ANNALS OF MEDICINE

OPEN CHANNELS

Do new cystic-fibrosis therapies hold the key to treating other genetic disorders?

BY JEROME GROOPMAN

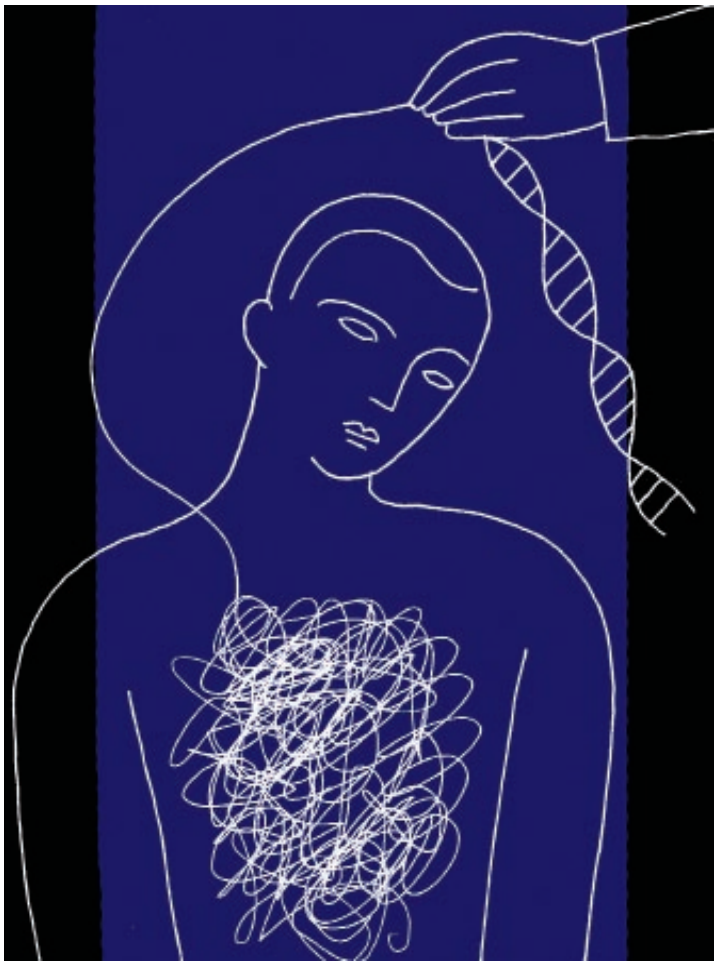
When Chrissy Falletti was born, in 1975, she seemed healthy, but soon her father, a physician in Youngstown, Ohio, and her mother, a nurse, observed that she was losing weight. At six weeks old, Falletti was admitted to Rainbow Babies & Children's Hospital, in Cleveland. As part of her evaluation, she underwent a sweat test—electrodes were strapped to her forearms, and her perspiration was collected on a thin paper filter. The test showed abnormally elevated amounts of chloride, a component of the salt in sweat, which indicated a diagnosis of cystic fibrosis. Falletti's mother quit her job to oversee her care. "No one in my family had cystic fibrosis, but my parents knew what to expect," Falletti, now thirty-three, told me when we spoke by phone recently. "Parents want to fix everything. As a doctor and a nurse, they are trained to fix people. They tried to keep positive, but they had a child who couldn't be fixed."

Cystic fibrosis is the most common fatal genetic disorder in North America among Caucasians; some thirty thousand Americans have the disease, and about ten million Americans are silent carriers of the defective cystic-fibrosis gene. The normal gene produces a protein called CFTR, which channels chloride ions out of the cells that line the body's cavities and surfaces. When the gene is mutated, the chloride ions cannot be moved, and regular body secretions become thick and congested. As the gastrointestinal tract and the pancreas become blocked, the absorption of fats and certain vitamins slows, starving the body of essential nutrients. (Sometimes the intestine must be opened surgically to remove a large blockage and help restore digestion.) In the lungs, the trachea, and the sinuses, the mucus becomes as thick as overcooked oatmeal, and the caked mucus provides a fertile field for bacterial growth. The mucus also impairs the function of the cilia, the fine hairlike projections on cells that are crucial to cleaning the airways. On average, people with cystic fibrosis lose two per cent of their lung function each year. Although antibiotics and pancreatic enzymes (which improve fat and vitamin absorption) have helped extend the average life span of patients into their late thirties, cystic fibrosis remains a uniformly fatal disease, with death often occurring as a result of pneumonia or respiratory failure.

Since childhood, Falletti has had a daily regimen that includes more than fifteen medicines and treatments. "Many days are exhausting," she said. Twice a day, she uses an apparatus that resembles a life vest; hoses inflate the vest at specified frequencies and pressures to vibrate the chest wall and mobilize the mucus. At night, she does postural draining, a sequence of twelve inverted positions during which a partner claps her upper back, her sides, and the front of her chest in order to loosen the mucus. "It takes about three hours out of my day just to clear my airway," Falletti explained. Even so, she has led an active life; until she was sixteen, she competed in gymnastics at state and national levels. A first-grade teacher now and married to an optician, she is keenly aware that she is approaching the age at which most people with the disorder die. She has suffered repeated bouts of pneumonia and a near-fatal collapse of one lung, which required emergency care, and she struggles to maintain a healthy weight. By 2007, her lung function had fallen to as low as fifty per cent of normal. "Because my lung function is so low, I am told that I shouldn't have children, which makes me really sad, because my husband doesn't have the gene and we really wanted kids," Falletti said. Two of her close friends, also cystic-fibrosis patients, have died; so has a fellow sufferer and teacher from a nearby town, whose achievements she admired. "That really hit home," she

said. "It is heartbreaking to imagine my husband by himself."

Last year, Chrissy was among a group of patients with cystic fibrosis who took part in a study of an experimental oral drug, produced by Vertex Pharmaceuticals, that is designed to restore function to the abnormal protein and permit the movement of chloride. "On the Vertex study, I just felt completely different," Falletti said. "My sister said she used to know when I entered church because she could hear my cough. Then, when I was on the study, my nephew told me, 'I can't tell when you come in anymore.'" Within two weeks, her lung function had improved from a baseline of about fifty per cent to just over sixty per cent. She gained almost ten pounds, and after twenty-eight days her lung function had increased by eighteen per cent over all. The clinical trial ended, and all patients came off the therapy. Within a week, Chrissy's lung function had begun to decline. "I call the cystic-fibrosis center all the time, asking, 'Do you have any news?'" she said. "When you've finally felt what normal feels like, you kind of realize it's not that much fun being your abnormal self."



Some drugs seem to restore a mutated protein to its normal function.

ANTHONY RUSSO

Last October, at the North American Cystic Fibrosis Conference, held in Orlando, Florida, Dr. Frank Accurso, of the Children's Hospital in Aurora, Colorado, presented the results of the first two parts of the Vertex trial, which was placebo-controlled. (I served on a scientific-advisory board at Vertex from 1991 to 2000. I have no current connection to or financial interest in the company.) Of the nineteen patients who participated in the second part of the study, those who received a specific dose of the drug demonstrated a renewed ability to transport chloride and markedly improved lung function, with an average gain of more than ten per cent. Vertex is launching a long-term, placebo-controlled trial that could lead to F.D.A. approval this year. And, while this drug targets a very specific form of cystic fibrosis that occurs in only four per cent of patients, the fact that similar drugs—which are also intended to address the basic defect in the faulty CFTR protein and which have shown promising results in initial laboratory trials—are being developed by Vertex and other companies seems to signal a new treatment paradigm.

In the past, drugs have been developed that inhibited proteins that were toxic to cells or that stimulated proteins to counterbalance the symptoms from diseased proteins. But Vertex's drugs are among the first that appear to be able to restore the normal function of a mutated protein like the one that causes cystic fibrosis. Researchers caution that further studies are needed, but the convergence of several drugs performing comparable functions might mean an entirely new approach to pharmaceutical research—with the potential to be applied to a wide range of genetic disorders, from Duchenne muscular dystrophy to Huntington's disease and certain kinds of cancer.

The characteristics of cystic fibrosis have long been recognized, if not always understood. In medieval times, a baby who tasted "salty" when kissed would be predicted to die under an evil hex; the autopsy of an emaciated, "bewitched" eleven-year-old girl in 1595 in Leiden, the Netherlands, revealed that the pancreas was swollen, hard, and gleaming white. The name of the disease describes this effect: fluid-filled cavities, or cysts; and prominent scarring, or fibrosis. Dr. Dorothy Hansine Andersen, a pediatrician at Columbia University's Babies & Children's Hospital, in New York City, was the first to identify cystic fibrosis as a disease, in 1935. She also first hypothesized that the disease was a recessive disorder, and was the first doctor to treat children with pancreatic enzymes to improve their digestion.

In 1985, researchers in the United States and Canada discovered that the cystic-fibrosis gene was situated on the seventh chromosome. Two years earlier, Dr. Francis Collins, at Yale, had devised a technique to find a gene by "jumping" over segments of chromosomes. "It's like looking for a burned-out light bulb in the basement of somebody's house," Collins told me. "You search the whole United States, and then you narrow it down to the right state, maybe even the right county, but you still have an awful lot of territory to search through for the burned-out light bulb. You needed a method that would allow you to do a house-to-house search—ideally, one that would allow you to start searching multiple city blocks at once. And that's essentially what chromosome jumping allowed."

The technology could identify mutations—the burned-out light bulb—in

any genetic disorder, but Collins chose to focus on cystic fibrosis, in part because of a young woman he had treated as an intern, in 1977. "I became interested in the condition, and frustrated when I tried to learn more about its genesis, and there was nothing there," he said. In 1984, Collins moved to the University of Michigan, and in 1987 he began to collaborate with Lap-Chee Tsui and Jack Riordan, researchers in Toronto, to find the exact nature of the cystic-fibrosis mutation. Two years later, the researchers identified the gene and determined its function. Their findings were published as a series of three papers in the journal *Science*.

The discovery of the gene set off a wave of euphoria, with many people convinced that cystic fibrosis would soon be cured. Collins recalled, "You know the affected organs, the lungs and pancreas, you deliver the normal gene, and, bingo! You'd be done." The N.I.H. spent millions of dollars setting up gene-therapy centers to attempt just that. In 1990, a year after the gene was discovered, researchers published papers on successful laboratory tests in prestigious journals like *Nature* and *Cell*. The New York *Times* reported, "Scientists have cured cystic fibrosis cells in the laboratory by inserting a healthy version of the gene that causes the disease, an unexpectedly swift advance that left researchers almost giddy with delight. The results throw open the door to using human gene therapy to treat the deadly respiratory disorder, the most common fatal genetic ailment in the United States." Yet, despite the successes in the laboratory, patients had an immune reaction to the procedure, rejecting the delivery system that held the normal gene.

For those who had invested their hopes in the therapy, the disappointment was devastating. Last November, I visited the Cystic Fibrosis Clinic at Children's Hospital Boston with Dr. Ahmet Uluer, a pediatric pneumologist and the director of the adult cystic-fibrosis program. The mood was sombre: a few days earlier, Dean Barnett, a conservative columnist and political activist in Boston, who had cystic fibrosis, had died, at the age of forty-one. In one examination room, Sabrina Kelley, a twelve-year-old patient, and her father, Brian, a Massachusetts state trooper, were waiting for the doctor. Sabrina had been running a fever for several days; during the previous year, she had spent weeks on intravenous antibiotics for sinus infections and lung infections. "Sabrina was born in 1996, when it was all about gene therapy," Brian Kelley said. He paused and looked at Dr. Uluer. "You doctors were supposed to fix it," he said. Kelley has been following the trials of the new drugs, but, he told me, "I have no expectation."

Dr. Robert Beall, a biochemist who had worked at the N.I.H., joined the Cystic Fibrosis Foundation in 1980 as executive vice-president for medical affairs, and became the president and C.E.O. in 1994. He was determined to find a new approach to treating the underlying genetic defect in cystic fibrosis. "I was on a long plane ride and catching up on reading," he recalled. "There was an article in *Nature Medicine* on high-throughput screening—using automated systems like robots to test hundreds of thousands of compounds in search of a drug." Beall sought funding to apply this new technology to cystic-fibrosis research, and in 1998, he approached Roger Tsien, a biologist who was working on ways to track proteins and monitor signals in cells using fluorescence. (Tsien won a Nobel Prize in Chemistry last year for this work.) Tsien had co-founded a biotech company, Aurora Biosciences, in San Diego, which utilized the high-throughput-screening technology.

Realizing that the research would be intensive, and that its results would have a relatively small market, Beall arranged for the foundation to provide Aurora with an initial grant of two million dollars. "Basically, we just wanted to see if they could get going," Beall said. The company formatted a system to screen for the chemical compounds that facilitated chloride transport, but it would need a larger subsidy in order to continue with the research. In 1999, Beall approached the Gates Foundation. William Gates, Sr., then the head of the foundation, interviewed Beall about the unprecedented idea of correcting a mutated protein with a drug that could be taken orally, would work throughout the body, and would restore functioning of diseased organs. "We sat around his living-room table and wrote a proposal," Beall told me. "They called us two weeks later and said they were giving us twenty million dollars to start the Aurora project." The Cystic Fibrosis Foundation contributed seventeen million dollars, from the sale of patent rights to an aerosolized antibiotic. (The foundation often acts as an investor in cystic-fibrosis research done by for-profit companies, and sometimes retains the rights to patents on any drugs produced from such research.) "We knocked the socks off the biotech world," Beall said. A few years ago, Beall recruited Joseph O'Donnell, a successful businessman whose twelve-year-old son had died of the disease, and who had served on the foundation's board, to lead an effort to raise a hundred and seventy-five million dollars to contribute to more new drug development; so far, he has garnered a hundred and fifty-one million.

With more than sixteen hundred known mutations in the cystic-fibrosis gene, the process of finding drugs to address the disease's pathology seemed formidable. But researchers soon realized that these mutations could be grouped into categories. One category of mutations produces a protein that is able to insert itself into the membrane of the cell but contains a "rusty gate" that closes off the passage of chloride. Another group of mutations, the most common, prevents the protein from reaching the membrane. A third category has a so-called "nonsense mutation," which causes the cell to abruptly terminate production of the protein, making only a fragment.

With the grant from the Cystic Fibrosis Foundation, scientists on the Aurora team began screening more than half a million chemical compounds. Once a chemical was found to restore the movement of chloride, the team worked to modify the compound into potable forms that might serve as drugs. Eventually, Aurora identified several compounds that would either wedge open

the rusty gate (potentiators) or repair the protein's ability to reach the cell membrane (correctors). "It's sort of molecular origami," Paul Negulescu, who was one of the Aurora researchers, explained. "The CFTR protein has to fold in a certain order in a certain way to work properly, and the mutated cystic-fibrosis protein didn't fold quite right. Just a little tweak, we believe, at one phase of that folding process gets it to the right shape, or otherwise it's like a crumpled piece of paper you can throw away." The drug that Falletti took, known as VX-770, is a potentiator; Vertex Pharmaceuticals (which acquired Aurora in 2001) has also developed a corrector, VX-809, which has been tested in small studies of healthy volunteers and cystic-fibrosis patients, and began a larger clinical trial in cystic-fibrosis patients this year.

At the Vertex offices, in Cambridge, Joshua Boger, the C.E.O., and the cystic-fibrosis research team showed me two videos, taken through a microscope, of cells from the lungs of cystic-fibrosis patients. In the first video, a layer of cystic-fibrosis lung cells had uncoördinated, slowly moving cilia that were unable to move mucus off the cell surface. The second video was of cystic-fibrosis cells that had been exposed to a corrector compound. In these cells, the cilia eventually began to beat in a synchronized fashion, like palm fronds swaying back and forth in the wind, showing how bacteria and mucus could be moved from the airways.

Other researchers are developing drugs that operate in similar ways and are also yielding positive results. One such drug targets the nonsense category of mutation. Normal genes contain so-called "stop signs" at the end of their DNA blueprint in order to produce proteins of an appropriate length. The nonsense mutations in cystic fibrosis create an additional, premature stop sign, which arrests synthesis of CFTR; researchers had to find a way to bypass the abnormal stop sign while respecting the normal one. Worldwide, some fifteen per cent of all patients with cystic fibrosis have this kind of nonsense mutation, but more than sixty per cent of patients in Israel carry it. PTC Therapeutics, a New Jersey development company, screened more than a million compounds and chemically optimized these compounds for several years before finding one that masks the abnormal stop sign while letting the cell recognize the normal point of termination.

In a clinical study conducted by PTC in Israel, nineteen patients underwent three months of treatment. Typically, these patients coughed nearly six hundred and fifty times per day. (Healthy people usually cough fewer than sixteen times per day.) During treatment, the nineteen study participants had their rate of coughing reduced by about two hundred times each day. In a study in Europe, lung function improved: pretreatment biopsies of the patients' nasal tissue, which is similar to lung tissue, showed no CFTR protein; after treatment, the protein appeared in its normal place on the cell surface. The drug also restored chloride transport in the airways. Dr. Eitan Kerem, who directs the department of pediatrics at Hadassah Medical Center, in Jerusalem, and is the head of its cystic-fibrosis center, oversaw the pilot study in Israel. "We saw that it improved the physiology—that chloride now could be transported," Kerem said. "But we need a placebo-controlled trial to really know its benefit." An international, placebo-controlled study of two hundred cystic-fibrosis patients will begin in the coming months. "I don't expect to see a complete cure," Kerem, who plans to participate in the study, told me. "But if it can slow the progression of the disease, and patients can receive it at an early age, so they live much longer and much better, *dayenu*"—a Hebrew word meaning "it is enough for us," traditionally used at the Passover seder to express gratitude for the exodus from slavery in Egypt.

"We can't find a compromise between a life of quiet desperation and life in the fast lane."

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These drugs may also have ramifications beyond cystic fibrosis. The drug produced by PTC Therapeutics, for instance, was designed to override any nonsense mutation—and could therefore be applied to a wide number of genetic diseases. (Nearly twenty-five hundred such disorders have been catalogued.) So far, the research team at PTC has provided data that suggest that the drug can bypass the stop mutation in mice genetically engineered to have Duchenne muscular dystrophy, and pilot studies in patients with the disease have shown promising results.

Although the treatment of genetic disorders has long been considered too small a market for most pharmaceutical companies, an increasing number of biotech firms have found that genetic research can be used in broader ways than anticipated. By identifying common protein pathways, a number of researchers say, research conducted to develop drugs that treat relatively rare afflictions may also be applied to a spectrum of illnesses. Mark Murcko, Ver-

tex's chief technology officer, said, "We believe that misfolding diseases can be treated with small-molecule drugs"—these may be oral medications, like those developed for cystic fibrosis, which work throughout the body. Diseases associated with a misfolded protein include Alzheimer's and more commonly occurring diseases, such as cancers that have the mutated p53 gene, thought to be important in certain cancers of the colon, pancreas, and lungs. In malignancies, tumor-suppressor genes, which normally restrain cell growth, acquire mutations, from carcinogens like tobacco smoke and radiation exposure, that eliminate the genes' ability to halt growth. If the promise of corrector drugs like those in development for cystic fibrosis bears out, drugs could also be developed to restore the restraining function of the tumor-suppressor protein. Murcko is also interested in the future possibility that other drugs that facilitate chloride transport might be useful in enhancing the pulmonary function of patients with common lung diseases, such as chronic bronchitis from smoking.

This strategy—pursuing treatments for rare genetic disorders with the intention of finding ways to use them for more common ailments as well—has found adherents outside the biotech world. Dr. Mark Fishman, the president of the Novartis Institutes for BioMedical Research, the pharmaceutical research arm of Novartis, a major global health-care company, has shifted his institute's research program to focus on diseases with well-understood underpinnings, including rare genetic disorders. "If you understand the genetic mechanism, it becomes scientifically tractable to develop drugs," he told me. Novartis, for example, is developing a treatment for Muckle-Wells syndrome, an extremely rare auto-inflammatory disease, which also has the potential to provide relief for those who suffer from gout, rheumatoid arthritis, and some kinds of diabetes. Fishman has been heartened by the advances made by Vertex and PTC in treating cystic fibrosis. "This approach to restoring the protein clearly changes the way you look at the world of drug development," he said.

Francis Collins, who discovered the cystic-fibrosis gene and, until last year, was the director of the National Human Genome Research Institute, at the N.I.H., has helped to establish federal programs to use high-throughput-screening technology to target a variety of mutated proteins, inspired in part by the success in finding new treatments for cystic fibrosis. "If we recognize that most inherited disorders do not have a foundation with the resources cystic fibrosis had, is it realistic to imagine that the same path of drug development could be travelled for other genetic diseases?" he asked. In the absence of private funding, Collins has urged the federal government to fill in the breach. "We have a robot screening three hundred thousand compounds in a span of forty-eight hours, each compound at seven different concentrations," he told me. "There are four screening centers throughout the country. At N.I.H., there is the ability to do animal testing and to see if a compound is ready for human trials." He added, "We have a unique opportunity with this cystic-fibrosis story to figure out how we can help other diseases."

It seems likely that the treatment for cystic fibrosis will ultimately consist of combinations of these targeted therapies, much the way people with AIDS have gained longevity with multi-drug regimens. Because each parent, as a silent carrier, contributes one mutated gene, many children with the disease have two separate defects—for example, a misshapen CFTR mutation and a nonsense mutation, or a misshapen CFTR and a rusty-gate type of mutation. The clinical studies are still in the early stages, so neither the long-term benefits nor the potential side effects are known. Combining drugs might allow for synergy in restoring chloride movement, but it might also lead to toxic interactions, as with any combination therapy.

All the physicians and scientists I talked to who are working on the experimental agents emphasized the many unknown variables, but for the first time there is convincing evidence that the underlying defect in cystic fibrosis can be corrected. Dr. Susanna McColley, the director of the Cystic Fibrosis Center at Children's Memorial Hospital, in Chicago, who did not participate in the Vertex or the PTC studies, told me, "These drugs are absolutely for real. It's very striking, for example, that with the Vertex drug patients showed dramatic improvement and when it was stopped their lung function dropped back down." It was particularly surprising, McColley said, because most experts believed that these patients' lung function had been so destroyed over years of infection that they were incapable of such improvement. "The work with these drugs tells us that maybe we were not ambitious enough in our thinking," she added.

McColley did take part two years ago in studies of denufosol, a drug produced by the biotech company Inspire that works in a different way from the Vertex and PTC drugs, by stimulating an alternative-chloride channel and bypassing the effects of the genetic mutations entirely. The drug, which increases hydration of lungs and helps open airways, is inhaled. In the placebo-controlled trial, involving three hundred and fifty-two patients, those on the drug showed a meaningful improvement in lung function. The study was not limited to a particular category of mutation, and the patients continued their standard therapies, such as antibiotics, during the trial. Dr. Accurso, at the Denver Children's Hospital, acted as the lead principal investigator for denufosol's Phase III clinical trial; one of his patients, Maya Nibbe, a seven-year-old with cystic fibrosis, participated in the study. (Another large clinical study is already under way.) Maya takes some forty pills a day, receives aerosol treatments with bronchodilators, to open her airways, and uses Pulmozyme, an enzyme-based treatment, to thin and loosen the viscous mucus. "During the cold weather, she needed supplemental antibiotics about once every month," her mother, Jennifer Reinhardt, told me when we spoke by phone. While on the denufosol trial, which lasted forty-eight weeks, Maya needed such supplemental antibiotic therapy only once. She has been off the drug for eighteen months, and her lungs are now populated by resistant staphylococcus—MRSA—requiring two months of combination-antibiotic therapy. "It's really hard on her," Maya's mother said. "It's frustrating that we can't have it." ♦