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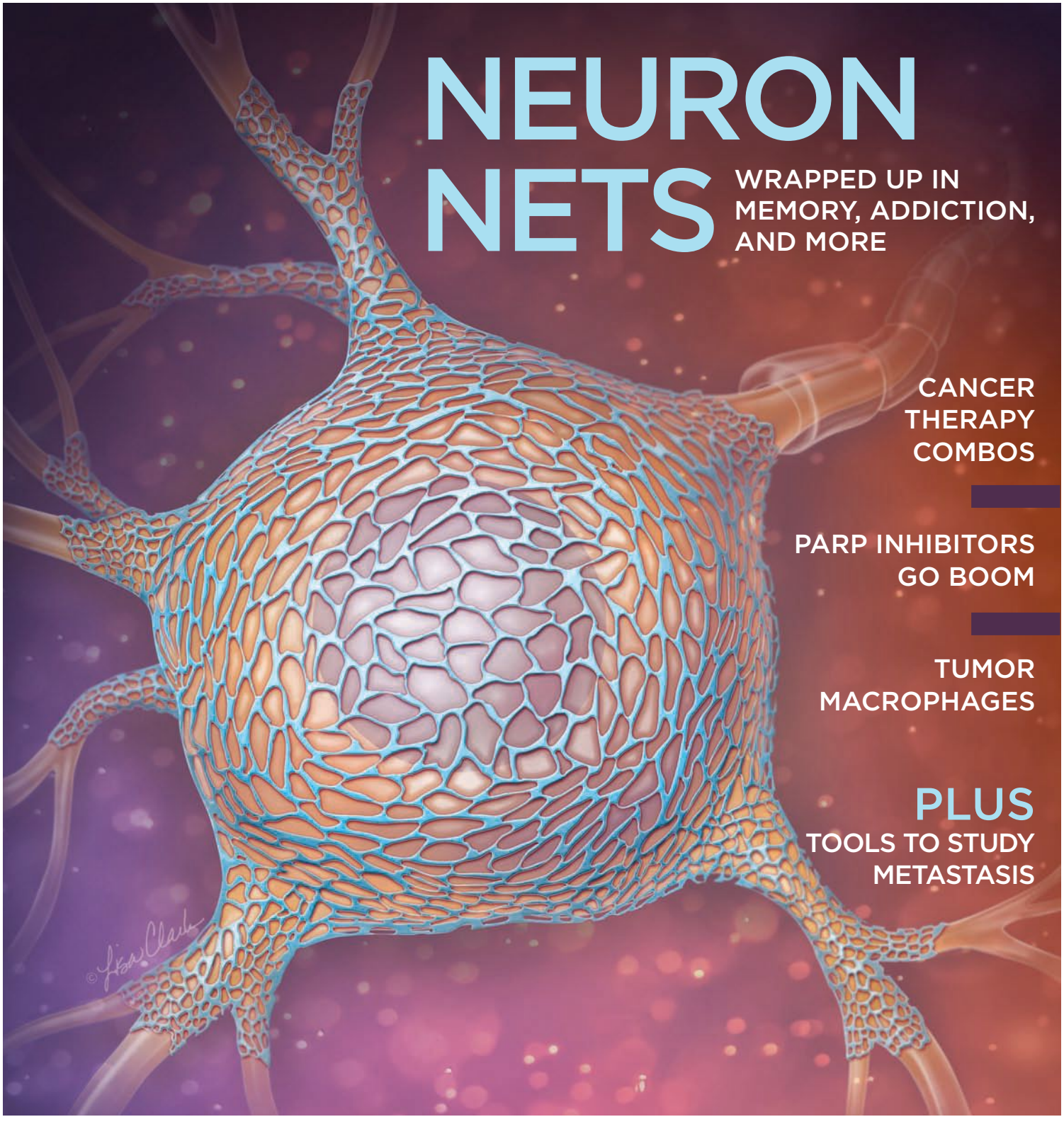

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Contents

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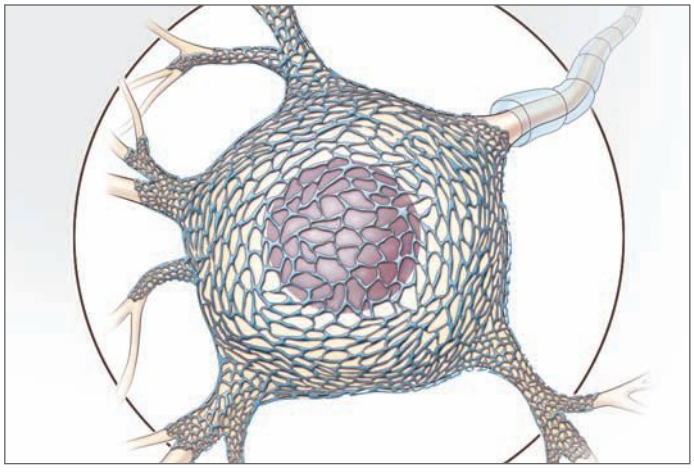
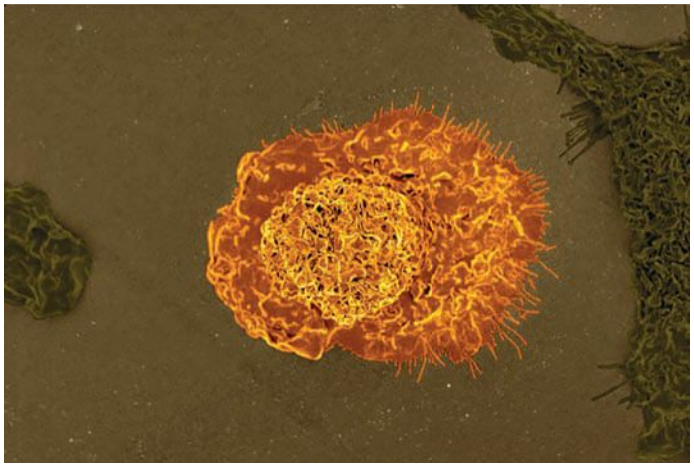




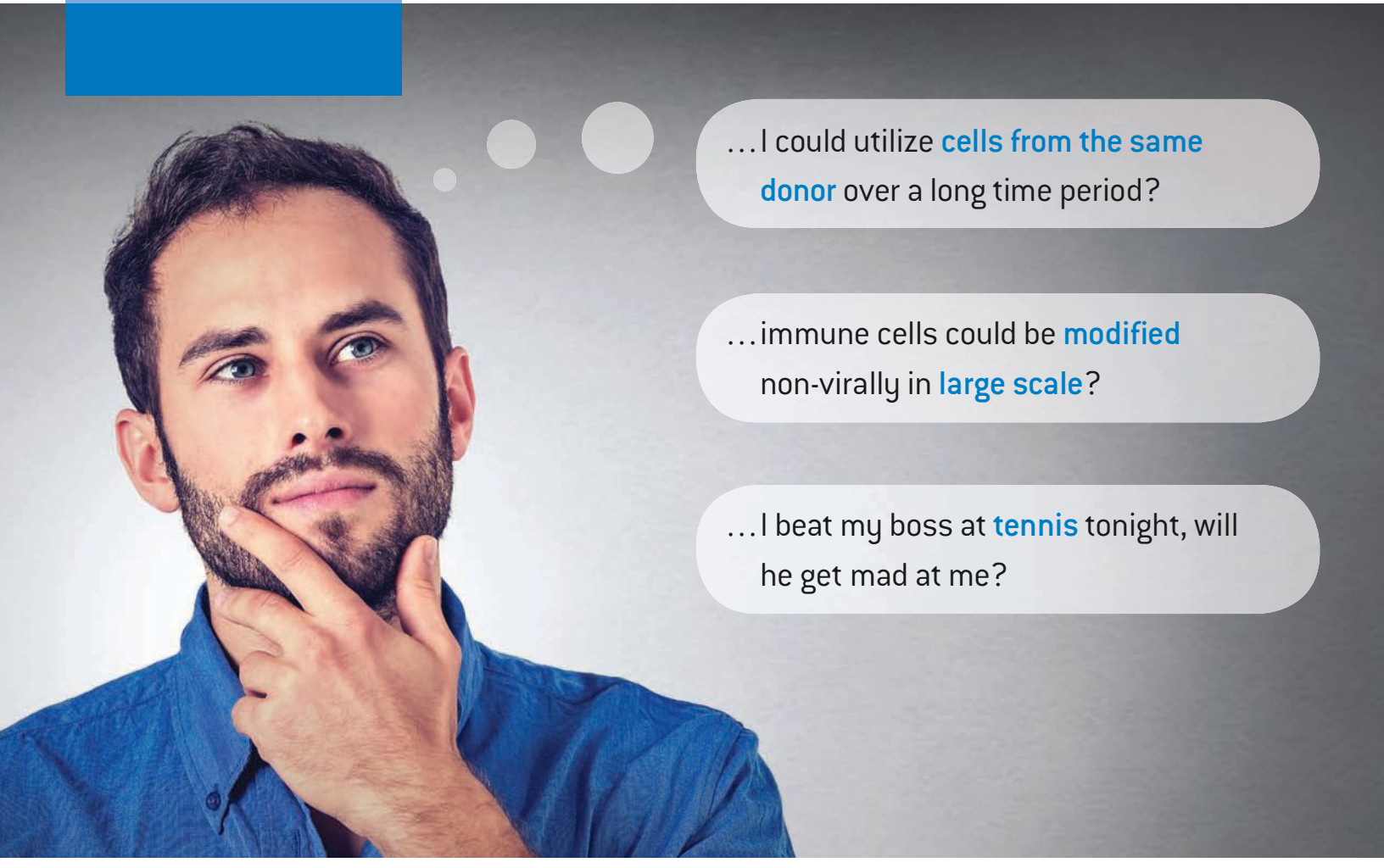
Features

ON THE COVER: ILLUSTRATION BY LISA CLARK

BioResearch

Lonza

What if...



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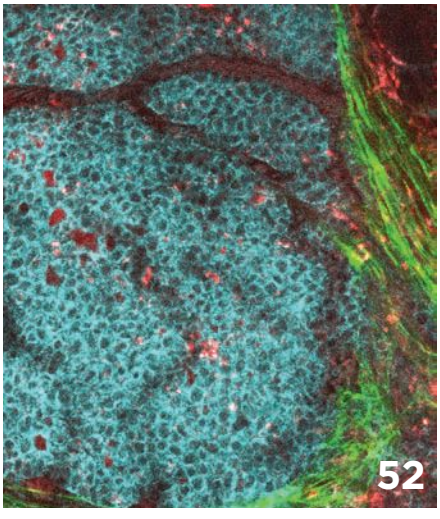

CORRECTIONS:
March 2018's "Undocumented Proteins" mistakenly stated that researchers scanned genomes for transcription initiation sites, when in fact they were scanned for translation initiation sites. *The Scientist* regrets the error.

P	U	P	I	L	■	O	S	M	O	S	I	S
U	■	A	■	O	■	S	■	A	■	E	■	U
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S	P	A	N	D	E	X	■	K	A	Y	A	K

Online Contents

THIS MONTH AT THE-SCIENTIST.COM:

VIDEO
Holy *Mola*
Watch ocean sunfish (*Mola mola*), a species that scientists track closely in the Mediterranean, feed on jellyfish off the coast of California.

VIDEO
Cancer's Complexity
Charles Swanton, researcher at the Francis Crick Institute and Cancer Research UK, explains the heterogeneity of tumors.

VIDEO
PARP Party
Learn about a new class of drugs, known as PARP inhibitors, that blocks DNA repair enzymes and targets hard-to-treat cancers.

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Coming in May

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

- Stories of rare-disease patients whose fundraising pushes gene replacement therapies forward
- The economics of rare-disease research and drug development
- How exome sequencing is helping answer questions for undiagnosed patients
- A big-picture look at rare diseases and the research enterprise surrounding them

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Contributors

"Like many kids, I was interested in animals," says **Daniela Carulli**, and she wanted to be a veterinarian when she got older. "Then I got allergic to animals when I grew up." She decided that she could study ecology instead and observe animal behavior from a distance. But during her undergraduate studies at the University of Turin, in her home town in Italy, Carulli took a comparative anatomy course and became fascinated by the complexity of the brain across different species. She went on to obtain a master's degree from the institution and then began looking for a place to pursue a PhD in neuroscience. After seeing a posting for an opening in Piergiorgio Strata's lab at the University of Turin, she applied and joined the group. With Strata, Carulli studied axon regeneration and plasticity. "They go hand in hand," she says. She went on to study the extracellular aspects of plasticity and axon regeneration during a postdoc at the University of Cambridge. For the past three years, Carulli, who is now an assistant professor at the University of Turin, has been on sabbatical as a visiting scientist at the Laboratory for Neuroregeneration at the Netherlands Institute for Neuroscience in Amsterdam. She writes about perineuronal nets, webs of tissue that surround some neurons, on pg. 40.

Barbara Lipska knows a lot about endurance. A lifelong runner, she has spent years training for marathons—and that training gave the neuroscientist the stamina she needed to get through many difficult times. Lipska lost her first husband to cancer nearly 40 years ago, while she was living in Warsaw, Poland. At the time, she says, cancer was a disease one had to suffer through alone, because of the stigma associated with it, and so they told no one about her husband's diagnosis. After moving to the United States in the 1980s, Lipska began working at the National Institutes of Health, where she later became the Director of the Human Brain Collection Core (HBCC). In 2009 doctors diagnosed her with breast cancer, and in 2012, with melanoma. Her marathon training helped her get through each radiation treatment: she says that in a marathon, you have to be able to keep moving forward, even when you think you can't. In 2015, Lipska faced down a brain tumor, which eventually led to vision loss and serious mental problems. But with the help of her family, doctors, and her own tenacity, she overcame that challenge as well. She writes about her harrowing brush with cancer and mental illness in her essay "Studying the Brain, Losing My Mind" on pg. 67.

Like many a science journalist, **Jim Daley**'s career path took a circuitous route—his perhaps more meandering than others. He grew up on the South Side of Chicago, and, having come from a long line of Chicago Public School teachers, he went to Morgan Park High School, rather than the one of the Catholic schools many kids in the neighborhood attended. Instead of going to college after graduating, he spent a few years waiting tables and attending anti-globalization protests around the country.

Eventually, Daley felt the tug of university and a career. It was a professor at Harold Washington College in Chicago, where Daley was taking a few general education courses, who finally pointed him to biology. "He told me, 'I'm going to make you a biologist,'" Daley recalls. The nudging worked. Daley went on to earn a biology degree from the University of Illinois at Chicago with a concentration on avian ecology, "with the interest of being a science writer, but I didn't know what that meant at the time," he says. "I didn't do anything with it after college because I fell in love with ecology." Daley didn't fall in love, however, with sacrificing animals for research, and he later quit working in a bird lab, opting instead for a series of technician positions in clinical research groups. After a few years, he revisited the idea of writing, launching a blog, freelancing for trade publications, and now interning at *The Scientist*. On page 23 of this issue, he writes about the chemical signals ants use to disinfect colony members carrying a pathogenic fungus. "It's like a superorganism with an immune system."

COURTESY OF DANIELA CARULLI; MIREK GORSKI

cancer research

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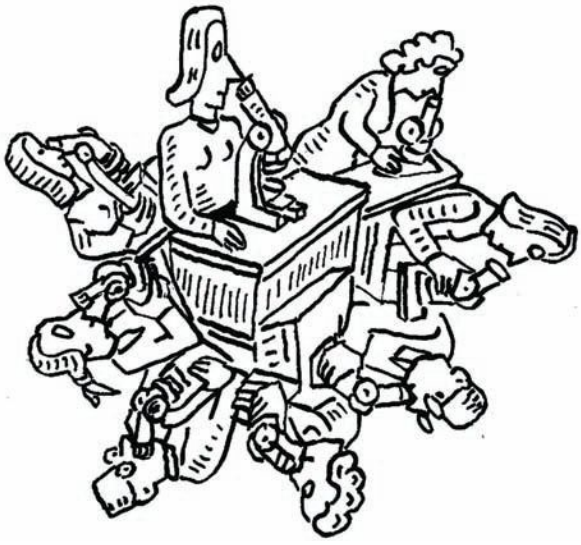

The research enterprise surrounding cancer spreads and changes as it explores multiple facets of the complex disease.

BY BOB GRANT

Sometimes it seems as though cancer research itself is metastasizing. Every day, we learn about new approaches to understand, prevent, and vanquish the many-headed monster that is cancer. But this is a beneficial, not a malignant, growth of knowledge and insight. The complexity in how we conceive of and learn about cancer mirrors the intricate—and on some levels still mysterious—workings of the disease. The efforts to beat cancer, which is actually a constellation of different diseases rather than a single malady, must spread and adapt to match the vagility of the foe.

In recent years, a fruitful strategy in the race to outpace cancer is to exploit the body's own compromised defenses to awaken and mount an attack against rapidly dividing cells. Previously, and per the current standard of care, doctors primarily attempted to eradicate cancer through exposure to harsh chemicals or damaging radiation. But now, the oncologist's toolkit has grown to include more-precise instruments in addition to these well-worn therapeutic cudgels.

In this issue of *The Scientist*, we explore a few of these exciting developments in cancer research. On page 46, author Amanda Keener dives into the peculiar behavior of macrophages, large immune cells that typically make their living gobbling up worn-out cellular components or invading pathogens in the body. For many years, scientists noticed that some cancer patients with a pronounced profusion of tumor-associated macrophages (TAMs) tended to experience enhanced tumor growth and poorer outcomes. But in the past decade, researchers have gained a still-emerging appreciation of the potentially therapeutic role of macrophages in cancer.

At least in some cancers, tumor cells lacking certain "don't eat me" signals are available to the rapacious appetites of macrophages. While the picture is by no means crystal clear—as is the case with much of cancer biology—the recent findings represent a potential foothold for new strategies that might add these immune cells to the arsenal of recently approved immunotherapies that already actively target tumors.

Interestingly, clinical trials that test the anti-cancer strategy of tamping down macrophage recruitment and activity and those that seek to enhance the tumor-fighting capacity of the immune cells are both underway. Only time and data will tell which approach proves more viable.

In another feature article, "Make Me a Match," on page 32, contributor Anna Azvolinsky runs down the state of knowledge regarding combining different cancer drugs to hit the disease at multiple points in its life cycle. The strategy has yielded promising combos, but surprisingly, the field arrives at these formulas using a variety of methods—some of which are less rigorous than others. "What's going on right now in early clinical development is that some companies look at their portfolio of agents, come up with a combination, and then pursue a scientific rationale of varying quality," University of Pennsylvania researcher Peter Adamson tells Azvolinsky.

More rationally designed combination generators are in the works. Hypothesis-driven modeling, relying upon an understanding of the multiple pathways that feed the development and spread of cancer, is producing interesting drug groupings that are wending their ways through clinical trials as well.

Beyond the treatment angle, cancer researchers are making strides in understanding basic principles in the behavior of cancerous cells. Metastasis is surprisingly understudied, according to Keener's Lab Tools piece on page 58. Again, science is pushing the envelope, coming up with new ways to model metastatic dynamics in living laboratory animals.

On a non-cancer note, we're proud to feature on this month's cover a beautiful illustration of a little-known biological phenomenon. Perineuronal nets, the subject of our April cover story, play a role in modulating how neurons are able to form connections, change with environmental inputs, and even store memories.

This issue of *The Scientist* reminds us that scientific knowledge has much room to grow and spread. From watching scientists propel basic biology along, learning ever more about life's seemingly boundless complexity, to observing researchers developing the treatments that may one day stamp out ills that have plagued humanity from time immemorial—how fascinating it is to watch it all unfold. ■

Editor-in-Chief
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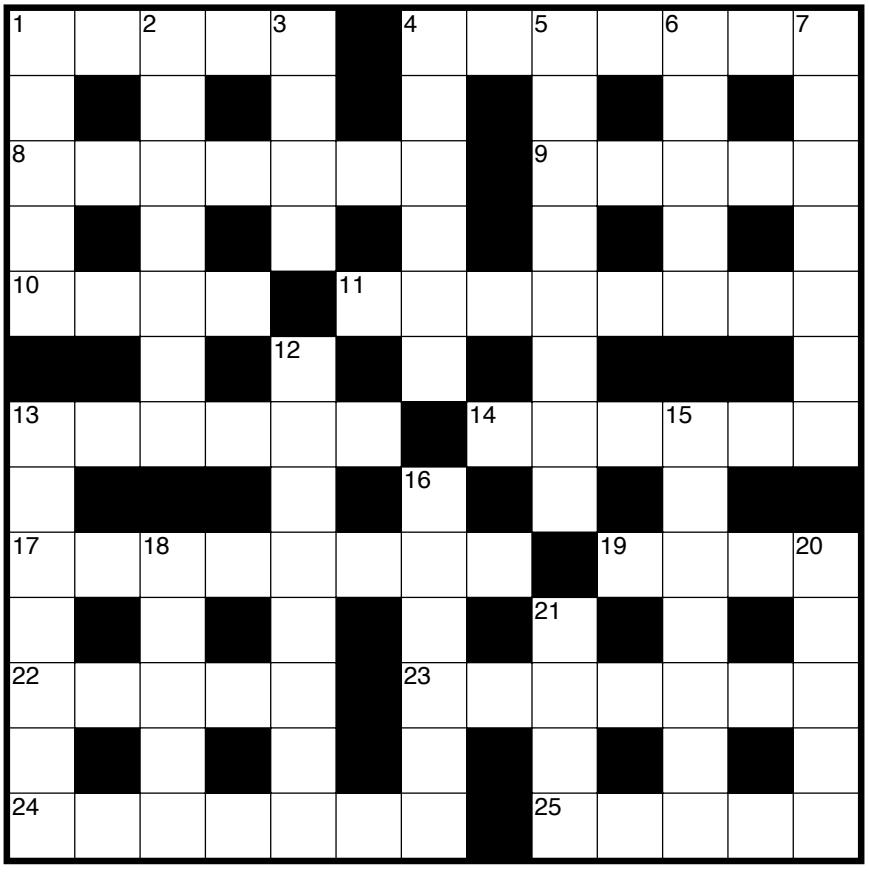
ANDRZEJ KRAUZE

Speaking of Science



Note: The answer grid will include every letter of the alphabet.

BY EMILY COX AND HENRY RATHVON

ACROSS

1. Student of optometry?
4. Movement through a membrane
8. Stinky mammal not really a feline
9. When a kiwi flies
10. Trees with some slippery members
11. Cyclotron inventor Ernest for whom element #103 is named
13. Firefly collection unit for a kid
14. Home tweet home?
17. See 13-Down
19. Viper's and vampire's point in common?
22. Flower cluster looking like a parasol
23. Underground rootstalk
24. Synthetic fiber a.k.a. Lycra
25. Palindromic craft of Inuit and Yupik

DOWN

1. Cocoon dwellers
2. Observatory operated by Caltech
3. What Scots call an inlet
4. Involving bones or skeletons
5. Growth in a brackish "swamp"
6. Number of human cervical vertebrae
7. Procedure once performed by barbers
12. Weasel, marten, mink, or wolverine
13. With 17-Across, marine explorer and pioneer of the Aqua-Lung (2 words)
15. Study of the structure of organisms and their parts
16. Array of numbers, as in a grid
18. Celestial shadow
20. Language of origin of "pterodactyl"
21. Hue of a salmon, often

Answer key on page 5

The organ looked like an overripe melon smashed by a sledgehammer, and was bleeding extensively. How could a gunshot wound have caused this much damage?

—**Heather Sher**, a radiologist in Broward County, Florida, writing in *The Atlantic* about treating victims from Marjory Stoneman Douglas High School who were shot with an AR-15 semiautomatic rifle (February 22)

Americans are more likely to die from a gunshot than from skin cancer or stomach cancer.

—Statistics published in ***The Week*** after the mass shooting at Marjory Stoneman Douglas High School in Florida, where 17 people, including children, were killed (February 15)

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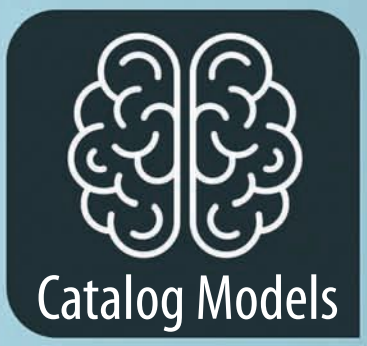

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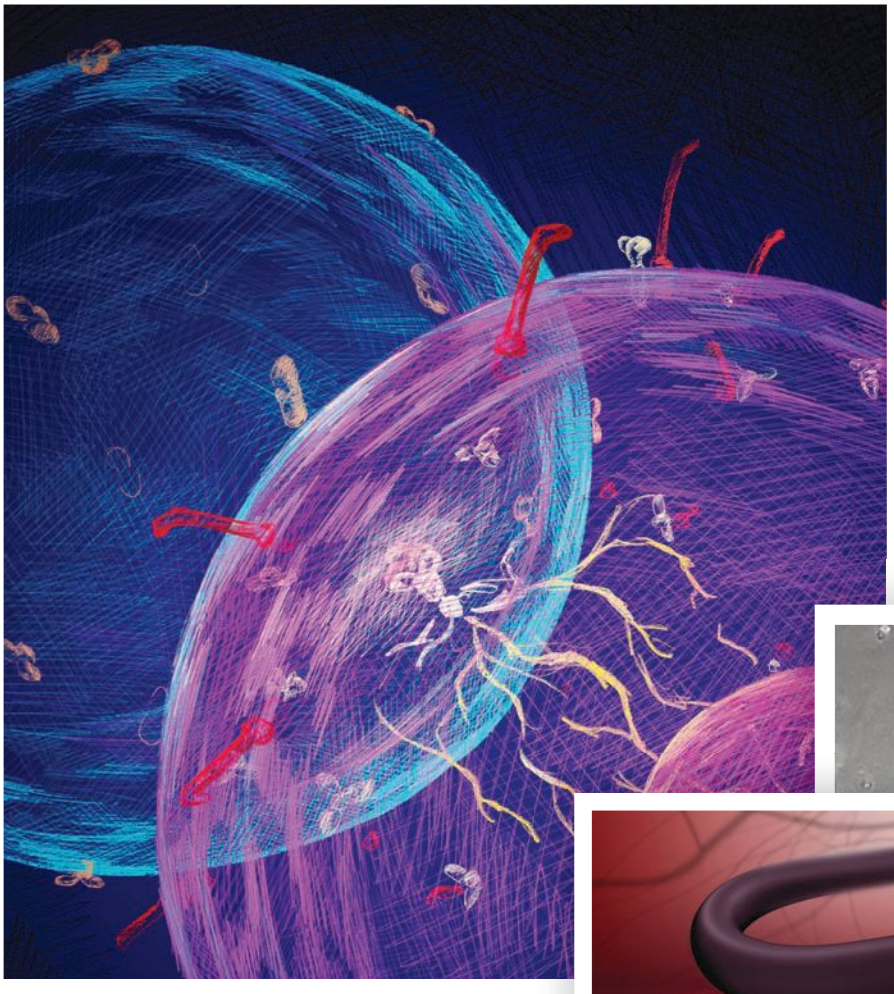
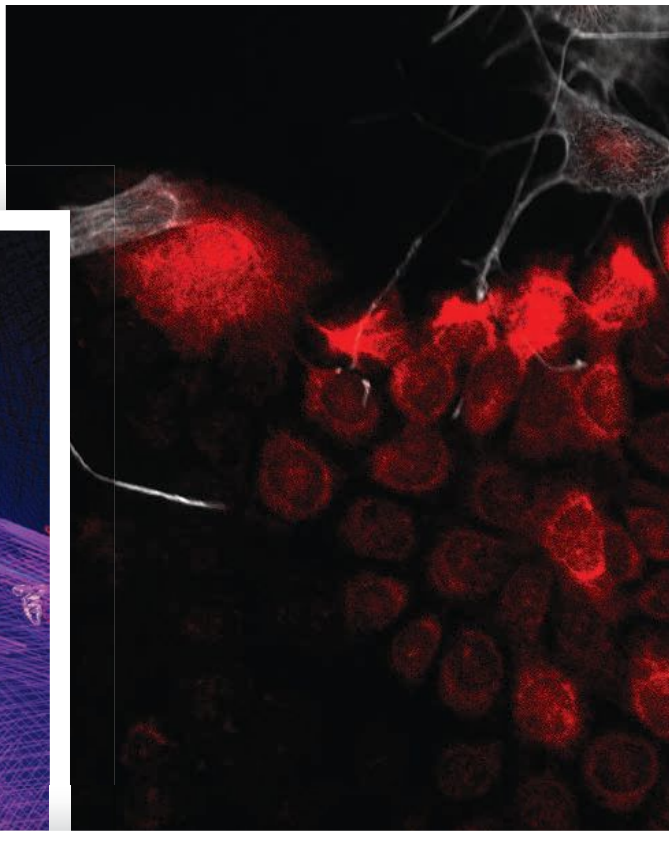
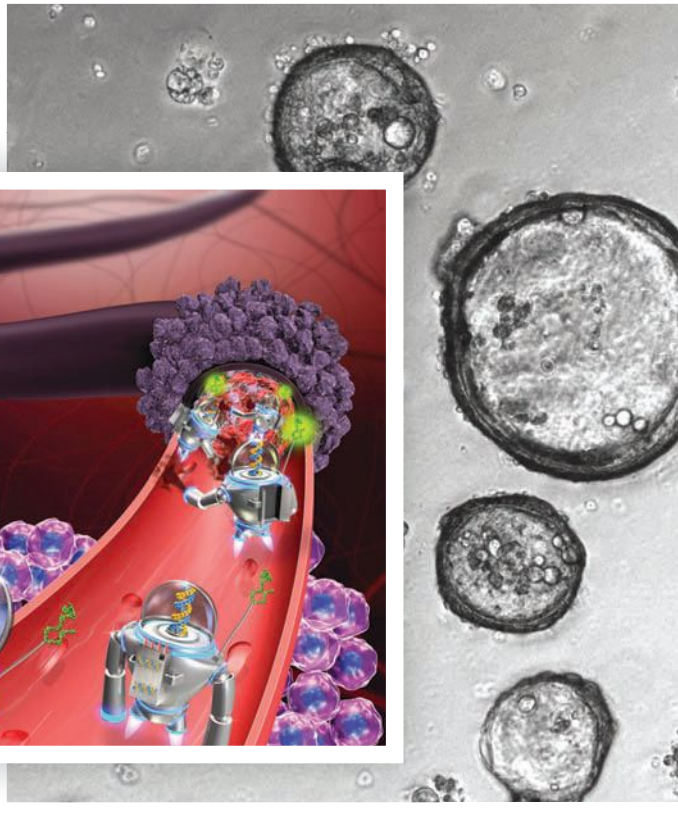
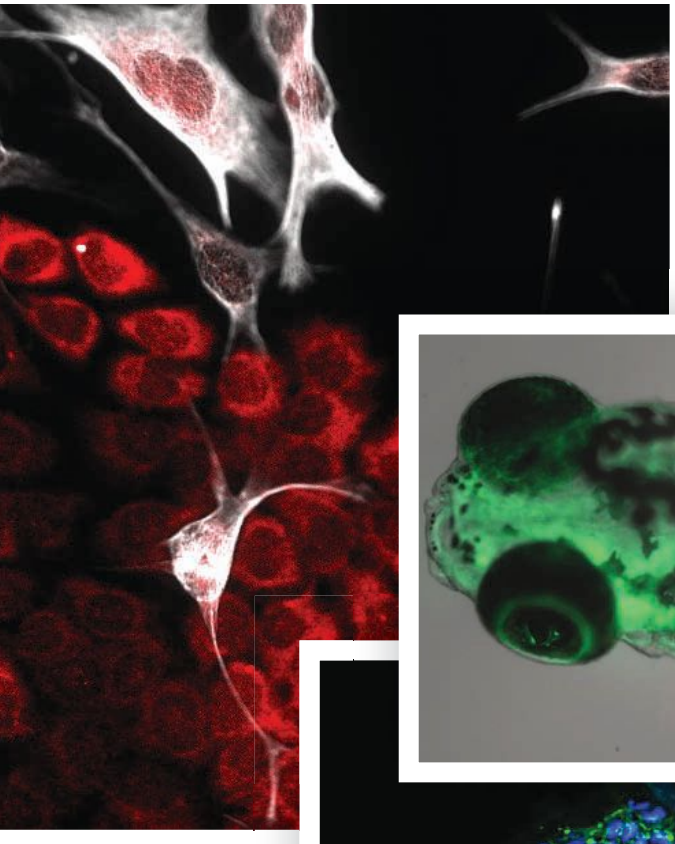






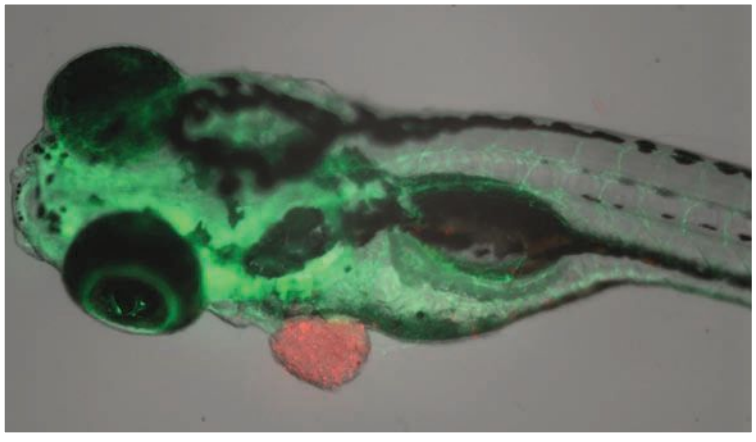
» ASSASSIN FOR HIRE
Researchers in Switzerland have made progress in designing non-immune cells that can target and attack tumor cells, as shown in this artist's depiction.
Posted: *November 13, 2017*

ROBODOCS »
Researchers use DNA origami to generate tiny mechanical devices that deliver a drug that cuts off the blood supply to tumors in mice.
Posted: *February 12, 2018*

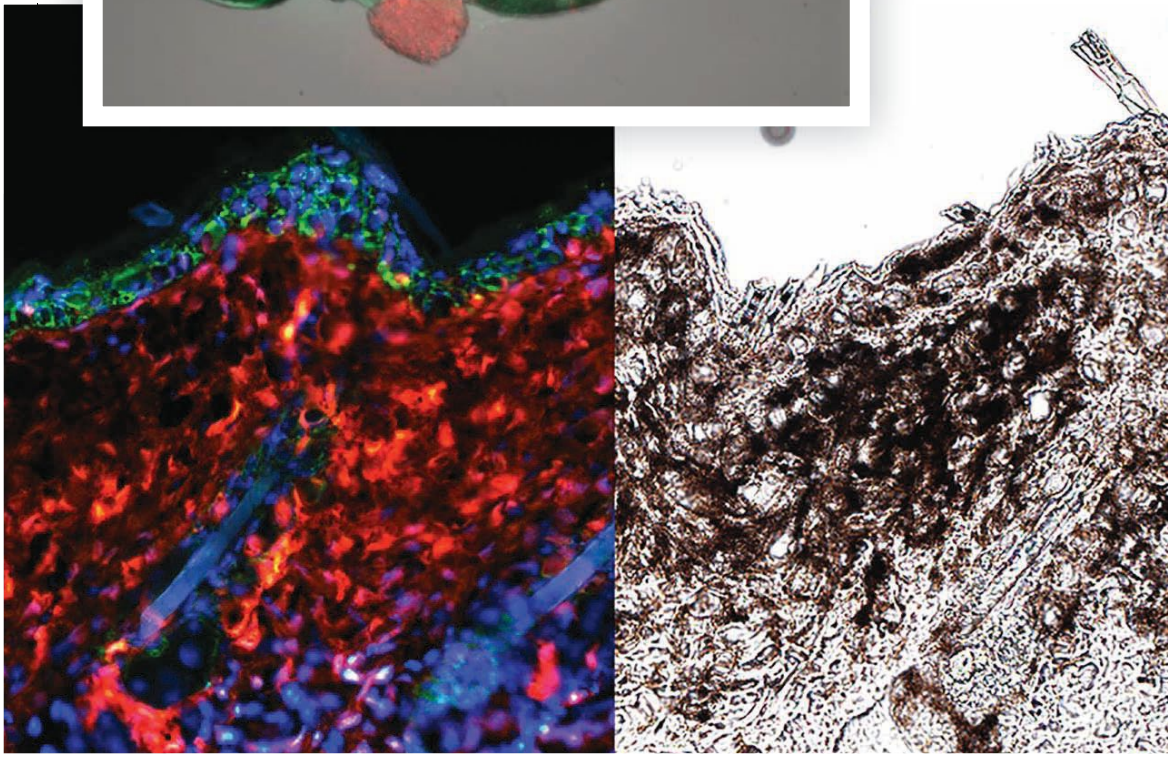


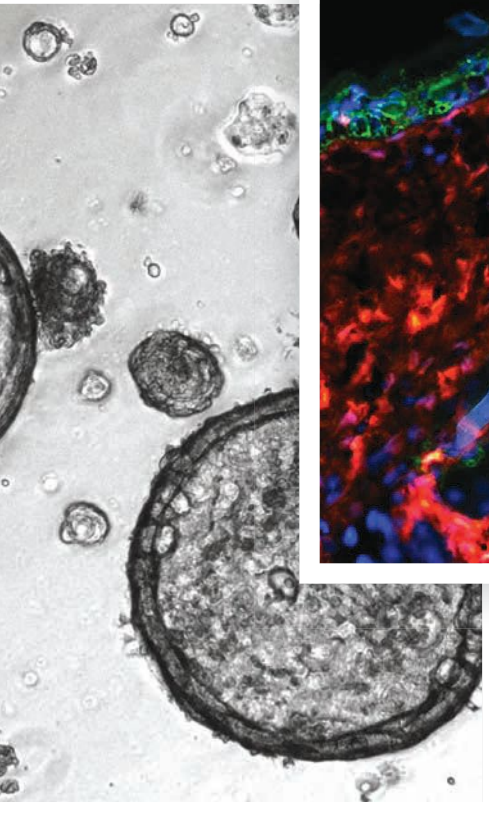
« MANIPULATIVE MELANOMAS
Early-stage melanoma cells (gray) alter proteins in nearby skin cells (red) to create a favorable environment for cancer progression.
Posted: *March 20, 2017*

» FISH AVATARS FOR CANCER
Zebrafish larvae transplanted with patients' tumors (red) respond as their human donors do to chemotherapy.
Posted: *September 11, 2017*



» SUNBURN
Melanoma (red and black on the left and right, respectively) in mouse melanocyte stem cells
Posted: *October 20, 2017*





« CANCER'S CRYSTAL BALLS
Testing treatments on mini tumors, such as these gastroesophageal cancer organoids, may save time in identifying which therapies work best.
Posted: *February 22, 2018*

Assassin for hire: Ryo Tachibana; **Robodocs:** Baoquan Ding and Hao Yan; **Manipulative Melanomas:** Department of Dermatology, University Hospital Erlangen, Translational Research Center, Germany; **Cancer's Crystal Balls:** George Vlachogiannis; **Fish Avatars for Cancer**: Rita Fior, Champalimaud Centre for the Unknown; **Sunburn:** Hyeongsun Moon and Andrew White, Cornell University

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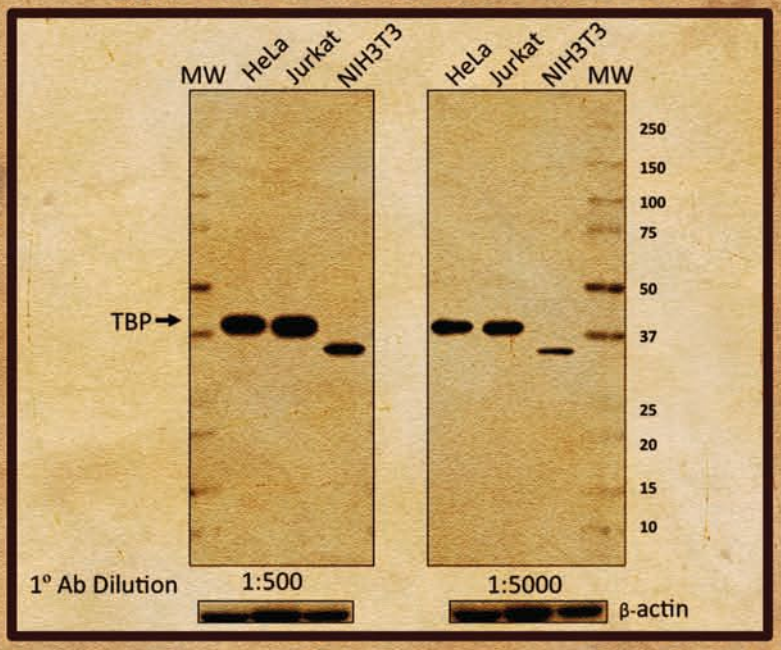

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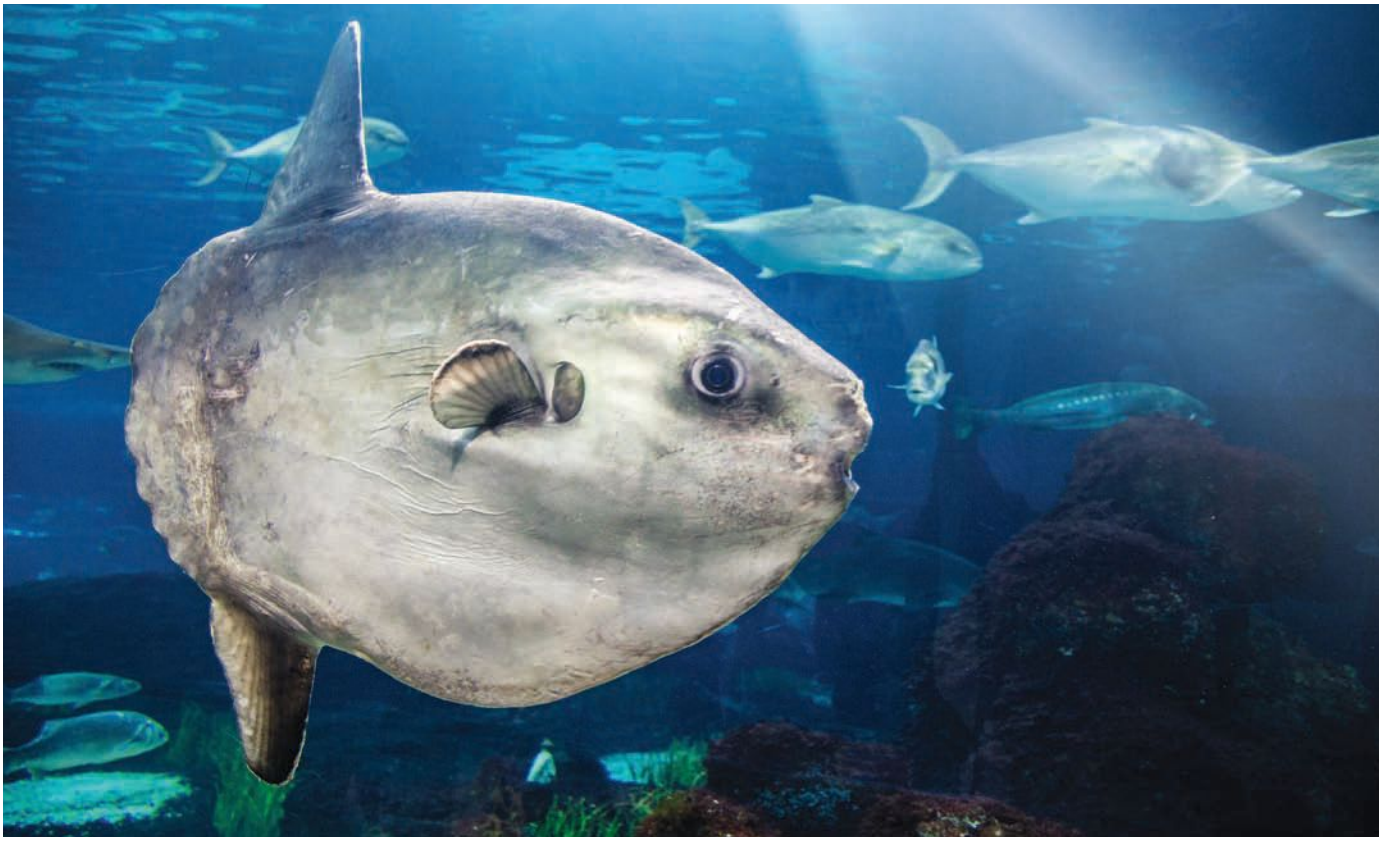

Slimy Seas

Off the coast of the French town Banyuls-sur-Mer, dozens of dorsal fins bob up and down in the water. A quick glance may suggest something menacing, but a longer look gives way to an even greater surprise: big, bizarre fish called *Mola mola*, from the Latin word for millstone. Commonly known as ocean sunfish, these behemoths tip the scales at up to 1,000 kilograms, making them one of the largest bony fishes alive today.

More intriguingly for the researchers who study them, some populations of ocean sunfish seem to be on the rise. "It's quite striking in the western Mediterranean. I was there diving last spring and there were so many sunfish," says David Grémillet, an expert on seabirds at the Center for Functional and Evolutionary Ecology in Montpellier, France, who has been fascinated with ocean sunfish since childhood. "People from the diving club there said that in recent years they had never seen that many."

Not much is known about these ocean giants. Strangely, given their massive size, sunfish appear to subsist on jellyfish—a relatively calorie-poor food. Although they're not highly sought after for human consumption compared with cod and salmon, their large, slow-moving bodies often get them nabbed as bycatch by fishermen. As a result, the International Union for Conservation of Nature has listed sunfish as vulnerable, making researchers keen to find out how many there are swimming slowly along in the world's oceans.

HOLY *MOLA*: The ocean sunfish can grow to more than two meters in length, with a mass of up to 1,000 kilograms.

To get a better estimate of the Mediterranean population, in 2011 and 2012 researchers from the University of La Rochelle took to the air in a slow, low-flying plane. Cruising about 180 meters above the water, at a speed of roughly 190 kilometers per hour, the surveyors could look down at the sea through bubble-like windows under the wings—though even then, to get good counts "you have to have really well-trained observers and a smooth sea," says Grémillet, who did not participate in the survey. All in all, the team spotted up to 475 ocean sunfish per 100 square kilometers—an order of magnitude higher than estimates for other seas.

When Grémillet saw the counts, he says he started to wonder how so many sunfish could be sustaining themselves in the relatively small confines of the Mediterranean, compared with the open ocean. So he turned to Craig White, an evolutionary physiologist at Monash University in Melbourne, Australia, for help running the numbers. "A hundred grams of milk chocolate contains a little over 500 calories, whereas a hundred grams of jellyfish contains less than one percent of that, around five calories," White tells *The Scientist* in an email. "In order to meet their daily energy requirements when consuming only jellyfish, sunfish must therefore consume very large amounts."

All the conditions are there for the rise of slime.

—David Grémillet, Center for Functional and Evolutionary Ecology

Last year, White and Grémillet calculated just how much jellyfish an average-size *Mola mola* spotted in the survey—that is, a 120-kilogram fish—would need to eat. The answer: more than half its body weight, or about 71 kilograms a day. Combining that information with population estimates of ocean sunfish from the aerial surveys, the researchers predicted that in the summer months the Mediterranean *Mola mola* population would eat roughly 20,774 tons of jellyfish a day (*Curr Biol*, 27:R1263–64, 2017). "That was really substantial," Grémillet tells *The Scientist*.

It was after calculating the ocean sunfish's jellyfish consumption that Grémillet realized that the team's approach might help resolve another ocean mystery, one linked to an idea dubbed "the rise of slime." This hypothesis, first posited by marine ecologist Jeremy Jackson and colleagues in 2001, suggests that due to a warming climate and overfishing, increasingly large numbers of jellyfish, along with algae blooms and other slimy blobs, will colonize the oceans (*Science*, 293:629-37). There are reasons to think this may already be happening in the Mediterranean, notes Grémillet. "Surface waters have been warming very quickly, and also there's a disappearance of lots of small fishes," he says. "All the conditions are there for the rise of slime."

This hypothesis has historically been difficult to test because jellyfish are difficult to see from the air, hindering large-scale population estimates. But sunfish, as the University of La Rochelle team had shown, are not. So in the paper that they published on the sunfish data at the end of last year, Grémillet and colleagues suggest that ocean sunfish might make good indicators of jellyfish populations, and, therefore, serve as a proxy for the rise of slime.

Natasha Phillips, a graduate student studying ocean sunfish at Queen's University Belfast, says the researchers have a "fascinating idea." But there's still a lot scientists don't know about *Mola mola*, such as whether or not the adult diet consists only of jellyfish. (In any case, the fish probably go right for the gonads; one of the cnidarians' most energy-rich parts, those are "caviar for ocean sunfish," Phillips says.) Recent research has shown that younger ocean sunfish have a more diverse diet, eating crustaceans and teleost fish as well as cnidarian species, so adults may, too (*Sci Rep*, 6:28762, 2016).

Tierney Thys, a marine biologist and *National Geographic* explorer, raises the same point. "Sunfish—both youngsters and adults—have a range of items that they eat—not just jellies. They are not obligatory jelly eaters," she writes in an email to *The Scientist*. What's more, jellyfish are "not a world of homogeneous slime, primed and poised to take over every ravaged ecosystem." Not all scientists are convinced that jellies will overrun warmed oceans, and as a group, Thys says, they are one of the most diverse animal phyla and a natural part of the ecosystem, so they are getting a bit of a bad rap. Still, she notes, it will certainly be important to monitor what is happening in the Mediterranean, specifically, identifying what the sunfish there are eating, which jellyfish species are there, and how both animals move and migrate.

Grémillet agrees that the team is far from proving that the abundance of ocean sunfish shows the rise-of-slime hypothesis to be correct. More aerial surveys of ocean sunfish are needed, which could happen this year. "We could repeat the counts and test the rise-of-slime hypothesis using sunfish," Grémillet says, explaining that an increase in sunfish numbers would likely indicate a corresponding increase in the fish's gelatinous foodstuff. "That's the whole idea."

—Ashley Yeager

Spermbots to the Rescue

A sperm's job is simple: Swim to an egg, and inject genetic material. The structure of a mammalian sperm cell reflects those basic functions, consisting primarily of a DNA-containing head and a rapidly beating tail. Recently, scientists at the Leibniz Institute for Solid State and Materials Research (IFW) Dresden in Germany decided to exploit this structure, plus these cells' natural inclination to travel through the female reproductive tract, for an unusual project. "We [thought], why not use these sperm cells as drug carriers?" says Mariana Medina-Sánchez, group leader of micro- and nanobiomedical engineering at IFW Dresden. The idea is less outlandish than it may sound. After all, "in the community [of researchers working on] micromotors or microswimmers, there were others using stem cells or bacteria to carry drugs."

Over the past decade, scientists and engineers have made significant steps toward realizing the vision of micro- and nanoscale robots that deliver therapeutics or aid diagnostics in the human body. Researchers now use both artificial and naturally occurring systems to create micromotors for biomedical purposes. The former includes those powered by chemicals, ultrasound, and magnetic fields, and the latter includes self-propelling motors, such as the flagella on bacteria or sperm. Some researchers

have even combined natural and artificial approaches—one of the earliest hybrid systems, for example, was created in 2000 by a group of researchers at Cornell University who tethered ATPase, an enzyme that spins as it catalyzes ATP, to a tiny metal propeller (*Science*, 24:1555-58).

Around five years ago, the researchers at IFW introduced their own system using sperm cells (*Adv Mater*, 25:6581-88, 2013). In the paper, the team proposed that sperm, paired with tiny, motorized harnesses that would give researchers control over where the cells swam, could be useful for a number of therapeutic applications, including assisted fertilization. A few years later, one of the study's coauthors, Oliver Schmidt, along with additional collaborators at IFW, successfully used the system—remotely controlled with magnetic fields—to lead immotile sperm to oocytes in an artificial fluidic channel that mimicked some of the physiological conditions in the female reproductive tract (*Nano Lett*, 16:555-61, 2016). "The idea was to counter one of the male infertility problems, which is low sperm count," says Medina-Sánchez. "It was to help these few sperm cells to reach the oocyte by coupling them with a magnetic harness."

This work set the stage for a second set of experiments, in which Medina-Sánchez and her colleagues decided to test their artificially motorized sperm cells as drug delivery systems for cervical cancer and other gynecological diseases—a job for which sperm are naturally equipped. In addition to being excellent swimmers, Medina-Sánchez says, the cells have a limited lifetime and don't proliferate and form colonies like bacteria. They also have the ability to fuse with somatic cells, which, she adds, is a big plus "because the sperm can fuse with cancer cells and deliver the drug inside them."

First, the team tested the sperm's ability to fight cancer cells by loading bovine sperm heads with doxorubicin hydrochloride, a chemotherapy drug, and placing them in a dish with cervical cancer cells cultured into spheroids that served as 3-D tumor models. The drug-loaded sperm, they found, were efficient killers: after 72 hours of treatment, they had destroyed almost 90 percent of the tumor cells (*ACS Nano*, 12:327-37, 2018).

Then, to test whether drug-loaded bovine sperm could be steered toward a target, the team coupled single cells with tetrapod microstructures—iron-coated casings with tubular bodies and four arms, capable of being steered by a magnetic field. "The sperm can swim into the tetrapod and propel it forward," says Haifeng Xu, a PhD student at IFW. "By coating a magnetic layer on the tetrapod, we can guide the micromotor to the target."

Medina-Sánchez, Xu, and their colleagues tested the ability of the bionic sperm to swim through microfluidic channels to a cancer spheroid. Although the tetrapods slowed the sperm's swimming speed by around 43 percent, the researchers were able to successfully guide the hybrids toward the cancer cells using rotating magnets located approximately 10 centimeters away from the sample. When the micromotors reached the spheroid, a mechanical trigger released the sperm from the tetrapods, allowing them to fuse with the cancer cells and deliver the drug—and

We thought, why not use these sperm cells as drug carriers?

—Mariana Medina-Sánchez, IFW Dresden

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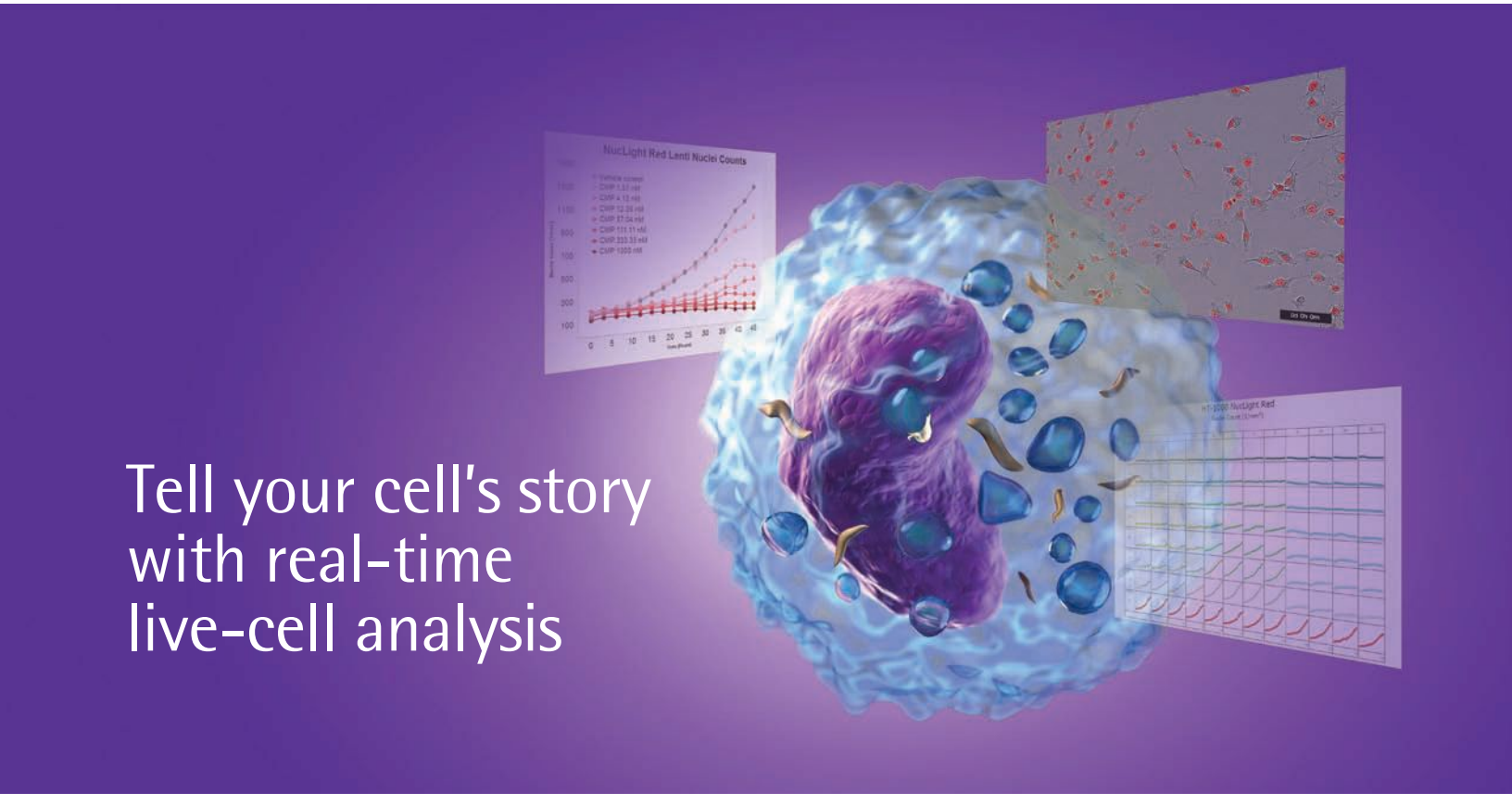

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after approximately eight hours, those cells had shrunk by around 40 percent.

"I was excited to see the work—I think it's a really nice next step, but we still have a lot more to do," Bradley Nelson, a professor of micro- and nanorobotics at ETH Zürich in Switzerland who didn't take part in this work, tells *The Scientist*. "Right now, you have to be able to know where the tumor cell is so you can steer the thing there. . . . Ultimately, you'd like it to be autonomous."

But while sperm have adapted to swim in the female reproductive tract, they may not necessarily be the optimal delivery vehicle to reach tumors, says Sylvain Martel, a nanorobotics researcher at Polytechnique Montréal in Canada. Unlike sperm, some bacteria possess the ability to sense low oxygen levels or fluctuations in pH, two properties found in actively dividing clusters of cancer cells. Martel and his colleagues previously reported a method using magneto-aerotactic bacteria—which, in their natural environment, swim along magnetic field lines toward areas with low-oxygen concentrations—to transport drug-loaded liposomes to tumors in mice (*Nat Nanotechnol*, 11:941-47, 2016).

Another important future consideration in using sperm as delivery vehicles will be how to avoid accidentally fertilizing an egg in the process of treating a patient, particularly because, according to Medina-Sánchez, sperm from humans would be better suited than those of other species to treat cancers in people. "We have thought about [this issue]," she says. "We believe that these treatments can be done, for example, when the woman is not ovulating."

There's still a long way to go before these sperm-driven hybrid micromotors will be tested in humans—to date, the system has only been investigated in vitro. Still, "this is a very good example of where hybrids are going," Martel says. "Right now, I'm not sure that the future for treating cancers in the reproductive tract will be this system, but I think it's an important step."

—Diana Kwon

Ant Acid

In a petri dish, three *Lasius neglectus* worker ants surround a cocooned pupa from their own colony's brood. Tearing into it with their mandibles, the ants remove the pupa from the cocoon, perforate its cuticle, and rip its body apart, dividing it between them. Finally, the workers apply formic acid from their mouths to the eviscerated pupa, leaving behind a heap of crumpled remains.

This is no random act of violence. According to Chris Pull, an evolutionary biologist at Royal Holloway, University of London, these ants were engaging in destructive disinfection, killing the pupa along with the ant-killing fungus, *Metarhizium brunneum*, that had infected it. The

BUG SPRAY: Worker ants tear apart and spray acid on a pupa in an act of destructive disinfection.

behavior evolved in social insects such as ants, honeybees, and termites to protect colonies from infected individuals, says Pull, with workers acting like immune cells called leukocytes that seek out and destroy infectious agents in mammalian bodies.

But just how the ants know which juveniles to kill, and which to spare, has been tricky to nail down. "We have all long wondered how ants can be so effective at their hygienic behavior," says Simon Tragust, a zoologist at Martin Luther University in Germany. Tragust researches how ants use antimicrobial secretions such as formic acid as a sort of disinfectant for the colony. Separately from Pull's group, he has reported finding *L. neglectus* worker ants grooming and spreading formic acid orally on pupae inoculated with *Metarhizium*, but he didn't identify how workers were choosing their targets (*Curr Biol* 23:76-82, 2013).

In 2012, as part of a PhD at the Institute of Science and Technology, Austria, Pull, along with graduate advisor and evolutionary biologist Sylvia Cremer, set out to get to the bottom of the mystery. After collecting thousands of ants, along with their queens and brood, from a supercolony of *L. neglectus* residing in northeastern Spain, Pull, Cremer, and colleagues exposed ant pupae in the lab to one of three dosages of fungal spores, or to water as a control. Then, they dropped worker ants into petri dishes with the pupae, and filmed their behavior. The ants were ruthless and effective: they reliably unpacked and disinfected pupae that had been exposed to the pathogen, while mostly leaving controls alone. "I was shocked by how efficient they were at preventing the fungus from growing," Pull says.

> I was shocked by how efficient they were.
>
> —Chris Pull, Royal Holloway, University of London

The team also noticed that workers were destroying infected pupae while the fungal infection was still in its incubation period, before it had become visible or contagious. Something other than the infectious agent was telling the workers that the ants were sick. Pull and his colleagues knew that ants communicate with their nestmates via chemical compounds called cuticular hydrocarbons (CHCs), and suspected that the infected pupae might be signaling workers in this manner.

To test the hypothesis, the researchers washed some of the infected pupae with a solvent to remove CHCs. Presented with these pupae, the worker ants carried out their disinfection routine 72 percent less often than when they were given infected pupae that were unwashed or had been rinsed with water. The researchers then used gas chromatography to confirm that infected pupae that hadn't been solvent treated had a unique chemical profile on their cuticles—a kind of "find-me/eat-me" signal, Pull notes, functionally similar to those released by apoptotic cells to attract phagocytic immune cells in the human body (*eLife*, 7:e32073, 2018).

While this sickness cue usually leads to the death of the infected individual by stimulating disinfection behavior in workers, it protects the rest of the colony, including egg-producing queens, from fatal infection, Pull says. "[The sick ants] are performing these behaviors and putting themselves at risk, but at the end of the day it's still to maximize genes which they carry, to ensure that genes they carry are being passed on to the next generation."

Provided the colony's queen survives to pass on her genes, the evolutionary fitness of every individual is maximized, explains Cremer. "In systems like this, selection acts on the level of the reproductive entity," favoring the evolution of collective defenses or "social immunity." The kind of altruistic chemical cues discovered by the team "could be very widespread" among social insects, Cremer adds. The CHCs that make up the "disinfect-me" signal for *L. neglectus* are upregulated not only in ants during fungal infection, but also in honeybees—which have similar colony dynamics—after viral infection or when injected with bits of bacterial cells, she notes.

Laurent Keller, a myrmecologist at the University of Lausanne who was not involved in the study, says the kind of altruism shown by the infected pupae in this study is not unlike that of a honeybee who will "sting another organism and give its life for the colony." But not all actions in social insect colonies are completely altruistic, he observes. He adds that he would like to hear more from Pull's team about how the signaling behavior initially began to evolve in ants. "That could be quite interesting," he says. "I think it's not an easy question."

Pull says he's now interested in examining destructive disinfection behaviors at the colony level. "Most of these experiments on disease defense in social insects are done in the lab, and we don't have a good idea of how they work in a whole colony," he says. He's curious to discover whether the speed of disinfection is linked to overall colony success, for example. "It would be really cool to look at how these behaviors scale up when you have a whole colony present."

—Jim Daley

CHRIS PULL

The Enemy Within

There are cancers with mutated genes, and then there's hypodiploid acute lymphoblastic leukemia (ALL). This rare subtype of ALL, a childhood leukemia, is characterized by deletions of whole chromosomes—and worse survival rates than other subtypes. More than 90 percent of ALL patients, but fewer than half of pediatric hypodiploid ALL patients, survive with treatment. In 2013, researchers at St. Jude Children's Research Hospital and colleagues looked into whether there was anything distinctive about the gene variants carried by patients with hypodiploid leukemia. Within a cohort they examined, they found, first, that the subtype itself has two subtypes; and then, in one of those, called low hypodiploid ALL, 91 percent of patients carried certain variants of the *TP53* gene, which codes for the tumor-suppressing protein p53 (*Nat Genetics*, 45:242-52, 2013).

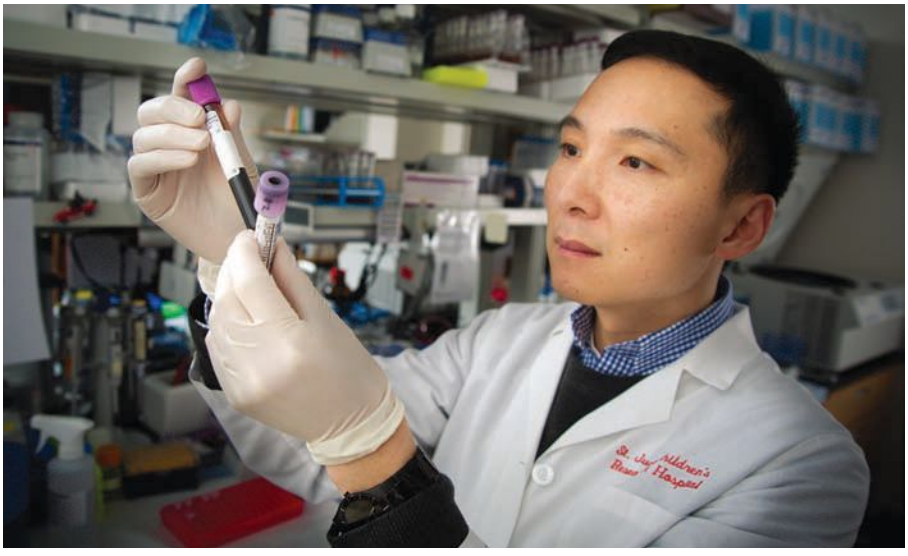
The finding made Jun Yang, a genetics researcher at St. Jude who wasn't involved in that study, wonder whether germline mutations in *TP53* might be implicated in more-common types of childhood leukemia too. To find out, he and colleagues combed through data from two clinical trials on 3,801 children with ALL. For each child, the researchers sequenced the coding regions of *TP53*; to find novel variants, they dug deeper by testing the alleles' effects on the transcriptional activity of downstream genes in vitro, and by using a computer model to predict whether the variants might be pathogenic.

Children who'd been diagnosed with ALL were five times more likely to carry at least one copy of a putatively pathogenic *TP53* mutation than were healthy controls, the researchers found. And among the children who'd had ALL, those with a pathogenic variant had a one in four chance of developing a second cancer in the five years after they went into remission—while those without such a variant had a greater than 99 percent chance of remaining cancer-free over that time (*J Clin Oncol*, 36:591-99, 2018).

Many families are very thankful for the possibility of cancer screening.

—Kim Nichols, St. Jude Children's Hospital

The team's results reinforce findings on *TP53* germline mutations that stretch back to the description of a rare, cancer-predisposing disorder known as Li-Fraumeni Syndrome (LFS) in 1969, says David Malkin, a pediatric oncologist at The Hospital for Sick Children and the University of Toronto who was not involved in the study but has collaborated with one of its authors. People with hereditary LFS are prone to develop a variety of cancers, often while relatively young; the syndrome has been linked to *TP53* mutations in some families. But Malkin contends the new study stops short of proving *TP53* mutations can cause ALL;



ST. JUDE/SETH DIXON

BIG SCREEN: Cancer researcher Jun Yang of St. Jude Children's Research Hospital and his colleagues combed through data on 3,801 children with ALL.

to do that, he tells *The Scientist*, researchers would need to show that the leukemia cells of patients with the mutations express the bad copy of *TP53*, and not the good one.

Yang says that the results suggest that physicians should consider alternative courses of action for cancer patients who carry such a mutation, particularly when it comes to administering potentially DNA-damaging therapies. "I think it will be important to think twice before you give radiation, especially total body radiation, to these children with *TP53* variants. You might have to explore other, less genotoxic therapy," he says. What's more, once the cancer goes into remission, these patients could be monitored closely in order to catch any second cancer early, he adds.

As it turns out, St. Jude began a program in 2014 to capitalize on knowledge about genetics to improve care for children with cancer. Every cancer patient at the hospital is offered a consultation at the institution's genetics clinic, explains the clinic director, Kim Nichols, who was a coauthor on the new paper. Those patients whose physical exam or family history indicates cancer predisposition variants are offered genetic testing. The testing offers peace of mind for families whose children's results come back negative for such variants, she says—only about one in 10 pediatric cancer patients carries a known predisposition variant. But for the families of those who do, "it's very difficult, because the molecular testing makes it a reality and no longer just a possibility," she says. Knowing he or she is at a relatively high risk of future cancers is "a reality that the child is going to need to live with lifelong."

Nichols concedes it's not always possible to avoid therapies that could raise the risk of a second cancer for patients with predisposition variants. "Sometimes you just have to do what you have to do to take care of the first cancer," she says. "My hope in the future is that by understanding how these predisposition genes do what they do, how mutations affect the function of the encoded protein, we can develop targeted therapies" that aren't as damaging.

In the meantime, doctors can take advantage of the knowledge that a patient has a predisposition gene by implementing an aggressive screening program for second cancers. "If you monitor [a patient with a predisposition variant], you're not going to prevent a second cancer from occurring, but if you pick it up earlier, it's so much more treatable," Nichols says. "The surgeries are usually easier, [and] oftentimes you may not need to use chemotherapy, or you can use less-intense chemotherapy." Depending on the gene variant, patients whose first cancer has gone into remission may need to come back every 3-12 months for these screens. In one study, led by Malkin, people carrying known *TP53* pathogenic mutations who opted for surveillance and were later diagnosed with cancer had an almost 90 percent five-year survival rate, while those who developed cancer after opting out of surveillance had a lower than 60 percent survival rate (*Lancet Oncol*, 17:1295-1305, 2016).

But much remains to be done on the basic-science front to better understand how to help people who carry predisposition variants. For example, Yang's study turned up 27 variants of unknown pathogenic significance in the *TP53* genes of ALL patients; his team plans to investigate their biological consequences. He also hopes to identify therapies that could improve the outcome for patients with *TP53* variants. The researchers also reported that many of the *TP53* germline mutations were found in those members of the cohort with low hypodiploid ALL—but questions remain, notes cancer genetics researcher Sabine Topka of Memorial Sloan Kettering Cancer Center (who was not involved in the study), including: "What is the significance of this very strong association of *TP53* variants and the hypodiploid phenotype of ALL? Is it because of the genomic instability that this hypodiploid phenotype arises, or is it a different causal relationship? That is not really clear."

Despite such uncertainties, families who find out one or more of their members carries a predisposition gene don't tend to succumb to fatalism, Nichols says. "My experience has been that families get over that initial distress, and many in the end find it empowering. They can now use this information to change what they're doing at home . . . [and] really try to make sure the family lives a healthy lifestyle. Many families are very thankful for the possibility of cancer screening."

—Shawna Williams

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TheScientist

Training Tomorrow's Bioinformaticians

With limited access to datasets, educating the next crop of biostatistical wizards is a steep uphill climb.

BY DAVID W. CRAIG

Precision medicine is founded on the premise of individualized medical decisions, practices, and treatments tailored to the unique genetic, epigenetic, proteomic, and clinical profiles of patients. Powered by next-generation sequencing technologies, the past five years have seen a burgeoning of patient data; just one of Illumina's NovaSeq machines, running two to three times a week, could conceivably generate a half trillion bases of sequencing data per year.

Yet for all the data science can produce, it is sorely lacking in the brainpower to analyze the information so it can be put to use. In particular, what are missing are master's-level scientists who could fill the massive skills gap that limits the field's ability to make new biomedical discoveries and translate them from the laboratory to the bedside.

Take, for example, those half trillion bases at our disposal. Excel is unable to open files larger than 1 million lines, and that tried-and-true spreadsheet software is the technological limit of many newly minted PhDs and postdocs when it comes to analyzing data. Or researchers may wish to merge public data with their own, a rather rudimentary task that can be challenging for experimentalists, most of whom are not trained in command-line environments. What happens, I have observed, is that trainees emerge from their studies able to differentiate complex calculus, but unable to complete the most basic biomedical data analyses.

While there are programs out there aiming to build up a workforce of bioinformaticians, the lack of educational resources is limiting the breadth of their training.

While there are programs out there aiming to build up a workforce of bioinformaticians, the lack of educational resources is limiting the breadth of their training.

Science needs more bioinformaticians

Discoveries don't tend to emerge from large datasets without complex analysis. Thus, the bioinformatician has become one of the most valued members of laboratories across academia, healthcare, and industry. And nowhere is the need more acute than within biomedical research.

I have spent the past decade leading undergrad and graduate research in a "damp laboratory"—a little bit dry lab and little bit wet lab—at the Keck School of Medicine of the University of Southern California (USC). My group melds molecular biology and bioinformatics to develop platforms for personalized medicine, and next-generation sequencing data management, analysis, and clinical genomic interpretation across several fields, including cancer and rare diseases. Ideally, each member of the group would be able to form a hypothesis, conduct an experiment, and do basic analysis, so that insights can occur quickly, without their significance getting lost in translation. But it's been difficult; I tried hiring PhDs in computer and data science, for instance, only to realize they lacked the

tremendous value that comes from several years of experience at the bench.

What I learned is that it is much easier to teach a biologist command-line programs such as BASH and statistical scripting languages such as R, and this can be accomplished in just a year or two with a handful of classes. These individuals understand the biological problems and how to apply the informatics solutions using or integrating existing tools. Such well-trained life scientists would be invaluable to any number of biomedical research labs. So why are these people so hard to come by?

With an estimated 183,000 life-science graduates competing for just 12,000 jobs in 2016, it's a Darwinian struggle for survival as a bachelor's-level biologist. Even those who are lucky enough to land lab jobs quickly reach a glass ceiling and might be better off as a barista, with average salaries for research assistants with bachelor's degrees hovering around $30,647, according to Glassdoor. As has been reported often, there is also a glut of life-science PhDs and postdocs.

The challenge of training the bioinformatics workforce

At USC, we are attempting to address the problem through a new master's degree program in translational biomedical informatics. One of its main objectives is to train those who are transitioning from the bench to the dry lab in academic, clinical, and pharmaceutical research settings. We want to provide students with practical and foundational skills in molecular biology, systems biology, structural biology, proteomics, genomic sequencing, and genomic tools and datasets. We hope they will leave the program able to implement, develop, and design analytical solutions for different health care applications, from prototyping to production. This also involves elements of project management, communication, and collaboration with computational and engineering colleagues.

However, we've come across an unexpected hurdle: a dearth of the data we need to train these students.

Aspiring healthcare bioinformaticians need to become familiar with the types of datasets they will be presented with in the labs in which they will work. But data from diseased patients is extremely hard to access for training purposes.

We want to provide students with practical and foundational skills in molecular biology, systems biology, structural biology, proteomics, genomic sequencing, and genomic tools and datasets.

Studying samples from healthy controls without a disease phenotype is no substitute for data on real conditions from actual patients; it would be like learning anatomy without a cadaver. A cancer cell looks nothing like its healthy counterpart, and neither do its genomic data. There may be chromosome deletions and duplications, swapped regions, modifications to methylation and expression, or integration of viruses such as HPV.

One of the primary obstacles limiting access to such substantive data is consent. Most trial protocols are not broad enough to include educational use. They specify research, and many resources, such as dbGAP and NIH Commons, even limit data use to lab staff under direct supervision of a PI.

There are exceptions, such as the Personal Genome Project (PGP), but they are few. The Texas Cancer Research Biobank (TCRB) Open Access Database is another promising example where specific efforts are being made to obtain consent from individuals with the goal of PGP-type open access, but within the context of relevant disease tissues. We need more.

At USC's Department of Translational Genomics, we are focused on ensuring that, as a major priority in studies where we seek consent from study participants, we are able to teach students using real data from studies of disease. It starts with basic scientists thinking about this in advance—something that, fortuitously, I had been considering even before leading the master's program at USC. For instance, we have been able to leverage our work publishing a melanoma line as a potential standard reference line for cancer, COLO-829. The detailed data from an analysis of single nucleotide polymorphisms, indels, structural variants, copy number variations, and transcriptomics are now incredible resources for our students. Another example is a series of synthetic fusions developed to validate our clinical RNA-seq pipeline that we published as an open-access resource for clinical validation. Now that I am at an educational institution, I'm thankful we put that out as a resource. Still, synthetic samples and a single cell line are only a starting point.

Much of the debate about data sharing has focused on the identifiability of genomic data and balancing privacy risks within the research community, leaving education as an afterthought. Let's reframe the conversation.

Because of the acute need for bioinformaticians now, we have not been focusing on the future. But we cannot neglect the need to build better training programs, incorporating real-world case studies using real data. We need to share primary data for educational use, and create broader consent protocols.

In 2011, Eric Green, the director of the National Human Genome Research Institute, wrote: "It is time to get serious about genomics education for all health care professionals."

It is time to get serious about providing the materials and the ability to train as well. ■

David W. Craig is Professor of Translational Genomics and Co-Director of the Institute of Translational Genomics at the Keck School of Medicine of the University of Southern California.

A Microfluidic Gizmo for Analyzing Pee

This device uses anchored nanowires to capture exosomes from urine for microRNA analysis.

BY RUTH WILLIAMS

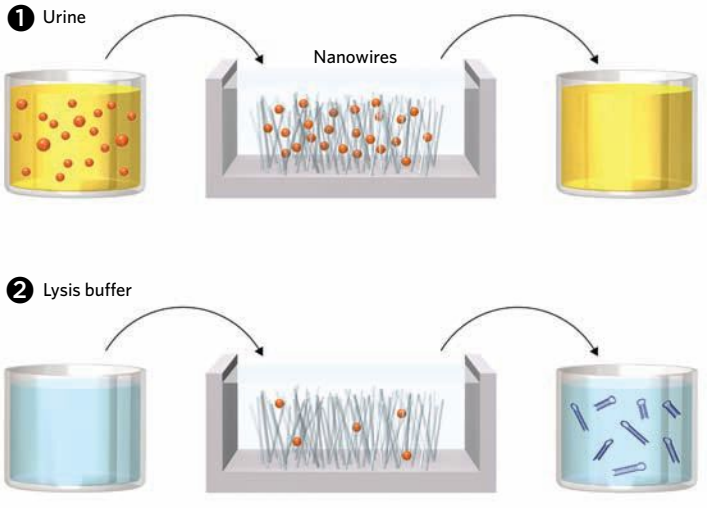
Exosomes are tiny membrane-bound packages that are released from practically every cell type and found in a wide range of body fluids. Containing RNAs, proteins, and other cell components, they are believed to be involved in communication between cells, and there's evidence that their abundance and content may change with disease state. Consequently, there is a growing interest in collecting and analyzing these vesicles for diagnostic purposes. Researchers who are interested in the diagnostic potential of microRNAs, for example, are especially keen to collect exosomes because the RNAs they contain degrade more slowly than free-floating RNAs.

"They are packed with important information," says Kai Wang of the Institute for Systems Biology in Seattle. But the problem is, "we actually don't have a good way to isolate them." This limits both basic research on exosomes and their clinical use, he explains.

The most commonly used method for extracting exosomes from body fluids is ultracentrifugation. But this requires large volumes and yields only small quantities, explains Johanna DiStefano of the Translational Genomics Research Institute (TGen) in Phoenix. A new device, developed by Takao Yasui of Nagoya University in Japan and colleagues, instead uses zinc oxide nanowires to isolate the vesicles. "Not only do they use a much lower volume . . . but they're getting a much higher exosome collection from that smaller volume," says DiStefano, who was not involved in the work. "It outperforms current methods."

The nanowires are approximately 100 nm wide and 2,000 nm long, and are held in place in a microfluidic chamber by a silicon-based organic polymer. The wires create a large, positively charged surface area, says Yasui, which the group hypothesized would be "a powerful tool" for collecting negatively charged exosomes.

They were right. Passing just one ml of urine through the device, followed by one ml of lysis buffer, enabled the team to collect and sequence small RNAs that yielded approximately threefold more microRNA species than the amount obtained by ultracentrifugation of 20 times more urine.



PEE IN, MICRORNAs OUT: 1 A small volume of urine is introduced into the microfluidic device, where positively charged zinc oxide nanowires attract and bind negatively charged exosomes. 2 Lysis buffer is introduced to the device to break open the exosomes and free the microRNAs, which are then collected for sequence analysis.

The team went on to analyze exosomes from healthy human subjects and patients with various cancers, and found differences in microRNA profiles. More tests will be needed to find out whether these differences are reproducible and informative, says DiStefano, but she adds the study is "a step in the right direction." (*Sci Adv,* 3:e1701133, 2017) ■

AT A GLANCE

EXOSOME EXTRACTION TECHNIQUE	MATERIALS COLLECTED	SAMPLE VOLUME	SAMPLE PROCESSING TIME	MICRORNA SPECIES IDENTIFIED
Ultracentrifugation	Exosomes	20 ml	300 mins	261 on average
Nanowire microfluidic device	Exosomes, microvesicles, and free-floating microRNAs	1 ml	40 mins	894 on average

Make Me a Match

Multidrug combinations for cancer are proving more effective than single drug therapies, but identifying promising pairings remains a challenge.

BY ANNA AZVOLINSKY

At the annual American Society of Clinical Oncology meeting last June, Bristol-Myers Squibb (BMS) researchers presented data on a cohort of patients not responding to the company's approved checkpoint inhibitor nivolumab (Opdivo). Layering on a novel immunotherapy antibody was effective in half of patients tested, the team reported, with no major increase in side effects compared to nivolumab alone.[1] Each of the therapies aims to unleash an immune cell–fueled tumor attack by targeting a molecule that normally suppresses T-cell activation—programmed death 1 (PD-1) in the case of nivolumab, and lymphocyte-activation gene 3 (LAG-3) in the case of the new, investigational antibody. The combination worked particularly well in patients whose T cells displayed LAG-3 on their surface. "We now have a population that we can sensitize to immunotherapy that was resistant to anti-PD-1 treatment," says Nils Lonberg, who heads the immune oncology and targeted drug discovery efforts at BMS in Redwood City, CA.

BMS has several newer checkpoint inhibitors, targeting other immune pathways, that trigger T cells to home in on tumors, and company researchers are accumulating data on combining each with nivolumab. "We focus on both innate and acquired immunity pathways to treat more patients with tumors we know can respond to immunotherapy, and also to open up other cancer types to immunotherapy," says Lonberg. "From basic-science principles, what we look for first in a combination are drugs with nonredundant mechanisms."

Other companies, including those with their own FDA-approved checkpoint inhibitors, such as AstraZeneca, Merck, and Roche, are taking similar approaches. The US Food and Drug Administration (FDA) approved the first checkpoint inhibitor antibody—ipilimumab (Yervoy), which targets cytotoxic T-lymphocyte antigen 4 (CTLA-4)—for advanced melanoma in 2011. Five other checkpoint inhibitor antibodies followed—six in total—targeting the PD-1 pathway for numerous cancer types. In 2015, the first and thus far only FDA-approved combination of two immuno-

therapies hit the US market: nivolumab plus ipilimumab for metastatic melanoma patients.

Combining multiple treatments for patients with recalcitrant cancers is not a new concept. Among the first pairings of anticancer drugs were two or more different chemotherapies. As drug companies developed additional types of cancer drugs, combinations of different modalities—including chemotherapy, radiation, targeted small molecules, and eventually immunotherapies—followed. (See illustration on page 36.) "There has long been a feeling that drug combinations will be needed to have the type of impact in cancer patient care that we would like to see," says David Hyman, a medical oncologist who specializes in early drug development at the Memorial Sloan Kettering Cancer Center in New York City.

But only a handful of cancer drug combos have so far been approved by the FDA, in part because many of the tested combinations were conceived largely at random—an inefficient approach given the dizzying number of approved and investigational therapies that could be combined. In fact, of the hundreds or even thousands of novel combos currently in clinical trials, many, if not most, were designed based on little more than convenience, depending on what drugs a company owns, says Charles Swanton, a cancer geneticist at The Francis Crick Institute in London. "My view is that there are too many trials, especially immunotherapy ones, being conducted in a serendipitous manner," he says. "It's more about, 'We've got these two cancer drugs, so let's put them together and see what happens.'"

Only recently have researchers adopted more-systematic approaches. One method that's growing in popularity is the use of high-throughput screens that allow researchers to quickly evaluate interactions between different cancer therapies to predict which might form a successful combo. Alternatively or in addition, some researchers are relying on knowledge of the underlying biology to determine which therapies are likely to make the strongest pairings, as is the case for BMS's checkpoint inhibitor combos. "We have to start from fundamental principles of tumor biology," says Swanton. "Once we know this information, then we can start to come up with rational combo approaches."

Only a handful of cancer drug combos have so far been approved by the FDA, in part because many of the tested combinations were conceived largely at random.

Ross Camidge, a thoracic oncologist at the University of Colorado Denver, agrees. "Our chances of successful combination therapies are only as good as the science going into the selection of the combinations."

Casting a wide net

In vitro screening of large numbers of drug combinations is one of the approaches to sort through a vast ocean of drug-pairing possibilities. In silico screening methods typically rely on compiling data generated by in vitro experiments and animal studies, then using the data as a basis for computer algorithms to predict promising interactions. But these methods are labor intensive and in vitro drug combination screening is also expensive, which is why they have not been widely adopted. "There are not many academic labs with the capability to do [large-scale] combination screens, and not many pharmaceutical companies are doing it either, for that matter," says Marc Ferrer, a researcher at the Chemical Genomics Center within the National Center for Advancing Translational Sciences.

To bypass the need for having libraries of drug compounds to physically pair, researchers have been taking advantage of genetic methods, including novel gene-editing techniques, to identify potential drug pairings that kill cancer cells. These approaches can be easier and less expensive than traditional cell-based drug combination screens using multiwall plates. Using guide RNAs to knock out pairs of genes using CRISPR, for example, Stanford University's Michael Bassik identified pairs of genetic targets that might encourage cancer cell death.[2] Researchers can then use databases to search for drugs that bind to and inhibit the proteins encoded by those gene pairs. Possible combos identified through such screening methods require validation in cell culture and animal experiments. With the CRISPR screen, "we're using a genetic proxy for a drug effect: pairs of genes versus pairs of drugs, which require extensive robotics, plates, lots of time and money," says Bassik. "We are making assumptions that there are specific drugs for those gene targets, which is often, but not always true."

In addition, some researchers are going directly to cell culture–based screens to identify promising combos. In 2009, Georgetown University pediatric oncologist and researcher Jeffrey Toretsky identified a novel small molecule that targets an oncogenic fusion protein, EWS-FLI1, found exclusively in Ewing sarcoma, a type of bone cancer. His lab pulled out the molecule from a biophysical screen that tested the ability of thousands of compounds to bind a recombinant EWS-FLI1 protein. Then, using cell culture, Toretsky's lab tested pairwise combinations of the small-molecule inhibitor with 69 generic cancer drugs. This second screen uncovered a synergy with the chemotherapy drug vincristine (Marqibo, Vincasar PFS),[3] a finding that Toretsky and his colleagues confirmed with in vivo data last year, showing that the combination thwarted tumor growth in two Ewing sarcoma xenograft mouse models.[4] The human version of the EWS-FLI1 inhibitor, called TK216, is now in a Phase 1 clinical trial for Ewing sarcoma, and the combination will also be tested, says Toretsky.

Such cell culture–based screens are able to relatively quickly parse through large numbers of potential combinations, says Toretsky. Traditionally, however, only chemotherapies, targeted small molecules, and certain targeted antibodies—not immunotherapies—could be screened using cell culture–based screens. "There are many cellular interactions that are not captured in a 2-D monolayer of cells," says Ferrer.

Because cancer drug combinations are showing promise in clinical trials, Ferrer and his colleagues are trying to devise more-dynamic in vivo screens that better mimic the tumor and its microenvironment. But this is no easy feat. In 2012, his team developed a way to systematically screen many cancer drugs using three-dimensional sphere cultures of tumor cells,[5] an approach that identified drug-combination effects that were drastically different than those measured in 2-D cultures.[6] Ferrer has used the high-throughput 3-D assay to test dose ranges of drug combinations, and he's now working to increase the complexity of the cultures by mixing tumor cells with cells from the tumor microenvironment, hoping to eventually include immune cells.

To further narrow the search, many researchers urge forethought on the front-end and reasoning on the backend, examining what is known about how certain drugs work and thinking about mechanisms that might pair well together. "The permutations of potential combinations are endless," says Samir Khleif of Augusta University's Georgia Cancer Center. "The best thing that we have in our hands is biology and logic." Khleif, for his part, is testing currently available immunotherapy drugs in various combinations in animal models based on hypotheses of what pathways might work well together to fight tumors.

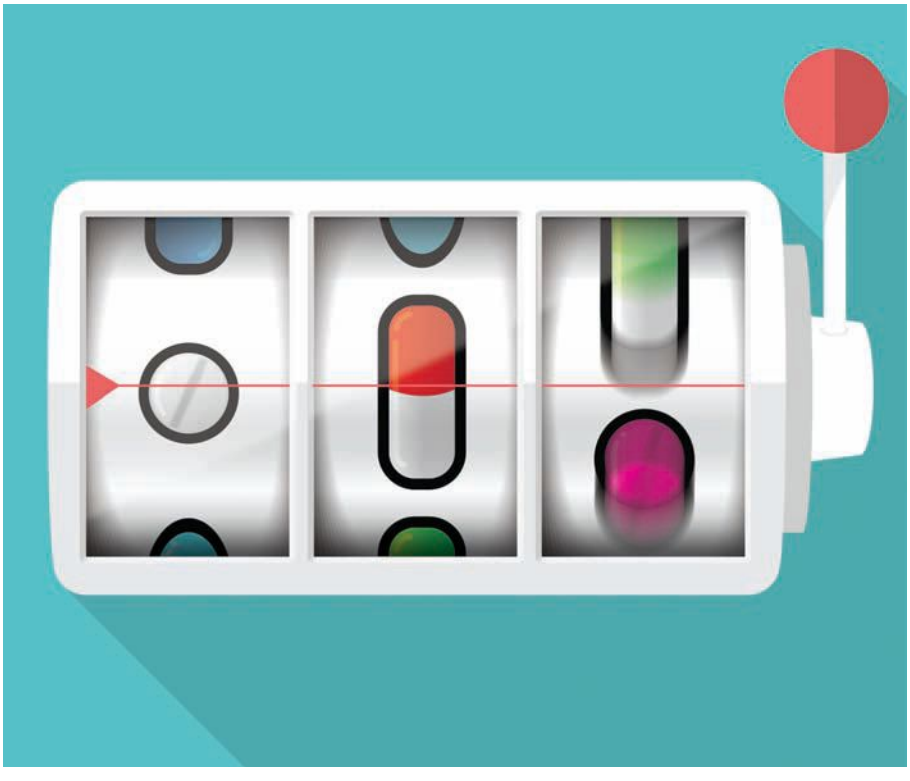
Back to biology

For a one-two punch aimed at two different mechanisms driving cancer cell survival, one approach is to go after two targets, each within a different signaling pathway. This is the strategy employed by the BMS researchers who paired the anti-LAG3 and anti-PD-1 checkpoint inhibitors that showed promise in combination in the recent clinical study. And the BMS team is not alone.

Last year, Karen Cichowski's lab at Harvard Medical School published results indicating that two targeted therapies, each of which binds to a molecule in a different pathway, can together cause enough oxidative stress in tumors in mice to kill cancers that are driven by the *Ras* oncogene.[7] The two oral drugs—one an inhibitor of the mechanistic target of rapamycin (mTOR) and the other an inhibitor of a histone deacetylase (HDAC)—are each individually approved for some tumor types. Several human trials, including a Phase 2 study in certain blood cancers, are testing the combination.

Other researchers are looking to harness such dual action in a single drug. Scientists at the Massachusetts division of Germany-based Merck KGaA are testing in mouse models a single antibody fusion protein, M7824, that simultaneously binds to the PD-1 ligand PD-L1 and traps transforming growth factor beta (TGF-β), a soluble cytokine protein that increases in abundance in patients with cancer. In results published earlier this year, the researchers reported that mice with breast and colorectal cancers treated with M7824 survived longer than those treated with either an anti-PD-L1 antibody or TGF-β trap binding alone.[8] M7824 is currently being tested in Phase 1 trials for advanced solid tumors.

Other drug combinations are born by pairing compounds that hit the same signaling pathway, to stave off resistance that can arise when treating with either drug alone. (See "How Cancers Evolve Drug Resistance," *The Scientist*, April 2017.) One such example is the small molecule trametinib, which was initially tested in combination with the already approved drug dabrafenib for patients with advanced melanoma. Both drugs target the Ras signaling pathway, which is a driver of cancerous growth in the 40 percent of melanoma tumors with an activating mutation in the *BRAF* gene. Dabrafenib



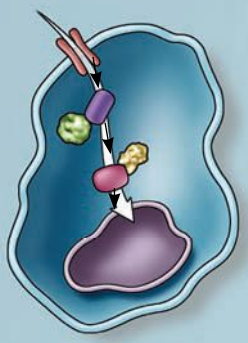
THE SCIENTIST STAFF

CANCER DRUG PAIRINGS

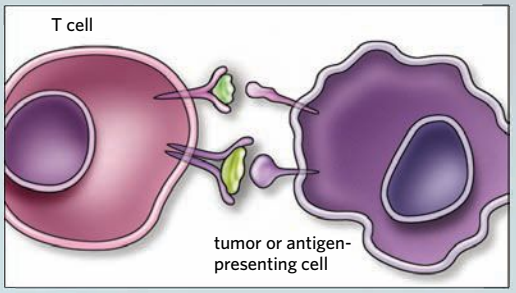
Among the first cancer drug combinations were mixtures of several chemotherapies that resulted in better and longer-lasting responses than individual drugs could deliver. Then came targeted therapies and immunotherapies, which were combined with chemotherapies and with each other to increase the proportion of patients who respond and the duration of those responses. While many cancer drug combinations were discovered by empirically testing opportunistic and random pairings, others were based on biological hypotheses that one drug could complement the other. Below are a few of the strategies behind recently successful and still investigational combos.

DOUBLING UP ON TARGETED THERAPY

Coadministering two targeted agents that work on different targets within the same signaling pathway is a way to stave off cancer resistance. Combining two targeted agents that block molecules within different pathways is another common strategy.



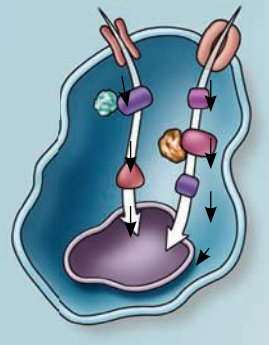
In 2014, the FDA approved the first combination: dabrafenib, a B-raf inhibitor, plus trametinib, a MEK inhibitor, for advanced melanoma. The two drugs target different molecules within the Ras signaling pathway.



DUAL CHECKPOINT INHIBITOR ANTIBODY COMBINATION: Combining two checkpoint inhibitors that target two different checkpoint pathways is one strategy to stimulate a greater and possibly more durable antitumor immune response.

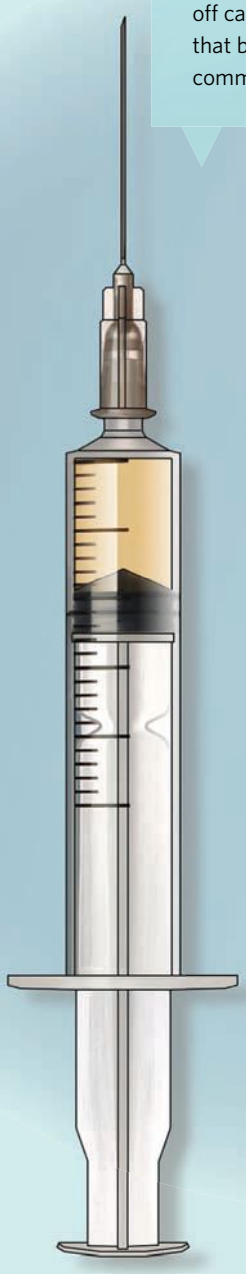
EXAMPLE: The only currently approved immunotherapy combination is ipilimumab plus nivolumab for metastatic melanoma. Other checkpoint inhibitor combinations are currently in clinical trials.

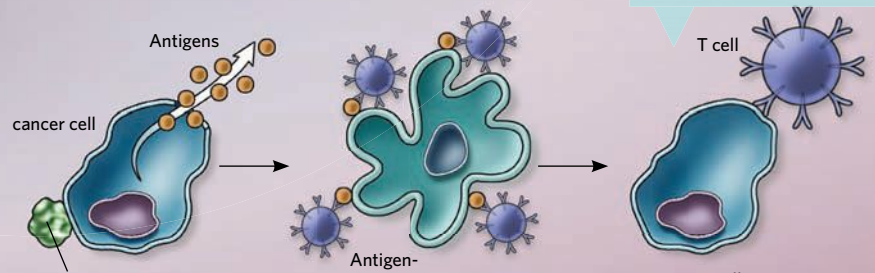
The combination of lenvatinib, an anti-VEGF oral drug, and everolimus, an oral mTOR inhibitor, was approved by the FDA for renal cell carcinoma in 2016. The drugs target two separate but cancer-linked signaling pathways that support tumor growth.



IMMUNOTHERAPY-CHEMOTHERAPY, RADIATION, OR TARGETED THERAPY

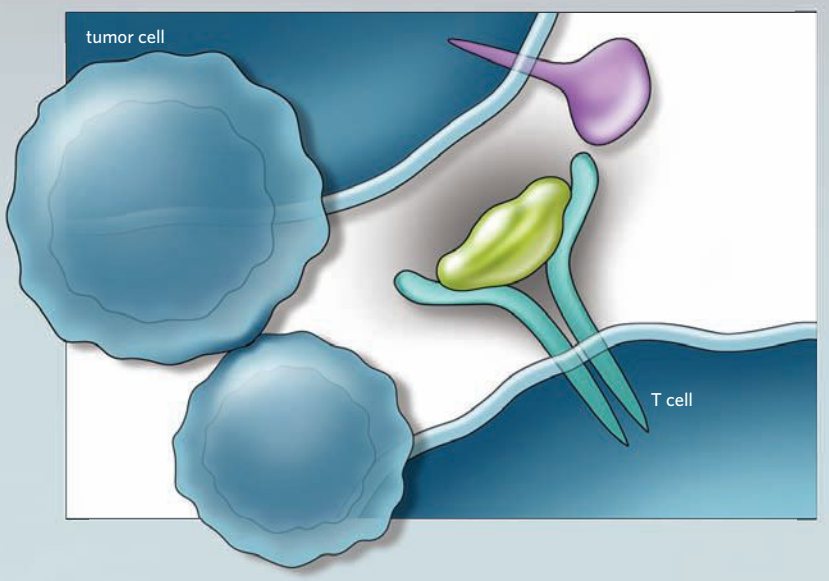
As chemotherapy, radiation, or targeted therapies kill cancer cells, neoantigens are released, helping the immune system recognize tumor cells. These therapies also minimize tumor burden, buying time for the immune system to act. Simultaneously, checkpoint inhibitors ramp up the immune response.





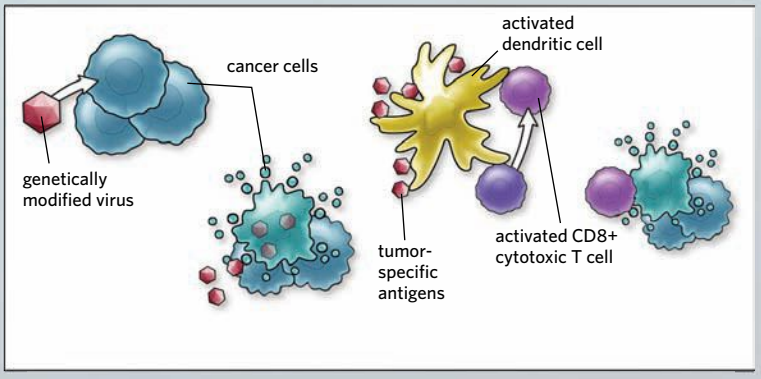
In 2017, the FDA approved the combination of the chemotherapies pemetrexed and carboplatin, plus the checkpoint inhibitor pembrolizumab, for advanced lung cancer.

IMMUNOTHERAPY-IMMUNOTHERAPY



CHECKPOINT INHIBITOR PLUS A CELL-BASED THERAPY: Also seen as a way to target two different pathways to amplify the immune response and potentially overcome resistance.

EXAMPLE: Still theoretical, with no combinations yet approved or in clinical trials



CHECKPOINT INHIBITOR PLUS A VIRAL VACCINE: A vaccine, in theory, should increase the presentation of cancer neoantigens to the immune system, bolstering the immune system's response to a checkpoint inhibitor.

EXAMPLE: The cancer vaccine talimogene laherparepvec, a genetically engineered herpes virus, plus ipilimumab is currently in a Phase 2 trial for advanced melanoma, with some positive preliminary data.

targets the B-raf protein itself, while trametinib targets MEK, a downstream kinase. The combination decreased the risk of death from melanoma by 31 percent compared with dabrafenib alone[9] and was approved by the FDA in January 2014. Recently, researchers at the Netherlands Cancer Institute uncovered two distinct populations of cells within drug-resistant melanomas. One consisted of cells expressing low levels of AXL, a receptor tyrosine kinase, and sensitive to B-raf and MEK inhibitors. The second population expressed high levels of AXL, was resistant to a B-raf plus MEK inhibitor combination, but was sensitive to a novel drug called an antibody-drug conjugate that binds to AXL on the surface of the tumor cells. The team showed that a triple combination targeting both cell populations was more effective than the standard combination, resulting in durable responses in patient-derived xenografts from resistant melanomas.[10]

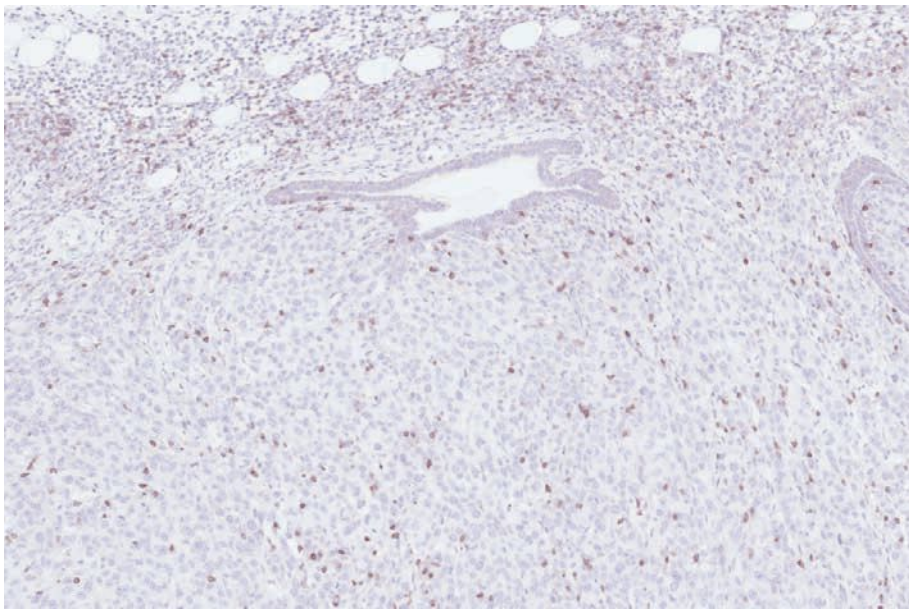
Sometimes the logic behind a potential drug combo is not as simple as targeting the pathways inside tumor cells. When it comes to immune checkpoint therapies, which don't target the tumor cells directly but rather the immune system, clinical studies have revealed that patients are most responsive if they have already started to mount an antitumor response. Thus, some researchers are now looking to layer additional drugs on top of an immunotherapy to transform a non-responsive immune system to a tumor-responsive one.

Earlier this year, for example, researchers at the University of Ottawa found they could slow tumor growth in mouse models of triple-negative breast cancer that are typically unresponsive to an immune checkpoint therapy by treating them with a Maraba rhabdovirus that sensitized the animals to an anti-PD-1 antibody.[11] And when the combination was coupled with tumor resection, up to 90 percent of the animals had zero evidence of disease. A version of the Maraba virus expressing the neoantigen MAGE-A3 is currently being tested in a Phase 2

clinical trial for patients with advanced lung cancer. Meanwhile, a group of U.K.-based researchers showed this year that a similar combination of an oncolytic human reovirus plus an anti-PD-1 antibody resulted in an antitumor response in mouse models of brain cancer.[12]

Even when researchers think they have a solid hypothesis for a two-drug combination, biology can throw them for a loop. One cautionary tale is that of an anti-PD-1 antibody plus an OX40 agonist that stimulates the proliferation and expansion of T cells. Two recent studies, from Bernard Fox's group at Oregon Health & Science University's Knight Cancer Institute and Khleif's lab at Augusta University, demonstrated that while, on paper, the combination should have been at least twice as effective in stimulating T cells as either treatment alone, it instead caused T cells to die in several mouse models of various tumor types. Indeed, two early-phase clinical trials combining OX40 and PD-1-targeting antibodies initiated prior to these publications have not panned out.[13,14]

As it turned out, the researchers were able to produce a synergistic effect, compared to an anti-PD-1 antibody alone, but only by giving the mice the OX40 antibody first, then treating them with the anti-PD-1 a few days later; reversing the order was ineffective. "Immunotherapy is the way of the future in cancer treatment, but the path is not straightforward," says Khleif. "When you treat with one immunotherapy, you are targeting an entire biological system, and the treatment changes that system in a way that adding a second immunotherapy results in an unexpected result."



MARIE-CLAUDE BOURGEOIS-DAIGNEAULT, OTTAWA HOSPITAL RESEARCH INSTITUTE

GOING VIRAL: Mouse triple-negative breast cancer tissue showing T cells (brown) recruited to the tumor site following treatment with an oncolytic Maraba virus.

Another major bottleneck stems not from biological limitations, but from the fact that biotech and pharmaceutical companies too often would rather test combinations only of molecules they have in-house, says Peter Adamson, professor of pediatrics at the University of Pennsylvania and the Children's Hospital of Philadelphia. The result is that many new cancer drug combos being trialed are still largely hap-

SAFETY ISSUES

Approved in 2015 for metastatic melanoma, the immunotherapy combination of the anti-CTLA-4 antibody ipilimumab and the anti-PD-1 antibody nivolumab increased the number of patients that responded to nivolumab alone by about 14 percent (*NEJM*, 373:23-34, 2015). But treatment-related side effects also increased with the combination of two immunotherapies, both of which can also unleash immune cells against healthy tissues. Specifically, 55 percent of patients who received the combination also experienced a greater number of serious treatment-related side effects—such as diarrhea and inflammation of the bowel—compared with 16 percent in the nivolumab-only group.

Layering multiple drugs typically increases the potential for side effects, adding to the challenges of developing promising treatment combos. In addition to the heightened risk of known side effects of either drug, pairing therapies can also uncover dangerous synergies not seen when a treatment is administered as a single drug. When the B-raf inhibitor vemurafenib was combined with ipilimumab in a Phase 1 clinical trial for advanced melanoma patients, for example, patients experienced high liver toxicity not seen with either drug alone, causing researchers to halt the study prematurely.

To make matters worse, such complications are often difficult to predict using animal models; unless a drug combination causes overt toxicity such as organ failure or significant immune-cell depletion in a mouse, the harmful effects of the pairing will likely only emerge in a clinical trial, says Joshua Brody of the Icahn School of Medicine at Mount Sinai in New York City. "Animal models do almost nothing to predict the safety profile of single drugs and drug combinations in humans."

Even when researchers think they have a solid hypothesis for a two-drug combination, biology can throw them for a loop.

hazard in nature, driven as much by commercial interests as by underlying biology and compelling preclinical data, he adds. "What's going on right now in early clinical development is that some companies look at their portfolio of agents, come up with a combination, and then pursue a scientific rationale of varying quality."

Personalizing combo therapies

Despite the challenges, the cancer research community continues to see drug combinations as the future of therapy. Most researchers agree that successfully reining in a cancer's growth and spread and extending patient survival will involve a barrage of multiple compounds. And this approach is spreading into the growing field of precision oncology, where researchers are looking in patients' genomes for clues to which therapies are most likely to be effective.

Several years ago, frustrated by the lack of FDA-approved treatments that offer lasting benefit and by an inability to find an appropriate clinical trial for many of her patients, oncologist Razelle Kurzrock, director of the Center for Personalized Cancer Therapy at the University of California, San Diego (UCSD), decided to start her own customized therapy trial. In her team's I-PREDICT (Investigation of Profile Related Evidence to Determine Individualized Cancer Therapy) study, patients often receive a custom two- or three-drug combination therapy—either FDA-approved drugs or experimental drugs from clinical trials—to target the specific mutations identified in their tumors.

According to Shumei Kato, a UCSD medical oncologist and a coinvestigator on the I-PREDICT trial, several thousand patients have been through genomic screening, and hundreds are receiving a customized combination of cancer drugs through the I-PREDICT or similar UCSD-led trials. And for some patients, it appears to be working.

On Mother's Day, 2017, Lisa Darner had muscle spasms and lost consciousness. At her local hospital in San Diego, physicians told her that she had suffered a grand mal seizure—and that she had cancer in several major organs, including the brain, which caused the seizure. She quickly received brain radiation therapy followed by standard chemotherapy for lung cancer, which her oncologists considered to be the most likely primary tumor.

While receiving nonspecific chemotherapy, Lisa opted to also have her tumor biopsy analyzed using a comprehensive genetic panel—not always part of routine cancer care—that homed in on two actionable mutations: an epidermal growth factor receptor (EGFR) gene amplification and an alteration in a cell cycle gene called *CDKN2A*. Kurzrock and her colleagues at UCSD's Moores Cancer Center came up with a triple drug combination including palbociclib (Ibrance), a cell cycle kinase inhibitor; a small molecule inhibitor of EGFR, erlotinib (Tarceva); and an antibody that also targeted EGFR, cetuximab (Erbitux). In August 2017, confined to a wheelchair because of her progressing disease, Darner started the custom combo as part of the I-PREDICT trial.

Aside from a rash (a side effect of the drugs), she responded well and is still on the treatment. "My tumors were still growing in August, but by October, scans showed everything was receding or has stabilized," she says. "There are places where you can't see a tumor anymore."

Unfortunately, not all patients are as lucky. "The patient has high expectations from their cancer treatment, but the reality is that it is not always that great," says Kato. "We're not saying this is for sure a better approach. It's a work in progress. But I think that continuing to do the standard-of-care approach, when it's known not to be beneficial, will not change outcomes for cancer patients. We need to try something different to see a different, better result." ■

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Inner Nets

Cellular wrappings called perineuronal nets control brain plasticity and are woven into memory and psychiatric disorders.

BY DANIELA CARULLI

In 1898, Camillo Golgi, an eminent Italian physician and pathologist, published a landmark paper on the structure of "nervous cells." In addition to the organelle that still bears his name, the Golgi apparatus, he described "a delicate covering" surrounding neurons' cell bodies and extending along their dendrites. That same year, another Italian researcher, Arturo Donaggio, observed that these coverings, now known as perineuronal nets (PNNs), had openings in them, through which, he correctly surmised, axon terminals from neighboring neurons make synapses.

Since then, however, PNNs have been largely neglected by the scientific community—especially after Santiago Ramón y Cajal, a fierce rival of Golgi (who would later share the Nobel Prize with him), dismissed them as a histological artifact. It wasn't until the 1970s, thanks to the improvement of histological techniques and the development of immunohistochemistry, that researchers confirmed the existence of PNNs around some types of neurons in the brain and spinal cord of many vertebrate species, including humans.

Composed of extracellular matrix (ECM) molecules, PNNs form during postnatal development, marking the end of what's known as the "critical period" of heightened brain plasticity. For a while after birth, the external environment has a profound effect on the wiring of neuronal circuits and, in turn, on the development of an organism's skills and behaviors, such as language, sensory processing, and emotional traits. But during childhood and adolescence, neuronal networks become more fixed, allowing the individual to retain the acquired functions. Evidence gathered over the past 15 years suggests that PNNs contribute to this

Increasingly, researchers are turning to PNNs as potential targets to enhance plasticity for the treatment of various diseases, from amblyopia to neurodegenerative diseases to psychiatric disorders such as schizophrenia and addiction.

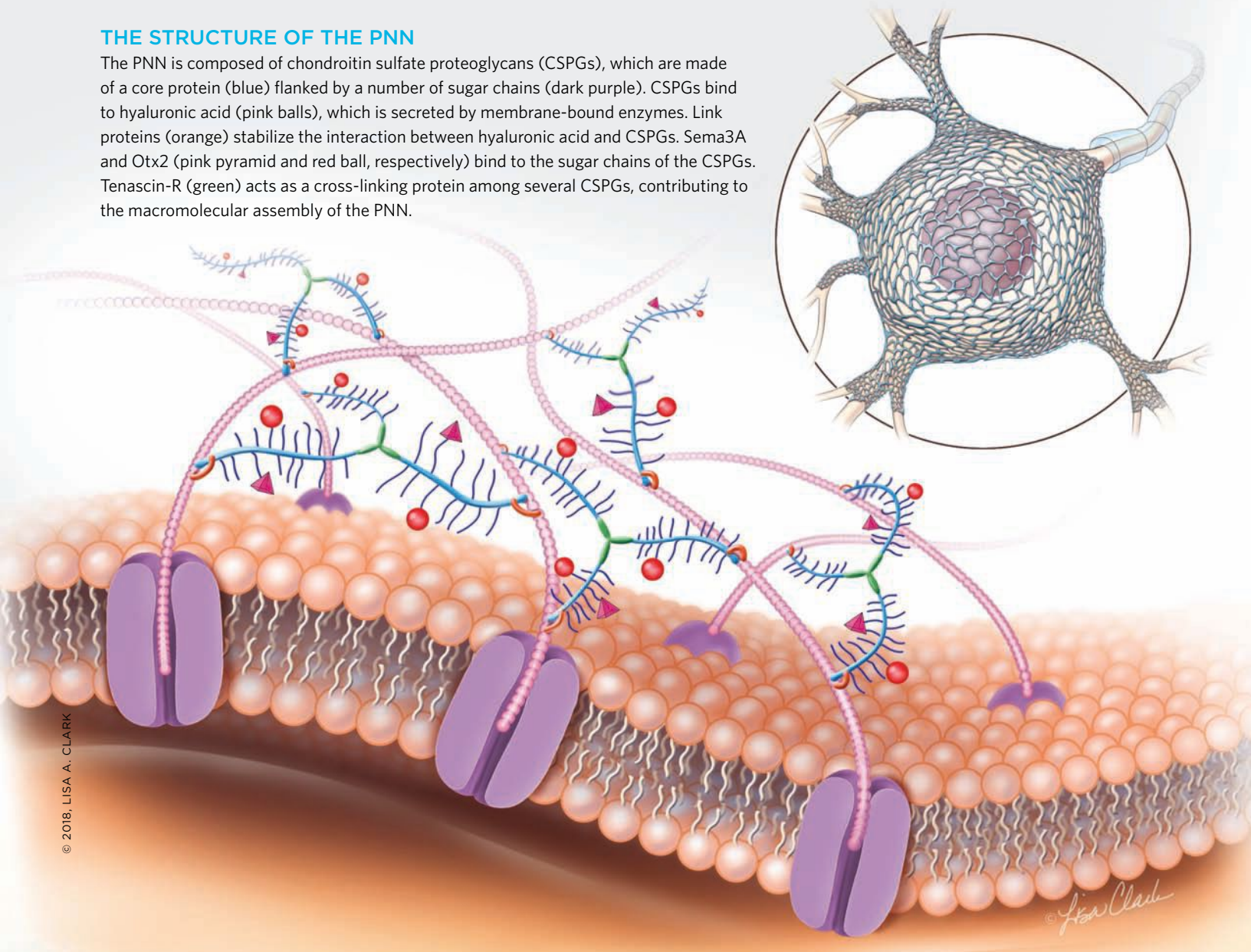
dation of the PNN via the application of the bacterial enzyme chondroitinase ABC gives similar results. In both cases, the removal of PNNs facilitates the induction of synaptic plasticity.[7]

In addition to resisting memory formation, PNNs may also be to blame for blocking memory destruction. Whereas young individuals can permanently erase a fear memory by extinction training—a form of learning involving associating the fear-inducing stimulus with neutral scenarios—adults exhibit fear behaviors that are resistant to erasure. These behaviors depend on the amygdala, where PNNs are present in adult, but not young, animals. Interestingly, in adult mice, PNN degradation in the amygdala by chondroitinase ABC reopens a critical period during which fear memories can be fully erased by extinction training.[8] In addition, PNNs in various cortical areas have recently been shown to be important for storage of fear memories, as their removal disrupts such memories.[9,10]

Currently, chondroitinase ABC is widely applied for removing PNNs in experimental animals, but it lacks specificity, causing a degradation of ECM molecules not only in PNNs but throughout CNS tissue. Researchers are looking for more subtle ways to manipulate the PNN in animal models in order to further understand their functions as well as

THE STRUCTURE OF THE PNN

The PNN is composed of chondroitin sulfate proteoglycans (CSPGs), which are made of a core protein (blue) flanked by a number of sugar chains (dark purple). CSPGs bind to hyaluronic acid (pink balls), which is secreted by membrane-bound enzymes. Link proteins (orange) stabilize the interaction between hyaluronic acid and CSPGs. Sema3A and Otx2 (pink pyramid and red ball, respectively) bind to the sugar chains of the CSPGs. Tenascin-R (green) acts as a cross-linking protein among several CSPGs, contributing to the macromolecular assembly of the PNN.



fixation in many brain areas, by stabilizing the existing contacts between neurons and repelling incoming axons.

Because limited neuronal plasticity underlies the irreversibility of many afflictions of the central nervous system (CNS), from stroke to spinal cord injury to neurodegenerative diseases, PNNs have been considered promising targets to enhance CNS repair. Moreover, they are increasingly recognized as important players in the regulation of memory processes.

PNNs may also play a supportive role in the normal functioning of the CNS. These coatings have been repeatedly observed around highly active neurons, and researchers have proposed that the structures provide a buffered, negatively charged environment that controls the diffusion of ions such as sodium, potassium, and calcium, thus serving as a rapid cation exchanger to support neuronal activity.[1] PNNs have also been shown to protect neurons from oxidative stress, as they limit the detrimental effect of excessive reactive oxygen species on neuronal function or survival. Indeed, enzymatic degradation of the PNNs renders neurons more susceptible to oxidative stress.[2]

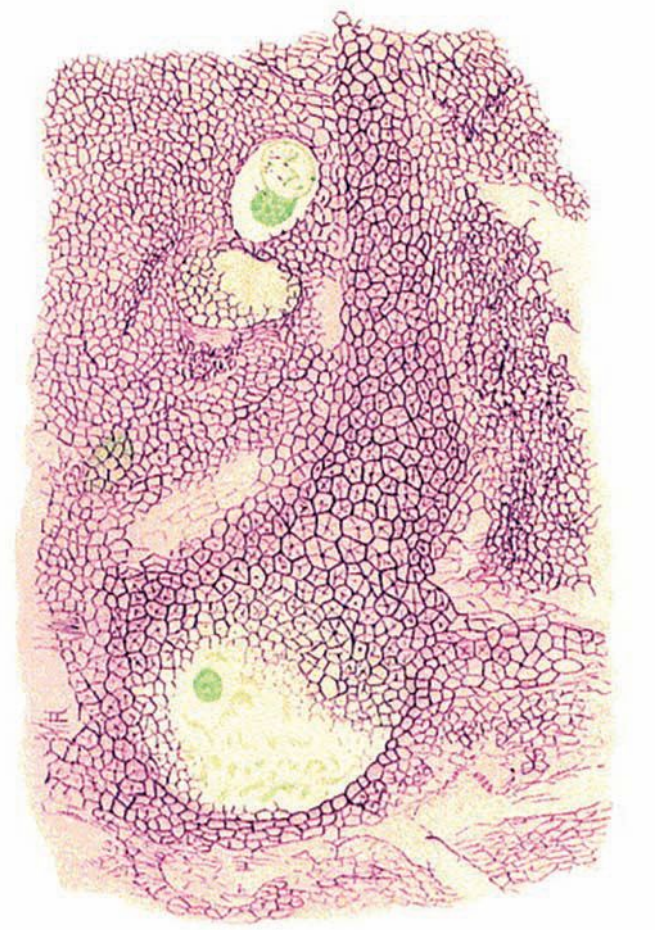
A lot of progress has been made in the last two decades toward illuminating the structural and functional properties of PNNs, defining their roles in CNS plasticity, and developing methods to manipulate them to increase plasticity, memory, and CNS repair. Still, how exactly PNNs work in the brain, and which precise mechanisms underlie their remodeling in physiological or pathological conditions, are still open questions.

PNNs and the plastic brain

PNNs' role in closing the critical period of brain plasticity is now well established. In 2010, for instance, my colleagues and I showed that knockout mice lacking a PNN component called *link protein* display a reduced formation of PNNs, and they maintain juvenile levels of plasticity throughout adulthood.[3]

Another example comes from rats with amblyopia, a neurodevelopmental disorder resulting from an imbalance between the neural signals coming from the two eyes during the critical period for visual development. Inputs from the right and left eyes compete when they first converge on neurons in the primary visual cortex, leading to a physiological and anatomical cortical representation of the relative inputs contributed by either eye. When one eye is deprived of visual input—for instance, due to a congenital cataract—individuals suffer a loss of cortical response to that eye and an overrepresentation of the input from the healthy eye, resulting in visual impairment. In adulthood, because the critical period is closed, vision will remain defective even if the cause of amblyopia is treated. The removal of PNNs in the visual cortex of adult rats, however, has proven effective in treating amblyopia.[4]

Under particular circumstances, such as enriched environmental stimulation, the adult brain regains certain levels of plasticity, and here too, PNNs appear to be important mediators. Adult amblyopic rats reared in a cage enriched with toys, ladders, and running wheels show a reduction of PNNs in the visual cortex and recover normal visual acuity after two to three weeks in this environment with no other treatment.[5]



COURTESY MARCO CELIO

MISH MASH MESH: A cell from the spinal cord of a dog, stained by the Italian neurologist Carlo Besta in 1910 and republished in *Trends in Neurosciences* in 1998 (21:510-15). The PNN's polygonal units are visible.

In 2016, my colleagues and I documented similar links between PNNs and plasticity in the vestibular system, which detects head position and acceleration, stabilizes gaze and body posture, and contributes to self-motion perception. Mice that have suffered permanent damage to the inner ear vestibular receptors generally show severe deficits in their posture and balance, but will improve over time. This improvement comes along with an initial decrease of PNNs in the areas of the brain stem that regulate vestibular functions, followed by a complete restoration of the PNNs after posture and balance strengthen.[6]

Beyond these examples of recovering from sensory deficiencies, the adult brain exhibits plastic tendencies during normal learning, and recent evidence points to the role of PNNs in memory formation and retention. In rodents, explicit memory—information that can be consciously recollected—can be assessed by a novel object recognition test: when animals are exposed to familiar and novel objects, they spend more time exploring the novel one. This type of memory requires synaptic plasticity in a specific area of the cerebral cortex. Mutant mice lacking link proteins, and thus having reduced PNNs, exhibit a prolonged memory for familiar objects, and degra-

to fine-tune neuronal plasticity. Additionally, while behavioral studies have clearly demonstrated the role of PNNs in mediating the plasticity of the brain, researchers still don't have a good grasp on the molecular details of these processes. Recently, revelations about the composition of PNNs have begun to yield clues.

PNN structure and the control of plasticity

A number of ECM molecules are present at higher concentrations within the PNN than in the rest of the extracellular space. The sugar hyaluronan serves as the backbone of PNN structure. Bound to hyaluronan are chondroitin sulfate proteoglycans (CSPGs). This binding is stabilized by the link proteins. CSPGs are composed of a core protein and attached sugar chains. Different sulfation patterns in the CSPG sugar chains create specific binding sites for a wide variety of molecules and receptors, affecting CSPG function. (See illustration on page 43.)

One mechanism by which PNNs control neuronal plasticity is the interaction between CSPGs and the homeoprotein Otx2. Homeoproteins are transcription factors that play major roles during embryonic development, controlling the organization of the vertebrate brain into distinct regions. Many homeoproteins also serve as paracrine signaling factors that shuttle between cells. In mice, experimentally reducing the capture of Otx2 by visual cortex neurons, which happens through binding to CSPGs, reduced PNN assembly, increased plasticity, and prompted the recovery of visual acuity in adult animals with amblyopia.[11] And research last year demonstrated Otx2's role in regulating PNNs' influence on the experience-dependent formation of tonotopic maps, the spatial arrangement of neurons according to their sound frequency responses, in the primary auditory cortex and the acquisition of acoustic preferences (which is mediated by the medial prefrontal cortex).[12]

Genetic studies have identified several ECM- and PNN-regulating molecules as potential contributors to the etiology of autism.

Another potential mediator of PNN-controlled plasticity is the axon guidance molecule Semaphorin 3A, a CSPG-binding molecule that is highly concentrated in the PNNs of distinct neuronal populations in the mature brain. Recent experiments have shown that neurons cultured on PNN sugars grow shorter neurites and that this inhibition is enhanced by the presence of Semaphorin 3A.[13] But the effect of Semaphorin 3A binding to the PNNs has yet to be determined. PNNs may also act through direct interactions with receptors for CSPGs on neurons, as occurs after a CNS injury, in which CSPGs upregulated in the injury site inhibit axon regrowth by binding to specific receptors. However, so far, no clear evidence exists about the presence of CSPG receptors on PNN-bearing neurons or synapses.

Although the mechanisms that allow PNNs to influence neuronal plasticity remain unclear, the effects of that influence are well documented. Increasingly, researchers are turning to PNNs as potential targets to enhance plasticity for the treatment of various diseases, from amblyopia to neurodegenerative diseases to psychiatric disorders such as schizophrenia and addiction.

Targeting the PNN to treat disease

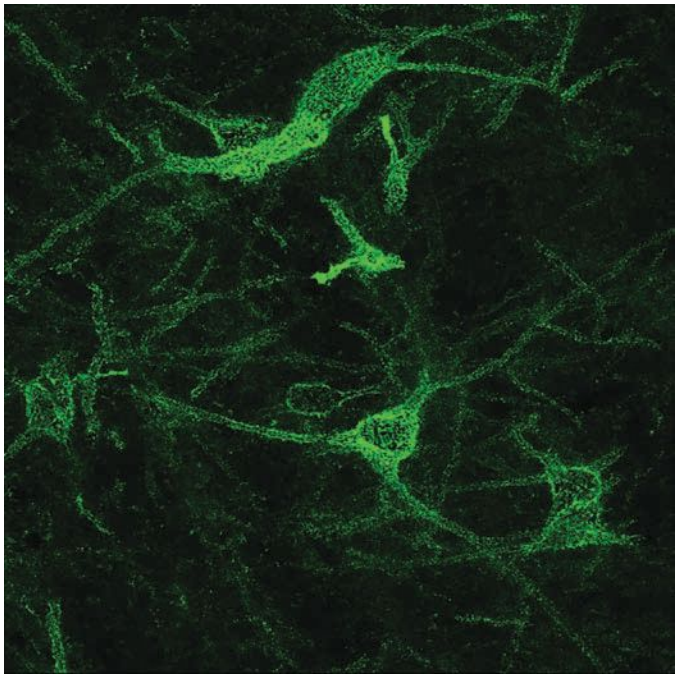
In accordance with findings showing a role for PNNs in memory, increasing evidence points to the involvement of PNNs in drug-associated memories. Environmental cues formerly associated with drug use (such as people or situations) can become strong triggers for drug-seeking behavior, contributing to the development of addiction. Therefore, disrupting these associations could aid in the treatment of addiction.

Studies on rodents show that PNNs in the dorsal cerebellar cortex might play an important role in the formation and maintenance of cocaine-associated memories,[14] and in the prefrontal cortex, PNNs are decreased after heroin self-administration but rapidly increased after reexposure to heroin-associated cues.[15] Conversely, degrading PNNs in the prefrontal cortex reduces the acquisition and maintenance of cocaine memory in rats,[16] while PNN degradation in the amygdala following an exposure to morphine, cocaine, or heroin inhibits relapse in the animals.[17] On the whole, PNNs appear to be necessary for creating and/or maintaining drug-related memories, and thus may serve as targets for weakening memories that drive relapse.

Experimentally degrading PNNs has also shown promise in treating various forms of brain damage. Research in rodents has demonstrated that the degradation of PNNs by chondroitinase induces the formation of new axon branches and synapses, for example, and improves specific functions after stroke, trauma, and spinal cord injury.[18] And in mouse models of Alzheimer's disease, in which memory formation is compromised, a chondroitinase injection into the brain can successfully restore the ability to form new memories, even in the presence of diffuse neuronal dysfunction and cell death.[19]

Sometimes it's not the presence of PNNs that is the problem, but rather aberrations in their structure. For instance, researchers have observed decreased densities of PNNs, or PNNs with degraded morphology, in brain areas responsible for complex cognitive functions, such as the frontal cortex and entorhinal cortex, in subjects with Alzheimer's disease, suggesting that neurons with PNN alterations might be vulnerable to cell death.

Abnormal PNNs have been observed in the postmortem brains of schizophrenia patients—specifically, in regions involved in emotion-related learning and associative sensory information processing such as the amygdala, entorhinal cortex, and prefrontal cortex. Researchers have linked mutations in the genes encoding CSPGs, Semaphorin 3A, and other components of the normal ECM such as integrins and remodeling enzymes to schizophrenia risk. Loss of PNNs may render neurons more



BUNDLED UP: PNNs, visualized by immunostaining, wrap around cells in a mouse brain.

susceptible to the excitotoxic effects of oxidative stress believed to occur in schizophrenia.[20]

Several other psychiatric disorders have also been linked to PNN abnormalities. For example, genetic studies have identified several ECM- and PNN-regulating molecules, including Semaphorin 3A, the hyaluronan surface receptor CD44, and Otx2, as potential contributors to the etiology of autism. And postmortem studies of bipolar patients have shown a marked decrease in specific sugars or proteins associated with PNNs in the amygdala. Scientists have also suggested that a variant of the gene encoding the CSPG neurocan could be a risk factor for the disorder. Consistently, neurocan knockout mice show manic-like behaviors. Furthermore, increased levels of matrix-degrading enzymes appear in blood samples from subjects with major depression, bipolar disorder in a depressed state, schizophrenia, and autism.[20]

Yet another brain disorder that might be affected by the state of PNNs is epilepsy, which is characterized by abnormal patterns of neuronal activity that cause convulsions, unusual emotions and sensations, and a loss of consciousness. PNNs are decreased in animal models of epilepsy, putatively allowing for synaptic reorganization, such as occurs following seizures. Conversely, PNN abnormalities may contribute to a susceptibility to seizures. For example, increased epileptic activity has been found in mice lacking an enzyme that helps synthesize hyaluronan.[20]

Although many questions remain unanswered, research has clearly demonstrated that targeting PNNs holds great promise for the treatment of several brain diseases. So far, no compounds that target PNNs are drug candidates, as they are too general in their disruption, but scientists are working to identify specific PNN components to zero in on. While finding noninvasive ways to precisely target PNNs in specific areas of the human brain still represents a challenge, researchers are hopeful that this could be a promising avenue for a variety of therapies in the coming decades.

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DANIELA CARULLI

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Double-Edged Swords

Macrophages play numerous roles within tumors, leaving cancer researchers with a choice: eliminate the cells or recruit them.

BY AMANDA B. KEENER

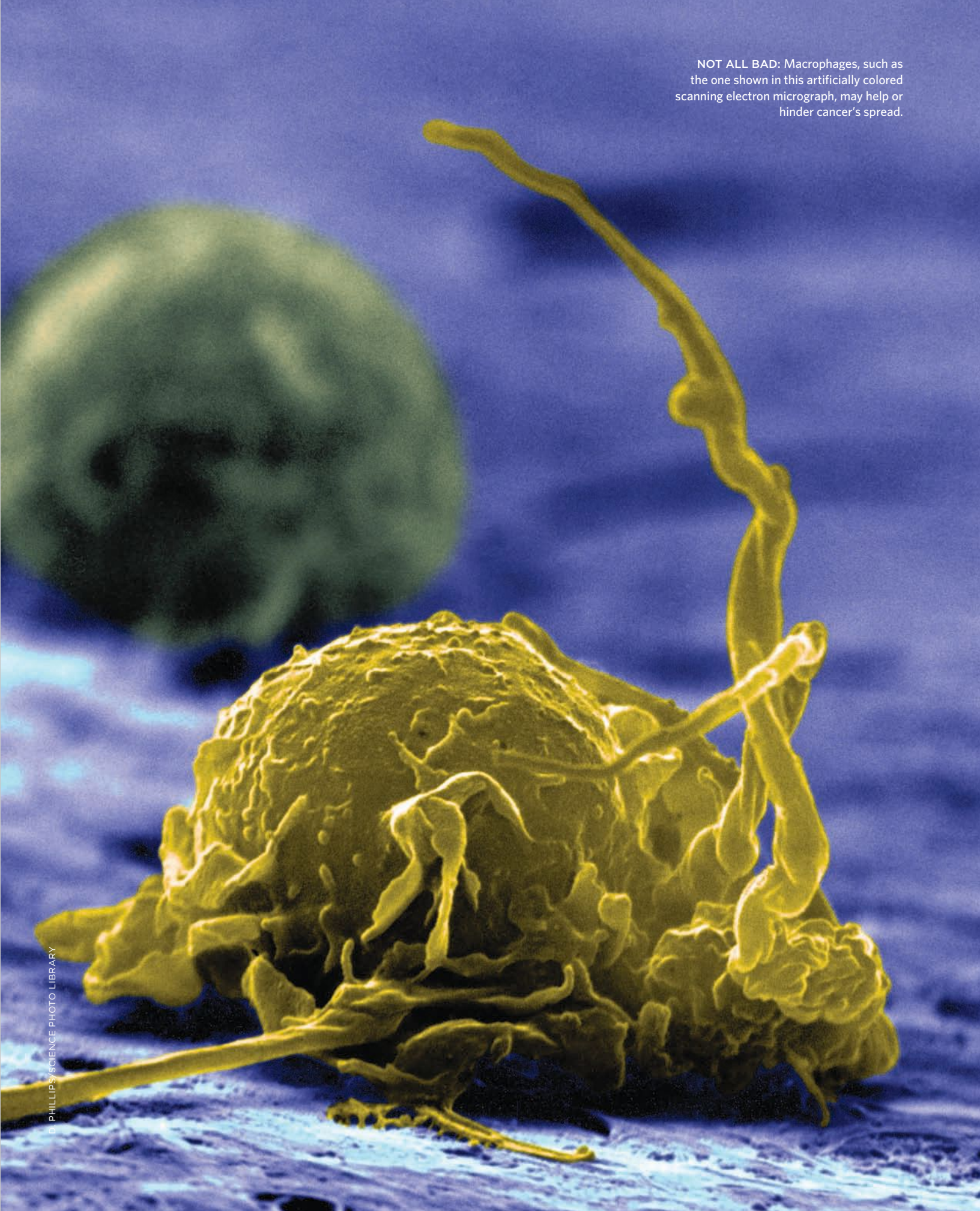
In the late 2000s, Stanford University stem cell biologist Irving Weissman wanted to understand how normal blood-forming stem cells differed from those that went on to seed a type of blood cancer called acute myelogenous leukemia (AML). Using bone marrow samples from AML patients who had survived the nuclear bombs dropped on Japan during World War II, his team identified the developmental stage at which blood-forming stem cells branch off to become cancerous and compared gene expression profiles between those cells and their counterparts from healthy bone marrow samples. The researchers found that the leukemia-forming stem cells highly expressed a gene encoding CD47, a surface molecule known for its role on normal, healthy cells as a "don't eat me" signal to phagocytosing macrophages. Weissman and his colleagues had no clue how CD47 had gotten onto cancer cells, but they couldn't ignore it. "The molecule was just staring us in the face," he says.

The researchers looked at stem cells from AML patients at the Stanford Medical Center to see if they also expressed CD47. "They all did," says Weissman. After demonstrating in cell culture experiments that macrophages only engulfed AML cells that did not display CD47 on their surface, Weissman's team grew human AML cells in five immune-deficient mice and treated the animals with an antibody against CD47.[1] In just two weeks, AML cells were nearly undetectable in the animals' blood, and had dropped by 60 percent in their bone marrow. "It was shocking," says Weissman, noting that four of the five mice were essentially cured. "We knew that we were on the track of a potential therapeutic."

In less than a decade, Weissman and his colleagues at Stanford have found CD47 on every type of cancer they've been able to get their hands on. Meanwhile, at least three biomedical companies, including the Stanford spin-off Forty Seven, Inc., have raised and invested tens of millions of dollars to test drugs that block the molecule. The approach seems so promising that last August, Rider and Victoria McDowell, inventors of the vitamin product Airborne, offered $10 million to anyone who would grant them access to an anti-CD47 therapeutic—none of which have yet been tested for pediatric use—to treat their 17-year-old son's brain tumor.

But back in 2008, when Weissman first tried to publish his work on how macrophages engulfed leukemia cells lacking CD47, his reviewers didn't buy it. Since the 1980s, cancer researchers have linked macrophages and macrophage-stimulating genes to tumor growth and poor outcomes for cancer patients, and the cells had been pegged as nothing but bad news when it came to cancer. In 1996, for example, researchers from the University of Oxford reported that women whose breast cancer biopsies contained a high density of macrophages were much more likely to succumb to the disease over the subsequent five years than those with low densities.[2] The same correlation was later confirmed in a dozen other types of cancer. These cells earned the name tumor-associated macrophages, or TAMs, and research focused on where they came from and how to block or deplete them. Weissman's data suggesting that macrophages could help defeat cancer just didn't fit.

"Several years ago, the idea was, 'Let's deplete these cells because they are bad,'" says Mikael Pittet, an immunologist at Harvard Medical School. Specifically, TAMs, which can make up as much as 50 percent of a tumor's mass, had been found to repress other immune cell activity, encourage blood and lymph vessel development to support growing tumors, and help cancer cells metastasize to new sites in the body. But over the past decade, some research has surfaced to support



NOT ALL BAD: Macrophages, such as the one shown in this artificially colored scanning electron micrograph, may help or hinder cancer's spread.

D. PHILLIPS/SCIENCE PHOTO LIBRARY

Weissman's conclusion that TAMs may have an upside, Pittet says. "I think now we are back to saying, 'Maybe it's just very complex.'"

Masters of metastasis

TAMs start out either as tissue-resident macrophages, which originate from the embryonic yolk sac and take on tissue-specific roles during development, or as monocytes, which are born in the bone marrow and circulate in blood until they are recruited to tissues throughout adulthood. Tumors secrete signaling molecules, such as colony-stimulating factor 1 (CSF-1) and CC chemokine ligand 2 (CCL2), that attract monocytes and tissue-resident macrophages and convert these cells to the cancer-supporting TAM phenotype.

In the mid-1990s, reports linking CSF-1 to cancer were rolling out one after the other, implicating macrophages as accomplices to tumors. But these studies didn't explain how the immune cells influenced the course of a particular cancer. To make sense of this growing literature, Jeffrey Pollard, a developmental biologist at the University of Edinburgh in Scotland, worked with colleagues to create a mouse model prone to developing breast cancer that also lacked the gene encoding CSF-1. The model revealed that the absence of CSF-1 had no effect on whether primary breast cancer tumors grew, but it did reduce the density of TAMs in the tumors and delayed metastasis.[3]

> I think now we are back to saying, "Maybe it's just very complex."
>
> —Mikael Pittet, Harvard Medical School

It made sense to Pollard that the link between macrophages and survival would have to do with cancer's spread. "The reason you die of cancer is metastasis," he says. Pollard's findings have since been repeated in several animal models of cancer, and CSF-1 signaling has become a popular target for developing cancer drugs. There are now more than a dozen ongoing clinical trials testing monoclonal antibodies and pharmacologic inhibitors that disrupt the pathway.

Pollard's team has also found that TAMs help tumors metastasize by supporting tumor angiogenesis, or new blood vessel development.[4] In addition to supplying conduits for oxygen, nutrients, and growth factors to support a tumor's development, angiogenesis lays out a path for metastatic cells. Other researchers have homed in on a specific subgroup of TAMs responsible for this angiogenesis, identified by their production of the protein Tie2.[5]

Tie2-producing TAMs also appear to act as chaperones for traveling tumor cells. While recording cell movement inside breast cancer tumors in live mice, John Condeelis and his team at Albert Einstein College of Medicine in New York recently found that Tie2$^+$ macrophages were always present as a cancer cell approached and entered a blood vessel. But the macrophages were doing more than just accompanying tumor cells. "When these macrophages and tumor cells started to approach the vasculature, they underwent this rather peculiar geometric transformation where they would form a pyramid-type structure on the vessel wall, and it had three cell types in it," Condeelis explains.

The pyramid always contained a Tie2$^+$ macrophage, a cancer cell overexpressing a protein called Mena, and a blood vessel endothelial cell, all three in contact with one another. This suite of cells, which the researchers called a tumor microenvironment of metastasis (TMEM), had to be present for tumors to metastasize.[6] In the TMEM, Tie2$^+$ TAMs make vascular endothelial growth factor (VEGF), which signals blood vessel endothelial cells to separate and allow cancer cells to slip into the bloodstream.[7] In healthy tissues, Condeelis says, similar structures develop at sites where new blood vessel branches will bud. In breast cancer, "instead of generating a branch on the vessel, you generate a doorway on the vessel."

Last year, the researchers reported that treating mice with breast or pancreatic tumors with an inhibitor of Tie2 called rebastinib kept Tie2$^+$ macrophages out of the tumors, reduced the ability of cancer cells to enter nearby blood vessels, and improved the efficacy of two chemotherapy drugs.[8] Condeelis has also licensed the use of TMEMs to the Boston-based company MetaStat, Inc. as a clinical cancer biomarker that predicts whether breast cancer patients will go on to develop a recurrence with metastatic tumors.[9]

TAMs also appear to play a role at sites on the receiving end of tumor metastasis. A population of macrophages distinguished by their expression of the VEGF receptor Flt1 are more likely to be found at sites of metastasis than in primary tumors, Pollard's team found. When cancer cells traveling in the blood attach to a blood vessel wall, these macrophages, dubbed metastasis-associated macrophages (MAMs), are already waiting on the other side. In a study published in 2009, Pollard and colleagues used fluorescence microscopy to track tumor cells injected into the blood vessels of mouse lung slices and watched as the cells made contact with MAMs across the vessel walls.[10] When the team depleted MAMs, the number of tumor cells that got across the vessel wall into the tissue dropped by 50 percent.

Helping cancer cells in and out of blood vessels are just some of the steps in a complex cascade of several rate-limiting events required for successful metastases, says Pollard, but in recent years it's become clear that "macrophages enhance those rates."

Tumor protectors

TAMs don't just help cancers spread; they can also help tumors survive attacks from the immune system, and from currently available treatments. For example, a group at the Oregon Health & Science University recently reported that TAMs secrete a cytokine called IL-10 that prevents dendritic cells from activating antitumor T-cell responses.[11] In some cancers, TAMs also produce transforming growth factor beta (TGF-β), which promotes survival of a subset of anti-inflammatory regulatory T cells, further blunting antitumor T-cell attacks.

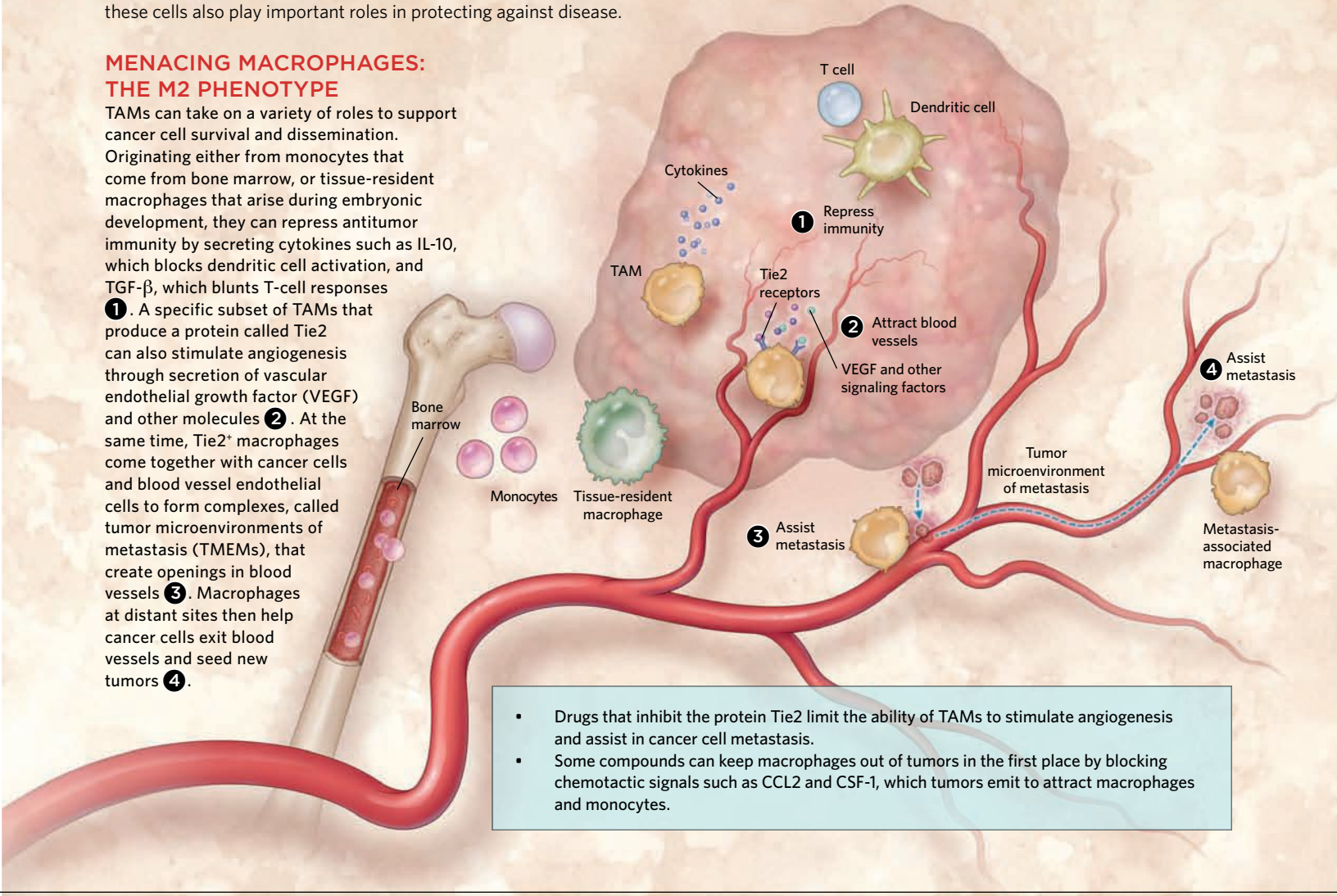
Damage caused by radiation and many types of chemotherapy can also stimulate an influx of new TAMs to tumors, where the cells release molecules that promote cancer survival. At least one molecule responsible for this protective effect, identified by cancer biologist Johanna Joyce and her team at the University of Lausanne in Switzerland in 2011, is a protein-chopping enzyme called cathepsin. The researchers reported that TAMs release cathepsin in response to chemotherapy drugs and that cathepsins reduced tumor cell death after treatment

TWO-FACED MACROPHAGES

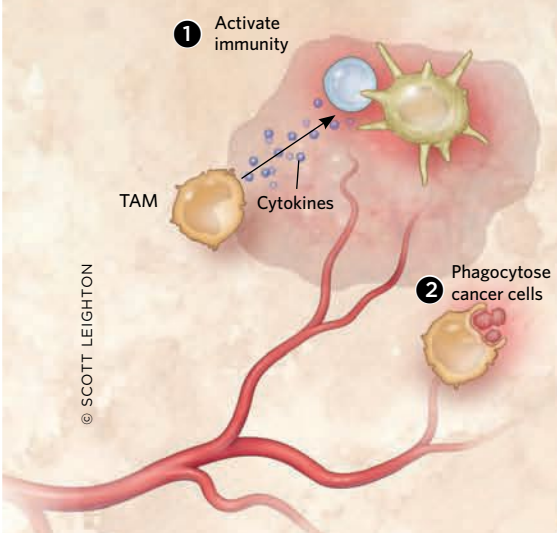
Tumors use chemokine signals to draw monocytes and tissue-resident macrophages into the tumor microenvironment, where the cells become tumor-associated macrophages (TAMs). Once believed to be wholly supportive of cancerous growth, these cells also play important roles in protecting against disease.

MENACING MACROPHAGES: THE M2 PHENOTYPE

TAMs can take on a variety of roles to support cancer cell survival and dissemination. Originating either from monocytes that come from bone marrow, or tissue-resident macrophages that arise during embryonic development, they can repress antitumor immunity by secreting cytokines such as IL-10, which blocks dendritic cell activation, and TGF-β, which blunts T-cell responses ❶. A specific subset of TAMs that produce a protein called Tie2 can also stimulate angiogenesis through secretion of vascular endothelial growth factor (VEGF) and other molecules ❷. At the same time, Tie2$^+$ macrophages come together with cancer cells and blood vessel endothelial cells to form complexes, called tumor microenvironments of metastasis (TMEMs), that create openings in blood vessels ❸. Macrophages at distant sites then help cancer cells exit blood vessels and seed new tumors ❹.



- Drugs that inhibit the protein Tie2 limit the ability of TAMs to stimulate angiogenesis and assist in cancer cell metastasis.
- Some compounds can keep macrophages out of tumors in the first place by blocking chemotactic signals such as CCL2 and CSF-1, which tumors emit to attract macrophages and monocytes.



TUMOR-KILLING TAMS: THE M1 PHENOTYPE

TAMs have the potential to aid antitumor immune responses by presenting cancer cell antigens to T cells and producing cytokines that activate dendritic cells and T cells ❶. Macrophages are also experts at phagocytosing and degrading foreign cells, including cancer cells ❷.

- Stimulation with cytokines or immune agonists can reprogram TAMs and coax them toward the proinflammatory, phagocytosing M1 phenotype. Lately, epigenome-altering drugs have also been used to skew TAM phenotypes toward M1.
- Antibodies and peptides that block the cancer cell "don't eat me" signal CD47 give TAMs free reign to phagocytose cancer cells. Blocking the inhibitory protein PD-1 on TAMs also increases the cells' phagocytic activity.

with the chemotherapy drug paclitaxel (Taxol). Last year, they found that as-yet unidentified TAM-secreted molecules interfere with the ability of paclitaxel to induce DNA damage and to block mitosis in cancer cells.[12]

TAMs may also thwart cancer immunotherapies. Recently, Harvard's Pittet and his team caught TAMs in the act of sequestering an anti-PD-1 treatment, which is meant to bind and activate tumor-killing T cells.[13] Using high-resolution imaging in live mice, Pittet's team found that within an hour of treatment, TAMs used antibody-binding receptors to steal the drug from the surface of T cells. When the researchers treated mice with antibodies that block the receptors on the TAMs before anti-PD-1 treatment, the drug remained on T cells at least twice as long, and the animals' tumors shrank more over 10 days of treatment.

With so many ways that TAMs protect tumors, it's no wonder many groups have found that blocking or depleting the cells in cancer models can improve T-cell responses and enhance the effect of cancer immunotherapy. For example, Mountain View, California–based biopharmaceutical company ChemoCentryx has developed a compound called CCX872, which blocks a receptor called CCR2 that monocytes use to find their way into areas of chronic inflammation. At the meeting of the American Association for Cancer Research last year, the company reported that in mice with pancreatic cancer, CCX872 treatment not only kept monocytes out of tumors, it also enhanced the animals' responses to anti-PD-1 therapy. The company's CEO Tom Schall says ChemoCentryx is currently designing another study to test this combination in humans. (See "Make Me a Match" on page 32.)

Drugs that target chemokine interactions have great potential for peeling away the immunosuppressive effects of TAMs and improving patient response rates to therapies that activate T cells, Schall says. "I think this is an idea whose time has come."

The good TAMs

Even as therapies that block TAM activity or prevent macrophage recruitment to tumors reach clinical trials, many researchers are not ready to give up on what macrophages may have to offer in the fight against cancer. Weissman's work on CD47 is a prime example of TAMs' cancer-killing potential.

DOUBLE AGENTS: TAMs with the M2 phenotype promote cancer progression, while M1 TAMs suppress it.

NIAID/FLICKR

Since his initial discoveries, Weissman has focused on macrophages' innate drive to eat damaged and dying cells, and he's found that many cancers display an "eat me" signal—a molecule called calreticulin, which marks the cells for phagocytosis.[14] But even if a cancer cell has "eat me" written all over it, presentation of CD47 can save it by engaging an inhibitory macrophage receptor called signal regulatory protein alpha (SIRPα). SIRPα blocks the molecular pathway that macrophages use to rearrange their structure and wrap themselves around the cells targeted for destruction. Weissman's team has published a suite of papers showing that masking the "don't eat me" signal can set macrophages loose against tumors in mouse models of AML, non-Hodgkin lymphoma, pancreatic cancer, and small cell lung cancer, as well as three different types of pediatric brain tumors. Like Forty Seven, Inc., Alexo Therapeutics and Trillium Therapeutics are preparing for Phase 2 trials of CD47-binding antibodies and fusion proteins, either alone or in combination with other drugs, including several immunotherapies.

Weissman says SIRPα is not the only gatekeeper molecule for macrophage phagocytosis. His group recently reported that some TAMs from human and mouse colon tumors display PD-1, the surface protein targeted by anti-PD-1 therapies to boost T-cell responses, and that these cells are worse at phagocytosing tumor cells than TAMs that don't display PD-1, suggesting that PD-1 may be a second TAM gatekeeper.[15] Indeed, the team reported that knocking out the PD-1 ligand in colon tumors in mice increased the phagocytosis activity of TAMs.

In some cases, Weissman argues, macrophages may aid cancer immunotherapies. For example, antibodies against CD20, which are used as an immunotherapy for some lymphomas, bind cancer cells in vitro and act as a tag that signals macrophages to engulf them.[16] And his team has found that anti-CD47 synergizes with immunotherapeutic antibodies against CD20, the breast cancer marker HER2, and the lung cancer marker epidermal growth factor receptor in mouse models of each cancer type.[17]

Pollard says there's no question that macrophages can participate in antitumor responses, "it's just that the tumors develop a way of polarizing or educating those macrophages to help [the tumors] rather than destroy them."

Recruit and re-educate

Many researchers are now taking advantage of macrophages' plasticity to re-educate the cells to work for the patient. One way to switch TAMs from what's known as the M2 phenotype, which promotes cancer growth, to the immune-boosting M1 phenotype is to provide the cells with proinflammatory stimuli, such as interferons or ligands for Toll-like receptors. Alternatively, researchers can directly target molecular switch proteins responsible for driving M2 characteristics, such as PI3-kinase and the transcription factor STAT3. In animal models, drugs that inhibit these molecules have successfully skewed TAMs toward M1 phenotypes and shrunk tumors.[18,19]

Taking a slightly different approach, a group at the Karolinska Institute in Sweden recently described success using a drug that targets a surface protein called macrophage receptor with collagenous structure (MARCO), which is preferentially displayed by immunosuppressive M2 TAMs from several types of mouse cancers and human melanoma and breast cancer. The researchers found that in a mouse model of breast cancer, the treatment shifted the balance of macrophages to favor M1s, promoted T

cell–dependent immune responses, restricted the size of the animals' tumors, and reduced the incidence of metastasis.[20]

Yet another strategy involves altering TAM epigenetics. Last year, a team led by researchers at Dana-Farber Cancer Institute in Boston and GlaxoSmithKline in Cambridge, Massachusetts, published findings concerning TMP195, a drug that inhibits histone deacetylases. Testing it in a mouse model of breast cancer, the team found that the treatment caused more macrophages to migrate into tumors, and that most of these cells did not take on an M2 phenotype; instead, they set to work phagocytosing cancer cells.[21] Although the role of histone acetylation in macrophage function remains unclear, "it seemed like these macrophages were converting to what could be an antitumor phenotype," says Dana-Farber immunologist Jennifer Guerriero. Sure enough, treatment with TMP195 alone or in combination with chemotherapy or anti-PD-1 antibodies significantly slowed tumor growth in the animals.

> It seemed like these macrophages were converting to what could be an antitumor phenotype.
>
> —Jennifer Guerriero, Dana-Farber Cancer Institute

As researchers strive to develop drugs that can shift tumors' macrophage makeup toward the M1 phenotype, however, they're learning that the M1/M2 distinction is a bit oversimplified. "M1 and M2 have been used for a long time now and have been a successful way to show how plastic the cells are," says Pittet. But M1 and M2 are extreme ends of a spectrum. "The in vivo reality is very different. There may be cells that have both phenotypes. There may be some that have neither phenotype but are still very important."

TAM function may also differ depending on the cells' location within a tumor or on whether they are derived from circulating monocytes or tissue-resident macrophages. Last year, a group at Washington University School of Medicine in St. Louis reported that TAMs from a mouse model of pancreatic cancer contained both, and that tissue-resident TAMs were more likely to contribute to tissue remodeling to facilitate tumor growth.[22] Joyce's team also recently reported that both bone marrow–derived macrophages and brain-resident microglia contribute to the TAM population within mouse and human brain cancers, but that the two cell types could be distinguished by their gene activation profiles.[23] "We know there are multiple different populations of [tumor] macrophages," she says. "They potentially have quite distinct functions."

Joyce adds that it's important to understand how these different TAM subsets influence responses to cancer therapies. For example, she says, it's possible that some drugs target multiple macrophage types when targeting just one might be better. "That's the challenge that we have going forward as a field."

Kaylee Schwertfeger, a pathologist at the University of Minnesota, agrees. "In order to harness their antitumor capabilities, we need to be able to understand the different subtypes in their contexts."

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The Literature

ONCOLOGY

Wolf in Sheep's Clothing

THE PAPER

S.F. Bakhoum et al., "Chromosomal instability drives metastasis through a cytosolic DNA response," *Nature*, 553:467-72, 2018.

Aneuploidy—the presence of abnormal numbers of chromosomes in a cell—is associated with cancer metastasis, but scientists have struggled to connect the mechanistic dots underlying the phenomenon. To explore the association, a team of researchers led by Lewis Cantley, a cancer biologist at Weill Cornell Medicine, and Sam Bakhoum, a radiation oncologist at Memorial Sloan Kettering Cancer Center, recently injected chromosomally unstable breast and lung cancer cells into mice, and saw that the cells were more likely to metastasize than cells in which chromosomal instability was suppressed. To the researchers' surprise, they also observed a heightened inflammatory response in the chromosomally unstable cells even before they were injected into the mice.

These findings led the scientists to examine whether the cells had an innate immune response to cytosolic DNA. Ongoing segregation errors in cancer cells can allow chromosomes to leak from the nucleus into the cytosol, forming "micronuclei" that expose naked DNA to the cytosol when they rupture. The research team first compared genomic integrity—a proxy for chromosomal stability—of primary tumors and metastases in data from a 2015 study, and found more instability in the metastases. They then transplanted metastatic cancer cells with chromosomal instability into mice, and found that an antiviral immune response called the cGAS-STING pathway was chronically switched on in the cells.

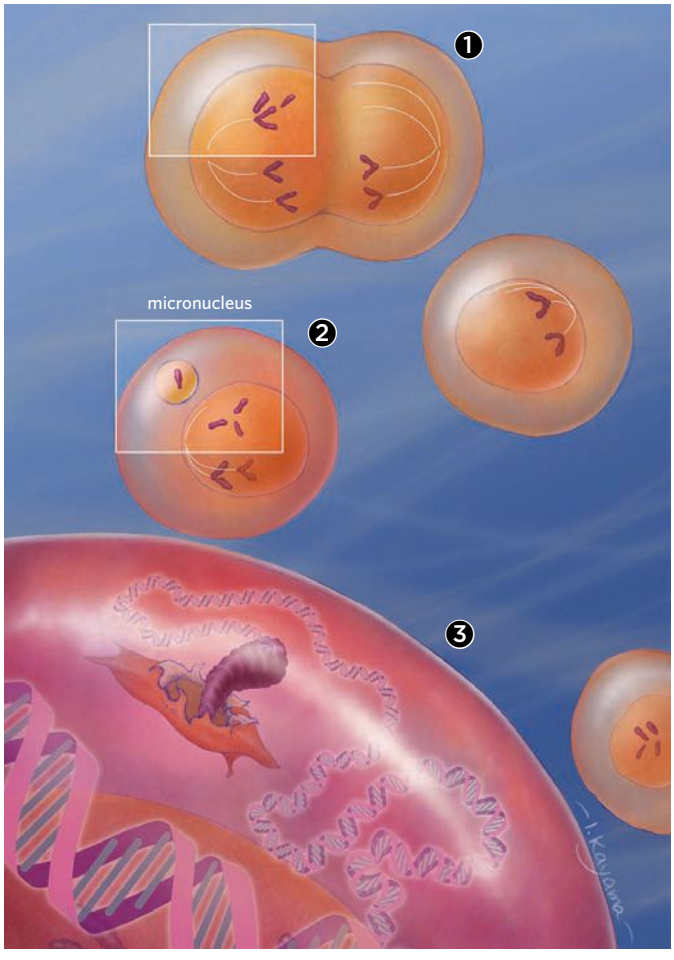
Normally, epithelial cells immediately die when cGAS-STING signals are expressed. But Bakhoum says metastatic cancer cells may adapt not just to survive the activation, but to use it to their advantage. That's because the same cytosolic DNA–activated pathway mediates the migration of macrophages and other immune cells to an area apparently under viral attack. This raises the possibility that cancer cells are "reacting to cytosolic DNA like immune cells rather than like normal epithelial cells," he says, enabling them to metastasize to distant sites.

"The analogy I like to use is [a] wolf in sheep's clothing," adds Cantley. Testing for cytosolic DNA in a primary tumor could "be predictive of who's going to metastasize," he says.

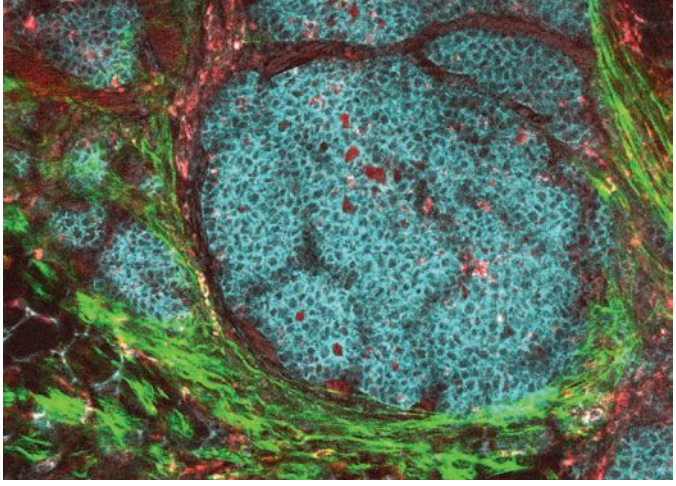
Virginia Tech cell biologist Daniela Cimini, who has collaborated with Bakhoum in the past but was not involved in this study, says it raises a red flag for one therapeutic avenue. "A lot of labs right now are focusing on possibly increasing chromosomal instability as a therapeutic strategy for cancer," because such instability appears to disrupt tumor formation and early progression, Cimini tells *The Scientist*. "If [chromosomal instability] promotes metastasis, now we're in trouble."

Cantley says the study may also have implications for the use of current treatments, such as radiation and chemotherapy, that also induce chromosomal instability. "We . . . have to be cognizant of the possibility that many of our therapies for primary tumors are probably actually increasing the probability of metastasis."

—Jim Daley



BREAKING FREE: When a chromosomally unstable cell divides, its chromosomes can become disordered during anaphase ❶. Errors in segregation can allow chromosomes to leak into the cytosol, where they form "micronuclei" ❷, which trigger an inflammatory response in the daughter cell ❸. This response can lead to metastasis.



GETTING DEFENSIVE: Tumors (cyan) create a cozy microenvironment to protect themselves from the immune system.

CANCER IMMUNOTHERAPY

Blocking the Signal

THE PAPER

Y. Nie et al., "Blockade of TNFR2 signaling enhances the immunotherapeutic effect of CpG ODN in a mouse model of colon cancer," *Sci Signaling*, 11:eaan0790, 2018.

DEFENSIVE PARAMETER

Cancers are notorious for creating a no-fly zone around themselves—called the immunosuppressive tumor microenvironment—that is hostile to immunotherapy treatments. Determining ways to turn off immunosuppressive actors such as tumor-infiltrating regulatory T cells (Tregs) is vital to making immunotherapies more effective.

FRIEND OR FOE?

Conventional wisdom has long held that tumor necrosis factor (TNF) receptor type II (TNFR2) downregulates Treg function, says Xin Chen, a cancer researcher at the University of Macau. But his group had found that TNFR2 in fact acts with TNF to activate, expand, and stabilize the most immunosuppressive type of Tregs, and that it tends to be highly expressed in invasive and metastatic lung cancers. In a new study, Chen and colleagues blocked TNFR2 on Tregs.

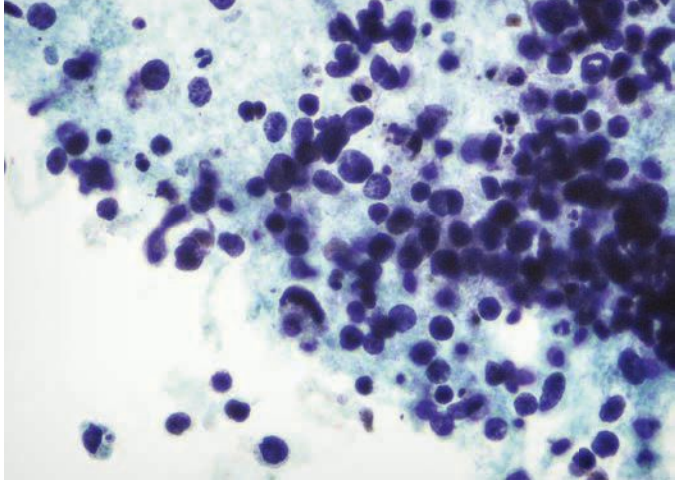
THE BLOCKADE

Chen and his colleagues then treated mice that had mouse colon tumor cells grafted under their skin with a TNFR2-blocking antibody called M861 and a low dose of CpG oligodeoxynucleotides (ODN), an anticancer drug. Compared with mice given just CpG ODN, those treated with both drugs had fewer Treg cells, a greater immune response to the tumor, and longer tumor-free survival.

DOUBLING UP

"[The researchers] show the immense value of inhibiting TNFR2 for getting better survival of murine tumors," says Denise Faustman, an immunobiology researcher at Massachusetts General Hospital. The study suggests, she says, that combining an immunostimulant with a drug that targets tumor-infiltrating Tregs "in effect, results in permanent tumor immunity."

—Jim Daley



DANGEROUS DIMERS: Linking mutant KRAS proteins with normal partners can make lung cancer (dark splotches) resistant to anticancer drugs.

ONCOLOGY

Deadly Combination

THE PAPER

C. Ambrogio et al., "KRAS dimerization impacts MEK inhibitor sensitivity and oncogenic activity of mutant KRAS," *Cell*, 172:857-68.e15, 2018.

BAD ACTOR

Genes in the RAS family regulate cell growth and differentiation, and mutations can render them oncogenic. One such proto-oncogene, *KRAS*, frequently turns up in human cancers, including lung cancer, and is associated with resistance to chemotherapies including MEK inhibitors.

PAIRING UP

Some proteins encoded by RAS genes appear to function as dimers—linked pairs of identical molecules. Pasi Jänne, a medical oncologist at Dana-Farber Cancer Institute, used a fluorescence resonance energy transfer (FRET) assay to find that the KRAS protein does, too. They then fashioned a mutant KRAS that was dimerization-deficient.

PARTNERS IN CRIME

Jänne and colleagues compared tumor development in mice with one copy of oncogenic KRAS and one copy of either wild-type KRAS or one that couldn't dimerize. The mice with dimerization-deficient KRAS fared much better, suggesting that oncogenic KRAS must dimerize with wild-type KRAS to function pathogenically.

GETTING IN THE MIDDLE

"Most of the efforts so far on KRAS-mutant cancers have focused on trying to directly target KRAS itself, which has been a challenge, or to target immediate KRAS effector pathways," says Jänne. Therapeutically targeting KRAS dimerization instead would be mutation-independent and pathway-specific, he says.

Marie Evangelista, an oncology researcher at Genentech, notes that the strategy comes with its own hurdles. "It's unclear whether there are going to be any small molecules that can target that interface" between KRAS monomers, she says. "We're going to need to have a better understanding of how that interface is formed to find out if there are any opportunities to really go after it."

—Jim Daley

JOSEPH SZULCZEWSKI, DAVID INMAN, KEVIN ELICEIRI, AND PATRICIA KEELY, CARBONE CANCER CENTER AT THE UNIV. OF WISCONSIN, NATIONAL CANCER INSTITUTE, NATIONAL INSTITUTES OF HEALTH; © ISTOCK.COM/OGPHOTO

Cancer Evolutionist

Motivated by his father's cancer diagnosis, Charles Swanton has been revealing the ways tumors evolve and why they are so difficult to treat.

BY ANNA AZVOLINSKY

In 1993, Charles Swanton's clinical training was set to commence. He had just finished his preclinical work at St. Bartholomew's and the Royal London Medical School when the 21-year-old opted to complete a yearlong cellular pathology program at University College London (UCL) Medical School and earn his bachelor's degree.

There, learning about prior discoveries in cell biology from his professors, Swanton got a taste for the pursuit of scientific discovery. "I vividly remember the first lecture. The professor was setting up the overhead projector and slides, and I was preparing for two hours of tedium. Then, 30 minutes into telling us about how cells move, I was completely mesmerized," recalls Swanton, now a cancer researcher at the Francis Crick Institute in London. The experience changed the course of his career: he wanted to not only treat patients but also make his own scientific discoveries.

I think it is harder to be a successful scientist without experiencing truly prolonged failures.

In the 1990s, researchers were beginning to understand the ways cells regulate transitions into the four phases of the cell cycle: G1, synthesis (S) phase, G2, and mitosis. One of the major figures moving this field forward was future Nobel Prize–winning geneticist Paul Nurse, who at that time was director of research at the Imperial Cancer Research Fund (ICRF, now Cancer Research UK). Having joined Nic Jones's group at ICRF as a UCL graduate student in 1994, Swanton heard Nurse give a lecture on this cell-cycle work. Swanton was stunned to learn how similar fundamental cellular processes were between humans and yeast, and how cell-cycle regulation is related to cancer development.

According to Swanton, his first 18 months in Jones's lab were "an unmitigated disaster." He had been trying to understand the functions of three related cyclin D family proteins, important for directing early cell-cycle transitions. But despite long days and late nights at the lab bench, he failed to produce any results. One Friday, Swanton recalls, his graduate advisor told him to hand in his midterm progress report on Monday morning. Soon after, with apprehension, he presented his failed experiments to his graduate committee, who recognized his efforts but also hinted that it might be time for Swanton to call it quits on his PhD degree.

"I remember thinking, 'I am in so deep already, and I enjoy being in the lab and addressing problems relevant to human disease. What have I got to lose by plowing on?'" His committee asked him what he wanted to do and, quick on his feet, Swanton thought of a new project so that he could continue his PhD.

Cyclins form complexes with cyclin-dependent kinases (CDKs), unique to each cell-cycle phase, and activate specific genes to drive cells through their cycles. Swanton proposed to explore how one such protein, cyclin D, interacts with proteins called p21 and p27 that bind to the cyclin-CDK complexes and can inhibit progression.

With the help of Jones, structural biologist Neil McDonald, and cancer researcher Gordon Peters, Swanton spent the summer of 1994 mutating every surface amino acid of the human cyclin D protein individually to create a library of cyclin D–mutated proteins. He then measured the ability of his 40 cyclin Ds to interact with the inhibitory proteins in vitro. One mutant could still bind to its protein-binding partner, a CDK, but not to wildtype p21 or p27, suggesting that the mutation resulted in an always-active complex that drove constant cell division—a hallmark of malignant cells. "This was my first and only result in two years and made me realize again how fun science was," says Swanton.

He then stumbled upon a paper that had identified a cyclin-like protein in a group of herpesviruses that could induce malignancy upon infecting mammalian cells. Swanton aligned the sequences of his mutated human protein and the viral one and found that they were nearly identical within the domain he had altered. Swanton decided to compare his mutated cyclin D to the viral protein in a test tube. He still remembers getting the result on a Saturday morning; the abilities of the two proteins to each bind to their CDK binding partners were the same, as were their inabilities to bind to either p21 or p27. "I called Nic on that Saturday to tell him the result. We both still talk about that phone call."

The work, published in *Nature*, presented a novel way mammalian virus proteins evolved, adapting to resemble those found in mammalian cells. In this case, the viral protein managed to deregulate the cell cycle and induce oncogenesis.

For Swanton, both the lows and highs of his graduate career were valuable. "I think it is harder to be a successful scientist without experiencing truly prolonged failures. Only when you've been through the terrible stuff do you learn to unravel a problem and develop resilience."

DISCOVERY ON THE BRAIN

Swanton was born in 1972 in Dorset, a southwest county in England on the coast of the English Channel, where his father was a cardiologist at a local hospital and his mother a historian. When he was two years old, his family moved to southwest London. "I was always into the outdoors—biking, cricket, and football—

THOMAS FARNETTI/WELLCOME IMAGES

CHARLES SWANTON

Chair, Personalised Cancer Medicine, University College London (UCL)
Co-Director, CR-UK UCL/Manchester Lung Cancer Centre of Excellence
Senior Group Leader, Translational Cancer Therapeutics Laboratory, Francis Crick Institute
Stand Up 2 Cancer Laura Ziskin Translational Cancer Research Prize (2015)
Fondazione San Salvatore Award for Cancer Research (Lugano, 2017)
Cancer Research UK Translational Cancer Research Prize (2017)

Greatest Hits

- Identified cyclins in herpesviruses that can stimulate continuous cell-cycle progression, providing a novel example of how viruses are able to hijack host machinery to induce malignancy in mammalian cells
- Discovered genes and cellular pathways that determine whether tumors are sensitive to certain chemotherapy drugs
- Demonstrated the prevalence of genetic heterogeneity and branched evolution within a single cancer by sequencing multiple regions from the same tumor
- Showed that the immune system can recognize trunk neoantigens found in all tumor cells and that this likely influences a patient's ability to respond to immune checkpoint inhibitor antibodies
- Revealed that the loss of the human leukocyte antigen (HLA) locus in lung cancers is a way these tumors evade the immune system and allow mutation expansion and branched evolution within tumor cells

although I was not good at either team sport," says Swanton. "One of the comments from a teacher in school went something along the lines of, 'Charlie's athletic contributions are rarely matched by his verbal ones.' I tended to talk a lot and not do very much," he says. "Some would say things haven't changed!"

Swanton also says he was not a great student, uninspired by the way even subjects he was interested in were taught. He liked biology but was keen on discovery and experiments rather than the scripted lectures and textbook material his teachers presented. In the evenings, Swanton liked to build with Legos and other building sets. "I think that science is a bit like Lego building. You build yourself an ever bigger and bigger model and hope that it remains standing," Swanton reflects.

After graduating from high school in 1990, Swanton took a year off and traveled. Afterward, he entered Bart's and the London School of Medicine and Dentistry and began his premed studies. His attraction to oncology was solidified in his first year, when his father was diagnosed with a high-grade B-cell lymphoma. After rounds of chemotherapy and radiation, Swanton's father was in remission, and 25 years later, still works in the UK's National Health Service at the age of 74. "It's really a remarkable story for 1991."

CANCER TREATMENT FAILURE

After receiving his PhD in 1998, Swanton went back to clinical training. Having developed "an addiction to the lab," he set out on the long path to becoming a physician-scientist.

Because of the many clinical training requirements in the U.K. Swanton didn't return to the lab bench for another seven years, practicing general medicine, surgery, and neurology before specializing in oncology. "I missed the bench massively," he says. "I enjoyed medicine, but it was treading water. I didn't feel we were making progress."

In 2004, he joined Julian Downward's lab at the Francis Crick Institute in part to understand why cancer patients become resistant to standard treatments. Using an RNA interference screen, Swanton and his colleagues identified a set of genes involved in the regulation of mitotic arrest and in the metabolism of ceramides, lipid molecules abundant in cell membranes that influence whether tumors are sensitive to certain chemotherapy agents. The work also showed that tumors with high chromosomal instability, which can lead to tumor cell diversity through chromosomal rearrangements, are least sensitive to these chemotherapies.

The project demonstrated to Swanton that lab work can inform why cancer drugs fail. "Cancer cells have this fast way of

gaining and losing whole chromosomes, adapting in the face of cancer therapy," says Swanton.

In 2008, Swanton set up his own lab at the Francis Crick Institute to study how chromosome instability can occur and how cancer cells can tolerate the genetic chaos that causes normal cells to self-destruct. (See "Wolf in Sheep's Clothing, page 52.) Again, his first project didn't go as well as he might have hoped.

THE EVOLUTION OF A CANCER

Swanton's new lab set out to identify specific genes that, when inhibited, result in the death of tumor cells that displayed aneuploidy, meaning they had more or less than the normal set of 46 chromosomes. But Swanton never found any such genes.

His first success came in 2012, when his lab provided an explanation of why cancer is such a difficult disease to eradicate. Swanton and his team took biopsies from four kidney cancer patients at various locations within the same tumors, and from metastases, at different times during their course of treatments. When the researchers sequenced the samples for genetic mutations and analyzed chromosome structure, they could trace the tumors' evolutionary histories, much as evolutionary biologists trace the origins of organisms back to their common ancestors based on fossils deposited in different geologic eras.

Swanton dubbed the founding mutations in the original tumor that persist in most tumor cells "trunk" mutations, and subsequent alterations, present in only a proportion of tumor cells, "branches." In all four patients, the investigators identified two lineages, one that seeded the metastasis from the original tumor and the other that allowed the original tumor to grow in place.

"It is very important in science not to claim you were the first in anything. The old adage that we stand on the shoulders of giants is so true. But I think the [tumor evolution] study was a bit of a wake-up call. People were relying very heavily on single-cell analysis to derive tumor information," says Swanton. "We showed that a single sample really dramatically underestimates the evolutionary complexity of a patient's disease." The research could also explain why informative cancer biomarkers are difficult to identify—the tumors transform and change too much—and why rapidly mutating tumors find ways to grow despite aggressive treatment.

Swanton's lab has since followed the work with comprehensive spatial and temporal genetic analyses of other tumor types, including colorectal cancer, showing the ways that faulty DNA replication promotes chromosomal instability in cancer. For Swanton, "these studies led to the idea that integrating genomics and cell biology could start to inform mechanisms of disease and ways to target those mechanisms with therapies."

A TURN TO IMMUNOTHERAPY

As the cancer field saw successes with immunotherapies that could boost patients' immune responses to cancer, Swanton turned to studying how heterogeneity within a tumor influences its interaction with the immune system. His lab demonstrated that neoantigens (mutated proteins unique to cancerous cells) present in most or all cells within a tumor are much more likely to be effectively recognized by the immune system. Additionally, the greater the number of these trunk neoantigens, the more likely the patient will respond to immune checkpoint inhibitor therapy, an antibody-based intervention that unleashes T cells to attack tumors.

Swanton is now focused on identifying the important trunk mutations present in most tumor cells. And, as a cofounder of Achilles Therapeutics, he's working to commercialize these discoveries into adoptive, cell-based therapies.

This past October, led by postdoc Nicholas McGranahan and graduate student Rachel Rosenthal, Swanton's lab found one way that lung cancers evolve to escape detection by the immune system. Forty percent of patient-derived tumors his team examined had tumor cells that stopped producing human leukocyte antigen (HLA)—a molecule necessary for the presentation of antigens on a cell's surface, to be recognized by immune cells. The loss of HLA often occurred relatively late in the tumors' evolution and resulted in an expansion of neoantigens within the tumors, predicted to bind to the lost HLA allele. If the immune system has no way to detect cancer-specific antigens, immune cells won't be able to mount an attack.

"This loss-of-HLA mechanism suggests that, when designing vaccines and cell-based therapies, we need to target antigens that are presented to the immune system," says Swanton. "The knowledge of which HLA molecules are lost will be critical to develop such effective therapies and choose the right antigens to target."

Swanton's goal is to map tumor evolution and adaptation over space and time. His plan is to sample thousands of tumors from hundreds of cancer patients across their disease course to track where immune checkpoint signaling molecules are distributed. The team will capture the tumor samples' genomic and transcriptomic data as well as the clinical outcomes and drug responses of each patient, starting with 842 participants and the already-collected tumor biopsies from 3,000 tumor regions among them. Those data are part of the Tracking Cancer Evolution through therapy (TRACERx) program, which recruited Swanton's patients with lung cancers. Swanton and his colleagues have expanded the program to include renal cancer and melanoma patients.

FINDING INSPIRATION

When not in the lab, Swanton spends time with his family, including his two daughters, ages 14 and 11, cycling, playing with their dog, or going to museums. His wife is an academic in gynecology, so dinner-table conversations typically turn to science and medicine.

Recently, Swanton gave a talk at his older daughter's school on his recent work that uncovered HLA loss as a way tumors avoid being recognized by the immune system. He mentioned that tumor cells will perish if they lose all six copies of their HLA genes.

"At the end, a girl stood up and asked whether we should be targeting the HLA molecule in the tumor as a way to kill tumors, since tumors cannot lose all of their HLA genes. Here is a young student that applied interest and logic to a problem and came up with a solution—one that, I must confess, I hadn't considered deeply enough until she asked me," says Swanton. "That was a wonderful light-bulb moment that I witnessed. I think there is a scientist in all of us that is just waiting to be inspired." ■

Ilana Chefetz: Cancer Adversary

Assistant Professor, Hormel Institute, University of Minnesota, Age: 40

BY JIM DALEY

Ilana Chefetz isn't someone who backs down from a challenge. During her mandatory service in the Israeli military police back in 1997, a male colleague working with her to investigate a motorbike theft opined that young women were not cut out to do police work. Chefetz remembers thinking, "I have to prove to this guy that girls can also arrest people and do whatever job is necessary." So when the pair finally tracked down the suspect, Chefetz jumped out of the car, grabbed the thief, and restrained him until more officers arrived.

Nowadays, Chefetz brings that same tenacity to her research. Although her current work is focused on identifying novel methods for treating ovarian cancer, this topic was far from Chefetz's mind while earning her bachelor's at Technion, the Israel Institute of Technology in Haifa. While looking for a "practical" subject with solid job prospects, Chefetz says, she decided to major in food engineering and biotechnology. This ultimately sparked her interest in biology, and led to her to continue to study those subjects for her master's degree, which she obtained at the same institution.

Chefetz stayed at the Israeli Institute of Technology for her PhD, but decided to switch to more clinically oriented research and joined dermatologist Eli Sprecher's lab to study familial tumoral calcinosis (FTC). At the time, researchers knew there was a genetic basis for the rare disease, which results in debilitating, tumor-like deposits of calcified phosphate in soft tissue, but the causative mutations had not yet been identified. By screening relatives of patients with FTC, Chefetz and Sprecher discovered that the condition was linked to mutations in *SAMD9*, a gene potentially involved in injury-associated inflammation.[1]

Motivated in part by a family history of cancer, Chefetz began studying cancer stem cells in 2009 as a postdoc at Yale University with Gil Mor, a reproductive sciences professor who focuses on ovarian cancer. At the time, the idea that stem cells were present in tumors was still new, Mor says, but the fact that Chefetz had come from another field allowed her to generate "all sorts of innovative ideas and a different perception of [cancer]." In one study, she discovered that inhibiting Aurora-A kinase, an enzyme involved in healthy cell proliferation that is often overexpressed in tumors, could decrease the spread of ovarian cancer in vitro.[2] In another project, she found that the transcription factor TWIST1, which regulates aspects of embryonic development, is constitutively degraded in human cancer cells.[3]

After her postdoc, Chefetz moved to the University of Michigan to work as a research fellow with oncologist Ron Buckanovich. There, in a quest to develop targeted therapies for ovarian cancer, she began studying necroptosis, a form of programmed cell death. "We used to think . . . necroptosis happens due to injury, ischemia, inflammation, and so on," Chefetz says. But recently, she adds, scientists realized that this process could also happen in a regulated manner—allowing researchers to probe this pathway to identify novel ways to combat cancer. "We would like to accelerate necroptosis to kill as many cancer stem cells [as possible]," she says. "I'm trying to learn all the downstream targets and find what inhibitors we can combine . . . to accelerate cell death."

Chefetz is continuing her search for necroptosis-based targeted therapies for ovarian cancer at the University of Minnesota, where she accepted an assistant professorship last year. "Ilana has taken a really creative approach [to fighting cancer]," says Costas Lyssiotis, a biochemist at the University of Michigan and one of Chefetz's collaborators.

Currently, she is investigating how inhibiting aldehyde dehydrogenase can kill ovarian cancer stem cells by inducing necroptosis. According to Mor, Chefetz's research is "really going to change the way we understand this disease." ■

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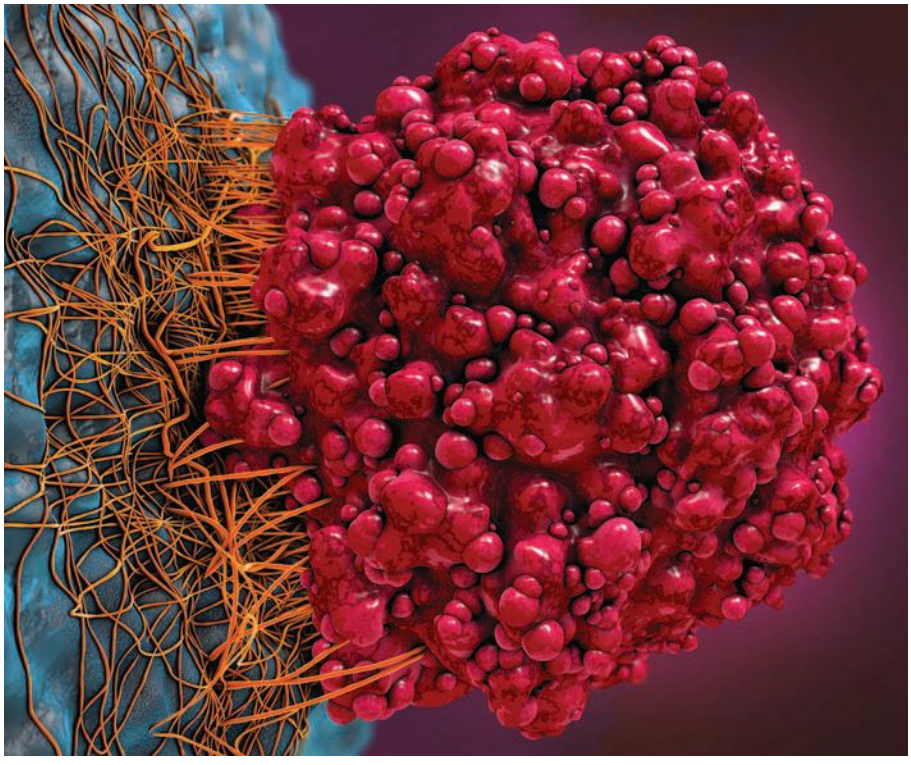
RAU + BARBER

Modeling Metastasis

Choosing the right models for studying cancer's spread

BY AMANDA B. KEENER



Although metastasis is responsible for 90 percent of cancer-related deaths, it's one of the least-studied aspects of cancer—perhaps because it is one of the trickiest to investigate.

Metastases can be established in mice either by injecting cancer cells into organs or the bloodstream, or by using animals genetically engineered to spontaneously develop tumors that then metastasize on their own. Injected cells may come from mutated cell lines, spontaneously grown animal tumors, or cancerous human tissues.

"Each one's profoundly different and all have potential value," says John Condeelis, who studies metastasis at Albert Einstein College of Medicine in New York. Yet the frequency of metastases in many animal models is low, and each method used to model the metastatic process can only recapitulate some of the steps.

The Scientist spoke to researchers confronting these challenges about how to choose a metastasis model and about new tools that are making it possible to study cancer's spread in more detail than ever before.

CELL LINE AND HUMAN CELL TRANSPLANTATION

Cell injection into the bloodstream or into an organ is the most common approach to modeling metastasis. Injected cells are useful for studying the effects of drugs on metastasis and the mechanisms that control how cells home. To avoid any bias in where cancer cells migrate, Sheila Singh, a cancer biologist at McMaster University in Ontario, injects malignant cells directly into the heart to give them access to the entire animal. However, most metastasis researchers agree that it's best practice to match injected cells to the organ in which they would have formed a primary tumor and wait for the cells to migrate to a secondary site from there. For example, metastatic melanoma cell lines can be injected into a mouse's subcutaneous skin layer and will subsequently migrate to the animal's lungs.

This approach can be hampered by the low frequency of metastatic events in mice. As few as 0.01 percent of cancer cells make it out of a primary tumor and seed a new one. But many commercially available cancer cell lines have already been enriched for the most highly metastatic cells through repeated injection into primary sites followed by harvesting from metastatic sites.

Both Condeelis and Singh caution that years or even decades of passaging cells in culture can introduce genetic changes and contamination that may alter the cells' behavior or identity (see "The Great Big Clean-Up," *The Scientist*, September 2015). One alternative to relying on commercial cell lines is creating new ones from primary animal or human tissues. "Those tumors tend to be more realistic," says Condeelis. Singh's group does this regularly, she says, but, "even then, our cells usually grow in culture for less than a year as opposed to ten or fifteen years."

The frequency of metastases in many animal models is low, and each method used to model the metastatic process can only recapitulate some of the steps.

COURTESY OF DAVID ENTENBERG AND JOHN S. CONDEELIS

OPEN WINDOW: The technique developed by John Condeelis and his colleagues makes it possible to attach a permanent window to a mouse's lung while allowing the animal to live out a normal life span.

Singh prefers to use patient-derived tumor xenograft (PDTX) models, in which human cancer cells are injected into animals. The process requires IRB approval and biosafety training, but she says it's worth the extra work because such experiments yield insight into characteristics specific to human metastatic cancer cells. "It's almost as if we're just using the mouse brain as an incubator for the tumor," she says. "We think that [using] human cells brings us one step closer to translation."

Singh's group receives human tumor tissue from brain cancer biopsies, which each yield about 5 million–10 million cancer cells. To enrich for tumor-initiating cells, the researchers either select for stem cells capable of forming spheres in culture or select for cells expressing a marker of tumor initiation called CD133.

In a study published last year, Singh's team injected human brain metastasis–initiating cells into mice and used RNA interference to identify two genes required for metastasis from the lung to the brain (*Acta Neuropathologica*, 134:923-40,

2017). They then examined tissue samples from primary lung tumors and found that one of the genes, *SPOCK1*, was only overexpressed in samples from patients whose lung cancer eventually spread to the brain, suggesting that the gene could be a biomarker for future metastasis.

PDTX recipient mice must be immunocompromised so that they don't reject patient cells. This limits the model's usefulness for studying immunotherapy drugs that work through activating T cells. To get around this, Singh says, it's possible to introduce matched human T cells along with tumor cells to initially test the efficacy of an immunotherapy drug, and then move into the more expensive humanized mouse models that have human immune systems.

SPONTANEOUS MODELS

For Condeelis, mice genetically altered to spontaneously develop tumors are the gold standard for studying the metastatic processes. These models offer insight into the earliest steps of metastasis, such as cancer cells' dissociation from primary tumors and entrance into the vasculature, as well as the roles of the microenvironment and the immune system in promoting metastasis. For example, Condeelis's team has described an important function of macrophages in escorting cancer cells to blood vessels and forming structures they call the tumor microenvironment of metastasis (*Cancer Discovery*, 5:932-43, 2015). The macrophages then release proteins that cause connections between blood vessel endothelial cells to loosen so that cancer cells can easily slip into the vasculature (see "Two-Faced Macrophages," page 49).

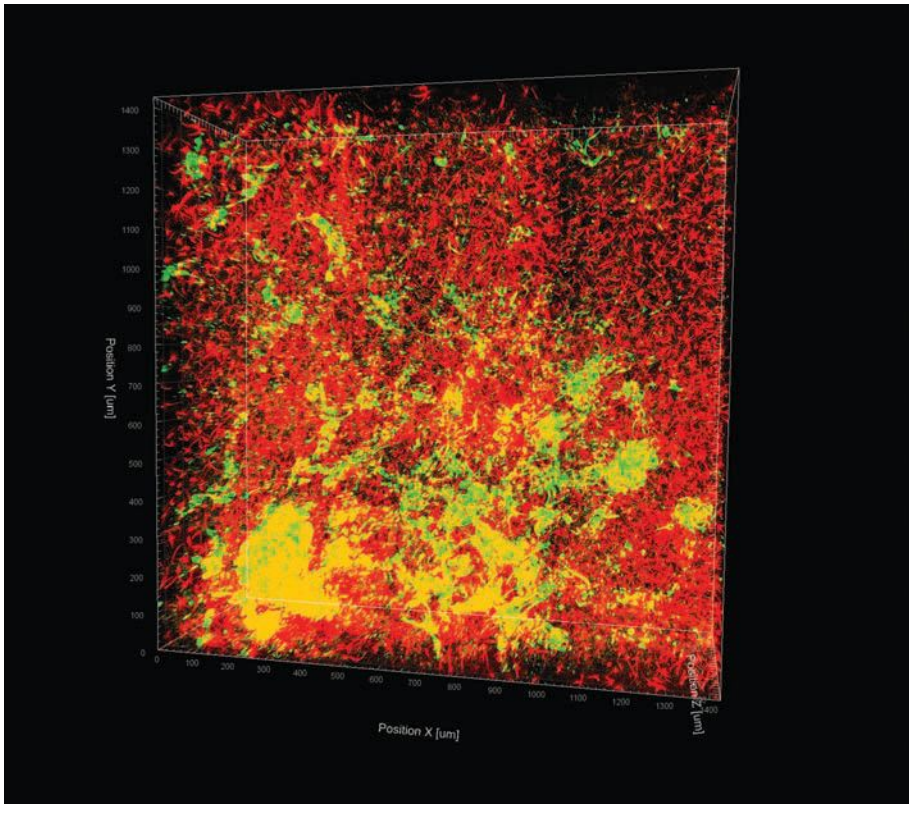
Several commercially available, genetically induced mouse lines exist for each tissue type, some of which develop more-aggressive cancers than others. There can also be variation in which organs cancer cells travel to among mouse lines with the same type of primary tumor, so it's important to choose a line that demonstrates metastasis to the organ of interest.

A downside of spontaneous models is that their tumors can kill an animal before they metastasize. This issue can be overcome by removing primary tumors once they reach 400-500 mm^3, which can mimic situations where metastases appear after surgical resection. With or without surgery, the time for a tumor to metastasize can vary greatly; some animals may develop metastases in a week, while others may take a month. Ultimately, a combination of approaches may be needed to validate findings. "In the end, all of these models are complementary," says Singh.

GETTING A VISUAL

Imaging plays a major role in metastasis research, regardless of the models used. Whether tracking individual cells' exits from primary tumors or measuring tumor volumes, choosing the right tools for the job is crucial. To get a broad view of metastases' growth and spread, many researchers apply the same techniques used for cancer patients, including computed tomography (CT), magnetic resonance imaging (MRI), positron emission tomography (PET), or single-photon emission tomography.

To count and analyze metastatic tumors in detail, says Siyuan Zhang, a tumor biologist at the University of Notre Dame in Indiana, many researchers harvest and section organs containing tumors. As a postdoc studying brain metastases, he found this method laborious and often inaccurate. For example, Zhang says, it's possible to count one tumor with a highly branched structure as several individual tumors. Now Zhang employs tissue clearing techniques, including one called CLARITY, that render tissues see-through while leaving structures intact (*Nature*, 497:332-37, 2013). Currently, he says, penetrating whole organs with antibodies to label tumors is the rate-limiting



IAN GULDNER AND SIYUAN ZHANG

SEEING CLEARLY: This 3-D reconstructed image shows astrocytes (red) and a breast cancer metastasis (yellow) within a mouse brain cleared with the CLARITY method.

step of the process and can take several weeks to complete. But the imaging is straightforward, requiring just a standard multiphoton microscope.

Zhang's biggest challenge is getting quantifiable information from three-dimensional imaging data. Teaming up with computational biologists, his lab recently developed a pipeline called spatial filtering-based background removal and multi-channel forest classifiers–based 3D reconstruction (SMART 3D) to quantify data from their whole-brain imaging experiments (*Sci Rep*, doi:10.1038/srep24201, 2016). Their algorithm, which is available on GitHub, segments foreground and background data, allowing researchers to resolve features of tumors that they can then quantify. For example, Zhang's team labeled brains with a marker of cell proliferation and compared features of highly proliferative metastatic tumors to those that were not actively growing. The scientists found that proliferation rates did not correlate with tumor size, and are now testing whether tumor proliferation is affected by environmental variables such as proximity to blood vessels. "It really takes imaging analysis beyond the descriptive—beyond the 'cool,'" says Zhang.

LIVING, BREATHING DATA

Fixed-tissue studies can only provide a snapshot of what's happening at one point in time. When studying the small-scale events of metastasis, such as cancer cell migration into and out of the vasculature, Condeelis says, "there's a real risk of making sweeping conclusions" based on endpoints alone. His team has developed techniques to image and track cancer cells in mammary tissue, abdominal organs, and brains of living mice, in real time. To study metastatic spread of breast cancer, they have now taken on one of the biggest challenges in live animal imaging—the lungs.

There are several obstacles to imaging lungs in live mice, "not least of which is the extraordinary mechanical movement of the lungs," says Condeelis. Mice take 120 breaths per minute, driving corresponding changes in lung shape and volume. To deal with this, researchers usually perform a terminal surgery that involves making a hole in an animal's rib cage and using a vacuum to hold the lung against a clear window. After imaging, the animal must be sacrificed, meaning each stage of metastasis must be imaged in a different animal.

It really takes imaging analysis beyond the descriptive—beyond the 'cool.'

—Siyuan Zhang , University of Notre Dame

Along with a team of engineers and surgeons, Condeelis's group recently developed a method to attach a permanent window to the lung tissue using a surgical adhesive. The window's rim attaches to the intact rib cage, but the rest of it moves as the lung moves. After the surgery, mice live comfortably for a normal lifespan. "In that single animal, you can put together a metastasis progression map," says Condeelis.

One of the first things Condeelis's team saw using this model was that when tumor cells arrive in the lungs, they immediately form a tumor microenvironment of metastasis structures, which the team had previously only seen in blood vessels surrounding primary tumors. This suggests that even before the metastatic tumor has grown to a clinically detectable size, it starts to spread.

Condeelis's group has trained a handful of postdocs and surgeons so far in the lung window procedure—an educational process that can take up to three months. He hopes to license the technique to a microscopy vendor that could offer training to customers so that the method could become more widespread. Live animal imaging, he says, can help resolve some of the outstanding questions in metastasis biology. ■

Targeting Cancer's Achilles Heel

Inhibitors of the PARP family of enzymes are making gains against historically hard-to-treat cancers.

BY VICKI BROWER

In 2005, researchers in the U.K. struck upon a new way to kill cancer cells. A London-based team led by Alan Ashworth, currently head of the University of California, San Francisco's cancer center, was working with cells harboring *BRCA* mutations—genetic perturbations that predispose humans to breast and other cancers. BRCA1 and BRCA2 proteins are part of the cell's homologous recombination (HR) machinery, and help repair double-strand breaks in DNA. When they are dysfunctional, cells accumulate mutations.

Ashworth and his team wondered whether *BRCA1* or *BRCA2* (*BRCA1/2*) mutations, in addition to making a cell susceptible to cancer, also made that cell more vulnerable in the event of further damage to its DNA repair machinery. So the researchers tried targeting a different pathway in these cells—one that repairs single-strand DNA breaks, and is mediated by a family of enzymes called poly(ADP-ribose) polymerases (PARPs).

At the time, small-molecule inhibitors of PARP enzymes were being tested as a way to increase the sensitivity of cancer cells to chemotherapy and radiotherapy. But when the researchers blocked PARP proteins in cells harboring *BRCA* mutations, the results were striking: the cells died on the spot. "They found an exquisite sensitivity—as much as 1,000 times greater, in *BRCA1/2*-mutant cell lines and xenografts—to PARP inhibition, compared with *BRCA* wildtype cells," says Timothy Yap, a cancer researcher at the University of Texas MD Anderson Cancer Center in Houston, who was not involved in the work, but receives funding from PARP inhibitor developers such as Pfizer and AstraZeneca.

This one-two punch—in which the loss of PARP and BRCA proteins, but not either one alone, is enough to kill the cell—is known as synthetic lethality, and its role in the 2005 findings "gave the impetus for PARP inhibitors to be tested in trials as single agents" in *BRCA*-mutated tumors, notes Christopher Lord, a cancer researcher specializing in genomics at the Institute of Cancer Research, London who holds patents on PARP inhibitors and has received payments for work with AstraZeneca and other drug developers. That same year, AstraZeneca began trials with a PARP inhibitor called olaparib (Lynparza); the drug was approved by the Food and Drug Administration (FDA) in late 2014 for advanced, pretreated *BRCA*-mutated ovarian cancer, and just this January, the same compound became the first drug to be approved for *BRCA*-mutated breast cancer.

A flurry of recent studies with PARP inhibitors have shown that the drugs can also kill cancer cells that harbor mutations in other genes involved in DNA repair processes. Such findings offer the hope of improving the prognoses of treatment-resistant cancers, including pancreatic and prostate cancer, and are changing the way researchers view these diseases.

Of course, it's not been all successes. The recent failures of several Phase 3 clinical trials have revealed cracks in

researchers' understanding of PARP inhibitors' exact mode of action. But with three PARP inhibitors FDA-approved, and at least four more in development, the sector is booming, and companies are jockeying for deals. Last July, Bristol-Myers Squibb announced plans to test Clovis Oncology's PARP inhibitor rucaparib (Rubraca) in combination with one of its own anticancer drugs in Phase 2 and 3 trials in multiple tumor types in the U.S. and Europe. The same month, Japan-based Takeda agreed to pay US pharma company Tesaro $100 million for the rights to its PARP inhibitor, niraparib (Zejula), approved in the U.S. a few months previously. In short, says Yale University radiologist Ranjit Bindra, "the PARP inhibition era is unbelievably exciting."

Beating back *BRCA* cancers

Ovarian cancer has historically proven stubbornly resistant to conventional treatment. But "recent trials are changing the landscape, and clinical practice, in ovarian cancer," says Shannon Westin, a gynecologic oncologist at MD Anderson Cancer Center, who has consulted for AstraZeneca, Clovis, and other PARP drug developers. In a study published last year, for example, olaparib held advanced disease in check for more than 19 months in patients with *BRCA1/2* mutations who had previously responded to platinum-based chemotherapy—more than three times longer than in patients taking a placebo (*Lancet*, 18:1274-84, 2017). The FDA approved olaparib last August for maintenance treatment to slow or prevent the return of disease in *BRCA*-mutated ovarian cancer. For women with *BRCA* mutations, who account for up to 15 percent of ovarian cancer patients, "the results are very impressive," Westin says.

Other PARP inhibitors are making gains against ovarian cancer, too. Following promising results across two Phase 2 trials, rucaparib received approval in December 2016 for patients with germline or somatic *BRCA* mutations who had received two or more previous chemotherapy treatment regimens. And last year's results from Clovis's Phase 3 trial found that, compared to a placebo, the drug more than tripled progression-free survival in women with *BRCA*-mutated tumors. "Rucaparib has really been a breakthrough in treatment for ovarian cancer," says Eileen Parkes, a clinical lecturer at the Centre for Cancer Research and Cell Biology at Queen's University Belfast, who was not involved in either study. Just last March, the FDA approved niraparib for maintenance treatment for multiple types of ovarian cancer in patients who had responded to platinum-based chemotherapy, making it the third PARP inhibitor to get the green light for that indication.

> PARP inhibitors have a clear benefit in *BRCA*-mutant disease.
>
> —Eileen Parkes, Queen's University Belfast

PARP inhibitors are also showing progress in the fight against *BRCA*-mutated breast cancers. At last summer's American Society of Clinical Oncology meeting, researchers from the University of Pennsylvania presented the results of a Phase 3 trial showing that, compared to chemotherapy, olaparib nearly doubled progression-free survival—to seven months—in patients with *HER2*-negative breast cancer with *BRCA* mutations (*NEJM*, 377:523-33, 2017). In light of these findings, the FDA extended olaparib's approval at the beginning of 2018 to include germline *BRCA*-positive, *HER2*-negative metastatic breast cancer for patients who have previously received chemotherapy—making the drug the first PARP inhibitor approved for breast cancer, and the first breast cancer therapy to target a germline *BRCA* mutation.

There are signs of more progress on the horizon. Pfizer is currently developing a "second generation" PARP inhibitor, talazoparib, which has shown much higher cancer cytotoxicity than rucaparib and olaparib in preclinical research. In a recent Phase 3 trial for multiple *BRCA*-mutated breast cancers, the drug significantly extended the time until relapse compared with standard chemotherapy. Patients also reported substantial improvements in their quality of life. Although there are some concerns about small (less than 2 percent) increases in the risk for complications such as myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML) with certain PARP inhibitors, overall, "PARP inhibitors have a clear benefit in *BRCA*-mutant disease," says Parkes, "and their toxicity profiles are much kinder than other currently available treatments."

From *BRCA* to BRCAness

Cancer cells harboring damage in other genes involved in DNA repair also appear to be vulnerable to the drugs. Scientists call this genetic vulnerability of certain tumors BRCAness. From a therapeutic perspective, "cancers that have BRCAness may also respond to similar therapeutic approaches as *BRCA*-mutated tumors," says Lord.

Exploiting BRCAness could significantly expand the range of patients treatable with PARP inhibitors. For example, while a little more than 20 percent of patients with high-grade serous ovarian carcinoma—the most common and most aggressive subtype of ovarian cancer—carry *BRCA* mutations, a further 30 percent have defects in other genes involved in the HR pathway, such as *PALB2*, *FANCD2*, and *RAD51*. All told, "we now have a broad population, about 40 percent of ovarian cancer patients, who will respond to these drugs," Westin says. Tumors with defects in yet other DNA repair genes such as *PTEN*, which is often mutated in brain, breast, and prostate cancers, are also considered to display BRCAness, opening up the possibility of treating these cancers with PARP inhibitors as well.

Recent clinical research suggests the strategy may be successful. Results from niraparib's 2016 Phase 3 trial signaled a watershed moment in PARP inhibitor development because it showed efficacy in all patients, regardless of *BRCA* status, indicating they likely had damage in other, unidentified DNA repair pathways. And following its 2017 trial, Clo-

vis announced that rucaparib worked almost as well in *BRCA*-wildtype ovarian cancer patients as it did in women with *BRCA* mutations.

Preclinical results show similar promise for other PARP inhibitors in targeting non-*BRCA* DNA-repair mutations. In patient-derived mouse xenografts with triple-negative breast cancer (TNBC), researchers at MD Anderson discovered that talazoparib produced shrinkage not only in tumors with *BRCA* mutations, but also in *BRCA*-wildtype tumors that had mutations in other HR genes such as *ATM* (*Clin Cancer Res*, 23:6468-77, 2017). For the study, Funda Meric-Bernstam and colleagues tested a number of anticancer drug types, including inhibitors of the mTOR pathway that regulates the cell cycle, but found that only talazoparib produced significant tumor regression. Meric-Bernstam, whose research is partly funded by AstraZeneca and other PARP inhibitor developers, tells *The Scientist* that the team is now developing newer models to home in on PARP inhibitor sensitivity in these and other cancers that might display BRCAness.

Bindra's group, meanwhile, recently uncovered an unexpected sensitivity to PARP inhibition in tumors harboring mutations in *IDH1* or *IDH2*, genes that code for enzymes involved in processing lipids and other molecules in the cell cytoplasm. Although the proteins are not directly involved in DNA repair, the team found that defective IDH enzymes produce a compound called 2-hydroxyglutarate that inhibits HR, conferring BRCAness on those cells. While IDH inhibitors have not been effective in *IDH*-mutated cancers such as glioma and acute myeloid leukemia, Bindra found in murine xenografts that these cancers did respond to treatment with olaparib (*Sci Transl Med*, 375:eaal2463, 2017). The findings offer a new path to treatment for these cancers using PARP inhibitors, he tells *The Scientist*. "Exploiting this DNA repair deficiency, rather than inhibiting the function of mutant IDH proteins, may be a better strategy for treating brain and other tumors with these mutations."

Mixing and matching

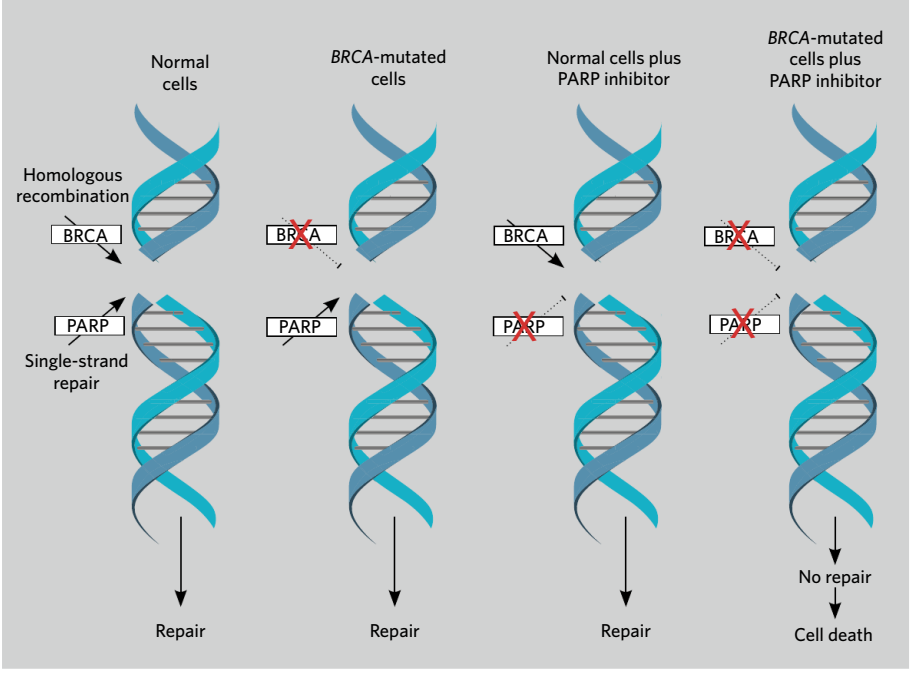
Cancer treatment almost always involves combining therapies to block multiple pathways and reduce resistance (see "Make Me a Match" on page 32). But combining PARP inhibitors with chemotherapy—usually the first-line treatment against cancer—has proven to be problematic, producing mixed results, with varying side effects, including bone marrow toxicity, says Westin. Instead of broad-effect chemotherapy drugs, "combinations with targeted drugs are most exciting," she says.

Last year, the University of Pennsylvania's Susan Domchek presented results of a Phase 2 trial on the combination of olaparib and the programmed death ligand-1 (PD-L1) inhibitor durvalumab, an immunotherapy. Around 80 percent of patients with pretreated germline *BRCA*- and *HER2*-negative metastatic breast cancer responded to the drugs, and 70 percent remained progression-free at 12 weeks. A Phase 2 trial is planned to test this same combination in TNBC patients. And Fatima Karzai of the National Cancer Institute (NCI) and colleagues reported a 50 percent response rate with the same two drugs in patients with castration-resistant metastatic prostate cancer. All patients with this type of cancer produce abundant amounts of PD-L1, and about 30 percent have germline or somatic mutations in DNA-repair genes, making the drug duo a logical choice.

Another Phase 2 trial sponsored by the NCI found that olaparib showed better antitumor activity in combination with the angiogenesis inhibitor cediranib than it did by itself. The pair is being tested in a larger study now, says James Doroshow, director of the Division of Cancer Treatment and Diagnosis at the NCI's Center for Cancer Research. Despite this progress, the combination of PARP inhibitors with other drugs is still in early stages, says Doroshow. "There is a lot of additional biology that needs to be explored before we can figure out which combinations will be best."

Focusing on efficacy

Researchers are working on better understanding that biology, but just what makes an effective PARP inhibitor is still an open question. Initially, the drugs were thought



DOUBLE WHAMMY: Cancer cells that lack functional BRCA proteins can still repair DNA damage via alternative pathways. But using drugs to inhibit another family of DNA repair proteins, the PARP enzymes, in *BRCA*-mutated cancers has proven to be a promising therapeutic strategy.

BASED ON *NEJM*, 361:189-91, 2009/*THE SCIENTIST* STAFF

only to work by blocking PARP enzymes' catalytic activity. However, in 2012, Yves Pommier, chief of the developmental therapeutics branch at the NCI's Center for Cancer Research and colleagues discovered a second mechanism of action, in which the inhibitors cause PARP enzymes to physically clump together on DNA and prevent repair (*Cancer Res*, 72:5588-99, 2012). Pommier and his colleagues found that this mechanism, which they named PARP trapping, was more deadly to cells than catalytic inhibition, and that different PARP inhibitors trap PARP-DNA complexes to different extents.

Some researchers hypothesize that, to be clinically effective, PARP inhibitors must have strong, dual mechanisms of action—that is, both catalytic and trapping activity. Pfizer's talazoparib, for example, is thought to derive its greater potency compared to previous PARP inhibitors by more successfully trapping PARP-DNA complexes than its predecessors, while simultaneously inhibiting the enzymes' catalytic activity. This argument was bolstered by the failure last spring of Phase 3 trials in TNBC and lung cancer patients of AbbVie's veliparib—a PARP inhibitor now known to only weakly trap DNA. The hypothesis is controversial, however, and ignores other benefits of "weaker" PARP inhibitors, says Doroshow. "The upside of veliparib's apparent 'weakness' is that it can be combined with chemotherapy and other drugs with less toxicity," he says. Indeed, in one trial, combining veliparib with chemotherapy drugs resulted in improved response rates in TNBC (*NEJM*, 375:23-34, 2016).

Some researchers hypothesize that, to be clinically effective, PARP inhibitors must have dual mechanisms of action.

Results such as these have highlighted questions about this still-evolving drug class, and some of the wrinkles to be ironed out in the future. For example, one key challenge researchers are now focusing on is finding effective biomarkers to identify which patients will benefit from which therapies and combinations. Nevertheless, the rapidly expanding number of trials using PARP inhibitors suggests that the drugs are therapeutically promising, leaving researchers hopeful that PARP inhibitors will change outcomes in additional patients with hard-to-treat cancers, notes Ohio State University cancer researcher David O'Malley, who has consulted for Clovis, AstraZeneca, and other drug developers. "We are starting to identify more and more patients who will markedly benefit from these drugs." ■

Vicki Brower is a New York City–based freelance writer specializing in biotechnology and medicine.

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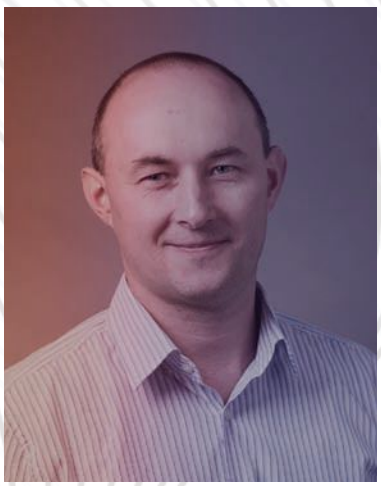

Talk to Alexey About:

Alexey has extensive expertise in both cell manufacturing and GMP-compliance clinical cell processing. If you have any questions about manufacturing CAR T-cell products, process development, cell processing and culture, or regulatory compliance, seek him out at lunch!

Learn more from Alexey's blog, Cell Trials.

Training: Alexey was trained as a general surgeon in Russia, where he also received a PhD in Transplantation Biology and Pathology. His postdoctoral work at Thomas Jefferson University and Children's Hospital of Philadelphia focused on hematology and stem-cell biology.

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Studying the Brain, Losing My Mind

Even as a neuroscientist, I didn't truly understand the experience of mental illness until it happened to me.

BY BARBARA LIPSKA WITH ELAINE MCARDLE

For more than 30 years as a neuroscientist, I (B.L.) have studied mental illness, in particular schizophrenia, a devastating disease that often makes it difficult for patients to distinguish between what is real and what is not. So it was with some irony that, three years ago, I myself ended up losing my mind, losing touch with what was happening around me. In the long run, I have come to see that terrifying journey, which I detail in my book, *The Neuroscientist Who Lost Her Mind*, as a gift, both personally and professionally.

In January 2015, two years after I became director of the Human Brain Collection Core at the National Institute of Mental Health (NIMH), I was diagnosed with brain metastatic melanoma and given four to seven months to live. But as an athlete and a breast cancer survivor, I had no intention of giving up easily. After brain surgery and radiation, I entered a clinical immunotherapy trial for patients with melanoma brain tumors at Georgetown University's Lombardi Comprehensive Cancer Center.

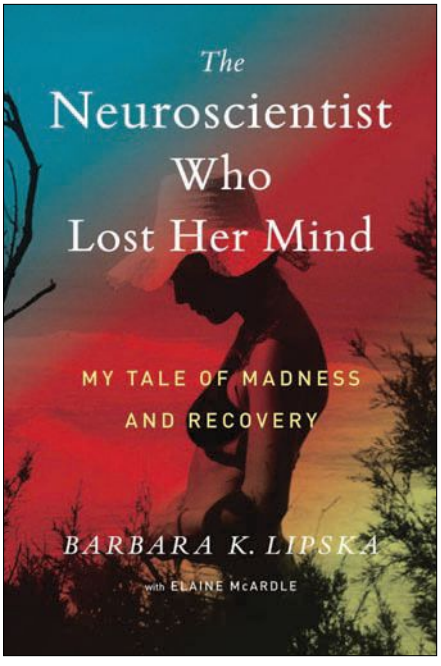
Throughout the trial, I continued to work full-time at my office in Bethesda, Maryland, putting in long days overseeing my large staff, reviewing scientific articles, and managing the surging demand from researchers across the country to use our brain tissue samples. But unbeknownst to me or anyone else, a full-scale war had erupted inside my brain. Even as the immunotherapy attacked the tumors that my doctors had irradiated, many new tumors were growing. My brain had become swollen and inflamed, and my frontal lobe function deteriorated rapidly. Soon my personality began to change.

At work, I found the minor shortcomings of my colleagues irritating. Instead of letting small things slide, as I normally would, I began to criticize the people I worked with frequently, just as I was doing at home with my husband and children. I became increasingly angry and suspicious of my family and my colleagues, certain that they were plotting against me. I began to struggle with reading and tasks that required sustained attention. I behaved in ways that were out of character, sending emails to my colleagues in all caps, the electronic version of shouting, and dispatching an odd, misspelled email to the organizers of a professional conference. One day after work, I couldn't find my car even though I parked it in the same spot every day, and I got lost going home. Increasingly, I was losing my memory—and my grip on reality.

Given what was happening in my brain, it's remarkable that I was functional at all. I soon learned that there were 15 new tumors in my brain as well as dramatic swelling and inflammation. Against all odds, I still believed that I would survive. After months of additional treatment, including more radiation and targeted therapy with drugs directly attacking melanoma cells, I did survive.

As the swelling decreased, my mind began to return. I started to remember some of the bizarre incidents and my out-of-character behavior, and to recognize what my family had been through. Despite all my years of research, it is my own suffering through that odd journey that truly taught me how the brain works—and how profoundly frightening it is when it stops working.

I am enormously grateful for the support of my colleagues at the NIMH, who believed in me and my recovery. I feel more deeply than ever the urgency of the work we are doing to find cures for mental illness. After enormous attention to and resources for cancer research, we have witnessed dramatic breakthroughs, which helped save my life. But the resources for research on mental illness lag far behind those devoted to other conditions. There is still so much we don't understand about the brain, and so few new drugs or other treatments to care for it. Armed with my new understanding of how it feels to go insane, I'm more focused than ever on helping to find cures.



Houghton Mifflin Harcourt, April 2018

Barbara Lipska is the director of the Human Brain Collection Core at the National Institute of Mental Health. Elaine McArdle is an award-winning journalist and coauthor of The Migraine Brain *(Free Press, 2008). Read an excerpt of* The Neuroscientist Who Lost Her Mind *at the-scientist.com.*

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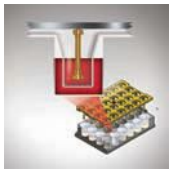

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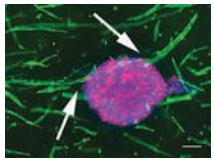

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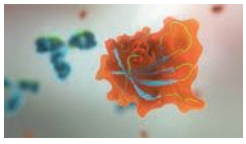

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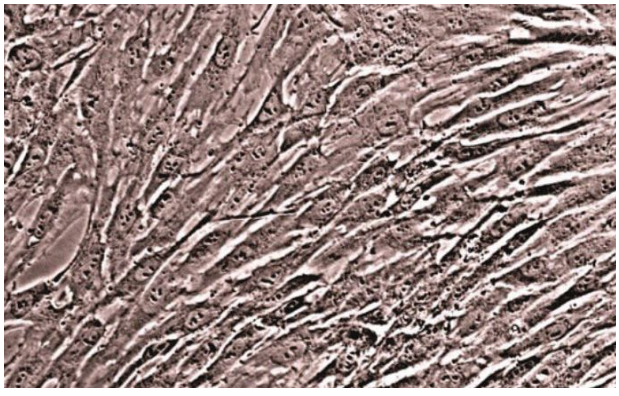

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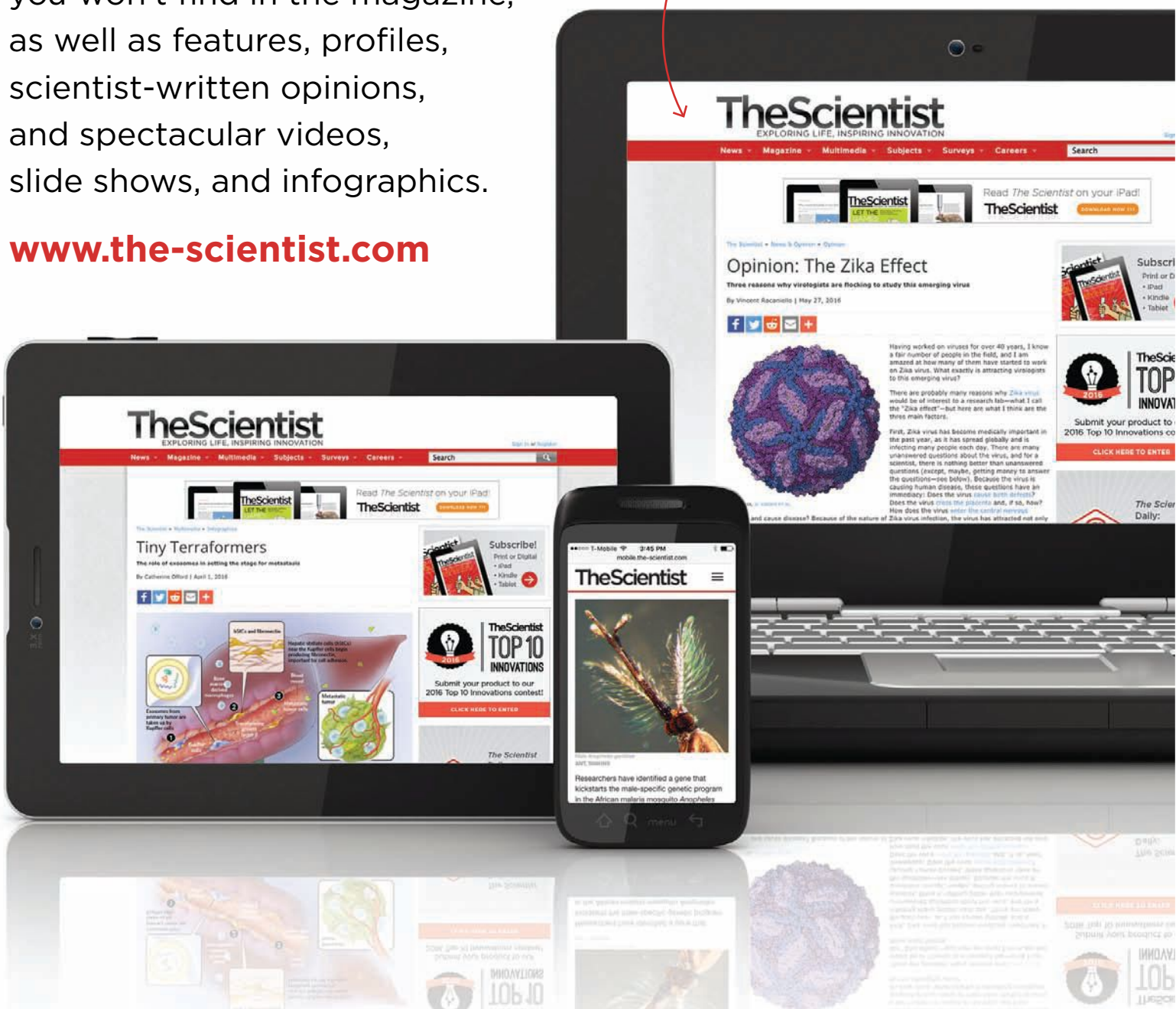

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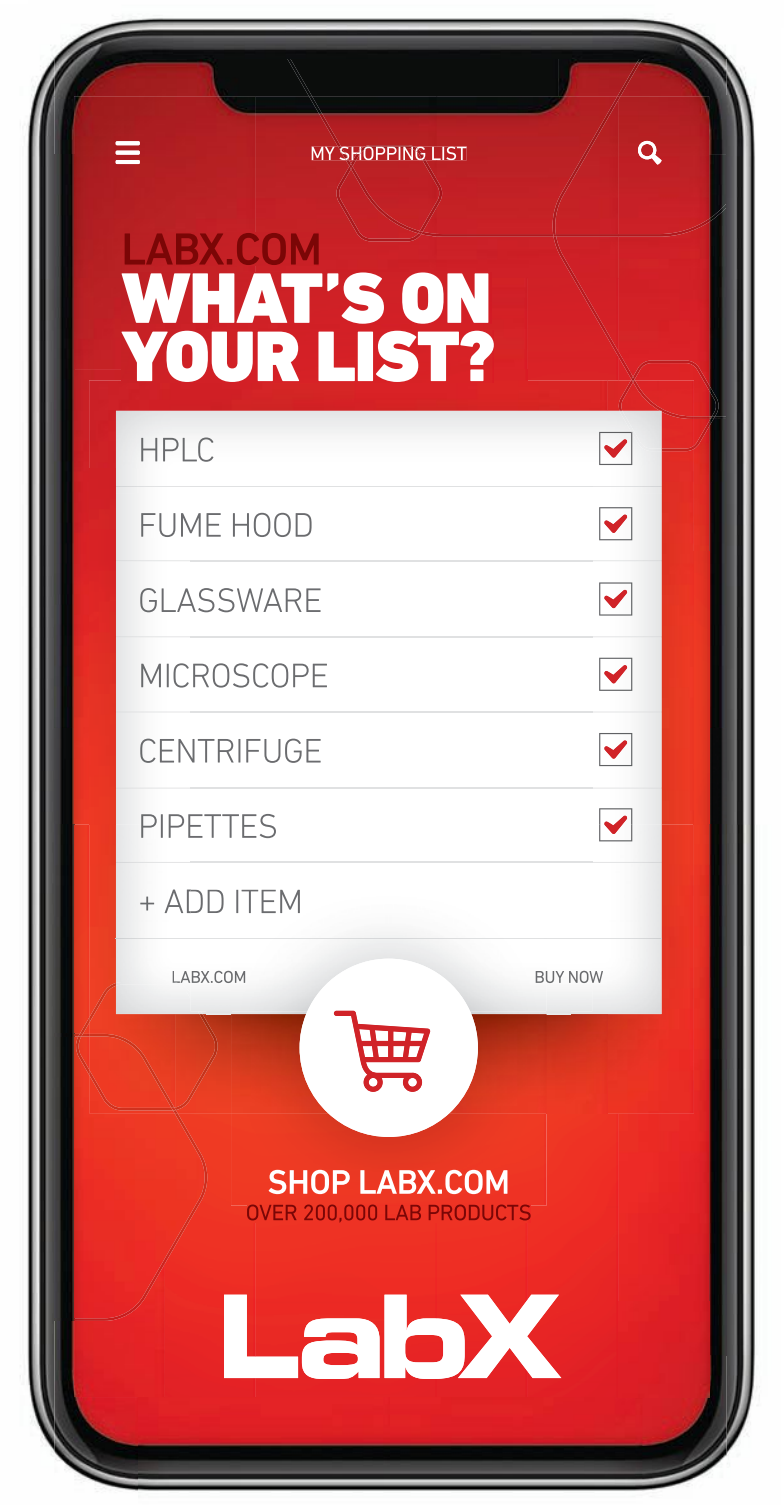

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TS WEBINARS

COMING**SOON** | Are All Neurodegenerative Diseases Made Equal?

Various neurodegenerative processes result in the development of diseases like Alzheimer's (AD), Parkinson's (PD), amyotrophic lateral sclerosis (ALS), and, arguably, multiple sclerosis (MS). Despite years of research, drug discovery initiatives, and promising clinical trials, these diseases remain incurable. But recent studies have suggested common mechanisms underlying these pathologies. Atypical protein assembly resulting in plaque formation is a common pathological finding in both AD and PD, while neuronal death is a primary (ALS) or secondary (MS) hallmark of disease. For a detailed look at the mechanisms that drive an array of neurodegenerative diseases, *The Scientist* is bringing together a panel of experts to share their research, discuss current therapeutic approaches, and offer their insights. Come engage with our panel and get the answers you seek.

RUDOLPH E. TANZI, PhD
Joseph. P. and Rose F. Kennedy Professor of Neurology
Harvard Medical School
Vice-Chair, Neurology; Director, Genetics and Aging Research Unit
Massachusetts General Hospital

GREGORY A. PETSKO, DPhil
Mahon Professor, Department of Neurology and Neuroscience
Director, Appel Alzheimer's Disease Research Institute
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BRUCE D. TRAPP, PhD
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TOPICS TO BE COVERED:

- The molecular and mechanistic similarities and differences between neurodegenerative diseases
- Whether primary and secondary neuro-degeneration distinctions are based on biology

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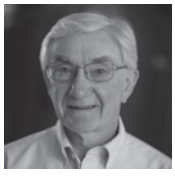
ON**DEMAND** | The Precision Medicine Revolution: CRISPR-Based Therapies

CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) gene-editing technology has been hailed as a breakthrough and has emerged as a new hope of precision medicine. Its potential as a treatment for numerous diseases, stretching from various cancer types to neurological diseases to lethal heritable disorders, has been well documented. But ongoing efforts aim to clarify the complex issues surrounding the legality and ethics of editing human genomes for therapeutic purposes. For a detailed look at the progress made toward CRISPR-mediated correction of human diseases and the continuing ethics debate, *The Scientist* brings together a panel of experts who will share their research, summarize the state of the science, and discuss the next steps for those looking to adopt the technique.



DANA CARROLL, PhD
Distinguished Professor, Department of Biochemistry
The University of Utah School of Medicine

JAMES DAHLMAN, PhD
Assistant Professor, Department of Biomedical Engineering
Georgia Institute of Technology and Emory School of Medicine

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TOPICS COVERED:

- CRISPR-generated model systems for in vivo study of a wide range of diseases
- Therapeutic applications of genome editing and their associated societal implications

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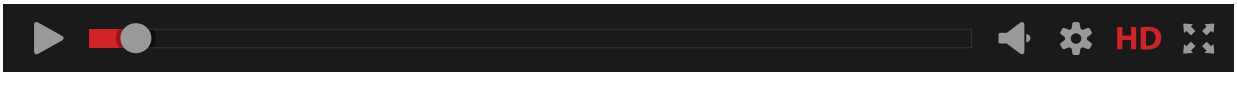

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COMING**SOON** | How to Utilize PDX Tumor Models to Evaluate Your Research or Preclinical Compounds

Tumor heterogeneity is a challenge in cancer research and drug development. Examination of cancer cell lines does not offer a comprehensive view of a tumor or tumor microenvironment. This is where it can be helpful to examine a patient derived xenograft (PDX) model of tumors. PDX models are typically implanted as dissociated cells or tumor fragments into immunodeficient mouse models which may then be used for in vivo efficacy studies. This could allow you to accelerate your oncology research or drug discovery and development programs, including applications for preclinical drug screening, preclinical combination therapeutics screening, identification and analysis of biomarkers. Horizon, the sponsor of this Lab Tools webinar, offers cancer model collections as ungrafted PDX suspensions or as comprehensive in vivo efficacy studies.

ANDREW BROWN, MS, MBA
Product Manager, In Vivo
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TOPICS TO BE COVERED:

- Selecting the right breast cancer or melanoma PDX line to support your research goals
- Constructing optimal experimental design and pre/post study characterization services

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ON**DEMAND** Bioanalytical Pharmacokinetics: The Intrinsic Value of Affimers

Antibodies represent the gold standard of affinity reagents used for regulated bioanalysis of therapeutic proteins. Over time, traditional antibodies have been refined to the point where they are specific, sensitive, and reasonably reliable. Yet, antibodies and natural ligands have their limitations and can present challenges with supply, stability, and batch-to-batch variation on assay performance. This webinar, sponsored by Avacta, demonstrates a high-performance Affimer assay detecting and quantifying the breast cancer drug trastuzumab, showing good sensitivity and increased dynamic range compared to the current antibody-based methods.

AMY REEVES, PhD
Assay Development Intern
Covance

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TOPICS COVERED:

- How antibody variability impedes pharmacokinetic studies
- What are Affimers, and when to adopt them for your R&D pipeline

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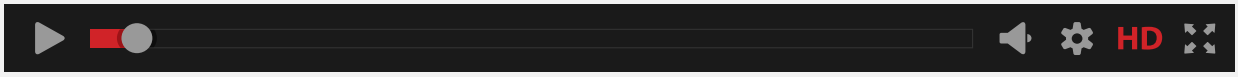


A Radical Intervention, 1894

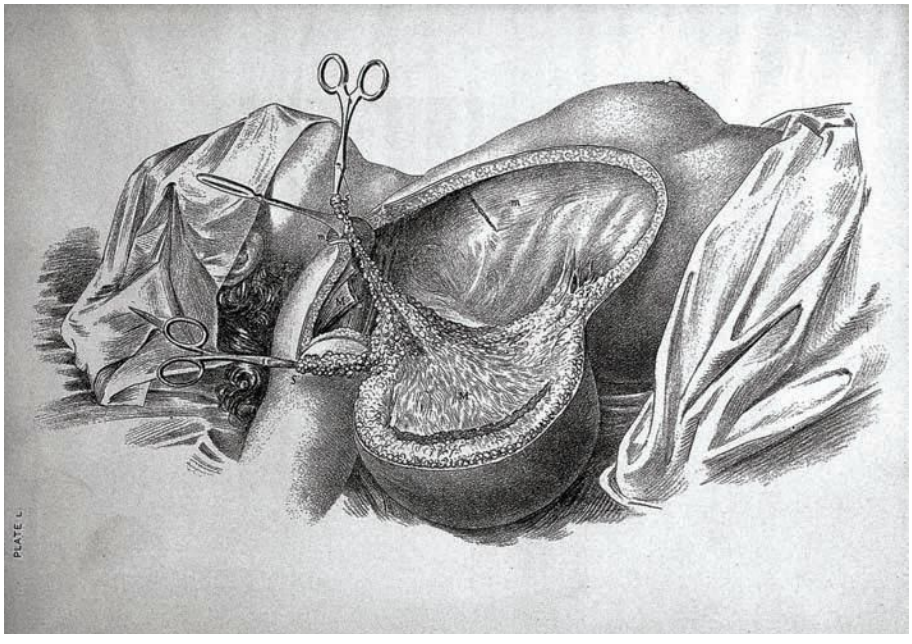
BY CATHERINE OFFORD

On May 28, 1889, a 38-year-old woman was placed on an operating table at the Johns Hopkins Hospital in Baltimore. Patient L.S. was married, with ten children, and had a cancerous tumor occupying most of her left breast. That day, and in subsequent operations over several months, her surgeon, William Halsted, painstakingly sliced out not only the tumor, but the pectoral muscle behind it, and the lymph nodes in her armpit. Then, he grafted skin—likely from her thigh—to patch up the gaping wound.

The procedure was unprecedented in the U.S. Although records of mastectomy-like procedures stretch back to at least the second century A.D., American physicians typically considered breast cancer patients doomed from the outset, and prescribed ointments and other less invasive measures. But L.S., or Case I, as she would be dubbed in Halsted's 1894 article on 50 such cases, marked the beginning of a sea change in breast cancer treatment. His "was a seminal paper of the day," says Kirby Bland, a surgeon and oncologist at the University of Alabama at Birmingham.

Halsted argued that excising a large volume of tissue could reduce cancer's spread, a poorly understood phenomenon at the time. Although L.S. died from cancer of the other breast less than two years after her operation, Halsted highlighted his approach's success in avoiding "local recurrence" of the disease. He also suggested that the procedure promoted patient survival (although his broader study only measured survival after three years—not much longer than the median survival time for untreated patients).

Halsted's conviction was contagious. By the early 20th century, his so-called "radical" or "complete" mastectomy had become the standard of care. For decades, surgeons followed his instructions, entirely removing the breast and associated tissue, and replacing skin with an unsightly graft—an outcome that caused considerable distress for patients, Bland says. Some also complained of disability in their arms after the operation—a concern Halsted dismissed in his paper noting that, with an average age of 55 years old, "these patients are old . . . They are no longer very active members of society."



THE CUT: William Halsted's "radical mastectomy" was believed for much of the 20th century to be the best defense against breast cancer. This illustration in his 1894 paper on the procedure depicts the removal of large volumes of tissue around the tumor. It would be decades before the medical community would begin to replace the procedure with more-targeted surgeries, together with cell-killing methods such as radiotherapy and chemotherapy.

Even in Halsted's time, some doctors doubted the procedure's efficacy. A contemporary, Rudolph Matas, wrote in 1898 that the terms "complete" and "radical" were "anatomical misnomers . . . and are illusory if used in the sense that they root out the evil with any degree of certainty."

In 1971, a team led by American surgeon Bernard Fisher began a large clinical trial comparing Halsted's mastectomy with smaller, lump-removing operations. The team found no significant differences in survival, prompting the gradual adoption of a less destructive, breast-conserving approach to surgery, together with newer treatments. "Today . . . the only time you use [a radical mastectomy] is when you have failed with chemotherapy or radiation," Bland says.

Still, with its focus on precision and aseptic techniques, Halsted's forward-looking work in surgery marked "a huge change in medical philosophy," Bland adds. Halsted himself concluded his 1894 paper by imploring surgeons in all fields to hone their operating skills, wryly noting that surgeons would someday "contemplate with astonishment some of the handy, happy-go-lucky methods for intestinal suture which are now so much in vogue." ■

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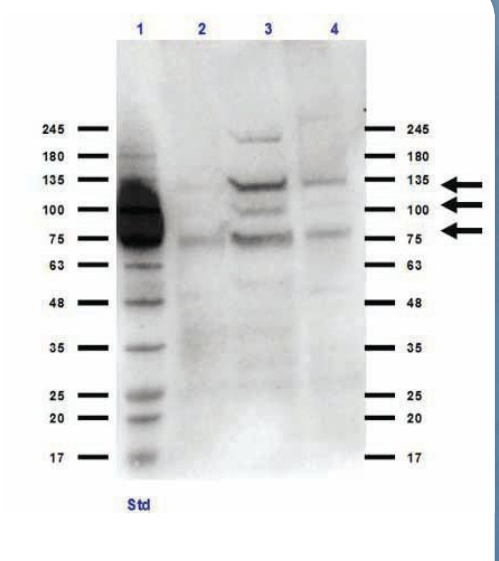

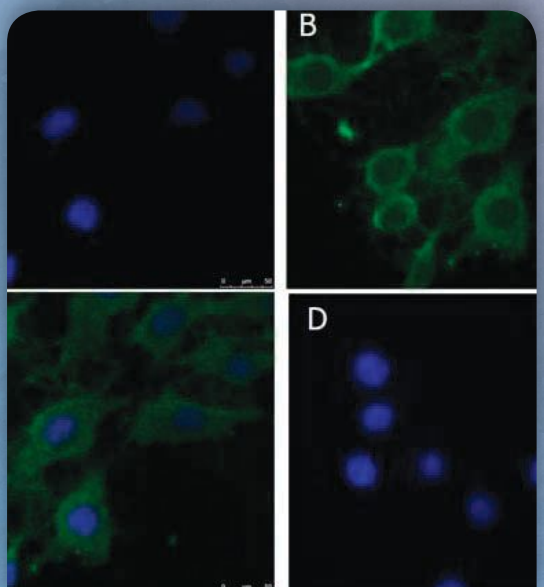

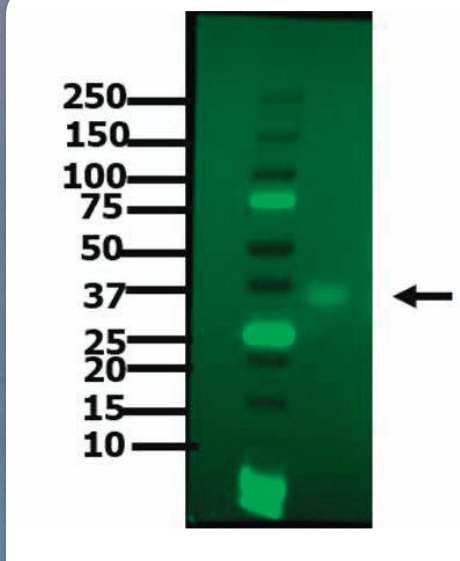

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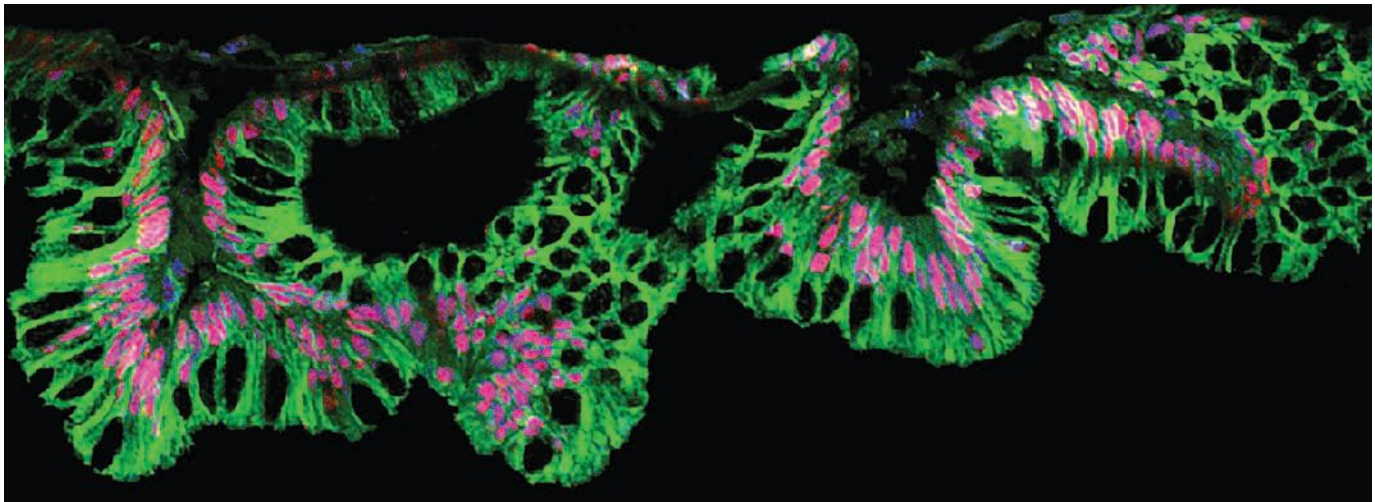

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