She was still moving her legs. Still bearing weight on them. They said, 'She's great, she's perfect. Take her home.' But then, at 13 months, all hell broke loose. All of a sudden she just wasn't moving her legs, and I'm like, 'What the heck happened?' It really took her overnight—that's what it felt like."

Emma was diagnosed with type 2 SMA, meaning her cells could make a small amount of usable "survival of motor neuron" (SMN) protein, but not enough to carry her very far in life. She was examined by Professor Darryl C. De Vivo, M.D., founding director of the Pediatric Neuromuscular Disease Center at Columbia. He explains that in SMA "like with so many other diseases, usually you have to lose about 50% of function before it becomes clinically apparent." In other words, Emma was losing motor neurons even as she met her first milestones. Now she was symptomatic—but there was a ray of hope. Dr. De Vivo suggested enrolling



A small portion of the chemical structure of ASO 10-27

says. The 18th exon in BRCA1 was skipped just as the 7th exon was in SMN2, in both cases with harmful effects. "The BRCA1 mutation was very rare, but now suddenly here's a more common disease I'm learning about in which all the patients have the same defect. It was readily apparent that if we could find a solution, it would apply to all SMA patients."

Toward a drug for SMA

Krainer's initial work on SMA was very basic. But within 3 years, he and postdoc Luca Cartegni had figured out a way to encourage the "inclusion" of exon 7 in SMN2's messenger RNA.

Before getting to that point, Krainer had the remarkable surprise of demonstrating that the splicing factor SRSF1—his first major scientific discovery at CSHL—was central to the pathology causing exon 7 to be skipped. His earlier work now placed him in position to understand why SRSF1 was not binding to the RNA, and why the 7th exon was being skipped over.

In 2003, Krainer and Cartegni tested an idea "that Luca proposed: we could make a synthetic molecule to bind to exon 7 and act as a surrogate for the [SRSF1] protein that was no longer binding there." Krainer and Cartegni invented a method called ESSENCE that promoted inclusion of missing exons in SMN2 and BRCA1.

This exciting result was noted by Dr. Frank Bennett, a leader of drug development at Isis Pharmaceuticals (since renamed Ionis Pharmaceuticals). "We reached out to Adrian, to see if there was an opportunity to collaborate. It was like two streams of basic research coming together," Bennett says.

Ionis had performed research to establish the first commercial platform for antisense drugs. In 2004, Ionis agreed to license ESSENCE from CSHL. Krainer and Bennett coordinated efforts to optimize the system. "Working with Adrian has been one of my most enjoyable collaborations," Bennett says. "It was almost magic the way things ended up working."

There were more surprises along the way. After Krainer's identification of SRSF1 as the missing splicing activator that caused exon 7 to be skipped, he realized that the corrective molecule—made up of two parts—was also able to correct exon skipping when stripped down to a short, "naked" sequence of RNA, called an antisense oligonucleotide, or ASO. This was key in the design of the drug now called nusinersen. Efforts began to synthesize an ASO with the greatest ability to promote inclusion of exon 7.

Another surprise was finding that it was possible to prevent exon 7 skipping by placing ASOs at various positions on either side of exon 7, not within it—in other words, within the "gibberish" segments, or introns, that were soon to be spliced out. The most promising of these, ASO 10-27, was chosen by Ionis for clinical development.

Krainer's team was determined to find out why the ASO worked so well. Here was their next surprise. The molecule, they discovered, attached to the RNA at a position normally occupied by a *repressor* of splicing. That repressor turned out to be hnRNPA1—the existence of which was the second major discovery of Krainer's CSHL career. "There's no reason in the world that both SRSF1 and hnRNPA1 would come into play in our much later SMA work," Krainer acknowledges. "Pure serendipity!" says Rich Roberts, who has experienced similar luck in his own illustrious career.

Yimin Hua was the Krainer lab postdoc who conducted the "screen" that identified ASO 10-27 and performed the crucial last phase of the preliminary work on the candidate drug, carrying it forward into animals. In April 2008, a paper by the team reported that ASO 10-27 injected into a mouse model of type 3 SMA corrected SMN2 splicing in motor neurons in the spinal cord and eliminated related pathology. Subsequent papers showed that the drug reversed symptoms of severe, type 1 SMA in mice.

Ionis received FDA permission to begin clinical trials in 2011. By early 2015 pivotal phase 3 trials were in progress, and in August, one of those trials, in infants, was ended early. The drug was effective enough in the company's eyes to justify providing it to all enrolled infants. In the fall, a new drug application was submitted to the FDA, seeking approval for the drug that began its life as ASO 10-27 in the Krainer lab.

"What all of this basic research has led to," sums up Roberts, "is the very first practical application in the clinic of our original discovery of splicing back in 1977. People have always asked me: 'Why was your splicing discovery important?' Well, now I can point to something everyone can appreciate. The nice thing is, if we can do it for this disease of splicing, we can do the same for others, too."

Peter Tarr

her in a clinical trial for a new drug, called nusinersen, to treat SMA.

There were two caveats. One was that Emma would have to wait until her second birthday to qualify. The other was that the trial was placebo-controlled. Some children would receive the drug immediately while others (selected at random) would receive placebo for a year before being eligible for the drug.

It takes courage to commit a child to a clinical trial. It helped to ask questions of Dr. Adrian Krainer, whose research at CSHL led to nusinersen. "He was very sweet and very hopeful, which was so important because I was in despair," Dianne says. She has since corresponded regularly with Krainer and others in the SMA research community.

Emma was enrolled in the trial but had to wait 6 months. "All during that time she was going through a swift regression. She got to a point where she wasn't able to sit up anymore and hold her bottle."

Her parents took her to Dr. De Vivo on her second birthday. "We were not waiting," Dianne says. She kept a diary:

First injection given March 3, 2015.
Second injection, end of March.
Third injection scheduled for May.

"It was after the second shot, but before the third," Dianne remembers. "I was in the bedroom; Emma was in the den. Now mind you, she can't move more than a few feet. All of a sudden, I hear her voice, getting closer and closer to me. What has she done? '*Emma?*' I say. Next thing I know, she's right beside me on the bedroom floor, right by the door. I was freaking out! I couldn't believe she had crawled all the way from the den."

Dianne's diary for **May 2015**: "*Something amazing is happening. Emma is regaining strength and endurance to crawl longer distances. She's also asking to stand and walk.*"

In June, Emma took her first steps, leaning against an ottoman. By September she took her first steps in a walker.

"She's my little fighter," Dianne says. "One of the little soldiers who's part of this battle to treat SMA or hopefully wipe it out."

By August of 2016 Emma was learning to use crutches to walk, and getting ready to begin preschool. Dr. De Vivo, whose team at Columbia's SMA Clinical Research Center in late 2012 administered the first dose of nusinersen ever given to a sick child, is thrilled to observe the results.

The impact of nusinersen has been "absolutely transformational," in De Vivo's view. The impact goes beyond SMA, he says, "to the whole field of rare diseases, particularly those that affect the developing nervous system and emerge postnatally."

Peter Tarr