SCIENTIFIC AMERICAN
Health & Medicine

Why Diets Fail

NEW RESEARCH ILLUMINATES WHY MOST DIETERS REGAIN LOST WEIGHT. IT'S EVEN MORE COMPLICATED THAN WE THOUGHT

February-March 2020

Plus:

CANNIBALISTIC CELLS IN YOUR BODY

GUT MICROBES MIGHT AID STROKE RECOVERY

BEHIND THE ZANTAC RECALL

WITH COVERAGE FROM nature

LIZ TORMES

The Science of Losing Battles

An old saying posits that the definition of insanity is doing the same thing over and over and expecting different results. If the $60-billion dieting industry is any indication, our society is steps away from a straitjacket. Despite copious evidence that most diets fail in the long term (beyond two years), many people repeatedly attempt to shrink their bodies, and the majority end up heavier than when they started. As Daniel Engber details in this issue, science is no closer to understanding why weight loss from dieting doesn't stick. What we know so far is that a complicated interplay of factors leads to scale bounceback–from levels of hormones such as the hunger hormone leptin to the shape and size of fat cells and hereditary genetics (see "Unexpected Clues Emerge about Why Diets Fail").

Elsewhere in this issue, Kendall Powell reports on a new path of research that is harnessing the innate competitive nature of cells with the hope for novel cancer treatments (see "Survival of the Fittest Cells"). And Robin Lloyd investigates harmful emissions from the plastics contained in so-called cured-in-place pipes, which are commonly used in sewer pipe renovation (see "Health Concerns Mount as More Old Sewer Pipes Are Lined with Plastic"). It never fails to surprise me that the science of health and medicine can touch nearly every human industry–from marketing diet shakes to the manufacture of construction materials. If we're lucky and wise, our discoveries will lead to improved health and welfare for everyone.

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Health & Medicine

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On the Cover

New research illuminates why most dieters regain lost weight. It's even more complicated than we thought.

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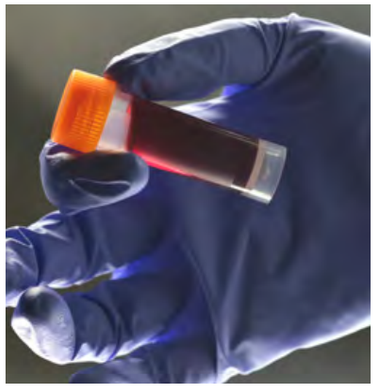

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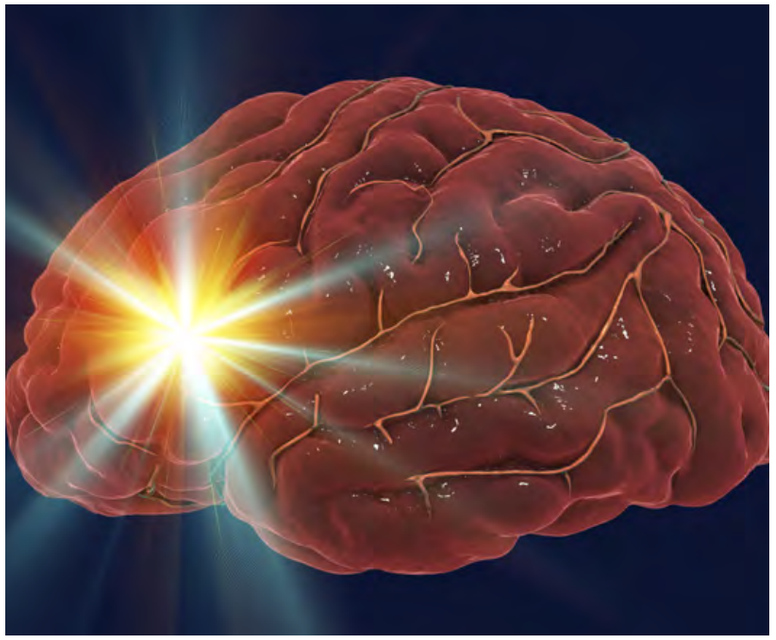

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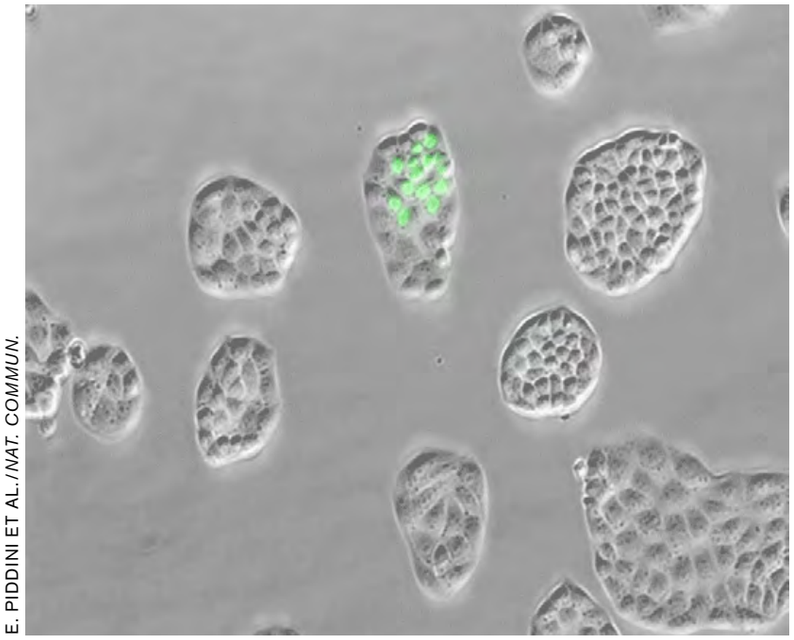

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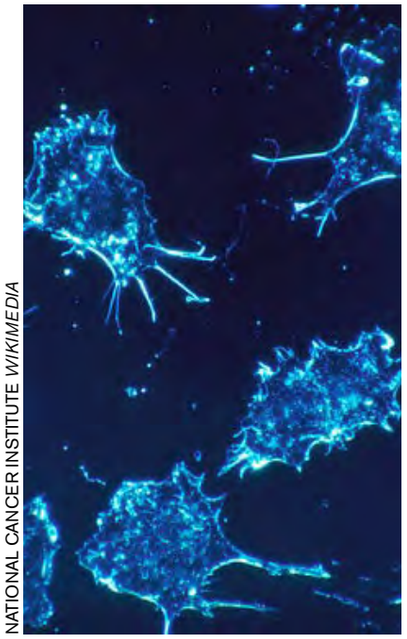

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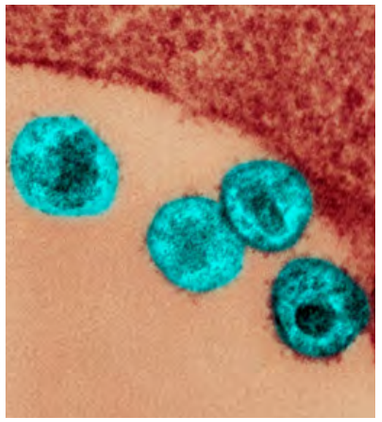

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NEWS

Why Do Some People Need Less Sleep? It's in Their DNA

U.C.S.F. researchers find a gene for flourishing with less shut-eye

We all wish we could get by on less sleep, but one father and son actually can—without suffering any health consequences and while actually performing on memory tests as well as, or better than, most people.

To understand this rare ability, researchers at the University of California, San Francisco, first identified a genetic mutation—in both individuals—that they thought might deserve the credit. Then the scientists intentionally made the same small genetic spelling mistake in mice. The mice also needed less sleep, remembered better and suffered no other ill effects, according to a study published in October 2019 in *Science Translational Medicine*.

SUNWOO JUNG *GETTY IMAGES*

Although a medication with the same benefits will not be available anytime soon—and might never materialize—the idea is incredibly appealing: take a pill that replicates whatever the father and son's body does and sleep less, with no negative repercussions.

"I find the concept of a gene product that might potentially provide protection against comorbid disorders of restricted sleep tantalizing," says Patrick Fuller, an associate professor of neurology at Harvard Medical School and Beth Israel Deaconess Medical Center in Boston, who was not involved with the work. "If true, this would indeed have 'potential therapeutic implications,' as well as provide another point of entry for exploring and answering the question 'Why do we sleep?,' which remains [one] of the greatest mysteries in neuroscience."

But as Jamie Zeitzer, an associate professor in the department of psychiatry and behavioral sciences at Stanford University, notes, "There often are trade-offs." Zeitzer says he worries that even if a drug like this could be produced without causing significant side effects, it would still have social consequences. Some individuals might be forced or pressured to take medication so they could work more hours. Even if people will not need as much sleep, they will still need downtime, he insists.

The study's senior author, Ying-Hui Fu, a professor of neurology at U.C.S.F., says it is far too early for such fantasies. Instead she is interested in better understanding the mechanisms of healthy sleep to help prevent diseases ranging from cancer to Alzheimer's.

"These people sleep more efficiently," she says of the father-son pair. "Whatever function sleep is doing for us, it takes us eight [hours to feel rested], but it takes them six or four hours. If we can figure out why they are more efficient, we can use that knowledge to help everybody to be more efficient."

The subjects, who live on the East Coast, reached out to Fu's team after hearing about a previous publication of its work. She would not reveal any more information about them to protect their privacy, except that they are fully rested after four to six hours of sleep instead of the more typical seven to nine. Also, Fu says, the duo and others with similar mutations are more optimistic, more active and better at multitasking than the average person. "They like to keep busy. They don't sit around wasting time," she says.

If most people sleep less than their body needs, that deficit will affect memory and performance, in addition to measures of health, Fu notes. Many think they can get away with five hours of sleep on weeknights and compensate for the loss on weekends—but few actually can. "Your perception is skewed, so you don't really know your performance is not as good," she says. "That's why people think [adequate sleep] doesn't matter. But actually, it does. If you test them, it's obvious."

Joking about her own academic experience, Fu adds, "All those nights that I stayed up to study, it would have been better to go to sleep." That's not true of the father and son, who genuinely needed just 5.5 and 4.3 hours of sleep each night, respectively, the new paper showed.

Stanford's Zeitzer praises the study's design, saying, "Starting with humans and going to rodents and then back is great." Mice, he adds, are not ideal role models because they regulate sleep differently than humans. And many individuals believe they are short sleepers but, when put in a lab, turn out to slumber the typical seven to nine hours.

People are naturally short sleepers if they rest a relatively brief time even when given the chance to sleep in on weekends or vacations. "If you get extra sleep when you have the opportunity, it's generally a good sign that you need more sleep," Zeitzer says.

Jerome Siegel, a professor of psychiatry at the University of California, Los Angeles, Center for Sleep Research, says he is comfortable with Fu's group's main finding: that the neuropeptide S receptor 1 (*NPSR1*) gene is important in regulating sleep. But it is likely only one small piece in a very complex process, he adds. And he is not convinced by the connection between sleep and memory the group claims. Sleep may have many functions, but there is no indication, he says, that needing less of it somehow boosts memory or cognition. "We consolidate memory while we sleep and while we're awake, even when we're anesthetized," he says. "It's not something that just occurs during sleep."

The mechanism of action of the newly discovered mutation is not entirely clear. Fu and her team used a molecular probe to explore how the protein made by the father and son's mutant *NPSR1* gene differs from that made by a normal gene. The mutation, they found, makes the receptor more sensitive and active. The specifics of that process, Fu says, still have to be worked out.

Fu and her collaborators previously discovered two other genes involved in sleep. They are continuing to explore the mechanisms behind these genes, she says, adding that the speed of their work would be faster if they had more financial support.

Fu says once she and her colleagues can find about 10 pieces of the genetic puzzle, "each piece can serve as a point to build upon. And hopefully, someday we can know the whole picture."

—Karen Weintraub

Office Workers May Be Breathing Potentially Harmful Compounds in Cosmetics

Some cosmetics and deodorants contain chemicals that, when released into the air, may pose a risk to human health

We often think of pollution as an outdoor problem. But many office workers are constantly breathing a complex soup of invisible airborne substances including ozone, carbon dioxide, particulate matter and volatile organic compounds (VOCs). VOCs are gases that can be released from molds, building materials, human metabolism—and personal care products such as lotions, deodorants, hair spray and cosmetics. Some VOCs have been linked to health effects, including fatigue; difficulty concentrating; eye, nose and throat irritation; and even cancer. Whether exposure to these substances in offices poses a significant risk to human health remains an open question, however.

Benjamin Franklin suspected the unhealthy effects of indoor air back in 1785. "I am persuaded that no common Air from without, is so unwholesome as the Air within a close Room, that has been often breath'd and not changed," he wrote in a letter to Dutch physician Jan Ingenhousz. Over the years scientists have tried to back up his claim, and recent research provides some support for it.

In one of the largest studies of its kind, researchers at Purdue University have now used a sophisticated system of sensors to measure the complex dynamics of VOCs in an office environment. The findings, presented in October at the American Association for Aerosol Research Conference in Portland, Ore., cannot prove that any one indoor air component causes health problems—but they could be used to design better-ventilated offices and advance research on the issue.

Sniffing Office Air

The study took place at Purdue's Living Labs, a simulated open office equipped with thousands of sensors, as well as an instrument called "The Nose," a highly sensitive mass spectrometer that can sniff out VOCs, ozone, carbon dioxide and aerosols. Researchers used temperature sensors embedded in office chairs to track the occupancy of 20 graduate students who spent their days working there.

GETTY IMAGES

Brandon Boor, an assistant professor of Civil Engineering at Purdue, and his team found that humans were the dominant source of VOCs in the model office's air. Nearly 2,000 such compounds can come from simply being alive: exhaled breath, sweat, saliva and the like. Concentrations of human-derived VOCs varied throughout the day in the experiment but usually peaked in mid-afternoon when occupancy was highest. VOC concentrations also depended on factors such as whether the office had recently been cleaned, whether someone had just applied a personal care product and how well the ventilation system was working.

Ozone gas from the outside air—which came in through the ventilation system—was highly reactive with indoor surfaces such as walls and furniture, and with VOCs left behind by occupants. The researchers found that the gas reacted with human skin oil to create new VOCs. It also reacted with chemicals called monoterpenes from a freshly peeled mandarin orange to form new, nanometer-sized ultrafine particles. (Monoterpenes can also

come from manufactured sources such as scented personal care products and cleaning fluids.)

The investigators further found that VOCs from personal care products peaked in the morning, when freshly deodorized graduate students arrived. A chemical called D5—found in thousands of such products—was detected at levels comparable to or greater than those of isoprene, one of the major VOCs in exhaled human breath, and was relatively high in the staff hangout area. The team also detected related compounds called D4 and D6, but these were found at much lower levels than D5.

"Our preliminary results suggest that similar amounts of isoprene and D5 can be released into the office air," Boor says. "The emissions of D5 are likely dependent on the amount and type of personal care products the occupants are wearing." He notes that results from his study apply only to this model office. His team is working on emission factors that may allow them to generalize their results to other settings.

Office workers may not have a lot of control over how much carbon dioxide their co-workers exhale, how much skin oil they produce or even whether they decide to peel an orange. But they do have some control over their own use of personal care products, says Carrie Redlich, a pulmonologist and director of the Occupational and Environmental Medicine Program at Yale School of Medicine. "If someone is symptomatic"—maybe they have a headache or their asthma is acting up—"in an environment where people are wearing a lot of perfumed products, the question is: Do we really need [these products]?" she asks. "I've seen enough patients who are very symptomatic in response to those [substances]. In some jobs, people may not be able to get up and walk away from what's triggering their symptoms, and it may really impact their ability to keep that job."

Researching the Chemical Risks

Some research suggests that compounds such as D4, D5 and D6—which are derived from silicone and called cyclic volatile methyl siloxanes (cVMSs)—could pose a risk to human health, although the vast majority of studies have been done on animals and are far from definitive. D4, D5 and D6 are all found in personal care products, and D5 is most abundant.

Animal studies have linked D4 to impaired fertility and both D4 and D5 to uterine cancer. But the animals were subjected to very high doses of the chemicals, for long durations and in highly unusual settings, according to Charles McKay, the former president of the American College of Medical Toxicology and current associate medical director of the Connecticut Poison Control Center at the University of Connecticut Health Center. "Those experimental conditions often have very little to do with human exposure to much, much lower doses," McKay says. "Studies did show uterine cancer issues in one animal model at very high doses, but I'm not sure that has any bearing on the human setting." (McKay has been retained previously by law firms representing pharmaceutical and medical device companies or their opponents, but with the exception of one case involving a car wax product, these cases were not related to cVMS compounds.)

Most of the animal studies have been sponsored by the silicone industry, and those that showed a connection with uterine cancer were done in rats. Industry representatives have argued the hormonal mechanism that may contribute to uterine cancer after exposure to D4 and D5 is different in rats than in humans, so studies of the former may not be relevant to humans for various reasons, usually published as half a dozen sponsored papers in special [perhaps paid] issues of journals that at least have a reputation of being close to industry," says Christoph Rücker, a chemist at Leuphana University Lüneburg in Germany and co-author of a review study about siloxanes.

Critics point out the industry ties and the dearth of independent studies. "As a common pattern, if facts of concern are found, the industry launches a firework of publications that try to downplay such results and to argue that the results of rat studies are not relevant

The European Union recently decided to regulate these compounds. Citing environmental risks, the E.U.'s REACH program has listed D4, D5 and D6 as substances of very high concern and labeled them as PBT (persistent, bioaccumulative and toxic) and vPvB (very persistent, very bioaccumulative). Starting after January 31, 2020, the E.U. will limit

D4 and D5 concentrations to 0.1 percent in wash-off products such as shower gels, shaving foams and shampoos. The E.U. has also proposed restricting D4, D5 and D6 in all consumer and professional products, such as dry-cleaning fluid. The silicone industry has sued the European Court of Justice over these actions.

Linda Loretz, chief toxicologist for the Personal Care Products Council (a national trade association), and Karluss Thomas, senior director of the Silicones Environmental, Health, and Safety Center (a subgroup of the American Chemistry Council that represents 90 percent of silicone chemical manufacturers in North America), point out the large body of research reviewed by regulatory bodies in a number of countries, including the U.S. Environmental Protection Agency. Loretz and Thomas say D4 and D5 do not pose risks to human health, although some of the research is inconclusive.

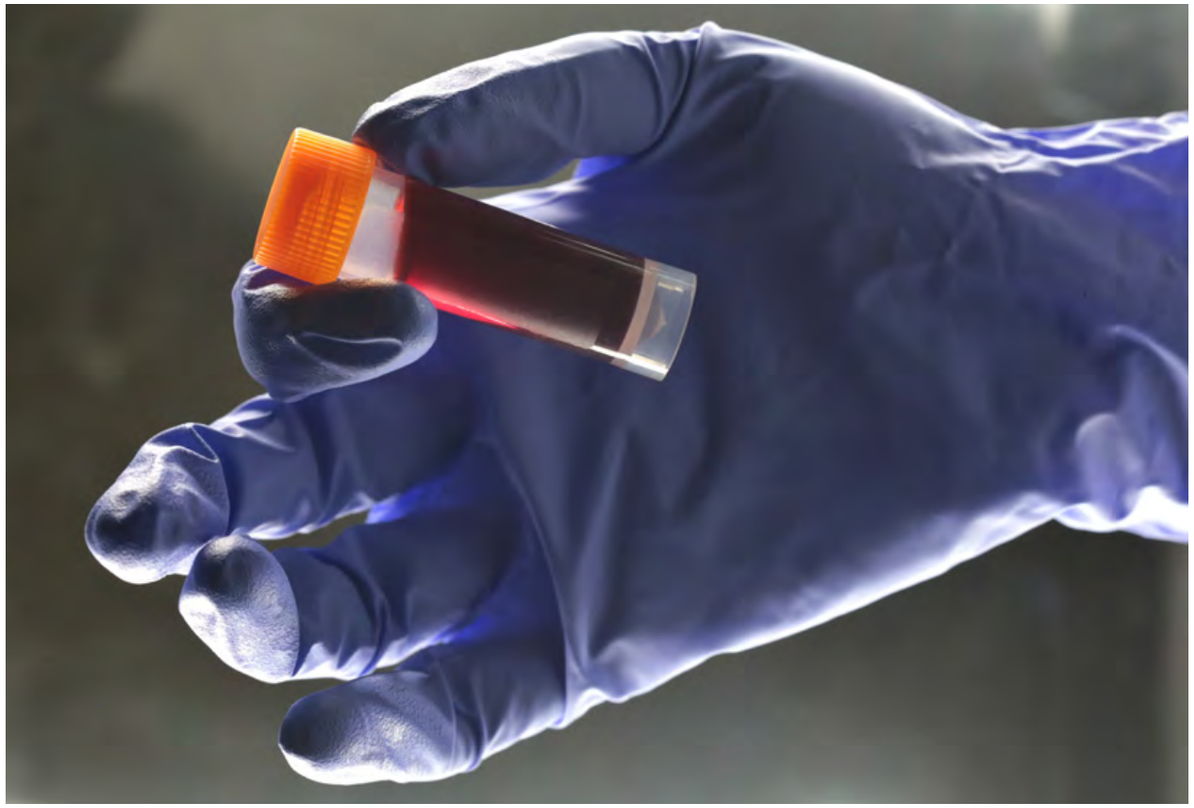
Studying cVMSs and human health is "complex" and "controversial," according to Rücker. Few studies have been conducted in humans, and not much research has been conducted in the past 10 years. "There are only [a handful] of experts on toxicity of siloxanes, and these are employees of the silicone industry," Rücker says. "The industry is free to publish or not to publish the results of their studies."

These compounds have been used in consumer products for almost 80 years. Children may have higher exposures than adults, with relatively high concentrations in baby products.

"Siloxanes are clearly one of the major contaminants in indoor air and dust, [which] form an important pathway of human exposure," says Kurunthachalam Kannan, deputy director of the Division of Environmental Health Sciences at New York State Department of Health's Wadsworth Center. But assessing risk to humans for these compounds is "sometimes politically sensitive," he says.

Whether or not these specific chemicals prove to be a risk, office workers could benefit from a little fresh air. Benjamin Franklin would throw open the windows, throw off his clothes and take so-called air baths. But if office heating, ventilation and air-conditioning systems are functioning adequately, people may not need to strip down. Their co-workers may thank them for it.

—Veronica Hackethal



DOUGLAS SACHA *GETTY IMAGES*

First New HIV Strain in 19 Years Identified

The surveillance of viral changes persists to keep the blood supply safe

A research group at the medical-device and health care giant Abbott has discovered a new strain of human immunodeficiency virus, or HIV—the first to be identified in 19 years. Abbott continues to look for potential new HIV strains to ensure that diagnostic tests for blood screening and detecting infectious diseases remain up to date, says Mary Rodgers, senior author of the paper announcing the finding and head of the company's Global Viral Surveillance Program.

The new strain, called HIV-1 group M subtype L, is extremely rare and can be detected by Abbott's current screening system, Rodgers says. The

company's tests screen more than 60 percent of the global blood supply, she adds, noting it must detect every strain and "has to be right every time."

In the early days of HIV/AIDS, in the 1980s and 1990s, some blood donors unaware that they had HIV added the virus to the blood supply. A large number of patients who needed regular blood transfusions—among them many with hemophilia—ended up contracting HIV and, often, dying. The supply has been essentially clear of HIV for years, and Rodgers says efforts such as Abbott's will help keep it that way.

The study, published in November in the *Journal of Acquired Immune Deficiency Syndromes,* serves as a reminder of the dangerous diversity of the HIV virus, says Jonah Sacha, a professor at the Vaccine and Gene Therapy Institute at Oregon Health & Science University, who was not involved in the new research.

"This tells us that the HIV epidemic is still ongoing and still evolving," he says. "The calling card of HIV is its diversity. That's what's defeated all of our attempts to create a vaccine." More than 37 million people live with HIV worldwide—the most ever recorded. "People think it's not a problem anymore, and we've got it under control. But, really, we don't," Sacha says.

"The calling card of HIV is its diversity. That's what's defeated all of our attempts to create a vaccine."
—Jonah Sacha

Antiretroviral drugs inhibit the virus's reproduction and spread, but they have significant side effects, he says. Even when drugs keep HIV under control, patients are at higher risk for blood cancer, cardiovascular complications and other problems.

The danger from the virus persists. A radically new viral strain could evade detection in the blood supply, avoid being controlled by drugs and render future vaccines ineffective, Sacha says. "Viruses break through all the time, and we're not ready to deal with them," he adds, "just like what happened with the original HIV."

But Michael Worobey, head of the department of ecology and evolutionary biology at the University of Arizona, who was also not involved in the recent study, is more sanguine. Worobey says it is not a surprise that there are a diverse number of HIV strains in Central Africa, which is where the disease originated. Identifying a new one does not add much to the knowledge of HIV, he says.

"It's actually misleading to describe genetic diversity from the [Democratic Republic of the] Congo as a new subtype," Worobey says, "because the only useful meaning of the term 'subtype' " would come from identification of a lineage of the virus that has spread significantly beyond Central Africa. Guidelines for classifying new strains of HIV were established in 2000. The recently discovered subtype belongs to the most common form of HIV, group M, which accounts for more than 90 percent of all HIV cases, Rodgers says.

Abbott created its surveillance program 25 years ago to track changes in HIV and hepatitis viruses. "We really need to be monitoring them to stay one step ahead of the virus," Rodgers says. The program now includes 78,000 samples from 45 countries. No other new subtypes have been characterized since 2000, she adds.

The most recent of the three samples used to identify HIV-1 group M subtype L has been sitting in an Abbott freezer since 2001. The amount of virus in the sample was too low to read back then, but new technology recently made it possible. Comparing that sequence with the others made available by the research community, Abbott researchers found two additional examples of the strain—in samples from 1983 and 1990, also from the Democratic Republic of the Congo, hinting that it has been around for a while. "Now that we know it exists, it'll change how we look for it," Rodgers says.

The company's tests focus on the part of the viral genome that does not change very much from generation to generation, which is why it was able to detect the new strain. The finding also suggests there are more strains to be found, Rodgers says. "The full diversity has not been characterized. We're going to continue to look."

—Karen Weintraub

Targeting Gut Microbes May Help Stroke Recovery

Growing evidence from mouse studies suggests that a healthy microbiome might improve poststroke outcomes

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When a clot blocks off circulation to the brain during an ischemic stroke, the loss of oxygen and nutrients can cause tissue to become damaged and die. Physicians have effective methods of clearing these occlusions: clot-busting proteins called tissue plasminogen activators and thrombectomy, a surgical technique. Removing the blockage is critical, but even after blood flow is restored, complications brought on by inflammation can lead to more cell death. Despite a decades-long search, scientists have yet to pinpoint effective ways of protecting the brain from poststroke damage. In recent years, a new potential player in stroke outcome has emerged: the microorganisms in our guts.

Some of the first findings linking gut microbes to stroke just appeared about three years ago. In one study, researchers in New York City reported that interrupting the diversity of intestinal flora in mice with antibiotics affected the amount of brain damage caused by stroke. Another investigation in rodents, conducted by a German team, demonstrated that strokes disrupted mouse microbiomes—and that the altered composition of gut microbes could worsen outcomes after stroke. That research meant "2016 was a fantastic year for the gut-brain axis in the stroke field," says Connie Wong, a stroke scientist at Monash University in Australia, who was not involved in the studies.

The work has continued. More recently, Venugopal Venna, a stroke researcher at the University of Texas Health Science Center at Houston, and his colleagues have been examining whether age-related changes to the microbiome affect recovery. "Stroke is mainly a disease of aging," Venna says. "Young people also get stroke but much less often." In a study reported in *Annals of Neurology* in 2018, Venna and his colleagues examined whether age-related changes to the microbiome would influence recovery in mice. The scientists first depleted the rodents' gut microbes with antibiotics, then used fecal pellets to introduce microbiota from either young or old animals. When the team induced ischemic strokes in the rodents a month later, it found that young mice with older microbiomes had worse outcomes than their counterparts with intestinal flora from younger animals. They had higher rates of mortality, greater neurological deficits, slower recovery of muscle strength and

movement, and increased levels of inflammatory molecules. Meanwhile elderly mice fared better with young microbiomes than with old ones.

The big question now is the mechanism mediating the microbiome's effect on stroke outcome, says Arthur Liesz, a neurologist at Ludwig Maximilian University of Munich, whose group authored the German 2016 study. Among the multiple possibilities, several researchers are currently investigating microbial metabolites. Venna's team has homed in on short-chain fatty acids, compounds generated via bacterial fermentation of fiber in the intestine. Its 2018 study showed that short-chain-fatty-acid levels were lower in animals with old microbiomes, so Venna and his colleagues hypothesized that these compounds might be involved in stroke recovery. To test this theory, they selected strains of gut bacteria that produced short-chain fatty acids and transplanted them into mice—and found that these microorganisms were enough to improve outcomes after stroke. Venna presented these unpublished results at October's Society for Neuroscience (SfN) meeting in Chicago.

Venna and others suspect that gut-microbe metabolites such as short-chain fatty acids may help keep the immune system—and thus inflammation—in check in healthy animals and that this equilibrium state is altered after stroke. Supporting this idea, Liesz's group also presented unpublished work at the SfN meeting demonstrating that, in rodents, both short-chain fatty acids and indoles—gut-microbe metabolites produced from digesting the amino acid tryptophan—modified the activity of immune cells following a stroke. "There are lots of questions that still need to be answered, but we're fascinated with the results so far," Venna says.

Most of the findings supporting the microbiome's role in stroke have stemmed from research in animals, so whether the benefits will carry over to humans remains to be seen. There are some small observational studies in people, but Liesz notes that larger, more long-term investigations are essential. To address this need, his lab is currently recruiting patients to procure their feces and blood to determine whether their intestinal flora and circulating metabolites reflect what is seen in mice. Although clinical trials are still far off, "I think many of us do think about treatments at this very early stage," Liesz says. Targeting the gut using methods such as probiotics or fecal transplants, he adds, "might be a very elegant way to treat a complex disease like stroke by not directly affecting the brain but using the microbiome as a way to sneak into the system."

"Extraordinary claims need extraordinary evidence. And I haven't seen extraordinary evidence, just extraordinary claims."
—Ulrich Dirnagl

Not everyone agrees that the evidence linking the microbiome to stroke outcome is compelling, however. Ulrich Dirnagl, a neurologist and stroke scientist at Charité University Hospital in Berlin, says that there are some major limitations in the research conducted to date. One key issue: because laboratory animals are raised in a very artificial environment—typically in clean cages and on a limited diet, for example—their microbiome does not accurately represent the diversity of gut microbes found in wild animals. For this reason, he explains, experiments conducted with lab mice may not be relevant to humans.

A paper published in August in *Science* supports Dirnagl's concerns. In that study, researchers generated so-called wildling mice by implanting embryos from lab animals into wild mice. Because of exposure to the wide range of microorganisms in their surrogate mothers, the wildlings had a microbiome that was a closer match to those found in natural environments. When the team tested two immune system–targeting drugs that had previously succeeded in lab mice but failed in humans, they found that the therapies were also unsuccessful in the wildlings. "The type of work that's done now by stroke researchers is really far off from real life," Dirnagl says, adding that there is also the issue of small sample sizes and a lack of replicated studies. "Extraordinary claims need extraordinary evidence. And I haven't seen extraordinary evidence, just extraordinary claims."

—Diana Kwon

HIV-Positive Babies Fare Better When Treatment Starts at Birth

Although not practical in many areas, the approach reveals clues to how the immune system battles the infection

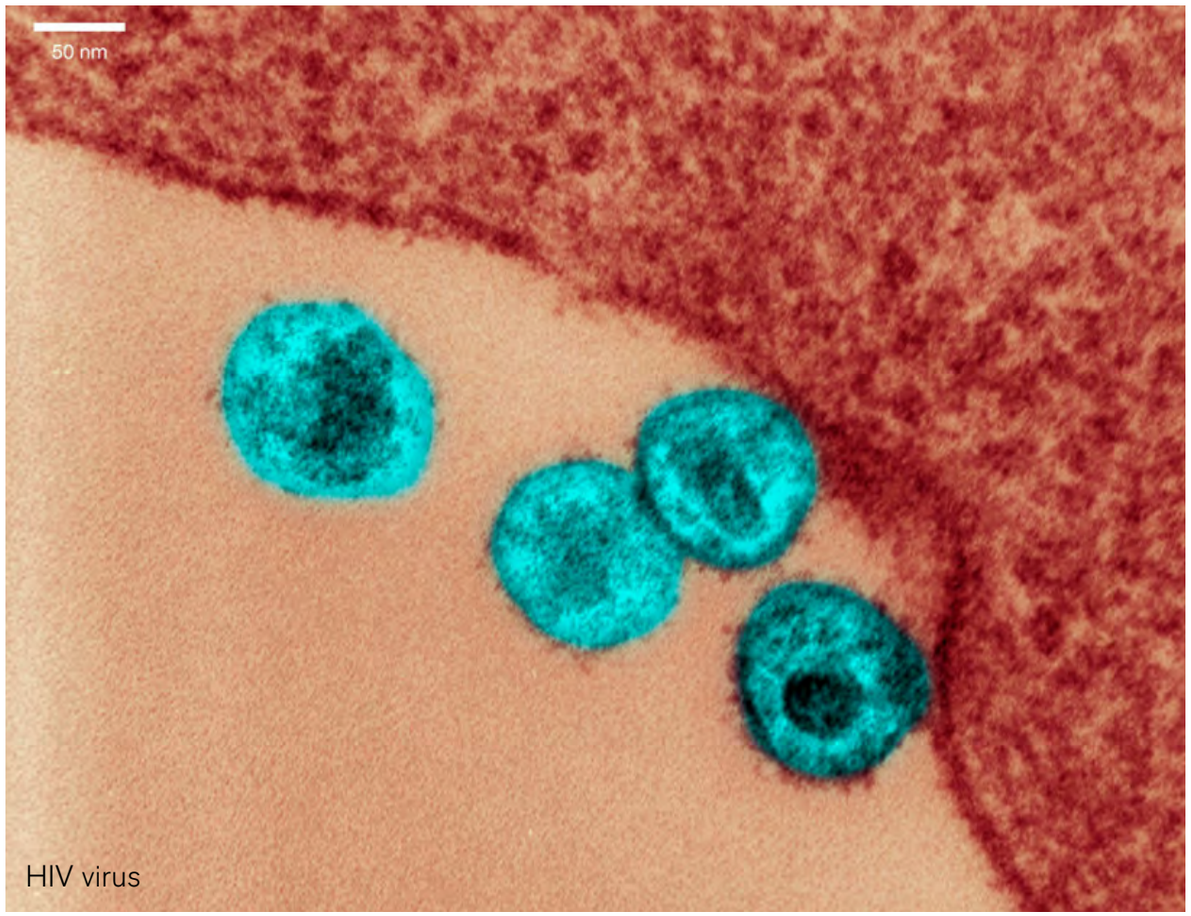
A newborn's immune system responds to HIV infection less effectively than a more mature one, so an HIV-positive baby should be started on antiretroviral therapy as soon after birth as possible, new research suggests.

Although treatment early in life was known to be advantageous, the study, published in November in *Science Translational Medicine,* shows the immune system's response in detail for the first time. The study could energize efforts to treat newborns with HIV, several experts say, and it may help pave the way for an eventual long-lasting treatment or even a cure.

In the study, 10 HIV-positive newborns in Botswana were started on antiretroviral therapy—the gold-standard treatment for HIV—within hours or days of birth instead of the more typical four months. If an HIV-positive pregnant woman is receiving treatment and the amount of virus in her body is well controlled, she will not pass the disease on to her baby, although the infant will have antibodies to HIV in his or her bloodstream. If the mother's disease is not well controlled, the baby may be born with HIV.

To look for HIV-positive babies, the team screened more than 10,000 newborns using very small amounts of blood. The researchers identified 40 who were HIV-positive and began treating them with a three-drug cocktail within days of birth. The study reported on 10 of those babies, who are now almost two years old, and compared them with HIV-positive babies who did not receive treatment until four months of age.

The early-treated babies fared much better in measures of viral levels in their bloodstream and lower levels of immune activity, which predicts the course of the disease, according to the study, which was conducted by a research team at the Ragon Institute of Massachusetts General Hospital, the Massachusetts Institute of Technology and Harvard University; Brigham and Women's Hospital; and the Botswana Harvard AIDS Institute Partnership in Botswana. The babies coped well with the drug regimen, with only one having to discontinue therapy because of side effects, said Roger Shapiro, a senior author of the paper and an immunologist at the Harvard T. H. Chan School of Public Health, in a news conference.



HIV virus

DOUGLAS SACHA *GETTY IMAGES*

The stakes are high for getting these babies treated, says Pat Flynn, an infectious-disease specialist at St. Jude Children's Research Hospital in Memphis, Tenn., who was not involved in the new study. HIV infection can have devastating neurological consequences, likely because of ongoing inflammation in the brain.

Every day between 300 and 500

babies in sub-Saharan Africa are infected with HIV, according to the study's authors, who cite data from the Joint United Nations Program on HIV/AIDS (UNAIDS). Up to half of them will die by age two if they do not receive antiretroviral therapy. Infants infected in utero face even worse outcomes than those infected during birth or breastfeeding, said Mathias Lichterfeld, a co-author and an infectious disease specialist at the Ragon Institute and Brigham and Women's, in the news conference. Putting all HIV-positive pregnant women on antiretroviral therapy is the best way to prevent them from passing the virus to their babies, but many such women face barriers to accessing treatment, Shapiro said.

Scientists have known since a study published in 2008 that treating HIV-positive babies as early as possible leads to better outcomes, but the new paper provides a "very comprehensive scientific rationale for why that is the case," says Sten Vermund, dean of the Yale School of Public Health and a pediatrician and infectious disease epidemiologist, who was not involved in the new research. "As soon as possible might be too late. We really would be better treating right at birth."

Compared with the immune system of an older baby or an adult, Vermund says, a newborn's immune system is much more immature but "developing at a breakneck pace." That's why infants are particularly vulnerable to intrauterine infections, which include toxoplasmosis, rubella, syphilis and Zika. And, he says, "HIV can be added to that list, given the findings of this study."

Unfortunately, Vermund says, it is unrealistic to think that most HIV-positive babies born in sub-Saharan Africa could be treated soon after birth. "The science is terrific," he says of the new paper, but it may not have much effect in the real world. "The clinical relevance in Africa is not at all obvious to me," Vermund adds.

In most countries in sub-Saharan Africa, infants are tested for HIV at four to six weeks of age, Shapiro said in the conference. This practice enables doctors to catch babies who are infected during pregnancy, at delivery or very early in life, but it misses the chance to start treatment immediately if the child is infected at birth. Adding a second test at birth—as South Africa now does—would be complicated and expensive, he conceded, but "that's really the direction that the rest of the world should be following."

Yet even something that is simple in the U.S.—such as drawing blood from a newborn, taking the blood to a lab and getting results back to the clinic and the family—remains "a major barrier to identifying those babies who are infected very early on," Flynn says. Instead it may make sense to determine women who are at high risk for transmitting HIV and put their infants on therapy even before the test results can be returned. But even then, maintaining stocks of antiretroviral drugs continues to be an issue in sub-Saharan Africa, she says, with funding streams to pay for medications being uncertain.

In the U.S., no more than about 50 babies are born each year to mothers who did not know they were HIV-positive, and they are generally identified at birth, Vermund says. The new study should "stimulate obstetricians and pediatricians to be especially aggressive" in promptly diagnosing and treating those newborns, Vermund says.

The research team plans to follow the babies and track how much viral "reservoir" they continue to carry. In a natural experiment in the U.S., the so-called Mississippi Baby was thought to be cured when her HIV remained undetectable for two years after stopping therapy. But then the disease rebounded, suggesting that early aggressive therapy is not a cure.

To improve long-term treatment of HIV-positive children, the researchers hope to put some of the babies on so-called broadly neutralizing antibodies—which can recognize and block many types of HIV from entering healthy cells. They want to see if, long term, these antibodies can substitute for the antiretroviral regimen, which is costly and cumbersome and comes with significant side effects.

Yvonne Maldonado, an expert in pediatric infectious diseases and epidemiology at Stanford University, who was not part of the new study, says the real benefit of the research may be not in how it impacts the care of newborns with HIV but rather in the insights it offers into the HIV reservoirs that remain in the body even during treatment. "This is really geared toward 'How do you get to the cure?' rather than 'How do you treat babies?' " she says.

—Karen Weintraub

What We Know about the Possible Carcinogen Found in Zantac

The popular heartburn drug may produce potentially unsafe levels of NDMA when its active ingredient breaks down

French drugmaker Sanofi recently announced a recall of over-the-counter Zantac, the widely used acid reflux medication, in the U.S. and Canada over concerns of possible contamination from a probable carcinogen. This action followed recalls by manufacturers and retailers of generic versions of the drug, called ranitidine. The recalls have prompted questions about whether the drugs' levels of a chemical called *N*-nitrosodimethylamine (NDMA)—which has been linked to cancer in animals—pose a more serious health risk than initially reported.

Several blood pressure medications, including the angiotensin receptor II blockers valsartan, losartan and irbesartan, were recalled last year over NDMA contamination. The U.S. Food and Drug Administration first flagged the possible contamination of ranitidine products last September. At that time, the FDA said the NDMA levels found in preliminary tests "barely exceed amounts you might expect to find in common foods." But the agency released a statement on October 2 calling the levels "unacceptable." Asked to elaborate, FDA spokesperson Jeremy Kahn said, "Although the FDA has detected NDMA in limited ranitidine samples at low levels, these levels still exceed what [the] FDA considers acceptable for these products."

The online pharmacy company Valisure first alerted the agency in June, after it said it detected concerning levels of NDMA in ranitidine medications during some routine testing. The company filed a detailed citizen petition to the FDA in September, alleging it had found "extremely high levels of [NDMA] ... in every lot tested, across multiple manufacturers and dosage forms of the drug ranitidine." The petition states that Valisure detected levels greater than three million nanograms per tablet—far exceeding the FDA's permissible daily intake of 96 nanograms.

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But these high levels may have been a result of the testing method Valisure used, which involves heating the sample. "That method is not suitable for testing ranitidine because heating the sample generates NDMA," the FDA said in its October 2 statement. Instead the agency recommends using one of two techniques: liquid chromatography–high-resolution mass spectrometry (LC-HRMS) or liquid chromatography–tandem mass spectrometry (LC-MS). Employing LC-HRMS, the FDA found much lower NDMA levels than Valisure had reported. "Valisure only showed detectable NDMA after exposing ranitidine to extreme artificial conditions—when they heated ranitidine to 266 degrees Fahrenheit [130 degrees Celsius] ... or when they added artificial nitrite far beyond what is ordinarily seen in humans," said Sanofi spokesperson Nicolas Kressmann in an e-mail. But Valisure says it developed a version of its testing technique that could detect NDMA even when samples

were only heated to 37 degrees C at conditions that more closely approximate those of the human body—in simulated gastric fluid with varying amounts of nitrites, which are found in foods such as processed meats. Neither Sanofi nor the FDA would comment on the specific NDMA levels they found using the latter's approved methods.

Sanofi has stated that it issued the voluntary recall as a precautionary measure. "Sanofi takes this issue very seriously, and we are currently conducting our own robust evaluations to ensure the safety of Zantac OTC, which has been used by consumers for over two decades," Kressmann says. The company is testing both the active ingredient ranitidine by itself and the finished product. "We have announced inconsistent preliminary test results of the active ingredient sourced in the U.S. and Canada products," said Sanofi spokesperson Ashleigh Koss in an e-mail. "At this time, we don't have any additional information to share about the specific test results."

"If you throw away these pills, [NDMA] can now enter the water supply."
—David Light

Tracing the Risks

In its petition, Valisure also claimed that the NDMA is likely formed as the result of an inherent instability of the ranitidine molecule. "We think the problem is much worse than contamination," says Valisure CEO David Light. He alleges that the drug itself may break down to form NDMA.

Some research indirectly supports this idea. A 2016 study at Stanford University gave 10 healthy volunteers 150 milligrams of Zantac and found that subsequent NDMA levels in their urine exceeded 47,000 nanograms. Because most of the NDMA would have been metabolized before reaching the urine, the actual amount in the body could have been much higher, the researchers wrote. And a 2004 study of people with peptic ulcers found that those who were taking either Zantac or another antacid, Tagamet (cimetidine), had a heightened risk of bladder cancer—but it did not distinguish between which of the two medications each subject was taking. (*Scientific American* sought comment from Tagamet's manufacturer, Prestige Brands, but did not receive a response by the time of publication.)

Sanofi notes that the 2016 study also used a method that involved exposing the samples to high temperatures and adding reference chemicals—both of which, the company says, could create NDMA. Sanofi also says that Zantac was not approved for sale in the U.S. until the 1980s and that nearly all of the ulcers reported in the 2004 study were formed before then. "Numerous studies since the 2004 study have shown the safety of ranitidine," Sanofi's Kressmann says. He cites a 2013 meta-analysis examining the link between acid-suppressing drugs—including H2 blockers such as ranitidine—and gastric cancer, which did not find a statistically significant association for the long-term use of H2 blockers. But the same analysis did find a statistically significant cancer risk within five years of use, and the authors concluded that "acid suppressive drugs are associated with an increased risk of gastric cancer." (They noted some limitations, however, including the fact that this conclusion was based on observational studies.)

Another potential concern is that if ranitidine breaks down into NDMA, it could enter the sewage-treatment system and contaminate drinking water. NDMA from rocket fuel is a known water contaminant, and Valisure's Light thinks the concentrations of this chemical in ranitidine medications could be large enough to pose a problem. "If you throw away these pills, [NDMA] can now enter the water supply," Light says. He encourages people to take their medicines back to their doctor or pharmacy to dispose of them safely.

A Complex Chemistry

Ranitidine has been widely used for decades. If it poses a risk to human health, how could that have gone unnoticed for so long? Light alleges that there were some limitations in early safety studies involving Zantac in the 1980s. Glaxo—a company that eventually merged into GlaxoSmithKline (GSK)—Zantac's

original manufacturer, published a study of ranitidine's metabolites in urine in 1981, but Light says that study appears not to have looked for NDMA. Glaxo published another study in 1987 that tested the stomach contents of people taking ranitidine, concluding that there was "no significant increase" in the concentration of nitrosamines, a group of chemicals—many of them carcinogenic—that includes NDMA. But Light says the detection method used in that paper was designed for food products and does not directly measure nitrosamines. In addition, the study discarded all stomach samples that contained ranitidine because they could have "falsely high" concentrations of nitrosamines, so any NDMA produced by the breakdown of ranitidine would not have been detected.

In a statement to *Scientific American*, GSK says it had considered the potential formation of nitrosamines in the body—during ranitidine's development, during its regulatory review and in subsequent studies. Scientists had hypothesized that any drugs that raised the stomach's pH could increase the growth of bacteria that produce nitrites, which could interact with chemicals called amines to produce nitrosamines. Although several studies did find that taking ranitidine could increase the concentration of nitrites in the stomach—and at least one found a statistically significant increase in nitrosamines—that does not mean they cause cancer, GSK says. The company adds that ranitidine was not carcinogenic in studies of rodents whose diet and bacterial metabolism were similar to those of humans and claims that "extensive pharmacovigilance monitoring, regular safety reviews and substantive epidemiological studies have not linked ranitidine to raised cancer risks."

Further, the issue of nitrosamine formation in the body "is fundamentally different to the current regulatory interest in the presence of NDMA in drug substance and drug product," says GSK, which has issued a recall of its generic version of Zantac. "The reason for the current precautionary recall of ranitidine is due to an emerging finding that some sources of drug substance and therefore drug product may contain very small amounts of nitrosamine. While the manufacturers, suppliers and regulatory authorities clarify the root cause of this issue, we have stopped supply and recalled product from the market as a precautionary measure."

Nevertheless, the recalls suggest a level of caution may be justified. The FDA says that consumers taking over-the-counter medications containing ranitidine could consider using other approved drugs and that patients who are taking prescription ranitidine and want to stop should consult their doctors about alternative options. The agency has asked ranitidine manufacturers to test their own products for NDMA and to send it samples of them. Kahn says the agency "continues to test ranitidine samples and will provide information as it becomes available."

—*Tanya Lewis*

Unexpected Clues Emerge about Why Diets Fail

The physiology of weight regain still baffles scientists, but surprising insights have come to light

By Daniel Engber

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Why is it so hard to lose weight and keep it off?

Daniel Engber is a science writer based in New York City.

For a moment, several years ago, it looked like we had an answer. In May 2016 the *New York Times* ran a front-page story on the findings from a study out of the U.S. National Institute of Diabetes and Digestive and Kidney Diseases: 14 reality show contestants had been tracked for half a dozen years after appearing on the program *The Biggest Loser*. Through dieting and very intensive exercise, each had lost at least 50 pounds during their time on the television series—and a couple had shed more than 200—but the follow-up study found they'd regained about two thirds of what they'd lost, on average. A handful ended up even heavier than when they first appeared on the television program.

This weight rebound came as no surprise. The tendency of dieters' bodies to creep back toward prior weights has been among the most reliable and replicable results in the study of weight-loss interventions. Research suggests that roughly 80 percent of people who shed a significant portion of their body fat will not maintain that degree of weight loss for 12 months, and, according to one meta-analysis of intervention studies, dieters regain, on average, more than half of what they lose within two years. Meanwhile follow-up care that is meant to stave off this backsliding via behavioral or lifestyle interventions appears to be effective only at the margins: across several dozen randomized trials, the benefits of these programs—in terms of minimizing regain—were pretty small at two years and undetectable thereafter. In short, we've known for quite some time that while it's hard to lose weight, it's even harder to keep it off.

The *Biggest Loser* study didn't just recapitulate this disheartening rule of thumb, however. It appeared to offer something more—an explanation, of a sort, for why the weight rebound might be happening. When the researchers at the National Institute of Diabetes and Digestive Kidney Diseases, led by physiologist Kevin Hall, examined the contestants six years after the show ended, they noticed major changes to the rates at which their bodies were expending energy. The contestants' resting metabolic rates had ended up much lower than expected, even taking stock of their smaller statures overall: most were burning at least 400 fewer calories than the researchers' model had predicted. Some initial dip in metabolic rate is a known side effect of weight loss, but Hall and his colleagues didn't realize that it would persist for so long, and to such a large extent.

"Dieters are at the mercy of their own bodies," explained the write-up in the *Times,* in a lightbulb formulation that helped to make the story one of the newspaper's top 10 most read of the year (just a few slots south of "Donald Trump Is Elected President"). For many readers, or dieters, this would be a way to sop frustration with a dour fact of physiology—and find solace in the revelation that shedding weight provokes a natural reflex to regain.

The *Biggest Loser* study only gestured at the underlying scientific problem, though. Yes, dieters are at the mercy of their bodies, but their reflex to regain could be undergirded by a wide array of mechanisms, such as flagging satiety hormones, adaptations in the microbiome of the gut and alterations to the makeup of their fat tissue. Changes to the metabolic rate may be thought of as one more factor on this list, as an outcome of a bunch of lower-level processes. In any case, the 2016 research, like other studies of this topic, has been nagged by a conundrum: how can you tell whether any single factor is in fact a cause of dieters regaining weight, as opposed to just a signal of their having gotten thinner in the first place?

That ambiguity shows up in the data from the reality show contestants. It's true that almost all of the dieters' resting metabolic rates had decreased across the follow-up and that this change would have seemed to favor weight rebounds, all else being equal. But it also seems that their metabolic slowing was not the primary driving force of anyone's weight regain. In fact, Hall and his colleagues found that the contestants who showed up

for testing six years later with the lowest metabolic rates were the same ones who actually had the most success in maintaining their weight loss. A lasting improvement to their exercise habits had allowed them to maintain a lower weight, and also apparently dampened their resting metabolic rates.

HORMONAL HEURISTICS

Ambiguities abound in the science of weight regain. One line of research, for example, looks at changes in circulating hormone levels in the aftermath of dieting. In a highly cited 2011 study published in the *New England Journal of Medicine,* Australian researchers put 50 overweight or obese people on a two-month diet of Optifast shakes and vegetables, yielding a total of about 500 calories per day. A year later, blood samples were collected from the patients for analysis of fasting and postprandial levels of ghrelin, leptin, peptide YY, amylin and other hormones.

The dieters had lost an average of 30 pounds during the initial intervention, and they then gained back about a dozen pounds over the months that followed, when they were given advice on healthy nutrition and exercise habits but were allowed to eat as they liked. Their endocrine markers showed a similar acute effect followed by a partial rebound. Levels of the satiety-inducing hormone leptin, for example, initially dropped by almost two thirds during weight loss when subjects were on the 500-calorie-per-day diet, but they remained more than one third reduced one year later, after all those months without dietary supervision. Similar patterns were seen for the other assays: across the board, it looked like dieting induced a rapid shift in hormone levels that would tend to favor increased appetite (and thus weight gain), and this effect would not return to baseline even after many months had passed.

Again, there was some murkiness regarding cause and effect. The study hinted that the drop in leptin levels, and other hormonal changes, might have been what spurred participants to gain back almost half of what they'd lost. But the hormonal changes could just as well have followed from the weight loss. Leptin levels in the plasma are known to drop during a very low-calorie diet, as well as when a person has been shedding fat. Contestants on *The Biggest Loser,* for example, saw their concentrations founder by almost 95 percent over the course of the weight-loss competition. That changes such as these might still be detectable, to some degree, 12 months down the road could just as well reflect the fact that the patients had maintained some degree of weight loss across that time, too.

"If you're the kind of person who can decrease your calorie intake and therefore lose a lot of weight, then you're going to experience the greatest decrease in leptin."
—Kevin Hall

That may be why follow-up attempts to predict the magnitude of a person's weight regain from the depth to which his or her leptin levels drop have been largely unsuccessful. Kevin Hall wonders if the correlation between these two variables might even end up the reverse of what you might expect, as he'd found for resting energy expenditure. "If you're the kind of person who can decrease your calorie intake and therefore lose a lot of weight," he says, "then you're going to experience the greatest decrease in leptin." Furthermore, he speculates, if you're the kind of person who is able to maintain that change in lifestyle, you'll also be the kind of person whose leptin levels stay reduced.

OUT OF SHAPE

A more comprehensive theory of weight regain, accounting for a broad array of mechanisms, may help address some of the confusion in this field. Researchers Marleen van Baak and Edwin Mariman of Maastricht University, for example, have proposed that the compensatory reflex begins with changes to the shape of fat cells. As these cells drain and shrink, their membranes pull away against the points of adhesion to the nearby extracellular matrix, creating mechanical stress. This in turn sets "a multitude of adaptations" in motion, they said in an interview, although the strength of these responses will differ across individuals.

According to their preliminary model, which is based on both in vitro studies of adipocytes and examinations of protein expression during and after weight loss, the mechanical tension that shedding weight creates at the fat-cell membranes inhibits further fat release and primes those cells to be filled again. At the same time, they theorize, caloric restriction may deprive adipose tissue of the energy it would need to relieve this stress through remodeling of the extracellular matrix. The stress response could also lead to changes in the adipocytes' secretion of leptin and other signaling proteins, as well as persistent inflammation in the aftermath of someone losing weight.

The Maastricht group has been looking to support this theory with data from the "Yo-Yo study": a randomized controlled trial of around 60 participants who were placed on either an intense crash diet (of 500 calories per day over five weeks) or a slower one (of 1,250 calories per day over 12 weeks). This was followed by a brief

weight-stabilization period (in which they received about as many calories as they would need to keep a constant weight) and then further check-ins for the next nine months. The team took biopsies of adipose tissue at the end of each study phase, measured changes to its gene activity and checked to see which, if any, might be correlated with weight regain. In a subgroup analysis of the crash-diet participants, they identified 15 genes related to the extracellular matrix and eight more associated with stress response.

Others are looking for answers in the genome. "At this point in time, people are still adding different pieces to the puzzle," says Jeanne McCaffery of the University of Connecticut. Her own puzzle piece relates to the question of inherited genetic risk for weight regain: "We were excited about the hypothesis that if you were genetically disposed to have a higher body weight, you'd put on weight again more quickly," she adds. But a genome-wide association study to determine whether genes that have been linked to the development of obesity might also be predictive of weight regain failed to turn up any positive results. That could be on account of its insufficient sample size, McCaffery explains. The study had about 3,000 people in the weight-loss condition, whereas similar studies of the genetics of obesity have been far larger in scope.

The one point on which nearly all researchers agree is that the physiology of weight regain, like the physiology of obesity itself, is almost certain to reflect a very complicated mix of factors ranging from genetics to behavior and the environment. That means we're unlikely to find any magic-bullet method for keeping pounds from coming back. Indeed, some degree of rebound may be more or less inevitable for the majority of dieters.

But even that news may not be as bad as it seems. Just last year, a team of researchers at the University of Alabama at Birmingham, led by David Allison, put out a rodent study of a provocative idea: what if there were lasting benefits to losing weight—even when that weight is almost certain to be regained? The researchers randomized 552 obese Black-6 mice into four groups: one set of animals ate a high-fat diet at will and remained obese; another two sets received either moderate or more extreme caloric restriction and stabilized at a "normal" or intermediate weight; and a fourth was put through several yo-yo cycles of restricted and ad libitum feed, losing weight and then gaining it right back.

At the end of the study, the mice that remained obese throughout the experiment had markedly increased mortality: they lived, on average, for just 21 months, as compared to the 26-month average life span of the mice that had been put on the most extreme diets and kept at a normal weight. More surprising was the fact that the yo-yo mice also gained longevity, by virtue of their weight cycling: they lived an average of 23 months, about the same as the mice that were kept under chronic, moderate calorie restriction.

In other words—at least for mice—it may be that weight regain does not cancel out all the benefits of dieting. Those who feel they are going around in circles may take some solace in this notion: even if your fat cells tug and twist your weight loss back to zero, that does not mean you have been pulled back to where you started.

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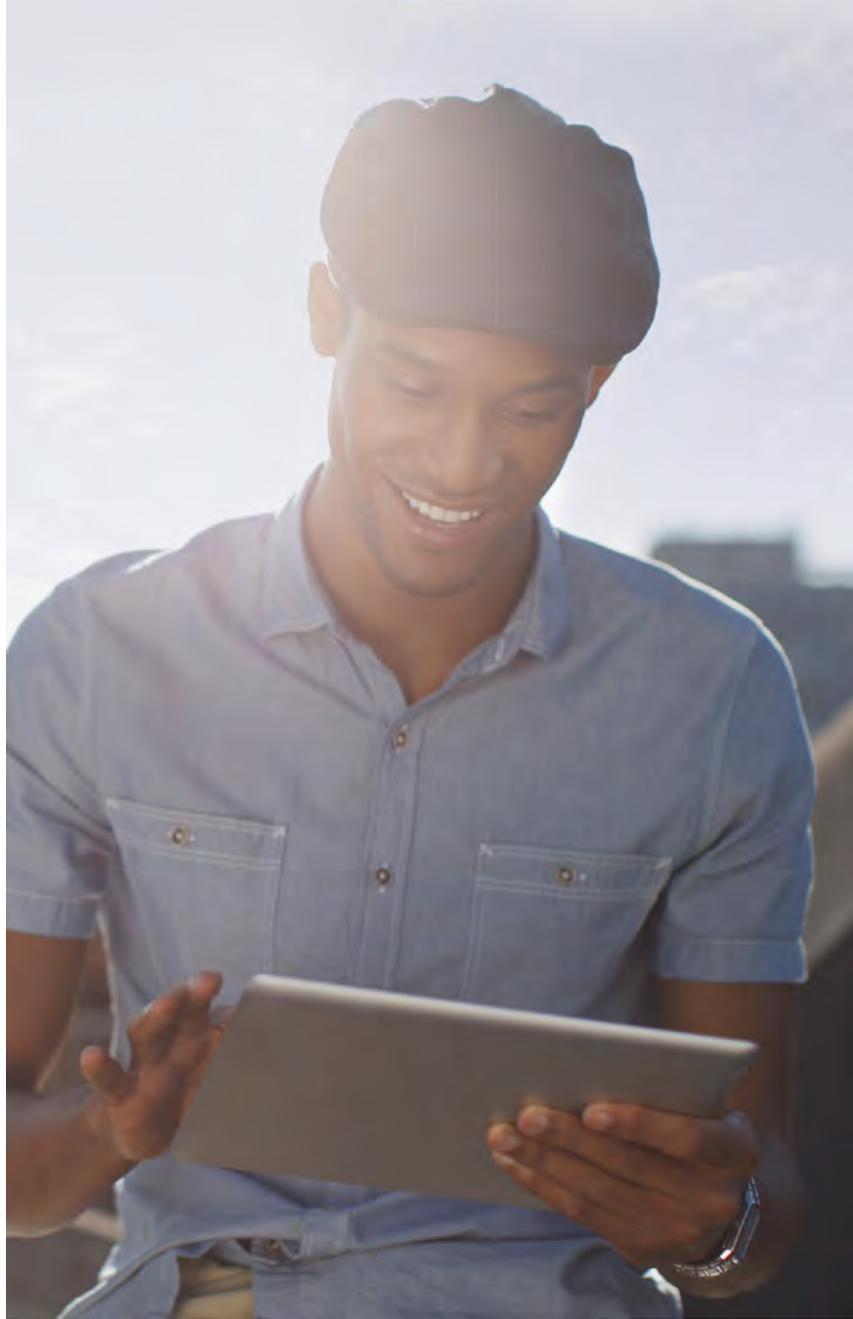


Survival of the Fittest Cells

Cells in the body don't always play nicely together. Could co-opting their competitive nature help to unlock cutting-edge therapies?

By Kendall Powell

ACAPULCO STUDIO

Kendall Powell is a science journalist in Boulder, Colo.

Yasuyuki Fujita has seen firsthand what happens when cells stop being polite and start getting real. He caught a glimpse of this harsh microscopic world when he switched on a cancer-causing gene called *Ras* in a few kidney cells in a dish. He expected to see the cancerous cells expanding and forming the beginnings of tumors among their neighbors. Instead, the neat, orderly neighbors armed themselves with filament proteins and started "poking, poking, poking," says Fujita, a cancer biologist at Hokkaido University in Sapporo, Japan. "The transformed cells were eliminated from the society of normal cells," he says—literally pushed out by the cells next door.

In the past two decades an explosion of similar discoveries has revealed squabbles, fights and all-out wars playing out on the cellular level. Known as cell competition, it works a bit like natural selection between species, in that fitter cells win out over their less fit neighbors. The phenomenon can act as quality control during an organism's development, as a defense against precancerous cells and as a key part of maintaining organs such as the skin, intestine and heart. Cells use a variety of ways to eliminate their rivals, from kicking them out of a tissue to inducing cell suicide or even engulfing them and cannibalizing their components. The observations reveal that the development and maintenance of tissues are much more chaotic processes than previously thought. "This is a radical departure from development as a preprogrammed set of rules that run like clockwork," says Thomas Zwaka, a stem cell biologist at the Icahn School of Medicine at Mount Sinai in New York City.

But questions abound as to how individual cells recognize and act on weaknesses in their neighbors. Labs have been diligently hunting for—and squabbling over—the potential markers for fitness and how they trigger competitive behaviors. These mechanisms could allow scientists to rein in the process or to help it along, which might lead to better methods for fighting cancer and combating disease and aging using regenerative medicine.

"Cell competition is on the global scientific map," says Eugenia Piddini, a cell biologist at the University of Bristol in England, who likens the buzz around this idea to the excitement that helped propel modern cancer immunotherapies. The better scientists understand competition, she says, the more likely it is that they will be able to use it therapeutically.

HISTORY REPEATS

During a blizzard that dumped more than 30 centimeters of snow in February 2019, biologists from about a dozen disciplines convened at a hotel at Lake Tahoe in California for the first major meeting devoted to cell competition.

"It was a zoo of researchers," says co-organizer Zwaka, and it included biologists who study flatworms that can regenerate their whole body from a single cell; geneticists attempting to make interspecies chimeras of mouse, monkey and rabbit embryos; and a keynote speaker who spoke about the terrible battles and cooperative campaigns waged in bacterial communities.

The snowbound attendees, about 150 in all, debated how and why cells size up their competition. And they celebrated the discovery that gave birth to the field.

In 1973 two Ph.D. students, Ginés Morata and Pedro Ripoll, were perfecting a way to track the various cell populations in a fruit-fly larva that would eventually develop into a wing. Working at the Spanish National Research Council's Biological Research Center in Madrid, they introduced a mutation called *Minute* into

a few select cells in the larva and left the rest of the cells unaltered.

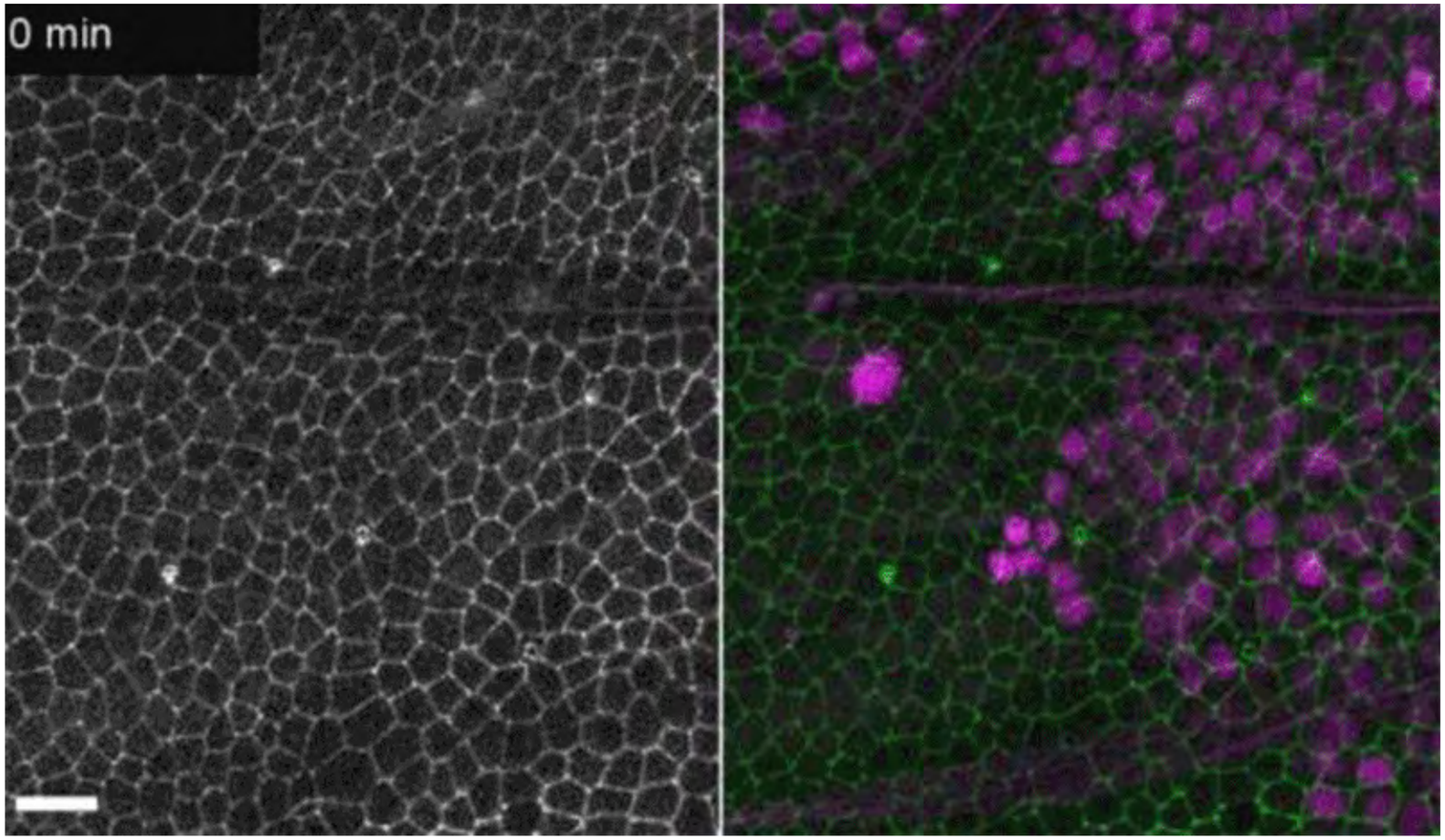
Knowing that *Minute* cells grow slower than their unaltered neighbors, the scientists expected to find some smaller cells amid the wild-type counterparts. “Instead, we found that the cells disappeared,” says Morata, now a developmental biologist at the Autonomous University of Madrid in Spain.

On their own, *Minute* cells can develop into a fly that is normal—except for the short, thin bristles on its body that give the mutation its name. But when mixed with wild-type cells in the larva, the cells simply vanished. “*Minute* cells were not able to compete with the more vigorous, metabolically active wild-type cells,” says Morata. They described the activity as cell competition. “It was a very surprising and interesting observation,” Morata says. But lacking the molecular tools to follow cell fates more closely, he and his colleagues let the finding simmer.

Twenty-six years later, postdocs Laura Johnston and Peter Gallant observed nearly the same phenomenon. Working with Bruce Edgar and Robert Eisenman, respectively, at the Fred Hutchinson Cancer Center in Seattle, Wash., they were studying a mutation in another fly gene, *Drosophila Myc* (*dMyc*), that also slows cell growth.

“There was a eureka moment when Peter and I realized that these *dMyc* mutant cells would disappear,” says Johnston, now a developmental biologist at Columbia University Irving Medical Center. They eventually showed that the mutant cells were forced to initiate a form of programmed cell death called apoptosis. “It was very clear that this was a competitive situation,” Johnston says.

Their 1999 paper ignited interest among scientists, including Morata. He jumped back into the fray with Eduardo Moreno, and they took advantage of modern molecular tools to repeat the *Minute* experiments. “The field blossomed from there,” says Johnston.



Fruit-fly cells with the oncogene *Ras* activated (*purple*) can outcompete neighboring wild-type cells (*green*).

Myc acts as a master controller of cell growth, and *Minute* encodes a key component needed for synthesizing proteins—so it’s not surprising that reduced expression of those proteins makes cells less fit. But the next finding took people by surprise. A pair of papers by Johnston and Moreno showed that cells with an extra copy of normal *dMyc* outcompeted wild-type cells. These fitter-than-wild-type cells came to be called “supercompetitors.”

The discovery of supercompetition emphasized that cell competition is about the relative fitness of a group of cells, says Zwaka. If one cell is falling behind, the entire group of neighbors could decide it has to go. But on the flip side, they can also sense that certain cells are better and should survive.

Cell competition wasn’t simply about getting rid of defects; it was about survival of the fittest, with the less fit “loser” cells dying and the “winners” proliferating. Importantly, competition was seen only when there was a mixture of genetically different cells, a phenomenon known as mosaicism. In this way, cell competition acts like a quality-control system, booting out undesirable cells during development.

VYING FOR VIABILITY

Fujita’s observation of the kicked-out kidney cells was one of the first hints that mammalian cells compete, too. Soon after that work was published, researchers started to observe competition forcing out mutant cells from

EDUARDO MORENO

various other tissue types such as skin, muscle and gut.

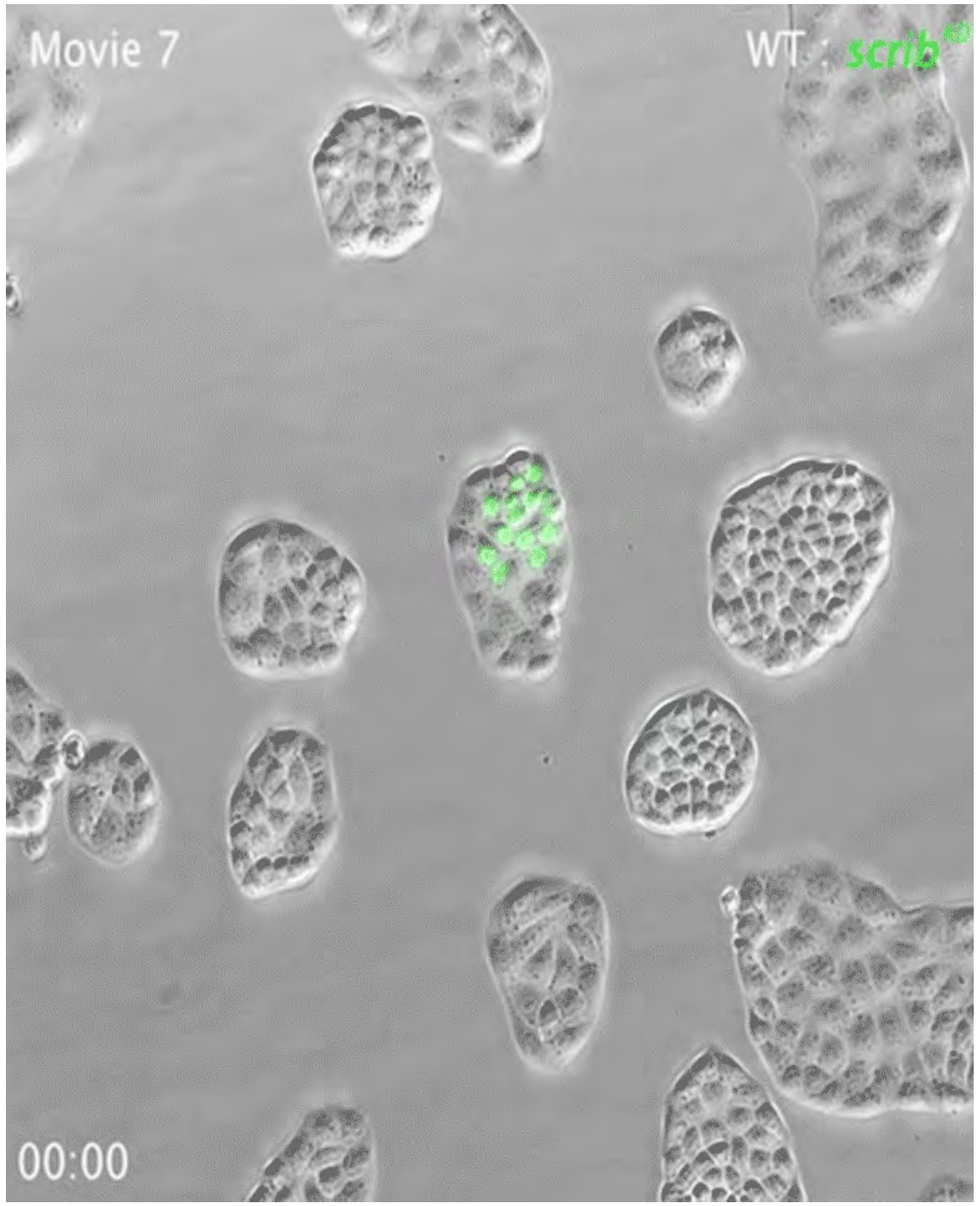
The next-most-obvious place to look for competing cells was the mammalian embryo. In 2013 Zwaka's team, and two other laboratories, probed mouse embryos at the earliest stage of development—those that have progressed just beyond a ball of cells. Zwaka's group made mouse embryonic stem cells (ESCs) with a supercompetitor mutation that lowered expression of p53, an important quality-control protein that normally puts the brakes on cell division. When these cells were put into a mouse embryo, they quickly took over and developed into a normal mouse. Similarly, Miguel Torres's lab at the National Center for Cardiovascular Research in Madrid showed that supercompetition could be induced in an early mouse embryo using slight overexpression of the mouse *Myc* gene.

By artificially creating losers or winners, researchers could force cell competition into play. But Torres's team, led by then-postdoc Cristina Clavería, also made the striking observation that *Myc* expression varied naturally in mouse ESCs. Cells in the embryo with approximately half the amount of the protein compared with their neighbors were dying by apoptosis. This was one of the first studies that strongly pointed to naturally arising cell competition.

SCULPTING TISSUES

The phenomenon also comes into play later on in embryonic development. In a study published in 2019, postdoc Stephanie Ellis at Elaine Fuchs's lab in Rockefeller University in New York City looked at mouse skin. During development, its surface area expands by a factor of 30 over the course of about a week. The cells within proliferate wildly—first as a single layer and later as multiple layers.

Ellis injected mouse embryos with a concoction that turns cells into genetic losers. She targeted a few cells present when the embryonic skin is a single layer thick and added a marker gene that made them glow red. Then she used time-lapse imaging to watch their grim fates: the skin cells popped out from the surface layer, broke up and disappeared. Later, she noticed the winner cells engulfing and clearing the losers' corpses.



Dog kidney cells that don't express the gene *Scribble* (*green*) become losers and are eliminated by wild-type cells through crowding and compaction.

Repeating the experiment at the multilayer stage, Ellis no longer saw the less fit skin cells perishing or being engulfed. Instead, the loser cells tended to differentiate and migrate into the outer layers of skin—where they acted as a barrier for a short time before being shed. The winner cells were more likely to remain behind in the bottom layer as stem cells.

This made sense. "Killing a cell is energetically expensive," says Ellis. A developing tissue, she says, might decide: "Why not just remove losers through differentiation?" Emi Nishimura's lab at the Tokyo Medical and Dental University in Japan found that competing stem cells in the aging tail skin of adult mice used the same pattern of asymmetrical divisions to eliminate stem cells with lower levels of a key structural collagen protein.

These experiments could provide guidance for scientists looking to harness stem cells to rejuvenate aging tissues and organs. Cell competition could either help or hurt such therapies: stem cells might outcompete older, less fit cells, or they might encounter a hostile neighborhood when transplanted into tissue. Understanding whether and how cell competition happens in adult tissue could help settle this matter.

Piddini admits that she was a little obsessed with the idea, and her group was part of a wave of researchers that proved cell competition does take place in adult organisms. To test the idea, she says, the team "genetically sprinkled" a mutated copy of *RPS3,* a gene functionally related to *Minute,* into some cells in the intestine of adult flies. Cells with the mutant copy were outcompet-

ed by their wild-type counterparts. It didn't matter whether the losers were the stem cells that maintain the gut or differentiated cells: all eventually perished.

Cristina Villa del Campo, a senior postdoc in the Torres lab, tested for adult competition in the mouse heart by introducing winner cardiac cells at eight to 10 weeks of age. Over the course of one year, she tracked the numbers of winner cells and wild-type losers and saw the loser population decline by about 40 percent.

"It was a slow replacement in the adult," Villa del Campo observes. "But even highly differentiated functional adult cells can sense that the less fit heart cells and eliminate them."

UNANSWERED QUESTIONS

Even with so many examples of cell competition playing out in different conditions, the field still faces a torrent of unanswered questions. One big puzzle is how cells in a group sense fitness. "Maybe cells are recognizing chemical differences, or physical differences, or differences in cell-membrane composition," says Fujita, who adds that labs have found evidence for all three.

His filament-poking kidney-cell experiments suggest that cell–cell contact is needed. Others have seen chemical-fitness signals that seem to be short-range, traveling up to eight cell diameters. Exactly which molecules are responsible for this signaling—either secreted chemicals or physical tags—is the subject of intense debate and investigation.

Both Johnston and Zwaka have turned up signals associated with immune surveillance. Johnston's group identified molecules that typically call immune cells to swarm in and engulf foreign invaders and that were driving death in losers. Normal cells express low levels of these death signals at all times. But in a competitive mix, winners flooded their loser neighbors with the signal, which pushed them to kill themselves.

"There was a eureka moment when Peter and I realized that these *dMyc* mutant cells would disappear."

—Laura Johnston

Zwaka proposes that cells might assess each other's health by sniffing out the general signals or debris that cells shed. It's akin to smelling the steaks that your neighbor is grilling for dinner and concluding that they must be doing well.

Or it could be as simple as seeing which flag your neighbor is flying. Moreno heads his own group now at the Champalimaud Center for the Unknown in Lisbon, Portugal, which discovered a membrane-spanning protein called Flower. In humans, the protein can take four forms, each displaying its own characteristic structure on the outer cell surface. Two signal "I'm a winner" and the other two signal "I'm a loser" to nearby cells, says Moreno.

Some human cancer cells fly the Flower winner signals, which might enhance their survival. Experiments in Moreno's lab showed that silencing the winning flags on tumors slowed the cells' growth and made them susceptible to chemotherapy.

Some researchers, however, dispute the importance of the Flower tags. Moreno acknowledges that they are not present in all cell-competition situations.

HEALTHY COMPETITION

Cracking the mechanics of competition will be key if researchers want to use it to improve cell-based cancer or regenerative therapies.

There are tantalizing hints that cell competition might already protect against cancer. Findings made in the past few years reveal that human skin, esophageal and lung cells show high levels of mosaicism. Approximately one quarter of skin cells, for example, harbor many precancerous mutations that only rarely turn into tumors.

It is unclear what gives cancerous cells the advantage when tumors do form. If researchers can learn how to subdue supercompetitors or blunt cancer cells' ability to compete, they might be able to turn that against cancer.

Conversely, stem cells might need to gain a competitive edge if they are to replace aged or diseased tissue for an organ makeover. Villa del Campo says that clinicians are already considering how to enhance patient-derived cardiac stem cells to efficiently replace cells that have been damaged by heart attacks or disease.

What started as modest observations in minuscule fruit-fly larvae have exposed the primal cellular battles that could usher in a new era of cell-based medicine. The process has scientists buzzing, but it remains mysterious.

"Cell competition might be a general process to remove any undesirable cell that should not be there," says Morata, after returning from a one-day meeting in Lausanne, Switzerland, devoted to competition last September.

Now 74, he's thrilled that work he essentially shelved more than 40 years ago is gaining new life and that the competition is heating up. "It's really exciting."

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CRISPR-Edited Babies Arrived, and Regulators Are Still Racing to Catch Up

One year after the world learned of He Jiankui's editing of twins, gaps in rules remain

By Alisa Opar

ANDREW BROOKES *GETTY IMAGES*

Alisa Opar is a science writer based in Missoula, Mont.

LAST NOVEMBER A CHINESE SCIENTIST PROVOKED A GLOBAL outcry when he announced that he had helped create the world's first genome-edited babies. Scientists swiftly and severely condemned Southern University of Science and Technology's He Jiankui for bypassing some safety and ethics checks. The revelation also prompted intense discussion about what should be done to block the next gene-editing rogue. Since then, various groups, including two major international organizations, have begun developing new regulatory frameworks to govern human genome editing. Meanwhile debate has also swirled about whether there's an immediate need to prohibit gene editing in clinical research.

When He used the popular CRISPR-Cas9 tool to try to disable the molecular pathway that HIV uses to infect cells in twin girls when they were embryos, there was no existing international moratorium against creating CRISPR babies, or penalties in China for doing so. Warnings had emerged from gene-editing conferences, but apparently they were not clear or emphatic enough. He, for instance, maintained that he'd followed the best practices set forth in 2017 by a panel of leading U.S. scientists and ethicists, checking all the boxes related to safety and oversight. His work represented a significant leap in germline gene editing, which introduces heritable changes and therefore has the potential to stamp out rare, devastating genetic diseases such as cystic fibrosis and muscular dystrophy. Yet serious concerns abound about off-target effects.

"The science is not ready; that is not even an issue," says Victor Dzau, director of the U.S. National Academy of Medicine. Right now, he says, it would be irresponsible to move ahead with clinical germline editing. "The silver lining is that the world was awakened by the conduct of Dr. He, and we are all working very, very hard with all good intentions to make sure that this doesn't happen again—not in the fashion that He did it. And that someday, if and when the technology is ready—and I think all of us are very bullish about this technology—that it will be helping humankind in the right way, knowing the risks and knowing the benefits."

To that end, the U.S. National Academies of Medicine and Science and the U.K.'s Royal Society have come together to assemble representatives from 10 countries to develop a framework that identifies scientific, medical and ethical requirements for the clinical use of human germline genome editing. "We're not going to tell the public whether they should use the technology," Dzau says, "but rather whether it's safe, the risks involved, and how it could be used." In August 2019, at the first of the committee's two planned meetings, members received reports on the current status of the science during a day-long public session. The final report will be published next spring.

An advisory committee convened by the World Health Organization, meanwhile, is taking a broader view in its development of global standards for governance and oversight. Its 18-member panel of interdisciplinary scientists, bioethicists and experts in law, geopolitics and technology futurism is looking at all gene editing—both germline changes, which are passed on to future generations, and somatic changes, which aren't. So far the group has recommended that the WHO create an open registry of all studies of clinical applications of genome editing. A working group is fleshing out the details, including which research should be submitted—preclinical as well as clinical, for instance—and how to ensure that publishers and research funders require scientists' participation. Its final report, which will take the National Academies'

and Royal Society's findings into account, will come out after the final meeting next summer.

"It's been moving slowly," says Alta Charo, a panel member and a bioethicist at the University of Wisconsin–Madison, of the groups' progress, chalking it up in part to bureaucracy at the WHO and the challenge of the academies' creating a new kind of collaboration. "I can only hope now these committees move more quickly."

When the WHO does make its recommendations on the criteria required for safe, ethical gene editing, individual nations will decide whether and how to adopt the regulatory framework and to enforce any laws created as a result of them. In countries with sophisticated regulatory systems, such as the U.S. and Japan, or impoverished nations without the resources to support such research, that likely won't be an issue, Charo says; the "million-dollar question" is whether mid-resource countries with traditionally lax enforcement will police gene editing. That wasn't the case, she points out, with unproven clinical stem cell research that took off in Mexico, Singapore, Ukraine and elsewhere. The WHO may be in a position, she says, to help spur more enthusiasm for enforcement in such places.

Although stricter enforcement could deter mavericks in years to come, the lack of clear rules in the interim won't stop the next scientist from using CRISPR to edit the germline in babies in the meanwhile. In June Russian scientist Denis Rebrikov announced his intention to create more CRISPR babies, and it's widely thought that he's hardly likely to be the only one considering doing so.

"It has a certain appeal because it conveys a strong message. But it also doesn't have a clear path in terms of what it means and how you implement it."

—Margaret Hamburg

FRAMING WHAT'S FORBIDDEN

At the time He made his controversial claim, China had laws that prohibited the creation of CRISPR babies, and the practice is either directly or indirectly outlawed in about 30 other countries. Several nations, including the U.K., Japan, Canada and China, have express bans on gene editing in human embryos that will be used for reproduction. China tightened its regulations last March, creating penalties for breaking the rules. Now scientists face up to $15,000 in fines and a five-year research ban; institutions that violate the regulations risk fines, blacklisting on grant applications and loss of their medical licenses.

The U.S. does not have an explicit ban, but federal regulations restrict germline editing. The U.S. National Institutes of Health cannot fund any research in which an embryo's genome is edited, and the Food and Drug Administration, which regulates all gene therapies used in patients, can't consider clinical-trial applications for any human germline genome editing. In some states, nonclinical research is legal, but it must be funded by nonfederal sources.

The rules are murky in many countries. Russia, for instance, has a law that prohibits genetic engineering under most circumstances, but it's unclear how the rules would be enforced with regard to gene-edited embryos or babies. Rebrikov, a molecular biologist in Moscow who intends to seek approval from three government agencies for his experiments to create HIV-protected babies, told *Nature* last June that he was tempted to push ahead with the work while the government hashes out regulations, but he has since backtracked.

The following month the WHO issued a statement widely viewed as a rebuke. "Human germline genome editing poses unique and unprecedented ethical and technical challenges," said director-general Tedros Adhanom Ghebreyesus. "Regulatory authorities in all countries should not allow any further work in this area until its implications have been properly considered."

The WHO gene-editing expert advisory committee made that interim recommendation, which stops short of calling for a moratorium—something many scientists have advocated for. "When you call for a moratorium, it immediately then raises another set of questions that are harder to answer: Who has the authority to put a moratorium in place? How do you enforce it? How do you determine when the moratorium is stopped?" says Margaret Hamburg, the committee's co-chair and a former head of the FDA. "It has a certain appeal because it conveys a strong message. But it also doesn't have a clear path in terms of what it means and how you implement it."

A moratorium is, by definition, a temporary prohibition of an activity. The approach has been used previously to take a time-out when cutting-edge, powerful science has been at risk of outpacing ethical guidance, public acceptance or the law.

In some instances, a government imposes the freeze. In 1988, for instance, when researchers began transplanting fetal cells into the brains of adults with Parkinson's disease, the public balked and the Reagan administra-

tion declared a temporary moratorium on U.S. federal funding for such experiments; it remained in place until 1993, when the Clinton administration lifted it. And in 2014, following mishaps at federal labs—one handling anthrax and one handling avian flu—the U.S. government halted, for a then-undetermined amount of time, funding for gain-of-function experiments, in which viruses are genetically altered in ways that could make them more contagious, more deadly or both. Three years later the moratorium was lifted when a formal process for evaluating whether the experiments should receive federal funding was put into place. No researchers are known to have broken these moratoriums, which had the significant force of the federal government behind them.

Other times, scientists themselves have pushed pause. In 2012 leading researchers from the Netherlands, the U.K., the U.S. and other countries voluntarily halted certain types of experiments involving the H5N1 avian influenza virus so that scientists, government officials and the public could debate the need for the research and impose new safety measures. They initially expected a 60-day hiatus but extended it indefinitely as discussions about how to proceed intensified. After a year, and following a two-day international meeting to discuss their progress, 40 researchers declared in a letter published in *Nature* and *Science* that the studies should restart in countries that had hammered out criteria for H5N1-virus-transmission research.

The groundwork for the H5N1-research moratorium was laid out decades earlier, when rapid advances in recombinant-DNA research sparked fears that a dangerous new pathogen might be created. More than 100 leading molecular biologists from around the globe voluntarily hit pause on many types of experiments using recombinant-DNA technology for about a year beginning in July 1974. Then they, along with a few journalists and policymakers, gathered in Asilomar, Calif., to draft safety regulations governing genetic engineering. Those recommendations quickly became the basis for rules adopted across the globe, and "Asilomar" became shorthand for scientists acting in a socially responsible manner.

"Making a claim that the scientists and technologists who are leading development of these technologies also should be the ones to decide how they should or shouldn't be used, I think that that's highly problematic."
—Benjamin Hurlbut

Asilomar has been invoked as a touchstone by numerous scientists who support a gene-editing moratorium. Yet there are concerns about researchers taking the lead. "Making a claim that the scientists and technologists who are leading development of these technologies also should be the ones to decide how they should or shouldn't be used, I think that that's highly problematic," says Benjamin Hurlbut, a biomedical historian at Arizona State University. "Technical expertise doesn't mean that you have expertise about what's good and bad for humanity."

It's already a complicated matter for an administration or group of scientists to decide when to lift a moratorium. Everyone interviewed for this article said it's important to get societal input on whether, when and how the research should be done. How exactly to do that, and to weigh whether public consensus would support ending a moratorium, is unclear.

TAKING A BREAK

Although there is widespread support for a germline-editing moratorium, there is also broad disagreement about the specifics—whether it should be voluntary or mandatory, for instance, and who should institute it. "I believe that many of the people calling for a moratorium are doing it with different ideas in mind of what that is," Charo says.

The most detailed plan to date was published in a Commentary in *Nature* in March 2019. Scientists and ethicists from seven nations called for a fixed period, perhaps five years, during which no clinical uses of germline would be allowed. The authors envision voluntary compliance by individual nations, which would retain sovereignty over scientific enterprises within their borders. "As well as allowing for discussions about the technical, scientific, medical, societal, ethical and moral issues that must be considered before germline editing is permitted, this period would provide time to establish an international framework," they wrote.

After that, countries would have to undertake more steps before starting any experiments, including a comment period of perhaps two years to discuss the pros and cons, and determining whether there's broad societal consensus in the particular nation. As for how that consensus might be reached, the authors point to the Global Genome Editing Observatory proposed by Hurlbut and others. It would be an international network of

scholars and organizations, similar to those established for climate change and human rights, that would facilitate diverse public conversations.

The NIH supported the call. So did the European Society of Human Reproduction and Embryology and the European Society of Human Genetics, says Guido de Wert, professor of biomedical ethics at Maastricht University in the Netherlands and lead author of the two groups' joint position paper on germline genome editing. (They support basic and preclinical research in this area and note that while clinical experiments might be an important intervention in the future, at present they would be "totally premature.") The WHO, the likely body to facilitate the proposed moratorium, thus far has instead called upon each country to keep its scientists in check.

In April another group of scientists and industry representatives urged the U.S. government to take the lead in instituting a binding global moratorium on germline genome editing. In a letter to U.S. Department of Health and Human Services secretary Alex Azar, orchestrated by the American Society of Gene & Cell Therapy (ASGCT), the 62 signees from five countries called for a ban on clinical research "unless and until diverse stakeholders have the opportunity to broadly and deeply discuss and reach a societal consensus on these challenges." Azar has not responded.

The ASGCT, meanwhile, is forging ahead with efforts to help further such discussion. On November 6, 2019, the group hosted a public workshop at which attendees discussed ethical, societal and policy issues in germline gene editing. Speakers included Hamburg and Francis Collins of the NIH. "What should the boundaries be?" is one of the big questions, says ASGCT's executive director David Barrett. "It's something that should be inclusive of bioethicists, researchers, clinicians, but we also think it's necessary to include patients and their advocates in the discussion, and other individuals who can represent diverse views in society, to make sure that it is inclusive of a discussion as possible."

Such open conversation to understand varying viewpoints is essential, says Jennifer Doudna, a University of California, Berkeley, molecular biologist who pioneered the CRISPR-Cas9 genome-editing system. And that's why she doesn't support a moratorium. "I think even the word 'moratorium' implies that you're not going to proceed to discuss the topic. And I think that would be a big mistake," she says. "Rather than squelching discussion of this topic, we should actively encourage it."

What's more, a global moratorium might not hold much sway in some countries. "In Russia, it would be unlikely that all scientists would listen to whatever U.S., or U.S.-backed, scientists have to say," says biologist Konstantin Severinov, who works both at Moscow's Skolkovo Institute of Science and Technology and at Rutgers University in New Jersey. "People will do it in spite of the international regulatory efforts."

As for Rebrikov, he told *Nature* in October 2019 that he has pushed back his plan to implant gene-edited embryos until he gets approval from the Ministry of Health of the Russian Federation.

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Editor's Note: On December 30, a Chinese court sentenced He Jiankui to three years in prison for conducting an "illegal medical practice." Chinese authorities found that He's team had falsified regulatory paperwork.

Health Concerns Mount as More Old Sewer Pipes Are Lined with Plastic

Residents near renovation sites claim noxious emissions from pipe inserts are making them sick

By Robin Lloyd

GETTY IMAGES

Robin Lloyd is a science writer based in New York City and a contributing editor at *Scientific American*.

In early 2019 Nicole Davis arrived at one of the San Antonio, Tex., offices of the audiology practice she co-owns, ready to see the day's patients. But on entering her office, Davis says, she quickly noticed a noxious odor that smelled like paint thinner. Her eyes started burning. By noon she felt nauseated and dizzy, with the burning sensation spreading to her nose and throat. Her mouth went numb. Co-workers in the building told Davis that they felt ill, too. By the evening, she says, she was vomiting.

Two days later Davis received an e-mail from an employee for a construction firm that was doing work that week on municipal pipes below street level near the building. The employee apologized in the e-mail for Davis's "recent experience" and attached a technical document describing the hazards and health risks associated with materials used to make plastic in the pipe project. The e-mail and attachment do not state that the work caused the odor or Davis's reaction.

The company was renovating an underground sewer pipe with a widely and increasingly used technique called cured-in-place pipes. A felt or composite sleeve is saturated, typically with a polyester or vinyl ester resin. Workers thread the sleeve through an underground pipe and then inflate and heat it, often with steam or hot water. The sleeve hardens to form a continuous plastic liner along the old pipe's inner walls. The technique is less expensive and takes less time than fully replacing old sewer-system pipes and stormwater culverts.

Davis has recovered from most of what she says her doctor told her were neurological effects from a chemical exposure. But she says she did not receive any advance notification of the work or of any associated odors or potential hazards, and she thinks she should have been notified. When she sought information from local and regional public health authorities about the health risks noted in the technical document and any treatment she might need, she hit dead ends with local and regional public health authorities, she says. The construction company did not reply to repeated attempts by *Scientific American* to obtain comment for this story.

Davis's experience reflects, in part, the scarcity of reliable, industry-independent research and public health advice about potential risks associated with the cured-in-place pipe, or CIPP, method. The practice has grown steadily in the past two decades, with more than 35,000 miles of the liners installed worldwide, according to a 2017 market report by BCC Research. CIPP is the most popular method among a group of pipe-renovation techniques that require minimal digging as compared with excavating an old pipe and replacing it. With billions of dollars spent and loaned annually in the U.S. alone to restore deteriorating pipes, the market for lower-cost renovation approaches is forecast to remain strong for several years.

Many residents say they are notified before these CIPP sites pop up, but some say they are not. The notifications typically state that the work is harmless. But accumulating evidence calls those claims into question. In many cases, the public receives incomplete, incorrect or scientifically unfounded information about potential health risks associated with CIPP emissions, emerging evidence suggests.

Andrew Whelton, a civil and environmental engineer, and his colleagues at Purdue University have collected details on more than 100 incidents spanning 29 U.S. states in the past 15 years in which people have raised concerns or called their fire department about odors and emissions from CIPP. Children have been mentioned in news stories and other reports in more than a dozen of

those 100 cases, including a September 2019 incident in Seneca Falls, N.Y., in which middle school students reportedly felt sick from a CIPP job several hundred feet from their classroom. In some cases, symptoms persist, and residents relocate. Nancy Hoback of Salem, Va., says it took several weeks to feel better last year after claiming to have been exposed to emissions from a pipe-lining job she measured to be more than about 700 yards (640 meters) from her house. She says she experienced burning in her mucous membranes, headaches, dizziness, difficulty swallowing and shortness of breath.

WORRISOME EXPOSURES

Peer-reviewed research published in the past few years has started to clarify the complexity of CIPP emissions questions. Studies by Whelton's group have revealed that jobs at study sites, where installers used steam to harden the resin, released a mixture of vaporized and liquid droplets of organic compounds and water, as well as particles of partially hardened resin, into the air. The compounds include hazardous air pollutants such as styrene and methylene chloride, as well as dibutyl phthalate, which some studies have identified as an endocrine disruptor. But other emitted compounds vary, possibly depending on the type of resin used and other operational differences. Styrene, which causes neurological effects, is classified as "reasonably anticipated to be a human carcinogen" by the U.S. National Toxicology Program. And methylene chloride is considered a potential, probable or reasonably anticipated carcinogen by various federal agencies.

A handful of other independent studies—including one published in January 2019 by the National Institute for Occupational Safety and Health, a U.S. federal agency that does research and makes safety recommendations to prevent worker injuries and illness—have identified airborne styrene levels at CIPP work sites that exceed worker safety thresholds set by the U.S. Occupational Safety and Health Administration.

"We are at a very preliminary stage with a lot of the investigations of the health hazards associated with this. But then the question is, 'Well, how do you get more people doing the science related to this?'"

—Jonathan Shannahan

Two years ago a worker died on the job while inside an underground pipe being renovated with CIPP. An autopsy seen by this writer stated that the cause of death was drowning but that styrene toxicity contributed to it. The incident prompted an OSHA investigation. As a result, the company paid $55,000 in penalties, in part for exposing employees to levels of airborne styrene exceeding the agency's worker safety limits. Many photographs show CIPP workers who are not using respirators that could protect them from inhaling emissions, raising questions about the safety culture at job sites.

PROTECTING THE PUBLIC

Measures designed to protect workers have limited relevance for members of the public. People not doing the jobs are usually farther away from emissions. Some are in structures such as homes, schools or workplaces, which sounds safer. But airborne emissions make their way directly into buildings, as environmental and occupational health specialist James Morrison found in late 2004. At the time, he worked with the Wisconsin Department of Health and Family Services and was tasked by the federal Agency for Toxic Substances and Disease Registry (ATSDR) to figure out what happened to sicken office workers in a building in Milwaukee that was formerly a brewery. Emissions from a nearby CIPP job got indoors through cracks in the building's foundation and irritated workers to the point that they evacuated, according to a report by Morrison and his colleagues. The report called the initial exposures a public health hazard. In telephone interviews in recent weeks, Morrison confirmed the incident and report details.

Chemical-exposure safety standards for the public are stricter than those for workers, Morrison says, for two primary reasons. For one, exposed workers are ensured recovery periods when the workday ends and on weekends, whereas public exposures can persist around the clock. In addition, worker safety standards are designed to protect a population of adult workers who are presumed to be healthy, whereas public safety standards are designed to go farther, to protect children, seniors and others who may be more medically vulnerable to chemical exposures, Morrison says. Contractors and those who hire them should have a plan in place to refer people who feel sick to public health authorities, he adds.

Thresholds for airborne exposures of residents to some of the dozens of chemicals identified by Whelton's group as present or emitted into the air at CIPP sites are set by the ATSDR, as well as by the EPA. But these thresholds are not public health regulations. They are meant to serve as reference information for health care workers or others who assess cases brought to their attention.

TOXICOLOGY STUDIES

Last summer some of the first findings to delve into the human health implications of exposures to CIPP

emissions were published in the journal *Inhalation Toxicology*. The results came from a study of lab-grown mouse cells, which researchers often use to determine whether tests should be done on human cells and then lab animals. Study senior author Jonathan Shannahan, a toxicologist and a member of Whelton's research team at Purdue, exposed lung immune and tissue cells to condensed emissions collected at three different CIPP work sites. The idea was to see which and how many cells died, but the results varied a fair amount by job site and concentrations of emissions, Shannahan says. Similarly, the team found alterations in gene expression and protein production in exposed cells, some of which are associated with changes in cancer, inflammation and injuries or with abnormal function in organs. Here again, the results differed from site to site, by the type of cells exposed, and by the genes and proteins examined.

The findings show the potential for adverse health effects in humans, Shannahan says. Yet the details of the link remain murky. "Is it related to every CIPP work site? We don't know. Is it related to the majority of them? We don't know," he says. The effect of these emissions on people may also vary by genetic profile, age and underlying health, including the strength of one's immune system.

A trade organization for CIPP and other methods of pipe repair, the National Association of Sewer Service Companies (NASSCO), has also taken steps to study the safety of airborne emissions for workers and the public. Last year it contracted researchers at Louisiana Tech University to collect, measure and model the dispersion distances of emissions of styrene and other chemicals at several installation sites. The researchers outlined results in a December 17 Webinar, saying that details would be published online in January 2020. At some of the sampled sites, if the measured levels of styrene in the air persisted, they could have exceeded safety thresholds for the general public or for workers as set by EPA, NIOSH or OSHA, according to findings presented by Elizabeth C. Matthews, the study's primary investigator. During an interview in October 2019, Lynn Osborn, the organization's technical director, said, "At NASSCO, our general emphasis is on safety. It's always up front." The trade group, which in 2017 released updated guidelines for the safe use and handling of styrene-based resins in CIPP, has made new preliminary recommendations in response to the study, co-investigator John C. Matthews said during the Webinar. The recommendations state that workers entering trucks or units that transport liners should wear protective gear depending on the results of air monitoring done after the trucks' doors are opened.

Rather than being stymied by variations at CIPP jobs, Shannahan and Whelton are now making a lab setting for controlled experiments—a chamber where they will create a cured-in-place pipe with two commonly used types of resin and then identify and measure how emissions vary. Later on they will look for markers of liver and lung inflammation and stress, as well as other blood chemistry, in male and female mice exposed to emissions in the chamber. One goal of the project—which is funded by the National Institutes of Health—is to inform any future measures that could ensure that cured-in-place pipes are made in a safe and efficient way that also protects public health.

The scientific evidence so far may not present a clear case that CIPP work poses a risk to the public. But the number of cases involving people who raised concerns or felt sick from emissions, combined with the Purdue team's early toxicology results, make the topic one that deserves more attention.

"We are at a very preliminary stage with a lot of the investigations of the health hazards associated with this. But then the question is, 'Well, how do you get more people doing the science related to this?'" Shannahan says. "Sometimes what it takes is people just being told about it."

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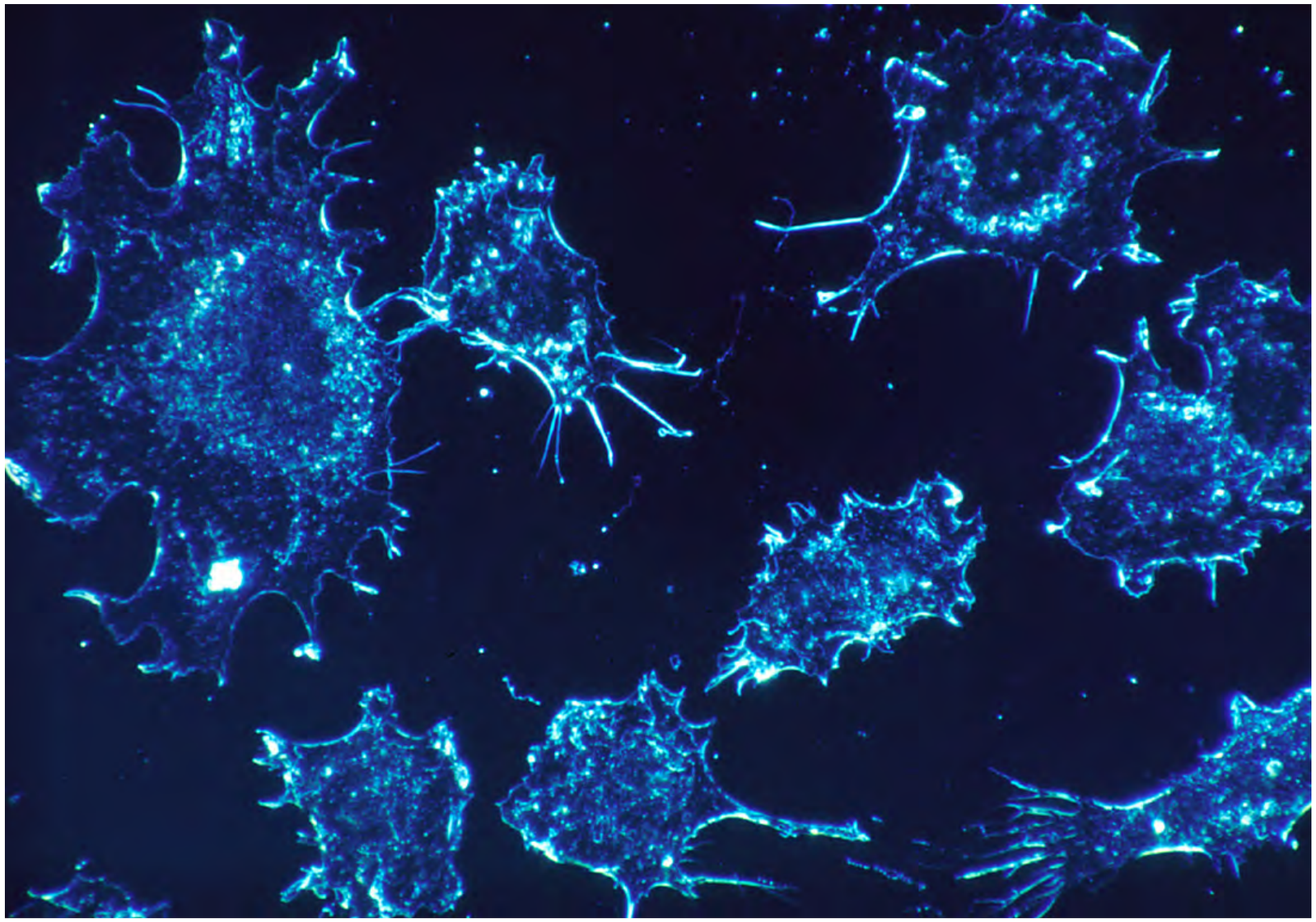
MEDICINE

Behind the Scenes of a Radical New Cancer Cure

I've now treated several patients with the new cancer gene therapy called CAR-T, but there's still a lot to learn



NATIONAL CANCER INSTITUTE WIKIMEDIA

An unexpected early-morning phone call from the hospital is never good news. When Joy Johnson answered, her first thought was that Sharon Birzer, her partner of 15 years, was dead. Her fears were amplified by the voice on the other end refusing to confirm or deny it. Just "come in and talk to one of the doctors," she remembers the voice saying.

Johnson knew this was a real possibility. A few weeks earlier she and Birzer sat in the exam room of a lymphoma specialist at Stanford University. Birzer's cancer had grown and fast—first during one type of chemotherapy, then through a second. Out of standard options, Birzer's local oncologist had referred her for a novel treatment called chimeric antigen receptor T cell therapy—or CAR-T. Birzer and Johnson knew that the treatment was risky. They were warned there was a chance of death. There was also a chance of serious complications such as multiorgan failure and neurological impairment. But it was like warning a drowning person that her lifeboat could have problems. Without treatment, the chance of Birzer's death was all but certain. She signed the consent form.

Johnson hung up the phone that early morning and sped to the hospital. She met with a doctor and two chaplains in a windowless room in the cancer ward, where happy photos of cancer "alumni" smiled down from the walls. This is getting

worse and worse, Johnson thought. As she remembers it, the doctor went through the time line of what happened for 10 minutes, explaining how Birzer became sicker and sicker, before Johnson interrupted with the thought splitting her world in two: "I need you to tell me whether she's alive or dead."

Birzer wasn't dead. But she was far from okay. The ordeal began with Birzer speaking gibberish. Then came seizures so severe there was concern she wouldn't be able to breathe on her own. When it took a few different medications to stop Birzer from seizing, her doctors sedated her, put a breathing tube down her throat and connected her to a ventilator. Now she was unconscious and in the intensive care unit (ICU).

Birzer was one of the early patients to receive CAR-T, a radical new therapy to treat cancer. It involved removing Birzer's own blood, filtering it for immune cells called T cells and genetically engineering those cells to recognize and attack her lymphoma. CAR-T made history in 2017 as the first FDA-approved gene therapy to treat any disease. After three to six months of follow-up, the trials that led to approval showed response rates of 80 percent and above in aggressive leukemias and lymphomas that had resisted chemotherapy. Patients on the brink of death were coming back to life.

This is something I often dream of seeing but rarely do. As a doctor who treats cancer, I think a lot about how to frame new treatments to my patients. I never want to give false hope. But the uncertainty inherent to my field also cautions me against closing the door on optimism prematurely. We take it as a point of pride that no field of medicine evolves as rapidly as cancer—the FDA approves dozens of new treatments a year. One of my biggest challenges is staying up to date on every development and teasing apart what should —and shouldn't—change my practice. I am often a mediator for my patients, tempering theoretical promises with everyday realism. To accept a research finding into medical practice, I prefer slow steps showing me proof of concept, safety and efficacy.

CAR-T, nearly three decades in the making, systematically cleared these hurdles. Not only did the product work, its approach was also unique among cancer treatments. Unlike our usual advances, this wasn't a matter of prescribing an old drug for a new disease or remixing known medications. CAR-T isn't even a drug. This is a one-time infusion giving a person a better version of her own immune system. When the FDA approved its use, it wasn't a question of whether my hospital would be involved but of how we could stay ahead. We weren't alone.

Today two FDA-approved CAR-T products called Kymriah and Yescarta are available in more than 100 hospitals collectively across the U.S. Hundreds of clinical trials are tinkering with dosages, patient populations and types of cancer. Some medical centers are manufacturing the cells on-site.

The FDA approved CAR-T with a drug safety program called a Risk Evaluation and Mitigation Strategy (REMS). As I cared for these patients, I quickly realized the FDA's concerns. Of the 10 or so patients I've treated, more than half developed strange neurologic side effects ranging from headaches to difficulty speaking to seizures to falling unconscious. We scrambled to learn how to manage the side effects in real time.

Johnson and Birzer, whom I didn't treat personally but spoke to at length for this article, understood this better than most. Both had worked in quality control for a blood bank and were medically savvier than the average patient. They accepted a medical system with a learning curve. They were fine with hearing "I don't know." Signing up for a trailblazing treatment meant going along for the ride. Twists and bumps were par for the course.

* * *

Cancer, by definition, means something has gone very wrong within—a cell has malfunctioned and multiplied. The philosophy for fighting cancer has been, for the most part, creating and bringing in treatments from outside the body. That's how we got to the most common modern approaches: chemotherapy (administering drugs to kill cancer), radiation (using high-energy beams to kill cancer) and surgery (cutting cancer out with a scalpel and other tools). Next came the genetics revolution, with a focus on creating drugs that target a precise genetic mutation separating a cancer cell from a normal one. But cancers are genetically complex, with legions of mutations and the talent to develop new ones. It's rare to have that one magic bullet.

Over the past decade or so, our approach has shifted. Instead of fighting cancer from the outside, we are increasingly turning in. The human

body is already marvelously equipped to recognize and attack invaders, from the common cold to food poisoning, even if the invaders are ones the body has never seen before. Cancer doesn't belong either. But since cancer cells come from normal ones, they've developed clever camouflage to trick and evade the immune system. The 2018 Nobel Prize in Physiology or Medicine was jointly awarded to two researchers for their work in immunotherapy, a class of medications devoted to wiping out the camouflage and restoring the immune system's upper hand. As I once watched a fellow oncologist describe it to a patient, "I'm not treating you. You are treating you."

What if we could go one step further? What if we could genetically engineer a patient's own immune cells to spot and fight cancer, as a sort of "best hits" of genetic therapy and immunotherapy?

Enter CAR-T. The technology uses T cells, which are like the bouncers of the immune system. T cells survey the body and make sure everything belongs. CAR-T involves removing a person's T cells from her blood and using a disarmed virus to deliver new genetic material to the cells. The new genes given to the T cells help them make two types of proteins. The first—giving the technology its name—is a CAR, which sits on the T cell's surface and binds to a protein on the tumor cell's surface, like a lock and key. The second serves as the T cell's caffeine jolt, rousing it to activate. Once the genetic engineering part is done, the T cells are prodded to multiply by being placed on a rocking device that feeds them nutrients while filtering their wastes. When the

As I once watched a fellow oncologist describe it to a patient, "I'm not treating you. You are treating you."

cells reach a high enough number—a typical "dose" ranges from hundreds of thousands to hundreds of millions—they are formidable enough to go back into the patient. Once inside, the cancer provokes the new cells to replicate even more. After one week a typical expansion means multiplying by about another 1,000-fold.

Practically, it looks like this: A person comes in for an appointment. She has a catheter placed in a vein, perhaps in her arm or her chest, that connects to a large, whirring machine which pulls in her blood and separates it into its components. The medical team sets the T cells aside to freeze while the rest of the blood circulates back into the patient in a closed loop. Then the hospital ships the cells frozen to the relevant pharmaceutical company's headquarters or transports them to a lab on-site, where thawing and manufacturing takes from a few days to a few weeks. When the cells are ready, the patient undergoes about three days of chemotherapy to kill both cancer and normal cells, making room for the millions of new cells and eradicating normal immune players that could jeopardize their existence. She then gets a day or two to rest. When the new cells are infused back into her blood, we call that Day 0.

* * *

I remember the first time I watched a patient get his Day 0 infusion. It felt anticlimactic. The entire process took about 15 minutes. The CAR-T cells are invisible to the naked eye, housed in a small plastic bag containing clear liquid.

"That's it?" my patient asked when the nurse said it was over. The infusion part is easy. The hard part is everything that comes next.

Once the cells are in, they can't turn off. That this may cause collateral damage was evident from the start. In 2009—working in parallel with other researchers at Memorial Sloan Kettering Cancer Center in New York and the National Cancer Institute in Maryland—oncologists at the University of Pennsylvania opened a clinical trial for CAR-T in human leukemia patients. (Carl June, who led the CAR-T development, did not respond to Undark's interview request.) Of the first three patients who got CAR-T infusions, two achieved complete remission—but nearly died in the process. The first was a retired corrections officer named Bill Ludwig, who developed extremely high fevers and went into multiorgan failure requiring time in the ICU. At the time, the medical teams had no idea why it was happening or how to stop it. But time passed. Ludwig got better. Then came the truly incredible part: his cancer was gone.

With only philanthropic support, the trial ran out of funding. Of the eligible patients they intended to treat, the Penn doctors only treated three. So they published the results of one patient in the *New England Journal of Medicine* and presented

the outcomes of all three patients, including Ludwig, at a cancer conference anyway. From there, the money poured in. Based on the results, the Swiss pharmaceutical company Novartis licensed the rights of the therapy.

The next year, six-year-old Emily Whitehead was on the brink of death when she became the first child to receive CAR-T. She also became extremely ill in the ICU, and her cancer was also eventually cured. Her media-savvy parents helped bring her story public, making her the poster child for CAR-T. In 2014 the FDA granted CAR-T a breakthrough therapy designation to expedite the development of extremely promising therapies. By 2017 a larger trial had given the treatment to 75 children and young adults with a type of leukemia—B cell acute lymphoblastic leukemia—that failed to respond to chemotherapy. Eighty-one percent had no sign of cancer after three months.

In August 2017 the FDA approved a CAR-T treatment as the first gene therapy in the U.S. The decision was unanimous. The Oncologic Drugs Advisory Committee, a branch of the FDA that reviews new cancer products, voted 10 to zero in favor of Kymriah. Committee members called the responses "remarkable" and "potentially paradigm changing." When the announcement broke, a crowd formed in the medical education center of Penn Medicine, made up of ecstatic faculty and staff. There were banners and T-shirts. "A remarkable thing happened" was the tagline, above a cartoon image of a heroic T cell. Two months later, in October 2017, the FDA approved a second CAR-T formulation called Yescarta from Kite Pharma, a subsidiary of Gilead Sciences, to treat an aggressive blood cancer in adults called diffuse large B-cell lymphoma, the trial of which had shown a 54 percent complete-response rate, meaning all signs of cancer had disappeared. In May 2018, Kymriah was approved to treat adults with non-Hodgkin lymphoma.

That year the American Society of Clinical Oncology named CAR-T the Advance of the Year, beating out immunotherapy, which had won two years in a row. When I attended the American Society of Hematology meeting in December 2018, CAR-T stole the show. Trying to get into CAR-T talks felt like trying to get a photo with a celebrity. Running five minutes late to one session meant facing closed doors. Others were standing room only. With every slide, it became difficult to see over a sea of smartphones snapping photos. At one session I found a seat next to the oncologist from my hospital who treated Birzer. "Look," she nudged me. "Do you see all these 'nonmember' badges?" I turned. Members were doctors like us who treated blood cancers. I couldn't imagine who else would want to be here. "Who are they?" I asked. "Investors," she said. It felt obvious the moment she said it.

For patients, the dreaded "c" word is cancer. For oncologists, it's "cure." When patients ask, I've noticed how we gently steer the conversation toward safer lingo. We talk about keeping the cancer in check. "Cure" is a dangerous word, used only when so much time has passed from a cancer's diagnosis that we can be reasonably certain it's gone. But that line is arbitrary. We celebrate therapies that add weeks or months because the diseases are pugnacious, the biology diverse, and the threat of relapse looming. Oncologists are a tempered group, or so I've learned, finding inspiration in slow, incremental change.

This was completely different. These were patients who would have otherwise died, and the trials were boasting that 54 to 81 percent were cancer-free upon initial follow-up. PET scans showed tumors that had speckled an entire body melt away. Bone marrow biopsies were clear, with even the most sensitive testing unable to detect disease.

The dreaded word was being tossed around—could this be the cure we've always wanted?

* * *

When a new drug gets FDA approval, it makes its way into clinical practice, swiftly and often with little fanfare. Under the drug safety program REMS, hospitals offering CAR-T were obligated to undergo special training to monitor and manage side effects. As hospitals worked to create CAR-T programs, oncologists like me made the all too familiar transition from first-time user to expert.

It was May 2018 when I rotated through my hospital's unit and cared for my first patients on CAR-T. As I covered 24-hour shifts, I quickly learned that whether I would sleep that night depended on how many CAR-T patients I was covering. With each treatment, it felt like we were pouring gasoline on the fire of patients' immune systems. Some developed high fevers and their blood pressure plummeted, mimicking a serious infection. But there was no infection to be found.

When resuscitation with fluids couldn't maintain my patients' blood pressure, I sent them to the ICU, where they required intensive support to supply blood to their critical organs.

We now have a name for this effect—cytokine release syndrome—that occurs in more than half of patients who receive CAR-T, starting with Ludwig and Whitehead. The syndrome is the collateral damage of an immune system on the highest possible alert. This was first seen with other types of immunotherapy, but CAR-T took its severity to a new level. Usually starting the week after CAR-T, cytokine release syndrome can range from simple fevers to multiorgan failure affecting the liver, kidneys, heart, and more. The activated T cells make and recruit other immune players called cytokines to join in the fight. Cytokines then recruit more immune cells. Unlike in the early trials at Penn, we now have two medicines to dampen the effect. Steroids calm the immune system in general, while a medication called tocilizumab, used to treat autoimmune disorders such as rheumatoid arthritis, blocks cytokines specifically.

Fortuity was behind the idea of tocilizumab: When Emily Whitehead, the first child to receive CAR-T, developed cytokine release syndrome, her medical team noted that her blood contained high levels of a cytokine called interleukin 6. Carl June thought of his own daughter, who had juvenile rheumatoid arthritis and was on a new FDA-approved medication that suppressed the same cytokine. The team tried the drug, tocilizumab, in Whitehead. It worked.

Still, we were cautious in our early treatments. The symptoms of cytokine release syndrome mimic the symptoms of severe infection. If this were infection, medicines that dampen a patient's immune system would be the opposite of what you'd want to give. There was another concern: Would these medications dampen the anticancer activity too? We didn't know. Whenever a CAR-T patient spiked a fever, I struggled with the question—is it cytokine release syndrome, or is it infection? I often played it safe and covered all bases, starting antibiotics and steroids at the same time. It was counterintuitive, like pressing both heat and ice on a strain or treating a patient simultaneously with fluids and diuretics.

The second side effect was even scarier: Patients stopped talking. Some, like Sharon Birzer, spoke gibberish or had violent seizures. Some couldn't interact at all, unable to follow simple commands like "squeeze my fingers." How? Why? At hospitals across the nation, perfectly cognitively intact people who had signed up to treat their cancer were unable to ask what was happening.

Our nurses learned to ask a standardized list of questions to catch the effect, which we called neurotoxicity: Where are we? Who is the president? What is 100 minus 10? When the patients scored too low on these quizzes, the nurses called me to the bedside.

In turn, I relied heavily on a laminated booklet, made by other doctors who were using CAR-T, which we tacked to a bulletin board in our doctors' workroom. It contained a short chart noting how to score severity and what to do next. I flipped through the brightly color-coded pages telling me when to order a head CT scan to look for brain swelling and when to place scalp electrodes looking for seizures. Meanwhile, we formed new channels of communication. As I routinely called a handful of CAR-T specialists at my hospital in the middle of the night, national consortiums formed where specialists around the country shared their experiences. As we tweaked the instructions, we scribbled updates to the booklet in pen.

I wanted to know whether my experience was representative. I came across an abstract and conference talk that explored what happened to 277 patients who received CAR-T in the real world, so I e-mailed the lead author, Loretta Nastoupil, director of the department of lymphoma and myeloma at the University of Texas MD Anderson Cancer Center in Houston. Fortuitously, she was planning a trip to my university to give a talk that month. We met at a café, and I asked what her research had found. Compared with the earlier trials, the patients were much sicker, she said. Of the 277 patients, more than 40 percent wouldn't have been eligible for the very trials that got CAR-T approved. Was her team calling other centers for advice? "They were calling us," she said.

Patients included in clinical trials are carefully selected. They tend not to have other major medical problems, as we want them to survive whatever rigorous new therapy we put them through. Nastoupil admits some of it is arbitrary. Many criteria in the CAR-T trials were based on criteria that had been used in chemotherapy trials. "These become standard languages that apply to

all studies," she said, listing benchmarks like a patient's age, kidney function and platelet count. "But we have no idea whether criteria for chemotherapy would apply to cellular therapy."

Now, with blanket FDA approval comes clinical judgment. Patients want a chance. Oncologists want to give their patients a chance. Young, old, prior cancer, heart disease or liver disease—without strict trial criteria, anyone is fair game.

When I was making rounds at my hospital, I never wandered too far from these patients' rooms, medically prepared for them to crash at any moment. At the same time, early side effects made me optimistic. A bizarre truism in cancer is that side effects may bode well. They could mean the treatment is working. Cancer is usually a waiting game, requiring months to learn an answer. Patients and doctors alike seek clues, but the only real way to know is waiting: Will the next PET scan show anything? What are the biopsy results?

CAR-T was fundamentally different from other cancer treatments in that it worked fast. Birzer's first clue came just a few hours after her infusion. She developed pain in her lower back. She described it as feeling like she had menstrual cramps. A heavy burden of lymphoma lay in her uterus. Could the pain mean that the CAR-T cells had migrated to the right spot and started to work? Her medical team didn't know, but the lead doctor's instinct was that it was a good sign.

Two days later, her temperature shot up to 102. Her blood pressure dropped. The medical team diagnosed cytokine release syndrome, as though right on schedule, and gave her tocilizumab.

Every day, the nurses would ask her questions and have her write simple sentences on a slip of paper to monitor for neurotoxicity. By the fifth day, her answers changed. "She started saying things that were crazy," Johnson explained.

One of Birzer's sentences was "guinea pigs eat greens like hay and pizza." Birzer and Johnson owned two guinea pigs, so their diet would be something Birzer normally knew well. So Johnson tried to reason with her: "They don't eat pizza." And Birzer replied, "They do eat pizza, but only gluten-free."

Johnson remembers being struck by the certainty in her partner's delirium. Not only was Birzer confused, she was confident she was not. "She was doubling down on everything," Johnson described. "She was absolutely sure she was right."

Johnson vividly remembers the evening before the frightening early-morning phone call that brought her rushing back to the hospital. Birzer had said there was no point in Johnson staying overnight; she would only watch her be in pain. So Johnson went home. After she did, the doctor came by multiple times to evaluate Birzer. She was deteriorating—and fast. Her speech became more and more garbled. Soon she couldn't name simple objects and didn't know where she was. At 3 A.M., the doctor ordered a head CT to make sure Birzer wasn't bleeding into her brain.

Fortunately, she wasn't. But by 7 A.M. Birzer had stopped speaking altogether. Then she seized. Birzer's nurse was about to step out of the room when she noticed Birzer's arms and legs shaking. Her eyes stared vacantly and she wet the bed. The nurse called a code blue, and a team of more doctors and nurses ran over. Birzer was loaded with high-dose antiseizure medications through her IV. But she continued to seize. As nurses infused more medications into her IV, a doctor placed a breathing tube down her throat.

Birzer's saga poses the big question: Why does CAR-T cause seizures and other neurologic problems? No one seemed to know. My search of the published scientific literature was thin, but one woman's name kept cropping up. So I called her. Juliane Gust, a pediatric neurologist and scientist at Seattle Children's Hospital, told me her investigations of how CAR-T affects the brain were motivated by her own experiences. When the early CAR-T trials opened at her hospital in 2014, she and her colleagues began getting calls from oncologists about brain toxicities they knew nothing about. "Where are the papers?" she remembered thinking. "There was nothing."

Typically, the brain is protected by a collection of cells aptly named the blood-brain barrier. But with severe CAR-T neurotoxicity, research suggests, this defense breaks down. Gust explained that spinal taps on these patients show high levels of cytokines floating in the fluid surrounding the spine and brain. Some CAR-T cells circulate in the fluid too, she said, but these numbers do not correlate with sicker patients. CAR-T cells are even seen in the spinal fluid of patients without any symptoms.

What does this mean? Gust interprets it as a patient's symptoms having more to do with cytokines than the CAR-T cells. "Cytokine release syndrome is the number one risk factor" for

developing neurotoxicity over the next few days, she said. The mainstay for neurotoxicity is starting steroids as soon as possible. “In the beginning we didn’t manage as aggressively. We were worried about impairing the function of the CAR-T,” she added. “Now we give steroids right away.”

But the steroids don’t always work. Several doses of steroids didn’t prevent Birzer from seizing. The morning after Johnson’s alarming phone call, after the meeting at the hospital when she learned what had happened, a chaplain walked her from the conference room to the ICU. The first day, Johnson sat by her partner’s bedside while Birzer remained unconscious. By the next evening, she had woken up enough to breathe on her own. The doctors removed her breathing tube, and Birzer looked around. She had no idea who she was or where she was.

Birzer was like a newborn baby, confused and sometimes frightened by her surroundings. She frequently looked like she was about to say something, but she couldn’t find the words, despite the nurses' and Johnson’s encouragement. One day she spoke a few words. Eventually she learned her name. A few days later she recognized Johnson. Her life was coming back to her, though she was still suspicious of her reality. She accused the nurses of tricking her, for instance, when they told her Donald Trump was president.

She took cues from the adults around her on whether her actions were appropriate. The best example of this was her “I love you” phase. One day, she said it to Johnson in the hospital. A few nurses overheard it and commented on how sweet it was. Birzer was pleased with the reaction. So she turned to the nurse: “I love you!” And the person emptying the trash: “I love you!” Months later, she was having lunch with a friend who asked, “Do you remember when you told me you loved me?” Birzer said, “Well, I stand by that one.”

She accused the nurses of tricking her, for instance, when they told her Donald Trump was president.

When she got home, she needed a walker to help with her shakiness on her feet. When recounting her everyday interactions, she would swap in the wrong people, substituting a friend for someone else. She saw bugs that didn’t exist. She couldn’t hold a spoon or a cup steady. Johnson would try to slow her down, but Birzer was adamant she could eat and drink without help. “Then peas would fly in my face,” Johnson said.

Patients who experience neurotoxicity fall into one of three categories. The majority are impaired but then return to normal without long-term damage. A devastating handful, less than one percent, develop severe brain swelling and die. The rest fall into a minority that have lingering problems even months out. These are usually struggles to think up the right word, trouble concentrating, and weakness, often requiring long courses of rehabilitation and extra help at home.

As Birzer told me about her months of rehab, I thought of how she seemed to fall somewhere in the middle among the patients I’ve treated. On one end of the spectrum was the rancher who remained profoundly weak a year after his infusion. Before CAR-T, he walked across his ranch without issue; six months later, he needed a walker. Even with it, he fell on a near-weekly basis. On the other end was the retired teacher who couldn’t speak for a week—she would look around her ICU room and move her mouth as though trying her hardest—and then woke up as though nothing happened. She left the hospital and instantly resumed her life, which included a recent trip across the country. In hindsight, I remember how we worried more about giving the therapy to the teacher than to the rancher, as she seemed frailer. Outcomes like theirs leave me with a familiar humility I keep learning in new ways as a doctor: We often can’t predict how a patient will do. Our instincts can be just plain wrong.

I asked Gust if we have data to predict who will land in which group. While we can point to some risk factors—higher burdens of cancer, baseline cognitive problems before therapy—“the individual patient tells you nothing,” she confirmed.

So we wait.

* * *

Doctors like me who specialize in cancer regularly field heart-wrenching questions from patients. They have read about CAR-T in the news, and now they want to know: What about me? What about my cancer?

So, who gets CAR-T? That leads to the tougher question—who doesn’t? That depends on the type

of cancer and whether their insurance can pay.

CAR-T is approved to treat certain leukemias and lymphomas that come from the blood and bone marrow. Since the initial approval, researchers have also set up new CAR-T trials for all sorts of solid tumors from lung cancer to kidney cancer to sarcoma. But progress has been slow. While some promising findings are coming from the lab and in small numbers of patients on early-phase trials, nothing is yet approved in humans. The remarkable responses occurring in blood cancers just weren't happening in solid tumors.

Cancer is one word, but it's not one disease. "It's easier to prove why something works when it works than show why it doesn't work when it doesn't work," said Saar Gill, a hematologist and scientist at the University of Pennsylvania who co-founded a company called Carisma Therapeutics using CAR-T technology against solid tumors. That was his short answer, at least. The longer answer to why CAR-T hasn't worked in solid cancers involves what Gill believes are two main barriers. First, it's a trafficking problem. Leukemia cells tend to be easier targets; they bob through the bloodstream like buoys in an ocean. Solid tumors are more like trash islands. The cancer cells stick together and grow an assortment of supporting structures to hold the mound together. The first problem for CAR-T is that the T cells may not be able to penetrate the islands. Then, even if the T cells make it in, they're faced with a hostile environment and will likely die before they can work.

At Carisma, Gill and his colleagues look to get around these obstacles with a different immune cell called the macrophage. T cells are not the only players of the immune system, after all. Macrophages are gluttonous cells that recognize invaders and engulf them for destruction. But studies have shown they cluster in solid tumors in a way T cells don't. Gill hopes genetically engineered macrophages can be the stowaways that sneak into solid tumor and attack from the inside out.

Another big challenge, even for leukemias and lymphomas, is resistance, where the cancers learn to survive the CAR-T infusion. While many patients in the trials achieved remission after a month, we now have two years' worth of data, and the outlook isn't as rosy. For lymphoma, that number is closer to 40 percent. Patients celebrating cures initially are relapsing later. Why?

The CAR-T cells we use target a specific protein on cancer cells. But if the cancer no longer expresses that protein, that can be a big problem, and we're finding that's exactly what's happening. Through blood testing, we see that many patients who relapse lose the target.

Researchers are trying to regain the upper hand by designing CAR-Ts to target more than one receptor. It's an old idea in a new frame: an arms race between our medicines and the illnesses that can evolve to evade them. Too much medical precision in these cases is actually not what we want, as it makes it easier for cancer to pinpoint what's after it and develop an escape route. So, the reasoning goes, target multiple pieces at once. Confuse the cancer.

Then there's the other dreaded "c" word: Cost. Novartis's Kymriah runs up to $475,000, while Kite Pharma's Yescarta is $373,000. That price covers manufacturing and infusion. Not included is the minimum one-week hospital stay or any complications.

They are daunting numbers. Some limitations on health care we accept—maybe the patients are too sick; maybe they have the wrong disease. The wrong cost is not one we as a society look kindly upon. And drug companies shy away from that kind of attention.

Cost origins in medicine are notoriously murky. Novartis, confident in its technology, made an offer to offset the scrutiny in CAR-T. If the treatment didn't work after one month, the company said, it wouldn't send a bill.

Not everyone agrees that cost is an issue. Gill, for example, believes the concern is overhyped. It's not "a major issue," he told me over the phone. "Look, of course—[with] health care in this country, if you don't have insurance, then you're screwed. That is no different when it comes to CAR-T as it is for anything else," he said. The cost conversation must also put CAR-T in context. Gill went on to list what these patients would be doing otherwise—months of chemotherapy, bone marrow transplants, hospital stays for cancer-associated complications and the associated loss of income as patients and caregivers miss work. These could add up to far more than a one-time CAR-T infusion. A bone marrow transplant, for example, can cost from $100,000 to more than $300,000. The cancer-fighting drug blinatumomab, also used to treat relapsed leukemia, costs $178,000 a year. "Any discussion

of cost is completely irresponsible without weighing the other side of the equation," Gill said.

How the system will get on board is another question. Logistics will be an issue, Gill conceded. The first national Medicare policy for covering CAR-T was announced in August 2019, two years after the first product was approved. The Centers for Medicare and Medicaid Services has offered to reimburse a set rate for CAR-T-cell infusion, and while this figure was recently raised, it remains less than the total cost. Despite the expansion of medical uses, at some centers referrals for CAR-T are dropping as hospitals worry it's a net loss. And while most commercial insurers are covering CAR-T therapies, companies less accustomed to handling complex therapies can postpone approval. Ironically, the patients considering CAR-T are the ones for whom the window for treatment is narrowest. A delay of even a few weeks can mean the difference between a cure and hospice.

This, of course, poses a big problem. A breakthrough technology is only as good as its access. A major selling point of CAR-T—besides the efficacy—is its ease. It's a one-and-done treatment. Engineered T cells are intended to live indefinitely, constantly on the alert if cancer tries to come back. Compare that to chemotherapy or immunotherapy, which is months of infusions or a pill taken indefinitely. CAR-T is more akin to surgery: cut it out, pay the entire cost up front, and you're done.

Birzer was lucky in this respect. I asked her and Johnson if cost had factored into their decision to try CAR-T. They looked at each other. "It wasn't an issue," said Johnson. They remembered getting a statement in the mail for a large sum when they got home. But Birzer had good insurance. She didn't pay a cent.

* * *

One year after Birzer's infusion, I met her and Johnson at a coffee shop near their home in San Francisco. They had saved a table. Johnson had a newspaper open. Birzer already had her coffee, and I noticed her hand trembling as she brought it to her mouth. She described how she still struggles to find exactly the right words. She sometimes flings peas. But she's mostly back to normal, living her everyday life. She has even returned to her passion, performing stand-up comedy, though she admitted that at least for general audiences, "My jokes about cancer didn't kill."

People handed a devastating diagnosis don't spend most of their time dying. They are living, but with a heightened awareness of a time line the rest of us take for granted. They sip coffee, enjoy their hobbies and read the news while also getting their affairs in order and staying on the lookout, constantly, for the next treatment that could save them.

Hoping for a miracle and preparing to die are mutually compatible ideas. Many of my patients have become accustomed to living somewhere in that limbo. It is humbling to witness. They hold out hope for a plan A, however unlikely it may be, while also adjusting to the reality of a plan B. They live their lives, and they live in uncertainty.

I see patients in various stages of this limbo. In clinic, I met a man with multiple myeloma six months after a CAR-T trial had supposedly cured him. He came in with a big smile but then quietly began praying when it was time to view PET results. He asked how the other patients on the trial were doing, and I shared the stats. While percentages don't say anything about an individual experience, they're also all patients have to go on. When someone on the same treatment dies, it's shattering for everyone. Was one person the exception, or a harbinger of another's fate? Who is the outlier?

I look at these patients and think a sober truth: Before CAR-T, all would have been likely to die within six months. Now, imagine taking 40 percent and curing them. Sure, a naysayer might point out, it's only 40 percent. What's the hype if most still succumb to their cancer? But there was nothing close to that before CAR-T. I agree with how Gill described it: "I think CAR-T cells are like chemotherapy in the 1950s. They're not better than chemotherapy—they're just different." For an adversary as tough as cancer, we'll take any tool we can get.

There remain many questions. Can we use CAR-T earlier in a cancer's course? Lessen the side effects? Overcome resistance? Streamline manufacturing and reimbursement? Will it work in other cancers? Patients will sign up to answer.

For now, Birzer seems to be in the lucky 40 percent. Her one-year PET scan showed no cancer. I thought of our last coffee meeting, where I had asked if she ever worried she wouldn't return to normal. She didn't even pause. "If you're not dead," she said, "you're winning."

This article was originally published on Undark. Read the original article.

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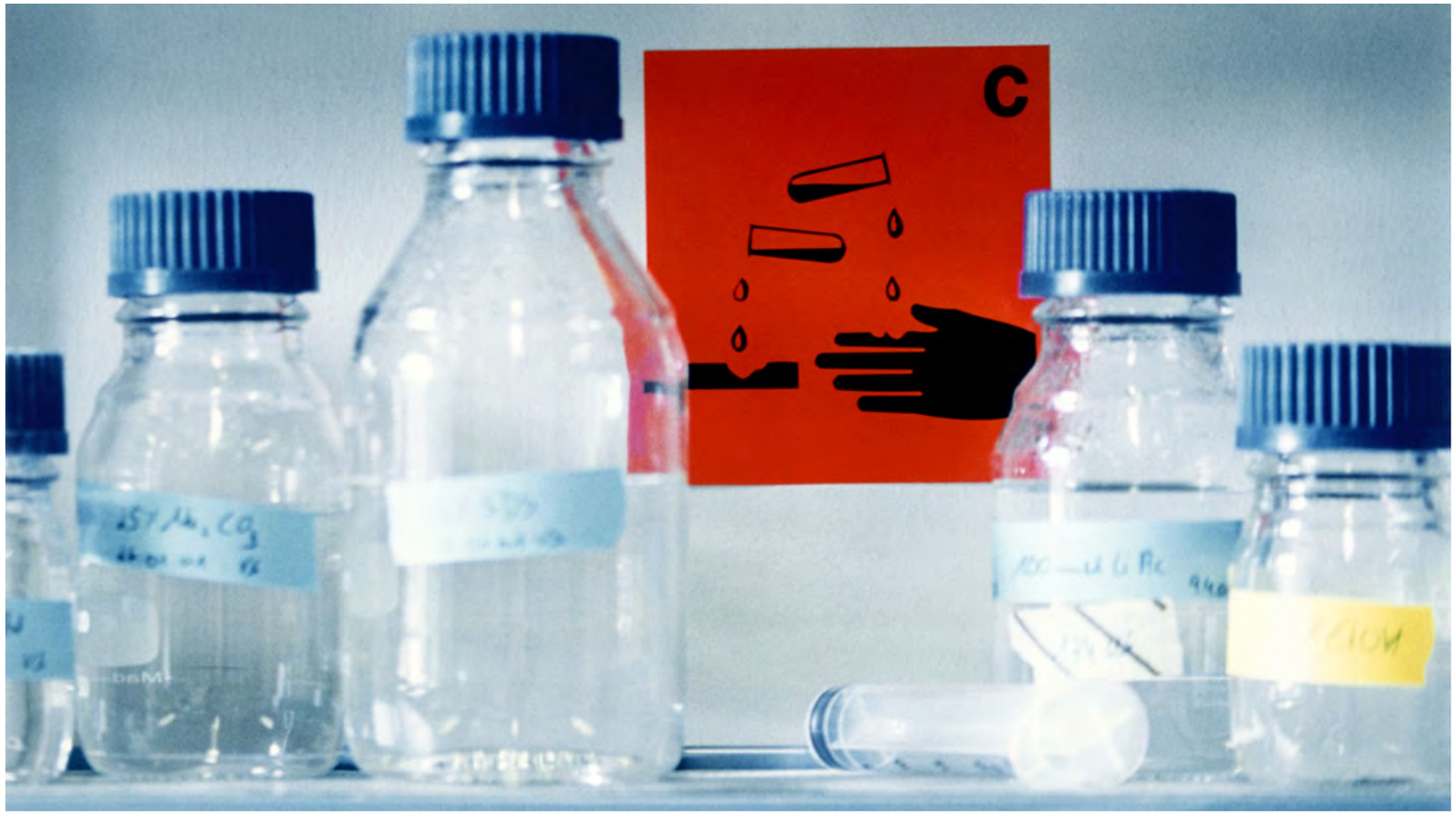
VOICES

Could Lab Work Be Affecting My Fertility?

I never considered that possibility before—but I am now



JOSH WESTRICH *GETTY IMAGES*

During a recent afternoon coffee break in our research group at MIT, one of my colleagues mentioned he was taking a break from working in the clean room for the next few months. He explained that he and his wife were trying to have a baby, and that he figured it was probably best to avoid touching chemicals for a while. In typical form, we all joked about discovering the side effects of our everyday lab chemicals later down the road, 20 years from now, when we might grow a third arm or get brain damage (a more realistic scenario). But everyone admitted to being clueless about the reproductive health effects of the solvents, resists and other chemicals that we handle on a daily basis as part of our research.

One particular chemical that came up was *N*-methyl-2-pyrrolidone, or NMP, a solvent that most of us use several days a week for standard nanofabrication tasks such as stripping resist and, frankly, treat as only slightly more dangerous than water. "I heard NMP makes women infertile," one co-worker mentioned. "Don't worry," another said to me, "the studies only showed infertility for male mice, I think, so you're good."

Later on, back at my computer, I scrolled through research papers claiming an alarming set of risks for NMP, including reports of fetotoxicity and reduced fertility in rats. One study attributed a lab worker's stillbirth to high levels of exposure to the chemical as part of her job. Hoping to find some advice on reproductive risks of other chemicals, I turned to the National Institute for Occupational Safety and Health's (NIOSH) Web site. Their page on female reproductive risks offered little comfort, acknowledging that "scientists are just now beginning to understand how reproductive hazards affect the female reproductive system" and that "most workplace chemicals have not been studied for reproductive effects."

Taking in this glaring admittance of ignorance, I wondered what other dangers we aren't aware of. Are the fertility risks different for women like me compared to our male colleagues? And will the chemicals I handle now as part of my graduate work rear their ugly heads in the future, affecting my ability to have children?

"A big misconception in my mind is that people rely on and think that their organization has policies in place that are protective," says Stephanie Chalupka, a professor of nursing at Worcester State University and a visiting scientist at Georgetown University. Chalupka studies risks of environmental and occupational chemical exposures, so I reached out to her to learn how reproductive hazards could be mitigated.

She explains that over 1,000 workplace chemicals have demonstrated reproductive effects on animals, but most have not been studied in humans. Organic solvents, for example, have been associated with menstrual disorders, fetal loss and birth defects in women, as well as reduced semen quality in men. The type, dose and duration of exposure can lead to drastically different results for both sexes. In men, some chemicals can alter the production, shape or genetic composition of sperm.

For pregnant women, exposures during the first trimester can lead to miscarriage or birth defects, while exposures later on during the pregnancy may be associated with neurodevelopmental issues and premature births. Some substances such as lead reveal their presence in the aftermath, building up in the mother's tissue for years until being released later during pregnancy or breastfeeding.

The workplace chemicals with documented reproductive effects account for only a tiny fraction of the 72 million unique chemicals registered by the American Chemical Society, the majority of which have not been tested for reproductive safety. With roughly 15,000 new substances added to the registry every day, the possibility of exposure to chemicals with unknown reproductive risks is constantly increasing.

Although it might seem obvious that a chemical should be tested for reproductive toxicity before being released to the public, most substances are not, and the reasons are, perhaps unsurprisingly, political.

Approximately 40,000 industrial and commercial chemicals, equating to a total production volume of 30,000 pounds per person per year in the U.S., are regulated by the Toxic Substances Control Act (TSCA), a piece of legislation dating back to 1976 that remained untouched for 40 years. TSCA has a legacy of being ineffective, giving breaks to chemical companies, such as requiring the EPA to account for the financial effects of forcing industry compliance with its regulations. This inadequacy famously led to the EPA's failed attempt to ban asbestos in 1989 and earned TSCA the reputation of allowing people to be "legally poisoned."

Although the policy was finally changed when the legislation was amended in 2016, I was shocked to learn that U.S. chemical safety regulations still lag far behind. When I spoke with Veena Singla, associate director of science and policy at the University of California, San Francisco's Program on Reproductive Health and the Environment, she explained that the European Union has a "no data, no market" policy whereby any new chemicals coming into the market must be accompanied by some minimum amount of toxicity data before they're allowed. As a result of heavy lobbying by chemical companies during the 2016 TSCA amendment, no equivalent minimum data policy exists in the U.S.

"In our political system, the way it works is those who have more money and more resources have more influence and more power," Singla tells me. "The chemical industry did not want a minimum-data-set requirement in the law, so we don't have one."

She also explains that although the amendment gave the EPA new authority to request toxicity data from companies to fill in chemical-safety gaps, it has yet to order a single test. "In our current scheme, having data is not rewarded. All these chemicals are presumed innocent until they're proven guilty, so there is zero incentive to fill in more information," Singla says.

The EPA has also struggled to act on proposed reforms under the new administration. For instance, Singla informs me that NMP, the chemical that first motivated my investigation, was supposed to be banned in certain consumer uses such as paint strippers because of its known toxicity. With the change of administration, however, the ban was never finalized.

As a woman in science, I am regularly inundated with questions and concerns about my fertility,

usually regarding how to plan having a family around an academic career, and reminders that my timeline is not as flexible as those of my male colleagues. But until I started investigating the risks of a chemical I've used heavily for over four years, I never considered that the substances I need to conduct my research could be taking that timeline away from me.

As scientists, we deserve to have the same level of rigor put into testing these chemicals as we put into applying them toward the research that advances our world. As people, we deserve to have legislation in place that protects us from harmful chemicals in the commercial products we use every day. Until those standards are met, we collectively face unknown fertility risks. In the meantime, I'll be returning to the lab, putting on my clean-room gloves and hoping for the best.

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