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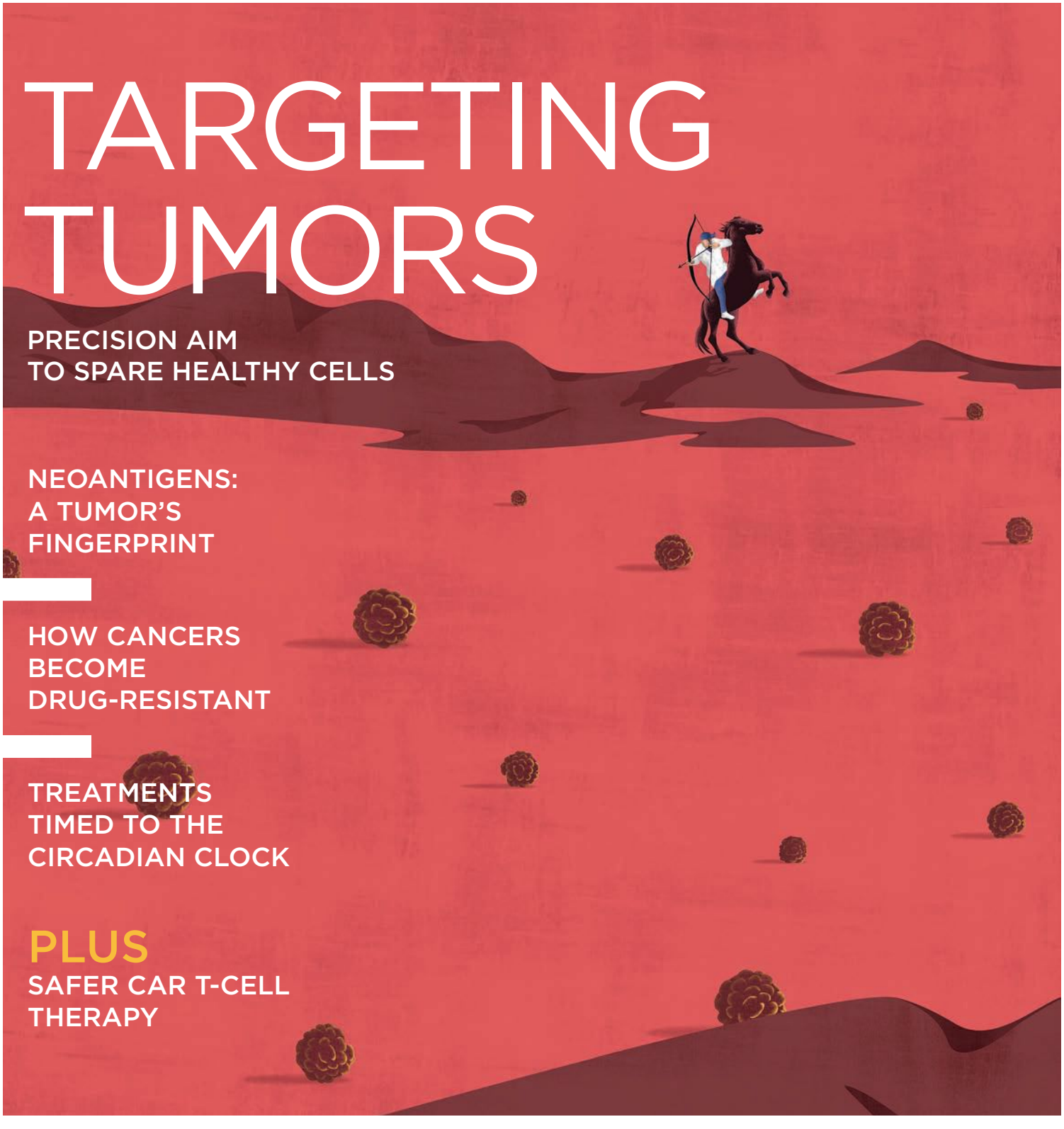

TARGETING TUMORS

PRECISION AIM TO SPARE HEALTHY CELLS

NEOANTIGENS: A TUMOR'S FINGERPRINT

HOW CANCERS BECOME DRUG-RESISTANT

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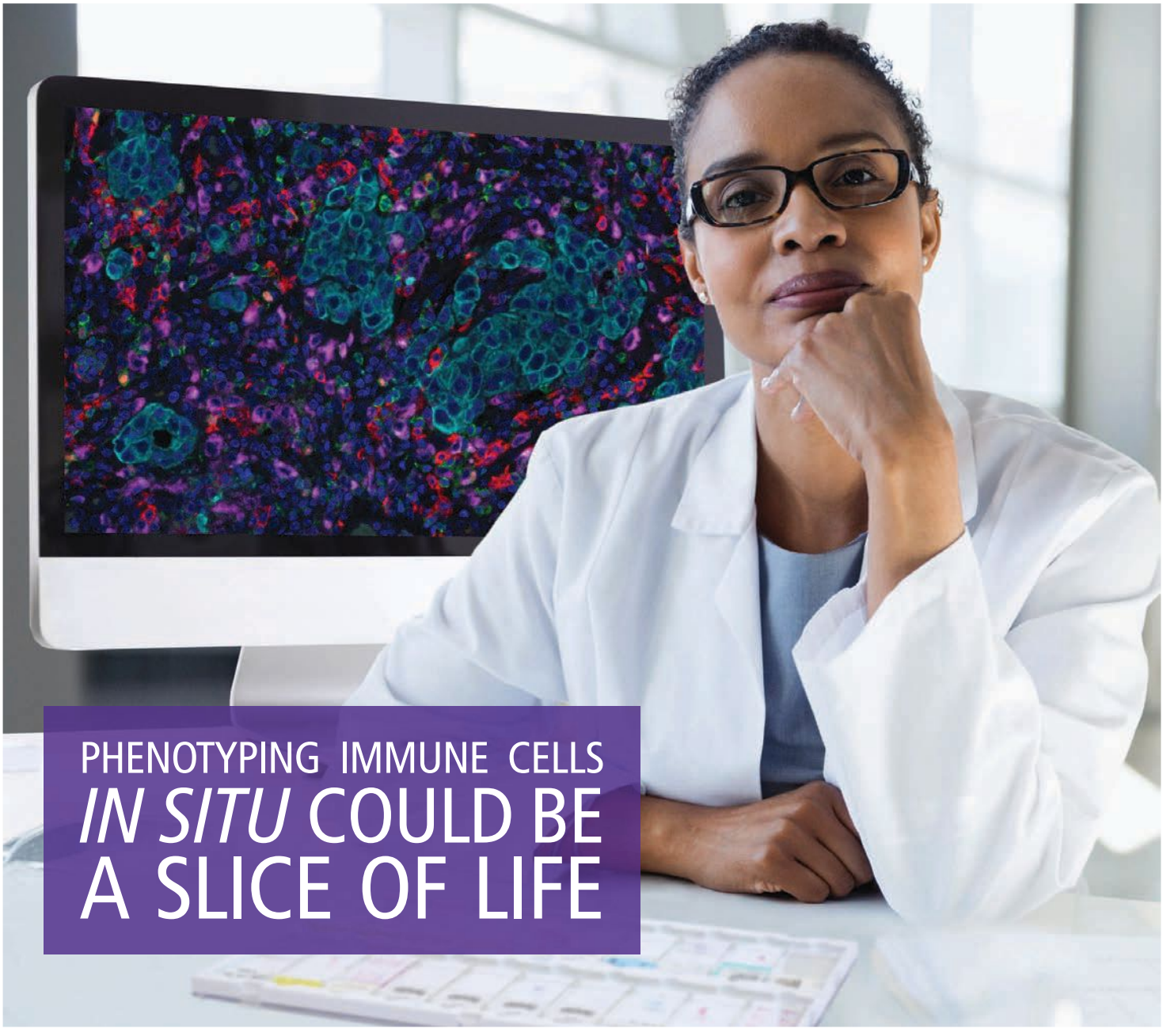

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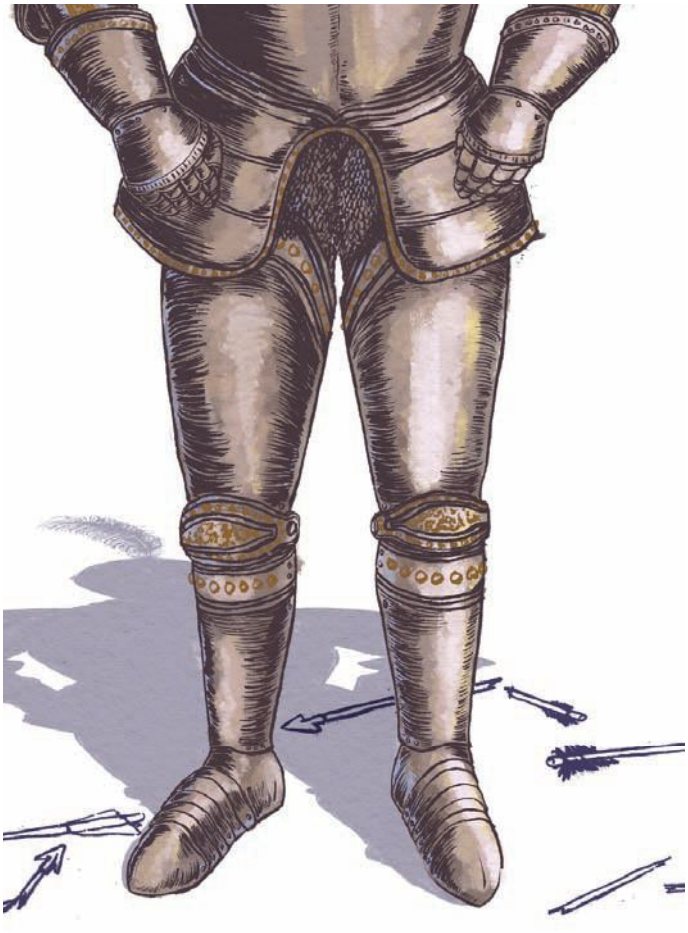

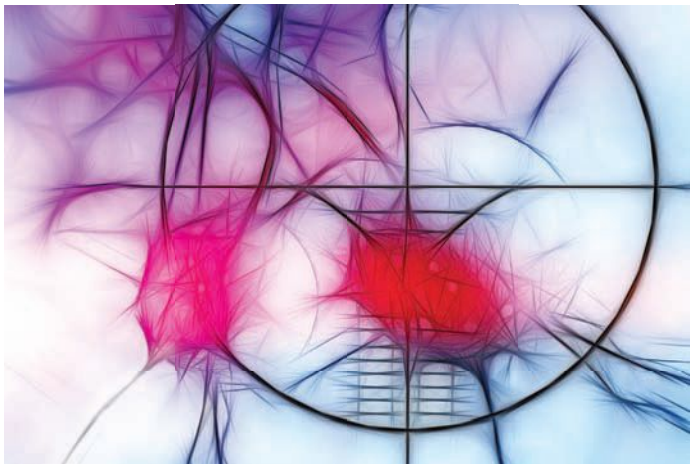

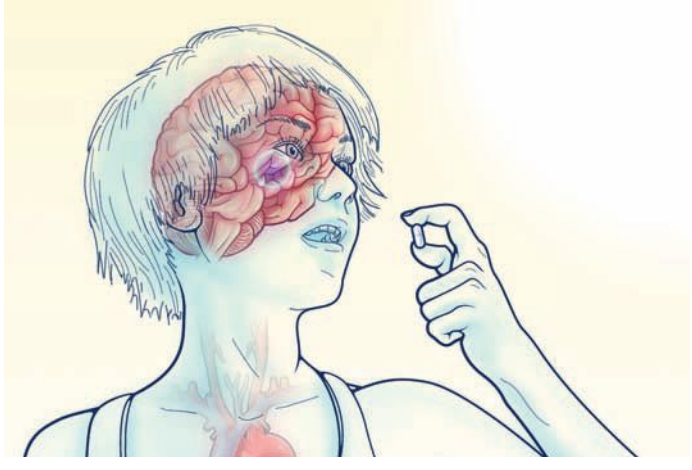

Features

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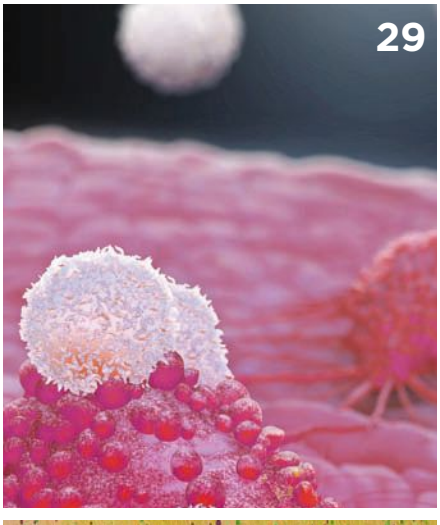
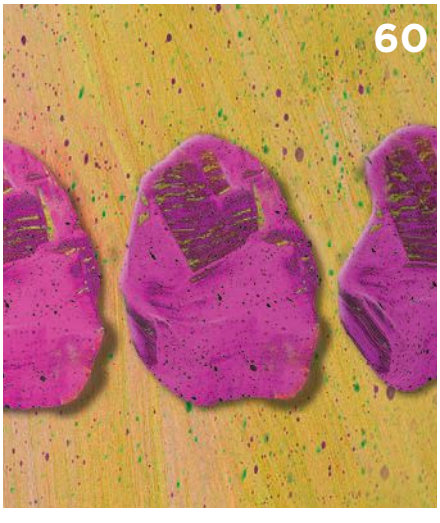




Online Contents

THIS MONTH AT THE-SCIENTIST.COM:

VIDEO
Cancer and Its Milieu
Lawrence Berkeley National Laboratory researcher and April profilee Mina Bissell explains the intricacies of how cancer takes hold in a body.

SLIDE SHOW
Wildlife Cams Gone Wild
See shots of human behavior captured by cameras meant to catch wildlife in the act.

VIDEO
Cancer Genomes
April Scientist to Watch Angela Brooks of the University of California, Santa Cruz, discusses her search to find vulnerabilities buried within the genomes of cancer cells.

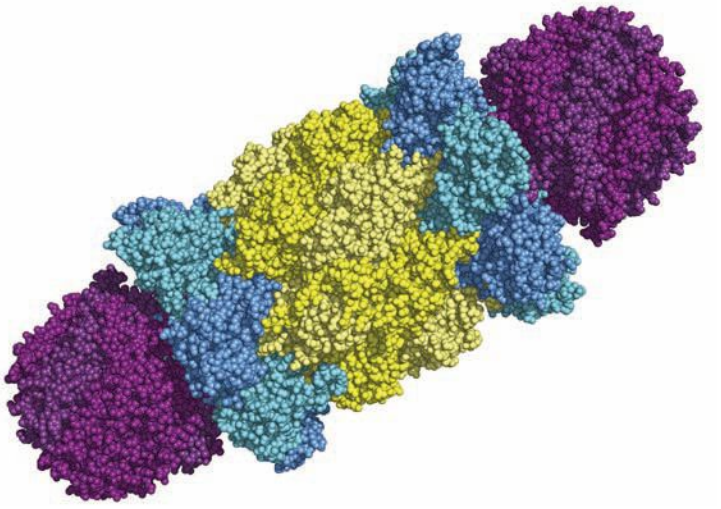
AS ALWAYS, FIND BREAKING NEWS EVERY DAY, AND LEAVE YOUR COMMENTS ON INDIVIDUAL STORIES ON OUR WEBSITE.

Coming in May

HERE'S WHAT YOU'LL FIND IN NEXT MONTH'S ISSUE:

- Proteasome structure and function
- Taking rapid evolution into account
- The human senses of body ownership and agency
- The responsibility of collaborators when research goes bad
- Machine-learning approaches in cell biology

AND MUCH MORE



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Contributors

Stephen Schoenberger was born in Los Angeles and was a musician before he became a scientist. After completing a bachelor's in microbiology and molecular genetics and a PhD in structural molecular biology at the University of California, Los Angeles, Schoenberger did a postdoctoral fellowship in immunohematology at Leiden University Medical Center. "This was in the 90s, and for the first time . . . we could vaccinate against cancer and really understand the target that was being recognized," he recalls. "And that was a very exciting area that I wanted to know more about." Schoenberger moved back to California after his postdoc to become a professor at the La Jolla Institute for Allergy and Immunology in San Diego, where he his lab currently investigates T-cell pathways and precision immunotherapies for cancer. "I want to be able to help develop therapies for cancer patients that are based on using their immune systems' responses to their own tumor antigens," says Schoenberger.

Ezra Cohen, the son of two physicians, grew up in Toronto. After completing his MD at the University of Toronto, Cohen decided to become a "small-town doc." "I came through medical school dreaming of doing everything—emergency rooms, obstetrics, small surgeries," Cohen recalls. Once he finished his residency, he moved to Orillia, a town of around 30,000 people just north of Toronto, and started a family practice. But Cohen soon realized he wanted to practice oncology instead. "I ended up getting interested in cancer and began to ask questions like: Why did this person get this particular type of cancer, and why did they respond to specific therapies?" To pursue his new passion, he moved to New York in 1996 to do a residency in internal medicine at the Long Island Jewish Medical Center. He followed this with an oncology fellowship at the University of Chicago, where he eventually joined the faculty, started a research lab, and directed the Head and Neck Cancer Program. In 2014, Cohen joined the faculty at the University of California, San Diego, where he helped build a cancer immunotherapy program. "I realized what they were doing in San Diego could make a large impact in the field," Cohen says.

Read their feature about the immunotherapeutic promise of targeting neoantigens on page 48.

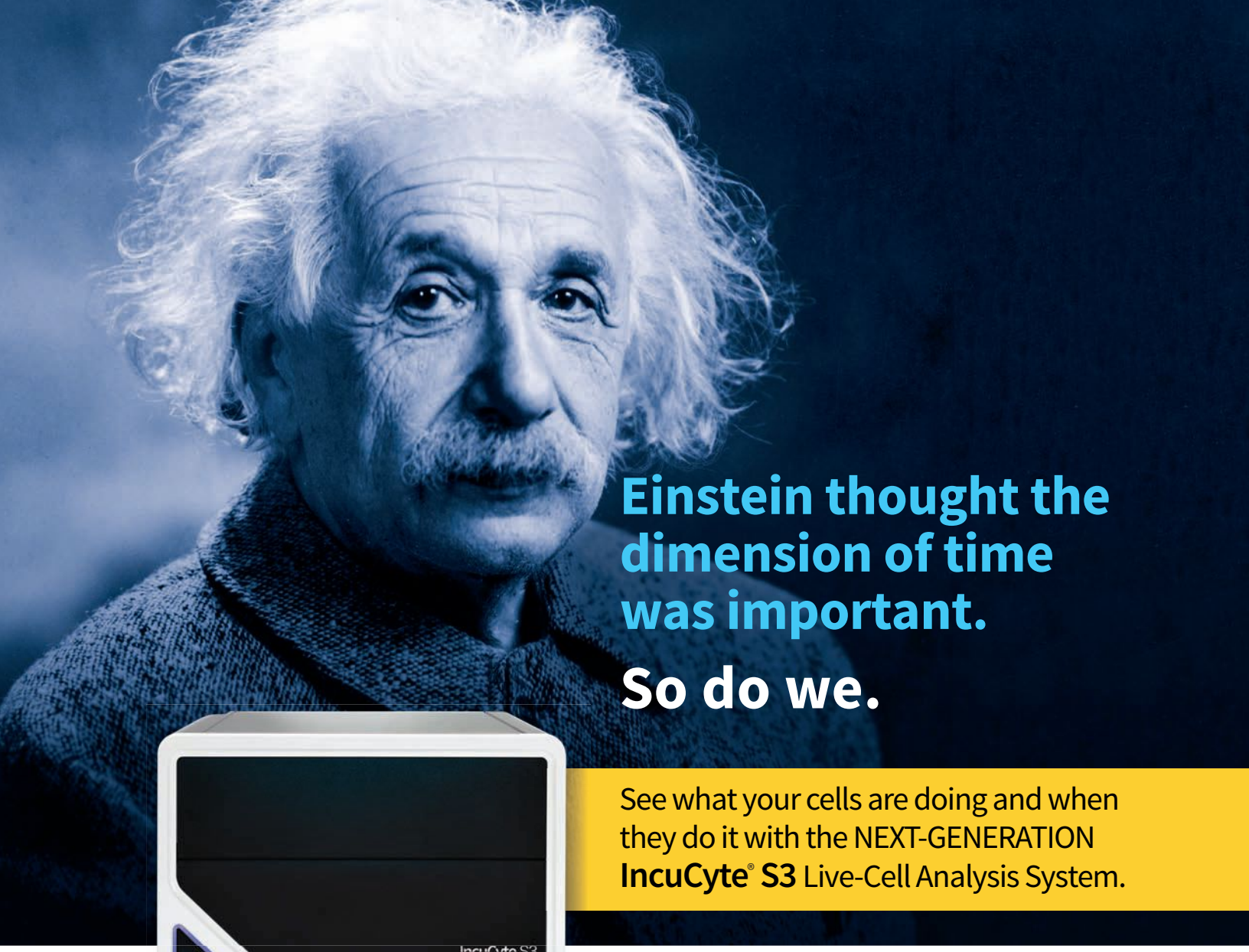
David Fabrizio, a Boston-area native, was drawn to the biological sciences from an early age. Fabrizio's mother died from breast cancer when he was a teenager, and "it was that experience that drew me toward the biology and medical community," he recalls. As an undergrad at the University of Vermont, Fabrizio focused on biological sciences and molecular genetics, and completed a thesis on HIV pathways. "[It] was a watershed moment for me, being able to work in a lab, getting hands-on knowledge, and seeing how science actually worked on the bench," he remembers. After completing his bachelor's, Fabrizio worked in a Harvard Medical School lab for two years before being offered a job at a biotech startup, Adnexus Therapeutics, in 2004. There, Fabrizio worked on a variety of preclinical drug-development projects, including checkpoint inhibitor immunotherapies. "Working on these drugs at the preclinical level, seeing them advance on into the clinic, and knowing the effect they were having on a subset of patients was really quite profound for me," he says. In 2013, Fabrizio joined another startup, AbVitro, where he focused on next-generation sequencing and neoantigen discovery. Two years later, he joined Foundation Medicine, where he currently leads the Cancer Immunotherapy group.

In a Critic at Large (page 29), Fabrizio urges the development of better biomarkers to guide the personalization of cancer immunotherapies.

While growing up in Orlando, Florida, **Wendy Whitman Cobb** regularly witnessed space shuttle launches. "That was a big influence on me as I got into political science," Whitman Cobb recalls. Although she had begun her undergraduate education at the University of Central Florida as a theatre major, Whitman Cobb decided to switch to political science once she discovered the school offered courses in space policy and history. She completed a PhD in political science at the University of Florida and based her first book, *Unbroken Government: Success and the Illusion of Failure in Policymaking*, on her doctoral dissertation. Whitman Cobb shifted her focus from space to health policy for her latest book, *The Politics of Cancer: Malignant Indifference*. "The most immediate influence for my most recent book was the fact that my dad is a cancer survivor and is currently being treated for it," says Whitman Cobb, now an assistant professor of political science at Cameron University in Lawton, Oklahoma. "I like looking at the things that people don't expect for politics to be involved in [but] are enmeshed in how we live our everyday lives," she says.

Her Reading Frames essay on the politics of cancer research and treatment can be found on page 77.

NICK ABADILLA PHOTOGRAPY; LLUVIA HIGUERA; FOUNDATION MEDICINE; CAMERON UNIVERSITY



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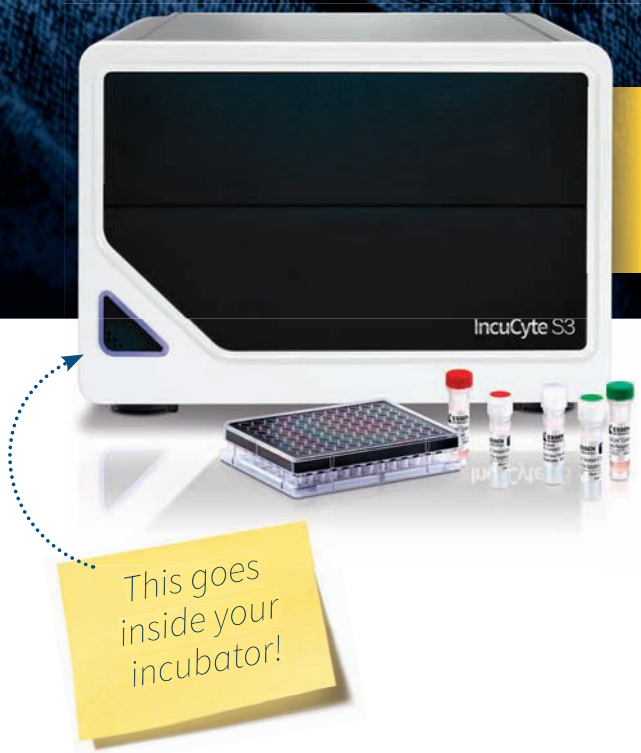

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Hitting It Out of the Park

Cancer can be as evasive and slippery as a spitball, but new immunotherapies are starting to connect.

BY MARY BETH ABERLIN

It's happened every spring for the last seven years: our annual issue dedicated to cancer research more or less coincides with the opening of the major-league baseball season. A recent *New York Times* article about revising the game to make it move faster (and hence be less boring) got me thinking about whether changing the rules governing this American pastime is an apt metaphor for what's now happening in cancer research.

The Oscar-nominated 1949 film *It Happens Every Spring* about a baseball nobody can hit also seems to contain a metaphorical nugget. The ball flies through a window into the laboratory of one Professor Simpson, where it acquires the ability to repel wood after a dunking in "methylethylpropylbutyl." Using the ball, Simpson goes on to pitch a major-league team all the way to the World Series.

Why do I see metaphors here? Cancer research is never boring, of course, but winning the treatment game has been plagued by drug resistance, difficulty preventing metastases, an inadequate understanding of the tumor milieu, and a black box of unknowns about the importance of some of the phenotypic alterations exhibited by malignant cells. For the past five years, however, an electrifying game-changer has been the attempt to harness patients' immune systems as anticancer cleanup hitters.

This issue of *The Scientist* takes a look at some exciting new research and preclinical advances, particularly as they pertain to extending the initial promise of various immunotherapies. In "Resist or Desist" (page 40), Anna Azvolinsky reports on a battle that never seems to be over—the fight against tumors' evolution of drug resistance. Genome sequencing has inspired ever-more-targeted and predictable molecular therapies, but still, "there's this constant chase of the next resistance mechanism and next therapy," says one investigator. The latest challenge is the development of resistance to new immunotherapies such as checkpoint inhibitors. Researchers are keen to understand the mechanisms of such resistance—even though it is less common than resistance to molecularly targeted therapies—given the extraordinary promise of turning a patient's own cells against cancer.

To that end, the identification of tumor-specific proteins, mutant gene products known as neoantigens, is a hot area. Cancer researchers Stephen Shoenberger and Ezra Cohen ("Seek and Destroy," page 48) write about how identifying the number and type of neoantigens in a particular cancer and selectively zeroing in on those mutant proteins that make the best T-cell targets has the potential to greatly improve the safety and accuracy of immunotherapies. One of the issue's Lab Tools ("Trunks and Branches," page 70) describes methods for analyzing single cells that make up the heterogeneous tumor to decipher its evolution.

A second Lab Tools ("Special Delivery," page 66) covers new efforts to carry several types of immune therapies directly to the tumor site using biocompatible polymers as molecular ferries. Bio Business (page 73) offers a summary of attempts to address safety problems that have arisen in recent CAR T-cell trials, and on page 39, you can read about a new method to refine immunotherapy by going after a subset of lymphocytes known as regulatory T cells, which suppress the immune response to tumor cells.

A Critic at Large (page 29) urges scientists to develop more biomarkers to identify the best immunotherapy treatment for each patient. And Reading Frames author Wendy Whitman Cobb discusses how politically motivated regulatory changes might affect those who treat and suffer from cancer (page 77).

With ever-improving immunotherapies, researchers stand a really good chance of knocking cancer out of the park—provided they can keep that ball from coating itself in resistance to the bat.

A final note: we mourn the late-February passing of Eugene Garfield, founder of *The Scientist* and truly a major-league player in science publication. ■

Editor-in-Chief
eic@the-scientist.com

ANDRZEJ KRAUZE

Speaking of Science

Everything he did, he was ahead of everybody in so many ways. He was a genius of a very special type. Not only because he had this incredible imagination and brain, but he had incredible tenacity and courage.

—**Vitek Tracz**, publisher of *Faculty of 1000* and former co-owner of *The Scientist*, on the passing of Eugene Garfield, the scientometrics pioneer who launched *The Scientist* in 1986 (February 27)

I think that measuring with precision human activity on the climate is something very challenging to do, and there's tremendous disagreement about the degree of impact, so no, I would not agree that it's a primary contributor to the global warming that we see.

—Newly confirmed US Environmental Protection Agency Administrator **Scott Pruitt**, on the CNBC program *Squawk Box* (March 9)

It is my hope that this new administration, once it gets organized—and I'm not being facetious—can be as committed and enthusiastic as we were. The only bipartisan thing left in America is the fight against cancer.

—Former Vice President **Joe Biden**, in a speech delivered at this year's South by Southwest festival in Austin, Texas, about the federal government's role in cancer research (March 12)

To succeed, the Trump administration needs to assume the leadership Biden was given previously as vice president to foster cooperation and collaboration among the various federal agencies and institutions with relevant resources, and to guide [the] moonshot in a direction that maximizes its value to public health.

—**Nancy Brinker**, founder of the Susan G. Komen breast cancer charity, and journalist **Eric Rosenthal**, writing in *The Hill* about how Donald Trump should pursue Barack Obama's cancer "moonshot" (February 15)

His treatment was much easier than what some people have to endure, but I've found that physicians who have a cancer experience understand the human side of things and how treatment impacts individuals and families.

—**Shelley Fuld Nasso**, CEO of the National Coalition for Cancer Survivorship, talking about Donald Trump's appointment of Scott Gottlieb—a physician, drug industry financier, and cancer survivor—to lead the US Food and Drug Administration (March 10)

It would allow employers to ask employees invasive questions about . . . genetic tests they and their families have undergone . . . [and] to impose stiff financial penalties on employees who choose to keep such information private, thus empowering employers to coerce their employees.

—**Nancy Cox**, president of the American Society of Human Genetics, in a letter delivered to the House Committee on Education and the Workforce a day before it approved HR 1313, a bill that would allow companies to require their workers to undergo genetic testing or pay fines (March 10)

ILLUSTRATION BY ERICA P. JOHNSON

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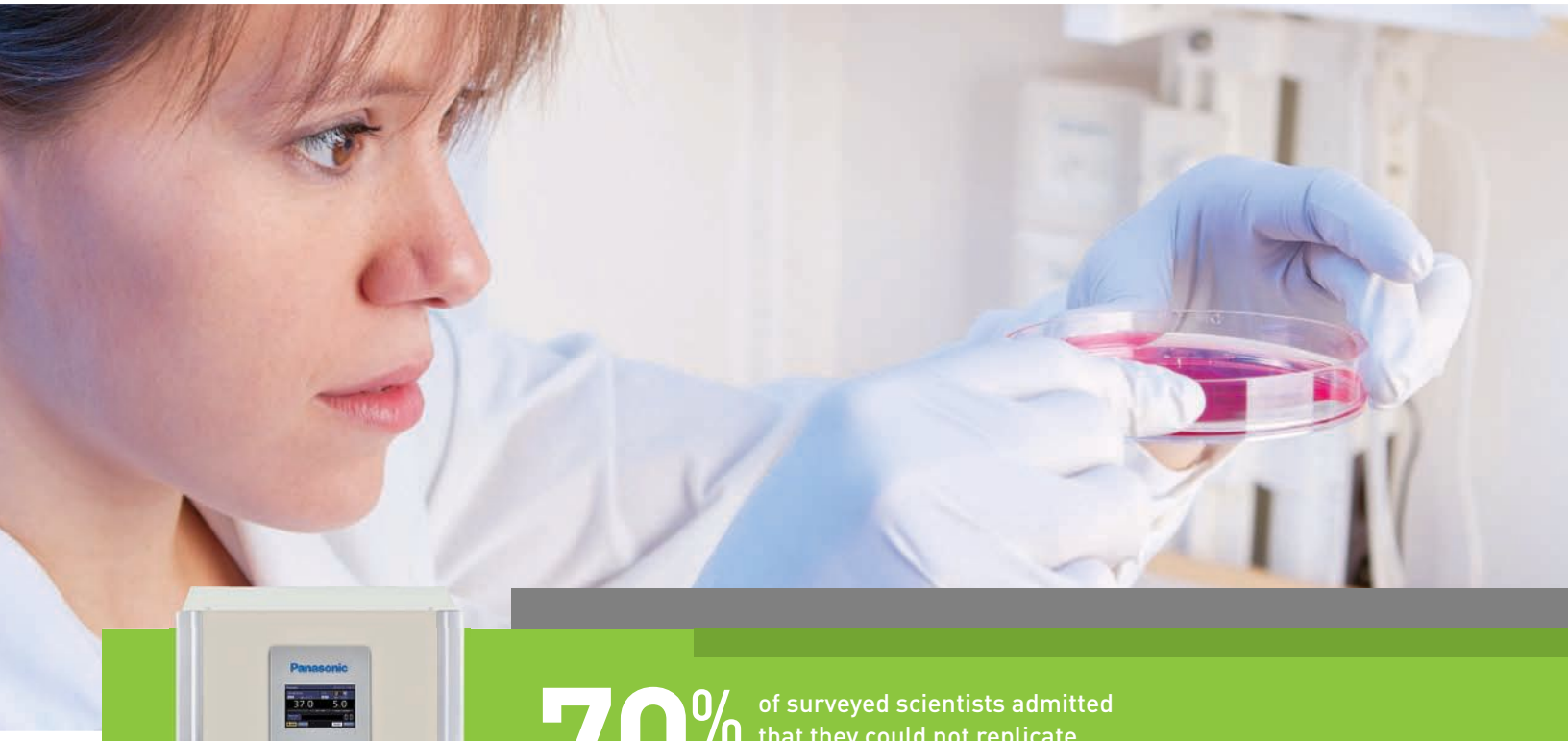

70% of surveyed scientists admitted that they could not replicate someone else's research.[1]

50% admitted that they couldn't replicate their own research.[1]

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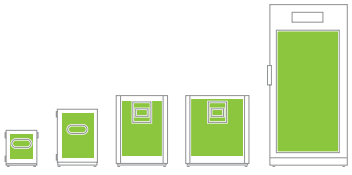

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1) Baker, Monya. "1,500 scientists lift the lid on reproducibility." Nature, no. 533 (May 26, 2016): 452-54. doi:10.1038/533452a.

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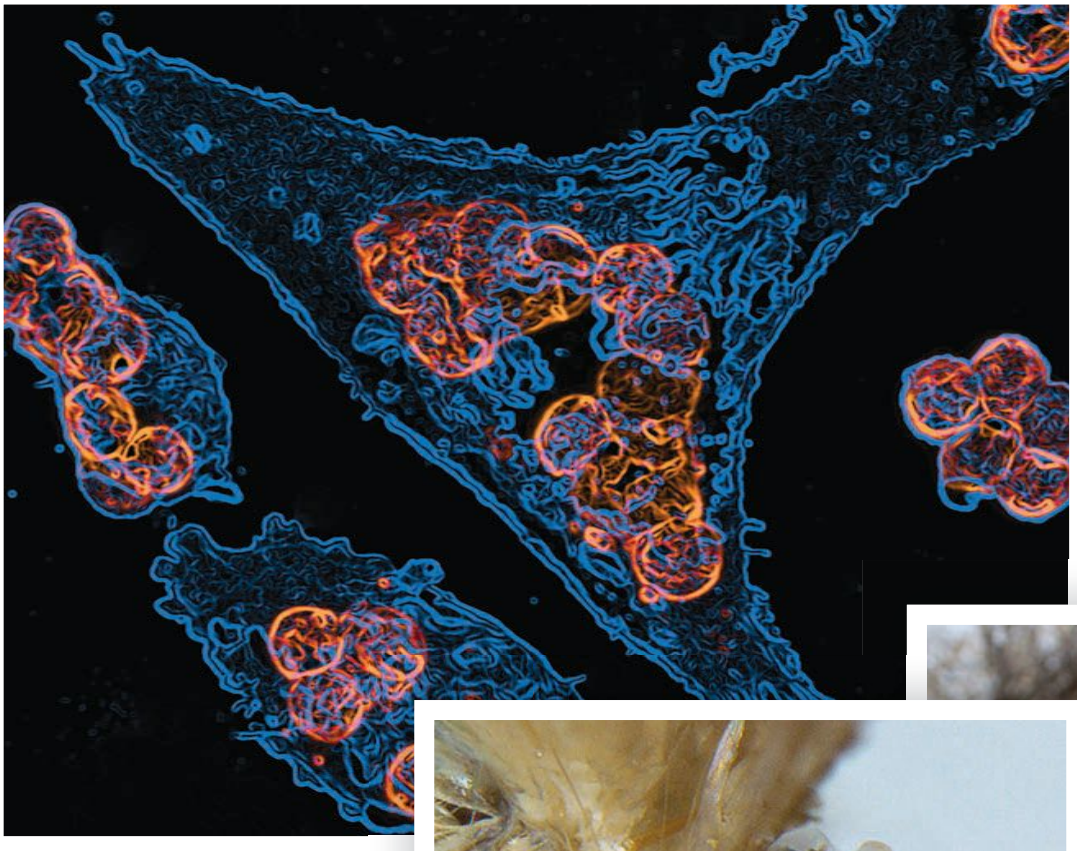

FEEDING TIME
Scientists are working to improve the abilities of therapeutic antibodies to flag cancer cells (orange) for destruction by macrophages (blue).
Posted: *January 10, 2017*

TRUMP BUG
Inspired by President Donald Trump's signature hairdo, a biologist named a new species of moth with yellowish-white scales on its head *Neopalpa donaldtrumpi*.
Posted: *January 19, 2017*

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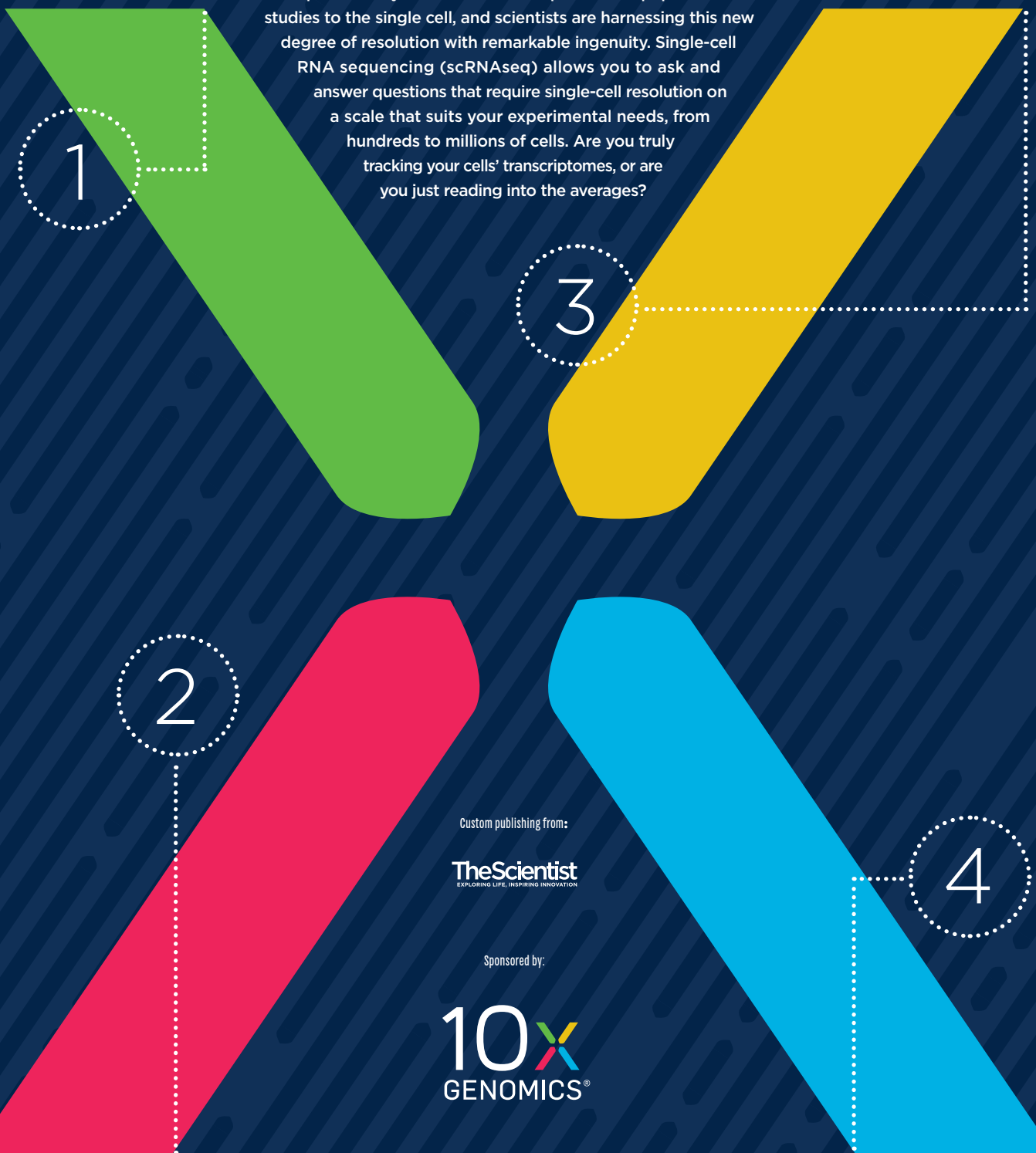

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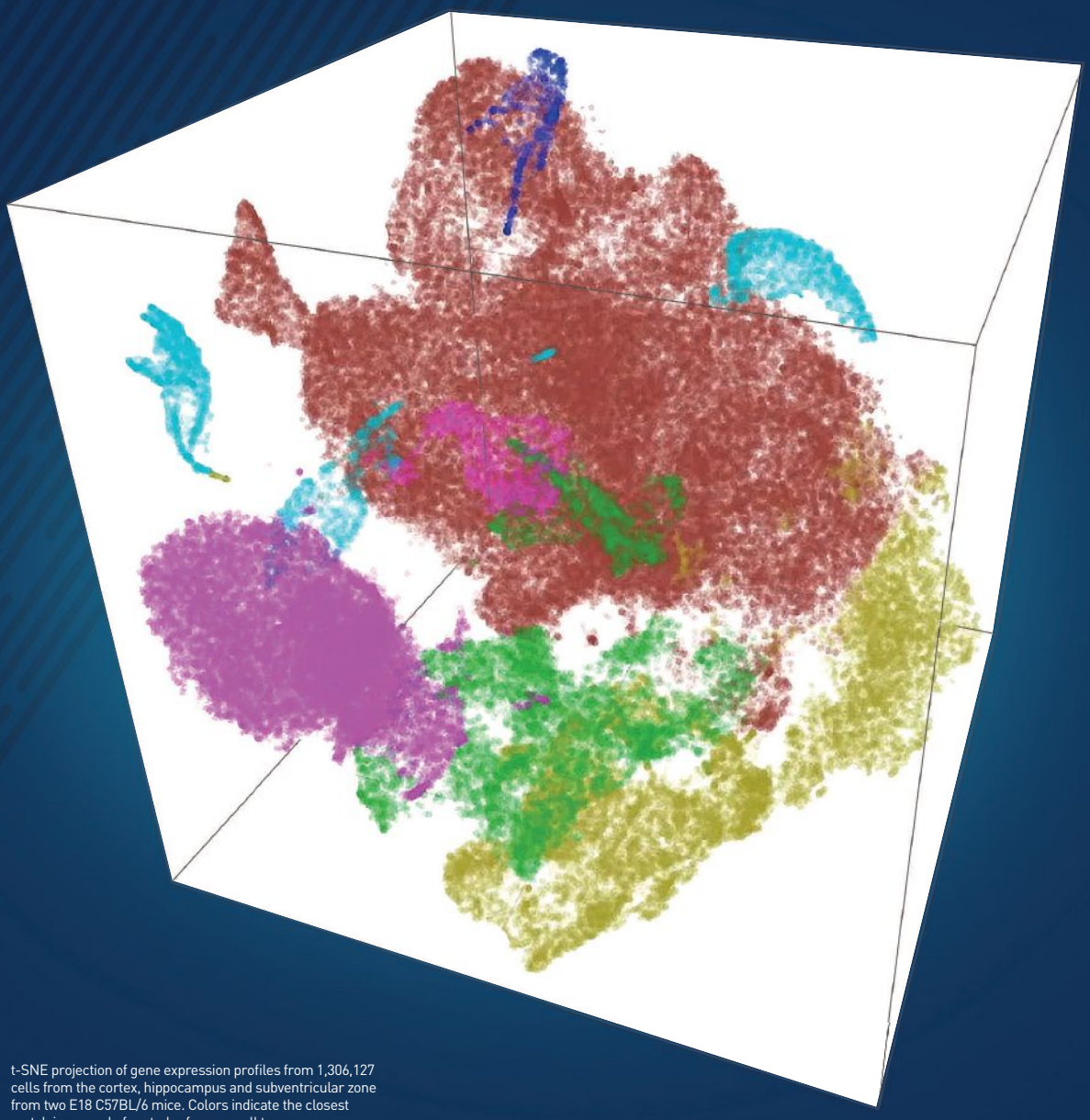

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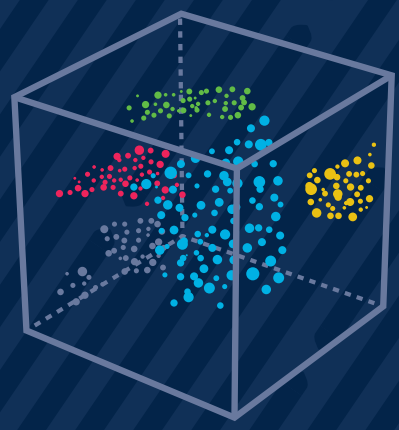

▲Relationship between cells by similarity of gene expression.

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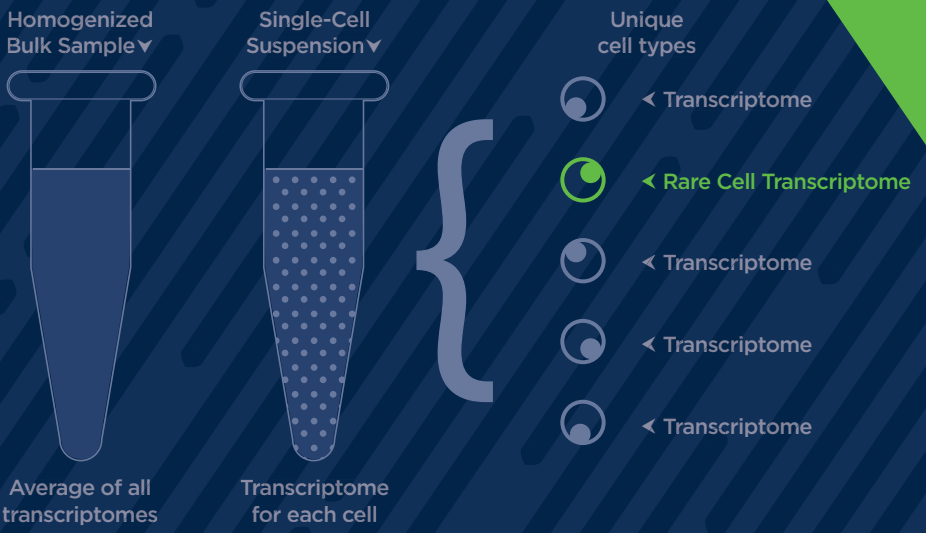

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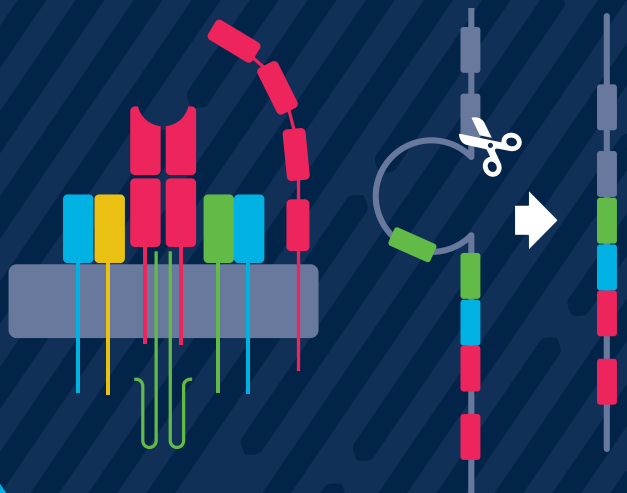

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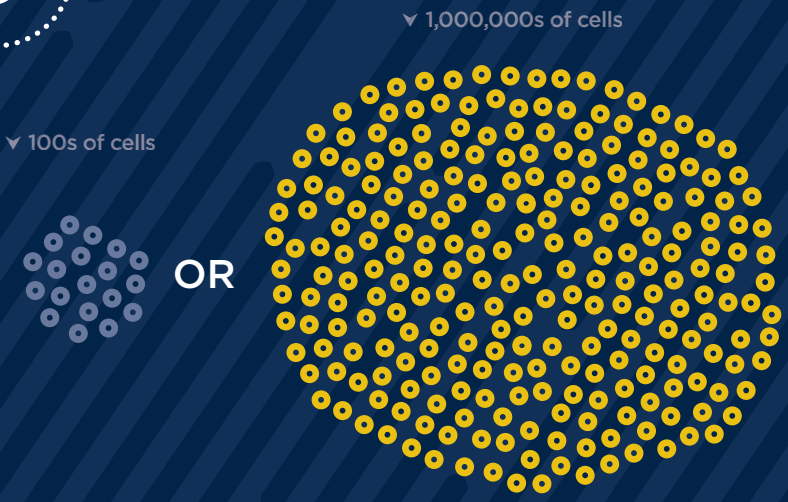

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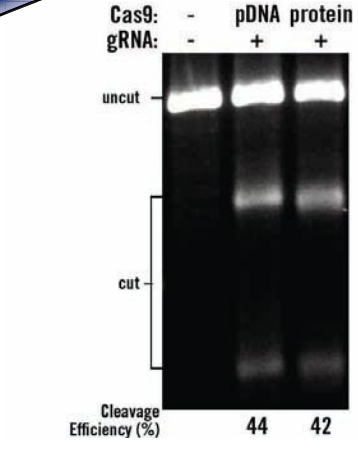

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Notebook

COURTESY OF RUAHA CARNIVORE PROJECT

I SEE LONDON, I SEE FRANCE: Barabaig women dance for a wildlife camera in their beaded undergarments.

Wild Ones

For more than a year starting in 2009, Amy Dickman had been trying to forge a relationship with members of a tribe in central Tanzania called the Barabaig. The University of Oxford zoologist was studying the killing of lions in the region, and wanted to learn more about clashes between carnivores and humans. "They said they weren't killing them," Dickman says. But the data suggested otherwise. "We found over 40 lion carcasses in 18 months. There were huge amounts of killing."

To learn more, she set up cameras in parks and wildlife management areas to monitor carnivores, and got much more than she bargained for.

One day, she was reviewing footage that appeared unremarkable at first glance: aardvark, aardvark, aardvark. Then unexpectedly, a few women walked into view, stripped down to their beaded underwear (an admitted point of fascination for Dickman and her colleagues) and began dancing for the camera. Immediately, Dickman started laughing. "It was a moment of lightness."

Although face-to-face relations with the Barabaig remained standoffish, the cameras became a sort of liaison. A little while after the dancers' performance, a group of men from the tribe paid a visit to the camera. They were drunk and goofing off, mooning the camera, when one gentleman fell over and face-planted right in front of the equipment. Later, when Dickman's group had a friendly encounter with some of the locals (they wanted to charge their cell phones using her equipment), she recognized the same guy and showed him the footage. Eventually, tensions eased, and she was able to get tribe members to discuss and implement strategies to end their lion hunting.

From Tanzania to Tennessee, wildlife cams have captured human behavior at its human-est. Just about any researcher who has a camera trap where people pass by has stories to tell about people getting back to nature.

Last year, an undergraduate student at Virginia Tech was reviewing tape from biol-

MANIMAL: An unidentified man scurried around naked at camera trap sites in the mountains of Virginia.

COURTESY OF MARCELA KELLY

ogist Marcella Kelly's camera traps near Mountain Lake Biological Station in Virginia when she came across images of a man, completely naked and scampering around on all fours. "He came up and pretended to bite the camera, which is weird because a lot of our animals do that," Kelly says. Perhaps even weirder was that this same fellow did his routine not once, but twice—and at cameras spaced one kilometer apart in the woods. "It was pretty hilarious," she says.

I think there's a lot more people naked in the woods than we've gotten pictures of.

—Roland Kays, North Carolina Museum of Natural Sciences

Other encounters between camera and human are less friendly. One time in Tanzania, a boy stole a camera, and Dickman retrieved footage of him running off while it kept filming. Another time, a couple destroyed a camera after it flashed while they were having sex. "They weren't really a couple, they were married to other people," Dickman says. It was "an illicit affair in the bush."

Last fall, Jesse Hacker, a graduate student at DePaul University in Chicago, visited a camera trap he had placed in the city's popular Lincoln Park. The equipment was trained on a large "puzzle box," about the size of a microwave, meant to attract birds and other animals and test their ability to solve the challenge of opening the box to get a food treat. When Hacker showed up, he found the box in pieces but the camera was unharmed. Dejected, he returned to the lab and set aside the video. "The next day I watched the footage, and it almost made up for the destruction of the box," he says with a laugh.

At about 3:30 a.m., a man walked up, crouched down to investigate the box, decided he wanted whatever was inside, and then went bonkers trying to break it open. He punched the Plexiglass, smashed the box, ripped off the solar panel and battery, and then wandered off into the night. But the best part of the scene, which, by the way, took place in late October: the vandal was wearing nothing but a white thong and black running shoes. "I couldn't begin to tell you what he was doing," says Hacker.

In another creepy encounter, Roland Kays, the head of the biodiversity research lab at the North Carolina Museum of Natural Sciences, recorded a man defecating in front of—or rather, for—the camera. "He took a crap while staring at the camera. It creeped the student out" who was logging the footage, Kays recalls.

Kays, who published a book on camera trap discoveries in 2016, says human encounters are part of the deal, and people's pri-

vacy should be respected, despite their odd behavior. He says researchers generally don't share footage of people. Some visitors—like the camera biter Kelly taped—are asking for their performance to be appreciated, he adds. (Kelly posted a selectively blurred photo of the man on Twitter, to the delight of many sympathetic camera trappers.)

But others, such as two people enjoying an au naturel hike in Virginia—Kays dubbed them Adam and Eve—were just peacefully doing their thing. Other than Eve's shoes, Kays says, the two were nude, "just strolling by, taking a walk in the buff." People ought to be respectful of the equipment, Kays adds, but otherwise, "they should be able to do what they want to do. . . . I think there's a lot more people naked in the woods than we've gotten pictures of." —Kerry Grens

Much Ado About Acrylamide

In 1997, construction workers digging an 8.7-km railway tunnel in Sweden began experiencing unusual symptoms: nausea, dizziness, and numbness in their fingers. Around the same time, people living near the dig site discovered paralyzed cows in adjacent pastures and dead fish floating in nearby pools. The cause of these mysterious ailments was a sealant called Rocha-Gil that the construction company had used to fill leaking cracks in the tunnel's walls. The sealant had contaminated the surrounding ground and surface water with a toxic chemical—acrylamide.

Faced with a national environmental scandal, the construction company abruptly halted its work and called in a group of researchers, including analytical chemist Margareta Törnqvist of Stockholm University, to examine the effects of acrylamide exposure on tunnel workers. Her study revealed something unexpected: people in the control group, who had not been exposed to the tunnel's toxic sealant, also had acrylamide in their blood.

At the time, acrylamide was primarily known as an industrial chemical, which earlier animal studies had revealed could cause a variety of cancers in rats at very high doses. Further investigations by Törnqvist and her colleagues revealed that acrylamide was also present in carbohydrate-rich foods that were prepared at high temperatures, such as chips, French fries, bread, and crackers (*J Agric Food Chem*, 50:4998-5006, 2002). Around the same time, Don Mottram, emeritus professor of chemistry at the University of Reading in the U.K., and his colleagues discovered that acrylamide was formed in the Maillard reaction, which browns and flavors certain foods, such as potatoes and bread (*Nature*, 419: 448-49, 2002).

Given the number of foods that contained acrylamide, we felt it was important to investigate whether eating the foods that contain acrylamide was enough to cause different types of cancer.

—Lorelei Mucci
Harvard T.H. Chan School of Public Health

The discovery that commonly consumed baked and fried products contained acrylamide, a known carcinogen in animals, prompted the Swedish National Food Administration to announce these findings to the public. "In Sweden, acrylamide was thought of as a poisonous compound that the tunnel workers were exposed to—there was so much written in the newspapers about that—then we found that acrylamide was in food and that [nearly] everyone was exposed," Törnqvist recalls. "That was a challenging risk-communication task."

Since 2002, various health authorities, including the US Food and Drug Administration and the European Food Safety Authority, have urged consumers to reduce the consumption of acrylamide-producing products. This January, the U.K.'s Food Standards Agency launched a "Go for Gold" campaign, urging consumers to avoid frying or baking starchy foods to a crisp. Despite campaigns like these, however, scientists have struggled to find

consistent evidence that acrylamide does, in fact, cause cancer in humans.

"When the findings came out from the Swedish Food Administration, it definitely raised concern all over the world," says Lorelei Mucci, an epidemiologist at the Harvard T.H. Chan School of Public Health who was then a research fellow at the Karolinska Institute in Stockholm. "Given the number of foods that contained acrylamide, we felt it was important to investigate whether eating the foods that contain acrylamide . . . was enough to cause different types of cancer."

Mucci and her colleagues looked for links between acrylamide intake and a variety of cancers (including bowel, bladder, and breast) in Swedish populations and found no significant associations (*Br J Cancer*, 88:84-89, 2003; *JAMA*, 293:1322-27, 2005). Similarly, many other studies, including investigations of large cohorts containing tens of thousands of people, have failed to find a correlation between dietary acrylamide and an increased risk for a variety of cancers, with two exceptions: ovarian and endometrial malignancies.

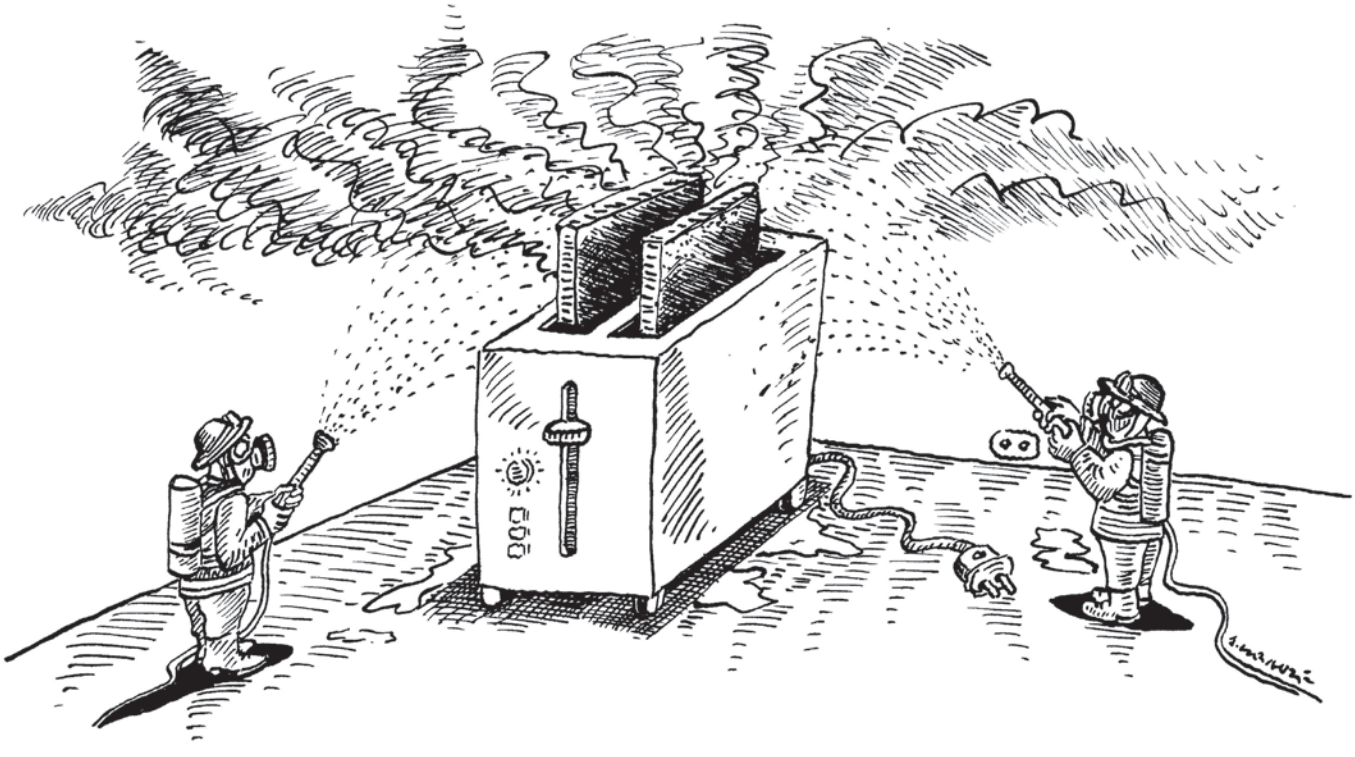
In 2007, Janneke Hogervorst, who was then a postdoctoral student in epidemiology at Maastricht University in the Netherlands, and colleagues reported that within a large Dutch cohort of 62,573 women, acrylamide intake was associated with an increased risk for endometrial and ovarian cancers (*Cancer Epidemiol Biomarkers Prev*, 16:2304-13, 2007). Around this time, Mucci collaborated with Kathryn Wilson, who was then a doctoral student in epidemiology at Harvard, to investigate this question in US populations. In 2010, they reported that their analysis of the acrylamide intake of 88,672 women also revealed increased risks for endometrial cancer and for serous tumors, a specific type of ovarian cancer (*Cancer Epidemiol Biomarkers Prev*, 19:2503-15, 2010).

Many subsequent studies, however, failed to find an increased risk for these types of cancer. One of the reasons it's been difficult to find consistent effects, says Hogervorst, is because "it's not so easy to estimate acrylamide intake through questionnaires."

Most of these early epidemiological studies relied on participants to complete surveys about the food that they had consumed. But these don't always provide a completely accurate estimate of a person's acrylamide intake, Hogervorst points out, because acrylamide content in food depends on the cooking process. In addition, she notes that some studies asked participants to indicate the amount of acrylamide-containing food they consumed, while others asked for frequency of consumption. Most studies have reported that the average daily intake of acrylamide through diet was less than 50 micrograms, which, according to Mucci, is on the order of 1,000 to 10,000 times lower than the levels tested in animal studies.

In a follow-up study, when Wilson and her team compared acrylamide levels in the blood of 263 women with ovarian cancer to control subjects from the same study cohorts, the increased risk for ovarian cancer they initially discovered disappeared (*Cancer Epidemiol Biomarkers Prev*, 22:653-60, 2013). "There are a lot of problems with trying to measure acrylamide intake with dietary questionnaires," Wilson says. "So the fact that when you look at an actual biomarker of acrylamide exposure and you don't see any [correlation with] ovarian cancer, I think kind of suggests that there really isn't an association."

Hogervorst thinks that more studies using biological markers are needed to confirm whether acrylamide really is carcinogenic in humans. Others, however, think it might be time to put the question of whether acrylamide causes cancer to rest. "When I review the literature, I take the



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findings from the epidemiology to suggest that dietary acrylamide does not increase the risk of cancer in humans," Mucci says. "So I think that given limited public health dollars, maybe those dollars would have a bigger impact on reducing cancer in the population through other means than reducing acrylamide in food."

—Diana Kwon

Monumental Maize

Jason Karl has been growing corn since he was a teenager. Starting in 1996, he began planting the crop on his family's farm in Olean, New York, and soon grew curious about how tall he could make it grow. So he started experimenting.

"Seeing how tall corn can grow comes down to internode length and quantity," Karl explains—in other words, the number of leaves a stalk has and the distance between those leaves. He learned early on that growing seedlings in a greenhouse greatly increases internode length, in part because the glass or plastic shifts the light spectrum reaching the plant's leaves. He also learned that certain strains of corn were "night-length reactive," meaning that the plant increases its number of internodes when grown in a light regimen of long days and short nights. Chiapas 234, an already-tall corn variety from southern Mexico, develops twice as many.

Karl carried on his corn-growing experiments at home while he was in college at Cornell University, a couple of hours' drive to the east. And at school, he had access to the university's library, which contained volumes upon volumes of scientific research on maize genetics. "Once I got into the literature, I could see there were mutations" that affected a corn plant's height, recalls Karl.

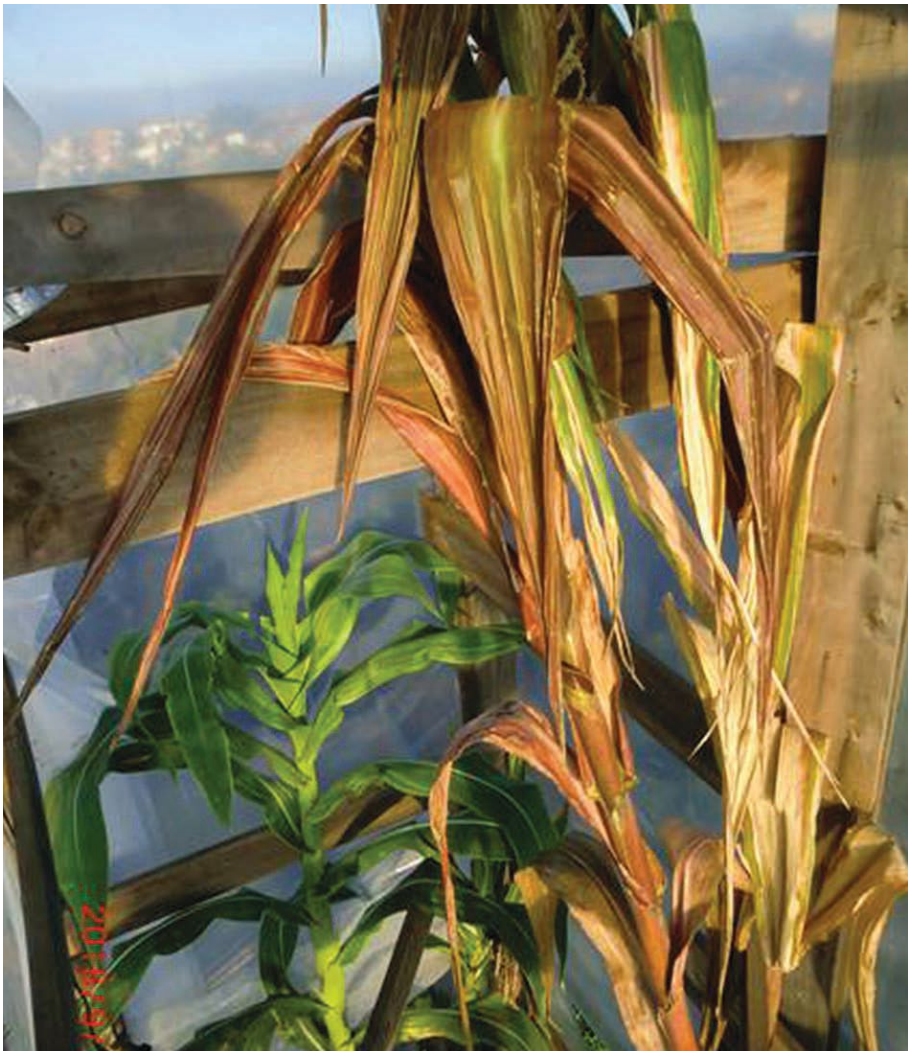
Karl found plenty of information on mutations that increased internode number. In the 1970s, for example, researchers had discovered a naturally occurring dominant mutation known as *Leafy* that adds extra leaves (and thus extra internodes). He also learned of *indeterminate* and *delayed flowering*, both recessive mutations that affect the same flowering pathway in a way that boosts the number of internodes. Karl figured he could use traditional breeding techniques to integrate these mutations into Chiapas 234.

"It's never been done before," he says. "No one would try it because it makes corn tall—too tall, people are not interested in super-tall corn. However, it's interesting for basic research. The questions I always had"—such as how the short night–driven increases in internode number interact with the greenhouse-triggered increases in internode length, and how both of these interact with height-linked mutations—"the literature didn't answer them."

> I've never seen anybody grow a 45-foot-tall corn plant; it's impressive. And he put together a cool set of genes in order to pull it off.
>
> —Edward Buckler, Cornell University

Over the years, Karl got his corn to grow taller and taller. At his family's farm, he grew the Chiapas 234 variety up to 35 feet tall, and that was just by manipulating environmental variables such as night length. That plant earned him the world record for tallest corn plant in 2011. Then, to push the plant taller yet, he bred a Chiapas corn plant with a mutant plant carrying the *Leafy* mutation and then back-bred that hybrid to the Chiapas for six generations to essentially place the *Leafy* mutation in the Chiapas genetic background.

But before he grew his new corn variety, there was one last environmental variable to consider: the growing season. Karl could only grow for about seven months in New York before the costs of heating the greenhouse became prohibitive. So last year, on his own dime, Karl moved from Olean to the central valley of Costa Rica. "In New York, you have to keep corn from freezing, whereas down here you can focus on trying to grow it out to completion to see what's happening," he says. Late last year, his efforts paid off. In a makeshift greenhouse setup designed to both encourage the plant's growth and support its stem as



ALL EARS: Jason Karl grows giant maize in specially constructed greenhouses in Costa Rica.

COURTESY OF JASON KARL

it climbed toward the sun, he grew a corn plant that measured 45 feet tall with more than 80 internodes—56 more internodes than unmodified Chiapas corn grown under normal conditions. (He regularly publishes his data in the *Maize Newsletter*.)

"What I think is so amazing about this tall plant is it really highlights how quickly a corn plant can turn the sun into carbon, into an incredibly tall plant," says Edward Buckler, a quantitative geneticist at Cornell University who in 2014 helped create a comprehensive map of maize height genetics (*Genetics*, doi:10.1534/genetics.113.159152). "I've never seen anybody grow a 45-foot-tall corn plant; it's impressive. And he put together a cool set of genes in order to pull it off. My hat is off to him."

Although farmers may not be interested in growing super-tall corn plants, which are unable to support themselves, Karl's work could have implications for increasing the height of other crops, says Sarah Hake of the University of California, Berkeley. "Most breeders would rather have their corn shorter so it doesn't fall over. But if this could be sugar cane or *Miscanthus* for biofuel or feeding to animals as a wet grass . . . beyond the science of it, which is interesting, there [are potential applications]."

Karl, though, plans to stick with corn. In addition to refining the growing conditions, he hopes to add some of the other mutations he's identified that might boost plant height. "Then having those stacked together, it should get up a bit higher," says Karl, who already has a 55-foot-tall plastic greenhouse constructed for his next big plant. —Jef Akst

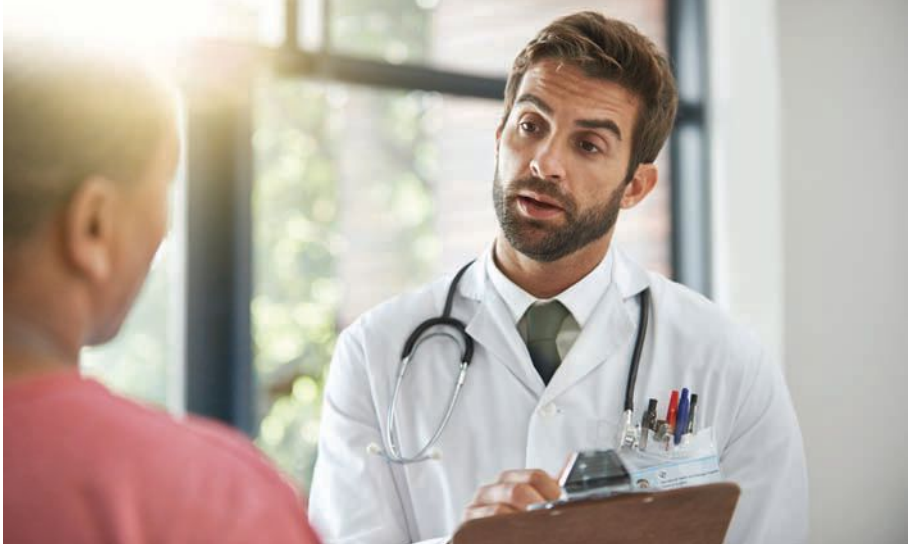
Tracking Cancer's Ups and Downs

The beginning of 2017 was a busy time for cancer awareness, with the World Health Organization (WHO) putting out its *Guide to Cancer Early Diagnosis* in anticipation of World Cancer Day on February 4, and the release of one of the most highly anticipated publications every year: The annual American Cancer Society (ACS) *Cancer Facts & Figures* report—a detailed study of population-based cancer incidence and mortality in the U.S. that teases apart trends across cancer types and demographics.

This year, "we found that the cancer death rate is continuing to decline and has in fact dropped by about 25 percent in the past couple of decades," says the report's lead author, Rebecca Siegel, strategic director of the ACS's Surveillance Information Services. "The reason that's important is that we've recently learned that the trends are no longer declining for many other leading causes of death," such as heart disease and stroke.

Using national data from 1999 to 2013 to build a mathematical model of mortality, the ACS researchers predicted that nearly 601,000 Americans will die from cancer in 2017—a daily average of about 1,650—with the most common causes being lung, colorectal, prostate, and breast cancers. However, the team also estimated that around 659,000 cancer deaths in women, and 1,484,000 in men, have been averted since 1991 due to declines in the death rate. "It's a measure of progress," says Kathy Cronin, deputy director of the Surveillance Research Program at the National Cancer Institute. "For many cancers, we see mortality decreasing."

Such decreases may be the result of improving treatment regimens or reduc-



It's a measure of progress. For many cancers, we see mortality decreasing.

—Kathy Cronin, Surveillance Research Program at the National Cancer Institute

tions in behavioral risk factors, Siegel notes. For example, rates of blood cancer and lymphoma survival have been boosted by the advent of precision medicine in recent years, and the decrease in incidence and death rates for lung cancer—a disease that now has an 18 percent five-year survival rate—probably has smoking cessation campaigns and the overall decrease in cigarettes' popularity to thank.

Rates of cancer screening also play an important role in driving trends in both incidence and mortality. For example, the declines in colorectal cancer incidence—down 3 percent per year from 2004 to 2013—and death rate probably reflect improvements in early detection, notes Scarlett Lin Gomez of the Cancer Prevention Institute of California. Colonoscopy use in adults over age 50 has tripled since 2000, so "polyps are detected and taken out before they have the opportunity to progress to cancer," she explains. "It's a good success story."

But more screening isn't always better, Siegel cautions, particularly in malignancies such as prostate cancer, for which incidence rate is strongly influenced by screening, and treatment carries significant side effects. "There have been rapid declines in the death rate for prostate cancer, and most people think that screening probably has contributed to that progress," she explains. "But there's so much over-diagnosis. . . . We have yet to find the happy middle, where we're detecting early the cases that would go on to cause harm, and we're not detecting [and overtreating] cases that would never have caused harm."

Despite overall declines, not all cancers follow the trend, and an important purpose of the ACS report is to identify areas that need more attention, says Cronin. These statistics "give you an idea if something is emerging and is potentially a problem," she explains. This year's report highlights that "some cancers, such as liver cancer, are increasing, and so that's an area that needs additional research."

The ACS team found that liver cancer incidence rose by 3 percent per year in women and 4 percent per year in men from 2004 to 2013, while the death rate in both sexes climbed by almost 3 percent per year from 2010 to 2014. Lin Gomez says that the increase is partially due to an aging population infected in a 1980s epidemic of hepatitis C virus—a known risk factor—and, potentially, upticks in other risk factors such as obesity. The report also indicates that the incidence of thyroid cancer and of melanoma is not yet decreasing substantially, she adds.

Meanwhile, a finer look at the data set reveals continuing disparity in cancer incidence and death rate between subsets of the American population. For example, men have a 20 percent overall higher incidence of cancer than women, and their mortality rate is 40 percent higher. This gender imbalance is not well understood, though Siegel says that a combination of gender-related risk factors, along with a "different mix" of cancer types in each group, likely explains some of the variation.

The disparities among different ethnicities are similarly striking. Although Siegel notes that "the heterogeneity within these large [ethnic] groups is substantial," breaking up the data reveals that both cancer incidence and death rates are highest in black populations and lowest in Asian Americans. Black Americans are also more likely than whites to be diagnosed with cancer once it has reached an advanced stage, and have lower survival rates at each stage of cancer progression.

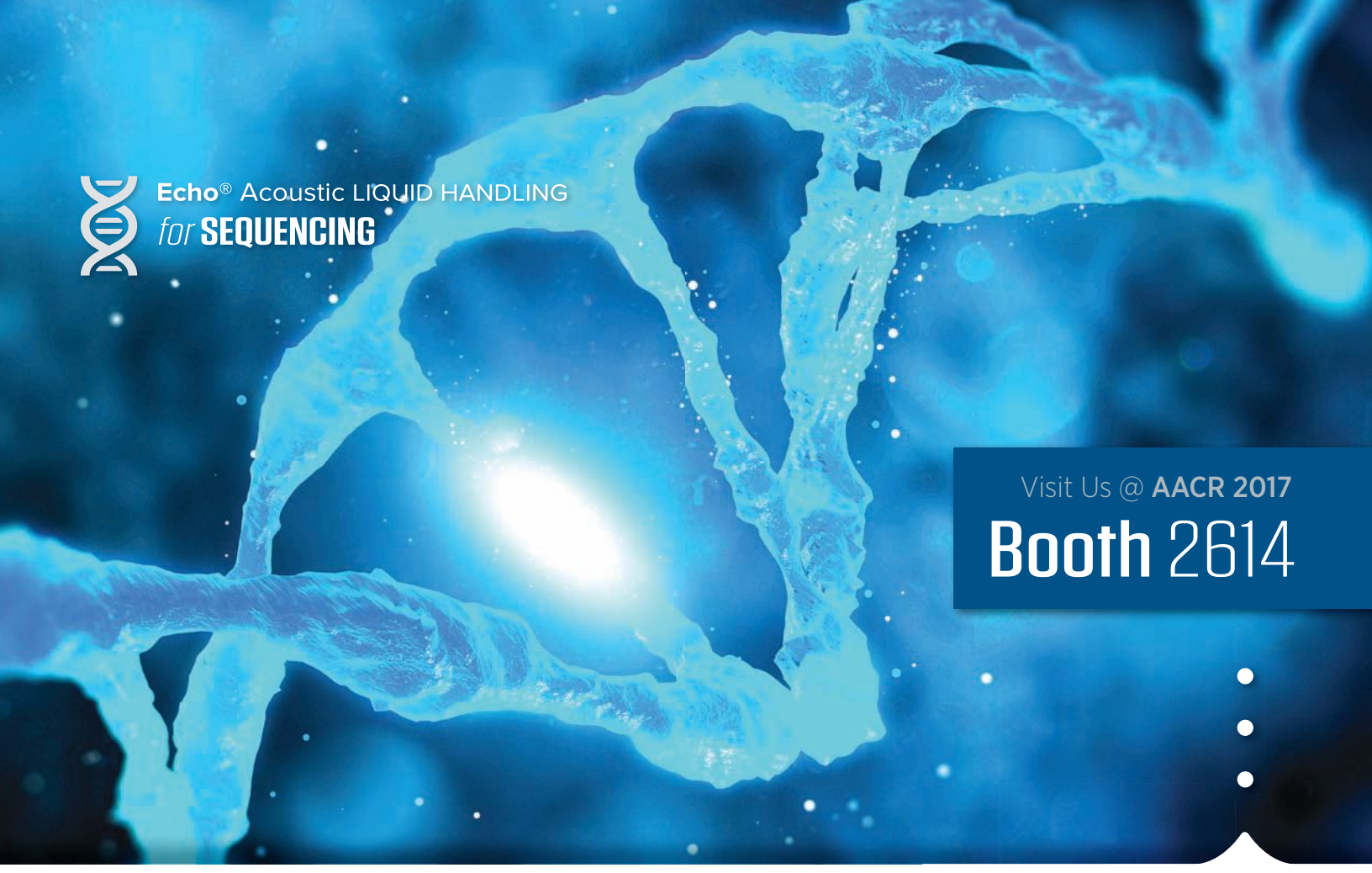
Like other studies, however, the ACS report suggests the gap between races is closing fast. "For example, around 1990, the overall cancer death rate in black men was almost 50 percent higher than it was in white men," says Siegel. "That has dropped to about 20 percent higher today." The decrease has been only slightly less dramatic in black women, falling from 20 percent higher than white women to 13 percent between 1998 and 2014.

Several factors contribute to this trend, Siegel explains, including substantial declines in smoking among black teenagers in the 1970s and recent improvements in access to treatment. "From 2010 to 2015, there's been a drop by half in the proportion of black people in the U.S. who do not have health insurance coverage," she says. "That's huge, huge progress."

Of course, there's more to be done, particularly with insurance coverage rates an issue of current political debate. "Patients with insurance are more likely to receive cancer screening and be diagnosed at an earlier stage," Johns Hopkins Medicine's Craig Pollack wrote in an email to *The Scientist*. "Maintaining and increasing access to affordable insurance coverage is crucial for getting patients the care they need."

"We know that people who don't have health insurance have higher cancer death rates," agrees Siegel, adding that ACS's advocacy arm, the Cancer Action Network, is a vocal supporter of the Affordable Care Act, which President Donald Trump and Republican legislators are trying to repeal. "We're about saving lives. So it's certainly a concern."

—Catherine Offord



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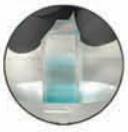

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A Fuller Picture

Measuring PD-L1 levels was a great start. Now we need to quantify more protein biomarkers, assess the tumor mutational landscape, and examine immune cell signatures.

BY DAVID FABRIZIO

Immunotherapy has revolutionized the fight against cancer. People who otherwise would have had little hope of survival are experiencing extraordinary comebacks when treated with a class of medicines called checkpoint inhibitors. The inhibitors disable a molecular disguise that cancer cells use to hide from the immune system, allowing a patient's own defenses to destroy tumors.

Although clearly transformative, these innovative treatments have faced challenges integrating within the framework of personalized oncology. Personalized treatments have traditionally been prescribed based on the presence of a single genetic vulnerability, but in immunotherapy the formula is not so simple. Each person's immune system is wired slightly differently, giving it a unique degree of sensitivity to cancer. Currently, only about 20 percent to 40 percent of patients respond to the most effective combination of two checkpoint inhibitor immunotherapies.

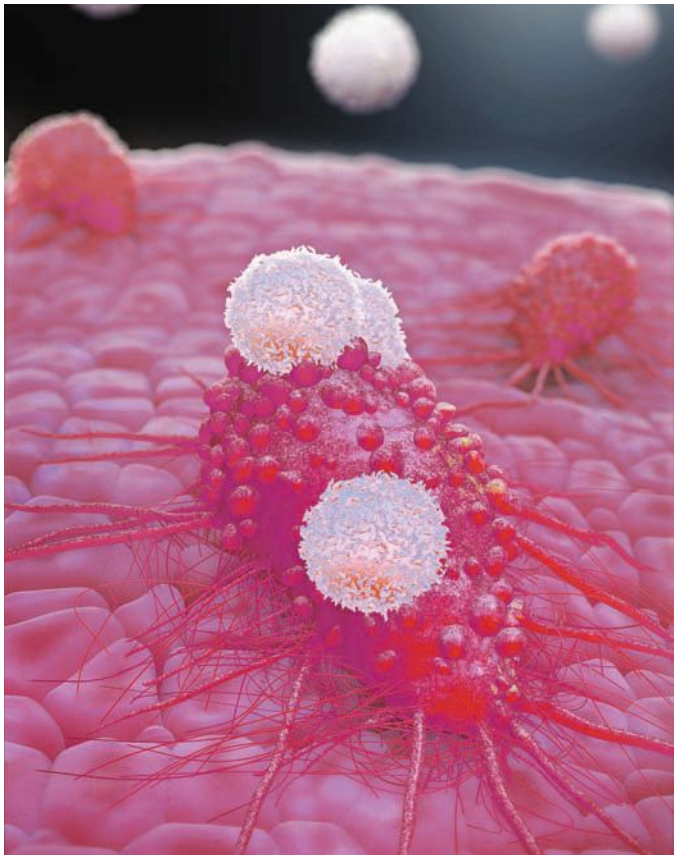
Determining who will benefit from immunotherapy is an enormous challenge. But by taking a comprehensive view—considering protein biomarkers, mutational changes, and immune cell signatures together—I believe we have a promising path forward.

Predicting success with PD-L1

The current standard for predicting responses to checkpoint inhibitor immunotherapies is through the measurement of a protein biomarker called PD-L1—one of the molecular cloaks that hide cancer cells from the immune system. Tumors that produce high levels of PD-L1 generally respond more favorably to anti-PD-1/anti-PD-L1 checkpoint inhibitor immunotherapies than do tumors with lower levels of PD-L1 (or none at all).

Despite its broad acceptance and application, PD-L1 abundance has proven an inconsistent biomarker because of its dynamic expression on both tumor and immune cells and a lack of standardization across PD-L1–detection tests. This biological variability and the subjective thresholds used to determine a positive outcome make it difficult to accurately stratify patients, and may actually affect whether a clinical trial succeeds. For example, if the threshold for PD-L1 expression level is set too low, then not enough people in a trial may respond to the treatment. Indeed, the recent failure of a lung cancer trial has been linked to the inclusion of patients with PD-L1 levels that were too low to make them likely candidates for response.

For immunotherapy, we will need to look at multiple biomarkers—in addition to PD-L1—to determine which approach is right for each patient and each tumor type.



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Getting quantitative

Fortunately, advances in next-generation sequencing, including the development of comprehensive genomic profiling (CGP), have enabled the discovery of new biomarkers that are more rigorous and quantitative, such as microsatellite instability (MSI) and tumor mutational burden (TMB). Rather than focusing on a single protein marker, CGP-measured MSI and TMB are based on quantifying the genomic changes associated with a given tumor.

MSI measures the accumulation of short, repeated sequences of DNA caused by defects in a specific type of DNA repair. In certain cases, "high" MSI can be a predictor for response to checkpoint inhibitor immunotherapy. For example, colorectal cancer patients who are MSI-high have been found more likely to respond to anti-PD-1 treatment.

TMB offers a quantitative measure of the total number of mutations in the coding regions of a tumor's genome. Tumor

cells that have higher levels of TMB—meaning they harbor more mutations—are believed to have more proteins on their surfaces that are distinct from those on a person's healthy cells. These mutated proteins, known as neoantigens, are like red flags that can activate the immune system. Therefore, having high TMB may lead to a more robust immune response to checkpoint inhibitors. (See "Seek and Destroy," page 48.)

There is a growing body of data demonstrating the utility of TMB as a predictive biomarker in advanced cancer. In fact, studies have shown that TMB can help predict responses to US Food and Drug Administration-approved checkpoint inhibitor immunotherapies across multiple tumor types, including lung cancer, melanoma, and bladder cancer. In a bladder cancer study, researchers found that TMB was superior to PD-L1 testing as a means of predicting and stratifying responses to an anti-PD-L1 immunotherapy.

Biological variability and the subjective thresholds used to determine a positive outcome make it difficult to accurately stratify patients.

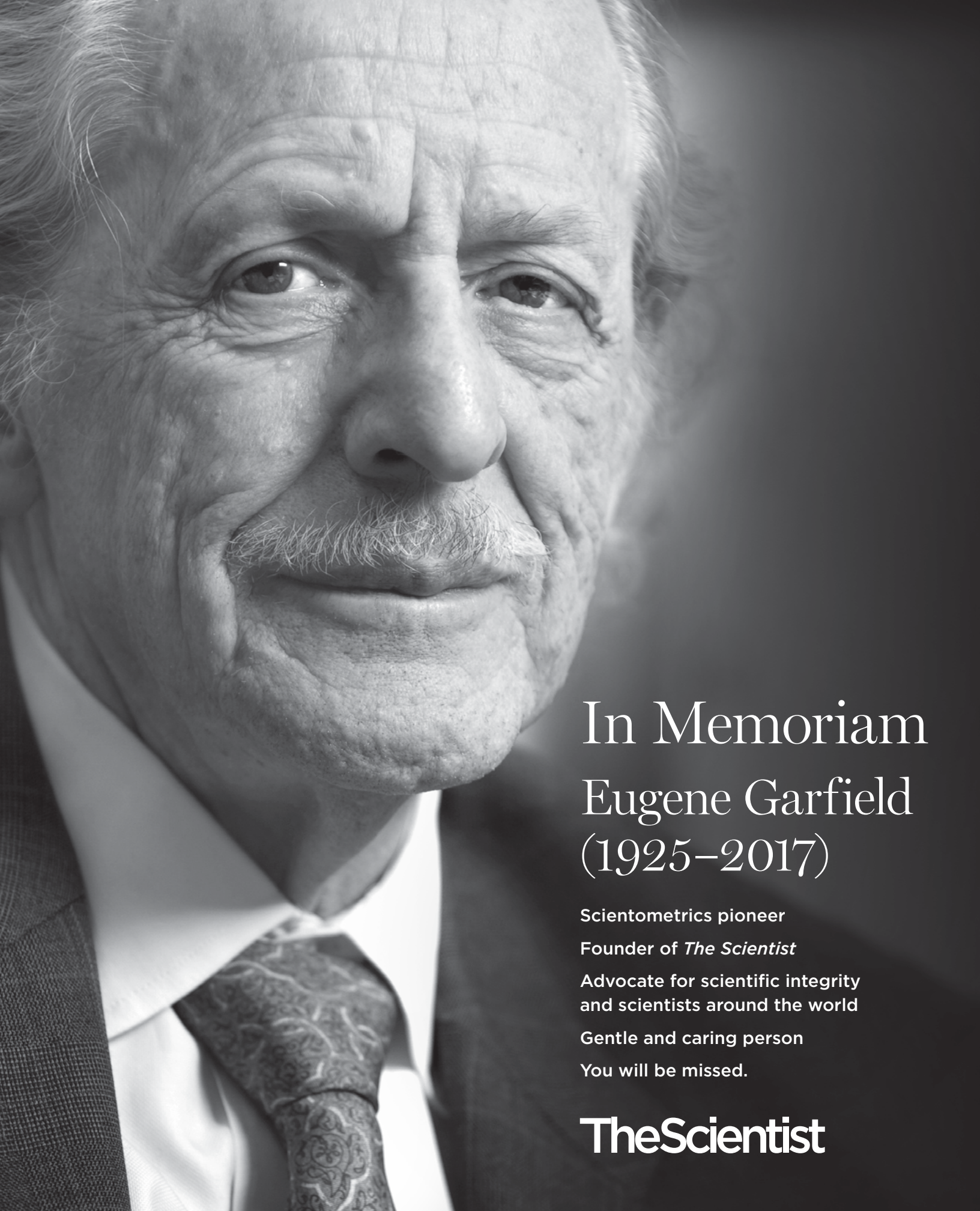
And while PD-L1 testing may be applicable only to checkpoint inhibitors that target the protein and its receptor, TMB has the potential to predict response and guide treatment to any cancer therapy that relies on immune activation, such as T-cell therapies or cancer vaccines. Together with comprehensive genomic profiling, TMB may provide an opportunity for physicians treating patients with advanced-stage cancers to find new treatment options.

The path forward

There is still a need for more diagnostic biomarkers in advanced cancer. One example that illustrates this need is triple-negative breast cancer. This malignancy is not very noticeable to the immune system, so many people have hypothesized that immunotherapies wouldn't work well. But it turns out that the presence of specific immune cells, called tumor-infiltrating lymphocytes, could help predict patient response to the combination of checkpoint inhibitor immunotherapy and chemotherapy, suggesting yet another predictive biomarker for immunotherapies.

While the incredible complexity of the human immune system poses a challenge to the development of biomarkers for immunotherapies, we are hopeful that we will continue to identify multiple biomarkers to guide the selection of the right treatment for the right patient. The potential for immunotherapies to change the cancer treatment landscape is clear. Now we need the right markers to help guide the way. ■

David Fabrizio is the cancer immunotherapy leader at Foundation Medicine, which offers a full suite of comprehensive genomic profiling assays to physicians.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New Traffic Cops on the Block

Bioactive fatty acids play a role in cancer metastasis.

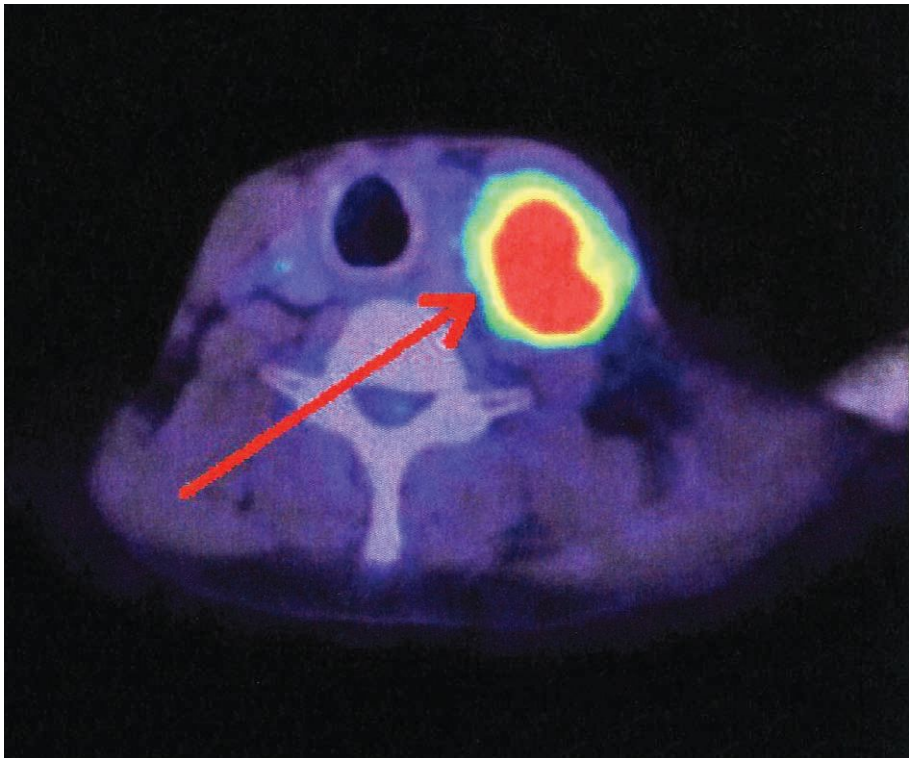
BY AMANDA B. KEENER

Although metastasis is the leading cause of death among people with cancer, researchers are stumped, for the most part, about which molecular signals trigger the exit of malignant cells from primary tumors to start new ones in other parts of the body. Two studies published in *Nature* earlier this year highlight roles in metastasis for an unexpected group of molecules—lipids.

"For many years, we were studying peptides and proteins," says Mariusz Ratajczak, a cell biologist at the University of Louisville who was not involved in the studies. "Now we are coming to bioactive lipids."

In the first study, published January 5, researchers at the Institute for Research in Biomedicine (IRB) in Barcelona reported that, in mice, human oral cancer cells that are most likely to migrate from primary tumors are marked by the surface protein, CD36—a scavenger receptor that binds fatty acids. The researchers initially identified the cells by examining genes upregulated in nondividing tumor cells, finding an increased expression of genes involved in lipid metabolism, transport, and storage—all processes downstream of CD36.

When the researchers knocked down CD36 with short hairpin RNA before injecting oral cancer cells into mice, they prevented the cells from seeding metastatic tumors in the lymph nodes of 80 percent to 100 percent of the animals without significantly changing the frequencies of primary tumors. "What's really cool here is that they showed that CD36 wasn't necessary for self-renewal, but was necessary for dissemination and metastasis," says Justin Lathia, a cell and molecular biologist at the Cleveland Clinic who was not involved in the work. This study, he adds, demonstrates that metastatic cells don't have to be cancer stem cells, which many researchers believe to be the case.



MONITORING METASTASES: Positron emission tomography (PET) scans like this one pinpoint the locations of metastatic tumors.

It also suggests that metastatic cells may have their own unique metabolic regulation. The IRB team demonstrated that feeding mice a high-fat diet increased the size and number of metastatic lymph node tumors. This effect was lost when CD36 was knocked down. The researchers generated the same effect when they pretreated the cancer cells in culture with a dietary fatty acid called palmitic acid. Lathia notes that while this finding could provide insight into the link between obesity and cancer, "human diets are far more complex than what we have here."

The researchers also used a CD36-blocking antibody to shrink lymph node metastases, and induce remission in 15 percent of treated mice. According to Lathia, CD36 may make a good drug target. Blocking the receptor could be helpful beyond oral cancers: the authors used public databases to examine CD36 expression levels in several tumor types, finding that the receptor's abundance correlated with metastasis.

The authors suggested that CD36 may give metastatic cells their edge by allowing them to utilize fatty acid oxidation as an efficient way to generate ATP. Kazuaki Takabe, a cancer biologist at Roswell Park Cancer Institute in Buffalo, New York, who was not involved in the study, wonders whether CD36 might also contribute the cells' preference for lymphatic vessels and lymph node

AKIRA KOUCHIYAMA/WIKIMEDIA COMMONS

microenvironments, where the receptors' ligands are more prevalent.

A second study, published January 12, highlighted such a role for lipid signaling in the tumor microenvironment. By inoculating a series of 810 knockout mouse lines with melanoma cells, a group at the Wellcome Trust Sanger Institute in Cambridge, U.K., found that when mice lacked a protein called Spns2, the cancer cells formed far fewer metastatic tumors in the animals' lungs. Spns2 transports the bioactive lipid sphingosine-1 phosphate (S1P) out of endothelial cells and into the blood. "Generally, the concentration is much higher in the blood than in the tissue," says Ratajczak. S1P forms a gradient that attracts immune cells and other cells from organs into blood and lymphatics.

In Spns2-deficient mice, this gradient was disrupted, which allowed higher concentrations of effector memory T cells to remain in the lungs and kill off melanoma cells that tried to take up residence. The researchers replicated this phenotype by both knocking out *spns2* in only lymphatic endothelial cells and by treating mice with a drug that increases S1P levels in lymphoid tissues.

Two studies highlight roles in metastasis for an unexpected group of molecules—lipids.

Coauthor Anneliese Speak, an immunologist at the Sanger Institute, says that before Spns2 could be considered a drug target, its roles in tumors versus healthy lung tissue must be teased apart. "We sought to focus solely on the host," she says. "The role of Spns2 in the tumor is confusing." For example, notes Speak, high S1P levels in tumors may encourage cancer survival.

"Lipids have many pleiotropic effects," says Ratajczak. "Sometimes it depends on concentration, sometimes it depends on context."

What both studies show, he adds, is that there are many potential ways lipids may regulate metastasis. Ratajczak's own group has found that at least two other bioactive lipids—ceramide-1 phosphate and lysophosphatidic acid—promote metastasis of rhabdomyosarcoma cells. "It's a very broad area," he says. "But we should go in this direction." ■

G. Pascual et al., "Targeting metastasis-initiating cells through the fatty acid receptor CD36," *Nature*, 541:41-45, 2017.

L. van der Weyden et al., "Genome-wide *in vivo* screen identifies novel host regulators of metastatic colonization," *Nature*, 541:233-236, 2017.

Amanda B. Keener is a freelance science writer and frequent contributor to The Scientist. *A version of this story was published at the-scientist.com on January 20, 2017.*

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ALEXANDER DROHAT, PhD
Associate Professor, Department of Biochemistry and Molecular Biology
University of Maryland School of Medicine

SAMUEL HONG, PhD
Postdoctoral Fellow, Department of Biochemistry
Emory University School of Medicine

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Many inflammatory disease processes induce fibroblast recruitment, leaving behind fibrosis in their wake; NASH is one such disease. The hallmark of NASH is fat deposition and fibrosis in the liver in the absence of excessive alcohol consumption, and the disease is closely tied to metabolic dysregulation. *The Scientist* is bringing together a panel of experts to discuss trends in cell-signaling analysis and molecular pharmacology that are informing the search for NASH therapies; to explore the challenges of identifying therapeutic targets for multifactorial, lifestyle-induced diseases; and to share the latest details of their NASH-directed R&D efforts. Attendees will have the opportunity to interact with the experts, ask questions, and seek advice on topics that are related to their research.

ARUN SANYAL, MBBS, PhD
Professor, Departments of Medicine, Physiology, and Molecular Pathology
Virginia Commonwealth University

MANUELA G. NEUMAN, MSc, PhD, FCAB
Professor, Department of Pharmacology and Toxicology, University of Toronto
CEO, In Vitro Drug Safety and Biotechnology
Banting Institute

THOMAS P. BURRIS, PhD
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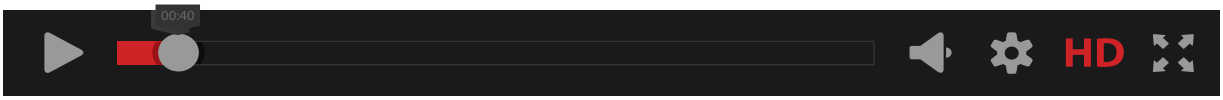

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ANDREA KASINSKI, PhD
William and Patty Miller Assistant Professor
Department of Biological Sciences
Purdue University

XIANSHUANG LIU, MD
Senior Scientist, Department of Neurology
Henry Ford Health System

TOPICS COVERED:

- The various roles of miRNAs in disease progression and recovery
- Steps for developing miRNA-based therapeutics

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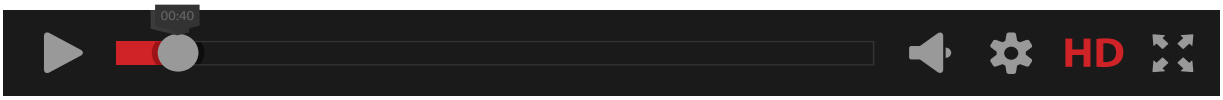
ONDEMAND | Novel Applications of Single-Cell Analysis: From Sequencing to Quantification of Nucleic Acids and Proteins

Single-cell analysis has long been the gold standard of methods for studying variation within a cell population, but interest in the technique has been dampened by low throughput and reproducibility challenges, until recently. Advances in technologies supporting single-cell analysis have enabled an explosion of new applications, from a new way to research pluripotent stem cell heterogeneity using single-cell RNA sequencing (scRNA-seq), to absolute quantification of mRNA and translated proteins within a cell. These methods all rely on digital droplet technology for improved reproducibility and higher throughput. *The Scientist* brings together a panel of experts to discuss their research, and to explore the technical advances available for single-cell analysis.



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MAROOF M. ADIL, PhD
Postdoctoral Scholar, Department
of Chemical & Biomolecular Engineering
University of California, Berkeley

CEM ALBAYRAK, PhD
Assistant Professor, Department of Chemical
and Biological Engineering
Koç University, Istanbul, Turkey

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- How digital droplet technology enables single-cell analyses

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Antibody Use Guidelines

1 Define the Intended Application

Determine the type of immunoassay required.

How is the target protein modified during sample preparation (e.g. denatured vs. native)?

How does the antibody identify the antigen in this application (e.g. cell type differentiation, protein localization, protein expression, or quantification)?

How does the abundance of target protein in native cells or tissues affect the immunoassay?

2 Understand the Antigen's Attributes

Confirm the proper nomenclature for the target protein.

Confirm the protein sequence & molecular weight for the target.

Identify any isoforms, variants or orthologs.

Identify PTMs & other protein processing.

Examine the presence of proteins closely related to the target protein.

Understand domains of interest within the target.

3 Establish Controls for Antigen Detection

Always include both positive and negative controls in assays.

Consider using recombinant proteins and/or lysates over-expressing the target.

Consider genetic manipulation, e.g. knockout models, CRISPR, siRNA.

Consider drugs to modulate target protein expression.

Consider using multiple lysates that display varying levels of target protein expression.

4 Review Antibody Vendor Information

Determine the type of immunogen used for antibody production.

Choose the proper 2° antibody based on the clonality, host, and isotype for the antibody.

Identify the product code, clone ID # and lot identification #.

Confirm validation parameters, e.g. sensitivity, specificity and reproducibility.

Confirm lot specific validation data pertains to the intended assay.

5 Perform In-house Validation

Determine data and protocols required to reproduce validation.

Independently validate antibody using your equipment, methods and materials.

Further optimize parameters, e.g. antibody concentration, buffer conditions, if necessary.

Include appropriate controls in your experiments to ensure sensitivity and specificity.

Commence with experimental research.

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- Use multiple lysates with known target protein expression levels and correlate predicted results with band intensity.

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Targeting Tumor Tregs

New monoclonal antibodies kill cancer-promoting immunosuppressive cells—and more—in vitro.

BY RUTH WILLIAMS

Tumors are adept at locally suppressing the body's immune system, creating a microenvironment that allows unchallenged survival and growth. One way they do this is by recruiting high numbers of regulatory T cells, a type of naturally immunosuppressive T cells known as Tregs or T_{regs}.

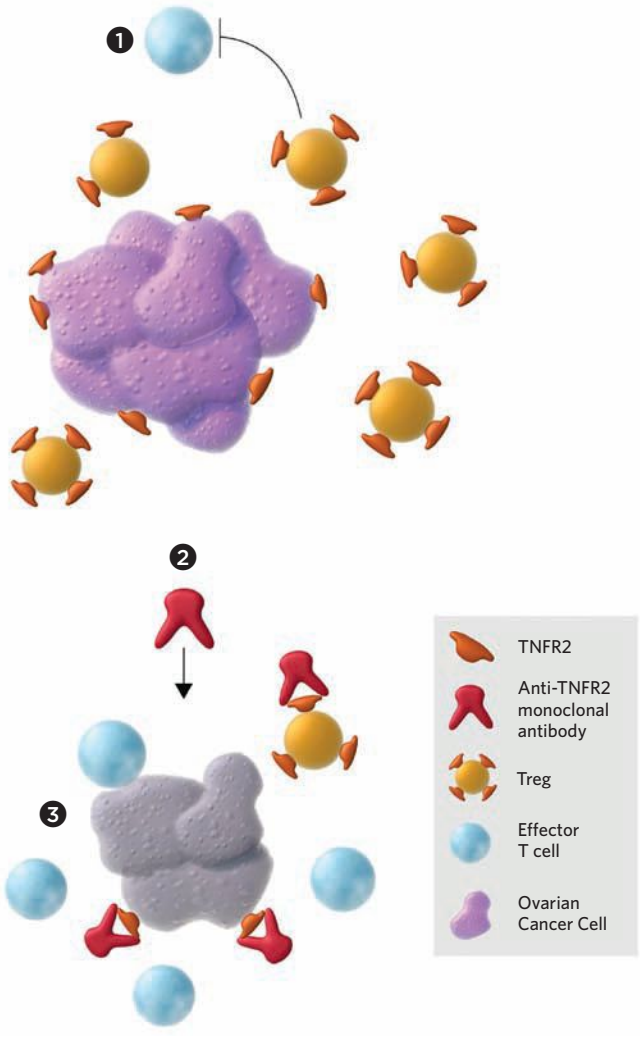
To counter this suppression, scientists are investigating ways to boost cancer patients' immune systems to encourage tumor destruction. But it's a delicate balance: too much immune activation and there's a risk of potentially lethal autoimmune disorders—as has been reported for some patients treated with the immune system–activating drugs ipilimumab (Yervoy) and nivolumab (Opdivo).

Searching for a more refined approach to immunotherapy, Denise Faustman and colleagues at Massachusetts General Hospital and Harvard Medical School have discovered that many tumors recruit a particularly potent type of Treg that expresses a receptor called tumor necrosis factor receptor 2 (TNFR2). These potent Tregs are rare in the rest of the body, but especially abundant within tumors. In some cases, cancer cells themselves express the receptor.

TNFR2 activation promotes Treg proliferation, explains Faustman, an activity that "has not gone unnoticed by tumors, and they're abusing the receptor in the same fashion—for preferential growth," she says.

Faustman therefore hypothesized that antibodies against TNFR2 should target tumor cells as well as Tregs. Sure enough, her group's newly developed antibodies killed both tumor-associated Tregs and ovarian cancer cells, in culture. "It's a double whammy," says Joost Oppenheim of the National Cancer Institute. What's more, non–tumor-associated Tregs were considerably less susceptible to the antibodies, which could reduce the risk of systemic toxicity.

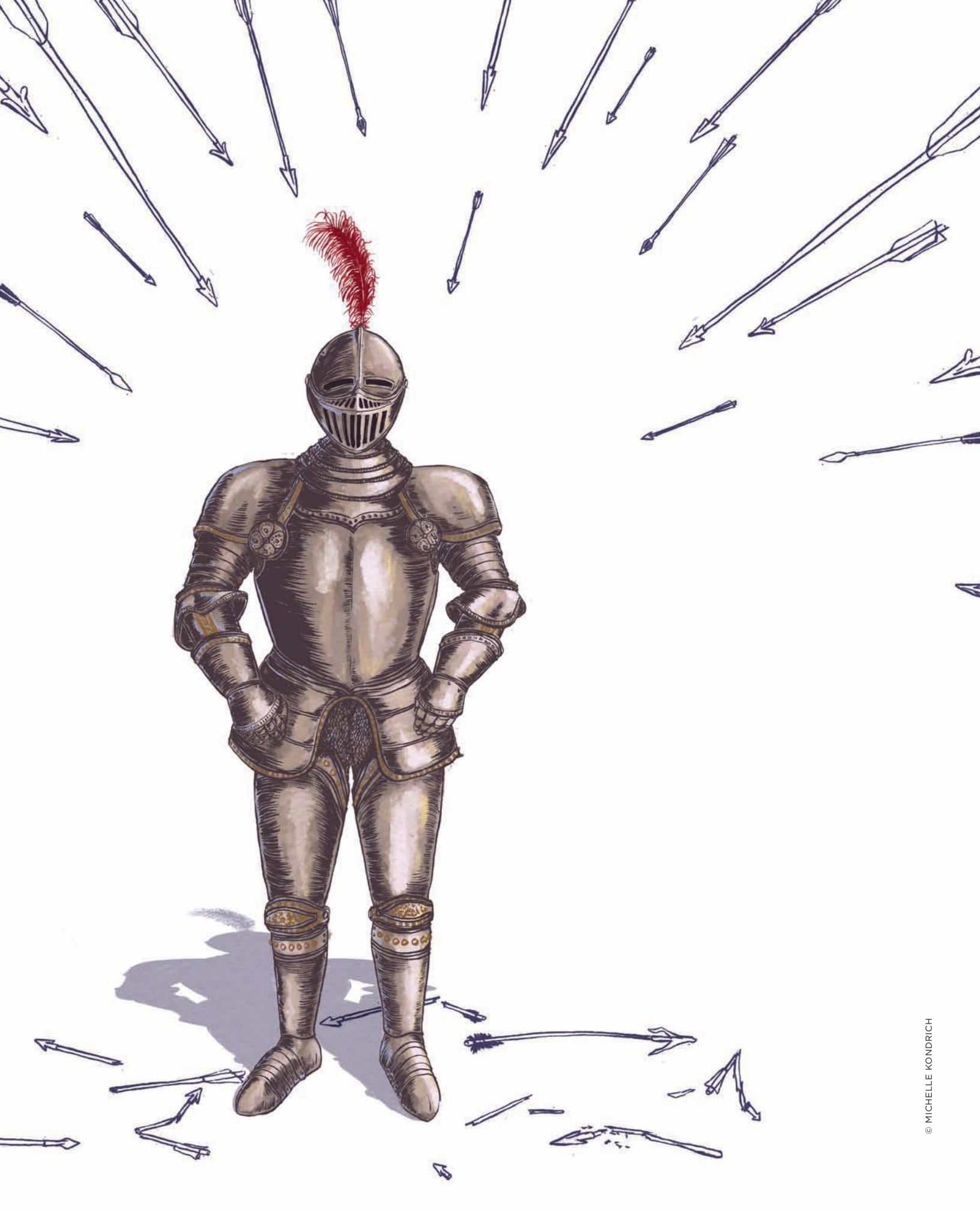
Faustman's team now plans to study other types of human tumor specimens to evaluate the breadth of the antibodies' cancer-killing capacity. But, ultimately, says Oppenheim, "the question is: How will anti-TNFR2 compare with better-documented [immune activators]? . . . That really can only be established by looking in vivo." (*Sci Signal*, 10:eaaf8608, 2017) ■



Tumors (purple cells) recruit abnormally high numbers of potently immune-suppressing Tregs, which repress effector T cells 1 and prevent cancer destruction. Addition of anti-TNFR2 monoclonal antibodies 2 targets and kills TNFR2-expressing Tregs, thereby boosting the activity of effector T cells, which attack the tumor 3. The antibodies can also directly kill tumor cells that express the TNFR2 receptor.

AT A GLANCE

IMMUNE-ACTIVATING MONOCLONAL ANTIBODY	ANTIBODY TARGET	USED TO TREAT	IMMUNE-RELATED ADVERSE EFFECTS
Anti-programmed cell death protein 1 (PD-1) (nivolumab)	PD-1 is expressed on the surface of T cells, where it downregulates the cell's activation, thus suppressing immunity.	Metastatic melanoma and some other late-stage cancers	Some patients have developed autoimmune disorders including myocarditis and pneumonitis.
Anti-TNFR2	TNFR2 is expressed on a subset of immunosuppressive Tregs and some cancer cells, where it promotes cell proliferation.	Not in clinical use	Experiments in mice show that deletion of TNFR2 does not lead to autoimmunity in the animals.



Resist or Desist

Researchers unravel the sophisticated ways cancers evade drugs designed to destroy them.

BY ANNA AZVOLINSKY

Receiving three separate courses of a new class of anticancer immunotherapy agents is not typical for a cancer patient, yet that is what retired Major League Baseball administrator Bill Murray, now 79, endured to treat his melanoma. "When I was told that I might be dying from melanoma, I thought I might as well go for it," says Murray. In 2011, Murray was given a round of a peptide-based vaccine plus nivolumab (Opdivo), a monoclonal antibody that targets the programmed cell death protein 1 (PD-1) displayed on the surface of T cells, as part of a clinical trial at the Moffitt Cancer Center in Florida. Unfortunately, this two-pronged attack—lasting 12 weeks—didn't work.

PD-1 is a signaling receptor on activated T cells that functions as an immune checkpoint, tamping down T cell activity when it detects its counterpart, PD-L1, on a tumor cell's surface. Blocking PD-1 was intended to keep Murray's T cells actively fighting the cancer. Because his tumors did not completely go away, Murray's doctor gave him ipilimumab (Yervoy), then a newly approved antibody, which binds cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), also expressed on the same T cells that express PD-1. Ipilimumab also serves as a checkpoint blockade releasing the checkpoint's break on the immune cells, allowing active T cells to attack cancer. Murray's tumors began to shrink after 12 weeks of treatment. After several more months, ipilimumab "essentially made his disease disappear," says Murray's oncologist Jeffrey Weber, now at New York University.

Five years later, Murray's cancer resurfaced. Weber decided to use the most powerful immune checkpoint inhibitor regimen on the market: a combination of nivolumab plus ipilimumab that was approved by the US Food and Drug Administration (FDA) in January 2016. Over the last nine months of 2016, Murray received four doses of the combo, and he will continue to receive nivolumab maintenance therapy for a total of 12 months.

So far, Murray says he is feeling fine, and he even flew from New York to Florida to spend the winter. But he will have to be regularly monitored for cancer over the coming years; there are never any guarantees that tumors won't return. While immunotherapies provide a better chance for a long-term and durable response, Murray's story highlights that even this new class of cancer treatments is susceptible to drug resistance, a problem that has plagued the field since the first chemotherapies were used in the United States in the 1940s.[1]

> I've been saying this for 15 years: beating cancer takes time, and we need more drugs.
>
> —Charles Sawyers
> Memorial Sloan Kettering Cancer Center

Just as bacteria evolve resistance to antibiotics, cancer cells evolve ways to evade even the best weapons in medicine's arsenal. Tumor cells employ numerous tactics—most of which remain unknown—to escape being killed by chemotherapeutic drugs, cytotoxic agents that indiscriminately kill both cancerous and noncancerous cells in the process of dividing. (See "Quest for Chemotherapy Biomarkers" on page 46.) When researchers began to study the genetic mutations (and, eventually, the entire genomes) of tumors, they identified some of the factors required for cancer cell proliferation and survival, including proteins involved in angiogenesis and signaling kinases that, when mutated, fuel tumor growth. This led to the development of drugs that directly bound and disrupted these fac-

tors. But even these so-called molecular-targeted therapies were plagued by resistance problems, with rapid tumor shrinkage often followed by regrowth of the cancer weeks or months later.

"I've been saying this for 15 years: [beating cancer] takes time, and we need more drugs," says Charles Sawyers of New York City's Memorial Sloan Kettering Cancer Center, where he chairs the Human Oncology and Pathogenesis Program.

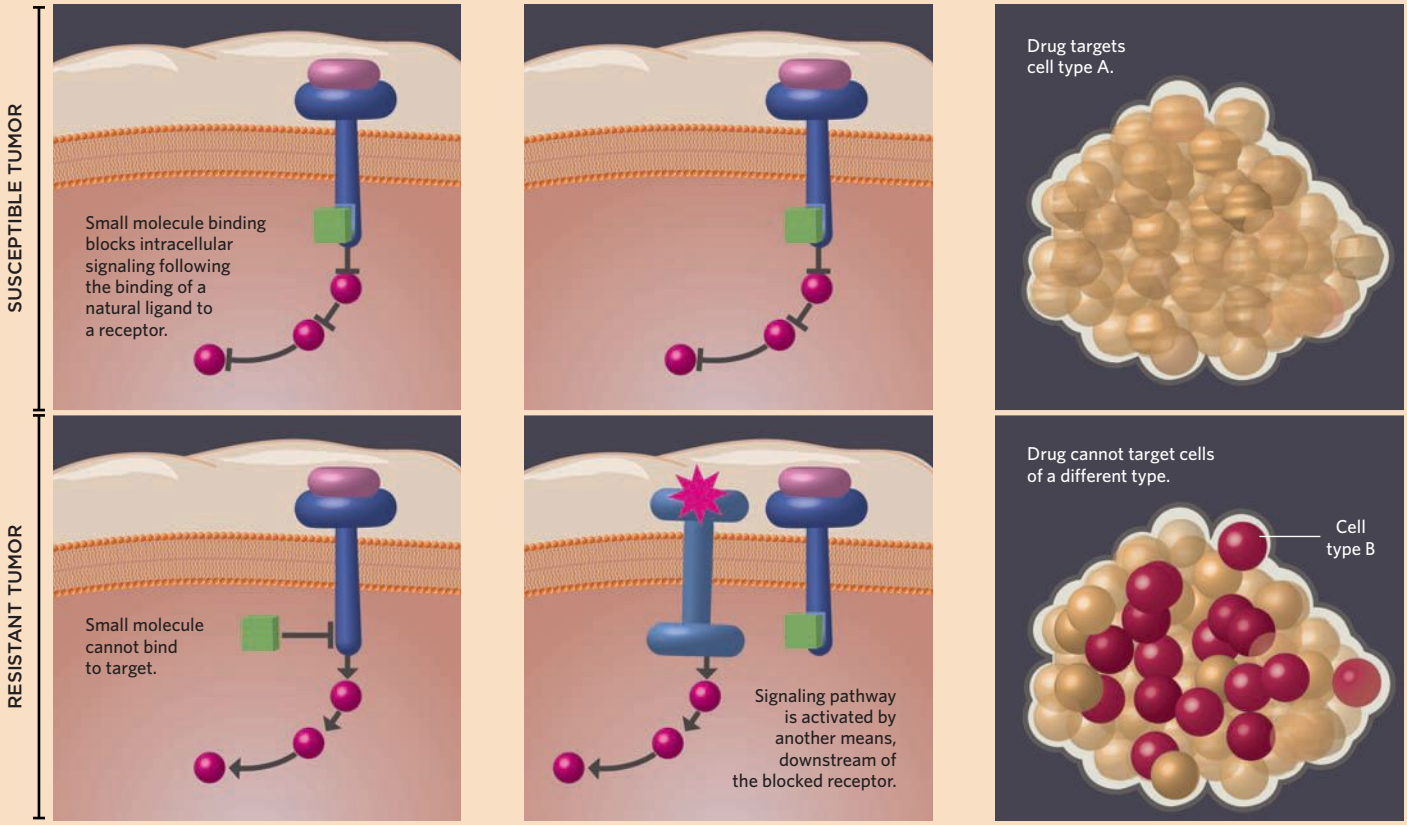
With the advent of checkpoint-inhibiting antibodies and other powerful immunotherapies, oncologists were optimistic that this new approach to cancer would be less evadable than chemotherapy or molecular-targeted therapies had proved to be. But as Murray's experience demonstrates, cancer finds a way. Researchers are now zeroing in on the types of resistance that can emerge following checkpoint inhibition, to determine when resistance is likely to arise and to design more-robust therapeutic strategies.

"The big question is whether it's the tumor cells that are becoming resistant, if the immune system is becoming dysfunctional, or a combination of both," says Jesse Zaretsky, an MD/PhD student at the University of California, Los Angeles (UCLA) who studies mechanisms of immunotherapy resistance in melanoma.

MECHANISMS OF ACQUIRED RESISTANCE

Cancers appear to be able to evolve resistance to many of the therapies doctors have tried. Resistance to chemotherapy likely encompasses a broad range of mechanisms having to do with DNA repair, cell cycle arrest, apoptotic pathways, and others, many of which are still unknown. When it comes to molecular-targeted agents and immunotherapies, however, research has nailed down some basic strategies.

MOLECULAR-TARGETED THERAPIES



Mutations within the target protein can prevent drug binding, or keep the protein active despite drug binding.

Mutations can restore cellular signaling by affecting a downstream gene or by activating a bypass pathway.

Tumor cells can lose characteristics of their typical cell type and acquire characteristics of a different lineage that does not depend on signaling blocked by the cancer drug.

Countering resistance to targeted therapy

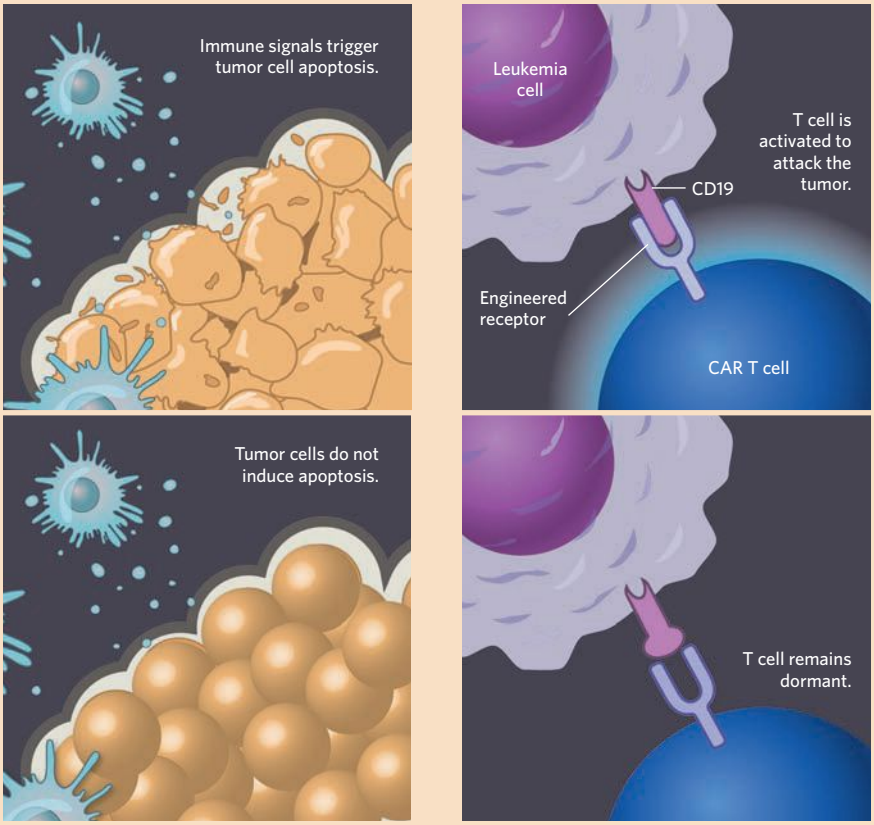
In contrast to the mostly obscure chemotherapy resistance mechanisms, genes and proteins that are likely to change in the evolution of resistance to molecular-targeted therapies are, by and large, predictable. In response, researchers can often devise rational strategies, such as prescribing combinations of drugs that block multiple steps of a tumor growth pathway, to boost a treatment's chance of successfully eliminating a patient's cancer.

One of the first examples of nailing down a mechanism of evolved resistance comes from the field of lung cancer. Approximately 10 percent of lung tumors harbor an activating mutation in the *epidermal growth factor receptor (EGFR)* gene, which encodes a cell-surface receptor that acts as an upstream activator of several pathways (including PI3K and MAP kinase, elements of which are mutated in many cancers). In 2003, the FDA approved the first EGFR inhibitor, gefitinib (Iressa), but within just a few months, some patients stopped responding to the treatment. Two years later, some of the patients whose tumors had become recalcitrant were found to have malignancies that harbored a novel mutation, a methionine-for-threonine substitution at amino acid 790 (T790M) that allowed for continued activation of EGFR despite EGFR inhibitor binding.[2,3]

"Back then, it was very novel to sequence a gene and find new, acquired mutations and then tell the story of how often these occurred in patients," says Geoffrey Oxnard, a medical oncologist at the Dana-Farber Cancer Institute in Boston who was involved in identifying mechanisms of acquired resistance to EGFR inhibitors. "It took years and years of collecting and sequencing patient tumor biopsies to understand that biology." Researchers now know that the vast majority of patients with metastatic EGFR-positive lung cancers eventually develop resistance, and about half those cases are due to a T790M mutation.[4]

In 2009, researchers developed an EGFR inhibitor that specifically targeted T790M mutation–harboring tumor cells,[5] and in 2015, osimertinib (Tagrisso) was approved for clinical use in patients with tumors that are resistant to the first-generation EGFR inhibitors, along with a test that specifically detects T790M mutations. In 2013, the FDA had approved a broader-spectrum tumor tissue test to check for the types of *EGFR* mutations present. "This is the first example of the FDA saying that a patient needs to have a biopsy to figure out the type of resistance in order to choose the next therapy," says Oxnard. "What's cool about this is the potential of science like this going from something discussed in the lab to something that oncologists are using to help treat their patients." (Last June, the agency approved a blood-based genetic test to detect the same mutations that now allows patients to be tested noninvasively.)

IMMUNOTHERAPIES



Mutations can render tumor cells less recognizable to the immune system or less responsive to molecular signals from immune cells.

Mutations and other changes alter the target protein. These can include altered splicing of the tumor target, which blocks recognition by the engineered T cell.

Unfortunately, the fight against cancer's evolution of resistance is far from over. The same year osimertinib was approved, Oxnard and his colleagues described a mutation, C797S, that rendered patient-derived lung cancer cell lines resistant to the new drug.[6] But just as swiftly, the researchers isolated a compound that can bind to this newly identified mutated protein and is effective in killing these tumors in mice.[7] "There's this constant chase of the next resistance mechanism and next therapy," says Oxnard.

Prostate cancers generally take a different tack in acquiring resistance. Typically treated with drugs that prevent the synthesis or activity of male hormones called androgens, prostate tumors can sometimes restore androgen signaling by activating factors downstream of a drug's inhibition. In contrast to EGFR-driven lung tumors treated with an EGFR inhibitor, in which resistance mutations arise within the gene encoding the drug's target, prostate cancer resistance finds a work-around by restoring downstream signaling, including changes in metabolic regulation.[8,9]

In January 2017, Sawyers and his colleagues identified a third mechanism by which a tumor can bypass a molecular-targeted therapy: a change in cell identity, or "lineage plasticity," as Sawyers calls it. His lab initially observed that an unexpectedly high proportion of sequenced metastatic prostate tumors that no longer responded to anti-androgen therapy had an inactivating mutation in the tumor suppressor gene, *p53*. But prior work had shown that a *p53* mutation alone does not confer hormone-based resistance, so the lab looked at the sequencing data more carefully and discovered two other mutations—in the *RB* and *PTEN* tumor suppressor genes; either of these, in combination with p53 inactivation, resulted in hormone therapy resistance in human cell lines and human prostate tumors engrafted in mice.[10] The reason for the resistance turned out to be a shift in cell identity—mutation of *RB* and *p53* led to overexpression of *Sox2*, which encodes a transcription factor necessary for self-renewal in embryonic stem cells.[11] Rather than luminal prostate cells, these doubly mutated cells "are in a multilineage state that does not rely on androgen signaling," Sawyers says. Lineage plasticity was also previously observed in EGFR-mutated lung cancer patients treated with EGFR inhibitors.[12]

"One way to look at these resistance mechanisms is to say, 'This is so depressing,'" Sawyers says. But on a positive note, he adds, if researchers understand how resistance arises, they may be able to overcome it. Two of these resistance mechanisms—the gene expression level changes and subtle lineage switch—are not a result of a genetic change within the tumor, but rather are epigenetic, and are therefore reversible, Sawyers says. By developing ways to modulate protein levels, "in theory we should be able to prevent [these resistance routes] or restore [drug sensitivity]."

Besides clinical and benchtop studies, scientists are turning to in silico methods to understand resistance and ways to combat it. Andrew Read, who studies the evolutionary genetics of disease at Penn State University, recently collaborated on a mathematical model to understand when it is best to use aggressive therapy to try to kill the entire lot of tumor cells or when tumor containment may be best for the patient's health.[13] "The key was to assume that there is competition between [drug-] resistant and sensitive tumor cells," explains Read. When a tumor rapidly mutates, an attack-all, swift approach may select for the extra-hardy, resistant cells that will take over the entire tumor. In these cases, keeping drug-sensitive, less-aggressive tumor cells in the mix may actually be advantageous for the patient.

> There's this constant chase of the next resistance mechanism and next therapy.
>
> —Geoffrey Oxnard
> Dana-Farber Cancer Institute

Picking apart immunotherapy resistance

While immunotherapies are the new kids on the cancer block, Murray and other patients are already forcing researchers to think about the evolution of resistance. Murray appears to have originally had what's called a "hot" tumor, says Weber—one that has been infiltrated with immune cells and proinflammatory molecules and is thus more likely to respond to a checkpoint inhibitor.[14] Murray's cancer initially retreated after ipilimumab treatment, then likely developed some type of adaptive resistance while retaining enough residual T cells within the tumor to respond to the third round of immune stimulation, Weber says.

The good news is that resistance in patients who initially respond to immunotherapy appears to be less frequent

than in patients treated with a targeted therapy, most of whom can be assured that their tumors will eventually become resistant despite treatment. "It's fair to say that resistance probably occurs less frequently with immunotherapy," says Walter Urba, an oncologist who specializes in immunotherapy at Providence Health & Services in Portland. But the fact that acquired resistance is infrequent among patients undergoing immunotherapies makes studying the underlying mechanisms difficult. "This makes the science of exploring and understanding resistance a bit of a challenge because, so far, the reports have only had single-digit numbers of patients," says Matthew Hellmann, an oncologist at the Memorial Sloan Kettering Cancer Center in New York City who specializes in lung cancer and immunotherapy.

To better understand the changes from pre- to posttreatment that may lead to immunotherapy resistance, researchers are beginning to sample immunotherapy-treated tumors in relapsing patients several years after their initial checkpoint inhibitor dose, searching for factors linked with a cancer's acquired resistance. In a first-of-its-kind study, Antoni Ribas, director of the tumor immunology program at UCLA, and his colleagues combed the whole exomes of metastatic melanoma tumor samples from four patients who had initially responded to the anti-PD-1 antibody pembrolizumab (Keytruda) and then stopped responding, their tumors beginning to grow again months or even years later.

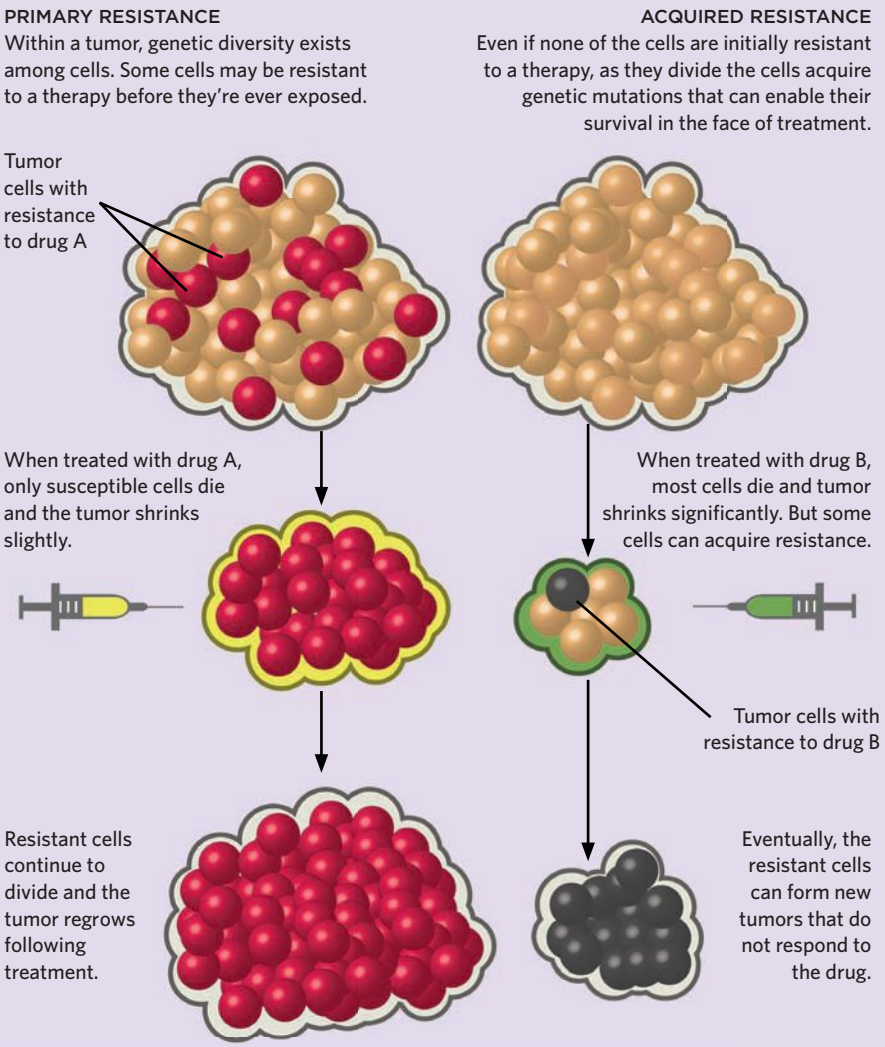
Two of the patients' posttreatment tumors, but not their pretreatment ones, had loss-of-function mutations in either the *Janus kinase 1* or *2 (JAK1* or *JAK2)* genes, which encode proteins that sense extracellular interferon gamma signaling and convert that into an intracellular response. The mutations likely rendered the tumor cells insensitive to interferon secreted by activated T cells, resulting in less tumor antigen presentation to the immune system and resistance to interferon-induced growth arrest.[15] In a third patient, acquired resistance was mapped to a mutation in the gene for the protein beta-2-microglobulin, which is necessary for cells to present major histocompatibility complex (MHC)-linked antigens on their surface; the loss of this gene thus enables tumor cells to hide out and elude recognition by T cells. The researchers did not find any mutations they could mechanistically link to resistance in the fourth patient, "but that's not to say that someone else won't find a mechanism that we just didn't recognize," says Zaretsky, one of the authors of the work.

"This study, where the authors showed that genetic mutations in key immune processing genes within the tumor can lead to immunotherapy resistance, has triggered the whole field to rethink how tumors adapt to prevent their own destruction by the immune system," says Ryan Sullivan, a translational researcher who specializes in melanoma at Massachusetts General Hospital.

Another immunotherapy, one that is nearing market approval, is also facing resistance problems: chimeric antigen receptor (CAR) T cells. For these therapies, researchers harvest T cells from a

PRIMARY VERSUS ACQUIRED RESISTANCE

Cancer cells can evolve ways to evade a drug's attack, or they may already be resistant prior to treatment.



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patient's blood, then modify the T cells, priming them for activation by tumor antigens, multiply the cells in the lab, and infuse them back into the patient. (See "Safety Belts" on page 73.) One such T-cell modification is the addition of an antibody-derived activating receptor to CD19, a protein normally found on the surface of B cells, including those in B-cell malignancies. In the case of T cells modified to target CD19-expressing leukemia cells, the majority of patients will initially respond to therapy, but about 30 percent of responders will soon relapse, seemingly with leukemia cells that no longer carry the surface marker.

But digging into how the cells are able to survive without CD19, which is thought to be required for B-cell growth, Andrei Thomas-Tikhonenko of the University of Pennsylvania discovered the "resistant cells are not actually CD19-negative." Rather, they express an alternative isoform of the protein that is missing the exon 2–encoded domain, which is where the antibody-derived CAR receptor binds CD19.[16] Thomas-Tikhonenko and his colleagues also found that levels of the splicing factor responsible for retaining exon 2 within the protein were lower in the relapsed leukemia cells compared with cells biopsied prior to treatment. The researchers are now trying to reverse this splicing regulation to force exon 2 inclusion, thus rendering the cells susceptible to anti-CD19 CAR T-cell therapies.

Of course, it's early days for understanding cancer's evasion of these types of treatments. "The mechanisms of resistance for immunotherapy are literally just being described now," says Jason Luke, a medical oncologist who conducts melanoma immunotherapy trials at the University of Chicago. For now, researchers continue to monitor the situation.[17] Luke is organizing a study in which biopsies of patients' tumors will be taken throughout their treatment on an anti-PD-1 antibody. Meanwhile, Sullivan at Mass General is sequencing melanoma tumors before and after anti-PD-1 treatment—the same approach used by the UCLA melanoma team. And researchers at Merck, which manufactures the anti-PD-1 antibody pembrolizumab, are also studying the changes that can convert a responding patient into a resistant one.

> **The big question is whether it's the tumor cells that are becoming resistant, if the immune system is becoming dysfunctional, or a combination of both.**
>
> —Jesse Zaretsky
> University of California, Los Angeles

Another study, led by Jennifer Wargo of the University of Texas MD Anderson Cancer Center in Houston, aimed to identify biomarkers of response to immune checkpoint inhibitors anti-CTLA-4 and anti-PD-1 antibodies. Response rates to these agents range from 10 to 50 percent, depending on tumor type. Her lab's recent work showed that the tumor's genetic makeup, its gene-expression profile, and the tumor microenvironment all influence melanoma patients' responsiveness to this type of immunotherapy, and that a biopsy early in the course of treatment rather than a pretreatment biopsy was most telling of whether a patient is likely to respond to the treatment.[18]

And in a follow-up study of tumor whole exome and T-cell receptor sequencing with the same cohort of melanoma patients that first received an anti-CTLA-4 and then an anti-PD1 antibody, Wargo and her team found that a high tumor mutational load and a genome-wide high copy number loss each independently predicted lack of response to the immunotherapies.[19] The team also found that if more of the patient's T cells carried receptors that bound the same target, that individual was more likely to respond to an anti-PD1 but not an anti-CTLA-4 antibody.

"It's a general theme we and other researchers are learning," says Wargo. "It's not a single biomarker that will give us the

QUEST FOR CHEMOTHERAPY BIOMARKERS

Despite advances in molecular-targeted therapies and immunotherapies, chemotherapy—the use of cytotoxic drugs to kill proliferating cells, both cancerous and noncancerous—is still a frontline treatment for many cancers. But researchers have been hampered by the lack of biomarkers that can predict whether a patient will be sensitive to a particular agent, or resistant, in which case the exposure to chemotherapy and its toxicity would be unwarranted. (See "Pharma Cooperates to Achieve Precision Medicine," *The Scientist,* February 2017.)

"It is striking to see how many studies there have been, and yet there is nothing in clinical practice," says Ken Olaussen of the Institut de Cancérologie Gustave Roussy in France. "The failure rate is huge."

The lack of reliable biomarkers stems partly from the fact that chemotherapy's reach is so broad, acting as a nonspecific cytotoxin that damages DNA. Tumor cells employ many tactics to avoid being killed when their DNA is extensively damaged, from altering their metabolism to prevent entry of the drug, to modifying DNA repair pathways and turning off apoptosis.

In 2005, following research demonstrating that post-surgery cisplatin chemotherapy improved survival in some lung cancer patients, Olaussen and his colleagues attempted to find a biomarker that correlated with treatment response. They focused on the expression of *ERCC1,* a gene involved in DNA repair, because such repair pathways are particularly active in tumor cells that are able to resist being killed by cisplatin. Staining patients' tumor samples with an ERCC1-targeted antibody, Olaussen and his colleagues found that tumors from patients who had minimal levels of ERCC1 protein appeared to derive a greater benefit from the chemotherapy (*N Engl J Med,* 355:983-91, 2006).

complete story on response. It's the combination of the genome, the host immune system, and even environmental factors like the microbiome."

Identifying mechanisms of immunotherapy resistance will not only help avoid such cases where patients stop responding to treatment, but will likely also shed light on so-called primary resistance, when a cancer never responds in the first place, says David Kaufman, the executive director of New Jersey–based Merck's oncology translational research division. Indeed, Ribas and his colleagues have already identified some of the same *JAK1* or *JAK2* mutations found in tumors with acquired resistance to checkpoint inhibitors in tumors of melanoma patients that never responded to this type of immunotherapy.[20] Moreover, these tumors did not express PD-L1, indicating that they may use a different checkpoint to disarm the immune system, which accounts for their lack of response to anti-PD-1 antibodies.

"Our finding that some patients can have these mutations before immunotherapy highlights that those tumors had already gone through a process . . . to avoid an immune response," says Ribas. "Understanding these mechanisms of resistance should allow us to start thinking about [personalizing] immunotherapies, the same way we think about it for targeted therapies for cancer." ■

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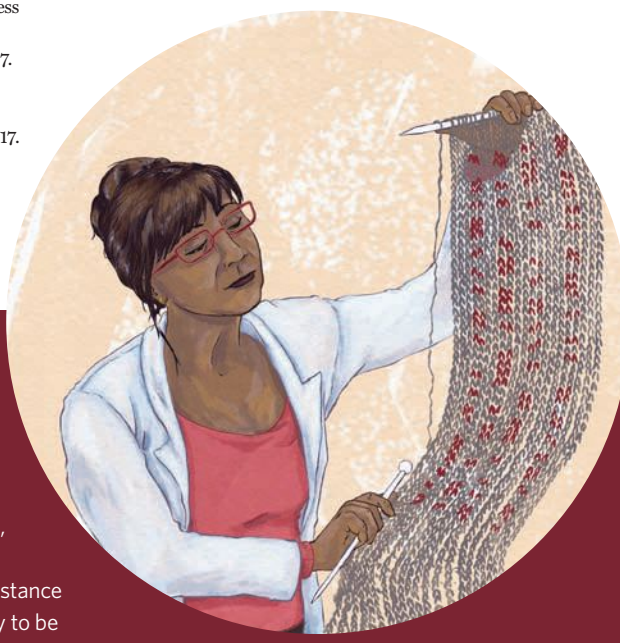

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"Sensitivity testing to match a cancer with a specific chemo has generally been a tough business. In 2006, this study made us think that we could now characterize lung tumors to decide who should get which chemotherapy," says medical oncologist Geoffrey Oxnard of the Dana-Farber Cancer Institute in Boston, who was not involved in the research. But attempts to validate ERCC1 protein levels as a biomarker in larger patient cohorts were unsuccessful, because no existing antibody could distinguish between the four different protein isoforms of ERCC1 found in cells, only one of which appeared to be relevant to cisplatin sensitivity in lung cancer patients (*N Engl J Med*, 368:1101-10, 2013).

Olaussen is not giving up. "We showed that the biology is not wrong but that we currently don't have the tools to measure *ERCC1* expression correctly," he says. "We're now trying to find new solutions where we combine functional assays or try to develop an antibody that works."

Others have moved on, though. For Oxnard, "the benefit for patients, even if we do find chemotherapy-resistance biomarkers, is likely to be modest." As a result, chemotherapy studies have been deprioritized in the context of bigger questions about immunotherapy and targeted therapies. "New studies are difficult," Olaussen says. Few studies include a no-treatment group, which is necessary to deduce changes linked specifically to chemotherapy, he explains, and "there is a general lack of funding to address these questions."

SEEK AND DESTROY

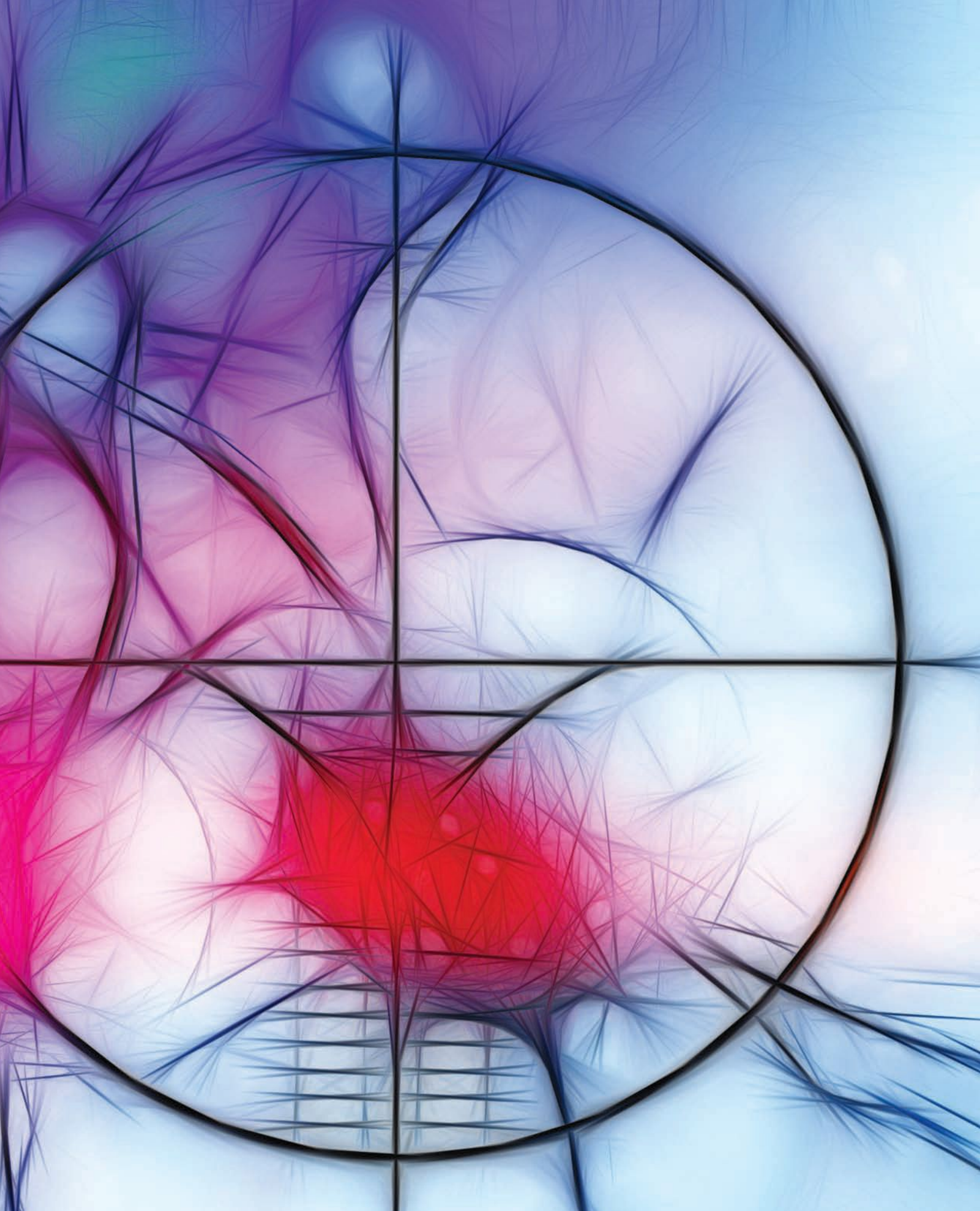
Tumors harbor the seeds of their own destruction, in the form of mutations that encode neoantigens recognized by T cells.

BY STEPHEN P. SCHOENBERGER AND EZRA COHEN

A cancer diagnosis often results in any number of relatively nonspecific treatments, such as surgery, radiation, or chemotherapy, all of which can destroy healthy tissue along with the tumor. Seeking approaches that could successfully eradicate tumors while avoiding such collateral damage from aggressive therapy, researchers have developed a number of treatments targeted to specific types of tumors and, more recently, a handful of therapies aimed at modulating the body's immune cells to more effectively fight its cancer. Mounting evidence suggests that such immunotherapies can effectively turn patients' own immune systems against the very molecules that distinguish the tumor from normal cells, allowing the body's T cells to serve as guided missiles that seek and destroy only the intended target.

This approach is based on the progressive mutational process that drives cancer evolution and generates antigens that are expressed exclusively in and on tumor cells. (See "Trunks and Branches" on page 70.) By training the immune system to target those tumor-specific antigens, called neoantigens, researchers hope to selectively eradicate the cancer cells while leaving healthy tissue unharmed.

Advances in genomic sequencing and bioinformatics over the past decade have synergized to produce a clearer picture of



the immune response to cancer and to move this concept from the laboratory to clinical practice. Hailed as nothing short of a revolution in oncology, immunotherapies have the potential to upend the field's standard of nonspecific, often damaging treatment regimens. Understanding the nature of cancer neoantigens is critical to continued development of these precision therapies.

Cancer as a disease of mutations

Cancer typically develops from a single cell that, as it divides into a clonal population, accumulates function-altering mutations in the genes that control cell growth, survival, and differentiation. This conceptual framework has become a central tenet of how the disease is both understood and treated.

The accumulation of mutations that control critical cellular functions is believed to occur throughout a normal cell's progression towards neoplasia—the stage at which a cell can be considered cancerous. In support of this notion, researchers have observed that early events in cancer development frequently involve loss-of-function mutations in DNA-repair proteins, thereby accelerating the rate of mutation accumulation in the tumor.[1]

Recent advances in both the cost and capacity of genomic sequencing and the development of powerful new computational methods for its analysis have enabled the mutational landscape of a number of histologically distinct tumors to be evaluated and cataloged. These efforts have revealed that a surprising range in the mutational burden exists among different tumor types, with those arising in mutagen-exposed tissues such as skin, lung, and bladder containing the greatest numbers, second only to those tumors lacking DNA mismatch repair (MMR) or proofreading functions, as occurs most commonly in certain subsets of colorectal and endometrial cancer.[2] While some of these mutations are in known "driver" oncogenes, the majority occur in genes whose functions play no obvious role in either establishing or maintaining the transformed state, and are collectively referred to as "passenger" mutations.

Both driver and passenger mutations can lead to the cell's production of tumor-specific neoantigens, which can be recognized by the T lymphocytes that are tasked with detecting foreign invaders in the body. T cells typically recognize short, linear peptides derived from proteins of intracellular and extracellular pathogens and presented on the major histocompatibility complex (MHC) molecules found at the surface of nearly all the cells in the body. While MHC-bound peptides that are derived from normal self proteins are largely ignored—a process known as tolerance—those that differ in sequence, even by a single amino acid, can be efficiently targeted for destruction by T cells.

Whereas the presentation of foreign peptides on the surface of cells infected with viral and bacterial pathogens is a well-studied phenomenon, only recently have researchers begun to consider the facts that tumor cells also display foreign molecules (in the form of mutated peptides) and that these antigens could be exploited for tumor control. Numerous preclinical animal-immunization studies have shown that both induced and spontaneous tumors possess varying degrees of intrinsic antigenicity, meaning that the immune system—specifically $CD4^+$ and $CD8^+$ T cells—can protect the animals from cancer development when they are rechallenged with the same tumor type. The earliest studies determined that the relevant antigens were those encoded by the viral oncogenes used to generate the experimental tumors, but subsequent work on spontaneous tumors showed that T cells can and do target mutated self proteins.[3] And recent studies in both mice and humans have documented the appearance of T cells specific for neoantigens expressed by a tumor.[4]

> Mounting evidence suggests that immunotherapies can effectively turn patients' own immune systems against the very molecules that distinguish the tumor from normal cells.

One type of immunotherapy in which a tumor's neoantigens are suspected to play a role is immune checkpoint inhibitors, which block inhibitory signals that would otherwise repress the body's cancer-fighting T cells. In both preclinical models and human cancer patients, administration of antibodies to block immune checkpoint pathways, including PD-1/PD-L1 and CTLA-4, can elicit strong antitumor T-cell responses. In 2015, several groups discovered that responsiveness to immune checkpoint blockade correlates with neoantigen load;[4,5] the more tumor-specific antigens the cancer cells have, the greater chance the body's army of T cells will include some lymphocytes with matching receptors. Similarly, a growing number of clinical studies testing the use of T-cell transfusions, also known as adoptive cellular therapy, have demonstrated that mutant gene products are the immunological targets of the transferred lymphocytes.

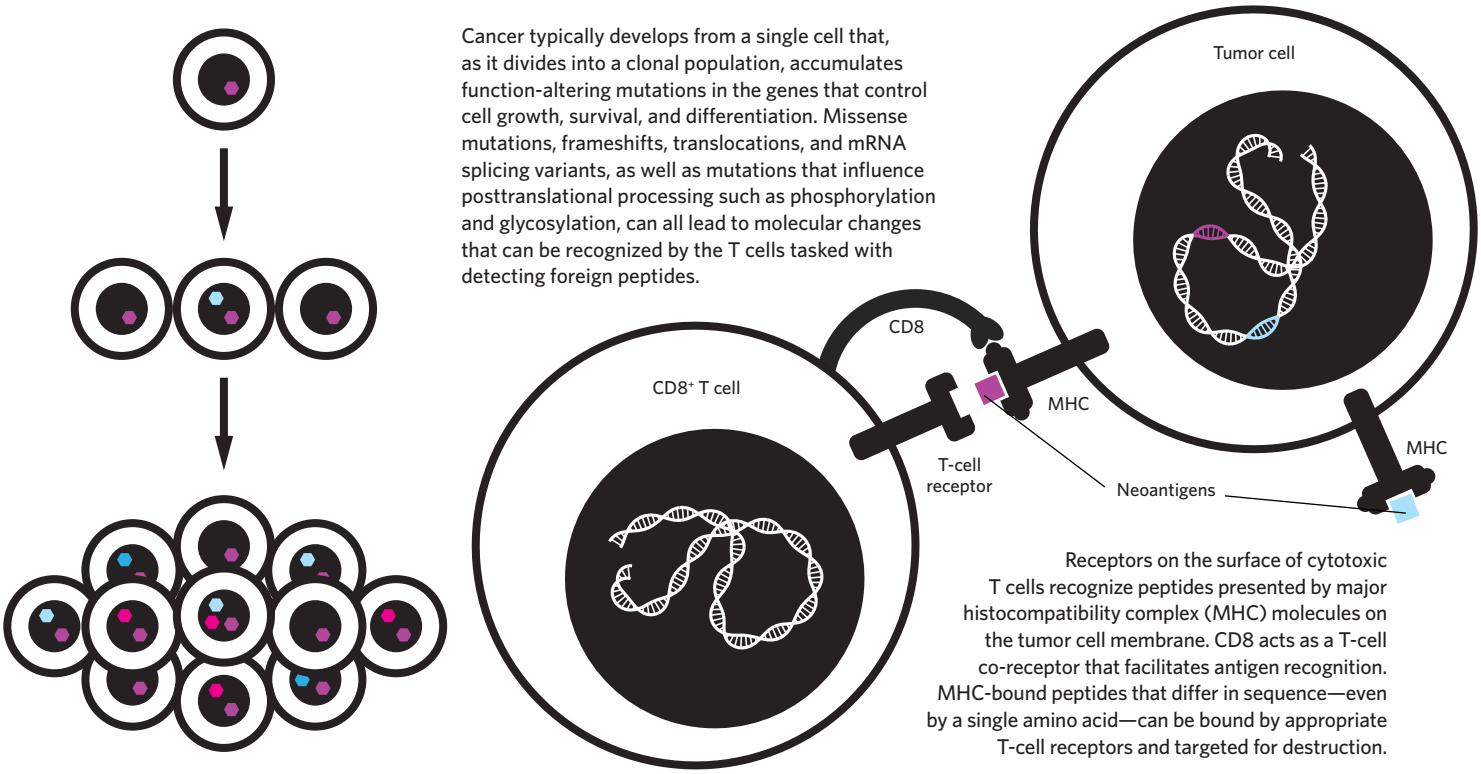
These fundamental studies and single-patient results have provided a compelling case for targeting neoantigens as a class across a range of cancers. The next key developments must be to rapidly identify unique cancer markers and train the immune system to effectively target them.

The rocky path to the clinic

Neoantigens derive from somatic mutations that produce modified or novel peptide sequences within a tumor cell's repertoire of expressed proteins. These include missense mutations, frameshifts, translocations, and mRNA splicing variants, as well as

TARGETING CANCER ANTIGENS

As tumor cells divide, they accrue mutations that result in modified or novel peptide sequences that are unique to the cancer. Known as neoantigens, these tumor-specific proteins could be the key to developing effective cancer therapies.



mutations that influence posttranslational processing, such as phosphorylation and glycosylation. All of these mutations can result in molecular changes that can be discriminated by an appropriate T-cell receptor.

Identification of tumor-expressed somatic mutations by sequencing is a relatively straightforward exercise that is increasingly within the grasp of most clinical research centers. The general strategy is to perform genomic or whole-exome sequencing of both a tumor and a reference genome (usually obtainable from peripheral lymphocytes or buccal swabs), as well as RNA sequencing to confirm that variants identified are indeed expressed in the tumor.

Predicting whether a patient will have an immune response to a particular mutation is challenging, however, as this depends not only on the presence of a suitable T cell within an individual's immune repertoire, but also on myriad factors pertaining to the mutant protein's ability to be processed and shuttled to the lymph nodes for interrogation by antigen-presenting cells. (See "Special Delivery" on page 66.) Nonetheless, researchers are now working to improve meth-

TUMOR MUTATION GLOSSARY

DRIVER MUTATIONS: Mutations in known oncogenes that help cells establish or maintain the transformed state
PASSENGER MUTATIONS: Mutations not known to play a causal role in the development or spread of cancer

Both driver and passenger mutations can lead to the cell's production of tumor-specific neoantigens.

TRUNK MUTATIONS: Mutations present in the majority of tumor cells
BRANCH MUTATIONS: Mutations that arise either late in the development of the cancer or in a select subclonal population

Therapies aim to target trunk mutations to effectively wipe out an entire tumor.

THE SCIENTIST STAFF

ods for identifying neoantigens in human cancer, in hopes of being able to develop personalized vaccine and cellular therapy approaches.

To this end, a number of computational tools have been developed to analyze a range of features thought to be relevant to a given peptide's ability to be a T-cell target. These include the amino acid sequence of the mutated peptide, its similarity to the corresponding wild-type sequence, its predicted ability to undergo proteolysis, and its predicted binding affinity to relevant MHC molecules. The success rate of these analyses in forecasting which somatic mutations can be neoantigen targets is, to date, less than impressive, however. As an alternative approach to neoantigen identification, researchers have used sensitive mass spectrometry techniques to define the spectrum of peptides bound to a tumor's surface MHC molecules. While this strategy has successfully identified neoantigen targets in murine tumors, its applicability to human cancers has yet to be established.[6]

Predicting whether a patient will have an immune response to a particular mutation is challenging.

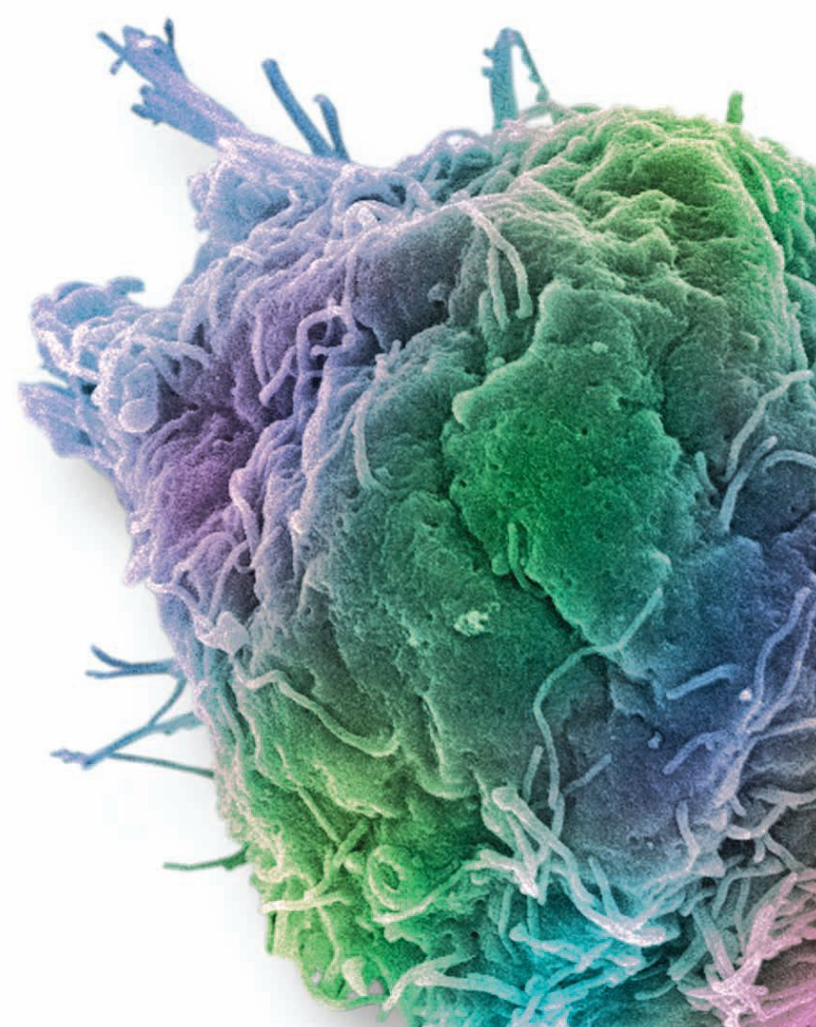
A third approach is to marry the empiricism and sequence analysis themes inherent in the first two, but instead of working purely in computational space, researchers test a patient's peripheral or tumor-infiltrating T cells for recognition of predicted neoantigens ex vivo. This has the advantage of confirming, rather than presuming, what the relevant targets are likely to be and allowing for the discovery of responses that would not have been evident from the computational models.

As our tools for the cellular- and molecular-level interrogation of tumors and for the identification of neoantigens continue to improve, other challenges to their development as therapeutics have become increasingly clear—namely, cancer's ability to adapt. Tumor cells' adaptations to maximize growth and therapeutic resistance likely represent the most significant impediment to neoantigen-guided precision immunotherapy. Tumors can counteract immune control via a number of extrinsic pathways of adaptive resistance, including those that exploit normal physiological pathways of immune suppression.[7] By eliminating the presentation of antigens, for example, such pathways can render the tumor invisible to the immune system. (See "Resist or Desist" on page 40.)

The genetic heterogeneity that results from tumor cells' evolution can also present a significant obstacle to neoantigen-focused immunotherapeutic strategies, as not all cells will carry the targeted antigens. Retrospective studies on patients who underwent checkpoint blockade immunotherapies have found that positive responses were associated with targeting clonally expressed neoantigens, which are present on most or all tumor cells. Treatments targeting subclonal mutations, on the other hand, tended to result in little or no response in the patients.[8]

As sequencing costs continue to decrease, research efforts should be aimed at capturing the clonal diversity of somatic mutations present within an individual patient over the course of his or her disease. In this way, clinicians can have a chance of identifying the mutations present in the majority of tumor sites (the "trunk" mutations) versus those that arise either late in the development of the cancer or in a select subclonal population (the "branch" mutations). Although it is tempting to imagine that driver mutations would, by virtue of their potent effects on enhancing self-renewal, more likely be found in the trunk than the branches of a tumor's mutational tree, it is just as likely that passenger mutations can provide the type of target coverage desired for an effective neoantigen-focused immunotherapy, in light of their greater number.

The wide variety in mutational burden among different cancers, however, may limit the number of instances in which this concept can be meaningfully tested. Cancers at extreme ends of the mutational burden spectrum may be less amenable, since

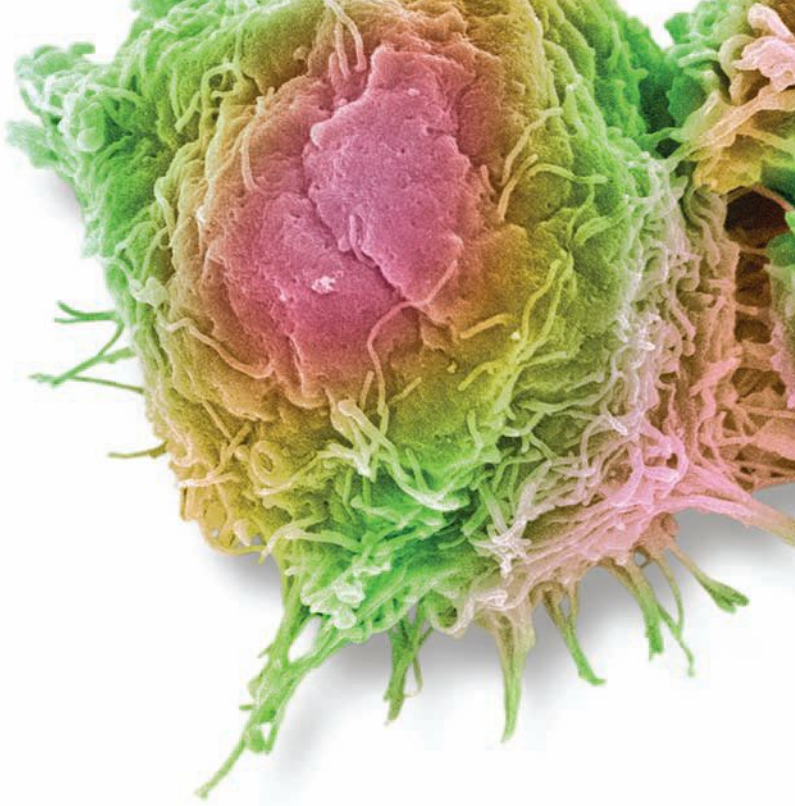


those with a low mutational load will provide few neoantigen targets, while those with a high mutational load will have too many to test.

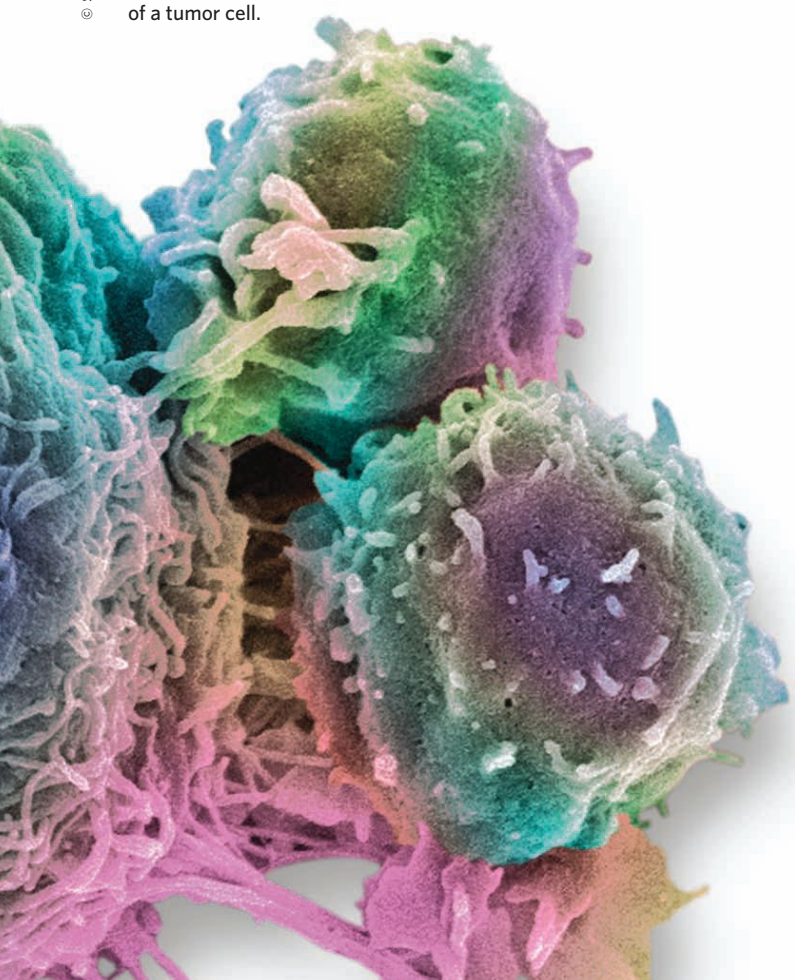
Early trials

Although it is still early in terms of clinical development, investigators have launched the first trials of neoantigen-guided immunotherapy, with methods ranging from peptide-loaded dendritic cells to lipoplexed mRNA being evaluated in Phase 1 clinical trials.[9,10] (See table on page 51.) A handful of trials are under way, and many more are being planned through numerous industry-academic collaborations. One hurdle for the routine clinical use of such personalized approaches will be to establish platforms to manufacture clinical-grade reagents for use in a single patient that are cost-effective and completed in a timely manner to avoid disease progression. This will clearly require an unprecedented degree of cooperation and alignment among clinicians, biopharmaceutical companies, and regulatory entities.

Given the personalized nature of a neoantigen-based vaccine, this strategy might be best employed when some cancer remains after prior treatment or in successfully treated cancers with a high rate of recurrence. More-aggressive therapies, such as the delivery of cancer-fighting T cells and checkpoint inhibitors to take the brakes off the immune system, will likely remain the better option for those with advanced disease and a high tumor burden. Nonetheless, it is unlikely that any monotherapy will be as effective as a combination. The pairing of two or more of these approaches could prove to be a synergistic intervention—one that provides a durable treatment benefit for the majority of cancer patients. ■



STUCK ON YOU: T cells (smaller cells) bind to antigens on the surface of a tumor cell.



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Cures by the Clock

For many diseases, timing treatments to circadian rhythms may make therapies more effective.

BY JYOTI MADHUSOODANAN

For three consecutive winters, starting in 2011, researchers at the University of Birmingham asked healthy men and women over the age of 65 to come in to clinics across the western Midlands in the U.K. for a seasonal influenza vaccination at specific times of day—either between 9 and 11 a.m., or between 3 and 5 p.m. Blood drawn a month later revealed that participants, who totaled nearly 300 over the three years, had higher levels of anti-flu antibodies if they'd received their vaccinations in the morning.[1] The results suggested that daily rhythms of people's bodies tweaked the vaccine's effectiveness. Lead author Anna Phillips Whittaker had suspected as much, after observing similar trends in her studies on behavioral factors such as exercise that affect vaccination responses, and in the wake of a growing body of literature suggesting that a little timing can go a long way when it comes to health.

Many hormones and immune signals are produced rhythmically in 24-hour cycles. Cortisol, for example, which is known to suppress inflammation and regulate certain T cell–mediated immune responses, peaks early in the morning and ebbs as the day progresses. Other facets of the immune system undergo similar cycles that could underlie the differences in antibody responses Phillips observed among people receiving the flu vaccine. Much more work is required to nail down the immune mechanisms responsible for such variation and exploit them appropriately, she says. But timing flu vaccine delivery would be straightforward to implement. "It's such a simple, low-risk intervention that's free to do, and could have massive implications for vulnerable populations."

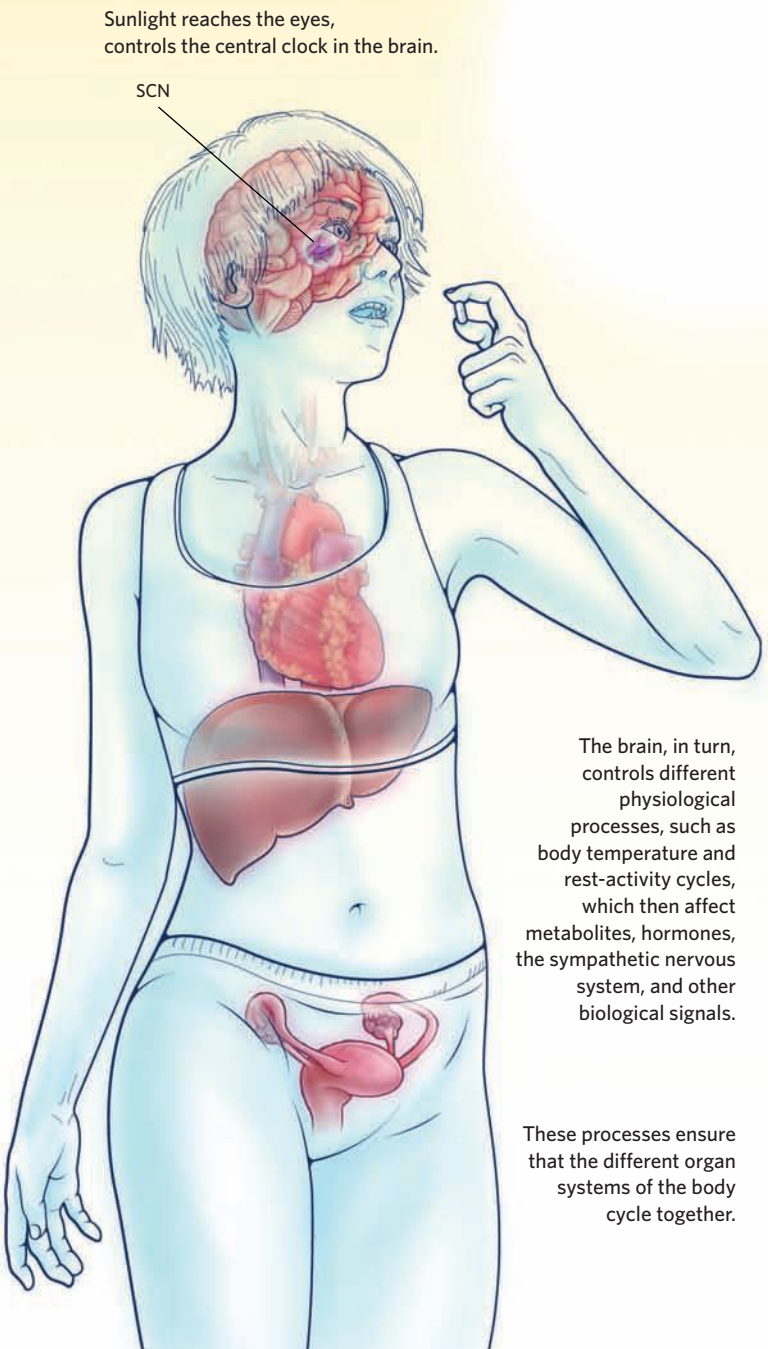
Across diseases, from cancer and cardiac ailments to allergies and arthritis, epidemiological data and clinical trials are revealing that timing medications to the body's internal clock could improve their effectiveness and reduce side effects. Although this concept, known as chronotherapy, has existed for at least 60 years, it has received little attention from physicians. But as biologists continue to unveil the molecular intricacies of cellular rhythms, they are beginning to realize just how pervasive the circadian clock's influence is. In a 2014 study of gene expression in mice, for example, researchers found periodic expression in conserved mammalian genes targeted by 56 of the top 100 best-selling drugs in the U.S., including aripiprazole (Abilify, an antipsychotic), esomeprazole (Nexium, for heartburn), and duloxetine (Cymbalta, for depression), even though most are not currently prescribed with suggested dosing times.[2]

But chronotherapy is gaining clinical traction, says University of Pennsylvania chronobiologist John Hogenesch, senior author on the 2014 study. "Now we have the groundwork to precisely understand a person's clock and leverage that information for better health," he says. "Because of the molecular work, we've opened new doors here. This [idea] is not coming from left field anymore."

Even so, researchers and clinicians working on chronotherapy still face skepticism, and implementing a new drug-delivery protocol or gaining regulatory approval from the US Food and Drug Administration (FDA) for time-of-day indications remains challenging. Thus, while the biomedical research community is starting to take notice of the body's internal rhythms, timed therapies are still the exception to the rule.

BY DAY OR BY NIGHT

The human body undergoes daily cycles in gene expression, protein levels, enzymatic activity, and overall function. Light is the strongest regulator of the central circadian rhythm. When light strikes a mammal's eyes, it triggers an electrical impulse that activates neurons in the suprachiasmatic nucleus (SCN), the seat of the brain's timekeeping machinery. The SCN sets the pace for neuronal and hormonal signals that regulate body temperature, feeding behavior, rest or activity, immune cell functions, and other daily activities, which in combination with direct signals from the SCN keep the body's peripheral organs ticking in synchrony.



The body's clock

By the 1970s, experimental data were piling up to support the idea that timing of exposure to toxins, X-rays, or drugs could alter the effects of these agents.[3] Researchers found that the rate-limiting enzyme regulating the synthesis of cholesterol in rats was most active at night, for example.[4] Within the next few years, researchers were examining cholesterol regulation in humans and testing the effects of administering cholesterol-lowering drugs at different times of day. Short-acting drugs such as simvastatin, which is still prescribed today, are most effective when taken at bedtime.[5] Accordingly, the FDA has long recommended taking such medications in the evening.

In the past 20 years, a slew of studies using genetic screens and genome-wide expression analyses have begun to establish the reasons for these and similar observations.[6] A group of approximately 20,000 neurons in a region of the hypothalamus called the suprachiasmatic nucleus (SCN) acts as a central timekeeper, while clock genes expressed around the body form self-regulating feedback loops that allow the body to keep time at the level of individual organs, tissues, or cells. Nerve impulses and hormonal cues initiated by SCN activity relay central timing information to peripheral clocks, and external cues including light, mealtimes, or temperature can alter peripheral clocks, which then send feedback to other systems in the body.

Mounting evidence indicates that keeping the body's cells synced up matters to the health of an organism. In 2007, based on epidemiological studies, the International Agency for Research on Cancer declared shift work, which causes circadian disruption, a carcinogen. Other studies have elucidated a link between immune cell activity and glucocorticoids—which are secreted in circadian patterns and regulate peripheral clocks—as well as a role for chronic stress in perturbing daily cycles in gene expression, which can alter immune, endocrine, and other functions.[7] This more precise knowledge is beginning to infiltrate the clinic, finally coming to the aid of physicians trying to more effectively time therapeutic interventions.

"There are thousands of studies since the 1970s, but little of that work was done in a mechanistic fashion," says Hogenesch. "Now we have a relatively complete picture of clock networks across the organism, and we can see actual genetic targets that are oscillating, so we can begin investigating the mechanisms underlying those observations."

Timing for tumors

Genes involved in cell division were among the earliest identified as being rhythmically expressed in both rodent models and human cells. In 1987, researchers studying ovarian cancers found that tumor cells synthesized DNA on a daily rhythm that typically peaked in the late morning hours, nearly 12 hours out of sync with nontumor cells.[8] This led the team to suggest that timing chemotherapy doses that target cells actively replicating their DNA might improve the drugs' effectiveness while reducing healthy-cell death.

Sure enough, over the past 30 years, experimental models and clinical trials have found that timing chemo regimens can significantly affect their toxicity and effectiveness. In animal studies of nearly 30 chemo drugs, tailoring dosing time to the medication's mode of action has been found to decrease toxic side effects and increase effectiveness.

In one study, rats that received the chemotherapy drug cisplatin at the time of day when their urinary output was highest (a correlate of other timed cycles in kidney metabolism) had fewer nephrotoxic effects, as measured in kidney function tests, than animals that received the doses at the time of minimum urinary output.[9] In another study, oxaliplatin chemotherapy caused fewer intestinal lesions and less bone marrow suppression in mice when given at night, possibly because DNA synthesis in murine bone marrow is highest during the day.[10]

Because rodents are nocturnal, however, the appropriate schedule changes as the experiments move into humans. And timing drug administration becomes even more complicated when patients are treated with combination therapies. But researchers are seeing success in human studies testing chronotherapy for cancer. In multiple clinical trials, they have found that patients with ovarian, endometrial, or metastatic bladder cancer who received doxorubicin at 6:00 a.m. and cisplatin 12 hours later experienced less toxicity and greater tumor response and survival than those who received the drugs in the reverse sequence.[11] Oncologist and biomedicine professor Francis Levi, a pioneer of chronobiology research now at the University of Warwick, has also shown repeatedly that patients experience better responses and fewer side effects from toxicity when drugs are administered at specific times of day.[12] "At this point we have conducted about 30 clinical trials," he says. "We have found that chronotherapy can improve survival up to fivefold and shrink tumors twice as much when compared to conventionally administered chemotherapy."

Now we have the groundwork to precisely understand a person's clock and leverage that information for better health.

—John Hogenesch, University of Pennsylvania

But circadian-timed chemotherapy only shows benefits for approximately half of patients in trials, Levi notes. One possible influence is gender, he says. In a meta-analysis of data from three Phase 3 trials, he and his colleagues found that nighttime chemotherapy improved survival in men but not women.[13] Phillips has also noticed gender-specific effects in studies of morning versus afternoon vaccinations, but only in younger populations, not among the elderly, so age may also play a role.

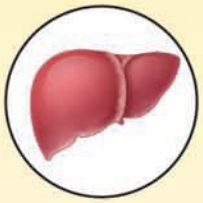
Another factor could be genetics. In 2014, a group of Israeli scientists found that in mice, glucocorticoid signaling—mediated by hormones that peak at night and taper off in the morning—suppressed levels of epidermal growth factor receptor (EGFR), which has been linked to tumor growth and migration. EGFR signals were stronger during the day when glucocorticoid levels were lowest; correspondingly, tumors in mice that were driven by EGFR mutations grew faster at this time. An EGFR inhibitor used to treat breast cancer slowed tumor growth more when given to the animals in the daytime than when the same dose was administered at night.[14]

The core clock genes themselves may also differ among individuals. In wild-type mice, researchers found a timing-dependent

TIMING TREATMENTS TO THE CLOCK

Regulated by peripheral clocks and interactions with other organs, many metabolic pathways in the body peak and ebb in specific circadian patterns. As a result, drugs targeting these pathways can work better when taken at particular times of day. Here are a few examples.

LIVER

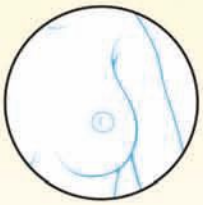


Condition: High cholesterol

Circadian pattern: Cholesterol synthesis is higher at night.

Timing treatments: The FDA recommends the short-acting statin simvastatin be taken in the evening.

BREAST



Condition: Breast cancer

Circadian pattern: In mice, glucocorticoid signaling, which suppresses levels of epidermal growth factor receptor (EGFR), peaks at night and tapers off in the morning, allowing EGFR signals to rise during the day.

Timing treatments: In an animal study, daytime doses of the EGFR inhibitor lapatinib were better at reducing tumor size.

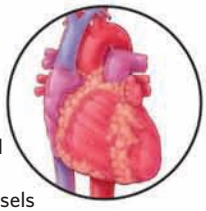
OVARY

Condition: Ovarian cancer

Circadian pattern: In dividing tumor cells, DNA replication peaks in the late morning, 12 hours out of sync with normal ovarian cells.

Timing treatments: In clinical trials, the DNA-damaging chemotherapy agent cisplatin caused fewer side effects and improved effectiveness when taken in the evenings.

CARDIOVASCULAR SYSTEM



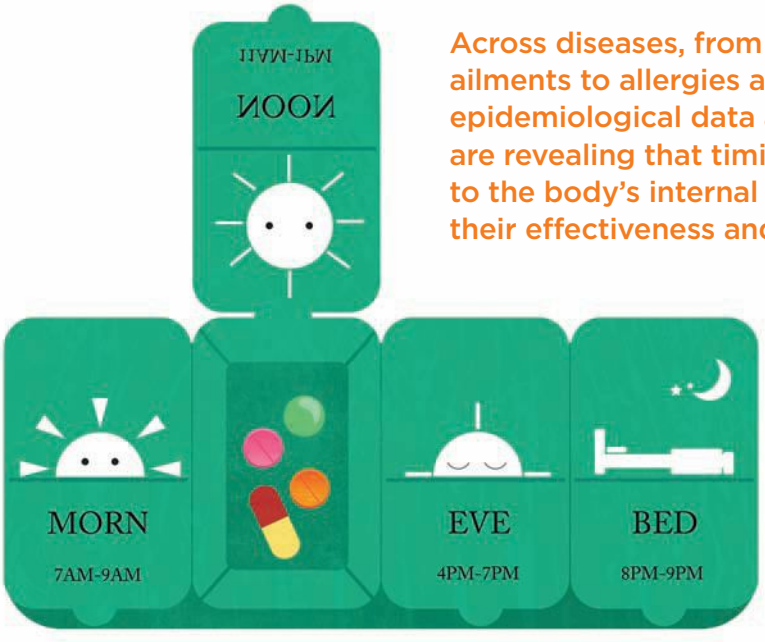
Condition: High blood pressure

Circadian pattern: Angiotensin-2 receptor levels are higher at night.

Timing treatments: Studies have suggested that bedtime doses of hypertension drugs that target this receptor and help blood vessels relax are more effective than morning doses.

response to the chemo drug cyclophosphamide, but they also found that animals lacking a circadian rhythm because of mutations in the clock genes *BMAL1* and *CLOCK* did not show a time-dependent response.[15] Another study by Levi and colleagues, using circadian gene expression data for 27 genes from mouse liver and human colon cancer cell lines, found that the optimal time to administer the chemotherapy agent irinotecan could be predicted based on a gene regulatory loop controlled by clock genes *BMAL1* and *REV-ERBα*. When *BMAL1* was silenced in vitro, irinotecan's timing-dependent effects vanished.[16]

Whatever the cause of the variation, researchers must now deal with it in a systematic way, Levi says. "Until a few years ago, our working hypothesis was to deliver chronotherapy to an average rhythmic pattern in a population, so all patients receive the exact same protocol," he says. "But interpatient differences could result in a marked improvement in some cases and none in others. This clearly indicates that we need to identify individual rhythms, analogous to what we're doing in personalized medicine now." (See "Getting Personal," *The Scientist,* February 2017.)



Across diseases, from cancer and cardiac ailments to allergies and arthritis, epidemiological data and clinical trials are revealing that timing medications to the body's internal clock could improve their effectiveness and reduce side effects.

Clocking other conditions

Even as chronotherapy was gaining recognition in the oncology research community, investigators realized that cancer was not the only disease likely to be affected by circadian cycles. Clinical trials in 1985 found that antihistamines were most effective when taken at night or early in the morning. Subsequent studies established that inhaling corticosteroids at bedtime, or using delayed-release prednisone formulations that allocated the medication to the body pre-dawn, were most effective at combating allergy symptoms.[17] Cardiovascular events were also recognized early on to cycle throughout the day, as doctors noticed that most patients admitted for heart attacks tended to experience their symptoms between 6:00 a.m. and noon.[18]

More recently, researchers have begun to capitalize on the body's link to the clock. In 2009, for example, after finding that blood pressure declines at bedtime and starts to rise early in the morning—in part because the angiotensin-2 receptor is maximally expressed at night—scientists discovered that patients with high blood pressure were better able to control their blood pressure and cardiovascular symptoms by taking angiotensin receptor blockers at night instead of in the morning.[19]

But the benefits of a bedtime dose don't extend to all blood pressure medications, St. Louis College of Pharmacy's Amy Drew points out. In 2014, pulling data from approximately 30 studies, Drew and her colleagues evaluated a range of hypertension medications for time-dependent effects. While angiotensin-2 receptor blockers were significantly more effective if taken at bedtime, other drugs, such as certain calcium channel and β-adrenergic blockers, didn't seem to have a clear benefit from being administered at a particular time of day.[20] Some treatments have a diuretic effect, which might disturb patients' sleep and make the medicine less effective, Drew says.

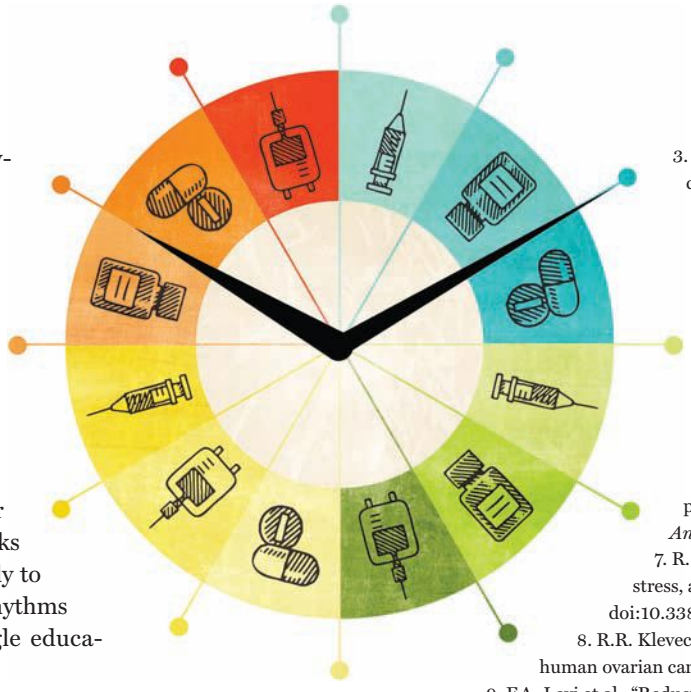
Nevertheless, the data for a time effect of angiotensin-2 receptor blockers is compelling, she adds. "Going through all this evidence, it allows you a certain comfort and confidence to say that if I do dose [angiotensin-2 receptor blockers] at bedtime, it's going to be more effective."

Implementing chronotherapy

Synchronizing medications to the circadian clock is easier said than done, as not everyone's rhythms are the same. And for patients who suffer poor sleep, reduced appetite, or fatigue that reduces their physical activity—common symptoms of many diseases—the clock itself often runs awry.

Researchers are now working to figure out how to normalize patients' circadian rhythms. In a small study of 32 patients with metastatic breast cancer, over-the-counter melatonin—often used to cope with jet lag or insomnia—was found to improve sleep quality and morning expression of circadian genes.[21] And preliminary results from an ongoing trial at Mount Sinai Hospital in New York suggest that exposure to bright white light can reduce disease-related fatigue in patients with breast cancer.[22]

Psychosocial support may also prove beneficial. In 1989, Stanford University psychiatrist David Spiegel and his colleagues reported that women with breast cancer who participated in group therapy sessions lived an average of 18 months longer than



patients who didn't receive psychosocial support to cope with their diagnosis.[23] Spiegel's team reported in a follow-up study that the effect was likely mediated by the endocrine stress response; when levels of cortisol followed a normal curve, cresting in the morning and ebbing by nightfall, patients lived longer.[24] In 2012, another group reported that breast cancer patients who received eight weeks of group therapy were more likely to have improved diurnal cortisol rhythms than those who received a single educational session.[25]

"We've learned enough now to know that there are relatively easy-to-do, low-risk things that may have an effect on disease outcomes," Spiegel says. "If you normalize your circadian rhythms, you'll certainly feel better, and you might just help your body," he adds. "I'd be surprised if there were any disease that didn't have some circadian component."

The trick now is to understand how time of day affects disease outcomes and treatment effects, and to respond accordingly—which may not be a slam dunk, says Hogenesch. "Many of these observations are in the scientific literature but not on drug labels." He and others aim to change that, beginning with the clinical trials necessary to demonstrate daily variations in a drug's effectiveness. A little timing could even rescue drugs that fell off the path to the clinic somewhere along the way, says Hogenesch, who consults with drug companies interested in putting chronotherapy into practice. "In the past when trials were done, time-of-day information was often not captured. It's very likely that drugs have failed not because they didn't work or the mechanisms were wrong, but simply because time of administration wasn't taken into account."

To chronobiologists, time is an often-overlooked aspect of precision medicine's mantra of finding the right drug for the right patient at the right dose. "The discussion is almost entirely focused on genetic precision, and not on all these aspects of physiology and behavior that are products of [the circadian] genetic network," says Hogenesch. "Time offers another way to be precise, and now the groundwork exists to precisely understand a person's clock and leverage that information for better health." ■

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The Literature

EDITOR'S CHOICE IN CANCER BIOLOGY

Melanoma on the Move

THE PAPER

P. Falletta et al., "Translation reprogramming is an evolutionarily conserved driver of phenotypic plasticity and therapeutic resistance in melanoma," *Genes Dev,* 31:18-33, 2017.

In melanoma, tumor cells generally adopt one of two phenotypes: proliferative or invasive. A switch from the first to the second often leads to metastasis and a poorer prognosis. But how this switch gets flipped has been a puzzle for some time—one that Colin Goding, a cancer biologist at Ludwig Oxford in the U.K., has been working on for more than a decade.

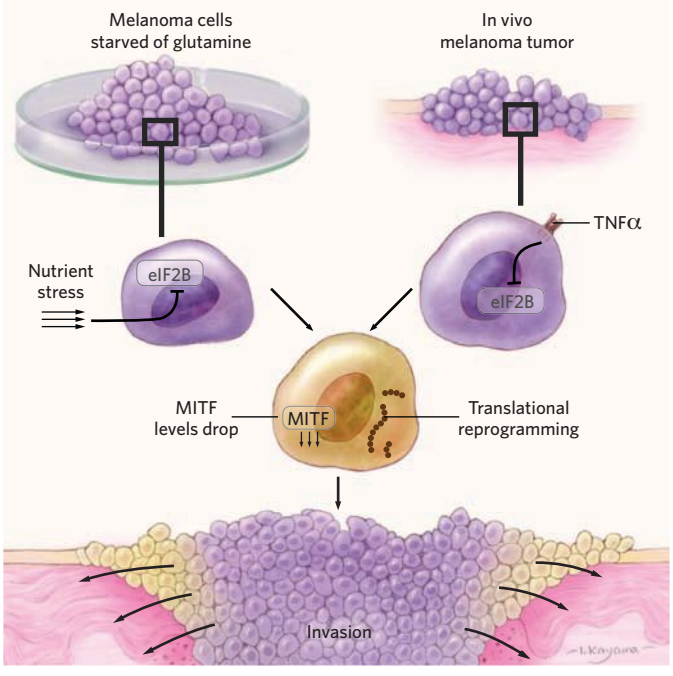
A recent clue came from his lab's discovery that human and mouse melanoma cells are particularly sensitive to glutamine, which is often low in melanoma tumor cores. Supplied with the amino acid, cultured cells ramped up levels of a transcription factor, MITF, associated with melanocyte proliferation. But when cells were starved of glutamine, MITF levels dropped and cells became invasive. "This got us thinking: Why is glutamine so important?" says Goding. "What's it doing?"

To find out, Goding and his colleagues took a closer look at gene-expression patterns from glutamine-starved cells. They found that, in addition to lowering MITF levels, starvation triggered large-scale translational reprogramming via inhibition of translation initiation factor eIF2B. Artificially inhibiting eIF2B—mimicking starvation—induced invasiveness in melanoma cells, while using drugs to render the protein insensitive to inhibition prevented invasiveness even in low-nutrient conditions.

This response is intuitive, Goding notes. "It's what other organisms do," he says. "Bacteria become invasive when they starve, yeast put out hyphae. Maybe invasion in general is a property of cells which are starving." Indeed, similar invasion-promoting reprogramming mechanisms, the researchers showed, operate in yeast, which lack MITF but possess eIF2B. Under nutrient stress, wild-type yeast invaded agar gel, while mutants with disrupted eIF2B interactions did not.

Although the findings suggest evolutionary conservation of invasion drivers, they don't tell the whole story; melanoma, Goding explains, can become invasive in vivo even in nutrient-abundant conditions. So the team began searching for signals in the tumor microenvironment that might trigger the same response, independent of food supply.

One candidate was tumor necrosis factor alpha (TNFα), a cytokine released by immune cells. The team discovered that, in culture, TNFα promoted an invasive phenotype very much resembling that of hungry cells—a result mirrored in gene-expression data sets from mouse models of melanoma. In effect, melanoma hijacked cells' intrinsic starvation response, Goding says, reprogramming them to migrate irrespective of nutrient levels.

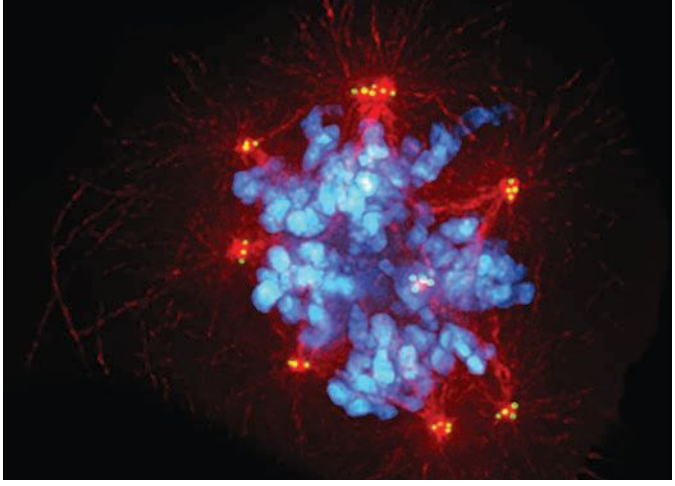


GOING AWOL: The tumor microenvironment can trigger an intrinsic starvation response that switches melanoma cells from a proliferative to an invasive state, according to work from researchers at Ludwig Oxford. In cell culture, nutrient stress leads to inhibition of translation factor eIF2B, triggering translational and transcriptional suppression of proliferation-associated protein MITF, plus large-scale translational reprogramming. The researchers show that TNFα, a cytokine released by immune cells in the tumor microenvironment, also triggers this pathway, suggesting an explanation for how melanoma cells become invasive in vivo even when food is abundant.

And that's not all. The team also found that cells with this invasive phenotype showed gene-expression profiles consistent with a poor response to certain immunotherapies—a result that may help to explain why some of these treatments are ineffective in a substantial number of patients.

The findings add to an "integrated picture" of melanoma progression, says Corine Bertolotto of the French National Institute of Health and Medical Research. Although steps in the pathway are still missing, "the authors are pulling together complex parts of the puzzle."

—Catherine Offord



OVERCROWDED: A cell with too many centrosomes (each with two centrioles; green), extra mitotic spindles (red), and abnormally segregated DNA (blue)

CANCER BIOLOGY

Divide and Conquer

THE PAPER

M.S. Levine et al., "Centrosome amplification is sufficient to promote spontaneous tumorigenesis in mammals," *Dev Cell,* 40:313-22, 2017.

THREE'S A CROWD

More than a century ago, the German biologist Theodor Boveri observed that cancer cells often had extra centrosomes, organelles essential for the segregation of chromosomes during mitosis. This raised a question that scientists have since puzzled over for decades: Is centrosome amplification a cause or effect of cancer?

CHICKEN OR EGG

In 2008, researchers found the first compelling evidence that extra centrosomes could drive tumor formation in flies. However, subsequent studies in mice failed to replicate the results, leading some to question the universality of extra centrosomes' effects.

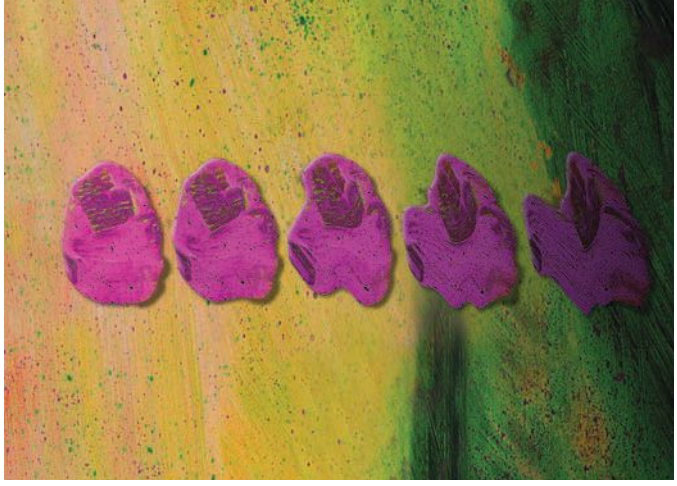
MASTER REGULATOR

In the latest study to investigate this link, Andrew Holland, a cancer researcher at Johns Hopkins University School of Medicine, and his colleagues genetically engineered mice to overexpress *Polo-like kinase 4 (Plk4)*, the "master regulator" of centrosome copy number. They found that once the mice were around eight months old, they began to develop a variety of tumors, including lymphomas and sarcomas. This study is "an important, clear piece of evidence for a long-standing idea," says David Pellman, a cell biologist at Harvard Medical School. Still, how much "chromosome segregation errors versus other effects of centrosome amplification [drives tumorigenesis] remains poorly understood."

SWEET SPOTS

Holland said he thinks his team's experiment succeeded because they were able to reach the "sweet spot of instability," given that too many centrosomes can be lethal. "It's nice now that both fly and mouse show that if you amplify centrosomes you can promote tumorigenesis," says the University of Oxford's Jordan Raff, a coauthor of the 2008 study, "but it's still not very clear how important that is for human cancers."

—Diana Kwon



TRANSFORMATIONS: An artistic representation of cancer progression in a cell, from normal to a leukemic state (from left to right)

CANCER BIOLOGY

In Sickness and Health

THE PAPER

A.G. Kotini et al., "Stage-specific human induced pluripotent stem cells map the progression of myeloid transformation to transplantable leukemia," *Cell Stem Cell,* 20:315-28.e7, 2017.

CANCER CONTINUUM

In recent years, cancer researchers have discovered that myeloid malignancies lie on a continuum of increasing severity, starting as precancerous mutations in blood cell precursors, then progressing to bone marrow disorders, and, finally, developing into acute myeloid leukemia.

TRANSITIONS

Eirini Papapetrou of the Icahn School of Medicine at Mount Sinai and colleagues followed disease progression by reprogramming cells from patients with various stages of myeloid malignancies, including premalignant cells, into pluripotent stem cells in their precancerous state. Then, by differentiating them back into blood cells, the team established cell lines representing specific stages of disease. "What we want to do now is understand the exact molecular/cellular events that drive the stepwise progression from normal cells to leukemic cells," says Papapetrou.

TRANSPLANTS

The researchers discovered that when they converted full-blown leukemia cells into stem cells and back into blood, they could transplant them into mice—a feat that had not been accomplished before with human blood cancer. "One of the holy grails of blood stem cell research is the ability to produce transplantable stem cells," says Stanford University's Ravindra Majeti, whose group concurrently reported transplantable leukemic cells using a similar approach (*Cell Stem Cell*, 20:329-44.e7, 2017). "It is one of the big hurdles limiting the translational applications for blood disease."

SOLVING THE PUZZLE

"One of the future goals is to figure out what it is that really can drive an engraftable cell," says Papapetrou's coauthor, Michael Kharas of Memorial Sloan Kettering Cancer Center. "We think that the leukemia-derived cells were able to maintain or acquire some aspect of the blood stem cell program that was not in the other cell types."

—Diana Kwon

BRAMWELL LAMBRUS, HOLLAND LAB; LEWIS LONG

PROFILE

Location, Location, Location

Since first proposing that a cell's function and biology depend on its surroundings, Mina Bissell continues to probe the role of the extracellular matrix.

BY ANNA AZVOLINSKY

"The reason I still travel and give talks, meet young scientists, and do interviews is that I see young people are inspired by my story of how I have persisted," says Mina Bissell of the Lawrence Berkeley National Laboratory (LBNL) in California. "I have been saying the same thing since 1981 and only in the last 15 or so years have many other scientists come around. But I never wanted to quit. If you are passionate and you have ideas leading to rigorous proof, you need to trust yourself."

Bissell, who is easily past retirement age, is not ready to retire. "I don't know what I would do with myself. My husband is essentially retired, and he is learning to play the fiddle and to speak French. But I think I would drive myself and my family crazy if I retired! . . . One of the biggest lessons I convey to others is the tremendous dignity that comes with work."

"I realized that when you put cells in culture, everything changed—the function, their shape. Clearly there was something that is missing in culture that is present in vivo."

In 1981, then a senior scientist at LBNL, Bissell challenged scientific dogma about how much cell culture studies can reveal about whole organisms, asserting that changes in gene expression and function in culture differ from patterns within tissues and organisms, and therefore, that microenvironment regulates cell function. The following year, she proposed that the insoluble extracellular matrix (ECM) outside of cells is in direct communication with the cell nucleus through both physical and chemical signaling, dubbing it the "dynamic reciprocity" model. Reaction to the proposal was lukewarm at best, and Bissell and her students and postdocs have continued to chip away at the naysayers' objections for the last 35 years, providing a steady stream of evidence for direct communication between cells and the ECM using the mammary gland as a model system.

For Bissell, having an opinion and voicing it is nothing new. She grew up in Iran, in close quarters with a large, highly educated multigenerational extended family (her father was the oldest of 10 children). Discussions and debates were the norm. "They were all intellectuals, empathetic and extremely passionate about literature, issues, and politics, and I was immersed in all of that," she says. From an early age, Bissell was curious, argumentative, and loved the sciences, which came easily to her. By 1979, at the tail end of the Iranian Revolution, Bissell had already built a life and a science career in the U.S., and although her parents and most of her extended family were back in Iran, she didn't see herself returning to her country of birth. Neither did her mother: "'Mina is not coming back, not even to visit,' I remember my mom saying, because she knew that I hadn't learned not to speak my mind and would upset someone. I was also very sensitive and had been raised to have a deep sense of justice, that people should not be mistreated. Because I was so outspoken, she knew I would argue and likely end up in jail." Instead, Bissell focused on her scientific pursuits: providing evidence for the idea that the seemingly inert ECM actually has important functions, guiding the biology of cells and tissues that surround it.

Here, Bissell discusses her mother's reaction to Bissell's becoming pregnant while in graduate school, her active choice not to research trendy topics, and her conviction that students need to question scientific dogma.

BRIGHT BISSELL

A drive to excel. Schoolwork came easily to Bissell. She received a medal from the Shah of Iran for being the top high school senior in the country and, in her last year of high school, she took an exam that earned her one of five scholarships from the Iranian government to partially cover the cost of attending university abroad. She applied to schools in the U.S., despite her "rudimentary English," and was accepted to Bryn Mawr College. "I left the county at barely 18 and all on my own," Bissell says. At Bryn Mawr, Bissell excelled in math and chemistry courses but anything involving English was a challenge. "I took a literature course with Ann Berthoff, one of my most favorite teachers, who is 93 now, and I struggled so much with the books she assigned, especially Faulkner. Still, I wish every teacher taught the way she did. She had so much passion; her drive and work ethic came out in every class. She was a huge influence on me."

Against the odds. After two years at Bryn Mawr, Bissell transferred to Radcliffe College, following her fiancé, who was a graduate student in political science at Harvard University. In 1963, she graduated with a chemistry major, got married, and, because her husband was still working towards his PhD, entered the bacterial genetics graduate program at Harvard Medical School. "I thought graduate school was better than medical school," says Bissell. "But I don't know why I chose microbiology."

Sitting in a lecture during her first year, she answered a technical microscopy question that made the teaching assistant, a member of Luigi Gorini's lab, take note. The postdoc told her to

MARILYN CHUNG/BERKELEY LAB

MINA BISSELL

Distinguished Scientist, Biological Systems and Engineering Division
Lawrence Berkeley National Laboratory, Berkeley, CA

Greatest Hits

- Over a period of 40 years, was instrumental in developing the field of tumor microenvironments
- Developed the concepts that phenotype is dominant over genotype, that context matters, and that cellular and tissue architecture relays messages to cells
- Used a "steady-state machine" she helped develop to show that the level of sugar in culture media determined whether chicken cells remained normal or displayed malignant metabolic patterns
- In her model of dynamic reciprocity, proposed that the extracellular matrix directly signals to the nucleus and chromatin biochemically and mechanically to regulate gene expression
- Developed three-dimensional culture techniques using basement membrane gels to study organ specificity in mammary organoids

come and meet Gorini, who was in his late 60s, and he decided to recruit her as a graduate student. A few months later, Bissell was visibly pregnant with her first child, and Gorini assumed she would be quitting graduate school. "'What would your mother say?' he asked me," Bissell recalls. "What my mother said, from Iran, was 'You're not quitting, are you?' Now how many mothers at that time would say that? But I came from a family and country where education is valued and expected; I was pregnant and going to school and my family saw nothing wrong with that."

Against the grain. In Gorini's lab, Bissell chose to probe the mechanism by which bacterial cells synthesize and excrete a proteinase. While the main focus of the lab, and the department, was studying bacterial resistance to antibiotics, Bissell's strategy was to avoid the pressure associated with this topic. "Even within the same department at Harvard, there was so much competition and friction that I wanted to work on something that wouldn't push me into that crowd," she says. The enzyme translation project was something that two prior postdocs in Gorini's lab had attempted, but they had failed to generate any publishable results. Bissell persevered for four years—but she could not confirm the model Gorini and his two former postdocs had developed for the mechanism by which this enzyme controlled its own synthesis from outside the bacteria.

Bissell was fascinated with the puzzle of how some proteins in a given bacterium get secreted, while most never exit the cell membrane or exert their functions only intracellularly. "I thought maybe the proteins that get out are synthesized in a different compartment and that a floppy version of the protein comes out of the membrane unfolded and then gets stabilized at the extracellular surface. Thanks to my chemistry background, I thought it might be calcium that acts as the stabilizer. But when I showed Gorini some of my results and explained the new model, he said, 'Mina, what do you think these proteins are, spaghetti? You will never make it in science.'" With the help of another professor in the department, Bissell devised a way to label the protein and measure its enzymatic activity, showing that only in the presence of calcium was the enzyme active. She proposed a new model of co-translational secretion to describe the process.

BISSELL BUBBLES

Questioning the dogma. Outside the lab, Bissell went through a divorce, became a single mother, and, in her final year of graduate school, married Montgomery (Monty) Bissell, a medical school student who was doing research in her department. "I had no opinion yet about what I wanted to study. I opened *The New York Times*

one day and saw an editorial by Harry Rubin, a biology professor in Berkeley, who had published a paper suggesting that the reason chicken embryo fibroblasts become malignant is that they secrete a particular protease. I quickly decided that I wanted to do a postdoc to isolate and study this protease," says Bissell. In 1970, the family moved to California, where Bissell began an American Cancer Society Fellowship in Rubin's lab at the University of California, Berkeley (UCB). Once she began her research, Bissell realized that that particular protease was an artifact created by cell lysis. They published the results, but now she needed something else to work on.

Revealing literature gaps. At UCB, Bissell became interested in cell culture techniques and how viral transformation changes metabolism, working with virus-transformed chicken cells to study how glucose metabolism differs from that of normal cells in culture. She began to read the literature on the Warburg effect—the observation that cancer cells produce energy by aerobic glycolysis and lactic acid fermentation rather than through the typical ATP-producing oxidative phosphorylation cycle. In typical fashion, Bissell quickly became critical of what she read: "The literature was a huge mess. No one was asking what relevance results of experiments from a monolayer of fibroblasts grown in 5 percent carbon dioxide and 20 percent oxygen had to what happens in the body. And no one seemed to be measuring both the input and the output of glucose metabolism." Bissell felt that those who criticized Warburg were performing poorly controlled experiments and decided to do a complete reanalysis of the Warburg effect on transformed chicken cells. She showed that with the same amount of glucose input, the level of lactate produced by transformed cells was always higher compared to nontransformed cells, independent of culture cell density.

Two years later, in 1974, now in her own laboratory at the LBNL, Bissell, along with James Bassham and colleagues, designed a steady-state machine to measure the kinetics of metabolism and other processes in cultured cells by keeping them in precise growing conditions, including constant temperature and pH, and in isolation from the outside environment. Using the device, Bissell's lab again confirmed that transformed cells rely more heavily on aerobic glycolysis for energy, but that the switch to this energy pathway did not result from the impairment of the hydrogen-transfer pathway. The results, says Bissell, went against the other half of Warburg's hypothesis: that the reason for the increased glycolysis is impaired hydrogen transfer.

Everything is in flux. Bissell's lab at the LBNL continued to study the metabolism of virus-transformed cells in culture. Some of their results from the late 1970s, including the role of cell shape in sugar transport and the potential of microtubules to influence cell growth, would later help shape Bissell's controversial 1982 proposal that the ECM directly communicates with cells, influencing their behavior and morphology. (See "May the Force Be With You," *The Scientist*, February 2016.) "I didn't know much about the ECM, but I had three postdocs, Richard Schwartz, Glenn Hall, and Joanne Emerman, who had worked with and thought about the components of the ECM. We observed that cells grown on a collagen gel more resembled the look of cells in vivo. It also occurred to me that in vivo, cells have a polarity that they don't have in culture. And I realized that when you put cells in culture, everything changed—the function, their shape. Clearly there was something that is missing in culture that is present in vivo," says Bissell.

She scoured the literature, the vast majority of it descriptive, for information about the biochemical components and structure of the molecules that made up the ECM, which at the time, was thought to be inert. In 1981, Bissell first proposed that gene expression, and therefore cell function, changes depending on context and that the cell's microenvironment influences these changes. That thinking led her to propose, in 1982, that the microenvironmental influence is the ECM, which both chemically and physically interacts with cells. According to Bissell's 'dynamic reciprocity' model, signals from the ECM traveled through transmembrane receptors to a cell's interior and nucleus, altering its gene expression. "I began to think that the ECM played a role in tissue and organ specificity, because the cells all had the same genetic material, but I realized that there is no constitutive gene expression, that the context changes and so do the cells."

BOLD BISSELL

Evidence builds. To provide evidence for the model, Bissell's lab developed 3-D culture techniques, allowing differentiation and creation of at least partial tissue architecture of the mammary gland in culture. "If the cellular and tissue architecture is so important, I thought we should be able to take a malignant cell and change its structure and make it normal and also vice versa," says Bissell. By the early 1980s, integrins—proteins that physically attach the ECM to the cell cytoskeleton—had been discovered. Valerie Weaver, a postdoc in Bissell's lab, showed that blocking integrins with an inhibitory antibody could revert the malignant phenotype of human breast cancer cells in 3-D culture. Then, in collaboration with Zena Werb, of the University of California, San Francisco, the labs showed that proteins called matrix metalloproteinases (MMPs), when upregulated, promote tumor formation, providing evidence that the ECM can encourage malignant transformation and proliferation. Six years later, the two labs revealed that signaling from the MMPs resulted in genomic instability in cells that led to malignancy.

Still at it. Bissell's lab is still buzzing with excitement, continuing to bolster the validity of her dynamic reciprocity model. "When I would give talks and say that laminin [a large extracellular protein that is a major component of the basement membrane] is as important as p53, people would laugh. We have been working on the story of what laminin does for the last eight years, and it is almost complete," says Bissell. "It probably will be considered one of my most important studies."

Paving a way. "I tell the people I train, don't listen to what the literature says, do your experiments and understand why you found something different—as long as you can reproduce the data and the process. I tell them to trust themselves." ■

SCIENTIST TO WATCH

Angela Brooks: Splicing Specialist

Assistant Professor, Department of Biomolecular Engineering
University of California, Santa Cruz. Age: 34

BY DIANA KWON

When Angela Brooks first saw *Gattaca*—a 1997 film about a futuristic society where humans are classified based on their genetic code—in high school, she was captivated by the possibilities hidden in the genome. "I've always been fascinated by the concept that every cell in your body has exactly the same DNA sequence but . . . can then have a different phenotype," Brooks says.

She was particularly drawn to alternative splicing, the process through which multiple proteins emerge from a single gene. This became the focus of her PhD research at the University of California, Berkeley, where she investigated the proteins regulating alternative splicing in fruit flies. As a grad student, Brooks was also involved in a number of other projects, including modENCODE, which was aimed at creating an encyclopedia of all the functional elements in the *Drosophila melanogaster* and *Caenorhabditis elegans* genomes. Brooks also created a program, JuncBASE (junction-based analysis of splicing events), to help analyze the large amounts of high-throughput sequencing data generated in these experiments by using techniques such as RNA-seq.[1]

"She did some beautiful work," says Steven Brenner, a computational genomicist at UC Berkeley and Brooks's PhD advisor, referencing a major project in which Brooks discovered that a splicing factor's regulatory map, which relates protein binding sites to their functions, is conserved between insects and mammals. "In order to do this, she developed new tools to be able to analyze RNA-seq, and was really an early person to harness that technology and show what its potential was."

In 2012, PhD in hand, Brooks decided to pursue her passion for human genomics and do a postdoc in Michael Meyerson's cancer genomics lab at the Dana-Farber Cancer Institute and the Broad Institute in Boston. There, Brooks studied proteins involved in splicing and human cancers and identified the cancerous effects of mutations in two genes: *U2AF1* in lung adenocarcinoma and acute myeloid leukemia[2] and *SF3B1* in chronic lymphocytic leukemia.[3]

During her postdoc, Brooks also collaborated with other Broad Institute researchers to develop a high-throughput phenotyping method called expression-based variant-impact phenotyping (eVIP), to identify mutations that lead to meaningful changes in gene expression.[4] "In a lot of ways, I think her most significant impact was in methods development," says Meyerson. "Both JuncBASE and eVIP are useful and powerful new methods."

Brooks has continued to explore alternative splicing in her own lab at the University of California, Santa Cruz, which she set up in the summer of 2015. Brooks ultimately hopes to one day "be able to directly sequence every RNA molecule and all the RNA modifications in a cell so you actually know [its entire] transcriptional output."

Beyond lab work, Brooks is also passionate about promoting diversity in research. "I think that's another big goal [I have], to increase the diversity of the data we have, but also [to help] include scientists from diverse populations to study these genomes," she says. ■

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Special Delivery

Using biocompatible polymers to carry cancer immune therapies directly to the tumor

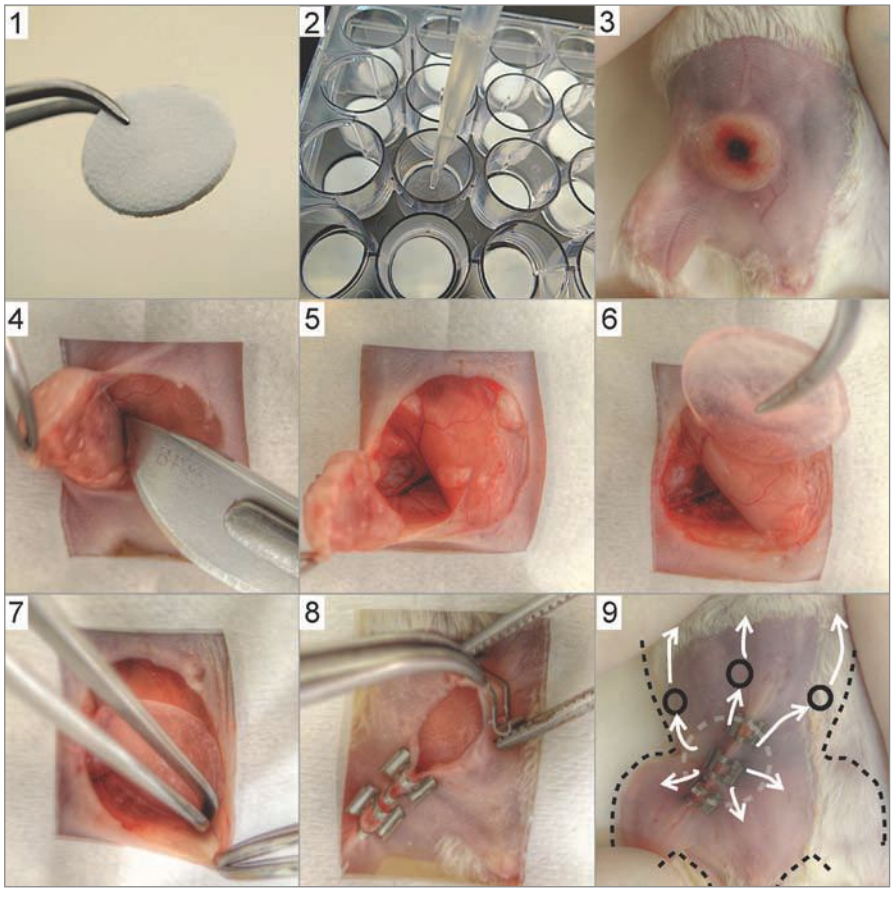
BY RACHEL BERKOWITZ

Some immunotherapies harness specially engineered patient-specific cells to fight tumors and bloodborne cancers. But traditional intravenous methods of administering such therapies still struggle to deliver the treatment. The targeted immune cells are often lost—destroyed in the bloodstream or eradicated by the hostile tumor microenvironment before they've served their purpose.

Biodegradable polymers could provide a solution to such delivery problems. From enhancing the repair of damaged spinal discs to releasing antibiotics that prevent infection after surgery, clinicians have loaded these so-called scaffolds with drugs and then implanted them near the treatment area. The scaffold dissolves after the drugs are released.

Scientists are also developing biocompatible scaffolds specifically designed to deliver tumor-reactive immune cells directly to a cancer site. These implants and gels create a safe local environment in which immune cells can proliferate precisely where needed, protected from factors secreted by the tumor cells themselves that hamper immune-cell function. Approaches can involve delivering T cells trained to attack a specific cancer or putting in place a dendritic cell–based vaccine that recruits and trains T cells in the lymph nodes. But creating a suitable scaffold that can be easily and effectively inserted into the patient poses material and bioengineering challenges. Generating a sufficiently large number of immune cells and ensuring that they do their job before the cancer does too much damage is also tricky.

The Scientist asked researchers who develop gel scaffolds for cancer immunotherapy how they're approaching these challenges. Here's what they said.



RIGHT SPOT AT RIGHT TIME: After removal of a mammary tumor from the breast of a mouse (3–4), a biocompatible gel scaffold loaded with T cells (1–2) is implanted at the resection site (6–9). There the scaffold acts as a reservoir, slowly releasing anticancer immune cells that attack residual tumor cells. (Black circles denote tumor-draining lymph nodes.)

COURTESY MATTHIAS STEPHAN

T-CELL TROJAN HORSE

RESEARCHER: Matthias Stephan, assistant member, Fred Hutchinson Cancer Research Center and assistant professor of oncology, University of Washington School of Medicine

PROBLEM: Only 5 percent of injected cancer-killing immune cells reach tumor sites in many clinical trials. Stephan wanted a tool that would deliver immune cells directly to the tumor site in the hope that these cells could shrink tumors to an operable size or clean up postsurgical malignant cells.

PROJECT: Stephan and his colleagues engineered mouse T cells to have an affinity for certain molecules on murine mammary tumors, training them to recognize and attack these proteins. They then loaded these T cells into a biodegradable polysaccharide gel and surgi-

cally positioned it at breast tumor sites in the animals (*Nature Biotechnol*, 33:97-101, 2015).

The main challenge was creating a friendly environment for the T cells. "If you get Walgreen's alginate wound dressing and load it with T cells, the T cells don't like it," says Stephan. He modified an alginate-based gel by adding peptides that mimic collagen fibers, along which lymphocytes migrate through the extracellular matrix. In addition to providing these "little handles to hold on to" so they could circulate properly within the gel, he also added stimulatory cues that tell the cells to proliferate and attack the tumor. So far, the approach has only been tested in animals, where the T cells expanded 10,000-fold after injection of the loaded gel scaffold at the tumor site and shrinking the mass within four days.

PROS/CONS: T cells infused into a patient's bloodstream are often rendered dysfunctional by the time they reach the tumor site. In contrast, Stephan's method directly delivers T cells to the cancer site. Because the delivery is localized, this treatment won't work against widely disseminated tumors, or against blood cancers such as leukemia/lymphoma, and placing it requires surgery. But it offers a powerful option for attacking an inoperable tumor, or cleaning up postsurgical tumor cells that could lead to relapse.

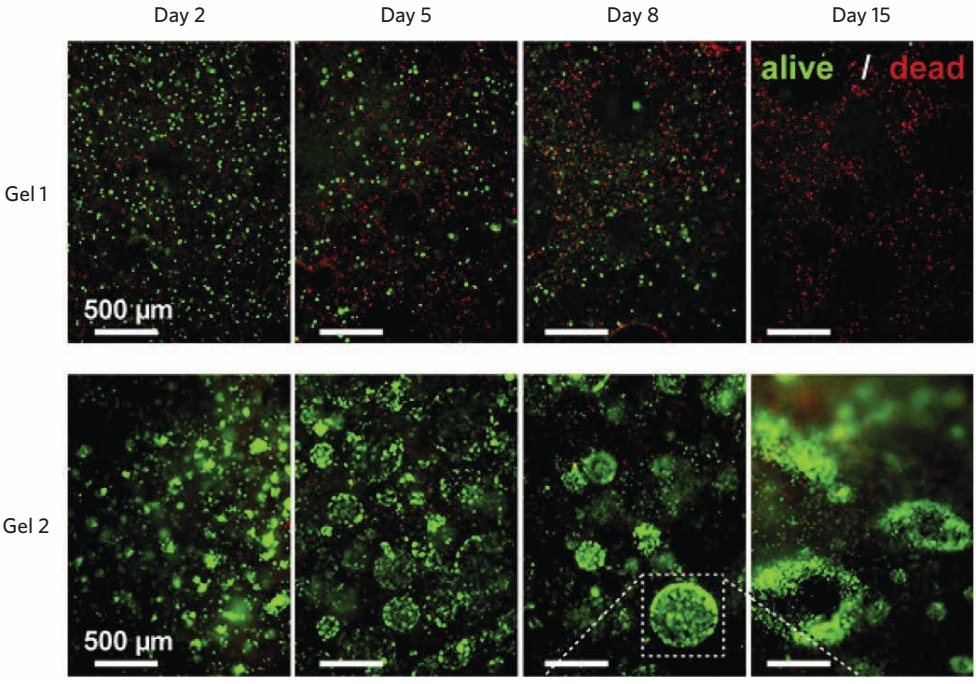
LOOKING FORWARD: The researchers are developing a biopolymer method that can deliver either active T cells or vaccines that use dead tumor cells as sources of antigens. These antigens prime the immune system against remaining tumor cells. Additionally, commercialization of off-the-shelf T cells may lead to a marketable biopolymer implant loaded with "frozen cells ready-to-go."

SURGERY-FREE IN VIVO T-CELL FACTORY

RESEARCHERS: Sophie Lerouge, professor of mechanical engineering, École de Technologie Supérieure, Montreal; Réjean Lapointe, professor of medicine, Université de Montréal

PROBLEM: Immunotherapy treatment of cancers depends on multiplying tumor-killing cells to extremely high numbers—a costly and time-intensive procedure. Lerouge and Lapointe sought a way to reduce the number of cells required for treatment, and to deliver them without surgery.

PROJECT: Lerouge and Lapointe developed an injectable, surgery-free scaffold consisting of a biodegradable polysaccharide mixed with gelling agents. To this mix they added activated T cells that had been isolated from patients' tumors and dosed with growth-stimulating factors. The resulting solution was liquid



LOCALLY PRODUCED: Microscopic examination of T cells encapsulated in two different gels helps to determine the optimal formulation to promote cell viability and proliferation of activated T cells. The gel is liquid at room temperature but hardens after injection at the tumor site.

at room temperature, facilitating injection, but at human body temperature it hardened to a hydrogel with pores large enough for T cells to escape from after they multiplied. "The main difficulty was finding the precise composition of the gelling agent to reach rapid gelation, good cohesion, biocompatibility, and macroporosity required for excellent T-cell growth," says Lerouge (*Biomaterials*, 75:237-49, 2016).

PROS/CONS: Because it is a liquid at room temperature, the T cell–containing "thermogel" can be injected directly into the tumor environment without surgery. Most currently used immunotherapies inject T cells intravenously. Local delivery also means potentially fewer T cells are required—around 10^7 to 10^8, compared to the 10^{11} needed for intravenously delivered immunotherapy—so the treatment can be produced more quickly and carries less threat of the side effects inherent in systemic delivery.

LOOKING FORWARD: The researchers are testing the gel in mice and assessing its effects on isolated human cells. One challenge with T-cell therapy, Lapointe notes, is that suppressive signals from the tumor itself can prevent T cells from doing their job. Compounds that block these signals, such as checkpoint inhibitors, could be added to the gel along with the T cells to boost effectiveness, the duo says.

IMMUNE CELL RECRUITMENT CENTERS

RESEARCHER: David Mooney, professor of bioengineering and core faculty member, Wyss Institute for Biologically Inspired Engineering, Harvard University; Omar Ali, Senior Scientist, Wyss Institute

PROBLEM: Preparing T cells outside the body for an infusion can be costly. Mooney wanted to find a way to train T cells in vivo while ensuring that the cells reach the desired tumor tissues.

PROJECT: Mooney's lab developed a vaccine composed of tumor antigens, cytokines, and oligonucleotide fragments that stimulates in situ dendritic cells to activate T cells resident in the lymph nodes. A polymer scaffold impregnated with the vaccine is surgically implanted under a mouse's skin, imitating an infection site where dendritic cells are programmed to train cytotoxic T cells, which then travel to the tumor site and kill malignant cells (*Science Transl Med*, doi:10.1126/scitranslmed.3000359, 2009).

The researchers initially developed a surgically implantable polymer scaffold, loaded with vaccine components, that recruits and activates dendritic cells; these then disperse to lymph nodes to create sustained T-cell immunity. The team later redesigned the scaffold to have shape-memory properties so that it collapses in a needle and re-establishes its structure once injected (*PNAS*, 109:19590-95, 2012). Their most recent scaffold delivery approach is a solution of mesoporous silica microrods that spontaneously assemble when injected subcutaneously into a mouse's flank. Like a pile of scattered matchsticks, they form a scaffold where immune cells can assemble, specialize, and grow. "As long as you have an antigen you can use as a vaccine, this system could be appropriate for a blood cancer," explains Ali (*Nature Biotechnol*, 33:64-72, 2015). The original version (for melanoma) is currently the only biomaterial-delivered cancer vaccine in clinical trials (clinicaltrials.gov/ct2/show/NCT01753089).

PROS/CONS: Recruiting and activating endogenous dendritic cells to prime T-cell immunity is significantly cheaper than growing these cells outside the body in cell culture. Also, this approach is systemic, so it can potentially work against blood cancers. However, patients must have a robust immune system, which may be compromised by cancer.

LOOKING FORWARD: The lab is now developing more-potent and injectable versions of their immunotherapy technologies.

SHAPE-CHANGING T-CELL RECRUITMENT

RESEARCHERS: Joon Haeng Rhee, professor of microbiology, and Vivek

A. MONETTE ET AL., *BIOMATERIALS*, 75:237-49, 2016. OPPOSITE PAGE: V. VERMA ET AL., *ONCOTARGET*, 7:39894-906, 2016

CHARTING THE TUMOR MICROENVIRONMENT:
NAVIGATING COMPLEX SYSTEM INTERPLAY

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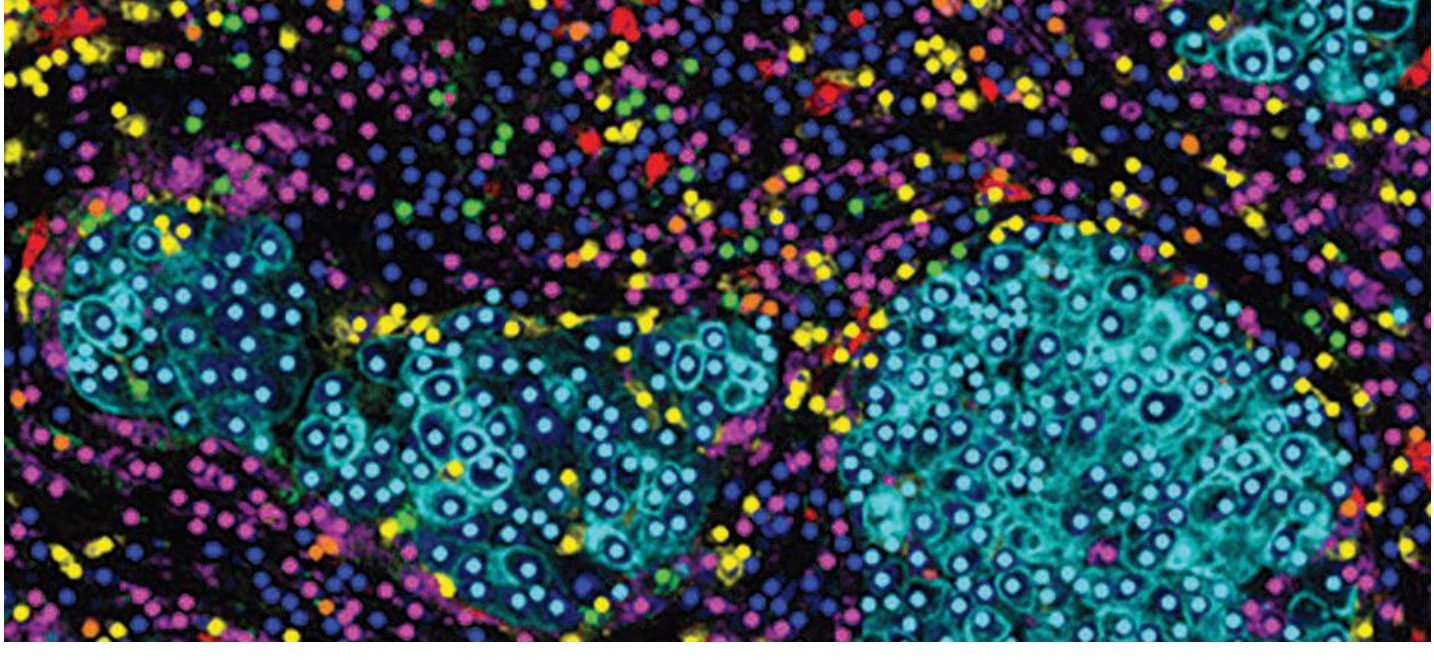

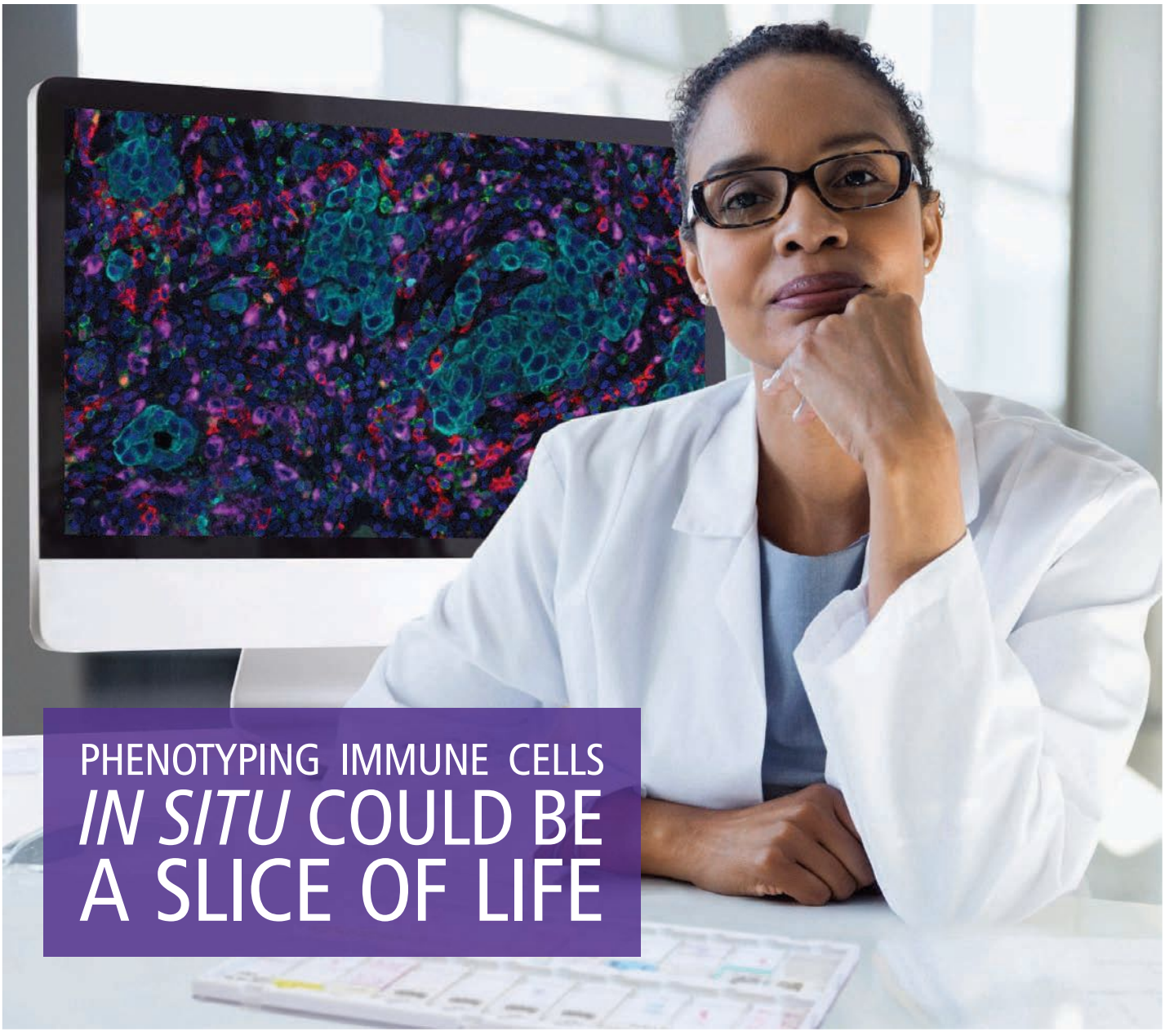

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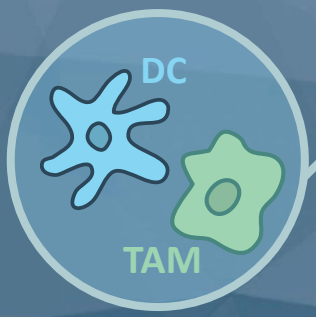
CHARTING THE TUMOR

NAVIGATING COMPLEX SYSTEM INTERPLAY

The tumor microenvironment is a complex network of cancer, immune, vascular, and stromal cells, generally characterized by extracellular-matrix (ECM) remodeling, immune suppression, and hypoxia due to poor vascularization.[1] These combine to create favorable conditions for cancer-cell survival, proliferation, and motility, resulting in tumor growth, invasion, and metastasis.[1] Understanding the components of the tumor microenvironment and their interplay will be essential to better targeting tumor growth and metastasis in the laboratory and clinic.



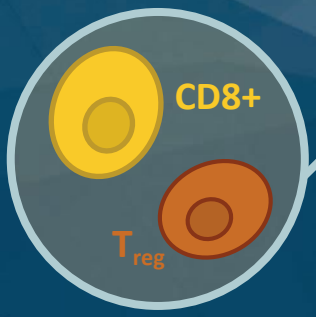
DENDRITIC CELLS AND MACROPHAGES

Dendritic Cells (DCs) (Markers: CD303, CD1c, CD141, CD14[10,16]) are required for CD8+ T-cell activation as antigen-presenting cells.[3] Hypoxia induces DC suppression of T-cell activity via PD-L1 production.[4]

Tumor-associated Macrophages (TAMs) (Markers: CD68, CD163, CD204[11]) are immunosuppressive cells, associated with poorer prognoses, that promote ECM remodelling and cancer-cell escape.[4-6]

FIBROBLASTS AND THE EXTRACELLULAR MATRIX

ECM structure influences cancer progression. Hypoxia promotes the recruitment and activation of cancer-associated fibroblasts (Markers: α-SMA, FAP[12]), increasing collagen deposition, which has been linked to mortality.[1] The microenvironment promotes ECM remodelling by stimulating matrix metalloproteinase secretion, facilitating cancer-cell proliferation and metastasis.[1,7]



T CELLS AND CHECKPOINT INHIBITORS

CD8+ cytotoxic-T cells are the primary effectors of tumor-cell death, restricting metastasis.[5] Evading these T cells is critical to net tumor growth.[3]

Hypoxia induces CD4+ regulatory T cell (T_{reg})-mediated CD8+ T-cell deactivation, resulting in CD8+ T-cell anergy and increased T_{reg} counts.[2,3] T-cell entry is physically impeded by the immunosuppressive tumor microenvironment, increased stroma density, and decreased endothelial adhesion protein expression.[2,3]

Tumor cells and T_{reg} cells express "checkpoint proteins" such as PD-1 and CTLA-4, which bind to CD8+ T cells, inactivating them. Specialized molecules called "checkpoint inhibitors" have been developed to prevent this interaction, thus reducing tumor-cell evasion and augmenting the CD8+ T cell response.[17,18] T_{reg} depletion, immunosuppressive-pathway downregulation, and T-cell stimulation also represent therapeutic options. A simultaneous multi-method approach yields optimal results.[3]

MICROENVIRONMENT:

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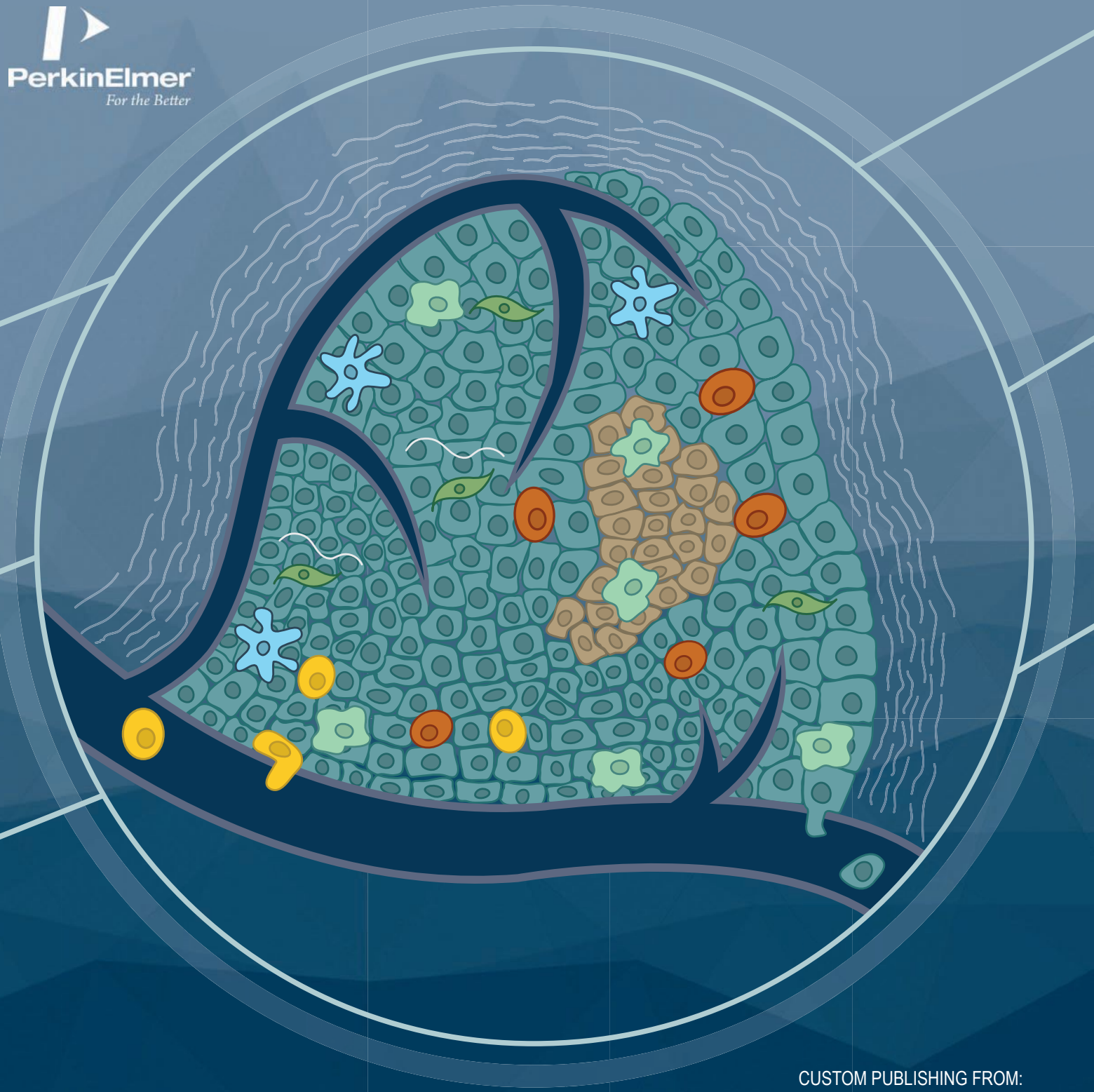

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HYPOXIA

Tumor hypoxia is caused by intercapillary distances exceeding O_2 diffusion range,[1] and can be marked by transcription-factor upregulation (e.g., HIF-1α[4]) or detected chemically using engineered probes.[15] Hypoxia stimulates ECM remodelling and fibrosis,[1] while hampering cell-mediated immunity[3] by promoting immunosuppressive phenotypes[4] and conferring increased cancer-cell resistance to effector-cell-mediated killing.[3]

THE VASCULATURE

Angiogenesis facilitates tumor growth and is stimulated by tumor cells and hypoxia. Tumor-stimulated angiogenic factors (e.g., VEGF, TGF-β, PDGF, endothelin[1,2,14]) can also limit immune-cell entry by downregulating endothelial-adhesion protein expression.[2] Endothelial cells also deactivate CD8+ T cells through PD-L1 and Fas ligand signaling.[2,8]

TUMOR INVASION AND METASTASIS

Tumor cells, either individually or collectively, invade the stroma and intravasate into the circulatory system.[9] They extravasate and initiate tumorigenesis at a different location. This process is termed "metastasis" and causes 90% of cancer-attributed deaths.[1]

The metastatic cascade exposes cancer cells to immune-system detection.[4,6] The tumor microenvironment counters this by hindering immunosurveillance,[6] altering ECM topography,[1] promoting angiogenesis, and recruiting TAMs.[4] These mechanisms facilitate cancer-cell evasion, motility, and escape.[1]

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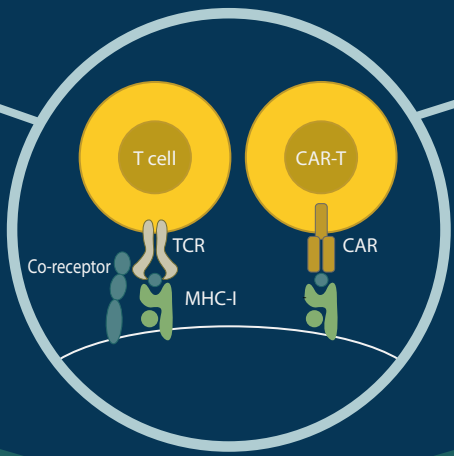
CHIMERIC ANTIGEN RECEPTOR (CAR)-T CELLS

CAR-T cells express synthetic T-cell receptors (TCRs), facilitating selective targeting of tumor-surface antigens. CAR-T cells have been successful in treating hematological cancers, but – to date – present less efficacy in solid tumors.[13] The tumor microenvironment limits CAR-T cells' therapeutic efficiency by downregulating T-cell trafficking and inducing dysfunction via immunosuppression mechanisms. Simultaneous cotherapy to alleviate these impediments using cytokines, chemokines, and/or antibodies is required for optimal therapeutic efficacy in solid tumors.[13]



QUANTITATING IMMUNE-CANCER INTERACTIONS

Immunohistochemistry is the conventional avenue for investigating the presence of various cell types, functional states, and protein expressions within tumor tissue. While quite effective for detecting one protein or one cell type at a time, the technique is limited in its capability to reveal specific cell-to-cell interactions occurring within the tumor microenvironment, especially interactions between immune cells and tumor cells. Flow cytometry of disaggregated tissues is often used when multiple proteins are needed to identify multiple cell types, but all spatial information is lost, thus important cellular arrangements and interactions cannot be assessed. Multispectral imaging coupled with multiplexed immunohistochemistry allows for the analysis of multiple protein expression signals within a single tissue section.[19,20] This opens up the exploration of specific cell-to-cell level mechanisms driving immune system-tumor interactions, and can be used as the basis for confirming drug method-of-action, for identifying new mechanisms to target, and potentially for future predictive tests in immuno-oncology.

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The Vectra Polaris system integrates high-throughput, seven-color multispectral imaging with whole-slide scanning in a simplified digital pathology workflow to support the quantification and analysis of tissue sections that are stained with multiple immunohistochemical stains. This helps scientists assess biomarkers that probe deeper into the understanding of the tumor microenvironment, by detecting multiple cell types, functional states, as well as spatial distributions.

"From basic research to clinical research studies, scientists continue to seek advanced imaging technologies to better analyze and understand disease mechanisms," said Jim Corbett, Executive Vice President and President, Discovery & Analytical Solutions, PerkinElmer. "The Vectra Polaris system is an innovative solution that helps further the exploration of new cancer immunotherapy approaches to help unlock the promise of precision medicine."

"PerkinElmer's multiplex IHC platform has addressed a critical need in immuno-oncology research to reveal the cell-level biology occurring in the tumor and its microenvironment that drives disease progression and response to immunotherapy," said Dr. Bernard A. Fox, PhD, Chief, Laboratory of Molecular and Tumor Immunology, Robert W. Franz Cancer Research Center in the Earle A. Chiles Research Institute at Providence Cancer Center (Oregon). "The development of the Vectra Polaris system has come at the right time, to support the transition from an exploratory research tool to a high throughput rugged high speed slide analysis research system that overlays PerkinElmer's unique multispectral technology on to a digital pathology workflow. I believe the Vectra technology will become the standard for tissue biomarker studies in immuno-oncology research and form the basis for tailoring cancer therapies of the future."

For more information on Vectra Polaris and our complete cancer research solutions, please visit **www.perkinelmer.com/AACR**

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Verma, postdoctoral researcher, Chonnam National University Medical School, South Korea

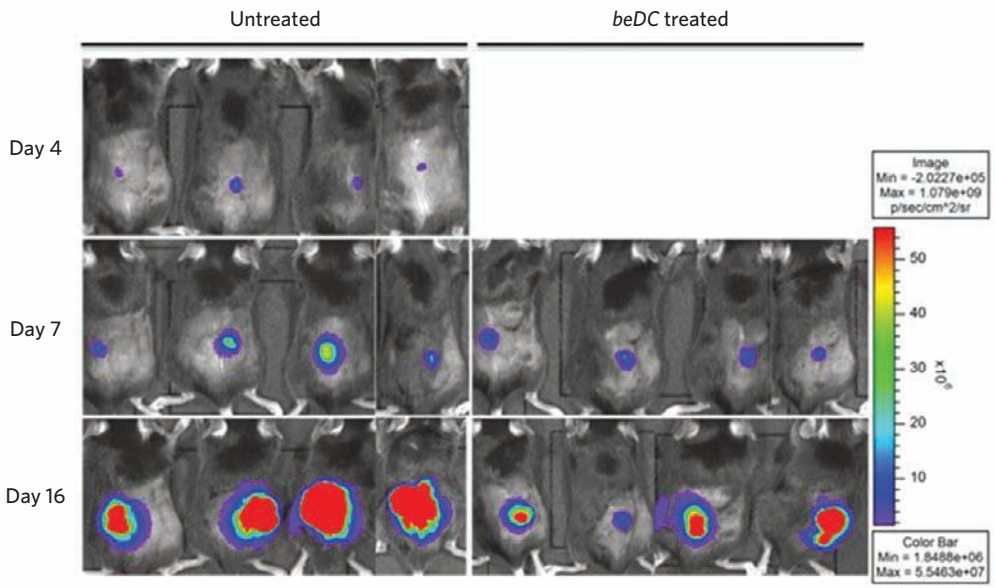
PROBLEM: Secondary tumors that form after surgery generally grow faster and are more immunosuppressive than primary tumors. Verma and Rhee wanted to create an easily delivered therapy that would remain active under such immunosuppressive conditions. They also wanted a material whose shape could be easily manipulated and didn't require extensive biochemical modification to incorporate dendritic cells.

PROJECT: The researchers set out to develop a moldable scaffold. In a preliminary mouse study, the first material they tried was a polymerized fibrin. This US Food and Drug Administration–approved, blood clot–forming protein easily binds immune cells directly to the scaffold. "We never looked further because it worked so well," says Verma.

To create the vaccine, they used the mouse's tumor tissue (melanoma) to "educate" dendritic cells in a simple blood sample. The dendritic cells were then loaded onto the scaffold, which was surgically implanted to snake along the irregular shape of tumors without interfering with tumor monitoring. Thus positioned, the dendritic cells could recruit and train cancer-fighting T cells. The vaccine shrank the secondary tumors by 50 percent to 70 percent (*Oncotarget*, 7:39894-906, 2016).

PROS/CONS: The implant's flat shape makes it easy to distinguish from tumor, and it is completely biodegradable. Like other locally implanted vaccines, it can induce a powerful immune reaction in the tumor vicinity. But the recipient's immune system must be robust for the vaccine to work. It also requires surgery.

FUTURE: Verma plans to generate an injectable version that can deliver the vaccine directly into lymph nodes inside or near the tumor. Here, T-cell education by the dendritic cells would happen right away because the two cell types would be in immediate contact. The team is also adding additional proteins to the scaffold that will help the vaccine remain active longer. ■



CANCER VACCINE: Surgically implanted fibrin scaffolds loaded with dendritic cells (beDC), primed by exposure to the mouse's melanoma, recruit and train the animal's T cells to fight the tumor.

Trunks and Branches

Analyzing single cell sequences to decipher the evolution of a tumor

BY AMBER DANCE

By the time a person arrives at the doctor's office with a tumor, a lot has already happened at the cellular—and genomic—level. That cancer sprang from one mutant cell that spawned a mass of cells with additional nucleotide changes and diverse phenotypes.

To understand the evolutionary history of a cancer, scientists are turning to single-cell sequencing. Conceptually, building a tumor's family tree is fairly simple: "Cells that have mutation A come before cells that have mutation A and mutation B," explains Aaron Diaz, a glioblastoma researcher at the University of California, San Francisco. Of course, most tumors are a bit more complicated than just two mutations. From the sequences of dozens of individual cells from the same tumor, computational algorithms build their best guess at how a person's cancer evolved. This gives researchers an idea of what mutations happened early versus late, how cells might have evolved drug resistance or the ability to metastasize, and what treatments might work best.

Each tumor will yield its own unique tree shape, notes Nicholas Navin, a genomicist at the MD Anderson Cancer Center in Houston. A long trunk with short branches, for example, would indicate the cells underwent genetic changes early on, then stabilized, yielding a fairly homogeneous tumor. In contrast, a short trunk with plenty of branching would suggest the tumor diversified continually after the cells first became cancerous.

The method of constructing tumor trees is akin to evolutionary biologists' strategies for constructing phylogenetic trees, and indeed, cancer biologists have borrowed computational tools from that field. However, single-cell sequencing brings its own special considerations. The major issue is that plenty of errors appear in single-cell data due to problems in amplifying or sequencing the individual genomes. Sequencing mistakes create false positives that look like a mutation, or obscure a true mutation. False-negative rates, in particular, can be high, sometimes topping 10 percent (*Genome Biol*, 17:86, 2016). In addition, some portions of a cell's DNA might be overrepresented; in other cases, genotypes may be missing.

Scientists working with these noisy data need specialized tools. In recent years, cancer researchers, computer scientists, and mathematicians have joined forces to design a handful of new algorithms customized for tumor studies. Some produce a phylogenetic tree, with each leaf representing a sequenced cell. Others collapse cells with similar sequences into clones, generating a tree that represents how those clones evolved. Still other trees display the individual mutations in the order they likely occurred. It's still early days, however, and there's certainly room for improvement in these tools, cautions Sohrab Shah, a senior scientist at the British Columbia Cancer Agency in Vancouver.

One factor that differentiates such algorithms is whether they make what's known as the infinite-sites assumption. This means they allow each genetic locus to mutate only once within a tumor, and no more, and never revert back to the wild-type sequence. While it seems like such multiple mutations should be unlikely, computational biologist Niko Beerenwinkel of ETH Zurich in Basel, Switzerland, recently posted a preprint to bioRxiv suggesting that recurrent mutations can and do occur, and not infrequently (*bioRxiv*, doi:10.1101/094722, 2016).

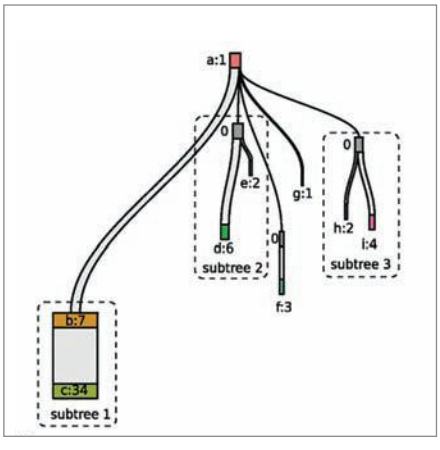
Here, *The Scientist* profiles four options to generate tumor trees based on single-nucleotide variations in single-cell sequences.

BITPHYLOGENY

AUTHORS: Niko Beerenwinkel, Associate Professor, ETH Zurich, Basel, Switzerland; Florian Markowetz, Senior Group Leader, University of Cambridge, U.K.

INPUT: Users give the program a matrix, with cells in rows and mutations in columns. For each spot in the matrix, they indicate a '1' if the mutation is present in that cell, a '0' if not. Missing data points are also allowed.

METHOD: BitPhylogeny uses an algorithm called Markov Chain Monte Carlo to search for the tree that would best match the data in the matrix. At the same time, it groups cells with similar genotypes into clones. The program can infer that certain clones must have existed in the past, even if they aren't in the tumor sample, because they are the likely last common ancestor of cells it's analyzing. It also computes the length of the branches on the tree—that is, how much relative time passed between mutation events (*Genome Biol*, 16:36, 2015).



TREE GROWING: BitPhylogeny created this clonal tree from the exome sequences of 58 blood cancer cells. Each node of the tree is a clone (a–i) and the numbers indicate how many cells are in that clone. Certain clones, with 0 cells, were inferred from the data to have existed.

K. YUAN ET AL., *GENOME BIOL*, 16:36, 2015

ERROR APPROACH: As it's developing possible trees, the program also estimates the error rate from the data matrix.

OUTPUT: BitPhylogeny builds a clonal tree, with each node being a group of genetically similar cells.

PROS

- In addition to single-nucleotide variants, BitPhylogeny can also analyze data on methylation of DNA, and in this case does not have to use the infinite-sites assumption, says Markowetz.
- It gives users information on the probability of individual branches occurring. Low-probability branches could have arisen from sequencing mistakes, says Beerenwinkel.

CONS

- For single-nucleotide variants, BitPhylogeny will use the infinite-sites assumption.
- Markov Chain Monte Carlo methods are slow; BitPhylogeny takes hours to generate a tree, depending on the complexity of data fed in.
- It does not distinguish between cells that are hetero- or homozygous for a given mutation.

SCITE

AUTHOR: Niko Beerenwinkel, Associate Professor, ETH Zurich, Basel, Switzerland

INPUT: As with BitPhylogeny, users provide a matrix of single cells and mutations.

METHOD: BitPhylogeny was inefficient, so Beerenwinkel and colleagues designed SCITE to be faster and more robust. It also uses Markov Chain Monte Carlo methods to search through possible trees for the one most likely to result from the input data, though compared to BitPhylogeny, SCITE offers fewer parameters users can tweak (*Genome Biol*, 17:86, 2016).

ERROR APPROACH: SCITE estimates the error rate from the data as it creates the tree, assuming the rate of false positives and false negatives are the same for all mutations.

OUTPUT: The program can create a phylogenetic tree, with each leaf representing a cell, or a mutation tree that lays out the order in which mutations occurred. It can decide which to produce based on the data input. For a large number of cells—say, hundreds—it's more efficient to make a mutation tree, explains Beerenwinkel; a phylogenetic tree is more efficient if the program has to deal with hundreds of mutations. Whether a given data set contains high numbers of mutations depends on the mutation rate in the tumor itself as well as which technology was used to obtain those sequences, says Beerenwinkel. Users can specify which kind of tree they'd prefer.

PRO

- SCITE is efficient. So far, the researchers find it takes only a few minutes to generate a tree from 60–100 cells, though the timing will depend on the complexity of each data set.

CONS

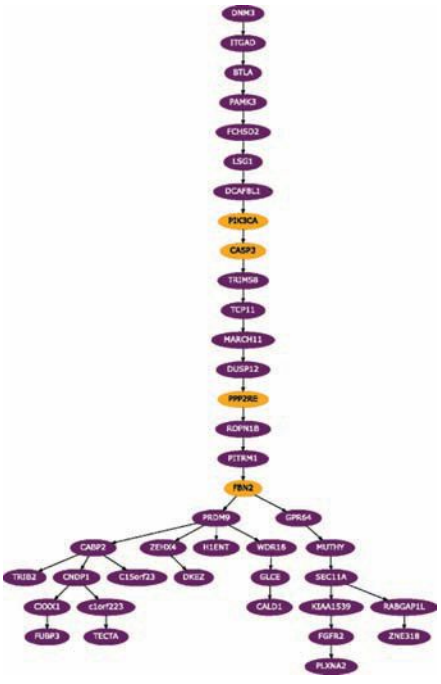
- The original SCITE algorithm makes the infinite-sites assumption, so it will be inaccurate if the same mutation happened independently in multiple cells within a tumor, and it assumes that any homozygosity for a mutation is due to a technical error. But in their recent *bioRxiv* paper on multiple mutation rates, Beerenwinkel and colleagues described an extension to SCITE that would allow for recurrent mutation.
- SCITE does not take into account mutations that are present in all cells or only in one cell.

ONCONEM

AUTHORS: Florian Markowetz, Senior Group Leader, University of Cambridge, U.K.; Edith Ross, Graduate Student, University of Cambridge

INPUT: Users input a matrix of cells and mutations along with the error rates in the sequencing data, if known.

METHOD: OncoNEM clusters individual cells into clones of similar genotypes. It uses what's called a neighborhood search to try out different trees and find one that would likely yield the observed sequencing data.



TUMOR EVOLUTION: SCITE generated this mutation tree from exome sequences of 47 cells from a breast tumor. Each node indicates a mutation that occurred as the tumor developed and differentiated. The yellow nodes indicate likely cancer drivers, because they cause amino acid changes in known cancer genes.

Like BitPhylogeny, it also infers the existence of clones that were likely present in the tumor at some point. However, the method is not too different from SCITE's approach, says Markowetz. "SCITE and OncoNEM are really, under the hood, the same model," he says (*Genome Biol*, 17:69, 2016).

ERROR APPROACH: If users don't input the error rates, OncoNEM can infer them from the data.

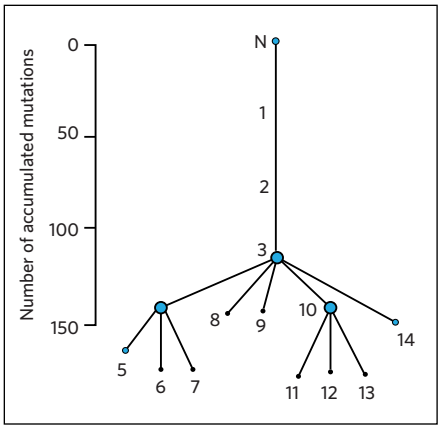
OUTPUT: Standard output is a clonal tree, but users can also ask the program to skip the clone-clustering step and produce a phylogenetic tree with single cells at leaves or branch points.

PRO

- It's fast for a relatively small number of cells. The data sets Ross has tried, including dozens of cells, so far yield a tree within minutes.

CONS

- It makes the infinite-sites assumption, so it can't take into account reversions



KIN GROUPS: OncoNEM determined this clonal tree from exome sequences of 44 bladder tumor cells. Each node represents a group of related or identical cells.

to wild type or multiple instances of the same mutation.
- It does not distinguish between cells that are hetero- or homozygous for the same mutation.

SIFIT

AUTHORS: Luay Nakhleh, Chair, Department of Computer Science, Rice University, Houston, Texas; Nicholas Navin, Associate Professor, MD Anderson Cancer Center, Houston

INPUT: The program can analyze either the full single-cell sequences or a matrix of single-nucleotide variants, along with error rates and the probability of a mutation across the genome. In addition, users can input data that distinguishes between cells that are heterozygous or homozygous for each mutation.

METHOD: Hamim Zafar, a graduate student at Rice, programmed SiFit with a maximum likelihood model to search through trees for the one most likely to explain the given data (*bioRxiv*, doi:10.1101/091595, 2016).

ERROR APPROACH: SiFit can estimate error rates from the data, if users don't input it.

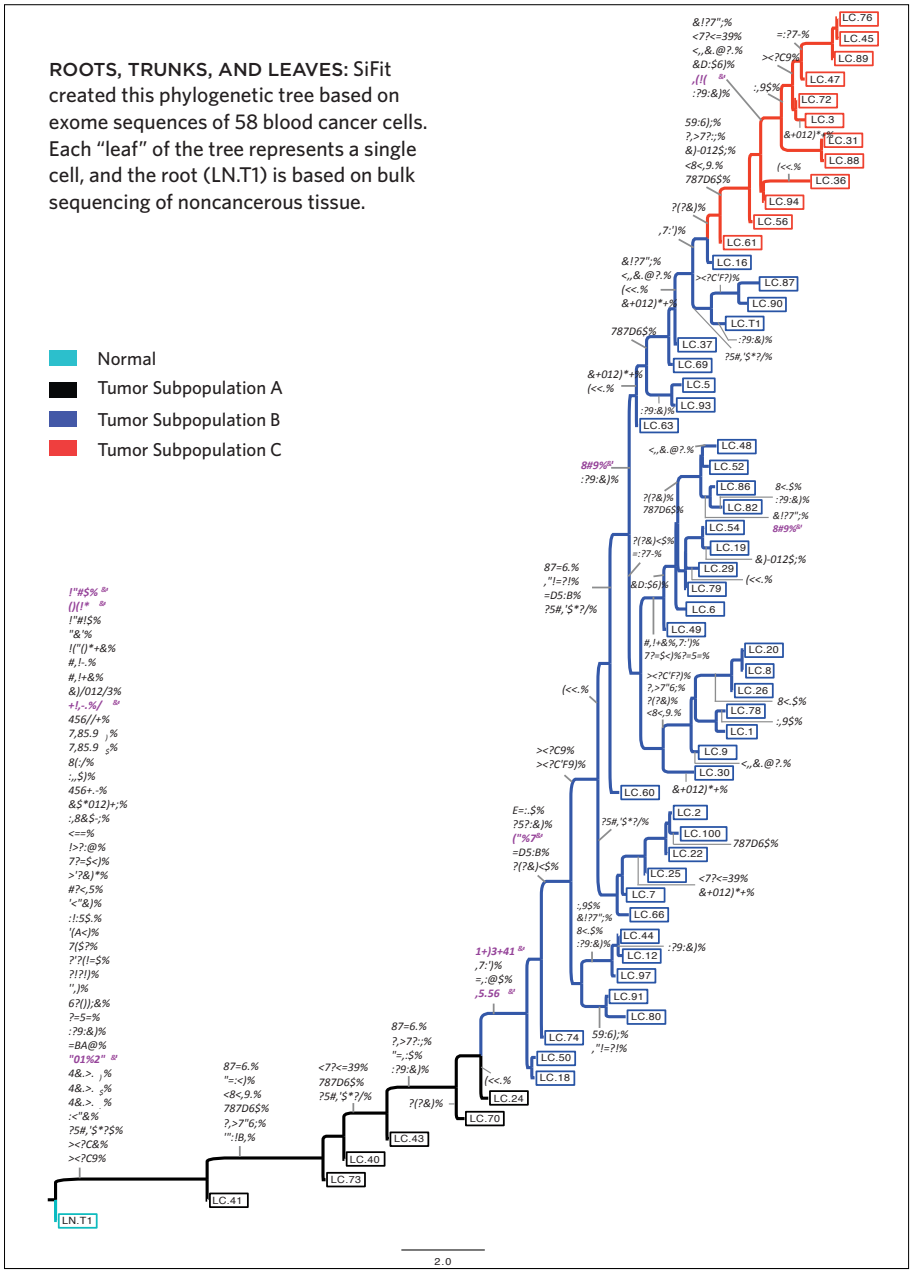
OUTPUT: SiFit builds a phylogenetic tree with each leaf representing a cell, but can also convert this into a mutation tree.

PRO

- Nakhleh and colleagues designed SiFit to avoid the infinite-sites assumption, so it allows for multiple occurrences of the same mutation, or reversion of a mutation to wild type.

CONS

- SiFit is computationally demanding, and never really "finishes" its analysis. "You can run it for days and weeks and months and you still have not explored every possible tree," says Nakhleh. Users typically set it to run for a given time, say, six or eight hours.
- Nakhleh is thinking about how to calculate and display the probabilities that an individual branch of the output tree is correct, which the program can't yet do, so scientists know how confident to be of each scenario. ■



H. ZAFAR ET AL., *BIORXIV*, DOI:10.1101/091595, 2016

Safety Belts

Following a spate of patient deaths in CAR T-cell therapy trials, researchers work to reduce the treatment's toxicity without sacrificing efficacy.

BY CATHERINE OFFORD

In 2015, biopharmaceutical company Juno Therapeutics launched a Phase 2 trial testing a therapy for adult relapsed and refractory acute lymphoblastic leukemia (ALL), a blood cancer that, with current treatments, only 10 percent of patients survive past five years. Developed in collaboration with researchers at Memorial Sloan Kettering Cancer Center, Juno's chimeric antigen receptor (CAR) T-cell therapy JCAR015 was engineered with a specific protein to help the immune cells recognize, and selectively kill, tumor cells displaying the CD19 antigen on their surface. Like other CAR T-cell products in development, the therapy had shown tantalizing potential, achieving remission in patients for whom other treatments had failed.

But in May 2016, things started to go terribly wrong; one of the 68 patients being treated with JCAR015 died from cerebral edema, a swelling of the brain. Then in July, another two patients died from the same condition and the trial was suspended. After appealing to the US Food and Drug Administration (FDA), Juno investigators recommenced the trial, omitting an accompanying chemotherapy drug they suspected was responsible for the adverse reactions—only to have the study halted again after two more deaths from cerebral edema in November.

The fatalities, widely reported and discussed, came as a blow to the field, with some investors and health care analysts questioning whether the company—and the FDA—had acted responsibly. By early 2017, Juno's shares had sunk to less than half of their value the previous summer, and in March, the company decided to call it quits on JCAR015 altogether, although it plans to continue development on other CD19 therapies.

JCAR015 is not the first CAR T-cell therapy to have been associated with patient deaths. In fact, even those trials considered a success sometimes have troubling safety profiles. For example, Novartis's lead candidate, the CD19-targeting CLT019, demonstrated a remarkable 82 percent remission rate in a 2016 trial with 50 children and young adults with ALL, but the treatment caused severe cytokine release syndrome (CRS)—a potentially life-threatening condition where large volumes of T cell–released cytokines trigger inflammation and fever—in nearly half of patients. And in September, Santa Monica, California–based Kite Pharma reported serious neurological side effects in one-third of its patients and CRS in one-fifth, as well as two patient deaths, during a trial of its lead CD19-targeting candidate KTE-C19, a therapy designed to treat non-Hodgkin lymphoma.

Although administration of steroids or antibodies targeting T-cell receptors can mitigate these side effects during treatment, concern over patient safety has grown to become "the theme of the field," says Sean McCarthy, CEO of CytomX Therapeutics, a San Francisco–based biotech developing CAR-based cancer therapies. "In general, in oncology, a higher level of toxicity has been accommodated by both physicians and patients," he adds, "but there's a limit."

Nevertheless, researchers remain excited about the promise of CAR T-cell therapies to fight many treatment-resistant cancers. (See "Resist or Desist" on page 40.) Even in the recent Juno trial, "many of the patients who were treated had really great responses," says Marcela Maus, director of cellular immunotherapy for cancer at Massachusetts General Hospital. And in late February, Kite announced that one-third of patients who'd received KTE-C19 showed no detectable cancer after six months.

As Novartis and Kite push for regulatory approval of their CD19-targeting CAR T-cell therapies this year, the field is striving to increase safety without sacrificing the treatments' ability to beat cancer. "These therapies are so powerful and have so much potential, we have to find a way to manage the toxicity," says Maus. "I would certainly not want to put the brakes on everything and say we have to go back to all animal models before we can go on."

Emergency brakes

While all CAR T-cell therapies tested thus far cause adverse reactions in at least some patients, certain treatments show greater toxicity than others, and understanding why has proven challenging. Different companies use different manufacturing processes,

clinical protocols, patient populations, and dosing regimens, making the identification of specific risk factors difficult. To help disentangle these issues, the FDA recently proposed establishing central databases to keep track of safety indications for CD19-targeting CAR T-cell therapies such as those being advanced by Juno, Kite, and Novartis. The hope is that combining trials' often small data sets could help researchers identify particular steps in development or administration that are linked to increased risks.

In the meantime, investigators are taking steps to improve patient safety during ongoing trials. "I think this point is being stressed by all companies at the moment," says Ronald Dudek, founder of early-stage CAR T-cell company Living Pharma and a consultant on CAR T-cell therapy development. One approach, he says, is simply getting better at recognizing common side effects and responding appropriately. "I look forward to some standardization of CRS monitoring technologies and some bona fide diagnostics that might help catch the syndrome at an earlier stage."

With this sort of real-time trial management in mind, several companies are investigating ways to mediate the action of CAR T cells during treatment, to rein in side effects before they become serious. A standard measure is to administer a general immunosuppressive drug such as tocilizumab to reduce inflammation—a method Dudek calls "the sledgehammer approach"—but researchers are now developing more sophisticated technologies to exercise finer control when hitting the immunological brakes.

In 2014, Houston-based Bellicum Pharmaceuticals pioneered a "suicide switch," CaspaCIDe, which can be engineered into T cells. The switch comprises an enzyme involved in programmed cell death (the gene for which is transduced into patients' T cells ex vivo), along with a small molecule activator, rimiducid. In the event of severe side effects in a patient, clinicians can administer rimiducid, triggering self-destruction of the modified T cells in as little as 30 minutes. The technology is incorporated in the company's lead candidate, BX-501, a therapy using partially matched donor T cells (from a parent, for example) that is currently being evaluated in children with blood disorders ranging from leukemia to sickle cell disease.

Other companies are exploring variations on the theme. Cellectis and Juno are both trialing treatments containing T-cell safety switches that rely on antibodies to trigger cell death. And Ziopharm Oncology has recently developed technology to tamp down the activity of the CAR T cells without killing them entirely, leaving open the possibility of reactivating the treatment if the patient is able.

"First-generation products didn't have switches," says Eric Ostertag, CEO at gene and cell therapy company Poseida Therapeutics. "But just about everyone I'm aware of is moving in that direction."

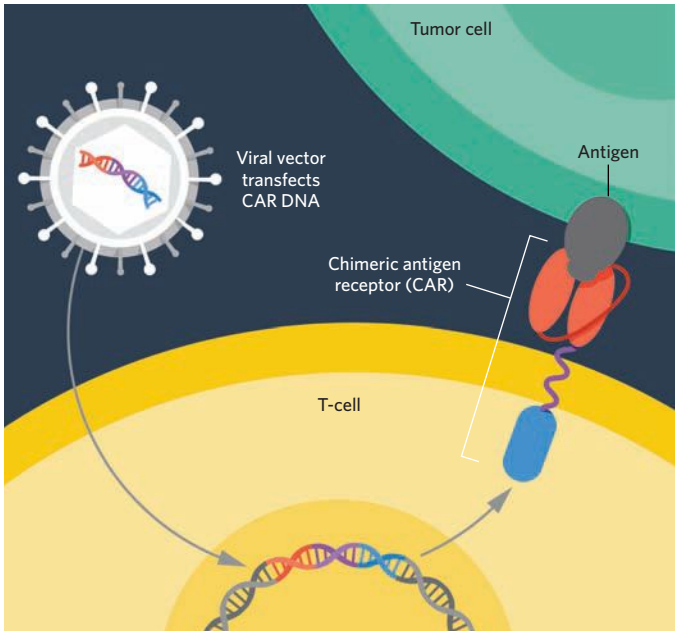
Engineering safer therapies

While managing CAR T-cell therapy toxicity could help keep already-designed treatments on their march to the clinic, many immunotherapy companies are also working to develop a new generation of therapies that are inherently safer, yet just as efficacious. "The goal is to separate the toxicity from the antitumor efficacy," says Dudek. "That's sort of the holy grail in this space."

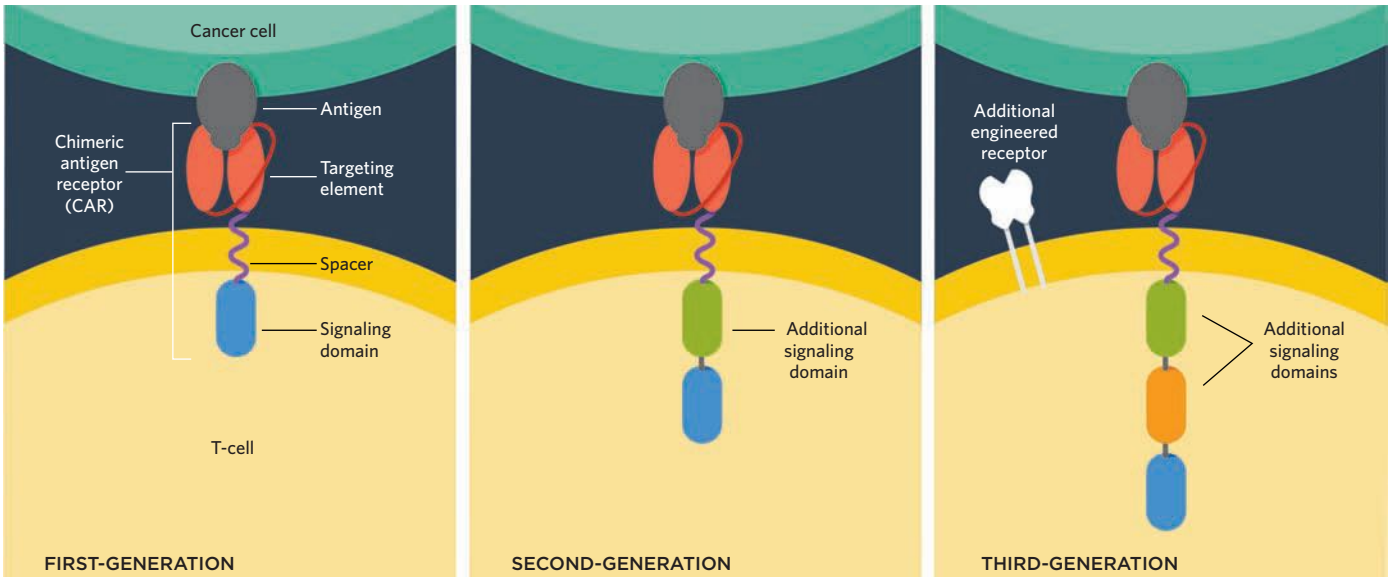
A key part of achieving this goal will be improving CAR specificity for target cells. With current therapies, the destruction of normal cells is often an unavoidable side effect when healthy tissue carries the same antigens as tumors; noncancerous B cells, for example, are often casualties in CD19-targeted therapies. While cell damage can be managed to an extent, these "on-target, off-tumor effects" can be fatal—particularly in solid tumors, where T cells are more likely to encounter target antigens on healthy as well as cancerous tissue. "The prediction is that it's going to be very difficult to treat solid-tumor patients with CAR T [cells], unless we can find antigens that are exquisitely localized to cancer tissue and not present at all on normal tissue," says McCarthy. "The reality is that there are very few, if any, such targets."

One approach to improving specificity is to engineer CARs with not one, but two antigen-binding domains. The resulting bispecific CARs could reduce off-target effects by requiring that two tumor antigens are present—or that one tumor antigen is present and a second, healthy-cell antigen is absent—before T-cell activity is stimulated. Such approaches have shown improved specificity in preclinical models of prostate cancer, and Juno states it has been developing bispecific technologies over the last couple of years.

Signals in the tumor microenvironment could also be exploited to help CAR T cells distinguish cancerous from healthy tissue. In January, Cellectis published a method to engineer CARs with oxygen-sensitive domains that render T cells ineffective unless they're in a hypoxic environment—a characteristic of up to 50 percent of solid tumors (*Sci Rep*, 7:39833, 2017). And CytomX's Probody technology masks the target-binding region of an antibody until it is bro-



LOCKED AND LOADED: T cells engineered to carry chimeric antigen receptors (CARs) on their surface can bind to tumor-specific antigens to target the cells for destruction.



IMPROVING CAR T CELLS: In addition to a specific cancer-targeting antibody, a transmembrane component, and a signaling domain that amplifies the activation of the T cells (left), new CAR T-cell technologies have added additional costimulatory domains within the cells (middle), engineered receptors (right), and even safety switches (not pictured) to improve targeting of the T-cell attack and minimize side effects.

ken down by proteases unique to the tumor microenvironment. "The technology is really designed to avoid binding to normal tissue," explains McCarthy. "Concentrating the active antibody in tumor tissue allows us to [lengthen] the therapeutic window, or create a therapeutic window where there may not be one."

Efficacy boost

Of course, as CAR T-cell therapies progress in development, it's not just safety profiles that researchers hope to improve; scientists also aim to further enhance the therapies' efficacy. Tweaking the genetics of T cells beyond adding CARs is a broad approach drawing more attention, reflected in several deals between CAR T-cell developers and biotechs employing the genome-editing tool CRISPR-Cas9. In 2015, Novartis announced a five-year collaboration with Intellia Therapeutics; soon after, Juno entered a five-year partnership with Editas Medicine. "What you're seeing is a logical marriage of gene-editing technologies to CAR T cells," says Dudek. "Companies have announced they're working on better-designed cells that work optimally in the hostile tumor microenvironment."

One application for genome editing is the deletion of genes that can dampen T cells' ability to fight tumors—a possibility being explored as part of the first US trial to test CRISPR's potential in humans, scheduled for early 2017. Designed by researchers at the University of Pennsylvania, the trial will include disrupting a gene coding the immune checkpoint protein PD-1, a T-cell surface receptor that tumors often exploit to dampen T-cell activity. While the protein can be targeted with inhibitors in the clinic, knocking it out genetically could increase the persistence of T-cell activity—though the approach deserves caution, notes Dudek. "Knocking out PD-1 might seem like a great idea, but you're taking the brakes off a very powerful locomotive, so you'd better be able to stop it if something goes wrong."

The precision of CRISPR could one day also be used to improve the delivery of the CAR genes themselves. Earlier this year, researchers at Memorial Sloan Kettering Cancer Center showed that, unlike viral delivery—which inserts the CAR gene randomly into T-cell DNA—CRISPR-mediated delivery can introduce a CAR gene at a specific location in the genome. T-cells created using this method showed higher potency, the researchers reported, and outperformed traditional CAR T cells in mice with ALL (*Nature*, doi:10.1038/nature21405, 2017).

Longer term, some companies are eyeing the possibility of moving beyond patient-specific products altogether, exploring the production of "off-the-shelf" CAR T cells from donated T cells. This approach has the potential to generate T-cell populations to be used in thousands of patients with lower risk of graft-versus-host disease, in which the recipient's immune system attacks the donor cells. Last year, Kite joined forces with the University of California, Los Angeles, to investigate relevant methods, while Juno partner Fate Therapeutics paired up with Sloan Kettering; just this February, Cellectis received approval from the FDA to begin Phase 1 trials of an off-the-shelf therapy for two types of blood cancer.

For now, while Juno scrutinizes its pipeline, all eyes are on CAR T-cell therapy frontrunners Kite and Novartis. If one or both of these companies' products gain approval in 2017—a prospect deemed likely by market analysts—"it's going to raise every CAR T cell's boat," notes Dudek, "as well as make this emerging field more real." Maus agrees: "It's going to be so exciting when the first cell therapy gets FDA approved," she says. "That's going to be a significant milestone."

And with such a variety of technologies trailing close behind, it's little wonder that the field remains optimistic, in spite of recent setbacks. "Patient deaths in clinical trials are of course the last thing we want to see," says CytomX's McCarthy. "But often, what we learn from these setbacks actually allows us then to move forward. I personally remain hugely optimistic for all these approaches in cancer immunotherapy, and I think that the best is very much yet to come." ■

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Trumping Cancer

Will President Trump and his antiregulation leanings help or hinder the effort to eradicate cancer?

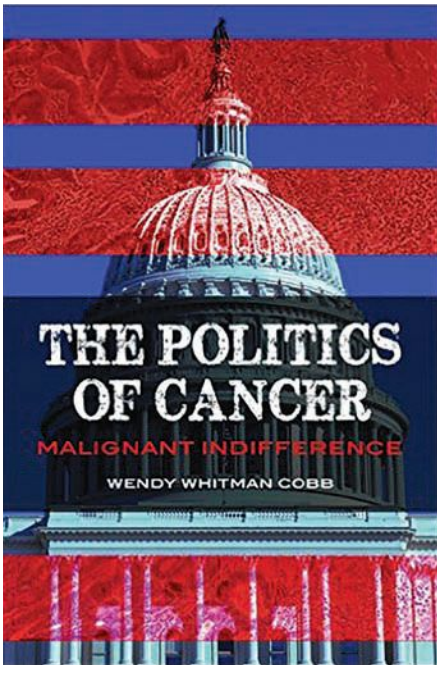
BY WENDY N. WHITMAN COBB

In his final State of the Union address in January 2016, President Barack Obama introduced a new "moonshot" effort aimed at treating and curing cancer. His Vice President, Joe Biden, having recently lost his son Beau to brain cancer, was put in charge of the project and immediately set out to remove barriers and improve communication between researchers, doctors, and patients. Biden's work helped lead to the December 2016 passage of the 21st Century Cures Act, which provided $1.8 billion for cancer research and changed US Food and Drug Administration (FDA) regulations to pave the way for faster approval of new cancer drugs. All of this occurred as the new Trump administration prepared to take power.

Unlike both Obama and Biden, President Donald Trump has not spoken publicly about cancer, either to relate experiences he's had personally or to address the politics surrounding the disease. The effectiveness of a broad push to cure cancer or even improve patient outcomes often benefits from the personal involvement of high-ranking officials such as the president or vice president, as I discuss in my new book, *The Politics of Cancer*. But such backing is unlikely to materialize under either President Trump or Vice President Mike Pence. Trump's stances on regulation and the FDA drug-approval process will, however, likely have a significant effect on both the researchers who study cancer and the patients who must live with it.

On the regulatory front, President Trump's executive order requiring that for every new regulation, two be done away with has the potential to imperil public health. Although most of the criticism leveled at Trump's take on government regulation in general and on environmental regulations in particular has involved climate change, rolling back some regulations could drastically increase the number of cancer cases. The agency in charge of promulgating many cancer-related regulations, the Environmental Protection Agency, has itself been targeted by the new administration. President Trump chose Oklahoma Attorney General Scott Pruitt to head the agency despite the fact that Pruitt has sued the EPA 13 times, claiming companies operating in his state were subjected to unfair and improper environmental regulations. According to the National Cancer Institute, between 4 percent and 19 percent of cancer cases can be linked to environmental factors. Given this connection between chemicals in the environment and carcinogenesis, removing regulatory barriers for pollutants and possible cancer-causing agents could result in a steep rise in the incidences of numerous cancers.

In addition to the potential for changes in the regulatory environment, President Trump, in a meeting with executives from pharmaceutical companies, expressed a desire to lower the approval burdens for new drugs, stating: "We're also gonna be streamlining the process so that from your standpoint, when you have a drug, you can actually get it approved if it works instead of waiting for many, many years." But speeding up this process could endanger lives by putting drugs on the market that may not be safe or any more effective that treatments already available. The types of wholesale regulatory changes the Trump administration desires appear motivated simply by a wish to deregulate instead of targeting specific regulatory burdens as the 21st Century Cures Act does.



Praeger, March 2017

The politics of cancer are such that changes in seemingly unrelated areas can have a significant impact not only on how we view and treat cancer but on how many lives are affected by the insidious disease. A holistic view of cancer treatment and cancer causation is necessary to protect millions of Americans. ■

Wendy N. Whitman Cobb is an assistant professor in the Department of Social Sciences at Cameron University in Lawton, Oklahoma. Read an excerpt of The Politics of Cancer: Malignant Indifference *at the-scientist.com.*

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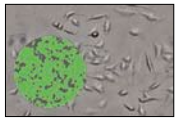

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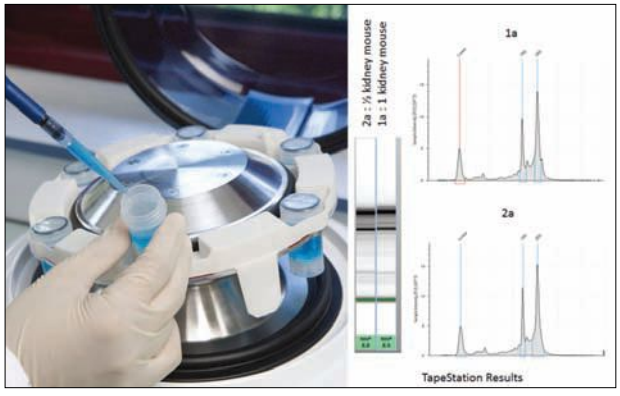

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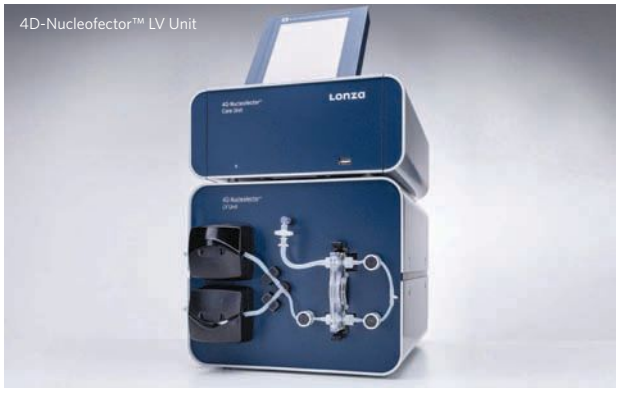

Genetic modification of human T cells holds huge potential for immunotherapy, particularly for cancer treatment. By introducing a modified T cell receptor (TCR) or a synthetic, chimeric antigen receptor (CAR), researchers can alter T cells to specifically recognize and target cancer cells.

Scientists are exploring different approaches for creating CAR-T cells for immunotherapy, using either viral or non-viral gene transfer methods. While viral approaches may provide higher efficiencies, non-viral systems offer greater flexibility and control, and could be a safer option for potential therapies. Non-viral, stable integration of the CAR gene can be achieved with the help of transposon/transposase-based systems like *Sleeping Beauty* or *piggyBac*. In this case, a CAR transposon plasmid or minicircle vector is co-transfected with a transposase vector or mRNA. Alternatively, CAR mRNA can be used providing a high level of CAR expression but only for a limited period of time. Transient expression of CARs in patients could be important from a safety aspect, as it allows monitoring for severe side effects or adverse reactions before beginning longer-term treatment.

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Cell Death August 15 - 19

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August 29 - September 2

Eukaryotic DNA Replication & Genome Maintenance
September 5 - 9

Annexins September 17 - 20

Stem Cell Biology September 25 - 29

Biology of Cancer: Microenvironment & Metastasis
October 10 - 14

Cancer Therapies & Clinical Outcomes October 14 - 15

Single Cell Analyses November 8 - 11

STATs: Importance in Basic & Clinical Cancer Research
November 15 - 18

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June 7 - 26

Metabolomics June 10 - 26

Single Cell Analysis June 30 - July 13

Chromatin, Epigenetics and Gene Expression
July 25 - August 14

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Searching for Cancer Cures, ca. 1950

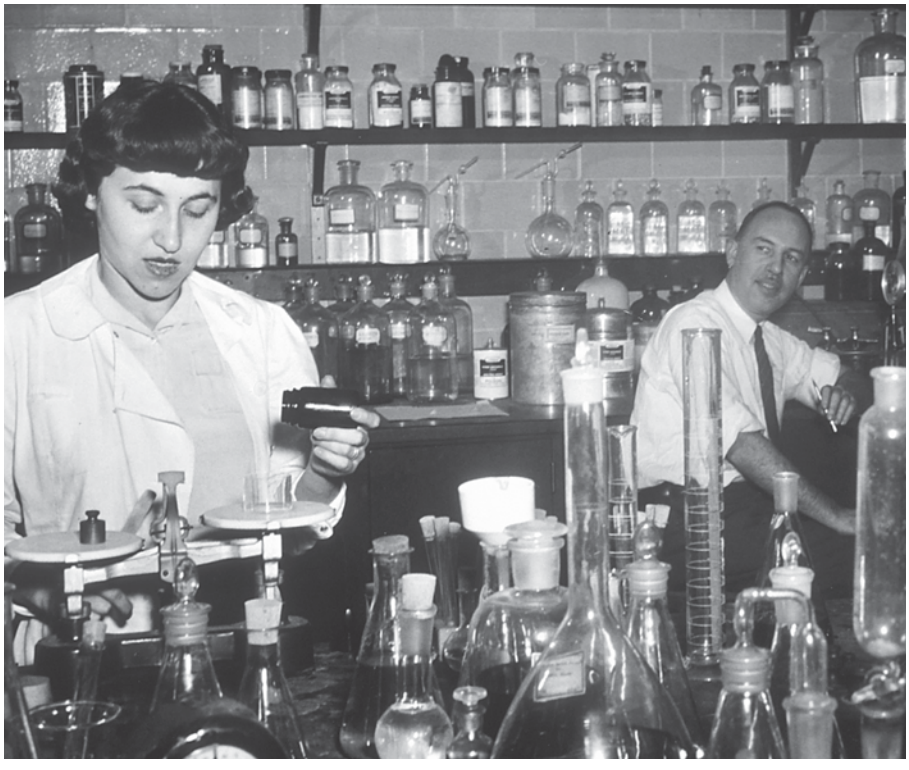
BY JEF AKST

Organic chemist Jonathan Hartwell joined the National Cancer Institute (NCI) to head up a natural products division in 1938, not long after the agency was founded. He'd read plenty of folklore about how plants had healed various ailments over the centuries, and wanted to identify compounds that might have chemotherapeutic potential. So he put out a call to researchers to collect plants from all over the world and send him extracts for testing.

In 1955, the NCI set up the Cancer Chemotherapy National Service Center to investigate all manner of compounds with potential cytotoxic activity, and in 1960, Hartwell helped broker a partnership with the United States Department of Agriculture (USDA) to have its botanists send plants to Hartwell's team for screening. Often, extracts that tested positive would be shipped to academic labs for purification of the active compound, which would then be sent back to NCI for retesting, says chemist David Newman, who led NCI's Natural Products Branch from 2005 to 2015.

Although cancer researchers had tested various chemicals and "potions" inspired by traditional medicines, Hartwell's methodical approach was novel, Newman adds. "People were working all over the world, but not in systematic ways."

The program soon began to yield fruit. For instance, in August 1962, USDA botanist Arthur Barclay collected bark from the Pacific yew tree (*Taxus brevifolia*) in Washington State, and work by the NCI group led to the eventual discovery and approval of taxol for treating ovarian cancer. Two analogs of camptothecin, a cytotoxic alkaloid isolated from the bark and stem of a Chinese xi shu, or "happy tree" (*Camptotheca acuminata*), are now used to treat a variety of cancers. And Hartwell's team helped develop vinblastine and vincristine, identified in crude extracts from rosy periwinkle (*Catharanthus roseus*), for the treatment of lymphoma and childhood leukemia, respectively, in the 1960s, effectively changing what had been death sentences into treatable cancers.



SCREEN TIME: By 1950, Jonathan Hartwell (right) and his team were regularly screening plant extracts sent to the National Cancer Institute by botanists. Initially, the NCI investigators tested the crude extracts in mice harboring natural mouse tumors. Over the next few decades, their methods evolved with the technology to include human cancer cell lines and immunosuppressed mice that carried human cancers. (Upon request from *The Scientist*, the NCI tried to identify the pharmacy technician in the photo, but could not find her name.)

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Hartwell supplemented what he and others were learning from their modern research with a massive literature search, including ancient Chinese, Egyptian, Greek, and Roman texts. "He was very interested in folkloric use of plants," says James Graham of the University of Illinois at Chicago (UIC). "He considered that a very useful strategy" for finding promising leads. (The happy tree is used as a cancer treatment in traditional Chinese medicine, for example.) Beginning in 1967, Hartwell compiled his research into a series of articles in the journal *Lloydia* (now the *Journal of Natural Products*) as well as into a book, *Plants Used Against Cancer* (1982). All told, the collection included information on more than 3,000 species of plants with alleged anticancer activity.

"If you look at the source of drugs used in cancer," Newman says, "60-plus percent of them are either a natural product, a modified natural product, or depend upon what is known as a natural product pharmacophore"—in other words, a synthetic version of a natural molecule. Hartwell was "the leader" in organizing the discovery of natural products to fight cancer, Newman adds. "He was a prophet before his time. Prior to that, nobody had really looked." ■

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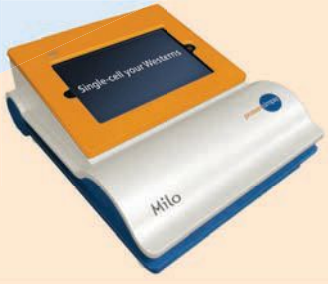

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