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Cracking the Alzheimer's Code

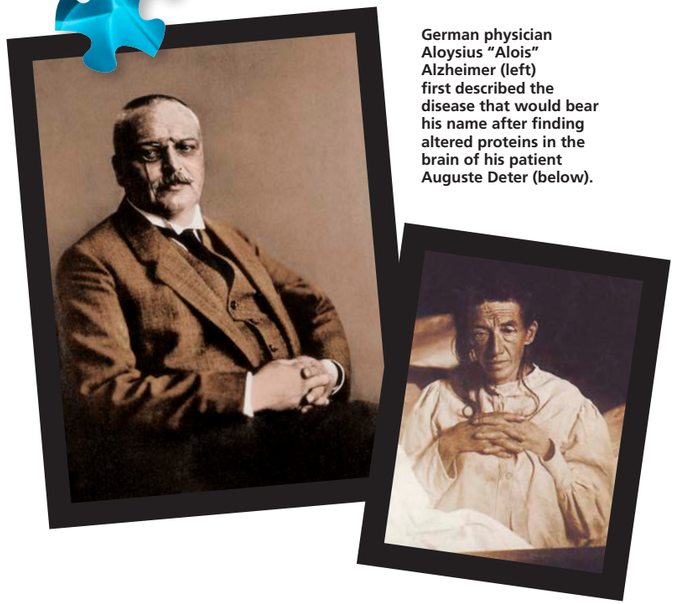
Are we close to conquering one of the most ***puzzling diseases*** of our time?

BY **LINDA MARSA**

More than a hundred years ago, German physician Alois Alzheimer did a grim postmortem analysis of the dementia-ravaged brain of one of his patients. He hoped to unmask the biological roots of her severe and rapid mental deterioration and bizarre mood swings.

Her name was Auguste Deter, and she was admitted to the Hospital for the Mentally Ill and Epileptics in Frankfurt in 1901. When Alzheimer first interviewed the 51-year-old woman, she was enveloped in a fog of confusion, and she exhibited delusional behavior: She was intensely jealous of her husband; she sometimes would start screaming, thinking people wanted to kill her; and she became wild and uncontrollable. She died five years later.

When Alzheimer examined thin slices of her brain under a microscope, he noticed that nestled right next to the labyrinth circuitry of healthy nerve cells were small clumps of hard, barnacle-like



German physician Aloysius "Alois" Alzheimer (left) first described the disease that would bear his name after finding altered proteins in the brain of his patient Auguste Deter (below).

bundles of proteins called amyloid plaques and that many of the fibers extending from the ends of the nerve cells — different proteins called tau — were thickened and tangled. This aberrant brain circuitry — the amyloid plaques and tau tangles — became the twin hallmarks of the disease that bears his name.

For years, scientists have struggled to understand how these proteins work. Why do they go haywire? What comes first — plaques or tangles? And which one is the miscreant that drives nerve degeneration? Are amyloids the toxic bad boys, or the tau tangles? Each camp has long had its devotees. But recent technological advances and a series of remarkable discoveries in the past two years have provided key clues about how Alzheimer's disease ravages the brain.

Although researchers are reluctant to utter the word *cure*, they're tantalizingly close to answering many of the questions that have stymied Alzheimer's research, and they're finding ways to prevent this devastating brain-wasting disease — or at least reduce the damage it causes. "Within our lifetimes, we will conquer Alzheimer's," says Anne Young, a Harvard neurologist and director of the MassGeneral Institute for Neurodegenerative Diseases in Boston. "It's an incredibly exciting time, and everything we dreamed of is coming to reality."

THE LOOMING AVALANCHE

More than 5 million Americans have Alzheimer's, and it's the underlying cause of 500,000 deaths each year. Barring a medical breakthrough, the incidence of the disease is expected to reach epidemic proportions as the nation's 76 million baby boomers move into old age. The number of Alzheimer's patients is expected to hit 100 million worldwide by 2050, including as many as 16 million in the U.S. Such huge demand for care would put unbearable strains on society and could bankrupt health care systems.

This looming avalanche of Alzheimer's has lent greater urgency to the search for treatments. In 2012, the Obama administration earmarked $156 million over two years to fund research, bringing the total to $530 million a year; the goal is to defeat Alzheimer's by 2025. That may seem ambitious, but there is now reason to believe this target may be within reach.

Until now, the quest for effective Alzheimer's treatments has been marked by costly and high-profile failures, mainly because we haven't focused on the right targets — and we're stepping in too late. Scientists believe that by the time the disease rears its ugly head, patients likely have sustained irreversible damage, which may explain why the drugs tested in clinical trials don't do much good.

But there is hope on the horizon: Scientists recently mapped out many of the neural pathways through which Alzheimer's develops. Powerful, new high-resolution visualization tools can peer deep inside a living brain, allowing scientists to track the cascade of events that leads to Alzheimer's. Genetic discoveries have shed light on the underlying biological mechanisms of the disease. Unpacking how these bits of renegade DNA work may provide more clues on how to stop the disease.

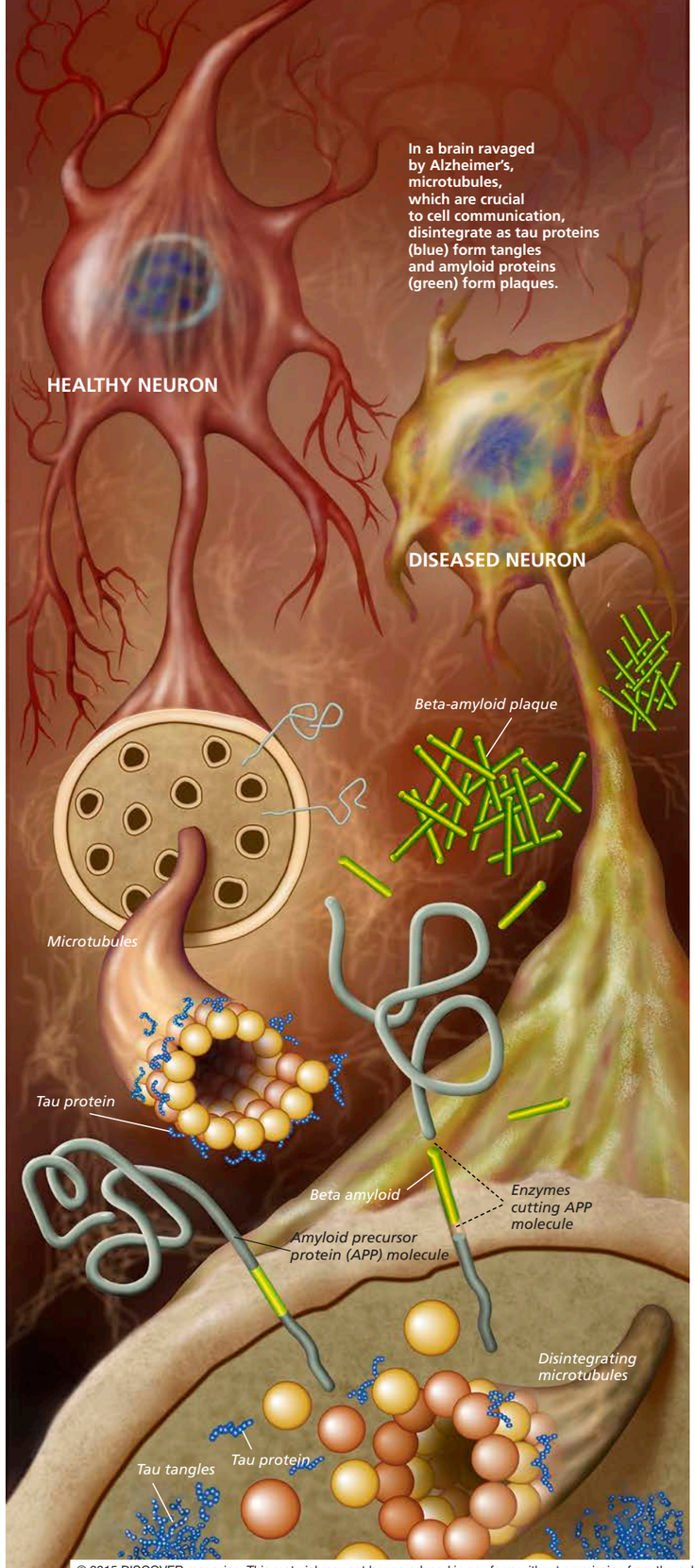
This deepened understanding of what drives neurological deterioration should lead to smarter therapies, while new diagnostic tools could allow intervention before too many neurons are destroyed. In the not-too-distant future, "we'll be able to identify those most at risk based on their genetics, do imaging tests to determine the onset and then institute therapies that nip it in the bud," says Rudolph Tanzi, a neurologist at Harvard Medical School. "That's the mantra: early prediction, early detection and early intervention."

But Alzheimer's is not divulging its secrets easily.

A DEADLY CASCADE

We now know that plaques are clusters of protein fragments called beta-amyloid peptides. They collect outside the nerve cells in the brain and disrupt the signaling system between neurons, blocking them from relaying messages. This communication breakdown explains why Alzheimer's patients suffer progressive memory loss, confusion and increasing difficulty doing daily tasks.

In contrast, the tau protein wreaks havoc *inside* nerve cells, causing



In a brain ravaged by Alzheimer's, microtubules, which are crucial to cell communication, disintegrate as tau proteins (blue) form tangles and amyloid proteins (green) form plaques.

THIS PAGE. FROM LEFT: JESSICA WILSON/SCIENCE SOURCE; PHOTOTAKE, INC./PHOTOTAKE. OPPOSITE: JAY SMITH

the accumulation of spaghetti-like strands that choke the neurons. This disrupts the flow of electrical signals that travel through the nerve cells, ultimately causing them to wither and die. The buildup of twisted tau deposits also destroys surrounding nerve cells and eventually wastes away vast swaths of brain tissue. Other vital structures, the axons and dendrites — known collectively as neurites — that project from the nerve cells and send and receive messages are dependent on this neural transport network. When this network is disrupted by tau tangles, it can trigger a chain reaction of damage in the memory and cognitive circuitry of the brain, accelerating mental deterioration.

Amyloid and tau are both naturally occurring proteins. The body produces amyloid plaques and then eliminates them in a normal biological process of decay and renewal. In some instances, amyloids may even serve a protective function; recent studies indicate they can act as molecular guardians that mute the body's autoimmune reactions. They also mop up the errant cells involved in inflammation after an injury. But for reasons that are yet unknown, when Alzheimer's develops, this protein divides improperly and creates a form called beta amyloid, which is toxic to neurons in the brain. When this happens, the brain generates plaques, which accumulate around the neurons and eventually disrupt the signaling system between brain cells.

Tau proteins, for their part, are vital to the structural integrity of neurons, acting like girders that stabilize synapses — the bridges that allow electrical impulses to cross between neurons. But at some point, tau goes astray, too, and the nerves lose their structural support, causing the cells to collapse and die. In fact, tau tangles may be the real culprits behind memory loss, since they're directly linked to cognitive deficits. People can have amyloid plaques and still function normally, but once they have tau tangles, dementia is evident.

"Tangles drive the dementia and drive the nerve degeneration," says Tanzi, who also is director of the Genetics and Aging Research Unit at Massachusetts General Hospital. "If you don't get tangles, you don't get the disease. Once tangles form, they spread like wildfire and keep spreading. But the biggest gaping hole in our understanding is, how does amyloid drive tangle formation?"

Scientists are beginning to find some clues, through genetic fingerprints and with technologies that allow researchers to watch plaques form.

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PEERING INSIDE THE BRAIN

Until recently, the only way to look at human plaques was by analyzing the brains of people who died from the disease — a challenge one scientist compared to looking at a car wreck and trying to puzzle out the accident's cause. We can't just simply peel away a living person's skull to watch what happens in real time or slice off samples to scrutinize under a microscope.

Although the postmortem studies of Alzheimer's patients revealed a mess of plaques and tangles, scientists knew they would need to figure out ways to study living brain tissue to unlock the disease's secrets. Traditional imaging techniques like MRI and positron emission tomography (PET) wouldn't work. They're good at providing snapshots as small as a millimeter and can tell us about brain activity, but they can't capture the formation of human plaques or tangles, which are about 100 microns, or a tenth of a millimeter — about twice the thickness of a cat's whisker.

So until recently, much of what scientists learned was gleaned by inference or from studying mouse models of the disease. But the lab animals are not good surrogates. In the past few years, however, powerful new visualization tools, coupled with novel tracer chemicals that can illuminate even the tiniest bits of brain tissue, have given scientists a glimpse into the inner workings of the human brain.

In 2012, the FDA approved Amyvid, a radioactive dye that attaches itself to amyloid proteins so they can be highlighted on a PET scan. Similar radioactive tracers also have come online. Using chemicals like these in combination with new imaging tools, such as the multi-photon confocal microscope, has enabled researchers to explore the minuscule world of the neuron and observe brain cells in action with far more precision. This microscope is powerful enough to detect objects as small as a single micron — one-thousandth of a millimeter — and plaques are about 10 microns across.

"You can see individual connections between neurons, and watch in real time images of plaque formation," says Bradley Hyman, a neurologist at Harvard Medical School.

TRACKING RENEGADE DNA

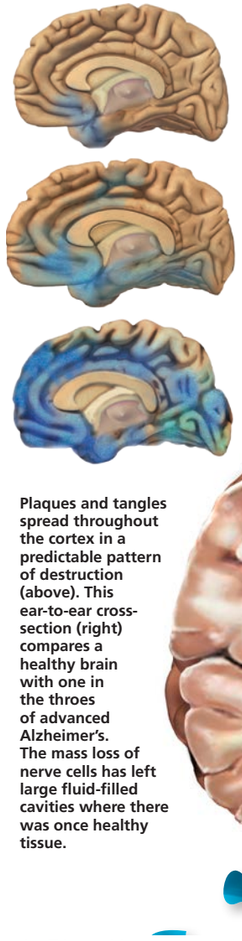
Using these new imaging tools, scientists can now track how errant genes associated with Alzheimer's behave. Although neural degeneration in Alzheimer's disease is a complicated process that happens over many years, the instigating event could be caused by just a few genes going awry. These snippets of renegade DNA are the research target.

Alzheimer's disease can tear through generations of families. In the early-onset form, it claims victims in the prime of their lives, in their 40s and early 50s. It envelops them in a fog of confusion and ultimately erodes relationships and memories. Scientists have spent much of the past three decades hunting down genes linked to this inherited form of the disease. In the past three years, they've uncovered more than a dozen of them.

Spotting the bits of mutant DNA that underlie Alzheimer's may soon lead to genetic tests that can tell whether someone is at higher risk so he or she can take preventive steps. They can also tell us about how the disease progresses.

"Every time you find a gene, you have a new tool to work with, and it gives us insights into the underlying mechanisms of disease," says Gerard Schellenberg, a pathologist at the University of Pennsylvania who chairs the Alzheimer's Disease Genetics Consortium, a collaborative effort of investigators from 44 universities and research centers.

Over the past decade, Schellenberg has spent a lot of time on the phone coaxing colleagues to do something they don't ordinarily do: pool their data rather than zealously hoard it in pursuit of a groundbreaking discovery. His years of gentle



Plaques and tangles spread throughout the cortex in a predictable pattern of destruction (above). This ear-to-ear cross-section (right) compares a healthy brain with one in the throes of advanced Alzheimer's. The mass loss of nerve cells has left large fluid-filled cavities where there was once healthy tissue.

LEFT: NATIONAL INSTITUTE ON AGING/NATIONAL INSTITUTES OF HEALTH. RIGHT: STACY JANNIS/ALZHEIMER'S ASSOCIATION

Spotting the bits of mutant DNA that underlie Alzheimer's may soon lead to genetic tests that can tell whether someone is at higher risk.

arm-twisting paid off in the creation of the consortium, which collected genetic data from more than 11,000 people with Alzheimer's and a nearly equal number of older people who showed no signs of dementia.

Using genotyping to determine genetic differences, the researchers began to see patterns emerge. They identified four new genes that were more common in those who had the disorder. When three other research groups from the United States and Europe added their patient profiles, swelling the overall numbers to 54,000, their findings were confirmed. The newly unmasked genes play a role in three distinctively different bodily functions, including systems that control inflammation and cholesterol and the regulation of how brain cells clean up toxic proteins. These discoveries build upon previous findings about genes linked to Alzheimer's, such as the *APOE-4* gene, which is a powerful marker for late-onset Alzheimer's disease; about 40 percent of those diagnosed have this DNA variant.

In fall 2013, this international consortium identified 11 more genes that increase the risk of developing Alzheimer's disease, bringing the total number of genes associated with the more common late-onset form of the disease to 21. This expanded collection helps paint a clearer picture of the factors that ratchet up risks. It also offers unprecedented glimpses of the biological pathways that drive the disorder.

Some of the newly spotted genes regulate how nerve cells talk to each other in the brain's memory centers, such as the hippocampus; others are related to immune response and inflammation. This discovery lends more weight to the growing evidence that inflammation — and our immune system's hyperactive reaction in mopping up the cellular debris left by the wayward plaques and tangles — plays a key role in the spread of the disease. "We're starting to connect the dots between the genes and the clinical symptoms," says Tanzi, who is involved in the work. "Plaques and tangles get the process going and nerve cells start to die. But it's not until inflammation kicks in that the process takes off like wildfire — and this is what drives the dementia."

Recent research also has illuminated how the deadly cascade that leads to brain atrophy is set in motion: The buildup of amyloid plaques sparks mutations in the genes that signal the formation of the renegade tau proteins. But which genes? To try to find out, Tanzi is studying the DNA of 3,000 families with multiple members affected by the late-onset form of the disease. The hope is to identify many, if not all, of the other genes and gene mutations that influence risk.

PLAN OF ATTACK

But the real heavy lifting comes with deciphering exactly what these genes do, and creating a mosaic with these bits and pieces that can provide a fuller picture of how the genetic variants work to cause destruction. A startling discovery in 2013 may contain a crucial puzzle piece that could lead to the development of therapies that can stop the disease in its tracks.

It began in 2008, when Harvard researchers fingered the *CD33* gene in a genome-wide dragnet of genes in families affected by Alzheimer's. At the time, they hadn't identified the gene's function. But in a postmortem analysis of brain tissue from patients with late-onset Alzheimer's, scientists noticed there was too much of the *CD33* gene in cells called microglia, which are dispatched by the immune system to repel foreign invaders. There seemed to be a direct relationship between the presence of *CD33* and the number of destructive amyloid plaques.

Normally, these microglia cells are beneficial. They clear out molecular debris, such as dead and dying nerve cells and deposits of the sticky amyloids that could impair brain function. But the microglia cells are hypervigilant and swing into action at the first sign of trouble. Somehow, when the microglia cells are armed with the renegade *CD33*, they go into overdrive, launching an indiscriminate search-and-destroy mission that strafes healthy neurons with friendly fire when too many amyloids start piling up.

"Instead of cleaning up when neurons die, the microglia assume they're under attack when they see too much cell death and kill nerve cells as collateral damage," says Tanzi, who led the research team. "It is now clear *CD33* is the main switch that triggers the microglia to change from a neuroprotective to a neurotoxic function." Finding a compound to block this rogue gene and turn it off, he believes, could stem the damage inflicted by the amyloids.

In fall 2013, Tanzi's group made what has been hailed as a "paradigm shifting" genetic discovery that helped settle the debate over whether it's amyloids or tau proteins that trigger late-onset Alzheimer's. After conducting experiments with a newly developed strain of mice that are good surrogates for Alzheimer's, they found that a gene called *ADAM10* makes an enzyme called alpha secretase, which prevents the formation of beta amyloid. But the mutant form of the *ADAM10*

gene did exactly the opposite: It blunted the action of the enzyme, resulting in the production of more of the toxic proteins. "People with this gene mutation usually get Alzheimer's at around age 70 because of the buildup of amyloids due to the reduced activity of the protective enzyme," says Tanzi. Researchers are now testing modulators of these enzymes in the lab to see if they can fix this defect and prevent plaque production.

But it's not just the bad actors that are being fingered. Researchers have also uncovered snippets of DNA that seem to *halt* the development of the brain disease, even in people whose genes put them at higher risk. Icelandic scientists studying the DNA of 1,795 of their citizens unearthed a rare errant gene — only 1 in 10,000 people of European descent carry the mutation — that slows production of plaques in the brain. They found that people who were 85 or older and didn't have Alzheimer's — or any decline in mental acuity, for that matter — had the mutant gene four to five times more often than those who had the disease. What the researchers found especially astonishing is that this was true even in octogenarians with two copies of the *APOE-4* gene, which is present in about 90 percent of 80-year-olds with Alzheimer's. About 25 of the people over age 85 had two copies of *APOE-4*, yet not one of them had the disease.

The next step is to figure out how the protective mutant gene works and then create a treatment that mimics its action. Accomplishing that feat would represent a giant leap toward a genuine cure. "The gene has been cloned, and we know it interferes with the production of toxic amyloid fragments," says Ralph Nixon, a professor of psychiatry and cell biology at New York University School of Medicine and a past chair of the Medical and Scientific Advisory Council of the Alzheimer's Association. "But we don't know precisely the mechanism by which it is operating — that's the work that needs to be done."

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HOPE ON THE TREATMENT FRONT

Advances in deciphering the genetics of Alzheimer's not only ferret out common DNA variants that more reliably predict risk, they also provide new clues to potential biological targets for drugs to prevent, stop or even reverse the disease. Until now, most promising treatments have fallen by the wayside. Drug companies have lost billions in Alzheimer's therapies that looked good in early research but failed in larger clinical trials, leading one report to characterize the field as "a graveyard of hope."

But those treatments were tested on people who already had symptoms of the disease. Recent studies in those with an inherited form of early Alzheimer's detected the presence of rogue amyloid proteins up to two decades before symptoms emerged, suggesting that we're intervening too late, when the damage is irreparable.

"That led to a search for individuals who are guaranteed by their genetics for developing Alzheimer's and identifying them at an age before they have symptoms," says Nixon. "Then we could treat the disease before it is like a locomotive that just can't be stopped."

In the past few years, two studies were launched to find out whether drugs that shrink plaques can halt the onset of the disease before symptoms appear in those genetically predisposed to develop Alzheimer's. One study, known as DIAN (Dominantly Inherited Alzheimer's Network), involves more than 260 people in the U.S., Britain, Germany and Australia. The other, the Alzheimer's Prevention Initiative, encompasses 300 members of an extended family of about 5,000 who live in Medellín, Colombia, and the surrounding mountain villages.

The DIAN study is composed of families with an inherited form of early Alzheimer's; many relatives have the disease or have died from it. People who harbor the mutant gene sometimes have symptoms in their 30s and 40s, usually around the same age that their parents started to show signs. On average, most develop symptoms before age 45 and are diagnosed with dementia by age 51. No one with this rogue DNA escapes this terrible fate.

DIAN participants were given one of two drugs, called gantenerumab and solanezumab. Both are antibodies that bind to amyloid proteins and help remove the toxic proteins from the brain before they have a chance to turn into plaques. In the study of the Colombian family members who develop early-onset Alzheimer's, scientists are testing another drug, crenezumab, which acts similarly to the other drugs. If these therapies can somehow halt or slow the development of memory loss, confusion or plaque formation, it might be possible to slow the progress of the disease.

Because the subjects in these closely watched studies are at such a high risk, researchers should know within the next few years if the experimental drugs will halt or delay the onset of the disease. "The characterization of these families has been so thorough, based on all their siblings and parents, that we know when any one person will start to develop cognitive deficits," says Ronald DeMattos, an Alzheimer's researcher at Indianapolis-based Eli Lilly and Co., which makes solanezumab and is co-sponsoring the DIAN trial. "If they progress more slowly than usual within that time frame, that would suggest these treatments are having their desired effect."


Rudolph Tanzi

But what about people who don't have a strong familial history of the disease? In the past year, two other long-term clinical trials began to test therapies that might either prevent or delay Alzheimer's in healthy volunteers who are not genetically at risk of developing the early-onset form of Alzheimer's. One study, called A4 (the anti-amyloid treatment in asymptomatic Alzheimer's trial), will test solanezumab in 1,000 cognitively normal people age 65 to 85, who have abnormally high levels of amyloid proteins. Participants will be selected based on the results of a PET brain scan to detect the presence of the haywire proteins.

The other trial, called the TOMORROW study, will give an FDA-approved diabetes drug, pioglitazone, to healthy volunteers to see if it can delay the first symptoms of cognitive impairment. The clinical trial will eventually encompass 5,000 participants who harbor two specific genes linked to Alzheimer's: *APOE* and *TOMM40*. An observational study conducted by German scientists in 2014 suggests that long-term use of this drug may protect against dementia. Using prescribing information from a German database, they looked at 145,000 adults 60 or older who did not have dementia. During follow-up six years later, they found that 10 percent of the cohort — about 14,000 people — developed dementia, but the risk was lower in those who were taking the diabetes medication. "People with diabetes may have an increased risk of Alzheimer's," says Keith Fargo, director of scientific programs and outreach at the Alzheimer's Association. "The drug may reduce these odds by controlling glucose metabolism and because it has an anti-inflammatory effect. Many researchers now believe inflammation plays a big role in Alzheimer's."

And in another advance, a research team led by Tanzi has concocted what they call "Alzheimer's in a dish." The team used human embryonic stem cells — which can transform into any cell of the body — and cultured them in a mixture of chemicals to grow human brain cells. Then they implanted Alzheimer's genes into the neurons, which obligingly began churning out the telltale clumps and tangles that characterize the disorder.

"Within six weeks, the nerve cells produced amyloids, and within eight weeks, we got tangles," says Tanzi. "This is a much better model than what we have, and it's faster and cheaper. We can now screen drugs within two months in a dish."

This discovery will solve one of the key stumbling blocks in devising therapies: the lack of a human model that can shed light on how the disease progresses. Scientists characterize the advance as a game changer that could dramatically accelerate the testing of new drug candidates.

It's likely that a combination of strategies will be needed to halt the disease's progression, using a cocktail of medications aimed at different targets in much the same way AIDS and many cancers are now treated.

"We've been operating under the assumption that a single drug would do the trick in terms of reversing the deficits," says Frank LaFerla, director of the Institute for Memory Impairments and Neurological Disorders at the University of California, Irvine. "But it's like trying to put out a raging fire with a bucket of water. Once a fire reaches that point, you need to bring out the firetrucks and helicopters." D

Linda Marsa *is a contributing editor for* Discover *and author of* Fevered: How a Hotter Planet Will Hurt Our Health and How We Can Save Ourselves (Rodale).

JOSH REYNOLDS