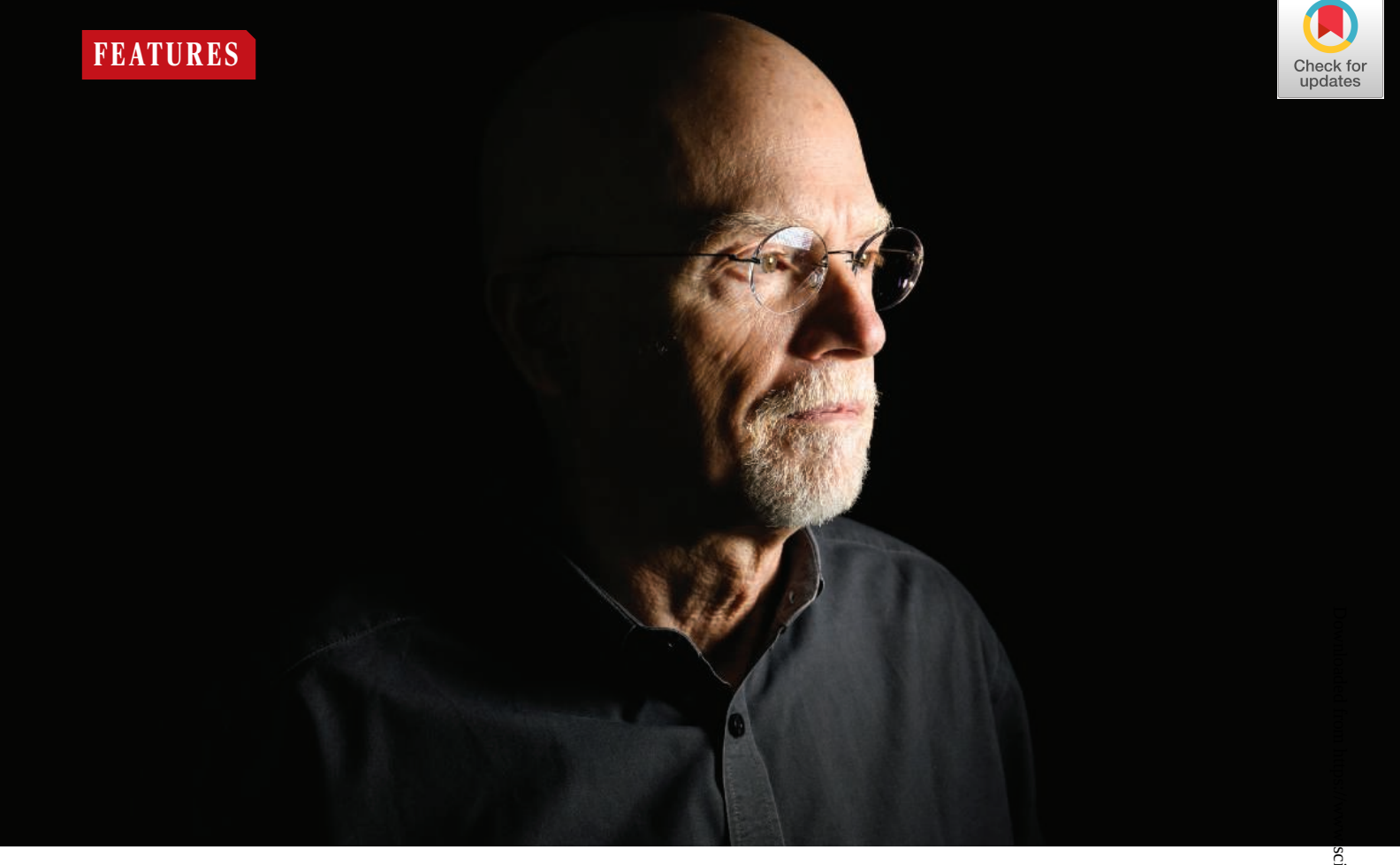


Tim Greenamyre sees new hope on the horizon for treating Parkinson's disease.

PHOTO: JUSTIN MERRIMAN

Several years ago, Tim Greenamyre, a neuroscientist and physician who directs the Pittsburgh Institute for Neurodegenerative Diseases at the University of Pittsburgh (Pitt), began to notice unsettling symptoms in his own body. He couldn't smell things. He was constipated. He was shouting and kicking in his sleep. His left arm didn't swing when he walked.

In July 2021, Greenamyre turned to a neurologist colleague to confirm the diagnosis he already suspected. He had Parkinson's disease, an illness he has devoted himself to treating and trying to cure. Over the course of his long and productive career, the 67-year-old has not only won the admiration of his patients and clinical colleagues, but also developed a widely used animal model of Parkinson's and contributed key insights into environmental triggers. That work exposed him to chemicals that induce the disease in rodents, a possible factor in his own illness.

"The irony is obvious," says Greenamyre, a shy man with a dry sense of humor and a penchant for practical jokes who, to the unpracticed eye, shows few if any signs of the disease. (For now, he says, medication is helping.)

For colleagues, the news has been shocking and heartbreaking. "I was so deeply affected I could not respond right away," says Laurie Sanders, a neuroscientist who studies Parkinson's at Duke University School of Medicine and is a former Greenamyre postdoc. "All I really honestly wanted to do was just drive to Pittsburgh and give him a hug."

Nearly all of Greenamyre's 200 or so Parkinson's patients, some of whom he has looked after for more than a decade, will learn of his diagnosis from this article, in which he is reluctantly making his condition public because it's becoming the subject of rumors. He worries it will distract them. "I want their visits to be focused on them, not on me," he says.

Compounding the irony, Greenamyre's diagnosis comes at a time of fresh optimism among Parkinson's researchers, who believe they might at last be closing in on treatments that could slow or stop the progression of the second most common neurodegenerative disease after Alzheimer's. Parkinson's disease afflicts roughly 1 million people in the United States, with nearly 90,000 new cases diagnosed each year (see graph, p. 452). Globally, more than 8.5 million people have the disease, which is the fastest growing neurological disease in the world by one estimate.

The classic motor symptoms of Parkinson's disease, described in 1817 by English surgeon James Parkinson, result from the degeneration of cells that produce the neurotransmitter dopamine in the substantia nigra, a midbrain area involved in movement control. This causes a variety of symptoms including, most commonly, tremors, muscle stiffness, and trouble with balance and coordination. As the disease advances, people may have difficulty speaking and initiating movement. Later, many develop dementia. Parkinson's itself doesn't kill, but its complications, particularly aspiration pneumonia due to difficulty swallowing, often do.

Dopamine, delivered in an oral drug combination of levodopa (L-Dopa) and carbidopa, has been the front-line therapy since it was approved in the U.S. in the 1970s, although it remains unavailable or unaffordable for many globally. The drug improves motor symptoms, but with time it begins to wear off more quickly, and sometimes-intolerable

TWIST OF FATE

A physician-scientist has probed Parkinson's disease for more than 30 years. Now, he has it

By **Meredith Wadman**

side effects—including involuntary, jerking movements called dyskinesia—frequently develop. Fifty years on, the failure of science to produce a therapy that stops the disease instead of targeting its symptoms has been painful and frustrating for patients and their families.

But now, "We are at an inflection point," says Todd Sherer, a neuroscientist and former Greenamyre postdoc who ran the Michael J. Fox Foundation for Parkinson's Research until 2021 and is now its chief mission officer. Such optimism is the outcome of decades of research, hastened by the revelations, beginning in 1997, of genetic mutations linked to the disease. Those discoveries opened the doors for scientists including Greenamyre to probe the disease's molecular mechanisms, and a gusher of publications resulted (see graph, p. 452). The new knowledge has in turn allowed companies to develop drugs and other experimental therapies that aim to slow, stop, or even prevent the disease (see graphic, p. 450). Today those therapies are entering clinical trials at a striking rate.

"There has been a shift because we are better understanding the disease and the targets," Sanders says, noting that more than 50 clinical trials attacking the roots of the disease are in progress. It's an extraordinary increase, she says, from the handful that were underway when she was starting out in the field 15 years ago.

Despite such hopeful signs, the anticipated breakthroughs may come too late for Greenamyre and his patients with Parkinson's. By the time the disease is diagnosed via a shaking hand or a dragging foot, it is thought to have been active under the radar for decades, causing the quieter symptoms such as constipation and loss of smell that Greenamyre experienced—and destroying roughly half of the dopamine-producing neurons in the substantia nigra. But Greenamyre remains determined to break the back of the disease.

"There's no good time to be diagnosed with Parkinson's disease," he said last fall when he won a $100,000 prize for research leadership from the Fox foundation. "But this is the best time in history to be diagnosed with Parkinson's disease."

GREENAMYRE GREW UP in New York's Westchester County, the son of a chemical engineer and a homemaker. In medical school at the University of Michigan, one of his first lecturers was physician Anne Buckingham Young, an enthusiastic new assistant professor whom Greenamyre recalls as a "force of nature." As Young described her Ph.D. work illuminating how the poison strychnine exerts its effects at receptors for the neurotransmitter glycine in the spinal cord, Greenamyre became entranced. "I thought it was so cool, the space she was in, where she could talk about basic pharmacological, physiological mechanisms that have clinical implications," he says. He joined her lab and developed a technique for visualizing neurotransmitter receptors in the brain, which he and Young used to investigate the mechanisms of Alzheimer's and Huntington diseases. He earned both an M.D. and a Ph.D., and by 1990, as he began running his own lab and seeing patients at a top-notch movement disorders clinic at the University of Rochester, he had co-authored 20 papers, two of them in *Science*.

He also began to study Parkinson's disease. Like others in the field, he was inspired by a 1983 *Science* paper that described a startling cluster of young people with sudden-onset Parkinson's who had turned up in Northern California hospitals. All had taken a street drug contaminated with a chemical called MPTP. Its toxic metabo-

lite, MPP+, had destroyed dopaminergic neurons in the substantia nigra, and scientists soon showed that MPP+ homes in on and inhibits complex I, the first enzyme in a crucial biochemical chain that converts food to energy in mitochondria, the tiny powerhouses inside cells. It was a tantalizing clue that mitochondrial damage might play a role in the disease.

Greenamyre and others knew MPP+ wasn't the only inhibitor of complex I. There were many, including some chemicals that studies were beginning to implicate in Parkinson's disease. But the classical inhibitor of this vital mitochondrial enzyme was a pesticide used by home vegetable gardeners called rotenone.

Rotenone was considered an "organic" insecticide because it was derived from the roots of certain plants; people also used it to kill fleas and ticks on their pets, and wildlife agencies used it to control invasive fish populations. But for Greenamyre, it was a way to plumb the mysteries of Parkinson's. By 1990, he had begun using radiolabeled rotenone, a fat-loving chemical that readily crosses biological membranes, including the blood-brain barrier, to map locations in the brain of complex I.

Around the same time, several labs found evidence in postmortem brain samples as well as platelets from patients that people with Parkinson's disease had defective complex I activity in their mitochondria. Greenamyre realized he might be able to mimic the disease in rats by exposing them to rotenone. He expected the chemical would addle mitochondria in every organ. And it did. But it had outsize toxic effects on just one cell type.

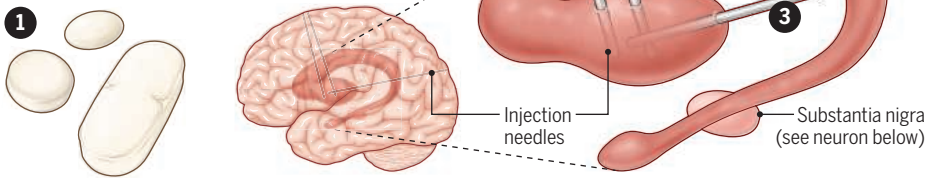
In 2000 Greenamyre, who by then was at Emory University, and his team published a seminal paper in *Nature Neuroscience*. They reported that giving rats a chronic intravenous infusion of rotenone selectively destroyed the same dopamine-producing neurons in the substantia nigra that degenerate in Parkinson's patients. Moreover, the surviving neurons contained fibrils, or tiny threads, of the protein alpha-synuclein, aggregated in clumps. Those aggregations closely resembled structures called Lewy bodies that are a signature of Parkinson's in human brains. What's more, the rats developed parkinsonian symptoms: unsteady movements and hunched postures, shaking paws and severe rigidity.

The work gave researchers the first animal model that captured both the classic motor symptoms and the hallmark pathology of the disease. It also increased suspicions that rotenone and other pesticides could trigger Parkinson's.

Taking aim at Parkinson's

Decades of research identifying genes and probing the biological mechanisms of Parkinson's disease have led to dozens of ongoing clinical trials that aim to slow or stop the disease. Some act on the dopamine-producing neurons in the substantia nigra that degenerate in Parkinson's. These cells project to nearby structures called the caudate nucleus and putamen.

Therapeutic strategies

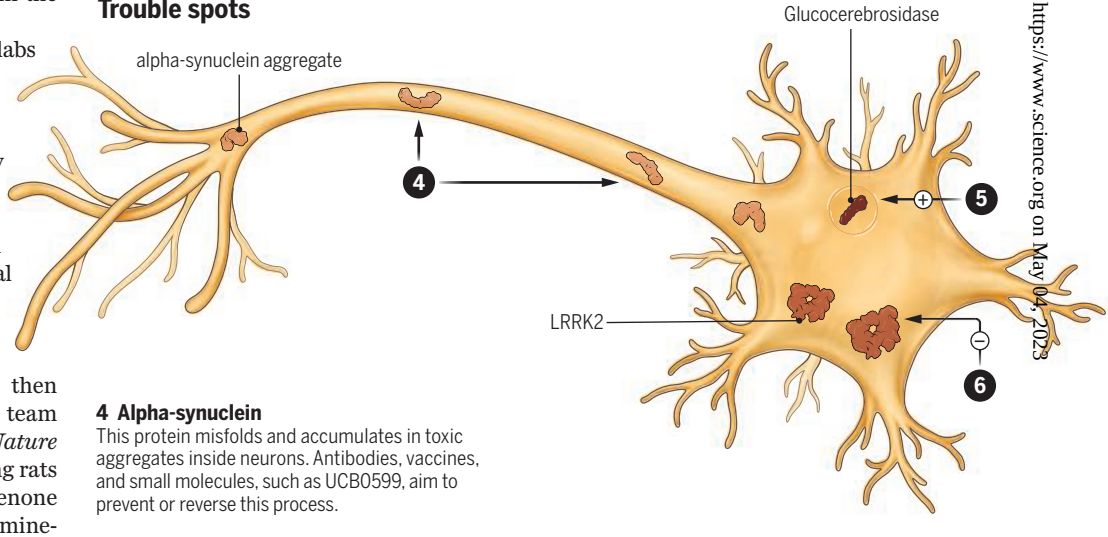


1 Oral and injectable drugs
Some drugs now in trials have specific targets within neurons (see below). Another approach repurposes self-injected diabetes drugs called GLP-1 receptor agonists. These may improve neuronal health by stimulating insulin secretion in the brain, which is often impaired in Parkinson's.

2 Gene therapies
This approach aims to boost survival or regeneration of dopaminergic neurons. One trial injects a nonreplicating virus carrying the gene for glial cell line–derived neurotrophic factor into the putamen.

3 Cell replacement therapies
An early trial is injecting healthy dopamine neurons derived from human embryonic stem cells into the putamen. Another approach uses mesenchymal stem cells to promote neuronal survival and regeneration.

Trouble spots



4 Alpha-synuclein
This protein misfolds and accumulates in toxic aggregates inside neurons. Antibodies, vaccines, and small molecules, such as UCB0599, aim to prevent or reverse this process.

5 Glucocerebrosidase
Suppressed activity of this enzyme in Parkinson's disease has been linked to accumulation of toxic alpha-synuclein. Researchers are trying gene therapy and drugs such as ambroxol, a repurposed cough medicine, to restore the activity.

6 LRRK2 (leucine-rich repeat kinase 2)
This enzyme is overactive in some patients, likely driving lysosomal damage, the buildup of toxic byproducts inside cells, and neurodegeneration. Drugs and synthetic genetic snippets called antisense oligonucleotides aim to tamp down LRRK2 activity.

GRAPHIC: A. MASTIN/*SCIENCE*

TODAY, GREENAMYRE wonders whether his decades of research with rotenone and similar compounds may have caused his disease. "Because we didn't know as much, we weren't as careful," he says. "And I got exposed to things, and particularly rotenone, quite a bit."

It's February, and Greenamyre is musing aloud over a lunch bowl of rice, spinach, and goat cheese in his sunlit office, which adjoins his lab in the heart of Pitt's medical campus downtown. He has just come from a lab meeting where neuroscientist Emily Rocha, a former postdoc, presented a gnarly challenge she had encountered in her research, and a stop to look over some newly collected images of dopamine neurons with grad student Matthew Keeney. Former grad students and postdocs say Greenamyre has an encouraging but hands-off style of mentoring. "He had

a great ability to let you explore but then he knew when to pull you back when you were going down a rabbit hole," Sherer recalls.

Over lunch, Greenamyre explains that rotenone has to be dissolved in a solvent like dimethyl sulfoxide (DMSO) to make an infusible solution. That solution would spill on his gloves from time to time, and "the DMSO takes it right through the gloves and into your skin and right through your skin." He didn't make much of it at the time, he says, because he and his colleagues didn't really think there was much danger. "Till we did a little research on it," he adds. (A high-quality epidemiological study published in 2011 associated rotenone use with a 2.5-fold increase in risk of developing Parkinson's disease in farmers and their spouses.)

If rotenone did play a role in causing Greenamyre's disease, it likely added to underlying genetic vulnerabilities. He is one of the 90% of people with so-called idiopathic disease: Their Parkinson's has no clear genetic cause but almost certainly results from some combination of ill-defined genetic susceptibilities and environmental triggers. Greenamyre, for example, once had red hair, which epidemiological studies have associated with an elevated risk of Parkinson's. (Why this might be isn't understood.)

About 10% of Parkinson's cases, however, are clearly due to mutations in specific genes. After a 1997 *Science* publication identified the first of them, in the gene for alpha-synuclein, many scientists focused on genes and only genes. Soon they unearthed more inherited mutations—in genes encoding a protein called Parkin, an enzyme called LRRK2, and another enzyme, glucocerebrosidase. Researchers began to drill down on how the mutated genes were doing their damage.

These discoveries coincided with a tricky time in Greenamyre's personal life. A difficult divorce led to his move from Emory to Pitt in 2004. He also faced funding woes. Greenamyre's primary funder at the time, the Picower Foundation, had invested tens of millions with Bernie Madoff, the notorious Ponzi scheme operator; it was all lost. "Anything from $20 on up is welcome," Greenamyre told *Neurology Today* in 2009 when asked how he would keep his 13-member lab afloat.

Despite such obstacles, Greenamyre has spent the past 20 years trying to define the critical mechanisms that destroy neurons in Parkinson's, rather than chasing new causative genes as many others have done. He has stayed fixed on the complicated dance between genes and the environment, with an emphasis on pesticides and solvents that interact with key Parkinson's-associated genes.

In recent years, Greenamyre has become laser-focused on *LRRK2* (pronounced "lark 2"), which stands for leucine-rich repeat kinase 2. The enzyme it encodes is a master traffic controller, regulating the movement of proteins and compartments called vesicles inside cells. Several mutations in the gene send the enzyme's activity into overdrive. That ultimately damages the function of lysosomes, the cellular trash collectors that degrade unwanted proteins, and the damage is thought to contribute to the disease.

Mutations in *LRRK2* account for about 3% to 4% of all cases of Parkinson's. But in 2021, Greenamyre's group, led by postdoc Briana de Miranda, now an assistant professor at the University of Alabama at Birmingham, found that toxins can mimic their effects. They reported that the solvent trichloroethylene, a mitochondrial toxin used in dry-cleaning and degreasing metal, ramped up LRRK2 enzyme activity and induced Parkinson-like pathology in the brains of older rats. The work has provided grist for an incipient class action lawsuit from people who decades ago drank contaminated water at the Marine Corps base at Camp Lejeune in North Carolina. They allege that trichloroethylene in that water caused their Parkinson's disease.

Greenamyre's decades of work on environmental toxins also helped form the basis for a lawsuit filed by the Fox foundation and others against the U.S. Environmental Protection Agency (EPA) after the agency in 2021 reapproved the widely used herbicide paraquat. Like rotenone, paraquat causes rodents to develop parkinsonian symptoms and pathology, and other researchers have used it to make animal models of the disease. EPA argued that with safety measures in place, paraquat's benefits in agriculture outweighed its risks to human health, saying it had found "insufficient epidemiological evidence" to suggest a causal link to Parkinson's. Greenamyre filed an amicus brief in the case, chiding EPA for discounting numerous epidemiological and animal studies linking paraquat exposure to Parkinson's. (At least 50 other countries have banned its use.)

Since Greenamyre's groundbreaking 2000 paper using rotenone to create Parkinson-like disease in rats, EPA has twice examined the safety of that pesticide as well. In 2007, it restricted rotenone's use to control of invasive fish species—and manufacturers voluntarily withdrew it from the market for residential home and garden use. Last year, EPA imposed further safety restrictions on its use in fish kills and reiterated that the occupational risks of rotenone exposure, driven by skin exposure, "are of concern." But the agency in 2022 also reviewed the literature and concluded there is "insufficient evidence" to suggest a causal link between rotenone and Parkinson's.

Other scientists say epidemiology studies and lab work by Greenamyre and others are persuasive. "He has made a really compelling case for rotenone and other pesticides as very significant and relevant triggers for idiopathic Parkinson's disease," says Malú Gámez Tansey, a neuroscientist at the University of Florida College of Medicine. "It was a very important contribution."

Tim Greenamyre as a teenager. Red hair may be a risk factor for Parkinson's.

PHOTO: J. TIMOTHY GREENAMYRE

IN A CLINIC in the next building over from his lab, Greenamyre has donned a white coat and mentally prepared his list of questions for Barbara Frieze, 71, a Parkinson's patient he has been looking after for nearly 10 years. "How's your puppy?" he asks, an easy opener. She confides that the puppy has kept up her spirits as her husband, who has dementia, declines in a nursing home. Frieze visits him every day, which makes exercising tough, she explains when he gently prods her about her activity. (Aerobic exercise probably slows the progression of motor symptoms, and Greenamyre himself works out 7 days a week.)

He goes on to ask Frieze about her sleep, her balance, her mood, and her tremors. The latter are painfully visible when she extends her arms straight in front of her; her left hand continues to shake as she walks down a nearby hall at Greenamyre's request.

Back in the exam room, he says: "For 11 years in, you're doing great. Your walking is really good. The only way I would know you had Parkinson's was the tremor."

"When I leave here, I feel hopeful," Frieze says. "If I'm feeling dubious about the Parkinson's and I come here, I always feel good leaving."

The next patient, Carl Maskiewicz, is a mostly retired accountant in his late 60s who attends boxing and yoga classes six times a week. He praises Greenamyre's patience and ability to explain things. "I think sometimes he has a better idea of what I'm going through than people that have Parkinson's," Maskiewicz says.

A third patient, Robert Hannan, calls in by Zoom from Florida. The 84-year-old former CEO of a drugstore chain has had the disease for 25 years. He takes dopamine every 2 hours now, but as the drug wears off, his words become slurred and the tremor in his left hand reappears.

On this February day, Greenamyre did not tell the patients he saw about his own diagnosis. He emailed them soon after, though, not wanting them to be blindsided when the news came out. "I told him he was in my thoughts and prayers," says Maskiewicz, who has been Greenamyre's patient for 9 years. "And I offered to chat, 'If you ever just want somebody to talk to'—because sometimes that's the difficult part of this disease."

Like the million other Americans with Parkinson's, Greenamyre's patients are all waiting for something beyond dopamine. "It's still a disease without a cure," Hannan says. "I keep asking every time I come: 'Is there something close?'"

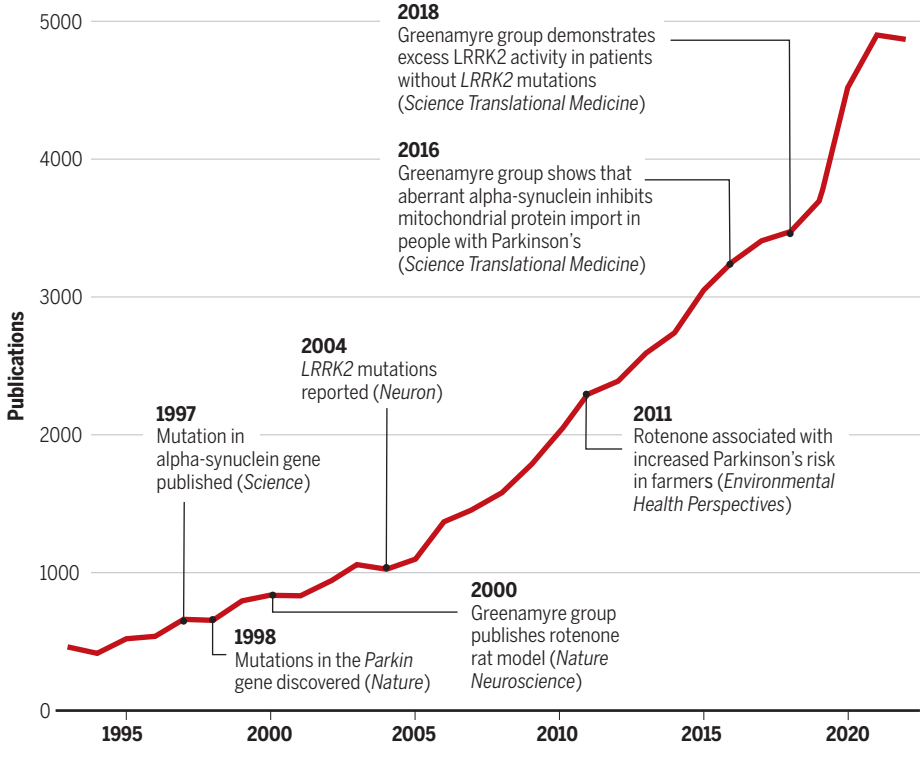
IN 2018, A NEW PAPER from Greenamyre's group, led by research scientist Roberto di Maio, pointed to one way forward. They had developed an assay to gauge LRRK2 enzyme activity in different kinds of brain cells and used it to show that the enzyme is overactive in dopamine-producing neurons in the substantia nigra—even in patients with no mutations in the gene.

Additional experiments revealed the underlying cascade of molecular events, culminating with the crippling of the cell's garbage disposal system. This, the team posited, resulted in accumulation of aberrant alpha-synuclein.

"This was the first data in actual patient tissues demonstrating … in patients with

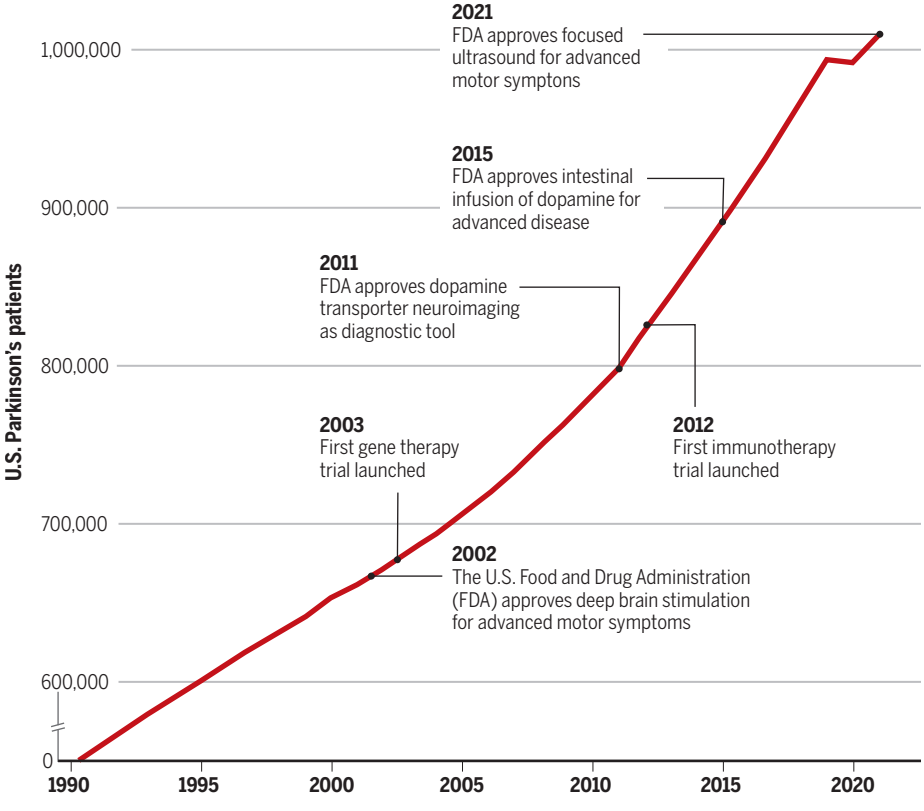
An explosion of research

After genetic discoveries opened new windows on Parkinson's, the number of papers probing its biology soared, as gauged by the number of PubMed publications with "Parkinson's" in their titles.



A growing problem

The aging of the United States has helped drive a big increase in Parkinson's prevalence, but the only approved therapies target symptoms rather than disease progression.



CREDITS: (GRAPHICS) D. AN-PHAM/*SCIENCE*; (DATA, BOTTOM GRAPHIC) PARKINSON'S FOUNDATION

idiopathic Parkinson's disease that they do have increased LRRK2 kinase activity," says Carole Ho, chief medical officer and head of development at Denali Therapeutics.

The paper strongly suggested inhibiting that activity might help many patients—and not just the 3% to 4% who harbor inherited *LRRK2* mutations. In rats given rotenone, the researchers found, an LRRK2 inhibitor blocked all the abnormal events typically caused by the pesticide. The finding gave new impetus to efforts by companies to test LRRK2 inhibitors in people; Denali's candidate is the furthest along. It has partnered with Biogen, which is now enrolling more than 1000 Parkinson's patients with and without *LRRK2* mutations to see whether an inhibitor of the enzyme slows progression of the disease.

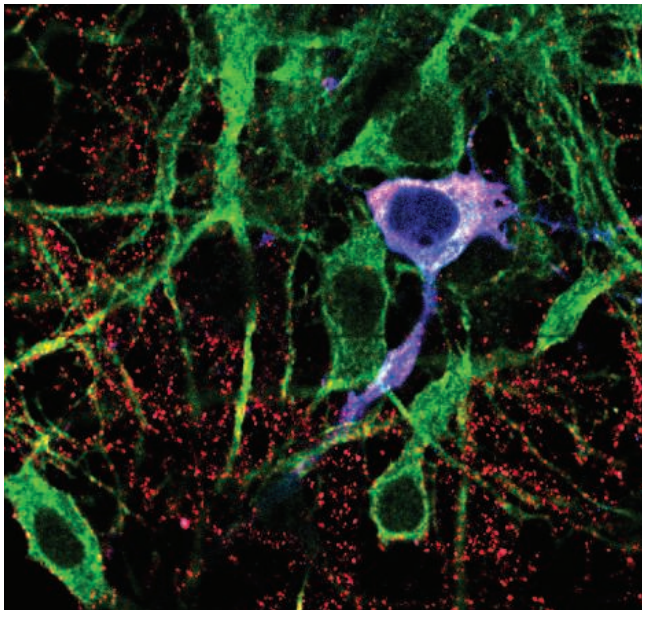
Labs and clinical trials around the world are pursuing other drugs, gene therapy, and stem cells to replace lost dopamine neurons. Some target demonstrated biological culprits, for instance boosting activity of the enzyme glucocerebrosidase, which can be suppressed in Parkinson's, or blocking aggregation of toxic alpha-synuclein. Others aim to give general ammunition to the brain, such as pharmacological tools for better repair and maintenance of dopaminergic neurons.

There have been early disappointments. In 2021 and 2022, respectively, Biogen and AbbVie pulled the plug on clinical trials of antibodies that attack alpha-synuclein. Roche and Prothena Biosciences are pressing ahead despite similarly disappointing trial results last summer showing that their monoclonal antibody, prasinezumab, had no meaningful impact on disease progression.

Many researchers hope such conspicuous failures will become rarer. The underlying biology of Parkinson's varies widely between subsets of patients, and efforts are afoot to match the right people to the right trials so that each therapy is tested in those most likely to respond to it.

Identifying at-risk people long before motor symptoms appear could also help. Antibodies to alpha-synuclein might still work, for example, if given early enough, before extensive neuron loss. The Fox foundation is running a huge, long-term study looking for imaging, biologic, and genetic markers that could catch people in the earliest stages of the disease. That study produced landmark results last month: A report in *The Lancet Neurology* found that a spinal tap test for misfolded alpha-synuclein in the fluid bathing the spinal cord accurately diagnosed Parkinson's 88% of the time, including identifying it before people started to have motor symptoms. Although the test's invasiveness makes it unlikely to be used routinely, it will be a valuable tool for sorting patients for clinical trials and probing the disease's biology, scientists say. And a similar blood test, if promising early results bear out, may be available by the time therapies are ready to treat early-stage disease.

AFTER MONTHS of worrying about his own lack of smell and other symptoms, Greenamyre finally turned to a trusted colleague, Edward Burton, a Pitt neurologist and neuroscientist who studies Parkinson's. Burton says he is sometimes burdened by his training; he analyzes people without meaning to, with an eye expertly attuned to the most subtle abnormalities of movement.



Oxidative damage (magenta) in a rat dopaminergic neuron (blue) after treatment with the pesticide rotenone.

In the summer of 2019, Burton was returning from a Gordon Research Conference on Parkinson's Disease that Greenamyre had chaired, and the pair was sitting in an airport lounge. Someone else asked Greenamyre a question, and Burton thought his friend's head turned toward the questioner just a shade too slowly.

Soon after, the COVID-19 pandemic descended and Burton did not see his colleague for the better part of 2 years. Then in July 2021, when both men were back in the lab and the clinic, Greenamyre asked Burton to meet confidentially with him.

"He was clearly quite worried," Burton recalls. (Greenamyre gave Burton permission to discuss his case for this article.) The sleep disturbances Greenamyre had been having for several years, rapid eye movement sleep behavior disorder (RBD), did not bode well. RBD is an early symptom of Parkinson's, but it can also be a harbinger of rarer, more rapidly progressive neurodegenerative diseases.

Burton listened carefully to his friend's account of his symptoms, then gave him the same motor tests that Greenamyre had given his own patients countless times. When Greenamyre tried to quickly open and close his index fingers and thumbs, his left hand lagged subtly. Burton had already noted in casual passings in the hall that his colleague's gait was asymmetrical; he was not swinging his left arm as he did his right.

"With it being somebody that I like very much and respect a great deal, examining him, every physical sign was like having my stomach ripped out," Burton recalls.

Greenamyre's symptoms and the slow progression of his illness led Burton to conclude this was likely Parkinson's and not something still worse. He started Greenamyre on dopamine therapy, and his symptoms rapidly improved, clinching the diagnosis.

"There was some slowness. And that totally changed," recalls Greenamyre's partner, Pitt neuroscientist Teresa Hastings. "His whole posture seemed to change once he started on the L-Dopa. It's like his muscles were tuned up."

As their interview ended that summer day in 2021, Greenamyre reached out to shake Burton's hand. "He thanked me for evaluating him," Burton says. "Tim has really handled this with dignity and aplomb."

IN A BREAK between patients in February, the typically reserved Greenamyre admitted to some worries about what comes next. "When I have some weird sensation or something—you know, everybody has these things. But now you wonder whether that's part of the disease … a tingling here or a tingling there."

Still, he remains optimistic, as he was when his patient Hannan asked whether a better therapy was finally coming into sight. "I think we're getting to the heart of it. But it's not ready for prime time," Greenamyre said. "Things are just beyond the horizon." ■

IMAGE: MATTHEW T. KEENEY/UNIVERSITY OF PITTSBURGH

Twist of fate

Meredith Wadman

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