

## TREATMENTS

# The big hope for Huntington's

*A quarter of a century after its discovery, researchers are finally unlocking ways to neutralize the gene behind Huntington's disease.*

BY LIAM DREW

In September 2014, at a meeting of the European Huntington's Disease Network, Sarah Tabrizi announced the launch of a drug trial. Tabrizi, a neurologist and director of the University College London Huntington's Disease Centre, would be working with Ionis Pharmaceuticals of Carlsbad, California, to test the safety and tolerability of a drug candidate called IONIS-HTT<sub>Rx</sub> in people for the first time. The drug had been designed to reduce the amount of protein being made by the gene that causes Huntington's disease.

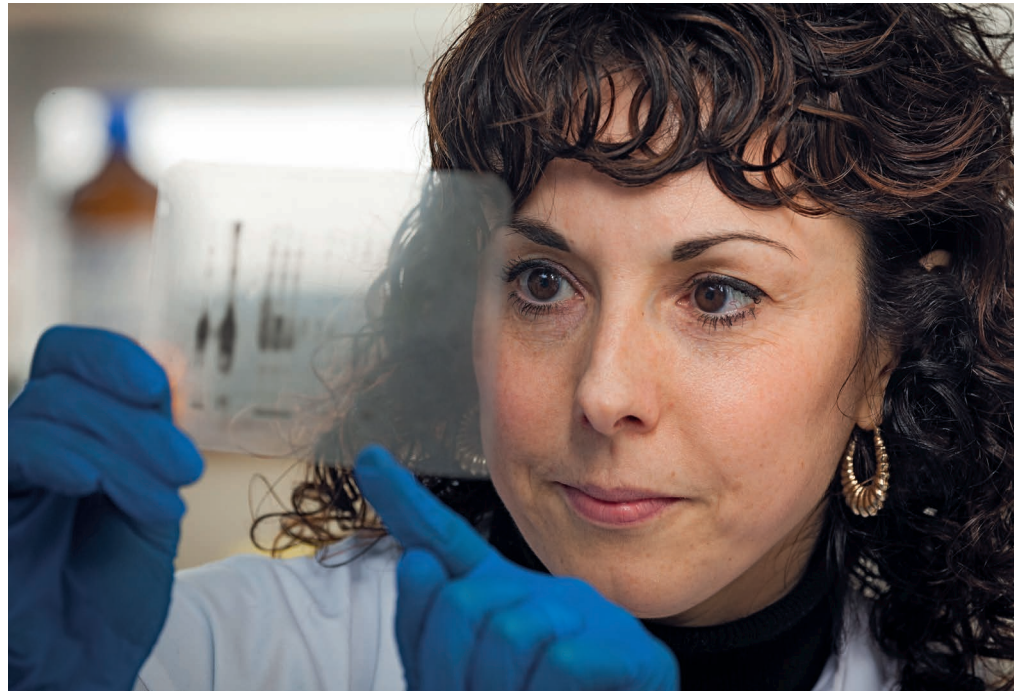
That gene is huntingtin (*HTT*). Inheriting just one mutated copy brings about a progressive neurodegeneration that typically begins in a person's forties. The condition's most distinctive symptom is involuntary, jerky limb movements. This is preceded by subtler psychiatric symptoms and followed by a disabling dementia.

IONIS-HTT<sub>Rx</sub> is an antisense oligonucleotide (ASO): an artificial chain of 12–25 nucleotides that is designed to prevent the production of protein from a specific gene. In the trial, people in the initial stages of Huntington's disease would receive four monthly injections of ASO directly into their cerebrospinal fluid (CSF) through a lumbar puncture. IONIS-HTT<sub>Rx</sub> was expected to diffuse into the brain, where it would suppress the production of the protein huntingtin in neurons. As well as ensuring that the drug had no adverse effects, Tabrizi and Ionis would use a new assay to measure levels of mutant huntingtin.

The Huntington's disease community was excited about the trial — a way of silencing *HTT* has been sought since the gene was discovered in 1993. But the path to using ASOs as treatments had been rocky and the brain is notoriously difficult to target with drugs. Tabrizi recalls that after her presentation, colleagues told her, "It'll never reach the brain. It's never going to work."

In December 2017, however, a press release revealed that the doubters had been wrong — the trial had been a success. And in March 2018, Tabrizi unveiled the resulting data at the final session of the 13th Annual Huntington's Disease Therapeutics Conference.

Her most important slide showed decreases in the level of mutant huntingtin in trial participants' CSF — indicative of reduced levels of the toxic protein in their brains — that were proportional to the amounts of drug the volunteers had received. At the two highest doses,



Sarah Tabrizi is helping to move innovative potential treatments for Huntington's disease into the clinic.

production of the protein had, on average, decreased by about 40%.

"People started crying," says Jeff Carroll, a neuroscientist who investigates Huntington's disease at Western Washington University in Bellingham. "Everybody who works in Huntington's disease long enough meets families and

***"It'll never reach the brain. It's never going to work."***

gets to know them, so it becomes very personal." Carroll's connection to his work runs particularly deep. He began his career in neuroscience after his mother was diagnosed with Huntington's disease. Then, in 2003, he discovered that he, too, had the mutation for the condition. Looking at Tabrizi's slide, Carroll thought, "This is a graph that is changing my life."

Tabrizi emphasizes that the trial did not show that IONIS-HTT<sub>Rx</sub> is able to treat Huntington's disease. It demonstrated only that the drug was safe and well-tolerated, and — crucially — that it engaged its target in the brain. "What we now have to do," she says, "is to move quickly forward to larger, longer trials to test whether the drug slows disease progression."

These trials will be run by pharmaceutical company Roche of Basel, Switzerland.

In 2013, it partnered with Ionis to develop IONIS-HTT<sub>Rx</sub> and, after the initial trial, Roche acquired the drug for US\$45 million. The companies will continue to collaborate. If all goes to plan, a large phase III trial of IONIS-HTT<sub>Rx</sub> will commence later in 2018.

Assessing where the project stands at present, Tabrizi says she has become fond of a quote from a speech by Winston Churchill: "Now, this is not the end. It is not even the beginning of the end. But it is, perhaps, the end of the beginning."

## THE BEGINNING OF THE BEGINNING

The molecular basis of ASO technology is the stuff of secondary-school textbooks. In double-stranded nucleic acids, the base guanine binds to cytosine, and thymine (in DNA) or uracil (in RNA) binds to adenine. This pairing enables DNA both to replicate and to supply cells with instructions for making protein.

ASOs are designed to be complementary to the messenger RNAs of specific genes, which act as templates for protein production. When a cell is flooded with a particular ASO, the ASO will bind to its target mRNA, preventing it from guiding protein synthesis.

Forty years ago, researchers at Harvard University in Cambridge, Massachusetts, demonstrated<sup>1</sup> that an ASO made of DNA

could stop the replication of a virus by blocking viral protein production. And a year later, in 1979, it was shown<sup>2</sup> that the binding of ASOs to mRNA not only prevented protein synthesis, but also triggered the degradation of mRNA.

Given that ASOs could, in principle, suppress the expression of any gene, these findings raised hopes of a fresh approach to the treatment of many diseases. And conditions such as Huntington's, caused by faulty genes that produce proteins with toxic effects, were seen as particularly promising targets.

There was, however, a considerable problem: DNA makes a lousy drug. A good drug tends to distribute evenly through the body, so that sufficient amounts reach the desired target. To achieve this, the drug must persist for a substantial amount of time. On this score, ASOs

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face a major problem when given to mammals, which produce high levels of enzymes called nucleases that digest nucleic acids.

Also, to be effective, ASOs must bind to target mRNAs both tightly and with specificity. Complementary base pairing means that ASOs have preferred-partner mRNAs. However, because they are highly charged molecules, ASOs can also bind to mRNAs to which they are not perfectly complementary — thereby affecting the expression of other genes — as well as to proteins. Both events could give rise to off-target effects and toxicity issues.

Making ASO-based treatments a reality has required the creation of molecules that retained nucleic acid's property of complementary base pairing, while otherwise overhauling ASO chemistry.

Ionis has been a central player in this pursuit since its foundation in 1989. Frank Bennett, vice-president of research, set up the company's laboratories and later led the development of IONIS-HTT<sub>Rx</sub>. He stresses that Ionis was “founded on an idea for a technology”. Unlike most start-ups, it did not license technology from elsewhere. And the technology that it, like other companies developing ASOs, has produced has undergone a substantial evolution.

Initially, the company focused on treatments for viral infections and cancer. It used first-generation ASOs, the backbones of which had already been chemically modified to differ from those of DNA, which fractionally increased their resistance to nucleases. In 1998, Ionis's drug fomivirsen (Vitravene) became the first ASO to be approved by the US Food and Drug Administration. It was injected into the eyes of people with AIDS to treat a viral infection that can cause blindness in those with compromised immune systems.

**Jeffrey Carroll's research is motivated by a personal connection to Huntington's disease.**

But after advances in treating HIV infection that maintained the immune response, the drug fell out of use. Other early ASOs were clinical failures. In the mid-1990s, several companies who had invested heavily in ASO technology withdrew from the field.

The frustration extended to basic research. The first published report of an attempt to suppress the production of huntingtin in mice using ASOs described a failed experiment<sup>3</sup>. That study's lead researcher, Ole Isacson, who works on neurodegeneration at the Harvard Stem Cell Institute in Cambridge, recalls the conflicted time that followed the description of *HTT* in 1993. “People were so enthralled by genetics,” he says. “They thought if you had the gene, you had the cure. Those of us with direct experience of working on disease models didn't get that feeling.”

Bennett concurs. The discovery of *HTT* piqued his interest in Huntington's disease, but “at the time, we weren't ready,” he says. Instead, Ionis focused on improving the stability of ASOs. Second-generation ASOs were developed in the late 1990s and early 2000s by incorporating further chemical modifications that increased resistance to digestion by nucleases. Isacson says that the enhanced stability of present ASOs, which can act for months, compared with the short-lived molecules that he used in 1997, is “one of the most remarkable improvements in technology that I've seen”. Only after Ionis had developed such ASOs did Bennett begin to tackle genetic disorders affecting the brain.

To do so, Ionis forged a collaboration, in 2003, with Don Cleveland, a neuroscientist at the University of California, San Diego, that aimed to treat a genetic form of amyotrophic lateral sclerosis, or motor-neuron disease. Then, in 2006, buoyed by progress they had made using mouse models of that condition, Ionis and Cleveland began to work on Huntington's disease. In 2012, they published studies<sup>4</sup> showing

ASOs that target *HTT* mRNA could reverse Huntington's-disease-like symptoms in mouse models of the condition, alongside a demonstration that ASOs downregulate huntingtin production in the brains of rhesus macaques.

After this proof-of-concept work, Ionis designed and validated an ASO that would work in people, established the best way to deliver it to the brain — optimizing the lumbar-puncture procedure, for example — and then determined the parts of the brain that the drug was most likely to reach. Finally, with Tabrizi, Roche and the CHDI Foundation (a US non-profit organization that funds research on Huntington's disease), it developed the assay<sup>5</sup> to track levels of mutant huntingtin.

**THE END OF THE BEGINNING**

The optimism that surrounds IONIS-HTT<sub>Rx</sub> stems from the well-established link between reduced levels of mutant huntingtin and improvements in symptoms in animal models of Huntington's disease. Yet some researchers are concerned that IONIS-HTT<sub>Rx</sub> suppresses not only the production of mutant huntingtin, but also synthesis of the normal protein.

This is because selectively targeting mRNA from the mutated copy of *HTT* — leaving mRNA from the normal copy untouched — poses a huge technical challenge. The mutation that causes Huntington's disease is an overlong run of the nucleotide triplet CAG (see page S36): normal *HTT* contains 17–35 consecutive such triplets, whereas in people with the condition, at least one copy of the gene has 36 or more in a row. Consequently, an ASO of around 20 nucleotides that targets CAG repeats would bind to both normal and disease-causing versions of *HTT* mRNA. And, problematically, more than 50 other human genes also contain 10 or more CAG repeats, which means that targeting the sequence could produce unwanted side effects.

An alternative approach to targeting *HTT*



RHYS LOGAN/WU COMMUNICATIONS AND MARKETING

mRNA is being developed by Wave Life Sciences in Cambridge, Massachusetts. Its strategy takes advantage of functionally insignificant differences that can often be found between a person's two copies of *HTT*. If their disease-causing gene differs from their normal copy by a single nucleotide — an A, for example, instead of a C — an ASO can be designed to target only the *HTT* mRNA containing the substituted nucleotide, a mutation known as a single nucleotide polymorphism (SNP). “We could make those SNPs into therapeutic targets for drugs,” says Paul Bolno, Wave's chief executive.

Wave was founded on an innovative means of manufacturing ASOs, in which the intrinsic symmetry — or ‘handedness’ — of each nucleotide is specified during ASO synthesis. But the company's SNP-targeting approach to Huntington's disease also requires technical innovation in genotyping. If a physician were to prescribe a drug that suppresses a gene on the basis of it containing a particular SNP, he or she would need to be certain that the SNP is in the patient's disease-causing version: inadvertently suppressing the normal copy while leaving mutated *HTT* unaffected could accelerate the disease. In conventional gene sequencing, DNA from a person's two copies of a gene is combined — it's possible to discover which mutations the person has, but not on which chromosome (of the pair) they are found. To determine that in Huntington's disease, the sequencing reaction must follow the same strand of DNA from the region of CAG repeats to the SNP that is being used to differentiate between versions of the gene. Wave says that its sequencing platform does exactly this. But to meet regulatory approval, the error rate will have to be essentially zero.

The company has now begun separate phase I trials of two ASOs that each target one of the two most common SNPs in mutated *HTT*. The approach represents a personalized route to treating Huntington's disease — only people with the targeted SNPs can benefit. Unfortunately, about 30% of those with the condition have neither SNP. Bolno says that Wave is looking for further SNP targets.

The need for specificity is contentious. Bolno points to studies in mice, in which switching off the production of normal huntingtin causes deleterious effects, as evidence that suppressing both forms of huntingtin in people might have unwanted consequences.

Ionis and Tabrizi, however, disagree. Although studies in mice show that normal huntingtin is crucial for early development, they emphasize that, in adult animals, its function is much less important. Besides, they point out, ASOs do not reduce levels of huntingtin to zero. They cite other studies in mice in which lowering but not totally removing huntingtin had no adverse effects. What's more, they say, rhesus macaques given IONIS-HTTRx for up to nine months showed no detrimental effects.

Both Ionis and Wave are working with Carroll to resolve this pivotal issue. Carroll

## RNA INTERFERENCE

### Another way to halt huntingtin

Antisense oligonucleotides (ASOs) are not the only way to suppress protein synthesis by targeting messenger RNAs. Cells already have an intrinsic mechanism for stifling mRNA that involves producing short molecules of RNA that are complementary to mRNAs. When a short RNA binds to an mRNA, protein synthesis is prevented through a phenomenon known as RNA interference (RNAi).

Most approaches to harnessing RNAi involve introducing a gene into cells that then constantly produces a desired short RNA to permanently suppress synthesis of a target protein. Three companies — Spark Therapeutics in Philadelphia, Pennsylvania; UniQure in Lexington, Massachusetts; and Voyager Therapeutics in Cambridge, Massachusetts — are pursuing RNAi approaches to treating Huntington's disease.

Each is developing a virus-based vector — which is incapable of replicating and therefore poses no threat to health — that delivers a gene encoding a short RNA that inhibits huntingtin production. However, this one-off genetic treatment raises potential safety concerns. Permanently

modifying tissues to produce RNA might reduce the need for repeated dosing, but the procedure cannot be reversed if side effects develop. And, unlike ASOs, which need only to be introduced to the cerebrospinal fluid, these vectors must be administered in the vicinity of the cells to be treated. In Huntington's disease, researchers have focused on the striatum — an area in the middle of the brain that is most-visibly affected by the condition.

Controversy exists as to whether suppressing huntingtin in the striatum alone will halt Huntington's disease. This brain structure has an important role in the condition's progression, and Pedro Gonzalez-Alegre, a neurologist at the University of Pennsylvania's Perelman School of Medicine in Philadelphia, thinks that “improving one key brain region will have benefits beyond that area itself”. But ultimately, he concedes, “we will answer this question only when we do it in humans.” Perhaps, he suggests, the strong RNA-based suppression of huntingtin in the striatum might be supplemented with ASOs to more modestly suppress the protein throughout the brain and body. **L.D.**

despairingly cites two almost identical studies<sup>6,7</sup> in mice that gave radically different results. “Right now, we don't know enough,” he says. “And you can only get so much from mice.”

Another question about the long-term future of ASOs concerns the plausibility of giving lumbar punctures to patients on a regular basis, potentially, for decades. ASOs that can cross the blood–brain barrier, which do not need to be introduced directly into the CSF, are still in early development. And there are methods other than a lumbar puncture for getting drugs into the CSF: some people with multiple sclerosis, for example, use implanted pumps for the task. Tabrizi says that finding an alternative delivery system is a “post-approval problem” — given that no current treatment stops the condition, she notes, people with Huntington's disease accept that they'll need to visit a hospital regularly to receive treatment.

Other potential genetic treatments hold the advantage of having to be administered only once. At the moment, the most viable such alternative is RNA interference (see ‘Another way to halt huntingtin’). Looking further ahead, the gene-editing tool CRISPR could correct *HTT* directly (S42). But although the enthusiasm that surrounds this technique is valid, the history of ASOs shows that much work is needed to move exciting ideas into the clinic.

“ASOs are reaching prime time,” says Tabrizi,

for Huntington's disease and, potentially, brain diseases in general. She can now smile about the doubters that she encountered on announcing the trial. “This is science,” she says. “When you're trying to develop new therapies, you're always going to have sceptics, and you have to just carry on with what you believe in. And I believed in this.”

Carroll, like many other researchers and people who have been touched by Huntington's disease, has been riding a fresh wave of hope since Tabrizi revealed results of the ASO trial in March. “Ever since I got my diagnosis,” he says, “I have operated under the assumption that I'll die at the same time my mum did — that I'll get sick when she did. Seeing that graph was the first time I've believed that it could be better.” ■

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