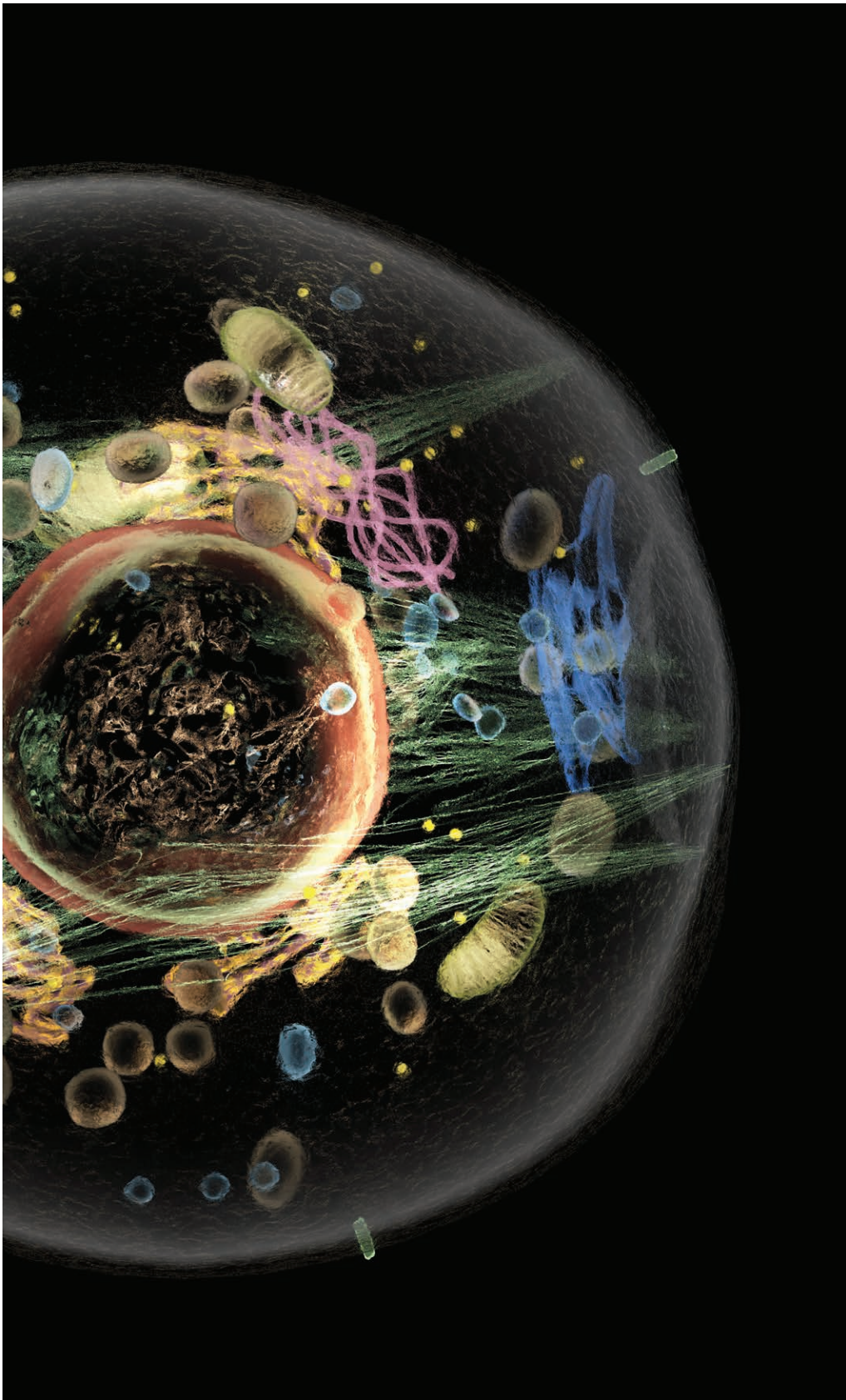
S T A N F O R D

M E D I C I N E

Issue 2 / 2024



special report

THE MAJESTIC CELL

How the smallest units of life determine our health

The company they keep

Neighboring cells influence whether tumors grow or perish

Cell engineering

Unlocking the possibilities for better health

Exploring the beautiful

Pursing mysteries and paradoxes of cells

A few of my favorite things

Researchers reveal the cells they most admire

The road to severe COVID

It's not what we thought

plus

Get rid of it

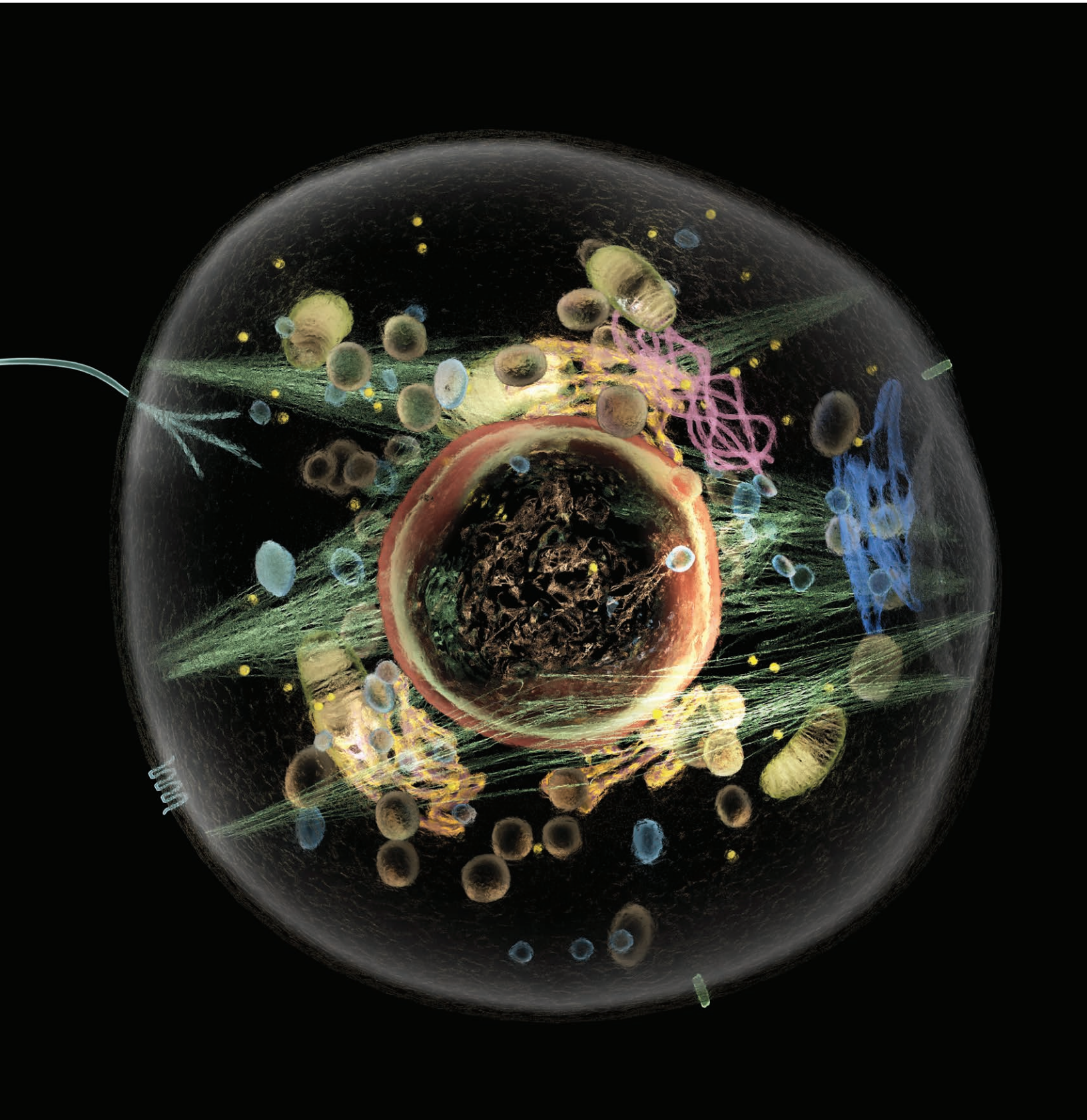
Knocking out this gene variant could be the key to an Alzheimer's treatment

Synthetic data

Illusion or medicine's latest AI bonanza?

STANFORD MEDICINE

Issue 2 / 2024



HIDDEN IN PLAIN SIGHT

HARM FROM ANEMIA DURING PREGNANCY IS PREVALENT, DISPROPORTIONATELY AFFECTING BLACK WOMEN

When I tell other physicians my area of research is anemia in pregnancy, I am met with a puzzled look. "But it's common," "It's treatable" and "Why is more research needed?" are comments I frequently receive.

These responses underscore the importance of the research. Despite being easily treated, anemia — the condition of having too few fully functional red blood cells — is a global problem for pregnant women. Typically caused by iron deficiency, about 40% of pregnant women worldwide experience anemia and are at increased risk of life-threatening repercussions.

Anemia rates are also high for pregnant people in the U.S., and they're highest for those who are Black, 21% of whom experience anemia — twice the rate of those who are white. Among those who are Black, the condition contributes to nearly a quarter of the severe medical conditions that arise during pregnancy, including sepsis, kidney failure, the need for blood transfusions and cardiac complications such as heart failure.

Irogue Igbinosa is leading efforts to develop interventions to reduce the high incidence of anemia among Black pregnant people.

Some common causes of iron deficiency are a lack of iron-rich foods and heavy menstrual bleeding, and it can usually be treated by diet and supplementing with iron. Yet our research shows that although anemia in pregnancy has increased in the past decades, only 50% of clinical providers routinely screen for the condition at the start of prenatal care. Too many women are unaware that they are anemic, which means they and their newborns bear the consequences.

So I ask: How can the tide be turned?

The solution starts with revamping the current — and flawed — norms in how we screen and treat anemia in pregnancy. To help bring this change about, I am studying the incidence of anemia and learning from those most affected in the U.S. — Black pregnant women. I'm a co-investigator for a National Institutes of Health-funded collaboration between Stanford Medicine's Obstetrics and Gynecology Department and the BLACK Wellness and Prosperity Center in California's Central Valley, conducting focus groups to understand the experiences Black pregnant patients had with care. Separately, I lead a team involved in providing nutrition education with the Santa Clara County Black Infant Health Program and Roots Community Health's San Jose clinic.

Participants in our studies, members of historically marginalized groups, universally expressed how symptoms were dismissed, information and education on anemia were minimal, nutritional advice was nonexistent, and treatment options were limited. Imagine fearing the high rates of stillbirths, hemorrhaging and other pregnancy complications you face as a Black pregnant woman and believing there is no way around it. In truth, discrimination, inequality and racism, not biology, are at the root.

Beyond understanding the barriers, we are creating solutions, finding ways to boost iron levels for those in need. With a grant from Stanford Medicine's Maternal and Child Health Research Institute, we are working with nutritionist Jocelyn Dubin at the Santa Clara County Public Health Department to increase access to information on nutrition, iron deficiency and anemia prevention in pregnancy through community health workers and doulas.

And I'm excited to be part of a Stanford Medicine-led effort funded by the NIH to reevaluate and revise how clinicians approach iron deficiency in pregnancy and resulting anemia from the first prenatal visit to the postpartum period. Ultimately, our goal is to improve birthing outcomes for all pregnant people.

So, yes, anemia is common and it is treatable. And we're finally paying attention. The hope is that other physicians will too. — IROGUE IGBINOSA, MD, *is a maternal-fetal medicine physician and an instructor of obstetrics and gynecology at Stanford Medicine.*

TIMOTHY ARCHIBALD

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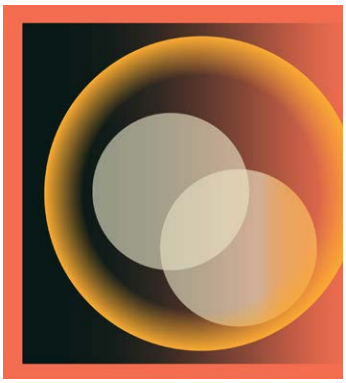
STANFORD MEDICINE

SPECIAL REPORT

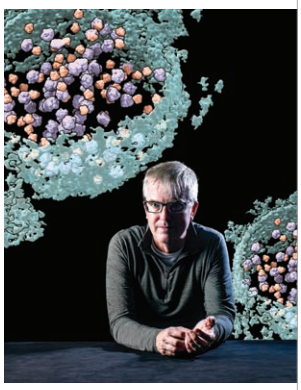
The majestic cell

How the smallest units of life determine our health



Scientists celebrate cells: 'Beautiful,' 'Unique,' 'Most insane thing I've ever seen.' page 28

Building a cell from scratch — and other cell-engineering quests page 10



The promise of synthetic data is great, but so are the risks. page 44

COVER: VIOLET FRANCES

letter from the dean

The definition of a cell — the smallest and most basic unit of life — belies its awesome power and astounding diversity. The trillions of cells in each human body represent more than 200 cell types that vary significantly in size and shape and enable a fascinating array of functions.

They are a veritable universe to explore, and this issue of *Stanford Medicine* magazine takes you on that journey of discovery.

Our guides for this journey are basic science researchers — explorers for whom the descriptor "basic" underplays the vital role that basic scientists play in filling the storehouse of human knowledge and advancing biomedicine.

Driving these researchers is one of the most powerful human traits: curiosity. For these individuals, wonder remains strong despite countless dead ends because the payoff — discovery and new knowledge — is the most precious of assets. New knowledge can be shared, and its value never diminishes. Indeed, discovery invariably leads to more discovery.

I am proud that Stanford Medicine supports and encourages researchers whose passions take them on open-ended explorations. From its founding as an academic medical center, Stanford Medicine has recognized the foundational importance of basic science research in its own right — and in the crucial role of advancing translational, clinical research and patient care.

Nearly every day, we see new evidence of the fruits of this commitment. Thanks to the tremendous scientific breakthroughs and technological advances of the past two decades, we are enjoying an unprecedented period of biomedical discovery. How unprecedented? Across multiple fields of study, our understanding of what is even possible is changing.

If you're like me, you will find your curiosity rekindled and your sense of wonder take flight as you read this issue. From synthetic biology's potential to fresh, exciting avenues of inquiry in oncology and immunology, you will dive deep into the universe of cells that are the human body. Enjoy the journey!

Sincerely,

Lloyd Minor, MD
Carl and Elizabeth Naumann Dean of Stanford School of Medicine
Vice President for Medical Affairs at Stanford University
Professor of Otolaryngology-Head & Neck Surgery

PHOTOGRAPH BY CAROLYN FONG

upfront

Ask AI

WHEN AN artificial intelligence algorithm takes a first pass at answering questions patients submit to health care providers, the clinicians report reduced work burden and fewer feelings of burnout, according to a study by Stanford Medicine researchers.

The results were published in March in *JAMA Network Open*. The study is an early demonstration of how integrating generative AI into health care workflows with a human in the loop can assist providers, said Michael Pfeffer, MD, chief information officer for Stanford Health Care and the School of Medicine. Clinicians could edit the responses before sharing them with patients.

"While multiple published studies show potential promise for generative AI in health care, this is among the first clinical uses to be rigorously evaluated — which is critical to assess real-world safety and usefulness," said Christopher Sharp, MD, the study's senior author and chief medical information officer at Stanford Medicine.

Get the worm

NIGHT OWLS have higher rates of psychological disorders than morning larks, according to a study by Stanford Medicine researchers, who found that people with a natural inclination to stay awake until the wee hours are at greater risk of poor mental health.

In a survey of nearly 75,000 adults, researchers compared the participants' preferred sleep timing, known as chronotype, with their sleep behavior. They found that, regardless of one's preferred bedtime, everyone benefits from turning in early. Morning larks and night owls alike tended to have higher rates of mental and behavioral disorders if they stayed up late.

The findings weren't expected. "There is a bunch of data out there indicating that living aligned to your chronotype is very important," said senior author Jamie Zeitzer, PhD, professor of psychiatry and behavioral sciences. "That was our expectation."

The study, published in May in *Psychiatry Research*, recommends lights out by 1 a.m.

Among the 73,888 participants, 19,065 self-identified as morning types, 6,844 as evening types and 47,979 as somewhere in the middle.

Morning types and evening types who went to sleep late had higher rates of mental health disorders, including depression and anxiety.

Night owls being true to their chronotype were 20% to 40% more likely to be diagnosed with a mental health disorder, compared with night owls on early or intermediate sleep schedules.

'THERE IS A BUNCH OF DATA OUT THERE INDICATING THAT LIVING ALIGNED TO YOUR CHRONOTYPE IS VERY IMPORTANT. THAT WAS OUR EXPECTATION.'

ILLUSTRATIONS BY PAUL WEARING

Virtual biopsy

STANFORD MEDICINE researchers have developed a technique for conducting a "virtual biopsy."

The researchers use lasers to harmlessly penetrate tissue and create a high-resolution, 3D reconstruction of the cells it contains. Then they make cross-sectional images that mimic those generated by a standard biopsy, in which a sample of tissue is sliced into thin layers and placed on a slide to be examined under a microscope.

The technique, described in a paper published in April in *Science Advances*, could be used to noninvasively scan the skin for unhealthy cells as well as provide rapid results on tissue biopsied conventionally. It could also reveal more information than do commonly used diagnostic approaches.

"We've not only created something that can replace the current gold-standard pathology slides for diagnosing many conditions, but we actually improved the resolution of these scans so much that we start to pick up information that would be extremely hard to see otherwise," said Adam de la Zerda, PhD, an associate professor of structural biology and the senior author of the paper.

The method was developed by Yonatan Winetraub, PhD, a former graduate student in the de la Zerda lab who now leads his own Stanford Medicine research lab.

In a traditional biopsy, tissue is sent to a pathologist, who slices it into thin layers. The pathologist then stains each layer with chemicals called hematoxylin and eosin (H&E), which makes cell patterns, shapes and structures easier to see. These slides are routinely used for diagnosing cancers and other diseases, but making them is labor intensive.

De la Zerda and Winetraub enhanced optical coherence tomography, which is typically used by ophthalmologists to image the back of the eye, so it would work in other organs. (OCT scans measure how light waves from a laser bounce off a tissue to create a rendering of its insides.)

"We kept improving and improving the quality of the image, letting us see smaller and smaller details of a tissue," de la Zerda said. "And we realized the OCT images we were creating were really getting very similar to the H&Es in terms of what they could show."

De la Zerda and his colleagues thought clinicians would be more apt to use OCT if the images looked familiar. So Winetraub turned to artificial intelligence to help convert the scans into flat images resembling H&E slides.

'WE KEPT IMPROVING AND IMPROVING THE QUALITY OF THE IMAGE, LETTING US SEE SMALLER AND SMALLER DETAILS OF A TISSUE.'

Strength in numbers

IN MARCH, Stanford Medicine became the first medical center nationwide to treat a patient with advanced melanoma using a new cell-based therapy called lifileucel. The first such therapy approved by the Food and Drug Administration for solid tumors, it offers hope to people with this deadly form of skin cancer that has metastasized — spread to other parts of the body — and resisted standard immunotherapies.

The treatment works by exploiting the body's natural cancer-fighting ability. Immune cells called tumor-infiltrating lymphocytes, or T cells, are harvested from the patient, then stimulated in the laboratory to multiply into billions of cancer-fighting cells. They're administered to the patient about a month later.

"These cells are naturally existing T cells that target multiple aspects of the existing tumor," said assistant professor of medicine Allison Betof Warner, MD, PhD, a member of the Stanford Cancer Institute. "Before now, there was no approved therapy for people with melanoma whose cancers had progressed after immunotherapy and/or targeted therapy."

Stanford Medicine is one of fewer than 30 medical centers around the country offering lifileucel treatment.

"We are very excited to move cell-based therapies beyond blood cancers," said David Miklos, MD, PhD, professor of medicine and chief of the Division of Blood and Marrow Transplantation and Cellular Therapy. "This has been a long time coming, but now we have a new standard of care for these patients."

Celebrating new hearts

IN 1984, Elizabeth "Lizzy" Craze, then 2, underwent a heart transplant at Stanford Medicine, making her among the youngest recipients on record at that time.

Now, as Stanford Medicine celebrates 50 years of pediatric heart transplants at the institution, Craze is marking 40 years of life with the same heart, far beyond the expected five to 10 years.

"There were very few of us who were transplanted in the early '80s and are still alive," said Craze, a college graduate who has married, launched a fulfilling career and started a family.

Stanford Medicine teams have completed more than 560 pediatric heart transplants since their first in 1974 for a teenager, and 98 pediatric lung and combined heart-lung transplants.

The success involved decades of collaboration between Stanford specialists, which led, for example, to the development of 3D imaging software to better match donor hearts with children who need them.

"Seeing our patients go from critically ill to living fulfilling lives is something I find gratifying, and it gives me a lot of optimism for the future of the field," said David Rosenthal, MD, director of the Pediatric Advanced Cardiac Therapies program at Stanford Medicine Children's Health.

"I definitely try to do everything I want to do and live all the life I can," Craze said. "I like to say yes to wild ideas."

DNA research disparities

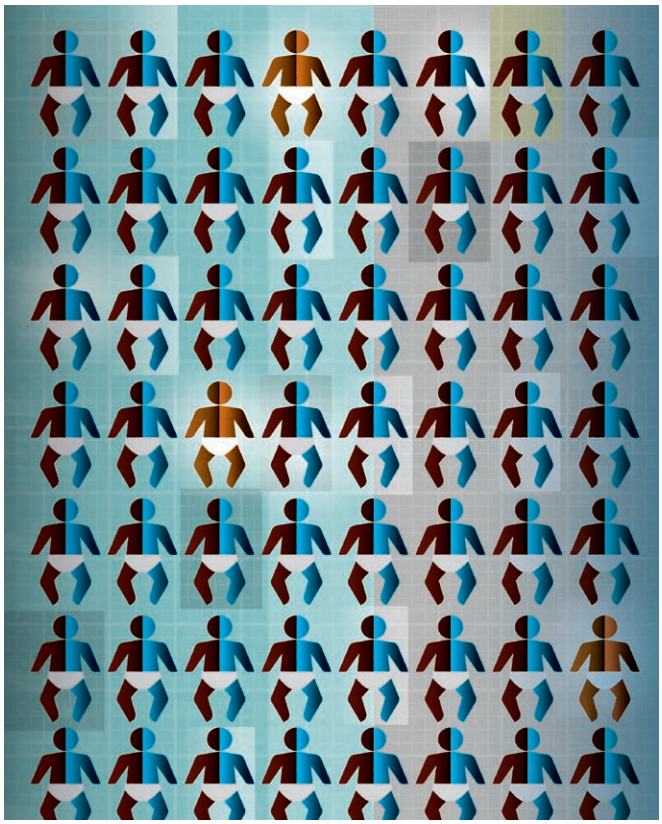
GENETIC SCREENING for metabolic disorders is more accurate for white than nonwhite newborns, a Stanford Medicine study found. The researchers described the problem and suggested corrective steps in the December 2023 study in *Genetics in Medicine*. The study, they said, showed the need to update DNA databases to reflect disease-causing gene variants in nonwhite populations who have historically had less access to DNA testing.

Conventional tests for metabolic disorders in newborns measure biochemicals in blood or urine, providing clear-cut diagnoses without relying on genetic information. But DNA tests have gained prominence in the past decade in part because they can help families not only learn a baby's diagnosis after birth but also get clues to the risk of disease during pregnancy.

To measure how well DNA tests discerned metabolic disease in nonwhite babies, the researchers compared the results of biochemical versus genetic screening of all infants referred to a metabolic genetic service over an 18-month period.

A total of 136 infants were referred based on results from a first-step biochemical screen of blood from a heel prick. Nineteen of them were diagnosed with metabolic diseases based on follow-up biochemical testing. Of those, 18 also underwent genetic testing. Ten of them had an ambiguous result: at least one variant of uncertain significance, a genetic change for which disease risk was not known. Of those 10, nine were of nonwhite ancestry.

"The reality is if you don't test people of nonwhite ancestry, it's this futile cycle: The lab's databases never diversify either," said the study's senior author, Christina Tise, MD, PhD, assistant professor of pediatrics.



Food therapy

ANTIPSYCHOTIC drugs can trigger insulin resistance and obesity, but a dietary intervention may help reverse these side effects.

A pilot study led by Stanford Medicine researchers found that a high-fat, low-carbohydrate diet not only restored metabolic health in patients on antipsychotic medications but also improved their psychiatric conditions.

The study was published in March in *Psychiatry Research*. The first author is Shebani Sethi, MD, associate professor of psychiatry and behavioral sciences, who coined the term metabolic psychiatry.

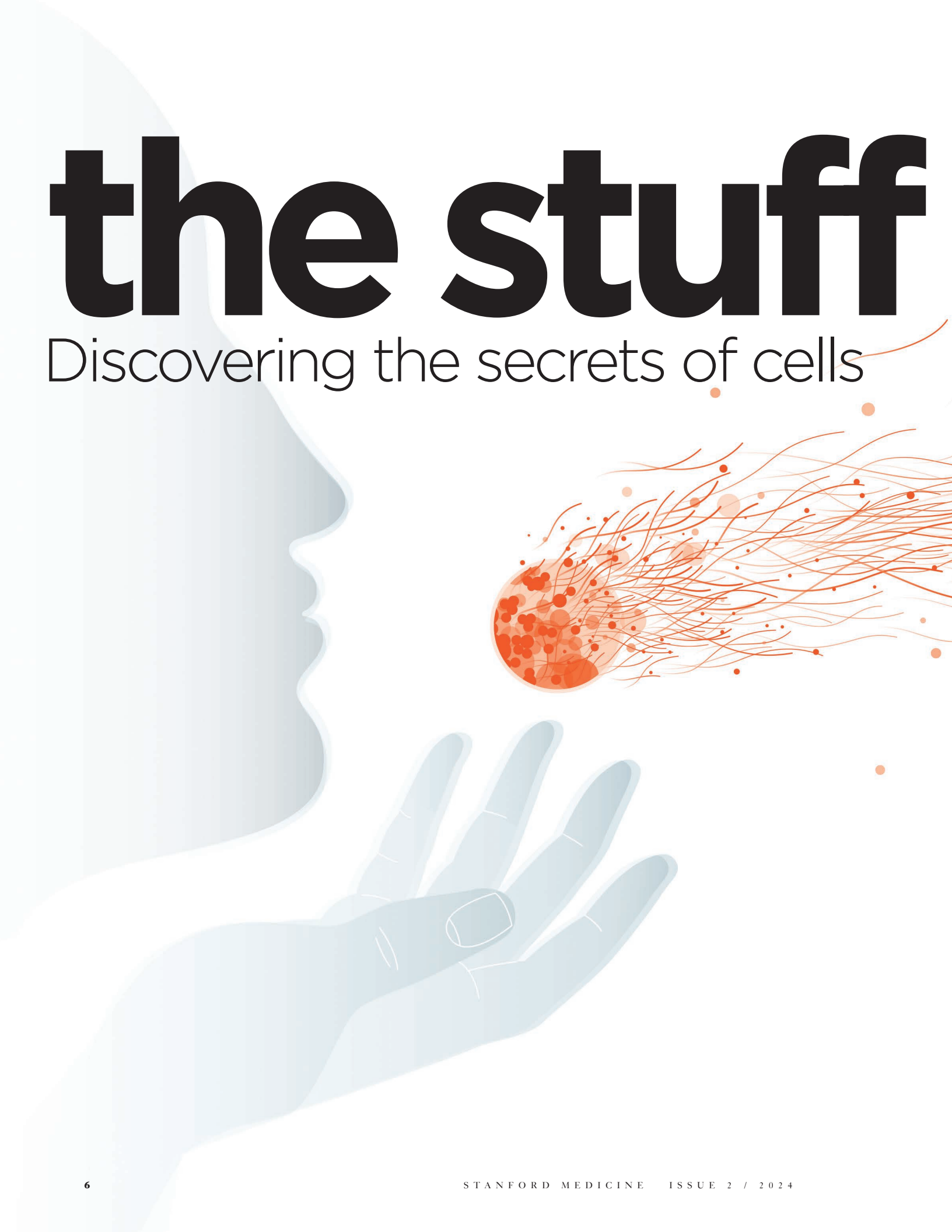
In a four-month pilot trial, the researchers followed 21 adults who were diagnosed with schizophrenia or bipolar disorder, took antipsychotic medications and had a metabolic abnormality, such as weight gain or insulin resistance. The participants were instructed to follow a ketogenic diet, with approximately 10% of their calories coming from carbohydrates, 30% from protein and 60% from fat.

Before the trial, 29% of the participants met the criteria for metabolic syndrome. After four months on a ketogenic diet, none of the participants had metabolic syndrome. On average, the participants improved 31% on a psychiatric rating of mental illness known as the clinical global impressions scale, with three-quarters of the group showing clinically meaningful improvement.

Overall, the participants also reported better sleep and greater life satisfaction.

the stuff

Discovering the secrets of cells



How the smallest units of life determine our health

# of life

Cells are among life's fundamental mysteries — perhaps *the* fundamental mystery, given that cells are life's most basic unit.

Yet as technology advances and biologists adopt new methods, cells' secrets are being revealed. At Stanford Medicine, the discoveries are shaping our understanding of biology and health and fueling new ways to treat disease.

Scientists can manipulate cells as never before, facilitating research and making new treatments possible. Genetically engineered immune cells, for example, are the basis of CAR-T cell therapy, a new therapy with high response rates for blood cancers, often leading to lasting remission for patients who have run out of other treatment options. • "It's a great time for cell biology," said Markus Covert, PhD, the Shriram Chair of the Department of Bioengineering. "It used to be that biology was what you went into if you loved science but were scared of math. That's changed. There's an influx of people who are intellectually ambidextrous, and the field has become more quantitative. That has broken cell biology wide open." • The field of biology got its start more than 350 years ago when, in 1665, scientist and expert microscopist Robert Hooke published his groundbreaking book, *Micrographia*, with engravings and descriptions of objects observed under magnification. When he examined a slice of cork, he saw boxlike structures that reminded him of monks' quarters — so he dubbed them cells. • Cells came into clearer focus when, in 1674, Antonie Van Leeuwen-

By Rosanne Spector

ILLUSTRATION BY HARRY CAMPBELL

hoek shook London's Royal Society with his letter detailing the first documented observation of live cells. Using a microscope he built himself, he studied water from a nearby lake and saw green streaks made up of rows of cells (probably the alga Spirogyra) as well as "very many little animalcules, whereof some were roundish, while others, a bit bigger, consisted of an oval."

Further microscopy studies led, in the 19th century, to the formulation of the cell theory, still recognized today. It holds that cells are the fundamental units of both plants and animals, that all cells are generated by existing cells, and that chromosomes in the cell's nucleus are responsible for heredity.

Today, biologists benefiting from vastly improved methods for studying living cells are making headway in fathoming the many millions of biochemical reactions that occur in a cell every second. Genomic sequencing, which took off in the early 2000s, has become a major tool, enabling scientists to identify the genetic transcripts in play and, with the help of other new technologies, watch the proteins and metabolites at work. Though these studies were first conducted on pooled batches, new methods target individual cells.

"There are so many new single cell techniques," said Denise Monack, PhD, the Martha Meier Weiland Professor in the School of Medicine and chair of the Department of Microbiology and Immunology. "I am finding spatial transcriptomics, which maps gene activity at the single cell level in tissues, to be particularly exciting because we gain so much more information about the relationship between cells as well as their location in tissue — which is crucial for understanding normal development and disease pathology."

Monack is using single-cell analysis, high-throughput screening and other tools of cell biology to ascertain how salmonella bacteria, including the serotype that causes typhoid fever, evade the immune system, persist inside of immune cells and finally transmit to new hosts.

Covert is using the reams of knowledge being produced by cell biologists to create computer models of the full gamut of a cell's biochemical processes — in other words, creating artificial life. In 2012, he and his team completed a model of one of the simplest bacteria, Mycoplasma genitalium, and have since simulated a colony of Escherichia coli. He's aiming to work his way up to modeling the behavior of mammalian cells that make up a tumor.

An important use for such models is to test-drive what happens when a cell is exposed to a drug or toxin or is given new genetic instructions — increasingly valuable as engineered cells are being applied as therapies.

"It's amazing," Covert said. "We're recognizing that a medicine doesn't have to be a molecule or protein. It can be a cell." **SM**

Contact Rosanne Spector at rspector1@stanford.edu

Nucleus:
The control center of the cell that contains the cell's genetic material, which is composed of DNA molecules. The DNA in the nucleus is packed into structures called chromosomes.

Ribosome:
Ribosomes are molecular machines that follow genetic instructions to build proteins. They can sometimes be picky about which genetic instructions they follow.

Mitochondrion:
Known as the cell's powerhouse, mitochondria generate energy through a process called cellular respiration. More than a billion years ago they were free-living bacteria and were engulfed by an ancestor of an animal cell, leading to a mutually beneficial relationship.

Endoplasmic reticulum (ER):
A network of membranes involved in the synthesis of proteins and fats. There are two types: smooth and rough. Smooth ER produces fats, like phospholipids used in cells' membranes, and plays a role in detoxifying drugs and toxins. Rough ER provides a platform for ribosomes to construct proteins. It's rough because ribosomes dot its surface.

Golgi apparatus:
Groups of flattened membrane-enclosed sacs that process, sort and deliver proteins and lipids to their proper destinations within the cell or for secretion outside of the cell.

Lysosome:
Membrane-bound sacs containing digestive enzymes that break down and recycle cellular waste and foreign materials. Think of them as the cell's garbage disposal or recycling center.

Cytoskeleton:
A network of protein filaments that provide structure, support and help in cell movement and division. It can quickly reorganize to change cell shape and enable motion.

Primary cilium:
A single, unbending hairlike structure that extends from the cell's surface. It serves as a cellular antenna for signal reception.

Cell membrane:
A flexible and dynamic wall that surrounds the cell's contents and controls what comes in and goes out. It's made of two layers of fat molecules (phospholipids) with their heads facing outward and their tails facing inward.

Receptor:
Proteins located on the cell membrane or within the cell that bind specific signaling molecules, such as hormones or neurotransmitters, triggering a cellular response. They are highly specific and selective.

Vacuole:
A sac that stores waste materials and aids in cell digestion and recycling.

Cytoplasm:
The gel-like substance enclosed by the cell membrane that houses the cell's structures. It's mainly composed of water, salts and organic molecules and is where many cellular activities occur.

a unit of life

The major structures of an animal cell at rest

ILLUSTRATION BY VIOLET FRANCES

A Nucleus

B Ribosome

C Mitochondrion

D Endoplasmic reticulum

E Golgi apparatus

F Lysosome

G Cytoskeleton

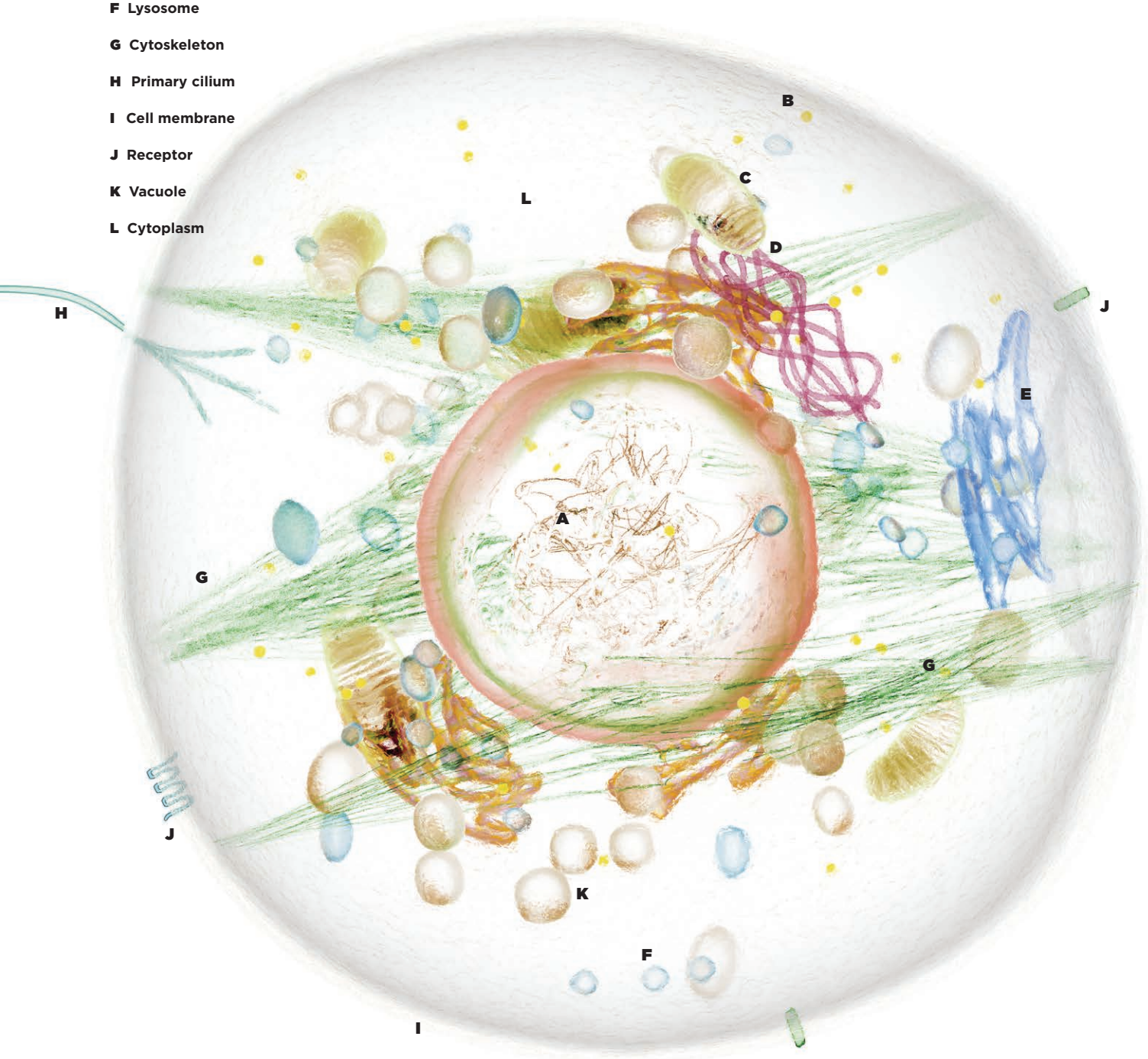
H Primary cilium

I Cell membrane

J Receptor

K Vacuole

L Cytoplasm



‘It’s amazing.

We’re recognizing that a medicine doesn’t have to be a molecule or protein.

It can be a cell.’

what the cell!

By Hanae Armitage

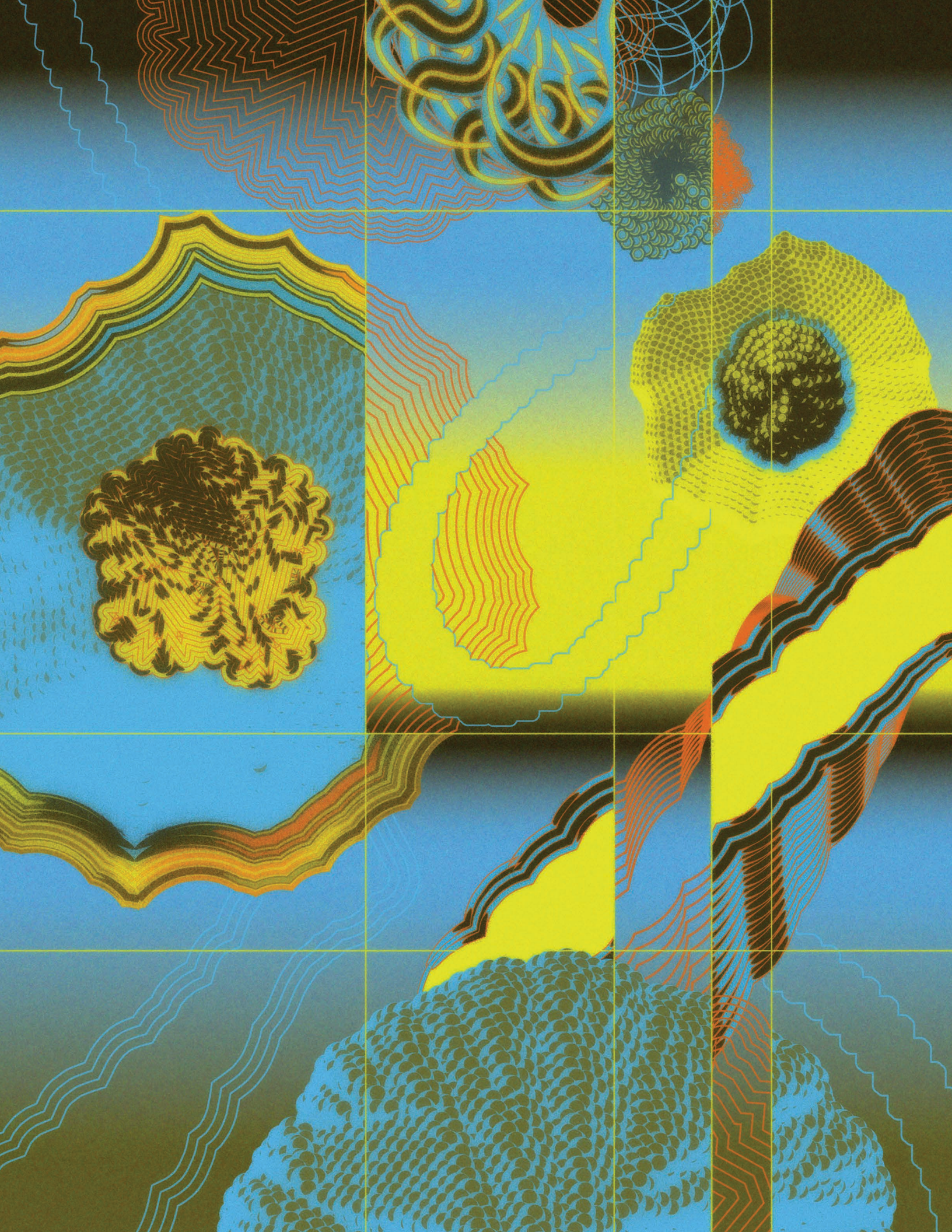
ILLUSTRATION BY PETRA PETERFFY
PHOTOGRAPHS BY TIMOTHY ARCHIBALD

Every cell is beholden to a phenomenon called cell fate, a sort of biological preset determined by genetic coding. Burgeoning cells take their developmental cues from a set of core genetic instructions that shape their structure and function and how they interact with other cells in the body.

To you or me, it's biological law. But to a group of researchers at Stanford Medicine, it's more of a suggestion. Unconstrained by the rules of evolution, these scientists are instead governed by a question: What if?

What if you could eat a vaccine? Or create a bacterium that could also detect and attack cancer? What if furniture could grow from a seed?

Though these types of questions may sound far-fetched, they consume the minds of researchers who specialize in a field known as cell engineering, which harnesses genetic manipulation to change the essence of a cell. That might mean reprogramming a cell to perform a function it isn't designed by nature to do, tinkering with its interior machinery or creating an entirely new type of cell.



"As a scientist and engineer, this idea of learning to build biology — to build with the components of living systems — really motivates me," said Michael Jewett, PhD, professor of bioengineering. "It's really fun to think about what we can learn from that process — like understanding how the biological world works — but then also apply what we learn to benefit society."

Those applications could take a multitude of shapes. Maybe it's building cells that churn out therapeutic drugs — one of Jewett's projects — or vaccines that stave off a bacterial infection, or enzymes that degrade harmful fungi in the rainforest.

The field of cell engineering is relatively new, but its seeds were planted in the 1950s when Nobelist Arthur Kornberg, MD, who came to Stanford Medicine in 1959, isolated the key enzyme used by cells to synthesize DNA. In the 1970s, researchers, including Stanford Medicine faculty, pioneered cutting and pasting DNA (Nobelist Paul Berg, PhD) and transplanting genes from one organism to another (Stanley Cohen, MD, at Stanford and Herbert Boyer, PhD, at UC San Francisco). This launched genetic engineering and the biotechnology industry. Cell engineering gained momentum about a decade ago, as genetic sequencing and manipulation advanced.

A cell engineer's approach to learning is generally to design, tinker and see what happens, said Drew Endy, PhD, the Martin Family University Fellow in Undergraduate Education.

"There has been, and continues to be, unbelievably beautiful scientific work to understand cells," Endy said. "The question for me is, what's next?"

Stanford Medicine researchers from a variety of disciplines are exploring that question. Some are retooling the insides of immune cells, others are digging out and redesigning the guts of a cell, and one is even building a cell from scratch.

Read on to discover how scientists are rethinking cell biology to benefit humanity.

Teaching cells to count

IN THE PAST COUPLE OF DECADES, cancer biologists have developed and refined a powerful new way to vanquish cancer cells floating in the bloodstream. During this treatment, known as chimeric antigen receptor cell therapy, or CAR-T cell therapy, a patient's immune cells are genetically modified to target specific cancer cells.

Naturally occurring T cells kill cancer cells based on recognition of a molecule on the cell's surface — an antigen — that acts like a name tag for tumor cells. With CAR-T cells, scientists remove a person's own T cells and hone their ability to identify specific tumor antigens, heightening their tumor-attacking capabilities. These cells are then returned to the patient. The therapy has so far proven to be a potent option — but only for blood cancers. The cancer-killing abilities of cell-based therapies like CAR-T generally don't extend to solid tumors.

"There are little to no unique markers of solid tumors," said Rogelio Hernandez-Lopez, PhD, assistant professor of bioengineering and of genetics. "Proteins that are known to be markers of cancers, such as HER2 or EGFR, are also shared with other tissues."

Unleashing T cells engineered to track and kill cells with those markers would wreak havoc on healthy tissues. But there's a saving grace that is the cornerstone of Hernandez-Lopez's research: Cancer cells are rich in these markers, and healthy cells are not. "What we're trying to do is teach these T cells to count and make a 'decision' to kill a cell based on the quantity of a particular marker," Hernandez-Lopez said. That kind of nuanced attack requires an entirely different set of instructions than those that naturally guide a T cell.

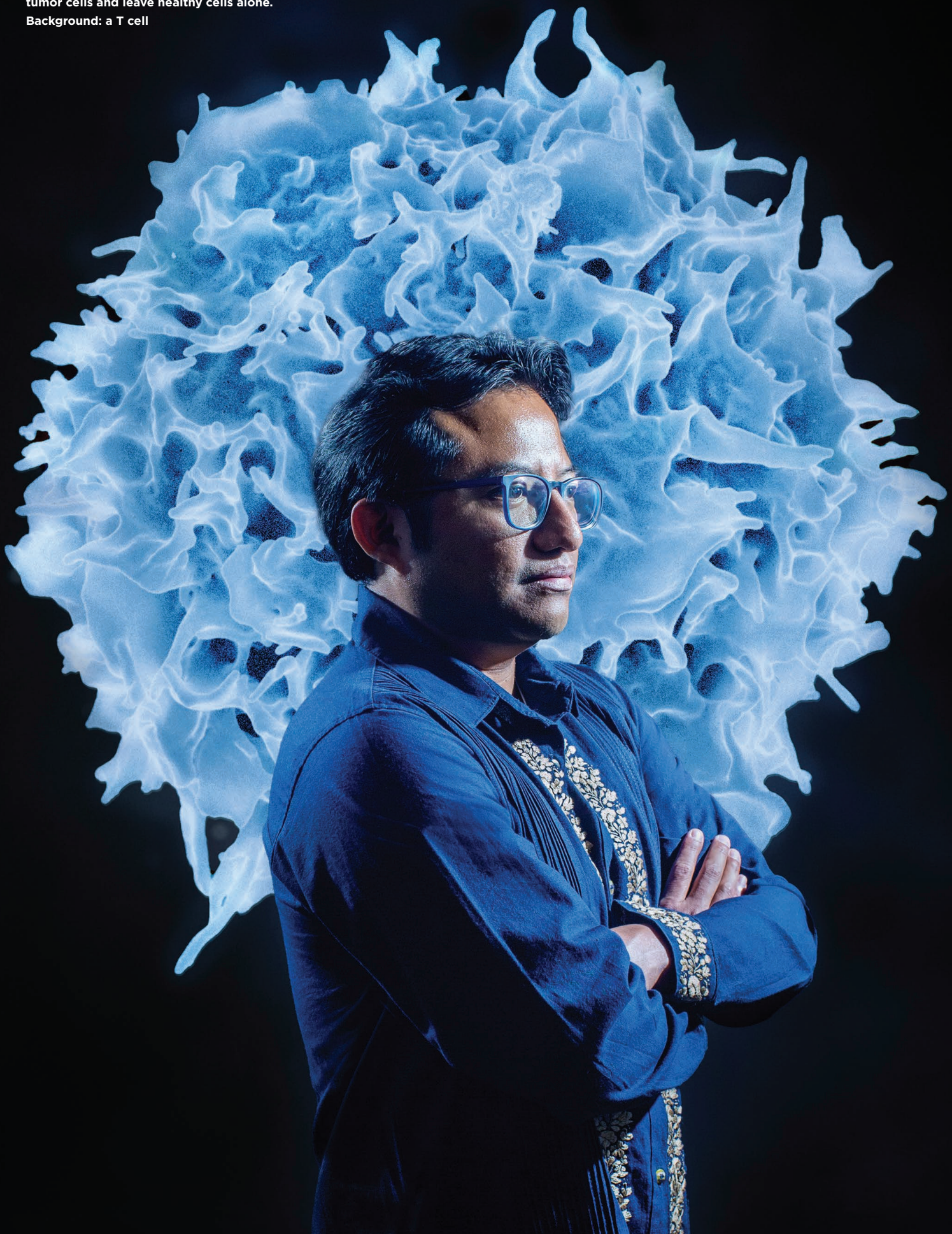
'As a scientist and engineer, this idea of learning to build biology — to build with the components of living systems — really motivates me.'

Hernandez-Lopez is engineering T cells that detect a high versus low abundance of specific markers, or antigens, by creating circuits of two types of synthetic receptors that embed into a T cell's surface.

One, called synthetic Notch, has "low-affinity binding," and when it latches onto its matched antigen, it stimulates the production of the other receptor — which, when bound to an antigen, sparks the T cell to attack. But here's the kicker: Low-affinity binding means the synthetic Notch receptor doesn't always latch onto its paired antigen. Only when that antigen is abundant does the circuit switch on.

That's what gives these T cells the ability to "count and decide." If the T cell detects healthy cells that harbor just a few antigens also carried by tumor cells, the T cell stays neutral. But if it detects a bunch of these antigens, the receptor latches onto the antigen, triggering a series of molecular steps that ultimately jolt the T cell into action.

Hernandez-Lopez's team is testing the synthetic receptor in mice, with early results showing promise for the approach.



ROGELIO HERNANDEZ-LOPEZ, PHD,
and his team are trying to teach T cells to seek and destroy only tumor cells and leave healthy cells alone.
Background: a T cell

HAWA RACINE THIAM, PHD,
is exploring whether an attack by overzealous immune cells called neutrophils can be quelled without stifling the ability to kill real threats. Background: neutrophils

Neutralizing the neutrophil

WHEN BACTERIA OR VIRUSES SNEAK INTO your bloodstream, a brigade of immune cells known as neutrophils attack, and they're equipped with a slew of molecular weaponry. They kill the infected cells by engulfing and destroying them; by releasing toxic chemicals toward them; or through a dramatic demonstration of demolition — like a microscopic supernova, a neutrophil can explode, spewing its DNA and enzymes that devour surrounding cells.

This process, known as NETosis (with NET standing for neutrophil extracellular traps), is unique to innate immune cells.

The cells' variety of attack modalities, while effective, can be too much. Neutrophils respond to signals of inflammation (which is one of the ways the immune system counters infection), but not all inflammation is caused by a microbe or virus.

Even in cases in which infection is not the culprit, such as in autoimmune diseases, neutrophils see the inflammatory signal as a summons, and they dutifully report to the ailing site.

But this time, their defense mechanisms worsen symptoms, exacerbating an already problematic situation. Hawa Racine Thiam, PhD, an assistant professor of bioengineering and of microbiology and immunology and a neutrophil expert (and enthusiast) is keenly aware of the problem.

Part of how neutrophils contribute to heightened inflammation comes down to some technical details of NETosis. "When the neutrophil 'nets,' it simultaneously releases DNA and cytotoxic proteins that can kill the pathogen," Thiam said. Those toxic molecules can inflict additional damage even after the invader has been killed.

Thiam is exploring whether it's possible to quell an overzealous neutrophil attack while maintaining its ability to kill off real threats. But to answer that question, she needs to know more about how NETosis plays out in the first place, one of the big goals of her research.

There are a few theories, one of which Thiam is testing. "For the cell to net there needs to be a breakdown of the nuclear membrane, and for that to happen we think the cell needs to generate force," she said.

She and others in the field suspect that push comes from genetic structures in the cell known as chromatin. Chromatin, the bundles of DNA and protein that form chromosomes, can change conformation, depending on the environment or cellular conditions.

"Think of a cord coiled up in a blown-up balloon," Thiam said. "If the cord unfurls and takes up more space, it puts pressure on the balloon, which can make it pop."

In a neutrophil, that is part of the process that ruptures the cell and expels pathogen-degrading enzymes.

"This is a working hypothesis that our early data supports," she said of the research. "But there's a lot more to understand." To that end, she's using genetic and biophysical experimentation to study how the cell bursts and to determine which components of the expelled content damage the host.

Building from the bottom up

IMAGINE YOU ARE AN alien presented with a chocolate layer cake. You're now asked to bake one from scratch.

How does one work backward from cake to ingredients (and the amounts necessary) to make the confection? Even broken into its component parts — cake, frosting, sprinkles — it's still not clear what the ingredients are nor how to blend them into that decadent baked good. And so ensues a lengthy process of trial-and-error experimentation.

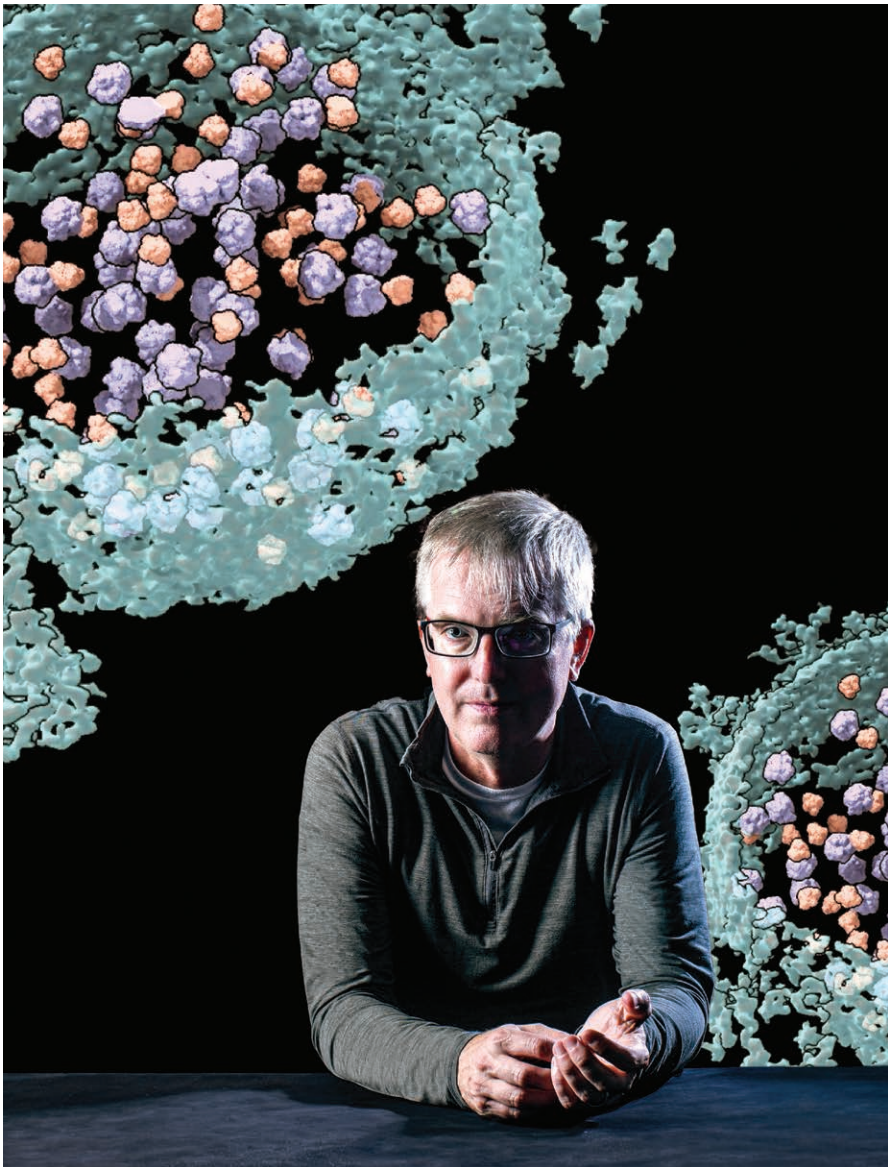
Such is Endy's conundrum — only it's not a cake. It's a cell. And it's much more complicated. Endy is on a mission to build a cell from scratch. After more than a decade of work by him and a crew of Stanford students, they have built a prototype, which he calls a "precursor cell."

Endy is on a mission to build a cell from scratch. After more than a decade of work by him and a crew of Stanford students, they have built a prototype, which he calls a 'precursor cell.'

A successful synthetic cell will need to satisfy a few generally agreed upon (though sometimes debated) criteria: compartmentalization, formed by some sort of lipid bilayer or the like; self replication, during which a cell duplicates its innards and divides on its own; and metabolism or energy production, to power the former.

Attempting to build a synthetic cell in Endy's lab starts with a commercially available set of molecules that, together, carry out one of the cell's main jobs — the synthesis of proteins. Endy's team has toyed with and manipulated this mixture to boost its capabilities and move it from test tube to a biological capsule, while maintaining its ability to create proteins.

That's not as simple as it might sound. So, to guide the process, Endy's group of researchers is implementing a model of



DREW ENDY, PHD,
says he hopes synthetic cells will be able to provide a foundation for solutions to threats including hunger and insufficient access to medicine. Background: cryogenic electron microscopy images of synthetic precursor cells

cellular behavior based in something called "colloidal hydrodynamics," to predict how a synthetic cell might form and react under certain laboratory conditions. Put together, experimentation and modeling have yielded some interesting molecular concoctions. But perhaps more impressively, some precursor cells are exhibiting core functions of a cell, churning out proteins when they are fed DNA.

The final step is visualization. Through a type of microscopy called cryogenic electron microscopy, or cryo-EM, which images frozen molecules by bombarding electrons at the specimen and measuring refraction, Endy and his team can glimpse the precursor cells they're making. Some look like cells — a lipid bilayer that surrounds machinery on the inside — but some go wonky, absorbing one another like a Russian nesting doll.

Either way, Endy is excited to see his lab's progress. "It's so heartening to see these things. It took six years for it to come together."

Endy hopes that his synthetic-cell building will fuel his larger goal as a bioengineer: the broad and accessible dissemination of bioengineering capabilities that can one day support solutions to the world's biggest threats, such as hunger, insufficient access to medicine in every country and climate change.

Synthetic cells, he hopes, will be able to generate new solutions. Exactly what they will look like isn't yet clear, but Endy believes his progress has laid a foundation. "That sets us up for the next generation of synthetic cell building," he said.

It's what's inside that counts

NOT ALL CELL-BASED engineering has to take place in a cell — that's the grounding philosophy of much of the work that comes out of Jewett's lab. What he cares about is on the inside. "We basically take cells, rip off their cell walls, collect the insides and build with that machinery, which has all the information necessary to support information flow in biology," Jewett said.

He's particularly focused on the ribosome — the little protein-making machines that operate inside cells and turn RNA into proteins, which then carry out a variety of biological functions. "We're trying to boot up ribosomes in a test tube," he said.

Unhoused ribosomes offer a lot of potential advantages. For instance, they could be shipped to faraway places (without the need to maintain the rest of the cell) where they could churn out proteins, which often are fundamental to therapeutics.

For now, synthetic ribosomes' potential remains to be realized — the current goal is to build a foundation that can be tweaked so that one day engineered ribosomes might assume new powers, such as the ability to create proteins in unnatural abundance or under extreme conditions.

For his experiments, Jewett often makes use of the ribosomes of the bacterium Escherichia coli, which are made of 54 different proteins and three strands of RNA. Together, those molecules translate RNA genetic templates into proteins, including those that make other ribosomes.

"Creating new, functional ribosomes in a test tube has really been a challenge, in part because it's kind of like the chicken or the egg paradox. The ribosome produces proteins that, in turn, are required to build ribosomes."

So far, Jewett and his team have figured out how to co-assemble all of the ribosomal proteins with the ribosomal RNA in a test tube and use that mixture to create new proteins. To get all the pieces working takes more than just swirling them all together, however. Biological processes require energy to be a self-sustaining system.

"It's like building a house. You need materials, you need energy and information," Jewett said.

"In this case, the information is DNA instead of house blueprints, the energy for the biological systems is the chemical compound adenosine triphosphate (or ATP) instead of human labor, and the materials are amino acids or nucleotides, rather than wood or brick."

But they've yet to have a ribosome beget another ribosome. "Our test tube ribosomes are good enough to make all of the chemical bonds necessary to synthesize another ribosome, but we're missing a step to get it to self-assemble," Jewett said. "Our next task is to figure out why."

Assistant to assassin

THERE'S A CODE SWITCH happening in the lab of Kyle Daniels, PhD, an assistant professor of genetics who has an express goal of coaxing out the unnatural side of immune cells, experimentally encouraging them to exhibit new capabilities. "We're engineering cells to get them to do things they don't normally do," he said. "If you can understand how to do that, you open up a whole world of possibilities in the future to tackle problems that we may not even know about yet."

Much of Daniels' work focuses on immune cells, with one project homing in on T cells, which are considered the cancer killers. Generally speaking, there are two types of T cells in the body: helper T cells, CD4, and killer T cells, CD8 — so named to denote the receptors embedded in the cells' outer surface. CD4 helper cells play an organizational role, stimulating other immune cells to do the dirty work and act against pathogens or tumor cells. CD8 cells, however, are natural born killers, built to destroy at the behest of CD4s. That, at least, is the traditional understanding of the role these cells have in human biology.

While T cells often function this way, Daniels and his team are using synthetic biology tools to reveal a recently discovered secret about both types of T cells: In every CD4 T cell, a killer lurks, and in every CD8, a mediator. But the hidden ability of CD4s to facilitate death has caught Daniels' attention the most. His team is creating a variety of synthetic receptors that can lodge in the outer layer of T cells and, when bound to the target molecule, guide the cells' behavior and activity.

Those receptors adhere to certain antigens, and their binding sets off a flurry of events that result in the T cell expelling toxic molecules that kill the cells around it.

"I think we assumed that the CD8 cells were doing all the

'We basically take cells, rip off their cell walls, collect the insides and build with that machinery, which has all the information necessary to support information flow in biology.'

MICHAEL JEWETT, PHD, and his team are trying to 'boot up ribosomes in a test tube' to expand their therapeutic potential. Background: a model of a ribosome

killing in our experiments, but it turns out if you have CD4 cells alone, they're really good at killing leukemias and lymphomas with the synthetic receptor," Daniels said.

And the modified CD4 cells can maintain their killing spree longer than CD8 cells that are modified with the same receptor. "It's been a big surprise to us."

His team has even found that, depending on the type of synthetic receptor, it can selectively activate a CD4 cell's killing program, triggering the same destruction of killer cells without tampering with the function of CD8 cells.

Exactly how these CD4s go from assistant to assassin is still a question. Are they equipped with the tools to kill all along? Or do the synthetic receptors reprogram a new pathway that generates its killing ability? Daniels is exploring that question in his lab.

He also hopes to test the engineered CD4 cells in mice as a next step. "We're seeing that CD4s might be a major driver of the killing. I think there's some appreciation of that, but I don't think it's most people's assumption," Daniels said. "It's clear that this is happening. Now we're trying to find cell signaling programs that maximize this effect so that we can really take advantage of it." **SM**

— Contact Hanae Armitage at harmitag@stanford.edu

KYLE DANIELS, PHD, and his team are engineering cancer-fighting T cells to 'get them to do things they don't normally do.' Background: a cancer cell and T cells.

the company they keep

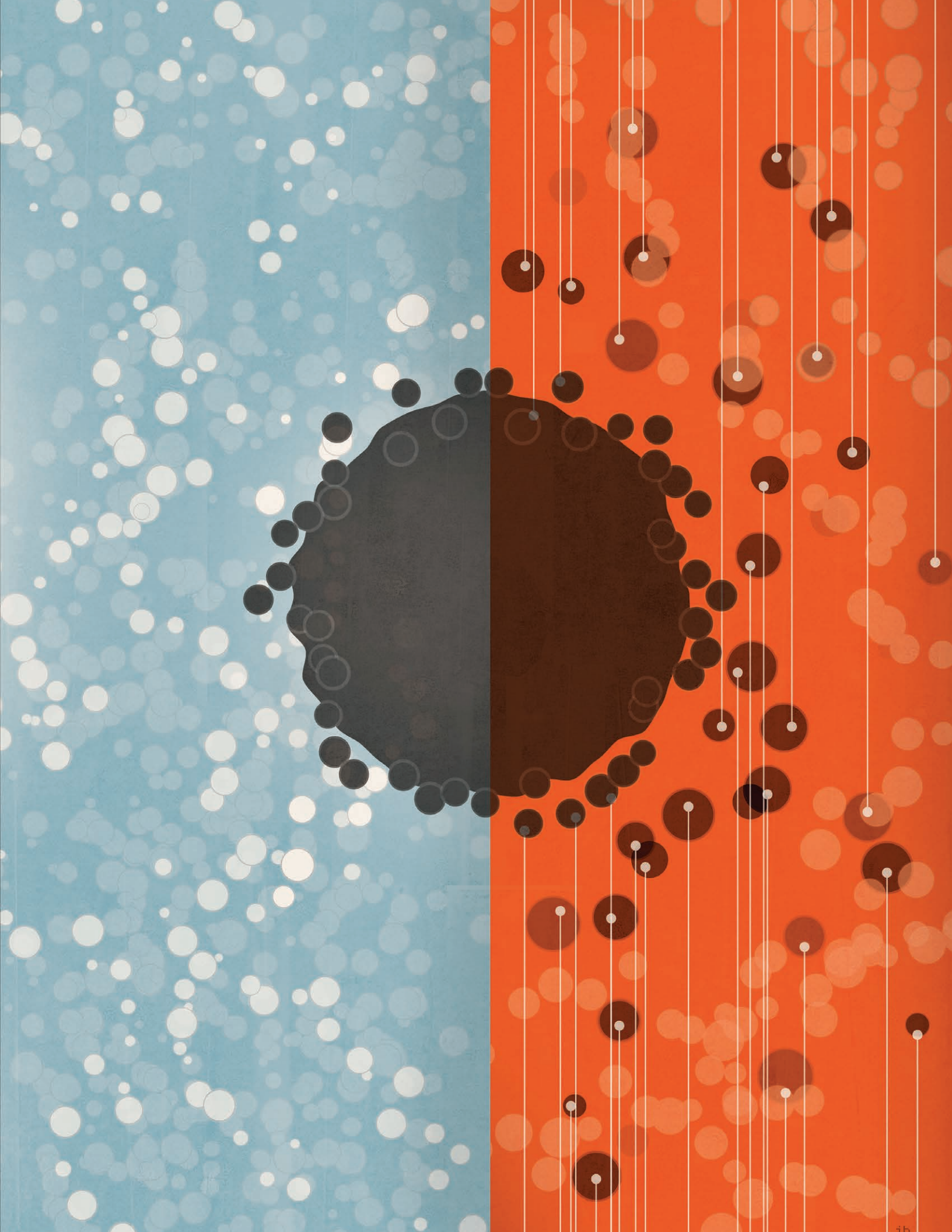
HOW NEIGHBORING CELLS INFLUENCE WHETHER TUMORS GROW OR PERISH

In 2020, Everett Moding, MD, PhD, an assistant professor in Stanford Medicine's radiation oncology department, noticed that some people with a rare cancer called soft-tissue sarcomas were cured with surgery and radiation while others saw their cancers quickly recur. "Two patients could have the same diagnosis and be treated the same way, but their cancers would respond very differently," he said. "And there was no effective way to predict who would have a poorer prognosis."

Around the same time, Magdalena Matusiak, PhD, then a postdoctoral student in the laboratory of professor of pathology Matt van de Rijn, MD, PhD, the Sabine Kohler, MD, Professor in Pathology, was growing frustrated with the traditional methods of predicting cancer cells' growth based primarily on mutations in their DNA. "In many instances, we can't explain tumor biology just by looking at mutations or gene expression," Matusiak said. "Ductal carcinoma in situ, a common early breast cancer, is not usually life threatening. But in about 1 in 4 patients, these cancers will become invasive for reasons we can't explain with conventional methods." ● Both young researchers turned for answers to a rapidly growing field defined by leaps in technology and machine learning that allow a close-up look at the thousands of interactions between cancer cells and the healthy cells and

By Krista Conger

ILLUSTRATION BY JOHN HERSEY



tissues in which they reside. This three-dimensional neighborhood is broadly defined as the tumor microenvironment, and our growing understanding of its importance relies heavily on studies of what's been called spatial biology.

It turns out that the company that cancer cells keep — and the way that company reacts to their presence — is critical to determining whether a new cancer grows, thrives and metastasizes to other parts of the body or is pounced upon and eliminated by the immune system.

"Cells don't exist in isolation," said assistant professor of biomedical data science Aaron Newman, PhD. "A cell's identity, its behavior, its characteristics depend on what other cells are around it in three-dimensional space and what those cells are doing. But even five years ago we didn't have a good way to identify these interactions. Now we can begin to assess aspects of this nuanced, community-specific biology."

Newman, a member of the Stanford Cancer Institute and a Chan Zuckerberg Biohub Investigator, is one of several Stanford Medicine scientists developing tools and techniques to collect and interpret dizzying amounts of data from human tumors to identify, on a cellular communications level, exactly who says what to whom, as well as where, when and why. It's a daunting task when you consider that a tumor the size of a small grape contains something on the order of 1 billion cells.

Some heavy hitters back this research, among them the National Cancer Institute, which in 2016 named the Human Tumor Atlas Network as one of the key research initiatives of its Cancer Moonshot — a program created to focus on areas of research deemed most likely to benefit cancer patients. The tumor atlas network aims to detail the evolution of the cellular and molecular interactions among healthy and diseased cells as a precancerous growth develops into full-blown cancer.

"It's really clear that a tumor is not just a collection of cancer cells," said Sylvia Plevritis, PhD, chair of Stanford Medicine's Department of Biomedical Data Science, the head of the Stanford Center for Cancer Systems Biology and the Stanford Cancer Institute's associate director of cancer AI. "In fact, some of the most difficult tumors to treat, like pancreatic tumors, are mostly noncancer cells. Techniques to study the spatial biology of tumors, like those developed in Aaron's lab and several others at Stanford including mine, are changing our understanding of cancer. Now, we can not only see what cell types are in the tumor but who their neighbors are and the molecular interactions that allow them to communicate and sustain each other."

In just a few years, researchers have gone from deciphering flat, stained slices of tumor tissue highlighting the gross anatomy of a tumor to parsing not just the precise cellular composition of small tumor samples but even identifying specific cellular neighborhoods and interactions that can determine health or disease. The insights are providing important clues to medical mysteries, like this one puzzling Moding and Matusiak: Why do some patients with what seem to be very similar cancers have better outcomes than others?

Proving the link between cancer cells and their surroundings

THE IDEA THAT THE CELLS and tissue surrounding a cancer cell may be as important as the cancer cell itself for determining whether the cancer cell thrives, divides and — eventually — metastasizes was first floated in 1863 when German physician Rudolf Virchow, MD, noted a connection between inflammation and cancer. In 1889, English surgeon Stephen Paget, FRCS, advanced his "seed and soil" hypothesis that the cellular environment within which a metastasizing cancer cell landed influenced whether it would flourish or die in its new location.

At that time, there were few ways to prove these hypotheses on a cellular level. Aspiring investigators pored over microscope slides holding thin slices of tissue stained a dull purple to delineate individual cells and structures. Researchers could only infer relationships among cells from a snapshot in time frozen on a two-dimensional grid — a bit like trying to predict how occupants of a high-rise spend their time by looking at the building's blueprints.

Decades later, in the late 1960s, scientists devised a way to attach color-changing proteins to antibodies that recognize and bind to specific cellular structures — vastly increasing the amount of information that could be garnered from a single slide. Now they could see the arrangement of furniture in individual rooms and predict the function of each space. But still, there was no inkling of how the cells communicated, or didn't, with one another in living tissue.

The floodgates started to open when genomic sequencing took off in the early 2000s. Soon researchers learned how to infer the cellular composition of a tumor by identifying the relative levels of RNA messages, or transcripts, expressed by the cells — first in bulk and then, almost incomprehensibly, at the level of individual cells. Suddenly, the high-rise blueprint shows not just rooms and furniture but also people and what was on their minds.

That's because, although most cells share a common vocabulary in the form of the genes encoded by their DNA, RNA messages are the genetic words a cell mutters to itself to accomplish a certain goal at a particular time. Single-cell RNA sequencing allows researchers to eavesdrop on these internal conversations.

Newman and his peers at Stanford Medicine have developed technologies that build on these earlier advances. One, CIBERSORTx, functions like an eerily accurate fortune teller,

predicting the various cell types in a bulk tissue sample based on the relative abundance and patterns of RNA messages in the sample. Another, EcoTyper, builds on this prediction to determine what the cell types are up to (a condition called cell state) and which other cells they are interacting with. The information allows researchers to build a picture of complex cellular neighborhoods called ecotypes within tumor tissue that hint at how the tumor is (or isn't) thriving.

"Spatial transcriptomics is a new technology that gives us information about gene expression and spatial location so we can understand the modular architecture of healthy and cancerous tissue," said Newman, the Institute for Stem Cell Biology and Regenerative Medicine Faculty Scholar. "In ecology, a species changes its characteristics and behavior in response to its local environment. Cells do this as well."

Most recently, another tool, CytoSPACE, developed in Newman's lab, maps these neighborhoods to precise locations in the tumor tissue, while also assessing the activity of all of each cell's 20,000 genes.

"Many times, if you just look at tumors as a bag of cells, your ability to predict a patient's prognosis is not great, even if you know how many of each cell type is in the sample," said associate professor of pathology Michael Angelo, MD, PhD. Angelo developed a way to visualize the locations of up to 50 individual proteins in a cell using a technique called MIBI-TOF. "But if you can incorporate where those cells are in the tumor, those predictions become much better. And they don't seem to have a whole lot to do with the tumor cells themselves," Angelo said. "The much more important angle is how the nontumor cells are responding to the presence of the cancer."

'MANY TIMES, IF YOU JUST LOOK AT TUMORS AS A BAG OF CELLS, YOUR ABILITY TO PREDICT A PATIENT'S PROGNOSIS IS NOT GREAT … . BUT IF YOU CAN INCORPORATE WHERE THOSE CELLS ARE IN THE TUMOR, THOSE PREDICTIONS BECOME MUCH BETTER.'

Importantly, the machine learning that drives each of these advances has no preconceptions about what it might find. By simply looking for patterns — this type of cell is likely to be found rubbing membranes with this other type of cell, but only when both are in a particular cell state, for example — the computers can identify interactions that defy expectations.

"When my lab started working with single-cell data of tumors, we kept finding fibroblasts coming up as really important," said Plevritis, the William M. Hume Professor in the School of Medicine. "Fibroblasts are most known for creating part of the skeleton that cells sit in and are one of the most understudied parts of a tumor, so it is very interesting and exciting to study this association."

Further studies in Plevritis' lab found that fibroblasts at the leading edge of a lung tumor had properties that stimulated cancer cells to invade surrounding tissue, while the fibroblasts in the interior appear to be more tumor suppressive.

New tools allow for deeper probes of archived cancer tissues and types

TAKEN TOGETHER, THESE technologies have given researchers, including Matusiak and Moding, valuable insight as to why people with the same type and stage of cancers can have such different outcomes.

Matusiak compared the location and activity of immune cells called macrophages in breast and colon cancers with healthy tissue. Prior to her study, researchers identified macrophages in tumor tissue by the presence of a protein that appears universally on all macrophages. Matusiak used single-cell RNA sequencing data to identify additional proteins that appear on only a subset of macrophages. She then found antibodies to these subset-specific proteins and used them to probe slides of tissue from colorectal and breast tumors.

She learned that macrophages are found in five distinct and very different cellular neighborhoods, or niches, within the tumors and that the macrophages were acting differently in each location.

"This was a big surprise," Matusiak said. "We were definitely not expecting to see such distinct and separate spatial regions."

For example, macrophages with a protein called IL4I1 on their surfaces were found in regions of high cellular turnover in both healthy and cancerous tissue — gobbling dead or dying cells. The presence of this class of macrophages correlated with a good response to immunotherapy in breast cancer patients and more favorable outcomes in people with colorectal cancers. In contrast, although macrophages with a protein called SPP1 were associated with tumor cell death, their presence in colorectal tumors correlated with poor outcomes.

"Now we have the first tools to really investigate macrophage biology in different tissues and cancer types in archived human tissue, including ductal carcinomas in situ," Matusiak said.

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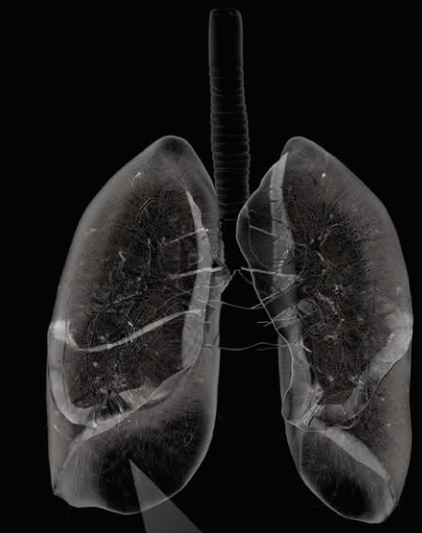
COVID'S unwitting enablers?

New research flags unexpected cells in lungs as a suspected source of severe COVID

BY BRUCE GOLDMAN



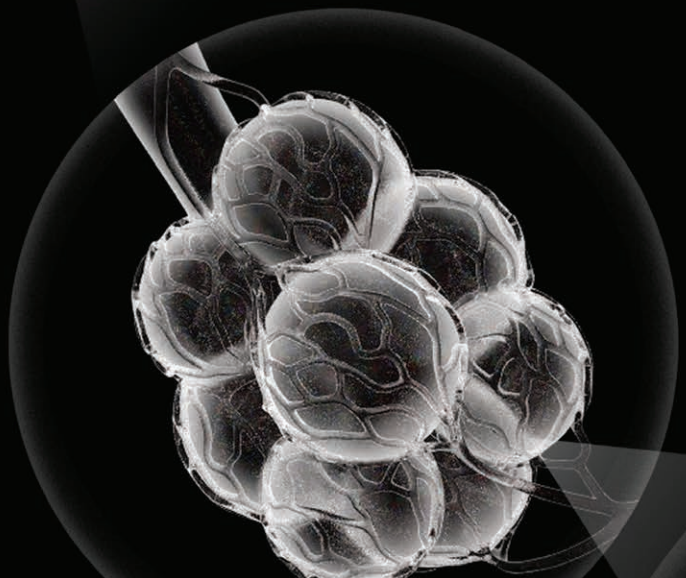
Mild COVID is manageable. Severe COVID can kill you. If we knew why a mild case turns into a severe one, it might help.

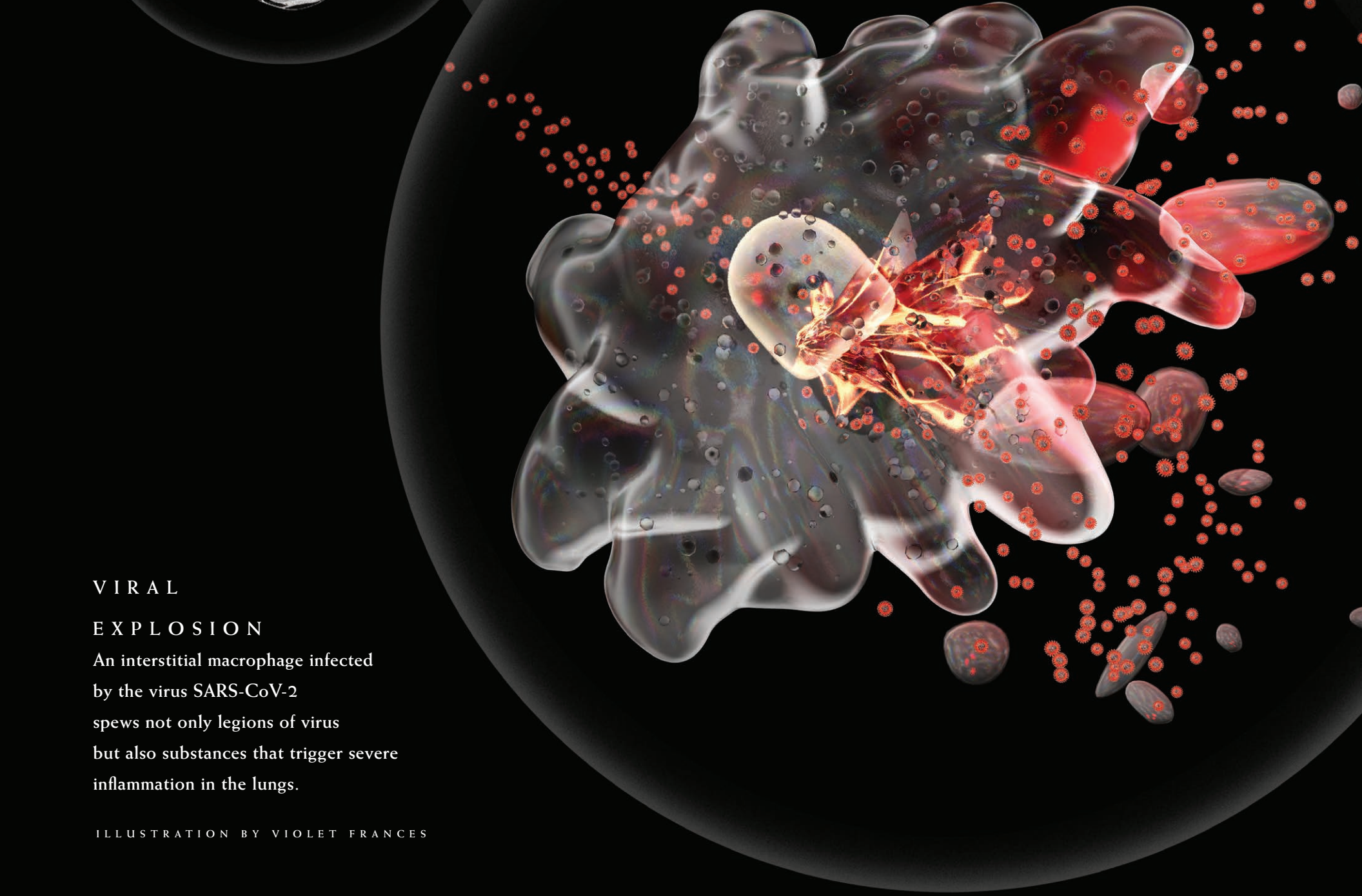
We could start with a look at your lungs, whose surface is squiggly, uneven terrain. That's because it's covered with minuscule, air-filled bubbles called alveoli. There are about 500 million alveoli in a pair of healthy adult lungs. The wall enclosing each tiny air pocket is just one cell thick.

Your lungs' bubbly contour makes their total surface area huge. If it could be spread out to form a flat sheet, that surface would coat the entire floor of a tennis court. That's great for the transfer of oxygen from the air you've just inhaled to the red blood cells that will carry their vital cargo to destinations throughout your body.

But that permeable surface's 1/100,000-inch thickness also makes it the most fragile of tissues: a mostly one-cell-thick border through which microbial pathogens can burrow, ride to their favorite targets and maybe, on the way, feast on the nutrient-rich buffet on offer in your bloodstream. Should anything — infection, inflammation or injury — disrupt that layer of lung-surface cells, what's on one side could leak out and what's on the other side could get in. Not good for your health.

That diaphanous border, however, doesn't go unpoliced. Specialized immune cells patrol both sides of it, scarfing down unwanted intruders and calling up reinforcements on active patrol nearby or snoozing in our bodies' barracks. We wouldn't last long without them.





VIRAL EXPLOSION

An interstitial macrophage infected by the virus SARS-CoV-2 spews not only legions of virus but also substances that trigger severe inflammation in the lungs.

ILLUSTRATION BY VIOLET FRANCES

HERE'S THE THING: We're finding out that the road to severe COVID may be paved with lung-resident immune cells we thought were our friends. And all the while, we've been pointing an accusing finger at other cells in our lungs that — although, OK, they may not exactly be perfectly innocent — are, relatively speaking, rank amateurs.

A type of immune cell known as an interstitial macrophage has recently been implicated in the critical transition from a merely bothersome COVID case to a potentially deadly one. Interstitial macrophages are situated deep in the lungs, ordinarily protecting that precious organ by, among other things, devouring viruses, bacteria, fungi and dust particles that make their way down our airways.

But as Stanford Medicine researchers showed in a study published in April in the *Journal of Experimental Medicine*, it's these very cells that, of all known types of cells composing lung tissue, are most susceptible to infection by SARS-CoV-2.

SARS-CoV-2-infected interstitial macrophages, the scientists have learned, once infected, can squirt out inflammatory and scar-tissue-inducing chemical signals, potentially paving the road to pneumonia and damaging the lungs to the point where the virus, along with those potent secreted substances, can break out of the lungs and wreak havoc throughout the body.

The surprising findings point to brand-new approaches to preventing a SARS-CoV-2 infection from stepping over the line beyond which a manageable disease becomes a life-threatening one. Indeed, they may explain why precisely targeted drugs called monoclonal antibodies meant to combat severe COVID didn't work well, if at all. When they did work, it was only when they were administered early in the course of infection, when the virus was infecting cells in the upper airways leading to the lungs but hadn't yet ensconced itself deep in lung tissue.

Monoclonal antibodies are designed to bind strongly to this or that specific feature on the surface of an invading virus, with the usual objective of blocking its ability to bind to its receptor on a target cell's surface. But when a "this" becomes a "that" because of a mutation that changes the shape of the viral surface, the antibody is out of a job, and a new one must be designed.

THE VIRUS SURPRISES

"WE'VE OVERTURNED a number of false assumptions about how the virus actually replicates in the human lung," said Catherine Blish, MD, PhD, the George E. and Lucy Becker Professor in Medicine II and associate dean for basic and translational research.

Blish is the co-senior author of the study, along with Mark Krasnow, MD, PhD, the Paul and Mildred Berg Professor and Executive Director of the Vera Moulton Wall Center for pulmonary vascular disease.

"The critical step, we think, is when the virus infects interstitial macrophages, triggering a massive inflammatory reaction that can flood the lungs and spread infection and inflammation to other organs," said Krasnow, a professor of biochemistry. Blocking that step, he said, could prove to be a major therapeutic advance. But there's a plot twist: The virus has an unusual way of getting inside these cells — a route drug developers have not yet learned how to block effectively — necessitating a new focus on that alternative mechanism, he added.

In a paper published in *Nature* in early 2020, Krasnow and his colleagues described a technique they'd worked out for isolating cells from fresh human lung tissue; dissociating the cells from one another; and characterizing them, one by one, on the basis of which genes within each cell were active and how much so. Using that technique, the Krasnow lab and collaborators were able to discern more than 50 distinct cell types, assembling an atlas of healthy lung cells.

'The critical step, we think, is when the virus infects interstitial macrophages, triggering a massive inflammatory reaction that can flood the lungs and spread infection and inflammation to other organs.'

"We'd just compiled this atlas when the COVID-19 pandemic hit," Krasnow said. Soon afterward, he learned that Blish and Arjun Rustagi, MD, PhD, then an instructor of infectious diseases, were building an ultra-safe facility where they could safely grow SARS-CoV-2 and infect cells with it.

A collaboration ensued. Krasnow and Blish and their associates obtained fresh healthy lung tissue excised from seven surgical patients and five deceased organ donors whose lungs were virus-free but weren't used in transplants. After infecting the lung tissue with SARS-CoV-2 and waiting one to three days for the infection to spread, the researchers separated and typed the cells to generate an infected-lung-cell atlas, analogous to the one Krasnow's team had created with healthy lung cells. They saw most of the cell types that Krasnow's team had identified in healthy lung tissue.

Now the scientists could compare pristine versus SARS-CoV-2-infected lung cells of the same cell type with one another: They wanted to know which cells the virus infected, how easily SARS-CoV-2 replicated in infected cells, and which

genes the infected cells cranked up or dialed down compared with their healthy counterparts' activity levels. They did this for each of the dozens of cell types they'd identified in both healthy and infected lungs.

"It was a straightforward experiment, and the questions we were asking were obvious," Krasnow said. "It was the answers we weren't prepared for."

WHERE AIR MEETS BLOOD

THE CELLS THE RESEARCHERS had expected to succumb most readily and ominously weren't the ones that did.

It's been assumed that the cells in the lungs that are most vulnerable to SARS-CoV-2 infection are those known as alveolar type 2 cells. That's because the surfaces of these cells, along with those of numerous other cell types in the heart, gut and other organs, sport many copies of a molecule known as ACE2. SARS-CoV-2 has been shown to be able to grab onto ACE2 and manipulate it in a way that allows the virus to maneuver its way into cells.

Alveolar type 2 cells are somewhat vulnerable to SARS-CoV-2, the scientists confirmed. But the cell types that were by far the most frequently infected turned out to be two varieties of a cell type called a macrophage.

The word "macrophage" comes from two Greek terms meaning, roughly, "big eater." This name is not unearned. The air we inhale carries not only oxygen but also, unfortunately, tiny airborne dirt particles, fungal spores, bacteria and viruses to our lungs. A macrophage earns its keep by, among other things, gobbling up these foreign bodies.

The airways leading to our lungs culminate in myriad alveoli, which are abutted by abundant capillaries. This interface, called the interstitium, is where oxygen in the air we breathe enters the bloodstream and is then distributed to the rest of the body.

The two kinds of SARS-CoV-2-susceptible lung-associated macrophages are positioned in two different places. Cells known as alveolar macrophages hang out by the billions patrolling the inner surfaces of the alveoli. As expected, SARS-CoV-2 can infect alveolar macrophages. Once infected, these cells smolder, producing and dribbling out some viral progeny at a casual pace but more or less keeping a stiff upper lip and maintaining their normal function. This behavior may allow them to feed SARS-CoV-2's progression by incubating and generating a steady supply of new viral particles that escape by stealth and penetrate the layer of cells enclosing the alveoli.

Interstitial macrophages, the cell type now revealed to also be infected by SARS-CoV-2, patrol the outer surface of the alveoli, where the rubber of oxygen meets the road of red blood cells. If an invading viral particle or other microbe manages to evade alveolar macrophages' vigilance, infect and punch through the layer of cells enclosing the alveoli — jeopardizing not only the lungs but also the rest of the body — interstitial macrophages are ready to jump in and protect the neighborhood.

At least, usually. But when an interstitial macrophage meets SARS-CoV-2, it's a different story. Rather than get eaten by the omnivorous immune cell, the virus infects it.

THE DEVIL'S SPATULA

AN INFECTED INTERSTITIAL macrophage doesn't just smolder; it catches on fire. The virus literally seizes the controls and takes over, hijacking the cell's protein- and nucleic-acid-making machinery. In the course of producing massive numbers of copies of itself, SARS-CoV-2 destroys the boundaries separating the cell nucleus from the rest of the cell, like the devil's spatula shattering, splattering and scattering the yolk of a raw egg. (This deformation is denoted by the hideous term "nuclear blebbing.") The cell's outer membrane explodes, allowing viral progeny to exit the spent macrophage and move on to mess up other cells.

But that's not all. In contrast to alveolar macrophages, infected interstitial macrophages pump out substances that signal other immune cells elsewhere in the body to head for the lungs. In a patient, Krasnow suggested, this would trigger an inflammatory influx of such cells. As the lungs fill with cells and fluid that accompany inflammation, oxygen exchange becomes impossible. The barrier maintaining alveolar integrity grows progressively damaged. Leakage of infected fluids from damaged alveoli propels viral progeny into the bloodstream, blasting the infection and inflammation to distant organs.

'It was a straightforward experiment, and the questions we were asking were obvious. It was the answers we weren't prepared for.'

Yet other substances released by SARS-CoV-2-infected interstitial macrophages stimulate the production of fibrous material in connective tissue, resulting in scarring of the lungs. In a living patient, the replacement of oxygen-permeable cells with scar tissue would further render the lungs incapable of executing oxygen exchange.

"We can't say that a lung cell sitting in a dish is going to get COVID," said Blish, a professor of infectious diseases and of

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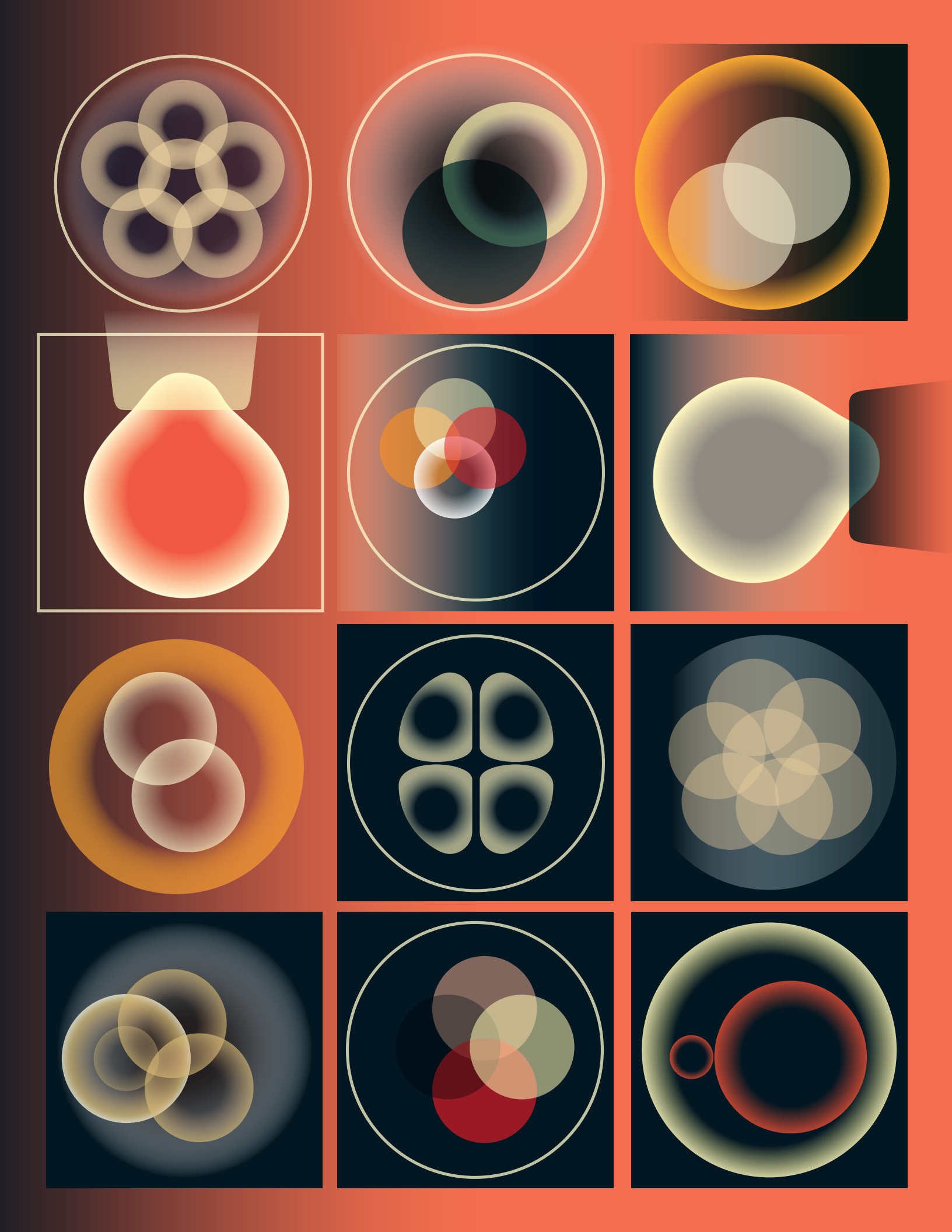


my favorite cell

Stanford Medicine researchers reveal the cell they most appreciate

By Krista Conger

ILLUSTRATION BY PAUL WEARING

Arguably every cell in your body matters, but which are the most interesting? The most mysterious, surprising or — yes — even the prettiest? These might seem impossible questions to answer, but when we asked several Stanford Medicine scientists to name their favorite cell and explain why, answers came easily — and ran the gamut.

With some creative license, we found that each of the cells listed could be assigned a range of attributes not out of place in a high school yearbook. But while some are easy to categorize — it seems obvious that "most ambitious" should be awarded to the stem cells that give rise to and maintain all the body's tissues — other categories are more competitive.

Should "best dressed" go to the stately, meticulously organized cells of the inner ear, or to the starburst beauty of the brain's Purkinje cells? "Most athletic" to the foot-soldier fibroblasts that do the heavy lifting to quickly form scars after injury, allowing our ancestors to sprint from danger or after prey? Or to the cells of the heart's sinoatrial node responsible for the electrical pulse that triggers every one of the around 2.5 billion heartbeats we experience throughout our lives?

Some of the cells aren't even human. A naturally occurring gut bacterium that digests fiber — usually from our diets, but it's not above chowing down on our intestinal lining when dietary fiber is scarce: "most likely to succeed"? A single-celled organism with

a dainty skirt that can transform into a shape-shifting multicellular colony at the drop of a hat: "most creative"?

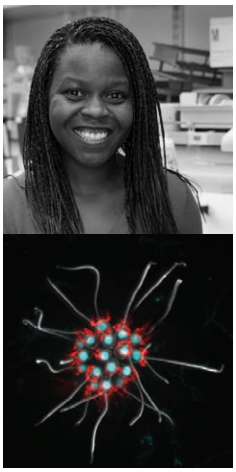
But while there's certainly room to quibble about each cell's specific category, what's clear is that every one of them deserves an overarching title of "researcher's pet." Each scientist spoke passionately about their favorite cell, often highlighting little-known facts or connections that have a vast impact on human evolution, development and health.

Florentine Rutaganira, PhD

Assistant professor of biochemistry and of developmental biology

Choanoflagellates

"We call them sperm with skirts," said Florentine Rutaganira of choanoflagellates — aquatic organisms that toggle between life as a single cell, scooping up and supping on bacteria with their distinctive collar surrounding a wiggly flagellum, and multicellular colonies that resemble tree-like chains, spherical rosettes and more.



A colony of choanoflagellates, the closest single-celled relatives to animals. The species is Barroeca monosierra. Nuclei are stained blue; flagella, white; and the collar structure, red.

"The cells themselves are really unique," Rutaganira said. "As someone who had previously only had exposure to mammalian cells, when I first saw them under the microscope I was like, 'This is the most insane thing I've ever seen.'"

Choanoflagellates are the closest living single-celled relatives to animals. An intriguingly large proportion of their relatively small genome is devoted to genes for protein kinases — molecules that play key intercellular signaling roles in mammals. Rutaganira launched her lab last year with the aim of learning whether and how the receptor protein kinases, which straddle the cell membrane, coordinate the choanoflagellate's ability to switch between one cell and many, and what that can tell us about how mammalian cells communicate.

Intriguingly, the shapes of colonies choanoflagellates form is governed by the type of bacteria to which they are exposed. "I've done this experiment probably 1,000 times," Rutaganira said. "You cohabitate these single-celled organisms with a specific type of bacteria, and you come in the next morning to find a beautiful set of colonies. It never gets old."

"Understanding this transition will give us better insight into what happens when things go wrong," Rutaganira said. "Cancer is essentially a breakdown in intercellular communication, and protein kinases are often mutated in cancer. Choanoflagellates are a good model system for studying these complex processes in a lab setting. And they're also cute."

Justin Sonnenburg, PhD

Professor of microbiology and immunology
and the Alex and Susie Algard Endowed Professor

Bacteroides thetaiotaomicron, or B. theta

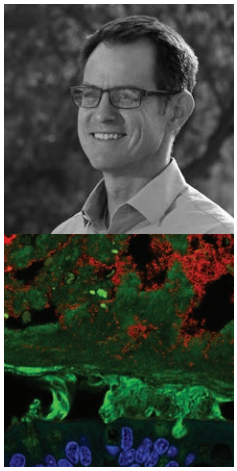
In the eyes of Justin Sonnenburg, B. theta is a magician. A magician in the form of a naturally occurring gut bacterium that munches on the indigestible fibers found in fruits, nuts and other carbohydrate-rich foods and transforms them into beneficial metabolites that keep our bodies running smoothly.

"There are probably hundreds or thousands of carbohydrate structures that we can't digest on our own," Sonnenburg said. "B. theta has many genes dedicated to digesting all sorts of fiber."

But if we don't eat enough fiber, the naturally occurring gut bacterium turns feral, feasting instead on the carbohydrate-rich mucus that lines the gut.

"Normally this lining keeps our gut microbes at a safe distance," Sonnenburg said. "As they say, good fences make good neighbors. But when B. theta starts digesting this lining, it may lead to inflammation and cause the gut lining to become leaky. Eating plenty of plant-based dietary fiber helps keep B. theta from eating us."

Sonnenburg studies the dynamics of the gut microbiome and whether diet or medical intervention can modulate its composition to prevent disease. B. theta was the first prominent gut bacterium to have its genome fully sequenced, in 2003, launching a full-scale study of the hundreds of bacterial species in our gut microbiome. Sonnenburg remembers the moment that B. theta's effect on the gut first captured his attention.



Bacteroides thetaiotaomicron (red) roams the gut, where it helpfully digests fiber. Without enough fiber to feed on, though, it can cause trouble by eating away at the gut's lining.

"It was 1996, and I read a paper in *Science* showing that if you colonized germ-free mice with B. theta, the lining of the gut began to produce a carbohydrate called fucose, which B. theta would then eat. It was almost like it was gardening the lining of the gut for its own food," Sonnenburg said. "This was one of a handful of studies around that time that made me think 'This is beyond science fiction.' It was amazing."

FLORENTINE RUTAGANIRA PORTRAIT BY ALISON YIN/HHMI, CELL IMAGE BY FLORENTINE RUTAGANIRA JUSTIN SONNENBURG PORTRAIT FROM STANFORD MEDICINE ARCHIVES, CELL IMAGE BY KRISTEN EARLE AND GABE BILLINGS

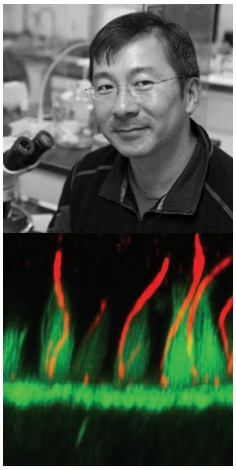
Alan Cheng, MD

Professor of otolaryngology and the Edward C. and Amy H. Sewall Professor in the School of Medicine

Hair cells of the inner ear

Deep inside your ear, past your eardrum, inside the shell-like cochlea, about 15,000 hair cells stand at attention. These cells sense motion, vibration and sound. Without them we couldn't hear our loved ones' voices, balance on one foot or thrill to the acceleration of a fast car. And when they're gone, they're gone.

"These cells are found only in the inner ear, and they don't regenerate naturally," Alan Cheng said. "They are critical to how we interact with our environment."



Filaments containing the protein actin (green) and filaments containing the protein alpha tubulin (red) bundle together in a hair cell. These cells, found in the inner ear, sense motion, vibration and sound.

Genetics, certain drugs, noise and aging all take their toll on hair cells. Cheng studies genetic reprogramming techniques to stimulate hair cell regeneration.

"Early in my career I fell in love with how hearing works and learned how little we can do to treat hearing loss, and I realized, 'Oh, it's the loss of these cells that is the major hurdle.' The interesting and fun part has been understanding how they work and what we can do to regenerate them." It doesn't hurt that the cells themselves are strikingly beautiful.

"They look like rows of statues on a checkerboard," Cheng said. "They stick straight up, all facing in the same direction. It's very organized and precise. And the cells themselves are gorgeous and elegant, like something you might find in an art magazine."

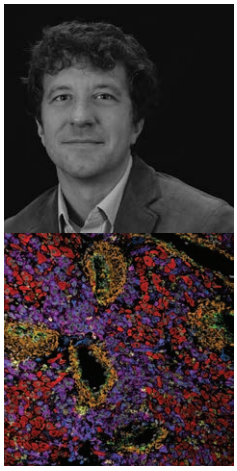
Michael Angelo, MD, PhD

Associate professor of pathology

Trophoblasts

Pregnancy is a tricky time. A fetus demands an ever-increasing amount of nutrients and blood flow from the mother. It also needs protection from a maternal immune system trained to attack genetically dissimilar cells.

Enter the trophoblast. These cells, with genetic material from both parents, arise from the outer layers of the developing embryo to form the placenta. As pregnancy progresses, trophoblasts burrow deeply into the uterine lining to remodel the maternal arteries that provide blood to the placenta to increase blood flow but not pressure. And they somehow do so without provoking an immune attack.



Trophoblasts (purple) have invaded the mother's endometrium and surrounded arteries, which they will eventually enter and transform into large vessels. Arteries appear as open areas bordered by green and yellow cells.

"These are dynamic, genetically foreign cells unlike any other cells in the body," Michael Angelo said. "They also contribute directly to two key properties of being human. The remodeling of these arteries is more extensive in humans than in nearly all other mammals and allows us to withstand the concentrated weight of a developing fetus when walking upright. The increased blood flow also allows for the longer gestations necessary to develop big brains before birth."

The uniquely human invasiveness of human trophoblasts also has a link to cancer. "That's what first got me hooked," Angelo said. "There's a striking correlation between the invasiveness of the placenta and the types of cancer an organism is likely to develop. That connection is pretty crazy. These are dynamic, genetically foreign cells unlike any other cells in the body."

Denise Monack, PhD

Professor of microbiology and immunology and the Martha Meier Weiland Professor in the School of Medicine

Salmonella Typhi

Most of us are familiar with the form of the Salmonella bacteria that cause severe food poisoning. While occasional nationwide produce recalls may have us side-eying our salad greens, there's an even more cunning relative that is, thankfully, rare in the United States: Salmonella Typhi. This species of the bacteria homes to and infects the lining of the gut where it spreads to deeper tissue, evading our immune systems and causing typhoid fever. Typhoid is endemic in India, Bangladesh and parts of Africa, and more than 200,000 people in the world die from it each year.

One reason Denise Monack thinks Salmonella Typhi is a "cool bug" is that it produces over 20 factors to manipulate the host's biological pathways to its advantage.

While some people experience a fever, abdominal pain and headaches

ALAN CHENG PORTRAIT BY NORBERT VON DER GROEBEN, CELL IMAGE BY TIAN WANG MICHAEL ANGELO PORTRAIT BY NORM CYR, CELL IMAGE BY INNA AVERBUKH DENISE MONACK PORTRAIT BY JESSICA MONACK, CELL IMAGE BY URFINGUSS VIA ISTOCK

that are hallmarks of the disease, others have no symptoms, unknowingly spreading the disease to others à la Typhoid Mary of New York in the early 1900s. These silent spreaders harbor Salmonella Typhi in clumps of white blood cells called granulomas lodged in the lymph nodes near the gut.

Denise Monack first became interested in Salmonella Typhi in the 1990s. "It has evolved a lot of tricks to escape the immune system."

One such trick allows it to not just survive being engulfed by immune cells called macrophages but also to thrive inside them, pulling and spinning biological levers and dials like a mad scientist to fashion a comfortable niche for itself inside these erstwhile killing machines.

"It is basically a tiny cell biologist, working from the inside of the macrophage," Monack said. "It produces over 20 factors to manipulate existing biological pathways to its advantage. It's a cool bug."

William Goodyer, MD, PhD

Assistant professor of pediatric cardiology and electrophysiology

Sinoatrial node cells

Nestled in the upper wall of the right atrium of the heart is a dime-sized, comma-shaped cluster of cells with arguably the most important job in the body: generating the electrical impulse that initiates every heartbeat. But these sinoatrial node cells are devilishly hard to identify with the naked eye.

"Damage to the node cells can be due to genetics or aging or diseases like heart failure," William Goodyer said. "But accidental damage during cardiac surgeries is not uncommon because surgeons can't see it and are therefore forced to estimate the location of the heart's conduction system."

A cluster of elongated cells in the heart known as sinoatrial node cells generates the electrical impulses that trigger each heartbeat. These sinoatrial node cells were isolated from a mouse.

If the cells are damaged, the only recourse is to implant an artificial pacemaker to keep the heart beating and the blood pumping.

"Many groups are trying to figure out how to reproduce these cells in a laboratory so that, in the future, we can repair damage by implanting a new sinoatrial node," Goodyer said.

But it's also important to try to prevent damage in the first place. In 2019, Goodyer mapped the entire electrical conduction system of the heart and developed dyes that can illuminate node cells and help surgeons steer clear.

Sinoatrial node cells are biological chameleons. They share many features with neurons, including their ability to generate and conduct electricity, which enables them to control the rhythms of the heart. Their unique characteristics proved a siren call early in Goodyer's career that he found impossible to ignore.

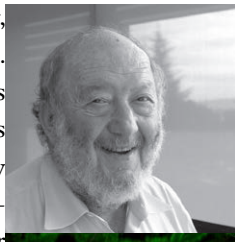
"I did my PhD research on the development of the pancreas," Goodyer said. "But I fell in love with the rhythm of the heart as a pediatric cardiology fellow, and it changed the trajectory of my career. It's a huge unmet medical need and a prime opportunity to help kids."

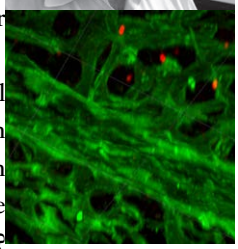
Irving Weissman, MD

Professor of pathology and of developmental biology, the Virginia and D.K. Ludwig Professor in Clinical Investigation in Cancer Research, and founder of the Stanford Institute for Stem Cell Biology and Regenerative Medicine

Hematopoietic stem cell

Imagine a child's drawing of a tree, trunk springing from the ground and larger limbs giving way to ever smaller branches and leaves. As an allegory for human development, the roots of the tree would represent a fertilized egg, the trunk is the embryonic stem cells. Moving upward, the different branches can be thought of as the various organs and tissues in the body. Nestled in every fork are tissue-specific stem cells dedicated to developing and maintaining their tissue or organ.





A bone-marrow section in which the blood-forming, hematopoietic stem cells (red) attach to the outer side of blood vessels (green).

Irving Weissman was a high school student in Montana in the 1950s when he began researching the biology of skin transplantation. How does the immune system identify and reject foreign cells? Where does the immune system even come from?

Decades of research, most at Stanford Medicine, led in the 1980s to Weissman's identification and isolation in mice and humans of the hematopoietic stem cell — the stem cell that can develop into all types of blood cells.

These stem cells are special. Their line of descendants is vast, and the relationships among the branches are far more complicated than depicted in a child's drawing. But Weissman dedicated his career to untangling these family ties to under-

WILLIAM GOODYER PORTRAIT FROM STANFORD MEDICINE ARCHIVES, CELL IMAGE COURTESY WILLIAM GOODYER IRVING WEISSMAN PORTRAIT BY NORBERT VON DER GROEBEN, CELL IMAGE BY JINYI XIAN

stand normal development and cancer — meticulously tracing back from leaf to branch to limb to trunk to identify the earliest mutations found in blood cancers like leukemia.

Weissman's decades of discovery highlight the importance of tissue-specific stems cells and have laid the foundation for new treatments for cancer, blood diseases and organ rejection.

"Stem cell biology is just taking off," Weissman said. "Every tissue has stem cells if you just look deeply enough. It's 2024 and we are just starting to realize the full clinical impact of these early discoveries."

"I always thought I should just follow my nose," Weissman said, "and if you get a result that is a little unexpected, explore it. I didn't start off focusing on hematopoietic stem cells, but I got there pretty fast. I was lucky."

Jennifer Raymond, PhD

Professor of neurobiology and the Berthold and Belle N. Guggenhime Professor II

Purkinje cells

Science is primarily a visual medium. Researchers peer at cells through a microscope, pore over stacks of data and observe the behavior of laboratory animals. Jennifer Raymond does all that. But she also gets to hear her favorite cells hard at work, their staticky cadence recorded through an electrode changing as the specialized neurons funnel electric impulses between the cerebellum and other brain areas.

"The cerebellum used to be thought of as controlling mostly motor skills, with the bulk of thinking and cognitive processing tasks assigned to the cerebral cortex," Raymond said. "But recently our understanding of roles played by the cerebellum has just exploded — language, fear, anxiety, navigation, you name it."

Perhaps not surprisingly, the cells are hyperactive compared with many of the brain's neurons — firing off as many as 100 impulses per second. They are also much larger and flatter than their peers, stacking their cell bodies atop one another to create an intricate — and, frankly, drop-dead gorgeous — signaling network of branching dendrites that gather input from about 100,000 to 200,000 other cells. In comparison, "normal" neurons hear from only about 10,000 other neurons.

This illustration of a Purkinje cell is part of a larger drawing on a whiteboard in Jennifer Raymond's lab. Postdoctoral researcher Negar Asadian drew it for Raymond on her birthday.

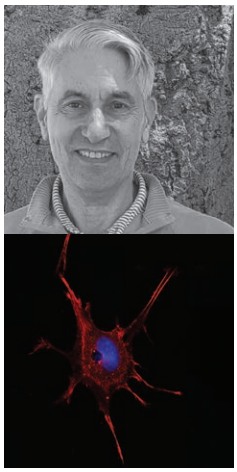
"Because everything that happens in the cerebellum is funneled through Purkinje cells, we know we are collecting all the output from the cerebellum when we place an electrode in this hub," Raymond said. "It's a simple circuit but, because it is highly evolutionarily conserved, we know it must reflect a fundamentally important type of computation and learning. It's so exciting to hear how the frequency of these impulses increase or decrease in real time as an animal learns."

Michael Longaker, MD

Professor of surgery and the Deane P. and Louise Mitchell Professor in the School of Medicine

Fibroblasts

"I call it scar wars," said Michael Longaker, of his fascination with fibroblasts — cells that secrete the proteins that fill the spaces between cells in normal development and wound healing. In humans, the latter often leaves a scar that can interfere with a tissue's normal function.



The image above shows an activated fibroblast in culture. Fibroblasts are the cells that fill spaces between cells in normal development and wound healing.

Humans are unique among mammals in their propensity to form large scars, but even that must be learned. Human embryos don't scar after fetal surgeries until the last trimester of gestation — a fact that piqued Longaker's interest as a postdoctoral scholar in the 1980s. Over the decades, he has studied how fibroblasts make scars, and whether there is a way to prevent scarring or remodel existing scars into normal tissue.

"Fibroblasts sense many aspects of their environment, including mechanical forces," Longaker said. "If we block that ability, we can heal without scarring."

Overenthusiastic fibroblasts are responsible for fibrosis, which is the development of fibrous connective tissue in response to injury. Longaker estimates that nearly half of all deaths each year are caused by some form of fibrosis in the guise of heart disease, lung disease, liver cirrhosis and more.

"Fibroblasts are fascinating cells that most people ignore," Longaker said. "In a way, they are foot soldiers, responding to physical and biological cues around them. But they have an enormous impact on human health." **SM**

— Contact Krista Conger at kristac@stanford.edu

JENNIFER RAYMOND PORTRAIT BY ELLA JAUREGUI, CELL IMAGE BY NEGAR ASADIAN MICHAEL LONGAKER PORTRAIT BY VIRGINIA FORD, CELL IMAGE BY ANNAH MORGAN

The worth of ‘worthless’ ideas

A connoisseur of cells explores the wonder

PHOTOGRAPHY BY
MANU PRAKASH, Prakash Lab
ILLUSTRATION BY
REBECCA CONTE, Prakash Lab

and beauty of the world

By Jennie Dusheck

MICROSCOPIC MYSTERIES UNFOLD

One question Manu Prakash is investigating is whether viruses cause debris drifting down from the ocean's upper waters to clump and fall more quickly (left). A recent discovery: a cell that uses an origami strategy to rapidly extend and contract (this page)

**‘Science is about understanding. …
So when you see something beautiful, there is something lurking behind it. It’s beautiful because of the mystery.’**

UNCOVERING LIFE’S DEEPEST SECRETS

On a 30-day cruise on the North Pacific Ocean, bioengineer Manu Prakash and his team sought out one-celled organisms they hope hold answers to marine viral pandemics. Below: the sampling apparatus

MANU PRAKASH, PHD, CAME INTO FOCUS on my laptop for a Zoom meeting. It was early June, and blue and white waves behind him caught my eye. Bright sun lit his face and jacket, and wind buffeted his curly hair. Then the blue horizon behind him slowly tilted, and I felt a hint of sea sickness.

For Prakash, it was the first week of a 30-day cruise off the coast of Northern California. In coming hours and days, the U.S. Navy research vessel Kilo Moana would follow the California Current, allowing the researchers to drag giant, ultrafine nets as deep as 1,000 meters and pull aboard ocean microorganisms. The microscopic beings wouldn't survive long out of the ocean, often dying in just a few hours, so Prakash and his colleagues had to hurry them to a shipboard laboratory for study. Prakash, an associate professor of bioengineering, was searching the North Pacific for extraordinary one-celled organisms that tell surprising stories about how life solves puzzles, survives and thrives in the deep dark ocean.

Many people believe research should always be in the service of new technology and the public good, to produce, for example, a vaccine or a better battery. Prakash rejects that idea out of hand. There is science, he said, and then — maybe — applications will come later. He's not against utility by any means, but he believes utility is not the point of science.

"Science is about understanding. It's centered around being able to explain this world. And so when you see something beautiful, there is something lurking behind it. It's beautiful because of the mystery. It's beautiful because it's almost outlandish."

That beauty and outlandishness fascinate Prakash. And he wants that joy, fascination and surprise for scientists of the future. Inspired by recreational mathemetics, he'd like to establish "recreational biology," a science of mysteries and paradoxes.

In June, Prakash was the Stanford lead on an ocean expedition funded by the National Science Foundation to study both marine pandemics and how viruses promote carbon sequestration in marine environments.

Back out on the research vessel, Prakash was staggering around with his laptop. A hundred seconds into the interview, he interrupted himself. "Oh, my goodness! This is insane right now!"

"What's happening?" I asked, safely at my desk.

"I think we have 12-foot waves and they are generating this massive plume. This boat is very special. It is a double-hulled boat. And the waves get trapped and it reverberates like an angry dragon!

"Do you hear the rumble at the back?" He paused. "Right there?"

I didn't. The Zoom app was helpfully blocking out the roar of the waves smashing between the two hulls of the big research catamaran, so I couldn't hear any of it. Unsatisfied, Prakash quickly recorded the roar on his phone and later emailed the recording to me.

CRUISING FOR ANSWERS

While studying marine pandemics and how marine viruses promote carbon sequestration, Prakash and his team came to understand that some deep-sea organisms might help keep climate change at bay. Above: Prakash (left) and Hannah Rosenblatt, PhD, on the Kilo Moana

Prakash, a fellow of the John D. and Catherine T. MacArthur Foundation, was embarking on his 17th voyage to study the strange and beautiful microorganisms that populate our oceans. Also aboard the 186-foot catamaran were four members of his lab from Stanford University, collaborators from around the world, plus a crew of 20 to run the ship and "keep us safe," as Prakash put it.

Prakash has an eclectic range of interests and inventions, including corralling tiny bubbles to execute computations in much the same way as a traditional electronic computer and a paper microscope that's cheap, portable and durable enough to be invaluable in any village in the world. Lately his research focus has been different kinds of protists — one-celled organisms that thrive in all kinds of damp places, from warm, shallow ponds to the near-freezing depths of the Pacific Ocean. Like big game hunters of the microbial world, Prakash and his students have snagged an amazing number of trophies.

In the past five years alone, these include:

• Tiny starfish larvae that can stir seawater in one pattern to bring nearby food closer and in another pattern to propel themselves toward better feeding grounds (Patiria miniata).

• Bacteria living in biofilms that feed a whole colony by gliding in spiral patterns to create flows of nutrients (Oscillatoria).

• Juvenile sea cucumbers able to form beautiful patterns of crystalline rocks in their skin (Apostichopus parvimensis).

• Bioluminescent cells that make half-kilometer-long journeys in the open ocean by increasing their volume six times, like tiny balloons floating through the water (Pyrocystis noctiluca).

• Colonies of cigar-shaped aquatic cells that can talk to one another by way of pressure waves and then, in unison, contract to release toxins that drive away predators (Spirostomum).

ANOTHER OF PRAKASH'S LONGTIME passions is the one-celled Lacrymaria olor. Under a microscope, the teardrop-shaped microorganism appears innocuously hiding among debris. But when it's ready to hunt, it whips out a long neck — up to 30 times the length of its body — and nabs other protists. Over and over, it extends and retracts its astounding neck while ambushing and engulfing prey. It would be as if your house cat suddenly extended a paw 50 feet down a hallway to seize a mouse.

How "Lacry," as Prakash calls the cells, could extend their necks so far at first seemed inexplicable. All cells are enclosed by a cell membrane that is flexible but not stretchy. Pull on it hard enough and it will tear like paper. That Lacry's cell membrane could stretch to 30 times the length of the cell was impossible. And there was another surprise. Lacrymaria olor's neck doesn't get thinner as it stretches out, as a rubber band would; it gets fatter, enabling it to engulf its often relatively hefty prey.

How was a single cell able to do this? For Prakash and his graduate student Eliott Flaum, one clue was a helical pattern of pleats just under the cell's membrane. When the helix of pleats unfold, the cell's neck extends — and with a girth that can accommodate morsels nearly as large as Lacry itself.

Lacry's beautiful, pleated neck is made of a helix of molecular filaments, capable of folding and unfolding, closing and opening. Prakash first realized how Lacry evolved the capacity to pull off this magic trick while he was on a trip to Japan with his kids. Lacry, he suddenly realized, had mastered the ancient Japanese art of folding paper, or origami.

Lacry's neck is the first known example of "cellular origami." As Prakash said, "It's the first time a single cell has been shown to 'invent' a new kind of origami to achieve this shape-shifting dance." Flaum's and Prakash's discovery inspired the lab to begin building a pleated surgical robot that can expand 100 times in length to reach far-flung corners inside the body.

In June, Flaum's and Prakash's seven years with Lacrymaria olor paid off with a paper in *Science* magazine and a beautiful image of Lacrymaria on the cover. By then, though, the entire lab crew was at sea, pulling miracles from the deep.

Whatever seems beautiful, intriguing or paradoxical in the microscopic world becomes Prakash's joyful obsession. For him, more than for many scientists, science is so much fun he thinks of it as play. But the joy comes with a tinge of feverish haste and a portent of loss. For one thing, cells don't live long out of the water. "When we're on board, I'm gonna throw a net out, and there is a fleeting moment of, I would say usually two or three hours, that there's a possibility that I might have these deep-water cells alive and I can watch them. And then it's gone, and I have to wait for that next fleeting moment."

One extraordinary finding this summer for Prakash was a single-celled organism the size of a small grape, the radiolarian Cytocladus, which had been last documented in 1898 during the Valdivia expedition and never before photographed or closely studied. Prakash spotted the "fuzzy grape" by chance among a haul of tiny shrimp and other plankton pulled from 600 meters below the Pacific's surface. Anxiously, he kept it alive for two days to study and then reluctantly dunked it in liquid nitrogen, flash freezing it for posterity. Weeks later, after obsessively filtering nearly 100,000 tons of water — as much water as might flow over Yosemite Falls on a spring morning — he found three more of the cells. With his first safely frozen, those three precious living cells occupied him day and night, all the way to the end of the cruise. Finally, as the Kilo Moana sailed under the Golden Gate Bridge into San Francisco Bay, Prakash

**‘I’m gonna throw a net out,
and there is a fleeting moment of … possibility that I might have
these deep-water cells alive and I can watch them.
And then it’s gone, and I have to wait
for that next fleeting moment.’**

THE LAB ABOARD THE KILO MOANA
Working at night in a lab equipped to study and capture images of microscopic organisms. Red light is less likely than full spectrum to damage living cells.

dropped the three extraordinarily rare cells into liquid nitrogen.

Not knowing if he or, really, anyone in the world would ever again see these remarkable deep-sea organisms, he sailed into the bay with a great sense of sadness.

Besides cells' short lives out of water, there's another reason for Prakash's persistent sense of haste. A senior fellow at the Stanford Woods Institute for the Environment, he's aware that many of the planet's most miraculous organisms are literally going extinct before we even know they are there, let alone know enough about them to appreciate their beauty and inventiveness.

At least some of their activity is deeply important to the entire world. On board the Kilo Moana this summer, Prakash came to understand that some of the deep-sea organisms he is studying are accelerating ocean carbon sequestration. Organisms we are barely aware of are helping keep climate change at bay.

SAVING THE WORLD, ONE CARBON ATOM AT A TIME

WE ALREADY KNOW THE WORLD'S oceans absorb vast amounts of carbon dioxide, keeping Earth's atmosphere from heating more than it already is. We also know that photosynthetic plankton in the ocean use sunlight to snatch carbon atoms from carbon dioxide and link the atoms into long carbon chains to form carbohydrates, fats or proteins — the building blocks of life. All those chains of carbon make up the mass of every cell.

In the ocean, once individual cells and other organisms die, they slowly sink to the bottom, so slowly that it might take a year for the carbon in a single cell to fall the 4 kilometers to the bottom of the sea. There it lies inert as sequestered carbon.

But cells often clump together and plummet through the sea much faster than individual cells ever could, reaching the bottom in weeks instead of months. "And that," said Prakash, "is how 30 to 40% of anthropogenic carbon is actually being taken up by the oceans. The ocean is both a great savior and a gigaton technology for carbon sequestration that already works."

But what makes the cells clump together?

Enter viruses! That's right, there are viruses in the ocean, just like on land, and they can infect everything from marine mammals to one-celled organisms.

Virus experts already knew that when viruses infect a cell, they need to be able to stick tightly to the cell. (When the infected cell bursts open later to release new viruses, those viruses need to *not* stick to the cell.) It was reasonable for the team to wonder if infected cells in the ocean might be stickier and more likely to form rapidly falling clumps, known as "marine snow."

"So that," said Prakash, "is what our expedition was about. We were studying how viral infection impacts the formation of these clumps." Prakash and colleagues tentatively hypothesize that stress induced by a viral infection might cause more snow formation.

Meanwhile, Prakash's lab has long studied other radiolarians — in part entranced by their breathtaking beauty and intricate crystalline structures. As fate would have it, many radiolarians turned up in the Kilo Moana's nets this summer. Because radiolarians have stone crystal skeletons, they are both lovely and incredibly heavy, at least for one-celled organisms. When they form marine snow, their weight and density make them fall even faster to the bottom of the sea, accelerating marine carbon sequestration. Prakash is excited to begin writing a paper describing how that works.

THE ART OF OBSERVATION

FOR A CELL BIOLOGIST, Prakash is a surprisingly enthusiastic advocate for field work. He and his lab members are always packing boxes of microscopes and flying these tools to expeditions around the world. When researchers study cells in the lab, he mused, "We strip away the relevant questions. We don't even know what questions to ask, right?" But out on the Pacific Ocean, with 12-foot waves tossing catamaran and researchers, the ocean environment speaks loudly. "Being here, knowing what the water looks like, I can think about, 'How the hell does this delicate, fragile cell survive in an ocean that looks like this?" Being out on the ocean, staring at living cells fresh from the wild sea, is bound to suggest thoughts and questions that might not come to mind in a quiet laboratory.

Prakash recently taught a Stanford University course titled The Art of Observation. "For a while now," he said, "I've been thinking about this notion that observation comes before ideas, even before any experiments."

The scientific method, as typically taught, emphasizes hypothesis testing, rushing past simply observing the world, rushing past just wondering why or how. Yet virtually all science begins with observing and mapping. Before the great discoveries of modern astronomy, we mapped objects in the sky and noticed, for example, that stars and planets were different. Only then could we begin to ask why. The first thing an ecologist wants to know about an ecosystem is what lives where. Likewise, geneticists spent years mapping thousands of genes on the chromosomes, even when they had no idea what the genes did.

Observation comes naturally to us. A 3-year-old will stare in wonder at a rabbit disappearing into a brush pile. But most adults learn to focus on our endless chores, and we often block out the wonders surrounding us. "The purpose of the course was partly to help students understand what observation means and the history of observation, but also, just practically, to teach

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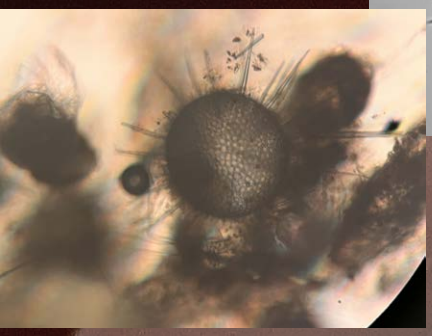
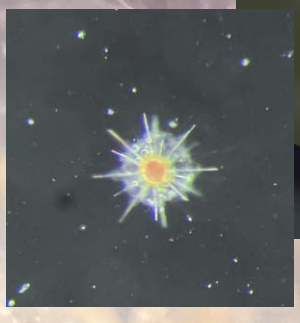
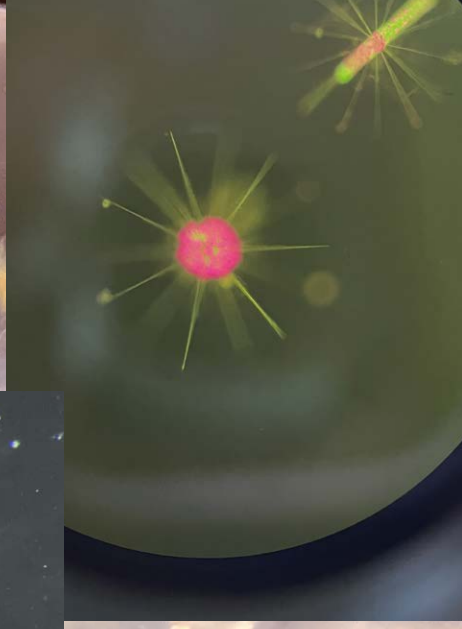


A group of radiolarians from the deep sea, California Current, Pacific Ocean

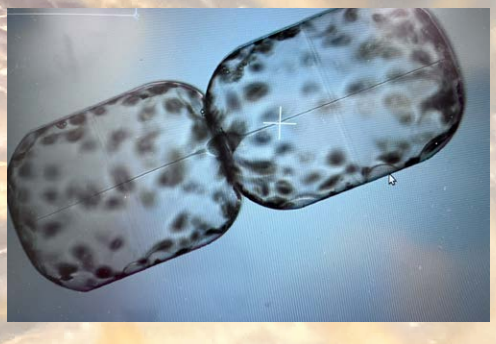
An acantharian, genus Lithoptera, collected in the Mediterranean surface waters near the coast of Naples, Italy





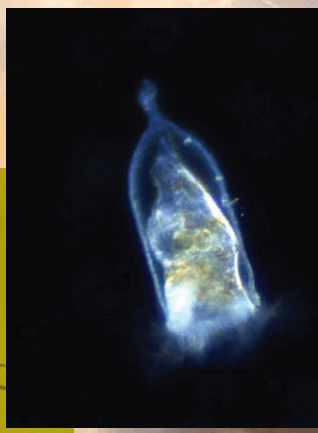
Left and above: Fluorescent acantharians collected in the Mediterranean near Villefranche, France

Tiny creatures drawn from the seas



A dividing diatom, genus Ethmodiscus, collected from surface waters in the Atlantic close to Bermuda

A motile tintinnid collected in the Monterey canyon



Background: A rare radiolarian, Cytocladus. This large cell, 10 centimeters across, was collected in June 2024 from the deep sea, California Current, Pacific Ocean.

A small acantharian, genus Lithoptera, with growing spicules, collected in the Mediterranean near Villefranche, France

why is a common gene variant bad for your brain?

The answer offers clues for a new kind of Alzheimer's drug

BY BRUCE GOLDMAN

PHOTOGRAPHY BY TIMOTHY ARCHIBALD

Alzheimer's disease's suspected causes are diverse, and its cures are, today, nonexistent. What's all but certain is that many who have the mental chops to wade through a detailed article about the disorder's drivers and demographics will nevertheless succumb to it someday.

With no cure available, despite numerous attempts to find one, researchers are looking down new roads for treatments. A recent discovery by Stanford Medicine neurologist Mike Greicius, MD, may help clear one of those roads for faster passage.

Gummy clumps, plaque-attack drugs and luck of the genetic draw

WHILE ITS MOST VISIBLE OUTWARD symptoms include memory loss and confusion, a key defining feature of Alzheimer's at the molecular level is the overabundance in patients' brains of a substance called A-beta, which aggregates into gummy clumps, collectively called amyloid plaque, situated between their nerve cells. These plaques begin showing up in the brain years before mental decline becomes noticeable.

So, hopes were high for a class of new drugs based on the idea that amyloid plaque is the smoking-gun cause — or at least one cause — of the slow, but steady, crumbling of memory that's one of Alzheimer's behavioral hallmarks.

But simply removing amyloid deposits, or plaque, from the brains of people with Alzheimer's disease hasn't been the game changer some thought it would be, leaving more than 6 million people with this condition and their caretakers and physicians looking for alternative treatments.

The recent failure of a slew of "plaque attack" drugs to provide clinically significant improvements in Alzheimer's patients' condition puts the spotlight on scientists who've been thinking outside the amyloid-plaque box. One of them is Greicius, who recently spearheaded a genetics study described in a paper published in January in *Neuron*. The study focuses on variants of a gene called APOE and ventures into the realm of personalized medicine: A drug that works for someone carrying one variant may not necessarily be effective against people carrying other variants.

At least one-fifth of all people on the planet are carrying a gene variant that predisposes them to Alzheimer's. Known as APOE4, it's one of four versions of a gene called APOE. Which APOE version you're carrying makes a big difference in your Alzheimer's risk.

Most people whose genome includes a copy of APOE4 don't wind up with an Alzheimer's diagnosis. But people with a single copy are at double or triple the risk for Alzheimer's compared with people who have two copies of the most common variant, APOE3. Those with two copies of APOE4 (one inherited maternally, the other paternally) develop Alzheimer's at more like 10 times the frequency that people with two APOE3 copies do.

"About 25% of people of European ancestry are APOE4 carriers, but this variant is present in 50% to 60% of Alzheimer's patients with European ancestry," said Greicius, the Iqbal Farrukh and Asad Jamal Professor and a professor of neurology and neurological sciences.

(A third, less-common variant, APOE2, is actually protective in comparison with APOE3. The fourth, APOE1, is so rare that fewer than 10 people carrying it have ever been identified.)

Of the people who develop Alzheimer's disease, the ones with an APOE4 copy tend to start showing symptoms

MIKE GREICIUS, MD

soonest — about five to 10 years earlier, on average, than those with two APOE3 copies.

"APOE4 starts the ball rolling well before it would normally start," Greicius said.

The unwanted connection

THE APOE4 VARIANT was first recognized in the 1970s as a risk factor in cardiovascular disease. In the early 1990s, studies directed by a Duke University neuroscientist, the late Allen Roses, PhD, showed that APOE4 also increased Alzheimer's risk. At the time, researchers were mainly laser-focused on amyloid plaque and A-beta — the protein snippet that aggregates to form these brain deposits — and were skeptical about any APOE4 connection to Alzheimer's. But now it's written in stone.

Yet, three decades later, nobody really understands why APOE variants differentially affect Alzheimer's risk. It's not even clear what the gene's protein product (designated "ApoE") does in the first place. Be that as it may, genes similar to APOE have been identified in all animals from amoebas to mammals, so you know it must be doing something important — it may be doing different things in our brains than what it's doing elsewhere in our bodies.

It is known that ApoE shuttles various fatty substances within and between cells, both inside and outside the brain, like passengers on a bus. And it's suspected to be involved in our immune response to infections, as some of the fatty acids it shuttles have antimicrobial properties.

That antimicrobial capacity, if it's for real, could help explain an intriguing ethnic distribution of APOE variants, whose prevalence and harmfulness seem to follow opposing geographic gradients.

Your likelihood of carrying APOE4 depends, in part, on where your ancestors came from. At least one copy of APOE4 in one's genome shows up in roughly 1 in every 3 people of African descent, for example; about 1 in 4 people of European descent; and a scant 1 in 10 (or even only 1 in 20, according to some research) Japanese people.

But APOE4 *risk* runs in the opposite direction. Among those of African descent, carrying a single APOE4 copy is barely observable as an Alzheimer's risk factor. For someone of European descent, having a single copy of APOE4 in one's genome translates to two to three times the risk of having two APOE3 copies. And Japanese people with a single copy of APOE4 are at five times the risk for Alzheimer's disease as their compatriots with two APOE3 copies. Having two APOE4 copies in your genome always boosts your risk, but much more so if you're Japanese, less so if you're of African ancestry.

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AI steps into the looking glass with synthetic data

Medical data scientists are using generative AI to create new data from scratch

BY ANDREW MYERS

ILLUSTRATION BY PETRA PETERFFY

A few years ago, generative artificial intelligence image technology — including Dall-E and Stable Diffusion — first emerged, allowing anyone to type in whimsical text prompts and produce original images, seemingly from thin air. Intrigued and curious, a group of students and postdocs training under Stanford Medicine radiologist Curtis Langlotz, MD, PhD, and computer scientist Akshay Chaudhari, PhD, decided to see what generative AI might do if asked to create a chest X-ray from scratch. So, they gave it a go.

"What they got back looked a little bit like a chest X-ray, but it really was not anything close to what we would think of as a clinically realistic X-ray," Langlotz said. "Then the students asked themselves: Can we make it better?"

That thought experiment led Langlotz, professor of radiology, medicine and biomedical data science, Chaudhari, assistant professor of radiology in the department's integrative biomedical imaging informatics section — and several of their students — to create the RoentGen text-to-image generative model for X-rays. The RoentGen model creates lifelike and convincing X-rays from medically relevant text prompts. Chaudhari and Langlotz published a paper describing RoentGen in August in *Nature Biomedical Engineering*.

"I can type in, 'Moderate bilateral pleural effusion and mild pulmonary edema,'" said Langlotz, offering up a concrete example he might ask in the course of his everyday work. "RoentGen will produce medically accurate X-ray images that are nearly indistinguishable from those taken from humans," even to the trained eyes of medical professionals.

Data from scratch

ROENTGEN is a glimpse into the future of medical AI in which a considerable share of data used to train new AI models are synthesized, and those models in turn churn out synthetic data to solve problems. This might include helping visualize inoperable cancers or sorting through potential drug candidates to identify only the most promising for further study.

The term synthetic data may sound like an oxymoron or even an impossibility, but to medical AI experts it is very real and very promising, even while introducing ethical and scientific gray areas.

Leaders of this emerging field say that synthetic data could enhance medical AI, helping flesh out incomplete datasets, supplementing data from key demographics to eliminate bias and addressing privacy concerns of patients who fear AI could reveal their personal medical histories — all in a single stroke.

Yet many leaders also urge a go-slow approach as the field evolves, saying medicine must wrestle with synthetic data's risks before it is too late. Over-reliance on synthetic data could breed a false sense of confidence, said Tina Hernandez-Boussard, PhD, an associate dean of research at the School of Medicine. Hernandez-Boussard and participants in Stanford's Responsible AI for Safe and Equitable Health (RAISE Health) initiative are among those helping researchers interested in synthetic data think deeply about the ethical and societal implications of this new field.

We talked with a few Stanford Medicine researchers who are tapping into the potential of synthetic data about how it is being used now, where it might lead and what remedies the field might have at its disposal to manage risks as this new frontier in medicine evolves.

X-ray vision

WHILE ROENTGEN is impressive in its ability to turn text into medically accurate X-ray images, Chaudhari noted that it is

so much more. RoentGen's synthetic X-rays could, for instance, be used to correct bias or address patient privacy concerns, he said. If a dataset lacks adequate representation of women, RoentGen can generate synthetic X-rays of female patients to fill gaps in the data. Similar approaches could address gender, socioeconomic, geographic, age and other demographic inequities. And because the images are not of any living person, synthetic data could help circumvent patient privacy concerns.

The generative model could also solve another challenge for medical AI — labeling — the time-consuming and expensive process done by highly trained medical professionals of annotating images to, in essence, tell AI what it is looking at. With patient permission, the RoentGen team trained their algorithm using a public library of more than 200,000 digitized X-rays, matching them against written electronic patient medical records to label their X-rays.

"We collected retrospective data from a hospital where the images already existed and where a trained radiologist had already written everything about the image," Chaudhari said. "No additional or specialized human labor was needed to create that generative model. Because we leveraged what's in the hospital already, it's the closest that we can get to having a free lunch labeling-wise."

Seeing the unseeable

DRUG DISCOVERY is another promising application of synthetic data and could be a boon in the study of rare, inoperable cancers where existing data is scant and biopsies can be dangerous or impossible to conduct. In one of many avenues of his cancer research, Olivier Gevaert, PhD, associate professor of medicine and of biomedical data science, studies an elusive type of inoperable, untreatable cancer of the brainstem.

Gevaert is using the generative powers of AI to test the effectiveness of new drugs on these cancers. With other, more accessible cancers, drug efficacy is verified by taking tissue samples from the patient to see if the drugs are killing tumors. At the brainstem, however, getting such biopsies is not possible. Instead, Gevaert uses generative AI to synthesize the biopsy slides from genetic data.

His latest model is RNA-CDM, which allows cancer researchers to manipulate the genes in a patient's RNA profile computationally, turning certain genes on and others off on a computer rather than in a person. RNA-CDM then creates synthetic biopsy slides, simulating the effect of new drugs on the unreachable, unseeable cancer. There are no real drugs being tested, no side effects for patients and no invasive biopsies necessary.

"Imagine if we now do this for all genes in the human genome and all drug candidates," Gevaert said. "We can do computer-based experiments and rank results according to what the investigator wants to see in the images, that is ... to see dead tumor cells ... and quickly sort through drug candidates." He and his coauthors described the method and how they tested it in *Nature Biomedical Engineering* in March.

Other targets, other applications

CANCER-KILLING DRUGS are but one avenue of drug discovery benefiting from synthetic data. James Zou, PhD, associ-

ate professor of biomedical data science, recently developed an AI model that can generate and reason about synthetic small molecules that have never been seen in nature. He used this approach to design potential new antibiotics at a time when antibiotic-resistant bacteria are a major concern for the medical community.

Using his model, Zou designed compounds to kill the bacterium Acinetobacter baumannii, a major source of drug-resistant infections. The outcome was not one or even a handful of new candidates, but 58 potential antibacterial drugs.

Zou's team then had those candidates manufactured for testing in lab mice. Six molecules proved to have low toxicity while showing promising antibacterial effects on A. baumannii and other pathogens. Zou and his collaborators described the approach in *Nature Machine Intelligence* in March.

Along another direction, Zou is using synthetic data to increase access to a promising but expensive new imaging technique that can analyze cancer cells and their immediate environment. The technology, CODEX, can detect 50 to 100 biomarkers in a tissue sample at once — each one a potential target for new drugs. "The technology is powerful, but it is slow and costs thousands or tens of thousands of dollars for a single patient, limiting clinical applications," Zou said.

Zou's answer is 7-UP, a fast, inexpensive synthetic approach that expands the data obtained through a less powerful imaging technology: multiplex immunofluorescence, or mIF. From tissue stained with just seven biomarkers and imaged via mIF, 7-UP builds a robust picture of 40 or more additional biomarkers that can be used to classify cell types and predict patient survival from various drug interventions.

"The generative AI does something almost like virtual reality for cancer, turning low-resolution data into this very rich data visualization on the computer. But it only costs a few dollars per sample," said Zou, who co-authored an article on the strategy in *PNAS Nexus* in June 2023. "It puts this promising technology within reach for more clinicians and researchers than ever."

Risks and rewards

PROMISE ASIDE, the concept of synthetic data seems risky to many. Hernandez-Boussard and her collaborators, including Arman Koul, a Stanford medical student, and Deborah Duran, PhD, senior adviser to the director at the National Institute on Minority Health and Health Disparities, are developing a framework to guide synthetic data research through an ethical and scientific minefield. The framework highlights the considerable risks while offering a measured pathway forward.

Over-reliance on AI could lead to what they call synthetic trust — a false sense of confidence in the models. Synthetic data could perpetuate biases rather than lessen them and produce nonexistent correlations that might lead to model degradation and misrepresentations that harm patients, they said.

"Generative AI is shown to preserve and, in some cases, worsen biases and inaccuracies in datasets," said Hernandez-Boussard, professor of medicine and of biomedical data sciences and surgery.

"We think a go-slow, cautious approach is warranted in using synthetic data to train clinical algorithms. We must ensure data integrity, fairness and transparency to promote equitable outcomes of all sectors of society in health care applications."

Synthetic data advocate Zou does not disagree and counseled vigilance in face of the risks. "We want to be extremely careful in evaluating the quality and potential biases in the synthetic data," he said. "It's really important for us to rigorously evaluate our models. Ask: Do I get to the same final outcomes and insights as if I would have done the same analysis on real data alone?"

"While we can use synthetic data for pretraining medical AI models," Chaudhari said, "it is critical to evaluate the performance of our methods on real datasets to understand what current gaps synthetic data can minimize and what gaps they maintain or even exacerbate. There is no shortcut to robust evaluation and validation."

Ground truths

LANGLOTZ CONCURRED. He is a co-lead of the faculty research council for the RAISE Health initiative — a collaboration between Stanford Medicine and the Stanford Institute for Human-Centered Artificial Intelligence to encourage ethical and responsible use of AI in biomedical research, education and patient care. The initiative, launched last year, is convening AI experts, stakeholders and decision makers to explore what it means to bring the technology into the medical realm and to define a structured framework for ethical health AI standards and safeguards. It is also curating high-quality tools and datasets to help guide ethical medical AI development.

"People have made very strong conclusions about the utility of synthetic data, but they don't quantify the quality of their underlying generative models," Chaudhari added, pointing out that an over-reliance on synthetic data produces a phenomenon known as model collapse. "If you train a model using real data and then use that model to create lots of synthetic data, only to train yet another model on the synthetic data alone, over time your model just falls apart."

To verify RoentGen's performance, for instance, Langlotz and Chaudhari asked two radiologists, one with seven years of experience reading chest X-rays and the other with nine, to conduct an audit of RoentGen's output. Those professionals reviewed and rated both real patient and synthetic X-rays for quality and accuracy. Additionally, they gauged RoentGen's alignment with highly specific medical language and concepts.

In reviewing those evaluations, the researchers found that the greatest uptick in classification performance was achieved when models were trained on a combination of real and synthetic data. They also noted that keeping humans in the evaluation loop is critical to improving results. Bottom line, Langlotz said: "The real test of any model is in whether your model gets at the ground truth. RoentGen does that."

Future directions

THESE STANFORD MEDICINE researchers point to several promising research avenues synthetic data might open, the most discussed being the "digital twin," a computational facsimile of a given patient upon which drugs and other interventions could be performed in silico — on the computer — without risk to the patient.

"We could run simulations on these digital twins or against disease models to rapidly figure out insights that can then be used to improve results for the real patients," said Zou, who is among several Stanford Medicine researchers, including Gevaert and Hernandez-Boussard, developing digital twins.

Another potential avenue of the future is the use of "synthetic arms" to supplement and speed clinical trials. Zou noted that pharmaceutical companies are already expediting drug discovery with digital surrogates. Instead of gathering 100 treatment patients for a trial, plus 100 controls, synthetic arms modeled on real patients from previous studies might be substituted for a large number of the controls, reducing time, effort and cost. "If you use 50 real controls and 50 digital arms, it could cut trial costs by a quarter," Zou pointed out.

On the potential of digital twins and synthetic arms, all the interviewed researchers concur — though Hernandez-Boussard does not classify digital twins or synthetic arms as "synthetic data" per se.

"We're using real patient data points. Only the outcomes are synthesized," Hernandez-Boussard said. "When you think about the cost of clinical trials, if you can reduce costs by a significant amount using digital twin or synthetic controls, that's a huge win for research, for medicine as a whole and for patients." **SM**

— Contact Andrew Myers at medmag@stanford.edu

THE COMPANY THEY KEEP

CONTINUED FROM PAGE 23

For his part, Moding was able to pinpoint three distinct ecotypes composed of 23 distinct cell states in nine cell types in soft tissue sarcomas from several hundred patients. In general, Moding found, patients whose tumors contained cellular communities with a high proportion of cancer-fighting immune cells fared significantly better than patients with tumors that had few immune cells and elevated levels of proteins involved in a signaling pathway called Hedgehog.

People whose tumors contained an intermediate number of immune cells and displayed elevated levels of RNA messages involved in two cancer-associated signaling pathways had the worst outcomes, but they were also much more likely to respond to immunotherapy than either of the previous two groups.

"These findings have led me to appreciate just how complex cancers are," Moding said. "We have to consider the interactions of the cancer cell with all the other cells in the tumor and in the body."

Untangling cell codependencies within view

So, what does this burgeoning alphabet soup of techniques mean for people with cancer? Will they lead to new clinical advances? Many researchers think so, but the timeline is difficult to predict because the questions are so complex.

"New techniques are helping us to conceptualize tumors as communities of cells with a network of interactions and multiple redundancies," Angelo said. "Interrupting one or two pathways is not going to break the system. These types of studies will help us to identify these mutually enforcing pathways and hit them from multiple angles."

"Now we're trying to untangle the codependencies," Newman said. "If I see a specific flavor of a certain kind of cell, do other flavors go along with it? Studies like Magdalena's and Everett's prove that these recurrent ecotypes exist. They are not just the fundamental units of tissue biology, but they are also functionally and clinically relevant."

"I remember 20 years ago when I was an assistant professor of radiology and the chair of the department, Professor Glazer, predicted that we would one day know the location of every molecule in the human body in three-dimensional space," Plevritis said. "At the time, it seemed futuristic. But we're getting there! This is actually within our reach now, which is amazing. Just amazing." **SM**

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COVID'S UNWITTING ENABLERS?

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microbiology and immunology. "But we suspect this may be the point where, in an actual patient, the infection transitions from manageable to severe."

Another point of entry

Compounding this unexpected finding is the discovery that SARS-CoV-2 uses a different route to infect interstitial macrophages than the one it uses to infect the other types.

While SARS-CoV-2 gains access to alveolar type 2 cells and alveolar macrophages by clinging to ACE2 receptors on those cells' surfaces, the virus breaks into interstitial macrophages using a different receptor these cells display. In the study, blocking SARS-CoV-2's binding to ACE2 protected the former cells but failed to dent the latter cells' susceptibility to SARS-CoV-2 infection.

"SARS-CoV-2 was not using ACE2 to get into interstitial macrophages," Krasnow said. "It enters via another receptor called CD209."

That would seem to explain why monoclonal antibodies developed specifically to block SARS-CoV-2/ACE2 interaction failed to mitigate or prevent severe COVID cases. To keep SARS-CoV-2 from binding to the alternate receptor on interstitial macrophages, those monoclonals would have to be reconfigured to aim at a brand-new bull's-eye.

It's time to find a whole new set of drugs that can hit that bull's-eye and impede SARS-CoV-2/CD209 binding. As in, pronto, Krasnow said.

Krasnow said he has heard from a potential European collaborator who's

developing molecules that block CD209 and would like to test these compounds' capacity to prevent SARS-CoV-2 from binding to interstitial macrophages.

With COVID-19 cases once again on the uptick, that sounds like a good idea. **SM**

— Contact Bruce Goldman at goldmanb@stanford.edu

THE WORTH OF 'WORTHLESS' IDEAS

CONTINUED FROM PAGE 40

them how to observe acutely. And you observe acutely when you immerse yourself in nature," Prakash said.

"I particularly like the small world, so I have acute powers of thinking about the small world." But others may notice the way plants behave or the way sunlight filters through water. His students made at least one new observation a day, writing down what they observed, sometimes with drawings. "When you've done it for two months, you start feeling like it's a practice. And then when you've done it for five years, you sort of feel like, oh, it's a part and parcel of your life." Prakash has been keeping a record of his observations for 20 years.

Sharing those observations is an important part of the practice, but it is often difficult, Prakash said. "I have some wild ideas that I am sometimes embarrassed to tell anybody," he laughed.

Prakash's dissertation topic at the Massachusetts Institute of Technology was one of his "embarrassing ideas." He had noticed the way bubbles avoid one another or merge depending on conditions, and it occurred to him that it would be possible to construct a computer circuit with bubbles. "Like literally bubbles. And it's something that I had thought about for a long time, but I would never tell anybody.

"But then I pursued it. And that was my PhD. I got my PhD," he said, with a trace of wonder in his voice.

Even at 44, Prakash still hesitates to share some of his wildest ideas with people outside his inner circle. He conceded, "It's easy to say it; it's hard to do."

Prioritizing wonder

IN SILICON VALLEY, investors say things like, "Ideas are worthless; execution is king." To the degree that ideas by themselves can't be copyrighted or patented, that is true. But ideas are the fuel that powers science (and, ultimately, technology).

Prakash argues for the worth of worthless ideas. Historically, he said, the most important ideas come from scientific backwaters, areas of knowledge that may have been ignored for decades. "The most important ideas lie in places where very little has been pursued so far. Curiosity is a way to take that leap."

Despite prioritizing wonder over utility, Prakash acknowledged that the fast pace of modern science leaves little room for rapt observation or the delight of noticing something completely unexpected. "The next big project is always around the corner. There's very little time to slow down, turn over rocks and just play. How do you write a grant about play? How do you write a grant about the most beautiful cell in the world? All scientists believe that if you pursue your curiosity, that's the primary way to make discoveries. But in practical terms, it is difficult.

"Just because it's difficult doesn't mean we don't do it. Play and curiosity are deep in our hearts," Prakash said. "But there are no awards for observation. It's just, you share it and lots of people build upon your observations and ideas. That's at the heart of the scientific pursuit."

Prakash calls his passion-driven research "recreational biology." "What if we could create an entire field that's associated with mysteries and paradoxes? Not because people 50 years ago were asking this question, so we have to continue that legacy, or because there is a disease that we are working on." What if instead of plodding, he asked, we unshackled biology from utility and said we are just going to work on puzzles?

Just days after returning from exploring the depths of the cold North Pacific, Prakash and his lab were off to the Atlantic Coast to teach recreational biology at the Marine Biological Laboratory at Woods Hole, in Cape Cod, Massachusetts. He was planning two weeks of play, with students joining from around the world in one of the oldest continuously running cell biology courses taught in the world. "I'm packing tomorrow. I'm also packing a whole bunch of organisms," he laughed. **SM**

— Contact Jennie Dusheck at medmag@stanford.edu

WHY IS A COMMON GENE VARIANT BAD FOR YOUR BRAIN?

CONTINUED FROM PAGE 43

APOE4's combined higher frequency but lower risk among people whose recent ancestors inhabited Africa — the continent where humans originated — suggests to biologists that APOE4 was the first APOE variant carried in humans. Some theorize that its initial importance was in combating infectious microbes, which abound in warmer climates. As humans migrated out of Africa to or through colder climes with less microbial exposure, the theory goes, other variants — first the now-dominant APOE3 and, later, the protective APOE2 — came along and, over time, became more common.

APOE4's power to boost the likelihood of Alzheimer's disease varies not only by ancestry but also by sex. Women of European descent between age 50 and 80 who carry one APOE4 copy and one APOE3 copy are at three or four times as much risk as those with two copies of APOE3, while same-age men with the same APOE status are at only marginally increased risk, according to a review Greicius co-authored in Neuron in 2019.

A no-brainer confronts a brain-teaser

SCIENTISTS AGREE that APOE4 is "bad" in the sense of hiking people's risk for cognitive decline in advanced age. But whether that's because ApoE4 — the protein for which APOE4 is a recipe — is an underachiever (not doing enough of some good thing it's supposed to be doing in the brain) or because ApoE4 itself is a bad actor (doing some bad

thing it's not supposed to be doing there) is an open question. Knowing the answer would tell researchers and drug developers whether their goal should be to punch it up or to tone it down — a key step toward finding a drug to deal with it.

That's what Greicius and his colleagues, including University of Washington professor of medicine Chang-En Yu, PhD, who was Greicius's co-senior author, set out to determine. For their study, they gained access to a giant registry of people with and without Alzheimer's whose genes had been carefully scrutinized for APOE status, then they zeroed in on people age 65 and older. Of the 56,684 people in this cohort, a fair number were APOE4 carriers — no surprises there — but precisely two carried an APOE4 copy that was so defective it couldn't direct the production of its correspondingly malfunctioning ApoE protein.

Those two people turned out to be carrying, along with a nonworking copy of APOE4, a perfectly normal APOE3 copy. Neither of them, despite their advanced years (one was 90 at the age of death, the other 79 and still alive at the time), had evidenced any signs of mental decline. To the contrary.

"They were in great shape," Greicius said. "I was shocked to learn that the 90-year-old, on postmortem inspection, had no appreciable buildup of beta-amyloid plaque in his brain."

The cerebrospinal fluid of the younger of the two was likewise devoid of any significant A-beta changes when last checked at age 76. (By age 75, two-thirds of even asymptomatic APOE3/APOE4 carriers — much less the ones diagnosed with cognitive symptoms of Alzheimer's disease — typically have abnormal A-beta levels in their cerebrospinal fluid.)

Evidently APOE4 wasn't simply too wimpy to get the job done; it was actually bad news. If you're carrying APOE4, it seems, you're better off if this gene variant isn't making any ApoE4 than if it is.

"This is the first human study to make a strong case that ApoE4 is toxic and that its loss may be protective," Greicius said.

He noted that a complete absence of ApoE activity could be damaging in peripheral organs such as the heart. "Rare cases have been found of people with zero functioning copies of any variant," Greicius said. They had very high cholesterol levels, he said.

But neither of these two broken-APOE4-carrying individuals, each of whom carried a working copy of APOE3, had sky-high cholesterol. "Apparently, one copy of out-of-order APOE4 doesn't hurt you," Greicius said.

The road ahead

SO FAR, NO GREAT small molecules that could be used as drugs have been shown to safely and selectively inhibit the production or activity of the problem protein, ApoE4. Finding such a finely discriminating drug could prove daunting.

But in the near term, it may not be necessary. A drug that knocks APOE down but not out, so ApoE production isn't entirely stamped out, might be safe. The robust health of the people in the new study who had only a single working copy of an APOE gene implies that, Greicius said.

Nor would a drug's inability to distinguish between different APOE variants pose a problem for treating those carrying two copies of APOE4 (2%-3% of all people), he observed.

The new research has begun to resolve scientists' uncertainty as to whether to put more muscle into ApoE4 or put it out of commission. That should give some direction to drug-development efforts, said Greicius, who is following up in a collaboration with other Stanford Medicine scientists to learn more about ApoE's interactions with other key fat-shuttling proteins and tease out differences in how ApoE4 and its alternatively numbered counterparts select which fatty substances they take aboard.

"Now, we know which way to go," he said.

ApoE4 is not a wimp. It's a cutthroat. Get rid of it. **SM**

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RECONSIDERED: BREAST CANCER ORIGINS AND RISK FACTORS

GENE SEQUENCES FROM PARENTS HAVE MORE IMPACT THAN RECOGNIZED IN DETERMINING BREAST CANCER'S COURSE

New research challenges the dogma that mutations arising during our lifetimes are the primary factors influencing whether we get cancer. They matter, but a wide variety of gene sequences we're born with may play a more decisive role than realized.

"This study unearthed a new class of biomarkers to forecast tumor progression and an entirely new way of understanding breast cancer origins," said Christina Curtis, PhD, the RZ Cao Professor and a professor of genetics and of biomedical data science. Curtis and postdoctoral scholar Kathleen Houlahan, PhD, described the research in May in *Science*.

Curtis has been interested in how cancers start since she was in high school, having lost family members to the disease. More recently, her parents were diagnosed with different cancers within one month of one another, when Curtis was juggling her work identifying the molecular basis of malignancy and metastasis with parenting her young children. Her father recovered. Her mother did not. "The whole experience impressed on me a renewed need to intercept earlier," Curtis said. "It's not enough to optimize therapy once a tumor has already spread. We have the tools to do more earlier."

Only a few high-profile cancer-associated mutations in genes like BRCA1 and BRCA2 are regularly used to predict cancer risk. These mutations have been associated with distinct subtypes of disease. The new findings suggest there are tens or maybe even hundreds of other gene variants that influence breast cancer development and progression.

The researchers looked at the relationship between oncogenes — normal genes that, when mutated, can free a cell from functioning normally — and an immune system intent on destroying developing cancers.

Their study zeroed in on small chunks of internal proteins that even healthy cells routinely display on their outer membranes — an outward display that reflects their inner style. Like fashion police, immune cells called T cells prowl the body and peruse these protein chunks (called epitopes), looking for any suspicious or overly flashy bling that might signal something amiss inside the cell.

Curtis and Houlahan wondered whether highly recognizable epitopes would be more likely to attract the attention of T cells than other, more modest, offerings (think golf-ball-sized, dangly turquoise earrings versus a simple stud). If so, a cell that inherited an oncogene producing a particularly flashy epitope might be less able to pull off its amplification — a cancer-associated mutation in which a cell ends up with multiple copies of an oncogene — without alerting the immune system. In other words, one pair of oversized turquoise earrings can be excused; five pairs might cause a patrolling fashionista T cell to switch from tutting to terminating.

The researchers studied nearly 6,000 breast tumors and found that people who inherited an oncogene that produced an immunologically gaudy epitope — and displayed it prominently — were significantly less likely to develop breast cancer subtypes in which that oncogene is amplified. But if they did manage to escape the roving immune cells early in their development, they tended to be more aggressive and have a poorer prognosis than their more subdued peers.

"Our findings not only explain which subtype of breast cancer an individual is likely to develop," Houlahan said, "but they also hint at how aggressive and prone to metastasizing that subtype will be."

The researchers envision a future in which the inherited genome is used to better tailor treatments to individual patients, including factoring in the risk of developing an invasive breast cancer. Such information, which can be obtained from a routine blood sample, can also be combined with other molecular features.

"We're examining other cancers through this new lens by integrating hereditary factors, immunity and acquired alterations to better forecast disease," Curtis said. BY KRISTA CONGER

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Anesthesia dreaming

PLEASANT DREAMS DURING SURGERY HELP SOME PEOPLE OVERCOME DEEP TRAUMA

Mare Lucas felt euphoric. From high above, she saw herself giving birth to her oldest son, Zane, who had died by suicide in 2017 at age 18.

His birth had been difficult, and his death brought Lucas lasting trauma — but now all she felt was overwhelming love and joy.

Then Lucas heard a voice over her right shoulder. "Hello Mare, can you hear my voice? Are you having happy dreams?" asked her anesthesiologist, Harrison Chow, MD. She was.

Chow adjusted the infusion of propofol, a common sedative used in surgical anesthesia, and carefully monitored her brain waves as Lucas fell back into her dream. Chow had become adept at tuning propofol to gently awaken patients from surgery, which eased their recovery. He'd also noticed that at a particular level of consciousness, patients often had pleasant dreams.

It was August 2022 and Lucas was undergoing routine surgery to remove a lump in her right breast.

She'd previously been diagnosed with post-traumatic stress disorder and still had terrifying nightmares about Zane's suicide. But the morning after the surgery, she awoke feeling a surprising sense of calm. Her anxiety felt manageable. And she vividly remembered the dreams she experienced while under anesthesia.

"There was something about the euphoria that came with this dream that somehow knocked my brain out of those trauma connections," Lucas said.

Chow and colleagues published a report in March in *The American Journal of Psychiatry* about two patients, including Lucas, whose trauma symptoms improved after anesthetic-induced dreaming during surgery.

The researchers suggested these dreams may work as an accelerated form of exposure therapy, allowing patients to process traumatic memories with a calm body and mind. They hope to develop the protocol into a therapy for psychiatric conditions.

"These cases are a profound demonstration that experience matters," said Boris Heifets, MD, PhD, assistant professor of anesthesiology, perioperative and pain medicine and co-senior author of the report with Chow. "And we have a unique way to deliver a transformative experience in a very safe manner." BY NINA BAI

ADOBE STOCK-CRAVENA

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