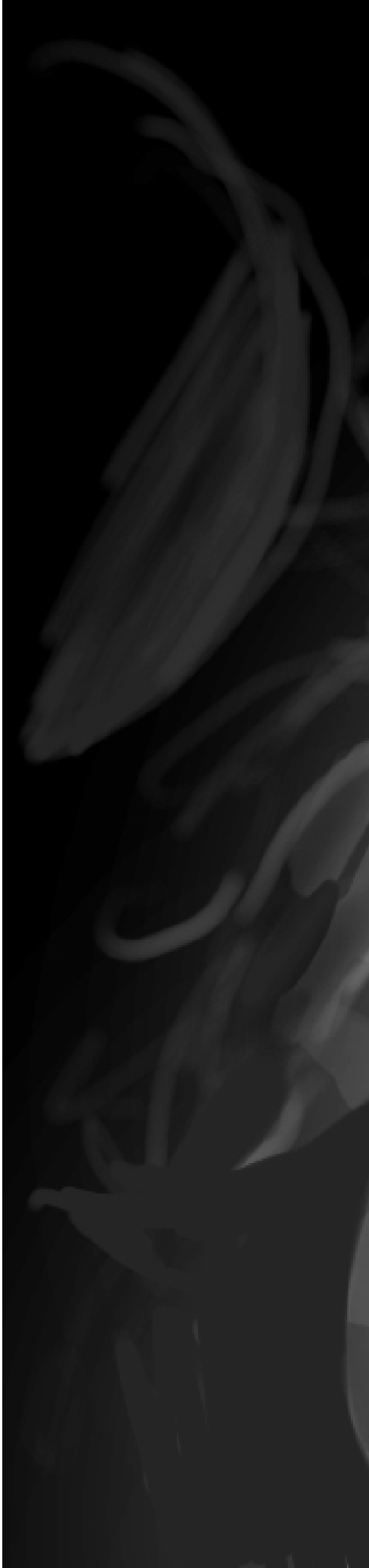
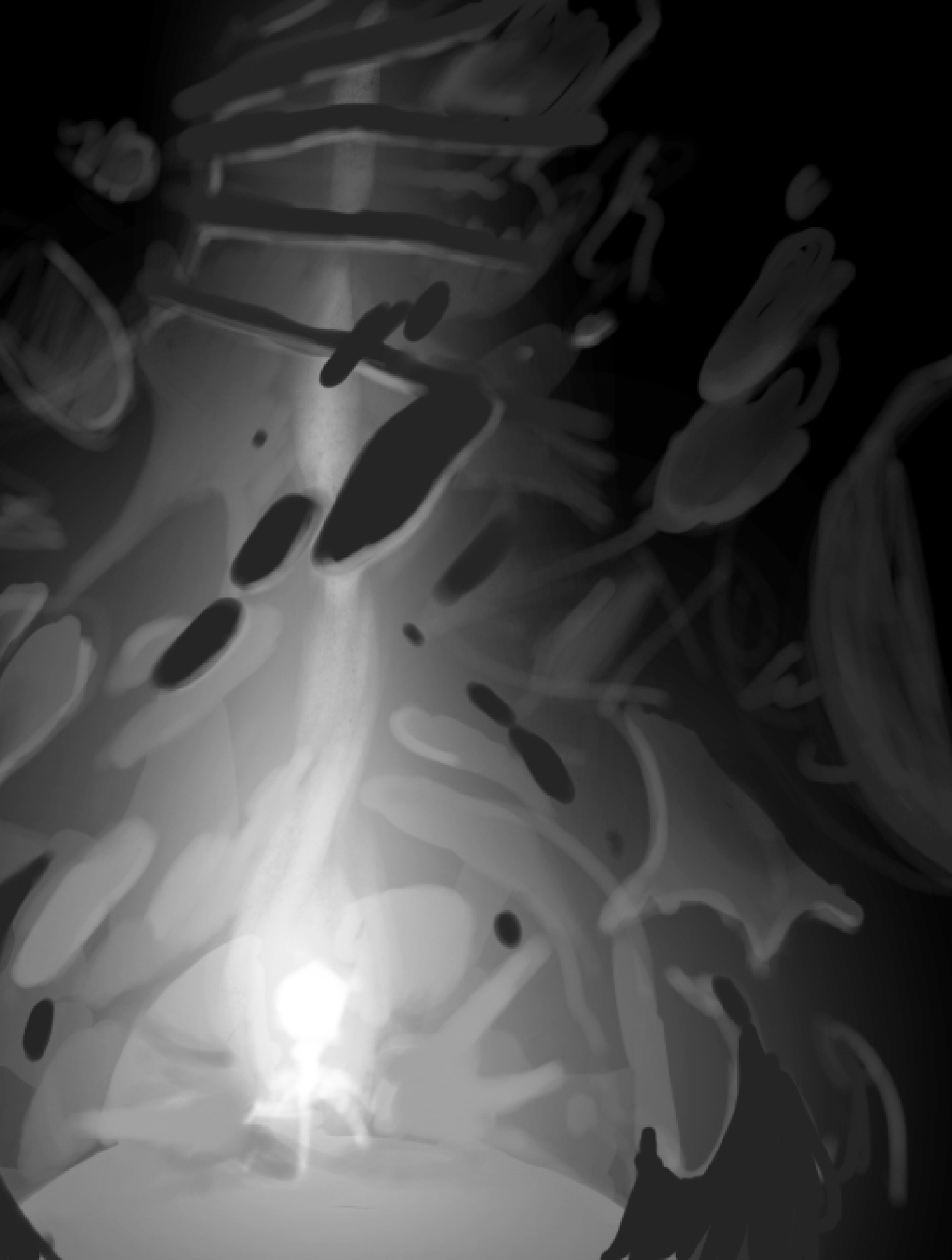
Is Phage Therapy Here to Stay

A treatment from World War I is making a comeback in the struggle to beat deadly multidrug-resistant infections

By Charles Schmidt

Illustration by Ashley Mackenzie





Charles Schmidt is a freelance journalist based in Portland, Me., covering health and the environment. He has written for us about dangerous contaminants in drinking water and about multigenerational effects from Agent Orange in Vietnam.

BOBBY BURGHOLZER HAS CYSTIC FIBROSIS, A GENETIC DISEASE THAT throughout his life has made him vulnerable to bacterial infections in his lungs. Until a few years ago antibiotics held his symptoms mostly at bay, but then the drugs stopped working as well, leaving the 40-year-old medical device salesman easily winded and discouraged. He had always tried to keep fit and played hockey, but he was finding it harder by the day to climb hills or stairs. As his condition worsened, Burgholzer worried about having a disease with no cure. He had a wife and young daughter he wanted to live for. So he started looking into alternative treatments, and one captured his attention: a virus called a bacteriophage.

IN BRIEF

Harmful bacteria are becoming ever more resistant to antibiotics. Physicians are turning to phages—viruses that infect bacteria—as a new line of attack.

Doctors are testing several different phage therapies in clinical trials, which kill bacteria in different ways.

Researchers will have to significantly reduce the time and cost needed to find the right phage to defeat a bacterium, if the therapies are to succeed commercially.

Phages, as they are known, are everywhere in nature. They replicate by invading bacteria and hijacking their reproductive machinery. Once inside a doomed cell, they multiply into the hundreds and then burst out, typically killing the cell in the process. Phages replicate *only* in bacteria. Microbiologists discovered phages in the 1910s, and physicians first used them therapeutically after World War I to treat patients with typhoid, dysentery, cholera and other bacterial illnesses. Later, during the 1939–1940 Winter War between the Soviet Union and Finland, use of the viruses reportedly reduced mortality from gangrene to a third among injured soldiers.

Treatments are still commercially available in former Eastern Bloc countries, but the approach fell out of favor in the West decades ago. In 1934 two Yale University physicians—Monroe Eaton and Stanhope Bayne-Jones—published an influential and dismissive review article claiming the clinical evidence that phages could cure bacterial infections was contradictory and inconclusive. They also accused companies that manufactured medicinal phages of deceiving the public. But the real end of phage therapy came in the 1940s as doctors widely adopted antibiotics, which were highly effective and inexpensive.

Phage therapy is not approved for use in humans in any Western market today. Research funding is meager. And although human studies in Eastern Europe have generated some encouraging results—particularly those from the Eliava Institute in Tbilisi, Georgia, the field's research epicenter—many Western scholars say the work does not meet their rigorous standards. Furthermore, a smattering of clinical trials in Western Europe and the U.S. have produced some high-profile failures.

Yet despite the historical skepticism, phage therapy is making a comeback. Attendance at scientific conferences on the treatment is skyrocketing. Regulators at the U.S. Food and Drug Administration and other health agencies are signaling renewed interest. More than a dozen Western companies are investing in the field. And a new wave of U.S. clinical trials launched this year. Why the excitement? Phage treatments have been curing patients with multidrug-resistant (MDR) infections that no longer respond to antibiotics. The FDA has allowed petitioning doctors to administer these experimental treatments on a "compassionate use" basis when they could show that their patients had no other options—exactly what Burgholzer was hoping to prove.

MDR infections are a rapidly growing public health nightmare. At least 700,000 people worldwide now die from these incurable maladies every year, and the United Nations predicts that number could rise to 10 million by 2050. In the meantime, the drug industry's antibiotic pipeline is running dry.

Like all viruses, phages are not really alive—they cannot grow, move or make energy. Instead they drift along until by chance they stick to bacteria. Unlike antibiotics, which kill a range of helpful bacteria as they kill the strains making a person sick, a phage attacks a single bacterial species, and perhaps a few of its closest relatives, and spares the rest of the microbiome. Most phages have an icosahedral head—like a die with 20 triangular faces. It contains the phage's genes and connects to a long neck that ends in a tail of fibers, which bind to receptors on a bacterium's cell wall. The phage then plunges a kind of syringe through the wall and injects its own genetic material, which co-opts the bacterium into making more phage copies. Other types of phages, not used medically, enter the same way but live dormantly, reproducing only when the cell divides.

Phages have co-evolved with bacteria for billions of years and are so widespread that they kill up to 40 percent of all the bacteria in the world's oceans every day, influencing marine oxygen production and perhaps even Earth's climate. The spotlight on phages as medical tools is getting brighter as technological advances make it possible to match the viruses to their targets with better accuracy. The few facilities that are technically able to provide phage therapy, under strict regulatory protocols, are being overwhelmed with requests.

Clinical trials underway are beginning to generate the high-quality data needed to convince regulators that phage therapy is viable, but considerable questions remain. The biggest is whether phage therapy can tackle infections on a large scale. Clinicians have to match phages to the specific pathogens in a patient's body; it is not clear whether they can do that cost-effectively and with the speed and efficiency needed to bring phages into routine use. Also problematic is a shortage of regulatory guidelines governing the production, testing and use of phage therapy. "But if it has the potential to save lives, then we as a society need to know whether it will work and how best to implement it," says Jeremy J. Barr, a microbiologist at Monash University in Melbourne, Australia. "The antibiotic-resistance crisis is too dire to not embrace phage therapy now."

TRADING VULNERABILITIES

BURGHOLZER LEARNED about phages by talking to other people with cystic fibrosis around the country. While scouring the Internet for more information, he came on a YouTube video made by phage researchers at Yale University. Soon he was corresponding with Benjamin Chan, a biologist in Yale's department of ecology and evolutionary biology. Since arriving there in 2013, Chan has accumulated a "library" of phages, harvested from sewage, soil and other natural sources, that he makes available to doctors at Yale New Haven Hospital and elsewhere.

Chan's first case, in 2016, was a resounding success. He isolated a phage from pond water, and doctors used it to cure Ali Khodadoust, a prominent eye surgeon. Khodadoust had been suffering from a raging MDR infection in his chest, a complication from open-heart surgery four years earlier. He was taking massive daily doses of antibiotics to try to fight his invading pathogen, the tenacious bacterium *Pseudomonas aeruginosa*. The virus Chan selected latches on to what is known as an efflux pump on the bacterial cell wall. The pumps expel antibiotics and are frequently found in drug-resistant bacteria. Most of the *P. aeruginosa* in Khodadoust's body had the pumps, and the phage killed them. The relatively few remaining *P. aeruginosa* faced an evolutionary trade-off: their lack of efflux pumps meant they survived the virus attack, but it made them defenseless against antibiotics. By taking the phages and antibiotics together, Khodadoust gradually recovered in just a few weeks. He died two years later, at age 82, from noninfectious illnesses.

After that first case, Chan supplied phages for nearly a dozen more experimental treatments at Yale, most involving cystic fibrosis patients with *P. aeruginosa* lung infections. He asked Burgholzer to send a sputum sample by overnight delivery so he could identify phages that might help.

I visited Chan at Yale last December, after the screening had begun. He was wearing a checkered oxford shirt, khakis and loafers, and before long he was calling me "dude," his preferred moniker. After chatting briefly in his office, we headed for an adjacent laboratory, where Chan showed me a petri dish. Burgholzer's bacteria had developed into a gray lawn spanning the dish, but two thin, clear rows cut across it. The bacteria that had been in those rows were all dead, Chan told me, killed by drips of a phage solution Burgholzer would soon be treated with. Burgholzer's infection was caused by three species of the bacterial genus *Achromobacter,* and Chan planned to select individual phages that could pick them off one by one—an approach known as sequential monophage therapy. "We're essentially playing chess in an antimicrobial war," Chan said. "We need to make calculated moves."

Chan hoped to induce an evolutionary trade-off similar to the one he believes worked for Khodadoust. Unable to find a phage that targets efflux pumps on *Achromobacter* bacteria, he instead selected one that targets a large protein called lipopolysaccharide (LPS) in the microbe's cell wall. LPS has side chains of molecules known as O antigens, which vary in length. The longer the chain, the better the bacteria's ability to resist not only antibiotics but also the host's immune system. Chan planned to kill the hardy long-chain strains with phages, leaving the weaker short-chain pathogens behind. In the best scenario, he said, a succession of phages would shift the bacterial population toward short-chain strains that might be more easily controlled by drugs and Burgholzer's own immune defenses. "Bacteria compete for real estate in the body," Chan said. "After large numbers of one species are suddenly killed by phage, in many cases, others move in." He wanted the new occupants to be less virulent than their predecessors.

Chan's boss, Paul Turner, has devoted his career to studying evolutionary trade-offs in the microbial world. A professor in Chan's department, he explained later on the day of my visit that phage treatments can work without completely ridding the body of a disease-causing bacteria. Especially when treating chronic conditions, doctors can use phages to selectively shape the population of the bad bacteria so it develops other vulnerabilities. "Should those vulnerabilities be toward antibiotics, then so much the better," he told me. Combining antibiotics with phages to achieve optimal effects for patients, he says, "makes it easier to move forward with phage therapy quickly."

I drove with Chan to Yale New Haven Hospital to watch as Burgholzer's phage treatment got underway. We took an elevator to the second floor, where we waited for Chan's clinical collaborator, Jonathan Koff, to arrive. A pulmonologist and director of the Adult Cystic Fibrosis Program, Koff soon came bounding in, a

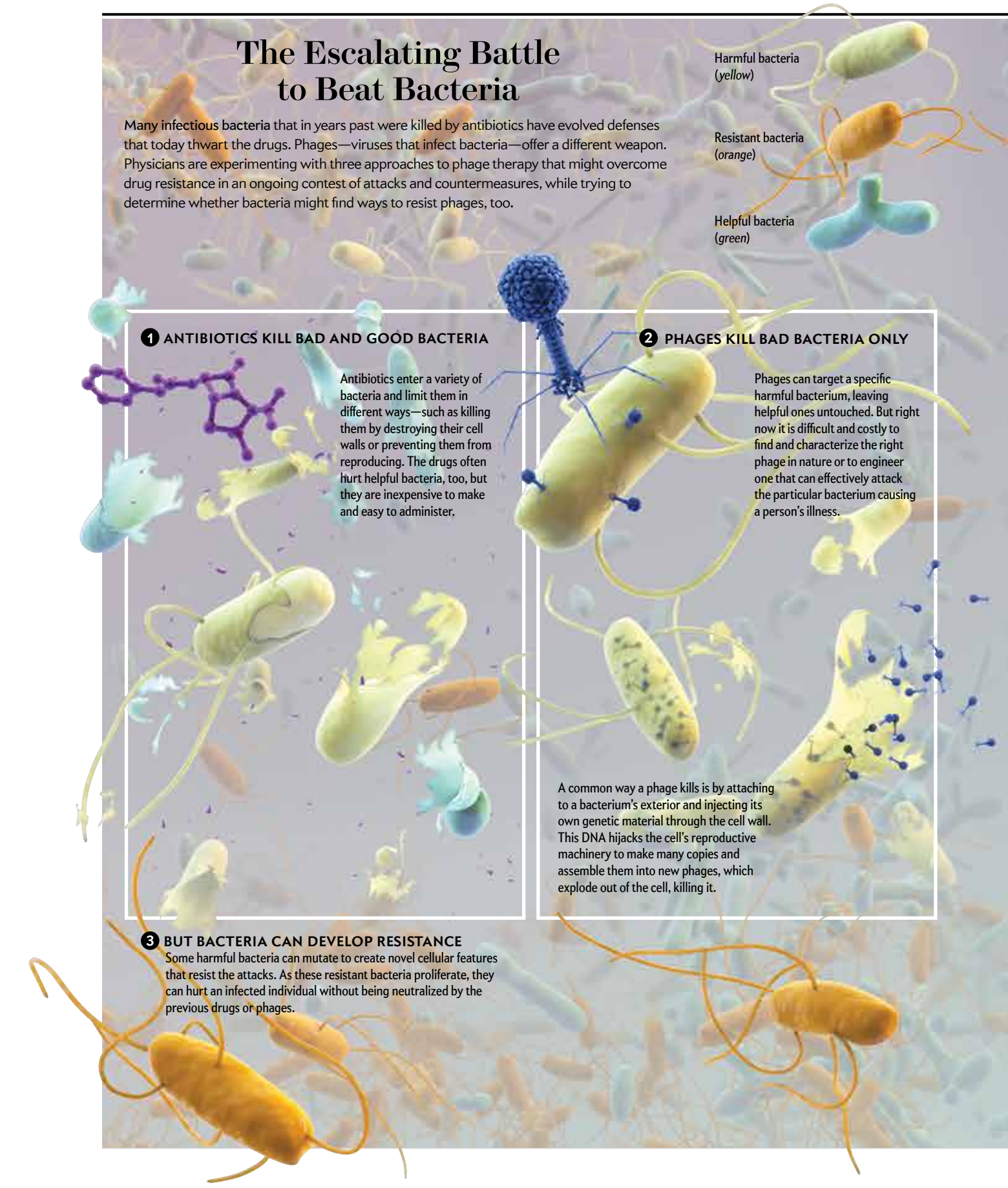
The Escalating Battle to Beat Bacteria

Many infectious bacteria that in years past were killed by antibiotics have evolved defenses that today thwart the drugs. Phages—viruses that infect bacteria—offer a different weapon. Physicians are experimenting with three approaches to phage therapy that might overcome drug resistance in an ongoing contest of attacks and countermeasures, while trying to determine whether bacteria might find ways to resist phages, too.

Harmful bacteria (*yellow*)

Resistant bacteria (*orange*)

Helpful bacteria (*green*)



1 ANTIBIOTICS KILL BAD AND GOOD BACTERIA

Antibiotics enter a variety of bacteria and limit them in different ways—such as killing them by destroying their cell walls or preventing them from reproducing. The drugs often hurt helpful bacteria, too, but they are inexpensive to make and easy to administer.

2 PHAGES KILL BAD BACTERIA ONLY

Phages can target a specific harmful bacterium, leaving helpful ones untouched. But right now it is difficult and costly to find and characterize the right phage in nature or to engineer one that can effectively attack the particular bacterium causing a person's illness.

A common way a phage kills is by attaching to a bacterium's exterior and injecting its own genetic material through the cell wall. This DNA hijacks the cell's reproductive machinery to make many copies and assemble them into new phages, which explode out of the cell, killing it.

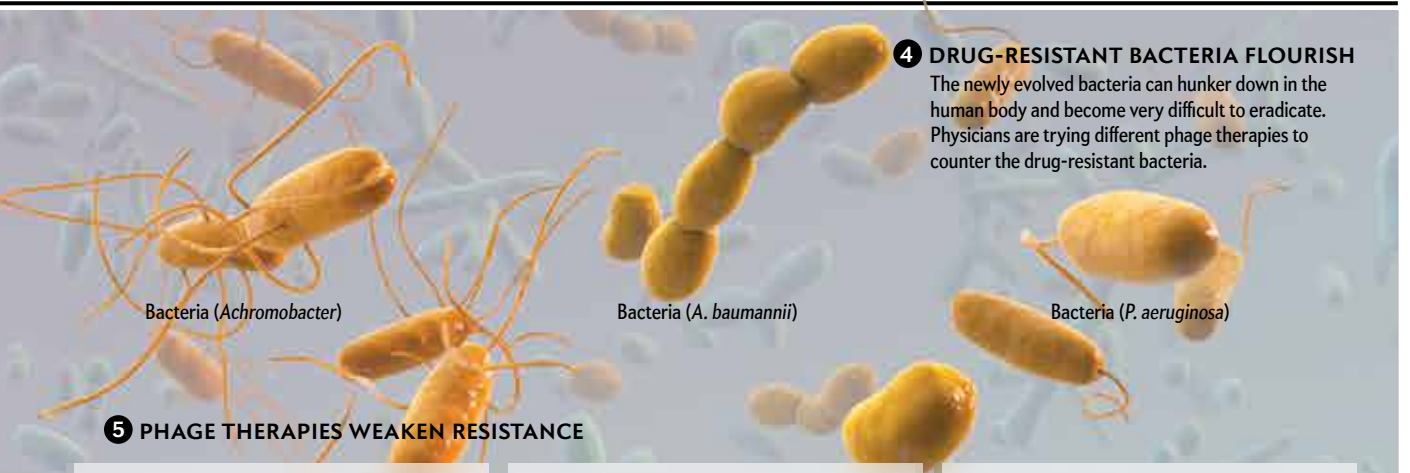
3 BUT BACTERIA CAN DEVELOP RESISTANCE

Some harmful bacteria can mutate to create novel cellular features that resist the attacks. As these resistant bacteria proliferate, they can hurt an infected individual without being neutralized by the previous drugs or phages.

Illustration by AXS Biomedical Animation Studio

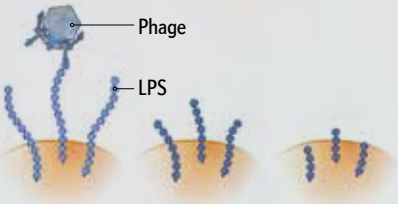
4 DRUG-RESISTANT BACTERIA FLOURISH

The newly evolved bacteria can hunker down in the human body and become very difficult to eradicate. Physicians are trying different phage therapies to counter the drug-resistant bacteria.

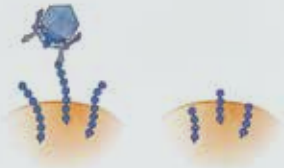


5 PHAGE THERAPIES WEAKEN RESISTANCE

Sequential monophage treatment



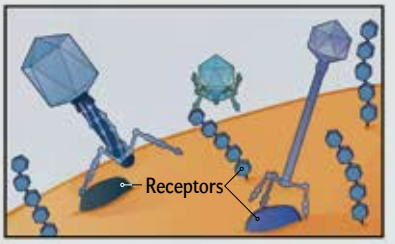
Phage 1 is given to a patient. It destroys *Achromobacter* species 1, which has long lipopolysaccharide (LPS) chains.

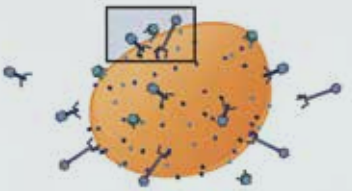


Phage 2 is then given to destroy *Achromobacter* species 2, which has moderately long LPS chains.

The immune system, which struggles against the longer-chain species, destroys the remaining short-chain *Achromobacter* species.

Phage cocktails

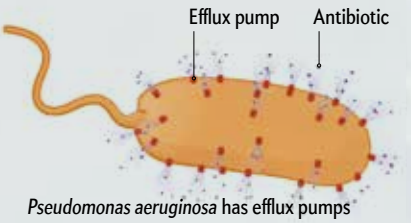




Several different phages are given simultaneously to a patient. Each phage targets a different receptor on *Acinetobacter baumannii*.

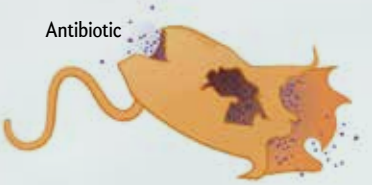
The *A. baumannii* cells cannot modify all types of receptors at once to resist the different phages and are killed.

Phage plus antibiotics



Pseudomonas aeruginosa has efflux pumps that expel antibiotics that sneak inside it.

Phages attach to the efflux pumps, shutting them down.



Antibiotics can now persist inside the *P. aeruginosa* cells and kill them.

6 BACTERIAL BALANCE IS RESTORED

By killing only harmful bacteria, phages allow helpful bacteria to dominate a person's microbiome—at least until bad bacteria evolve again.

knapsack slung over his shoulder. Burgholzer met the three of us in a treatment room and spoke with a rasp—the only outward sign of his disease. As Koff and Chan compared notes, he told me he wanted to stay healthy for his three-year-old daughter. When treatment time arrived, he tossed his cell phone to his wife. "Here, take a photo for my mother," he said with a grin. Then he raised a nebulizer over his mouth and nose and began inhaling a vaporized phage solution into his lungs.

PHAGE COCKTAILS

ACCORDING TO KOFF, sequential monophage therapy makes sense for treating cystic fibrosis and certain other chronic diseases that sequester bad bacteria in the body. When there is no proven way to eliminate the pathogens completely, he says, the tactic is to chip away at the harmful strains.

Some clinicians are choosing a different approach: They give patients multiple phages in a therapeutic cocktail, trying to knock out an infection completely by targeting a variety of bacterial resistance mechanisms simultaneously. Ideally, each phage in a cocktail will glom on to a different receptor, so if bacteria evolve resistance to one virus in the mixture, other viruses will keep up the attack.

Experts cannot say which of the phage therapies may win out. What is needed now are results from clinical trials that can help overcome residual skepticism.

Chan and Koff argue that phage interactions with bacteria are unpredictable and that when exposed to cocktails, pathogens might develop resistance to all the viruses in the mixture at once, which could limit future treatment options. "Splitting the cocktail into sequential treatments allows you to treat patients for longer durations," Koff says.

Jessica Sacher, co-founder of the Phage Directory, an independent platform for improving access to phages and phage expertise, says convincing arguments can be made for either method. "The science isn't there yet to say one is necessarily better than the other." She notes that cocktails might be more appropriate for acutely ill patients, who cannot always wait for doctors to develop a sequential strategy.

Urgency was paramount in the now famous case of Tom Patterson, a professor at the University of California, San Diego, who in 2016 was saved by phage cocktails after being stricken by an MDR infection during a trip to Egypt. The invader was *Acinetobacter baumannii,* a notoriously drug-resistant microbe that is common in Asia and is spreading steadily toward the West. Patterson was in multiorgan failure by the time doctors delivered mixtures of four viruses through a catheter into his abdomen and a fifth intravenously. The physicians treated him twice a day for four weeks, and he was cleared of infection within three months. He still needed extensive rehabilitation, but he remains healthy today.

The case drew worldwide media attention. The treating physicians were Robert Schooley, a friend of Patterson's and chief of infectious diseases at U.C. San Diego, and Patterson's wife, Steffanie Strathdee, then director of the university's Global Health Institute. Two years later, with an initial investment of $1.2 million, Schooley and Strathdee launched the Center for Innovative Phage Applications and Therapeutics at U.C. San Diego to fund clinical research and promote the field.

Each phage Patterson was treated with was screened for its ability to kill *A. baumannii* in infectious samples obtained from his body, using assays at the Naval Medical Research Center at Fort Detrick, Md., and at Texas A&M University. The assays can test hundreds of phages against bacterial pathogens simultaneously in just eight to 12 hours, according to Biswajit Biswas, chief of the bacteriophage division at the center, which supplied some of the phages used in Patterson's treatment. Biswas, who developed the assay and created the center's phage bank, says the assay allows new viruses to be easily swapped in to counter the onset of resistance. Patterson did develop resistance to his first cocktail within two weeks, prompting the navy to prepare a second one with longer-lasting effects. A company called Adaptive Phage Therapeutics in Gaithersburg, Md., has since licensed the navy's assay and its phage bank and will soon take them both into clinical trials in patients with urinary tract infections.

The navy assay checks only for bacterial cell death; it does not reveal which receptors are targeted. Whether cocktails should target known receptors is in debate. Ry Young, a phage geneticist at Texas A&M, who supplied viruses for Patterson, argues they should. "We don't even know if phages were responsible for his successful outcome," he says. "Our best guess is that phage treatment lowered his infectious load to a level where his immune system took over." The better approach to cocktails, Young says, is to combine three or four viruses targeting distinct receptors on the same bacterial strain. The odds of a bacterium evolving resistance to a single phage are about a million to one, he says, whereas the odds of it losing or developing mutant forms of receptors targeted by all the phages in a cocktail "are essentially zero." Furthermore, the identification of important receptors is critical if clinicians hope to make bacteria sensitive to antibiotics again.

Barr says scientists are working to identify the receptors targeted by Patterson's cocktails, but he disagrees on the need to identify the receptors prior to use. "It's an understandable viewpoint and a hot topic in the field," he says. "We know very little about these phages, and we need checks and balances before using them in therapy. Does that mean we need to identify host receptors? That is a huge amount of work currently, so I would say it's not required but definitely desirable."

ENGINEERED PHAGES

GIVEN THE VAGARY OF COCKTAILS, some researchers say phages should be genetically engineered to bind to specific receptors and also to kill bacteria in novel ways. The vast majority of

phages used thus far have been natural, harvested from the environment, but phage engineering is an emerging frontier with a new success story under its belt. Isabelle Carnell, a British teenager with cystic fibrosis, was suffering from life-threatening infections in her liver, limbs and torso after undergoing a double lung transplant in 2017. Her bacterial nemesis—*Mycobacterium abscessus*—was not responding to any antibiotics. Yet this year, in a first for the field, researchers from several institutions successfully treated the girl with an engineered cocktail of three phages. One naturally rips apart *M. abscessus* as it replicates. The other two also kill bacteria but not as completely, leaving 10 to 20 percent surviving the process. So the team, led by Graham Hatfull, a professor of biological sciences at the University of Pittsburgh, deleted a single gene from each of those two phages, turning them into engineered assassins. The cocktail of three phages cleared Carnell's infection within six months.

Researchers at Boston University first developed engineered phages in 2007. They coaxed one into producing an enzyme that more effectively degrades the sticky biofilms secreted by certain infectious bacteria for protection. Scientists have since modified phages to kill broader ranges of harmful bacteria or potentially to deliver drugs and vaccines to specific cells. These lab-designed viruses are also more patentable than natural phages, which makes them more desirable to drug companies. As if to underscore that point, a division of the pharmaceutical giant Johnson & Johnson struck a deal in January with Locus Biosciences, worth up to $818 million, to develop phages engineered with the gene-editing tool CRISPR.

Developing a phage therapy that is commercially viable will not be easy. Barr and other scientists point out that it takes a tremendous amount of time, money and effort to engineer a phage, and after all that the target bacteria might soon evolve resistance to it. Furthermore, regulatory approval for an engineered phage "could be a tough sell," says Barr, echoing the view of several scientists interviewed for this story. But FDA spokesperson Megan McSeveney, in an e-mail, claimed the agency does not distinguish between natural and engineered phages as long as therapeutic preparations are deemed safe.

FUTURE PROSPECTS

COMPANIES ARE NOW testing different ways to bring phages to broader markets. Some companies want to supply patients with personalized therapies matched specifically to their infections. That is the strategy at Adaptive Phage Therapeutics. The company's chief executive officer, Greg Merril, says assays used to screen the navy's phages against infectious samples could be offered at diagnostic labs and major medical centers worldwide. Phages effective against locally prevalent bacteria in each region could be supplied in kiosks, bottled in FDA-approved, ready-to-use vials. Merril says doctors could continually monitor treated patients for resistance, swapping in new phages as needed until the infections are under control. He estimates that the per-patient cost under the current compassionate-use system is approximately $50,000, an expense that should fall with economies of scale.

Other companies reject this personalized strategy in favor of fixed phage products more akin to commercial antibiotics. Armata Pharmaceuticals' lead product is a cocktail of three natural phages targeted at *Staphylococcus aureus* bacteria, the cause of common staph infections often contracted at hospitals. It is in clinical trials in patients who have infected mechanical heart pumps. Armata's plan is to monitor for treatment-resistant staph in the general population, then introduce new cocktails as needed, in much the same way that influenza vaccines are tuned every year to match the latest circulating strains. Pharmaceutical executives said it was too soon to estimate what the costs would be.

Experts still cannot say which of the current strategies—sequential monotherapy, cocktails, engineered phages, and general or personalized treatments—may ultimately win out, assuming any do. An optimal approach "might not even exist," says Barr, considering that "phage treatments in each case could depend on complicating issues, such as the target pathogen, the disease and the patient's medical history."

Phage therapy is still saddled by geopolitical biases, too, says Strathdee. What is really needed now, she says, are positive results from well-controlled clinical trials that can help overcome residual skepticism. Alan Davidson, a biochemist at the University of Toronto, speculates that within a decade phage therapy might be cheaper, easier and faster than it is today. He leans toward the engineering approach, saying sequencing the whole genome of a patient's bacteria and then synthesizing a phage to cure an infection could be quicker and less expensive "than screening the pathogens against a battery of viruses drawn from nature."

Meanwhile Burgholzer, who was self-administering phage therapy with a nebulizer at home until March 2019, has not yet experienced the clinical improvements he was hoping for. In March, Chan and Koff introduced a second phage targeted at another *Achromobacter* strain. By April the bacterial counts in Burgholzer's lungs had fallen by more than two orders of magnitude since the initial treatment began. "So it does appear we can pick off those strains successively," Koff told me. Yet Koff acknowledged that Burgholzer was not noticing a dramatic change in lung function. When I asked why, Koff responded, "We know a lot more about the phage we use against *P. aeruginosa* than we do about phages targeting *Achromobacter*." The ability to manipulate the infection "is less informed."

The next step, Koff says, will be to genetically sequence mucus samples from Burgholzer's lungs. "We really need to understand what's happening with his bacteria so we can get to the high level of sophistication we have with *P. aeruginosa*. Bobby is letting us take a chance to see if, at a minimum, we can help." Frustrated but still eager, Koff says, "Some patients respond better than others. We need to understand those dynamics." SA

MORE TO EXPLORE

Global Priority List of Antibiotic-Resistant Bacteria to Guide Research, Discovery, and Development of New Antibiotics. World Health Organization, 2017.

Engineered Bacteriophages for Treatment of a Patient with a Disseminated Drug-Resistant *Mycobacterium abscessus*. Rebekah M. Dedrick et al. in *Nature Medicine*, Vol. 25, pages 730–733; May 2019.

Phage Directory: https://phage.directory

FROM OUR ARCHIVES

Infectious Drug Resistance. Tsutomu Watanabe; December 1967.

scientificamerican.com/magazine/sa