FOILING DEADLY PRIONS

Can the course of fatal prion diseases be changed by removing the protein before it goes bad?

By **Meredith Wadman**

My friend Charlie Clark began complaining that his eyeglasses were faulty in September 2023. A trip to the optician didn't fix them; they were still "out of alignment," he said. The same month, his insomnia, a decades-old problem, became a torment. Still, at age 70, Charlie, a dogged journalist and prominent local historian in Arlington, Virginia, was thriving. He masterfully interviewed philanthropist David Rubenstein in front of a packed dinner audience at an Arlington country club; he put on his tuxedo for a library gala at Mount Vernon; and he cranked out, week after week, his "Our Man in Arlington" column for the *Falls Church News-Press*.

In September, "Life was good and happy and rich," his wife Ellen Clark remembers. (Ellen and her adult daughters agreed to have Charlie's story told in this article.)

In early October, Charlie suddenly couldn't remember what day of the week *60 Minutes* came on, after watching the TV program every Sunday night for 40 years. His sense of where things were in space grew warped: Out for a walk, he grabbed Ellen in a panic, thinking a passing car was swerving to hit them on the sidewalk.

On 22 October, Charlie was admitted to Virginia Hospital Center with increasing confusion and paranoia. Within days, he developed double vision and a left-sided tremor that soon became a rigid paralysis. In the deafening MRI machine, he thought the hospital was being bombed and cried: "We have to save the babies!"

He died on 15 November, after tremendous suffering. But until the end, Charlie displayed his trademark generosity of spirit. "Congratulations on solving the mystery," he said, holding his hand out to the doctor who informed him, 12 days before his death, that he had Creutzfeldt-Jakob disease (CJD).

CJD is the most prominent of the handful of diseases, all fatal, that result when prions, proteins of uncertain function that are abundant in the brain, misfold into an infective form that spreads widely. The aberrant proteins convert normal prions to their malevolent shape as they add them, like rungs on a ladder, to growing, ropy aggregates that destroy neurons, effectively chewing holes in the brain. (Prion diseases are officially called "transmissible spongiform encephalopathies," after their "spongiform" Swiss cheese patterns of brain damage.)

Prion diseases are rare or ultrarare. There are between one and two new cases per 1 million people annually, mostly in people older than 60. In the United States, that comes to about 500 cases diagnosed each year, the vast majority of them CJD (see table, below). Other animals have their prion diseases: scrapie in sheep and chronic wasting disease in deer and other cervids, which has been reported in 32 U.S states and five Canadian provinces. The most famous human form of prion disease, called variant CJD and acquired by eating beef from cattle with an analogous disease, caused an outbreak in the United Kingdom that peaked in the late 1990s and early 2000s. It has since faded thanks to herd surveillance, culling, and changes to cattle feeding practices.

Cases acquired from contaminated meat, blood transfusions, neurosurgical instruments and grafts now account for less than 1% of all prion disease. Between 10% and 15% arises from inherited genetic mutations. But most is sporadic, due to out-of-the-blue misfolding of normal protein potentially caused by spontaneous mutations, chemical changes after prions are made inside the cell—or both. The resulting diseases can look different clinically because prions, although all versions of the same protein, can adopt unique shapes with distinct biochemical properties that tend to attack different brain regions first. But all end in spread of the misfolded proteins, brain destruction, and death.

Ever since neurologist Stanley Prusiner of the University of California, San Francisco identified infectious prions as a cause of neurodegenerative disease in 1982, the quest for treatments has come up short. But on 4 January, a new era was inaugurated. At the University Hospitals Cleveland Medical Center, the first potential participant was screened in the first randomized, placebo-controlled trial targeting prion disease in a dozen years.

The trial will use a strategy already tested, with mixed success, against other neurodegenerative diseases: a snippet of synthetic DNA called an antisense oligonucleotide (ASO) that can reach the brain via an injection into the fluid that bathes the spine and destroy the messenger RNA (mRNA) vital to the production of a disease-causing protein. The ASO in the new trial, made by Ionis Pharmaceuticals and dubbed ION717, targets the mRNA encoding normal prion protein, without which the misfolded form can't arise.

Two other companies aim to remove normal prion protein by different methods. Sangamo Therapeutics is developing zinc finger proteins (ZFPs), tailored to bind to patient DNA and shut down expression of the gene for prion protein, called *PRNP*. These would be delivered as DNA encoding the ZFPs, packaged in a harmless virus and given as a one-time intravenous injection. Last week, the company reported promising results delivering ZFPs to the brains of nonhuman primates, and it hopes next year to file for permission to launch a human trial in the United Kingdom.

Meanwhile, Gate Bioscience is developing an oral drug that would block the production of prion protein in brain cells. If things go well, the company's small molecule is 3 to 5 years from the clinic, says CEO Jordi Mata-Fink (see graphic, p. 1287).

Still other groups are seeking to develop small molecules or antibodies that act later in the process, preventing prion protein from misfolding or limiting the damage of the pernicious form. But past attempts have disappointed, and the three firms leading the treatment race argue that the first and essential requirement is to snuff out prion protein before it's ever made. "It's three different approaches for the same therapeutic hypothesis: that eliminating prion protein will be meaningful in slowing, stopping, maybe even reversing the disease," Mata-Fink says. "It feels like the cusp of potentially a really, really, really big change."

"I've been in this field for 17 years. This is the first time I have a treatment trial to

Three forms of attack

Cases of prion disease acquired from eating infected beef made headlines, but less than 1% of prion disease is caused by external sources of infection, including surgeries and blood transfusions. The vast majority is sporadic Creutzfeldt-Jakob disease (CJD); it simply appears, usually later in life. Inherited mutations cause the rest.

CLASSIFICATION	NAME OF DISEASE(S)	NUMBER OF U.S. CASES PER YEAR	TYPICAL AGE OF ONSET	MECHANISM
Acquired	Variant CJD, iatrogenic CJD	≤1	Varies	Contaminated meat, neurosurgical instruments and grafts, blood transfusions
Genetic	CJD, fatal familial insomnia, Gerstmann-Sträussler-Scheinker syndrome	~50	Midlife	Dominant mutation in the gene encoding prion protein
Sporadic	CJD	~450	>60	Spontaneous conversion of normal to mutant protein

Zach Hoffman (left) with his father Paul and son Henry. Zach and Paul both have a rare genetic form of prion disease.

CREDITS: (DATA) U.S. CENTERS FOR DISEASE CONTROL AND PREVENTION; NATIONAL PRION DISEASE PATHOLOGY SURVEILLANCE CENTER; (PHOTO, OPPOSITE PAGE) BRIAN WIDDIS

offer a patient. … It's hard not to seem excited in front of the families," says Brian Appleby, a neuropsychiatrist who directs the National Prion Disease Pathology Surveillance Center at Case Western Reserve University and is the principal investigator at the Cleveland site of the Ionis trial.

If ION717 works, the payoff might extend beyond prion diseases. It has become clear in the past 2 decades that abnormal proteins propagate in a prionlike way in Alzheimer's disease, Parkinson's disease, and other maladies that destroy neurons. "The mechanisms are similar," says David Harris, a neuroscientist at the Boston University Medical Center who is working to identify small molecules to block prion misfolding. "And so there's every reason to think that therapies developed for prion disease could work for other neurodegenerative diseases."

THE IONIS trial's launch is arguably due, more than anything, to two people: Sonia Vallabh and Eric Minikel, a wife-and-husband team at the Broad Institute who have become superstars in the prion research community.

Vallabh was a 25-year-old student at Harvard Law School in the winter of 2010 when her mother, Kamni Vallabh, then 51, a hospitable, artistic woman who threw regular dosa-eating contests for Sonia and her cousins, started having double vision and rapidly losing weight. By June, after months of alarming cognitive deterioration, she was on life support. In December, a less-than-ideal diagnostic test—the only one available at that time—suggested CJD, and after that, life support was removed.

Ten months after Kamni's death, her daughter, by then a law school graduate working in consulting, received a devastating, revised diagnosis: Kamni had died of fatal familial insomnia (FFI), an ultrarare prion disease that commonly strikes in midlife. FFI is caused by a dominant mutation in the gene for prion protein, meaning there was a 50% chance that Sonia had inherited it. In that case, she would surely develop the disease with time. She got tested. She was positive.

In a story that is now widely told, Vallabh and Minikel, a transportation consultant, transformed their lives. They left their jobs and earned Ph.D.s in biomedical science at Harvard. They launched a shared lab at Broad. They began calling themselves "patient-scientists." Minikel refers to prion disease as "our disease."

Even as green Ph.D. students, they lit a fire under the research. "Sonia and Eric brought a unique urgency and therapeutic focus to the field," says Bryan Zeitler, senior director of gene regulation at Sangamo.

Prion scientist Byron Caughey at Rocky Mountain Laboratories, part of the National Institute of Allergy and Infectious Diseases, whose lab had recently developed a transformative diagnostic test for misfolded prion protein in the spinal fluid, took them in for training. At the time, Vallabh recalls, "I had never held a pipette."

Holly Kordasiewicz, now senior vice president of neurology research at Ionis, was also impressed. When the duo visited her at the company's Carlsbad, California, headquarters in 2014 to make an urgent pitch, Ionis was already deep into developing ASOs targeting neurodegenerative diseases; it had even developed mouse ASOs for acute prion disease a decade earlier. Slide deck in hand, Vallabh and Minikel bluntly told Kordasiewicz that the company should also turn its attention to preventing prion disease altogether. This was fully possible in mutation carriers, they argued.

Sonia Vallabh (right) and Eric Minikel changed careers to study prion disease after learning Vallabh carries a mutation that makes her certain to develop it eventually.

Their slides laid out a case that removing normal prion protein wasn't likely to be harmful. The healthy protein is anchored on the cell membranes of neurons and glia throughout the brain, although its best validated role is in maintaining myelination of peripheral nerves. It may also play roles in neural development, the establishment and proper functioning of synapses, and protecting neurons from cellular stress. But cattle, goats, and mice engineered to lack both copies of the gene for prion protein have been healthy and reproduced perfectly well. And without it, mice and goats have proved invulnerable when their brains are injected with infectious prion; cattle brain tissue examined in the lab has, too.

Vallabh and Minikel even had their own human data to show Kordasiewicz. With far-flung colleagues led by population geneticist Daniel MacArthur at Massachusetts General Hospital, they had analyzed genetic data from more than 600,000 people and identified several older adults with only one functioning prion protein gene. Although they had only half of their normal allotment of the protein, they were apparently unaffected.

Her interest piqued, Kordasiewicz then gave the couple a crash course in drug development, describing what Ionis would require to successfully develop an ASO to prevent prion disease. They would need studies showing mice treated with an ASO survived prion disease better than untreated mice. They would need a prion disease patient registry to speed enrollment in treatment trials. And to help identify participants in an eventual prevention trial, they would need a study of biomarkers in people who carry mutations for prion disease but have not developed symptoms. "They took that as a to-do list," Kordasiewicz recalls.

"Holly was with us in the weeds," Vallabh said one day in January as she and Minikel told their story. Interviewing the two of them is like speaking with a single, highly passionate, highly intelligent person. Their comments flow seamlessly into each other's, and they seem to be corralling every iota of time toward their singular purpose. Not a word or a gesture is wasted.

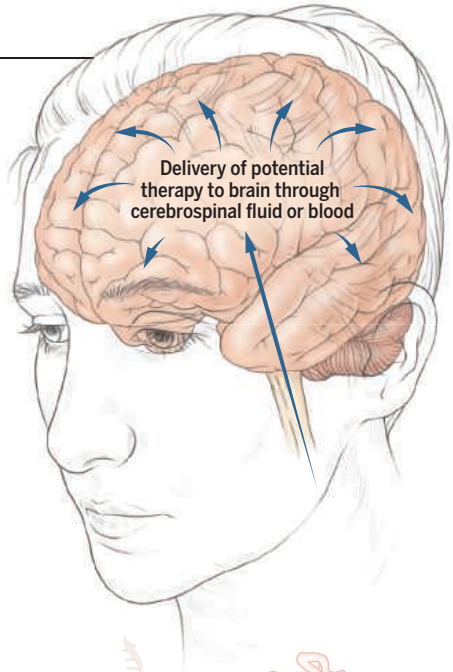
Ionis soon provided the duo with ASOs crafted to attack prion disease in mice. After they collaborated with Caughey's lab to conduct initial mouse studies, Ionis pharmacologist Curt Mazur flew to Boston to show them how to inject these into the mice's brains themselves. Another collaborator, Deb Cabin, who studies prion disease at the McLaughlin Research Institute, flew out from Great Falls, Montana, to instruct them on inoculating the mice with infectious prion.

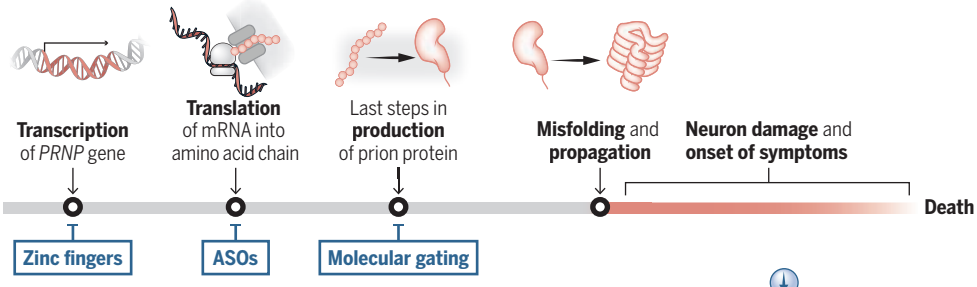
In 2019, Vallabh, Caughey, Minikel, and their colleagues at Rocky Mountain Labs,

PHOTO: MONICA JORGE/THE NEW YORK TIMES/REDUX

Cutting a protein pipeline

For years, scientists have tried unsuccessfully to stymie prion diseases by blocking the conversion of normal prion protein to its pernicious, misfolded form. But a new generation of experimental therapies for the fatal brain diseases has moved upstream, seeking to stop even the production of normal prion protein, encoded by the gene *PRNP*. Three drugs that are in development use different molecules and mechanisms, and take different routes to the brain.

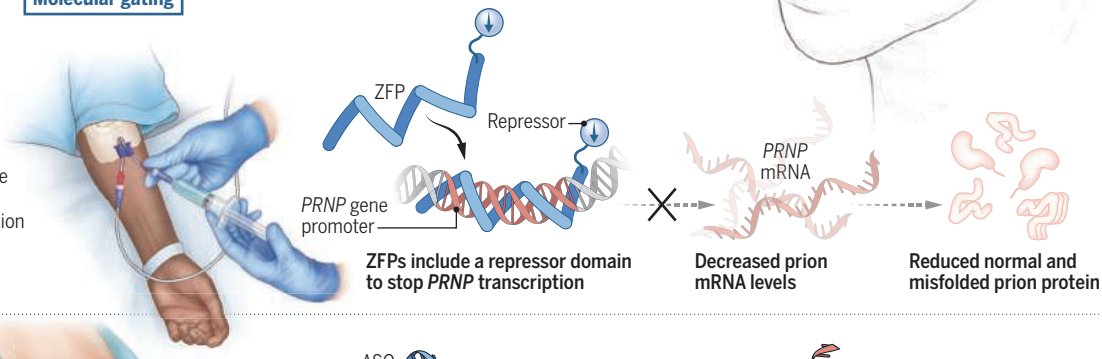




Zinc fingers

Repressing transcription

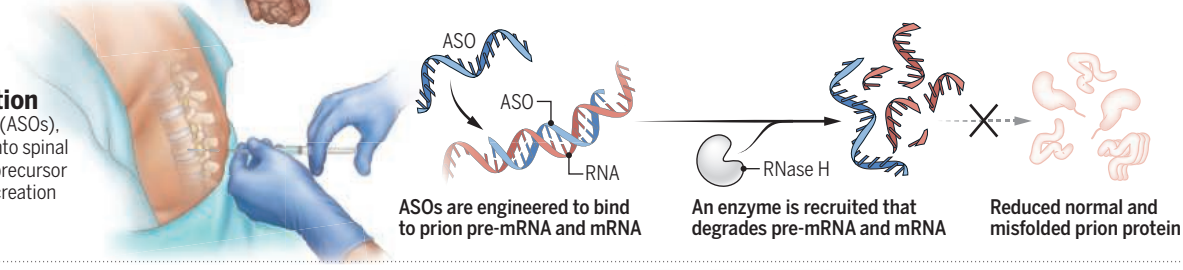
Zinc finger proteins (ZFPs), delivered as DNA encoding the proteins and packaged in a harmless virus, would be given once, intravenously. ZFPs bind to prion DNA, preventing its transcription to messenger RNA (mRNA).



ASOs

Preventing translation

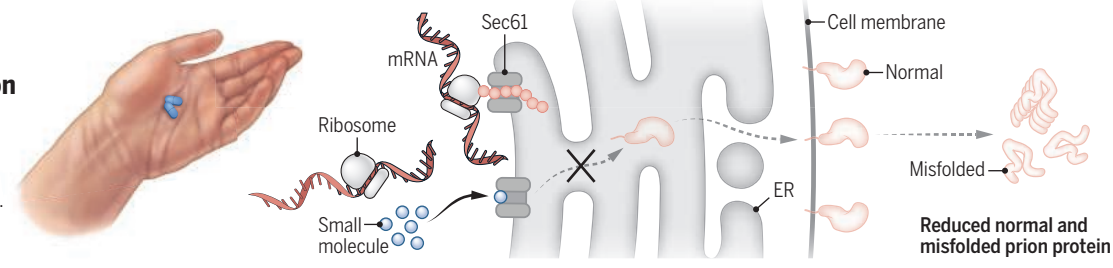
Antisense oligonucleotides (ASOs), given by repeat injections into spinal fluid, attack mRNA and its precursor (pre-mRNA), stopping the creation of prion protein.



Molecular gating

Stopping prion production

Small molecules given as pills would obstruct the sec61 protein channel where the ribosome feeds the prion's amino acid sequence into the endoplasmic reticulum (ER). This blocks the final stages of the protein's production.



GRAPHIC: N. BURGESS/*SCIENCE*

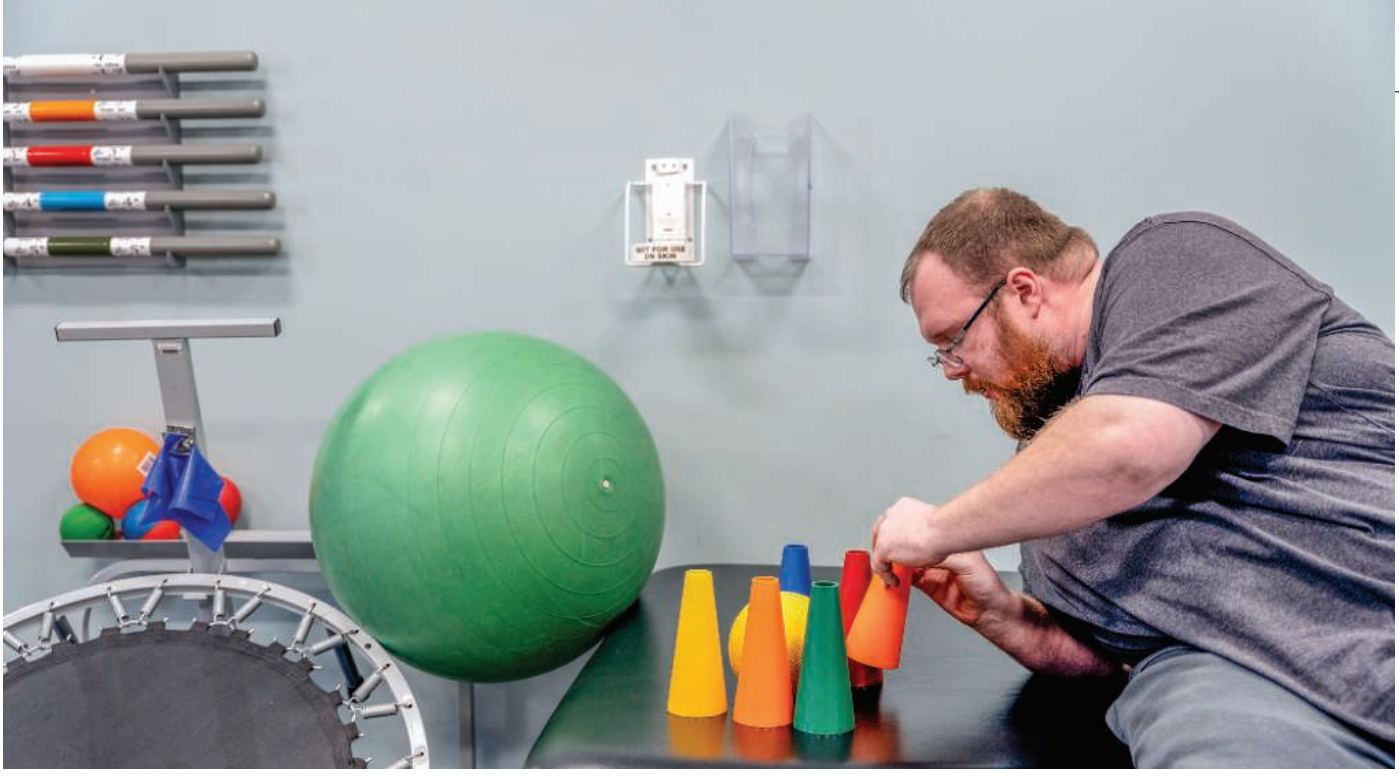
Ionis, and elsewhere reported encouraging results from the mouse studies in *JCI Insight*. The team found mice injected with ASOs survived 61% to 98% longer than control mice after being inoculated with infectious prions. Even mice not treated with the ASO until 120 days after inoculation—shortly before symptoms typically appear—lived 55% longer, suggesting the ASO was helpful even after significant amounts of toxic prion had already accumulated.

The couple and collaborators soon showed that the survival benefits were directly correlated with the degree of suppression of prion RNA in the animals' brains. They developed a test to accurately gauge levels of normal prion protein in the brains of humans and animals using spinal fluid samples—a tool that would be needed to show the impact of an ASO in human treatment trials. Ionis helped support this work; it has awarded the couple more than $1 million in funding since 2018. But they do not have equity in the company and do not stand to benefit financially if ION717 succeeds commercially.

Meanwhile, propelled by Vallabh and Minikel's successes and progress from its own internal research group, Ionis designed ASOs to treat people. When the ASO binds to the mRNA encoding normal prion protein, it summons an enzyme that destroys the mRNA. The ASO is then freed up to target another mRNA molecule. In 2021, the company selected a molecule for human testing, ION717, and soon won U.S Food and Drug Administration (FDA) approval to move into clinical trials.

"The Ionis thing is really a tribute to the work of Eric Minikel and Sonia Vallabh," Harris says. "It's a tribute to their persistence and determination."

PAUL HOFFMAN, 61, formerly a machinist at an injection molding company in Reed City, Michigan, began unaccountably losing his balance in the spring of 2020. He stumbled and had a hard time picking up his feet. He began sitting at a machine where he had



Zach Hoffman, pictured at a physical therapy appointment, has enrolled in Ionis Pharmaceuticals's clinical trial of antisense oligonucleotides to treat prion disease.

PHOTO: BRIAN WIDDIS

stood for 40 years. His physicians ruled out blood sugar problems, Lyme disease, Long Covid, and heavy metal poisoning, but couldn't come up with a diagnosis.

In 2021, his son, Zach Hoffman, a designer at the same company, started to have similar symptoms. When he sought medical help in the spring of 2023, his primary care provider promptly sent him to a geneticist. Test results arrived when Zach's wife Mackenzie Hoffman was 9 months pregnant with the couple's first child. Zach, 32, had Gerstmann-Sträussler-Scheinker (GSS) disease, an extremely rare, dominantly inherited prion disease that sets in as early as the fourth decade of life and is usually fatal in 2 to 10 years. (This slower time course is unusual in prion diseases.) Paul's own genetic test came back positive for GSS disease a few days before the birth of Zach and Mackenzie's son Henry, "a little ray of sunshine in the shitstorm," Mackenzie says.

In January, both men arrived at an examination room at University Hospitals in wheelchairs. There, Appleby and his team screened them for entry into the Ionis trial, doing neurological exams, running electrocardiograms, taking blood and urine, and asking them to repeat lists of words.

Paul, a big, bald man with a full gray mustache and beard, retired from his job in 2023. He says he's enrolling in the trial "to help everybody," but also in hopes it might touch his symptoms. "I'm all for it if it [makes] … my daily life just a little better," he manages, through sobs. Emotional lability is a common symptom of prion disease. The ready tears are "a complete flip" from Paul's predisease personality, notes his wife, Billi Hoffman.

Zach's motivation for participating is his son—"so that if he does have it, they can treat him." (If Henry has inherited the disease-causing mutation, his symptoms won't occur until adulthood; he can decide whether to get tested after he turns 18.)

The researchers plan to enroll 56 patients who are early in the course of prion disease in Cleveland and at study sites in Los Angeles; Barcelona, Spain; Boston; Göttingen, Germany; Edmonton, Canada; and elsewhere. Each patient will receive a 24-week series of spinal injections, some of them active ASO and some of them placebo, if they live that long; CJD patients typically survive just 4 to 6 months after symptoms appear. The trial, the company hopes, will conclude by October 2025.

Zach and Paul know this trial is only aimed at establishing the safety and appropriate dosing levels of the ASO. In addition to common and expected side effects with spinal injections, such as headache and back pain, the researchers will watch for numbness or weakness in the limbs, which mouse studies indicate could be a side effect of long-term antiprion therapy.

But in the face of rapidly progressive, uniformly fatal diseases, the risk-benefit ratio is small, says Sangamo's Zeitler, who is not involved in the trial. "The unmet need is extraordinary and … the science is very clear that there [are] no overt safety concerns," he says. "It seems like a good bet to make."

Some of Ionis's previous bets on ASOs have paid off. FDA granted approval in December 2016 for nusinersen, an Ionis drug licensed to Biogen that treats the neuromuscular disease spinal muscular atrophy (SMA). Eight years of real-world use later, it has conferred significant benefits, especially when given before children develop symptoms. And last year, FDA granted approval for an ASO targeting a rare genetic form of amyotrophic lateral sclerosis (ALS), developed by Ionis and licensed to Biogen. Ionis also published encouraging biomarker results from an early human trial using an ASO to attack the aberrant protein called tau in people with mild Alzheimer's.

But other Ionis ASOs targeting brain diseases have disappointed. In 2021, its ASO attacking Huntington disease failed in a late-stage clinical trial. In 2022, the company and its partner Biogen pulled the plug on an early human trial of an ASO targeting the most common known ALS mutation.

Qingzhong Kong, a molecular biologist at Case Western, says "the biggest problem" with ASOs is that spinal injections are invasive, often have uncomfortable side effects, and require weeks or months of repeated administration. (He is working to develop an alternate upstream strategy, a one-time intravenous injection of the gene for a small RNA molecule that would silence expression of the *PRNP* gene.) Getting the ASOs to spread throughout the brain is another challenge, he says. They penetrate the cerebral cortex readily but have more difficulty reaching deeper parts of the brain, such as

the striatum, which are also damaged by prion disease.

"We start getting less drug as you go into the deeper brain structures," Kordasiewicz acknowledges. But she adds that the ASO Ionis is developing for mild Alzheimer's, which is very similar in design to ION717, reversed buildup of misfolded tau protein throughout the brain, "giving us some confidence that we are targeting broadly."

If ION717 proves effective, treatment will likely be costly, as is typical for rare disease therapies. Ionis's SMA drug, nusinersen, has a list price of $750,000 for the first year of injections, and $375,000 annually after that. (The drug is typically given from early childhood.) An ASO for a still-rarer group of diseases with a much shorter course "will be priced accordingly," says Allison Bratzel, a senior research analyst who follows Ionis for investment banking firm Piper Sandler & Co. Bratzel notes that if ION717 is approved, Ionis will need to work to find patients while there is still time to treat them. On the other hand, she adds: "We've seen with rare diseases … once there's a treatment for it, doctors are more likely to look for it, and diagnosis rates go up."

IN JANUARY, in the small office Minikel shares with Vallabh at Broad, he was immersed in online conversation with a collaborator. "We obviously need more than one shot on goal," he said, leaning forward with trademark intensity.

Minikel and Vallabh are living that philosophy. Having propelled the Ionis trial into reality, they are now collaborating with Sangamo, testing the company's zinc finger reagents in mice as it prepares to file for regulatory approval to launch a human trial. They're also working with Gate Bioscience, whose CEO, Mata-Fink, says it was Minikel's blog, cureFFI.org, that led him to reach out to the duo and eventually commit his company to attacking prion disease. The startup's approach, called molecular gating, uses a small molecule that parks itself in the channel leading to the endoplasmic reticulum, where proteins destined to be secreted from the cell go through the final stages of manufacturing. The drug is intended to block the nascent chain of amino acids that gives rise to the prion protein from passing through. With no finished prions produced, misfolded prions have no substrate for their mischief.

Minikel and Vallabh have been "helping us understand the path forward and giving us a bit of a foothold in the disease," Mata-Fink says. He adds: "They're an incredible center node of this community."

Other researchers say blocking the production of normal prion protein may not be enough. For the 85% to 90% of people with sporadic disease who have no means of foreseeing its onset, "you are never going to completely prevent the conversion" of normal prion protein to its toxic cousin, Harris says. "You want to combine it with something that acts downstream."

Harris is searching for such an agent by probing just how misfolded prions wreak their damage. He has identified an enzyme, P38MAPK, part of a neuronal signaling pathway activated by the misshapen protein, which leads to the collapse of neurons' dendritic spines. Drug designers might target that enzyme or others in the pathway to protect neurons, he says.

John Collinge, a neurologist at University College London, has long been pursuing a downstream intervention. He showed in 2003 that antiprion antibodies prolonged survival in mice with prion disease. In a compassionate use study in six people published in 2022, an intravenous antibody treatment didn't noticeably alter their disease course. But two autopsies hinted that the antibodies might be reducing and clearing misfolded protein from the brain. "The evidence is clearly there that it's time to do an efficacy study," Collinge says, but no one in the private sector wants to fund a randomized, controlled trial. "There's barely a company I haven't spoken to over the past couple of years," he says. "Their business development team[s] … [all] say the numbers don't add up."

Even if the Ionis ASO succeeds at substantially lowering prion mRNA in symptomatic patients, Minikel concedes, "the [existing] protein has to clear according to its own half-life. And how many more half-lives is that patient going to be alive for? Or going to have quality of life left for?"

But the story might be different for the 10% to 15% of people who carry a mutation like Vallabh's. For them, starting a prion-removing therapy before symptoms ever show up is the obvious goal, the duo argues. Ionis is fully behind that approach, which would expand its market, given that a preventive therapy would be administered for years. "Our goal is to treat all patients with prion disease … patients who are gene carriers, as well as symptomatic patients," Kordasiewicz told investors on a call in October 2023.

Vallabh, who turned 40 this week, adds: "When you're sitting in [my] seat, the prospect of waiting until all the dominoes are falling and trying to chase after them … seems like madness."

Vallabh and Minikel are, at least, not carrying one weight that burdens the Hoffmans and other families. They used preimplantation genetic diagnosis to ensure that FFI has been rooted out of their family. Their children, now ages 4 and 6, do not carry their mother's mutation.

ON THE DAY I visited the Cleveland trial site, a list of genetic testing results from recently donated brains landed in Appleby's inbox. Case Western's prion pathology surveillance center is the only site in the country that receives, processes, and stores for research the brains of people who died of prion disease and chose to be organ donors—thousands of them so far. My friend Charlie Clark was now one of them.

For each new donor, Appleby reviews the results before they are communicated to surviving family members. The last report in his inbox that day was Charlie's. His family had given Appleby permission to discuss the results with me, but first he walked out of earshot and called Ellen Clark.

He told her that Charlie's disease had been sporadic, not genetic, so their daughters were not at risk of having inherited the disease. And he explained that Charlie had a naturally occurring version of the prion gene present in one-third to one-half of people of European descent that is associated with very rapid progression if a person spontaneously develops prion disease.

Ellen told Appleby some things about Charlie, too.

When he rejoined me, he said: "I didn't realize that he also was a journalist, and this [article] is like his ultimate contribution. That's kind of beautiful." ■

PHOTO: ELLEN CLARK

Charlie Clark near Arlington National Cemetery in 2021.