Henry Brady was diagnosed with cystic fibrosis at three weeks of age.

COURTESY OF KATIE BRADY

Chasing an inclusive cure

After three decades of false starts, gene therapy against cystic fibrosis is in new clinical trials — and there is even hope of a cure. **By Roxanne Khamsi**

When Katie Brady's son was born, everything seemed normal. The hospital staff pricked his heel to get blood for a routine newborn screening test, and she and her husband waited for the result without much concern. The couple had three children already — all girls — and the only thing that seemed to set Henry apart from his siblings at birth was the fact that he was a boy.

But in the days that followed he didn't gain as much weight as Brady's other children had done immediately after birth. Then the result from the blood test came back, and Brady's concern increased. "We got the call when he was six days old saying that his newborn screening came back inconclusive for cystic fibrosis," she says, referring to the often fatal genetic condition in which chloride can't flow in and out of cells normally, causing mucus build-up in the lungs that creates a breeding ground for fatal infections. When Henry was three weeks old, his family brought him back to the hospital, where doctors analysed his sweat. The test showed high levels of chloride, confirming everyone's fears. Henry had the disease.

The medical team took additional samples of Henry's blood to find out which mutations he carried in the gene at the root of his condition: cystic fibrosis transmembrane conductance regulator (*CFTR*). "We didn't find out his gene mutation until eight weeks later," Brady says. Although cystic fibrosis is one of the most common life-threatening genetic disorders, affecting an estimated 90,000 people worldwide, some *CFTR* mutations are more common than others. Moreover, individuals with cystic fibrosis have mutations in both of their *CFTR* genes, so different people have different combinations of mutations.

Henry turned out to have an ultra-rare mutation in both copies of his *CFTR* gene. "They've told us that there's maybe two other people in the country or the world who have his gene combination," Brady says.

Fresh options

The past decade has brought huge breakthroughs in drug treatments for cystic fibrosis (see page S2). In 2012, the US Food and Drug Administration (FDA) approved ivacaftor, sold under the brand name Kalydeco by Vertex Pharmaceuticals in Boston, Massachusetts. Ivacaftor was the first drug to treat the underlying cause of cystic fibrosis by rescuing the function of the protein made by *CFTR*, and more drugs that act in a similar way have arrived since then. In 2019, the FDA approved Vertex's Trikafta, a triple-drug combination of ivacaftor, elexacaftor and tezacaftor that increased lung function in people with cystic fibrosis by an average of 14% in clinical trials[1]. Although this sounds like only a minor improvement, patients say that they feel the difference with even small increases in lung function. Trikafta works for people with cystic fibrosis who have at least one copy of the F508del mutation in their *CFTR* genes. That accounts for around 90% of those with the condition.

However, Henry doesn't have that mutation, or any of the other handful of mutations that recent drugs have targeted. He is among those who have ultra-rare mutations in CFTR that prevent the gene from producing any protein whatsoever. Drugs can't rescue the protein, because it isn't there. "The medications that have come out will not help him at all," his mother says. For patients like Henry, genetic

therapies are the most promising hope for a healthy life.

Scientists have been trying for 30 years to wield gene therapy against cystic fibrosis. In past efforts, the viruses that they engineered to deliver the working copy of the gene into cells didn't work effectively. Now, thanks to better vectors and other innovations in delivering genetic sequences, gene-replacement therapies are nearing clinical trials, and the field is gaining momentum.

In October 2019, the Cystic Fibrosis Foundation, a non-profit organization in Bethesda, Maryland, announced US$500 million in funding over the next six years for research into treatments for cystic fibrosis, including gene-therapy approaches. And in April 2020, Vertex Pharmaceuticals said that it was partnering with biotechnology company Affinia Therapeutics in Waltham, Massachusetts, to develop gene therapies for cystic fibrosis.

Michael Boyle, chief executive of the Cystic Fibrosis Foundation, says that scientists should explore many different genetic therapies that might help people whose illness can't be treated with drugs such as Trikafta. "We want to have a lot of shots on goal in this last group," he says.

Gene-based therapies are already being approved for other diseases. In late 2017, for example, the FDA made headlines by approving an *in vivo* gene-replacement therapy called voretigene neparvovec, or Luxturna, which treats a rare form of inherited blindness.

Katie Brady says that doctors have changed their tune and are much more optimistic about gene-therapy options than they were when Henry was born. "When he was diagnosed five years ago, they said that science has come a long way and big things are coming, but they weren't nearly as hopeful as they are now," she says. "It's just a completely different world. We've been told in the last two years that they believe there will likely be something for him so he can live a normal life."

Going viral

Scientists first identified[2] the *CFTR* gene as the culprit behind cystic fibrosis in 1989. Just one year later, two different groups[3,4] independently showed that it was possible to introduce the gene into *ex vivo* cells using a viral vector, causing the cells to produce the CFTR protein. This proof of concept spurred research into gene therapy against cystic fibrosis using adenoviruses as viral vectors. Usually, adenoviruses would cause the common cold or other respiratory-tract infections, but the researchers used non-harmful versions and engineered them so that they carried the *CFTR* gene. In 1993, results from the first clinical trial of this approach were published[5].

The 1993 trial, conducted by a team that included biologists at the University of Iowa College of Medicine in Iowa City and at the biotechnology company Genzyme in Cambridge, Massachusetts, was an early attempt at a type of therapy called gene replacement. In this approach, the gene is introduced into a person's cells, but the sequence does not integrate into their DNA. But the results from this first gene-therapy trial for cystic fibrosis were lacklustre — as was the case with other trials over the next several years.

Scientists began worrying that the repeated administration of the adenovirus-based treatments caused the body to produce an immune response that neutralized the virus, rendering the treatment ineffective. Then tragedy struck. In 1999, an 18-year-old named Jesse Gelsinger underwent an adenovirus-based treatment for an inherited disorder. Four days later he died from a massive immune reaction called a cytokine storm.

"There will likely be something for him so he can live a normal life."

The field of gene therapy came to an abrupt halt. When work gradually resumed, the research community ditched adenoviruses and switched to adeno-associated viruses (AAVs), which were thought to cause a milder immune response. In February 1999, before Gelsinger's tragic death, a group at Stanford University School of Medicine in California had published the results from a clinical trial of ten patients with cystic fibrosis who were given gene therapy that used an AAV vector[6]. This trial, and subsequent ones, didn't produce a major improvement in symptoms. But nor did they seem to cause the massive immunological issues that arose with adenovirus vectors.

Drug companies are still pursuing AAVs for cystic fibrosis treatment. The gene-therapy company 4D Molecular Therapeutics in Emeryville, California, has several AAV-based therapies in preclinical development, and Spirovant, a gene-therapy firm in Philadelphia, Pennsylvania, is also pursuing this approach. David Schaffer, a bioengineer at the University of California, Berkeley, and a co-founder of 4D Molecular Therapeutics, says that the company uses an AAV that has been engineered to be more infectious (but still harmless) so it can reach more cells in the lungs. He says the firm is hoping to test this approach against cystic fibrosis in clinical trials in 2021. "We now have our lead AAV which is much, much better than what has been in the clinic so far for cystic fibrosis," Schaffer says.

AAV vectors do have size limitations. The small ones, which are best able to get into cells, can only carry gene sequences of up to around 4.7 kilobases. The *CFTR* gene sequence that is added into them is around 4.6 kilobases, which means there is almost no space remaining for scientists to incorporate additional sequences that can help to promote protein production from the gene. Such promoter sequences would theoretically boost production and make the vector better at raising CFTR protein levels. Spirovant chief executive Joan Lau says that her company has proprietary innovations to address this issue. "We have a functional *CFTR* that's a little shorter and a strong promoter that fits within the carrying capacity of the AAV," she says.

Spirovant and other companies are looking beyond AAVs as well, with a particular focus on lentiviruses. Eric Alton, a biologist at Imperial College London, explains that lentiviruses can carry a larger gene insertion than AAVs can. Lentiviruses also seem less likely to be neutralized and destroyed by the immune system than AAVs, adds Alton, who is coordinator of the UK Cystic Fibrosis Gene Therapy Consortium, which is made up of scientists from Imperial College London, the University of Oxford and the University of Edinburgh. Furthermore, because lentiviruses integrate into host cells' DNA, they might not need to be administered as often. The consortium has partnered with pharmaceutical maker Boehringer Ingelheim in Ingelheim, Germany, to pursue a lentivirus-based gene therapy against cystic fibrosis, which is at the preclinical stage.

Virus alternatives

Scientists have also looked beyond viral vectors to deliver gene-based therapies. For example, one approach has been to inject the *CFTR* gene directly, but with lipid molecules added around it to shield the genetic sequence from degradation. Trials with this approach date back to the early 1990s. But back then, the levels of protein production were not high enough to move the therapy forward — and what small effect it did produce didn't seem to last long enough. In 2015, the UK Cystic Fibrosis Gene Therapy Consortium took a liposome-based treatment as far as a phase IIb trial[7], but it only improved lung function by a few percentage points and never advanced to the market. Alton says that he and his colleagues are still working to make the liposome-based treatment more efficient. "We haven't set it aside," he says.

Rather than deliver the DNA code for the faulty *CFTR* gene, some groups are trying to

A scientist at Spirovant in Philadelphia, Pennsylvania, tests for chloride channel correction.

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deliver RNA to help people with cystic fibrosis. For example, the therapeutics company Translate Bio in Lexington, Massachusetts, is doing this using messenger RNA (mRNA). Normally, DNA in the cell nucleus is copied into mRNA, which then goes into the cell cytoplasm and serves as the template for protein production. Translate Bio skips the first step by directly delivering the mRNA for CFTR into lung cells. The company has launched a clinical trial in which at least 40 adults will be randomly assigned to receive this treatment or a placebo. In February 2020, the firm announced that the FDA had granted fast-track status to this treatment, meaning that Translate Bio will have more frequent meetings with the regulatory agency, which could decide to move the treatment more swiftly towards approval.

Another approach involving RNA comes from the biopharmaceutical company ReCode Therapeutics in Dallas, Texas. The method the company has developed relies on transfer RNA (tRNA) — short sequences that help to ferry amino acid molecules to the cellular machinery to produce proteins. Certain tRNA molecules also start and stop this protein-production process. Unfortunately, some cystic fibrosis mutations prematurely recruit the tRNA molecules that signal the production of CFTR protein to stop. ReCode's approach involves creating tRNA molecules that trick the machinery into continuing to make the CFTR protein. "The tRNA we're using is really similar to the natural tRNA," says David Lockhart, president of ReCode Therapeutics, but with a twist that enables it to keep the protein production going.

Other drug developers are working on so-called read-through approaches that encourage cells to ignore the premature stop signal in certain cystic fibrosis mutations. For example, Eloxx Pharmaceuticals in Waltham, Massachusetts, has designed a molecule that tricks the cellular machinery itself — known as the ribosome — to keep assembling the CFTR protein by ignoring stop messages from tRNA. The company halted its phase II trial of this therapy due to the COVID-19 pandemic, but it says it hopes to resume this clinical investigation soon.

Most of the recent buzz in the field of gene therapy for cystic fibrosis has surrounded gene editing with systems such as CRISPR–Cas9, a method in which the DNA of patients' cells is directly corrected in such a way that subsequent cells produced by replication carry a working version of the *CFTR* gene. (By contrast, treatments delivered by AAVs do not result in replacement genes being integrated into cells' DNA; in fact they often disappear over time owing to cell turnover.) The ultimate hope is that patients might one day receive a single CRISPR treatment that repairs their lung cells for life. This, say scientists, would be a true cure for cystic fibrosis.

Some of the most tantalizing evidence for CRISPR's promise comes from experiments with organoids — clusters of patients' cells that grow to have some of the characteristics of an organ. In August 2019, a team of scientists including molecular virologist Anna Cereseto at the University of Trento in Italy showed that CRISPR could edit out the mistakes in *CFTR* and cause the gene to produce functional proteins in organoids[8]. It also did this successfully in airway cells derived from cells taken from a person with cystic fibrosis. And in February 2020, scientists at the Hubrecht Institute in Utrecht in the Netherlands used a precise CRISPR approach called base editing to correct the mutation in the *CFTR* gene, and proved that it worked in organoids[9].

Path towards a cure

Worries remain about how best to deliver gene-based therapies for cystic fibrosis. The lungs diverge into smaller and smaller branches, and getting a therapy to reach the smallest, deepest parts of the lungs is tricky. "The greatest challenge is to deliver a vector to as many of the epithelial cells lining the airways as possible," says geneticist Chris Boyd at the University of Edinburgh in the UK.

For a gene-editing approach such as CRISPR (or even lentiviruses) to provide a true 'cure', it must reach the lung's stem cells, says Sriram Vaidyanathan, who researches stem-cell transplantation at Stanford University. "A durable gene therapy for cystic fibrosis," he says, "would need to correct disease-causing mutations in airway stem cells that give rise to the rest of the cells in the airway." Vaidyanathan was the lead author on a study published in December 2019 showing that a CRISPR approach could correct as many as half of the upper airway cells taken from people with cystic fibrosis[10].

Another concern is that CRISPR therapies might edit the wrong place in the genome. Some scientists say that such off-target effects could cause cancerous mutations.

Katie Brady says that her family would need reassurances that any gene-based therapy her son might receive in the future is safe. "We would want to be sure of what it's actually doing," she says. But Brady is avidly watching the progress being made with genetic treatments. "I know that's probably the route that we're likely going to have to go because his mutations are so rare. We are all for trying anything that might help Henry and have minimal side effects."

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Correction

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This Outlook article used the wrong pronoun when referring to Sriram Vaidyanathan.