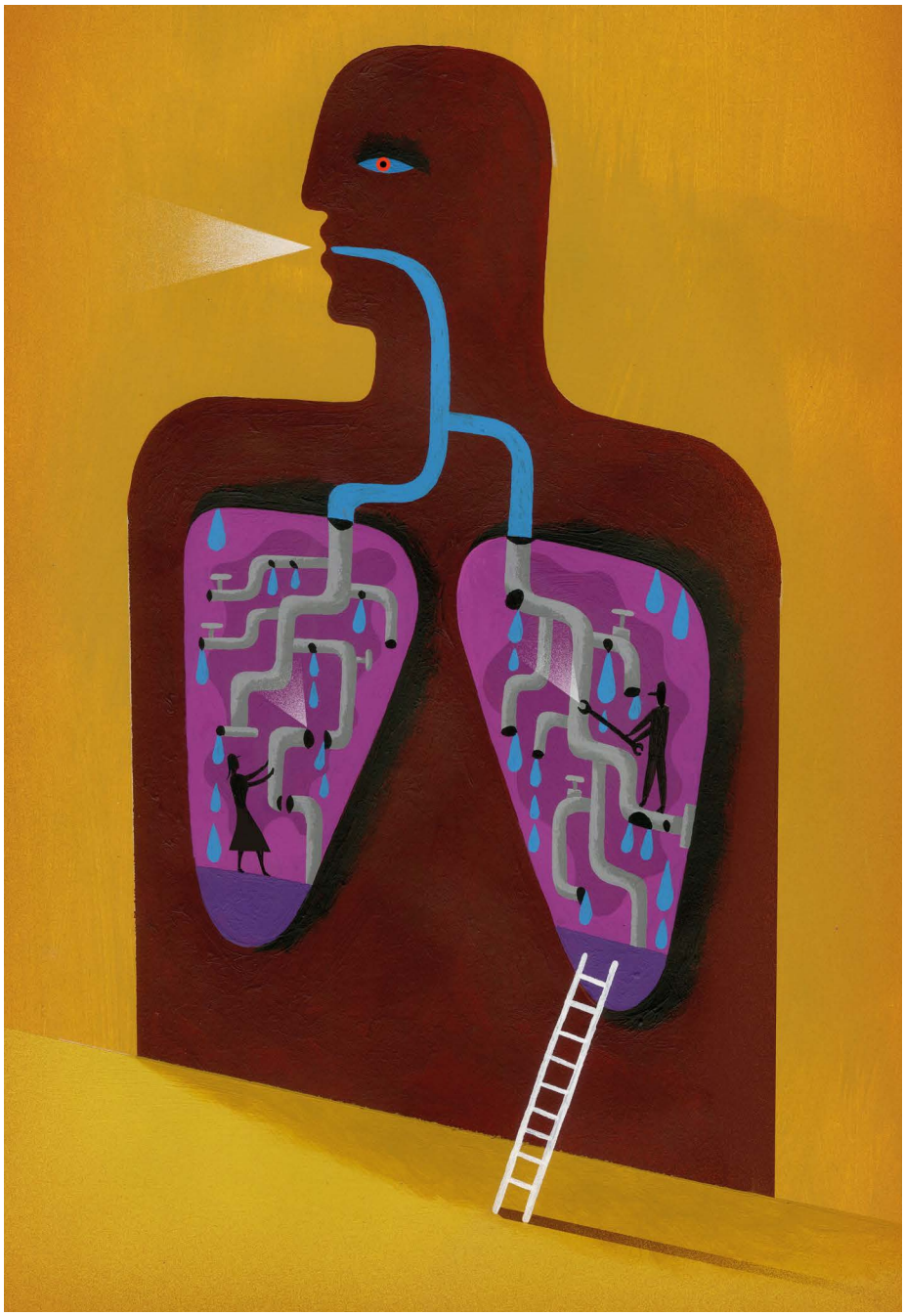


ILLUSTRATION BY RUSSELL COBB

Fix what's broken

Drugs that target specific mutations in the protein at the root of cystic fibrosis have supercharged treatments for the disease and spurred the search for further therapies. **By Sarah DeWeerdt**

Over the course of two decades spent developing treatments for the genetic lung disease cystic fibrosis, biologist Fredrick Van Goor has had hundreds of conversations with patients. But he remembers one in particular.

The discussion was about the genetics of cystic fibrosis, a disease that develops when a person inherits two faulty copies of the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene. This gene encodes the CFTR protein, which resides in the cell membrane and transports chloride and bicarbonate ions out of the cell. More than 2,000 variants of *CFTR* have been identified, and more than 350 of them are known to produce enough disruption in the protein's function to trigger the debilitating and life-shortening condition.

The focus of the conversation was the inevitable inequities of personalized medicine, which can be highly effective for people who meet certain criteria, but will leave others behind – as was the case for this patient. "He described it as being on a sinking ship, when all of the other lifeboats have left," Van Goor recalls. "That image has stuck with me."

Van Goor, the head of cystic fibrosis research at Vertex Pharmaceuticals in San Diego, California, had a major role in building the biggest lifeboat for cystic fibrosis yet: the blockbuster combination drug Trikafta. The drug achieved sales of US$420 million in the first 10 weeks after its launch in late 2019, far exceeding expectations.

"I think it has made a big difference," says Martina Gentzsch, a molecular biologist studying CFTR at the University of North Carolina at Chapel Hill. "The success in the clinic really justifies that this is an absolutely outstanding treatment." Trikafta is indicated for people with cystic fibrosis who carry at least one copy of a mutation known as F508del. This is the most common cystic fibrosis mutation, present in more than 80% of the over 90,000 people with cystic fibrosis worldwide.

The advent of cystic fibrosis therapies that target specific CFTR mutations has reshaped scientists' understanding of how to group the incredibly diverse mutations into classes, and has spurred the development of approaches to test the effectiveness of drugs. It is also inspiring efforts to find treatments for those patients who have been left behind – to expand the fleet of lifeboats.

Methodical work

Two decades ago, the only therapies for cystic fibrosis treated the symptoms of the disease, such as physical therapy to clear mucus from the lungs and antibiotics to treat infections. The Cystic Fibrosis Foundation, a non-profit

organization based in Bethesda, Maryland, wanted to encourage the development of treatments that would target the underlying cause of the disease. The foundation struck a deal with Aurora Biosciences, a San Diego company specializing in high-throughput screening, to look for compounds that might improve the function of defective proteins produced by faulty *CFTR* genes. When Vertex Pharmaceuticals, which has its headquarters in Boston, Massachusetts, bought the company in 2001, it kept the programme going, adding its own expertise in synthesizing molecules to expand the search for what are known as CFTR modulators.

> **"How do you get approval for medicines when there's only one or two people in the world with that mutation?"**

From today's vantage point, the approach looks like "a no-brainer", Van Goor says. But he remembers that at the time, the cystic fibrosis programme was known internally as "the fantasy project". No one knew whether it would be possible to find a molecule to reverse the effects of a particular mutation on protein function, because it had never been done before, for any disease.

Van Goor tells the story as one of methodical work resulting in steady, incremental progress — albeit not along an entirely straightforward path. The team knew it wanted a treatment that could rescue the function of F508del CFTR, because that would help the largest group of people with cystic fibrosis. But F508del is a complicated target.

Different *CFTR* mutations have different effects. Some prevent the synthesis of any protein at all or result in too little protein; others yield a protein that never makes it to its proper place in the cell membrane, doesn't work effectively when it gets there or doesn't remain there for as long as it should. Different categories of CFTR-modulator drugs target these different problems. Moreover, scientists are increasingly realizing that many mutations result in multiple categories of dysfunction.

In the case of F508del, the loss of a single amino acid about one-third of the way along the CFTR protein leads to two defects: the resulting protein has trouble making its way to the cell membrane, and the few copies of the protein that do get there don't work very well, because the portion of the protein that forms a channel across the cell membrane does not open and close properly.

So the Vertex team knew that it would need a combination of at least two drugs: a corrector to help stabilize the mutant F508del protein and shepherd it to the cell surface, and a potentiator to help it function once it gets there.

The researchers ended up solving the second problem first: they identified a potentiator, dubbed ivacaftor, that helps the channel in the CFTR protein to stay open. On its own, the molecule wouldn't be enough to restore the function of F508del CFTR. But it could make a difference for people with a mutation known as G551D, which only affects the opening and closing of the membrane channel.

In 2012, the US Food and Drug Administration (FDA) and the European Medicines Agency approved ivacaftor, sold under the trade name Kalydeco, for people with cystic fibrosis who have at least one copy of G551D. G551D accounts for just over 2% of all cystic fibrosis alleles, or forms of the *CFTR* gene. Still, here was proof that the 'fantasy project' might yield results in reality.

Mixing and matching

Next, the team turned its attention to correctors. The researchers identified a promising candidate, lumacaftor, that could act together with ivacaftor to improve the function of F508del CFTR. This combination therapy, called Orkambi, was approved in 2015 for people who have two copies of the F508del mutation — about 42% of people with cystic fibrosis worldwide. An improved corrector, tezacaftor, was then combined with the potentiator ivacaftor and approved in 2018 under the brand name Symdeko for the same population, as well as for those with one copy of F508del plus any of 17 other mutations on the second *CFTR* allele. Finally, to produce Trikafta, the Vertex team added a second corrector, elexacaftor, to the two drugs that made up Symdeko.

The two correctors in Trikafta work on different parts of the CFTR protein, and together they have a synergistic effect. The combination is so effective at improving the function of F508del CFTR that it can benefit anyone who carries only one copy of the F508del mutation, regardless of which mutation they carry on the other allele — letting another big group of people into the lifeboat.

"It's very promising. There's no question about it," says molecular biologist Gergely Lukacs. But Lukacs, who studies CFTR function at McGill University in Montreal, Canada, cautions that Trikafta's long-term benefits aren't yet clear, and says past experience suggests that expectations should be kept modest until more real-world data have been accumulated. For example, Kalydeco showed dramatic initial effects in people with G551D mutations, but has proved unable to halt the decline in lung function over the long term — perhaps because of pre-existing lung damage. Something similar could occur with Trikafta. "We have to wait and see," Lukacs says.

Vertex is currently conducting clinical trials of Trikafta in younger participants (the initial approval is for people aged 12 and older), as it has done with its previous cystic fibrosis drugs. The company's eventual aim is to have a suite of therapies that could be initiated in

VERTEX

To develop the drug Trikafta, Vertex scientists screened several million compounds.

infants soon after diagnosis — and thus, it is hoped, prevent lung damage from taking hold, and improve long-term outcomes (see page S6).

The company plans to keep its other cystic fibrosis drugs on the market, although it expects that most patients will eventually switch to Trikafta because of its potentially greater effectiveness. The Vertex team is also continuing to test further molecules that might become components of improved combination drugs in the future. A new version of ivacaftor is in phase II clinical trials, as is a potential corrector molecule.

Nor is Vertex the only company on the hunt for these drugs. The pharmaceuticals company AbbVie in Chicago, Illinois, is testing multiple potentiators and correctors in phase I and II clinical trials. Flatley Discovery Lab and Proteostasis Therapeutics, both in Boston, each have a potentiator and a corrector in human trials.

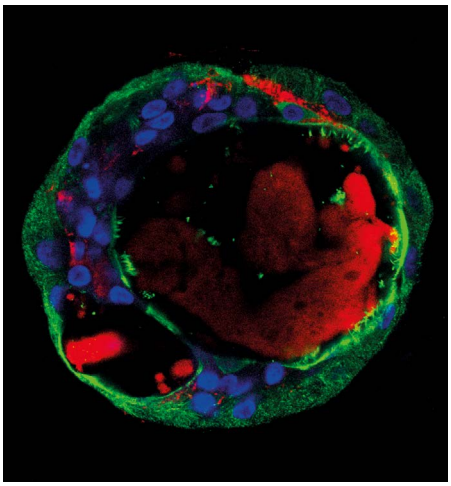
As correctors proliferate, understanding which part of the CFTR protein each corrector acts on will be increasingly important, says Lukacs. Scientists can design more-effective combination therapies, he explains, by including compounds that have different mechanisms of action or different binding sites.

Other types of CFTR modulator are also in development: amplifiers to enhance the flow of chloride ions through the CFTR channel, and stabilizers to extend the protein's lifespan in the cell membrane. Further development of these approaches could provide treatments for people with some of the rare cystic fibrosis mutations that don't respond to existing drugs — but few candidates in these classes have entered clinical trials.

A drug for every patient?

The effort to develop CFTR modulators, and especially the desire to find treatments for rare mutations, has shifted how scientists understand the mutations that cause the disease. Conventionally, these mutations are sorted into six classes on the basis of their effects on protein structure and function. But it turned out that not all mutations in the same class respond to the same CFTR modulator. Further complicating matters, sometimes mutations in different classes could nevertheless be targeted by the same drug.

"As we've gotten more and more aware of the different properties of CFTR, nothing is simple," says Garry Cutting, a clinical geneticist at Johns Hopkins University in Baltimore, Maryland. Cutting was one of several scientists who proposed classifying CFTR proteins as 'theratypes', a system that also incorporates how mutations respond to different CFTR modulators.



Cell testing alone has led to drug approval.

MARTINA GENTZSCH

The idea of theratyping was to "make it easier to make these drugs available for as many patients as possible, particularly if they're carrying a rare variant", Cutting says. Cutting directs CFTR2.org, a reference database run jointly by Johns Hopkins and the Cystic Fibrosis Foundation that documents CFTR variants. The database was first established to help with diagnosis, but has become a repository for theratyping information as well.

At the same time, researchers have come up with ways to test the effectiveness of drugs on rare mutations. Large-scale clinical trials are impractical in these cases, says Van Goor: "How do you get approval for medicines when there's only one or two people in the world with that particular mutation?" To get around this problem, commercial and academic researchers have used cell-culture systems to gather data on the susceptibility of rare mutations to different drugs. On the basis of *in vitro* data, Vertex has secured FDA approval for use of its drugs in people with several rare mutations — a first for the regulatory agency.

Such strategies have expanded the reach of some of Vertex's drugs. Kalydeco has been approved for 37 additional rare mutations that, like G551D, mainly affect channel opening and closing. The population eligible for Trikafta could expand in a similar way, Lukacs predicts.

Channelling new treatments

Still, even a full suite of CFTR modulators is likely to leave out one group of people with cystic fibrosis: the 7% or so who produce no CFTR protein at all.

Some potential treatments for this group might be useful for everyone with cystic fibrosis. Gene therapy promises a way to cure the disease by addressing the root cause (see page S12). There are also drugs that either enhance or inhibit the function of other proteins, besides CFTR, that transport chloride or sodium ions out of the cell. Improving the performance of these ion-channel proteins could help to compensate for the lack of functional CFTR.

Other approaches to help people who make no CFTR protein at all would be more mutation-specific. Many such mutations result in what's called a premature stop codon, which essentially writes 'The End' in the middle of the gene's protein-making instructions. This causes the ribosome, the cell's protein factory, to produce a truncated, non-functional protein.

Read-through drugs are one possible solution to this problem. These molecules induce the ribosome to skip over the errant stop signal and produce a slightly altered but full-length, functional protein.

The antibiotic drug gentamicin is known to modestly increase read-through, but it is too toxic for long-term use. Scientists are looking for safer candidates, but there is no systematic way of finding them. "There should be some, but there's not a lot of ways of tricking the ribosome," says Alexandre Hinzpeter, who studies protein-modulation therapies at the biomedical-research agency INSERM in Paris.

Finding a treatment for people with premature stop codons is also likely to require circumventing nonsense-mediated decay, a kind of cellular proofreading process that gets rid of aberrant protein-coding instructions, or transcripts, before they even reach the ribosome. But this requires careful calibration: disrupting that process too much could have dire effects for the overall function of the cell. "You have to find some way to protect your transcript that you're targeting but keep the rest of the cell with a normal amount of nonsense-mediated decay working," Hinzpeter says.

Many genetic diseases involve mutations that introduce premature stop codons. So in theory, a therapy developed for cystic fibrosis could also help people with other conditions, Hinzpeter says. However, this doesn't always pan out in practice. A read-through drug called ataluren has been approved for treatment of Duchenne muscular dystrophy, but failed in cystic fibrosis trials.

These problems mean that it might take as much time, effort and investment to find treatments for the last 10% of people with cystic fibrosis as for the first 90%, Van Goor says. But, he adds, echoing the resolve of both academic and pharmaceutical company scientists, "We can't leave anybody behind."

Sarah DeWeerdt is a freelance writer in Seattle, Washington.