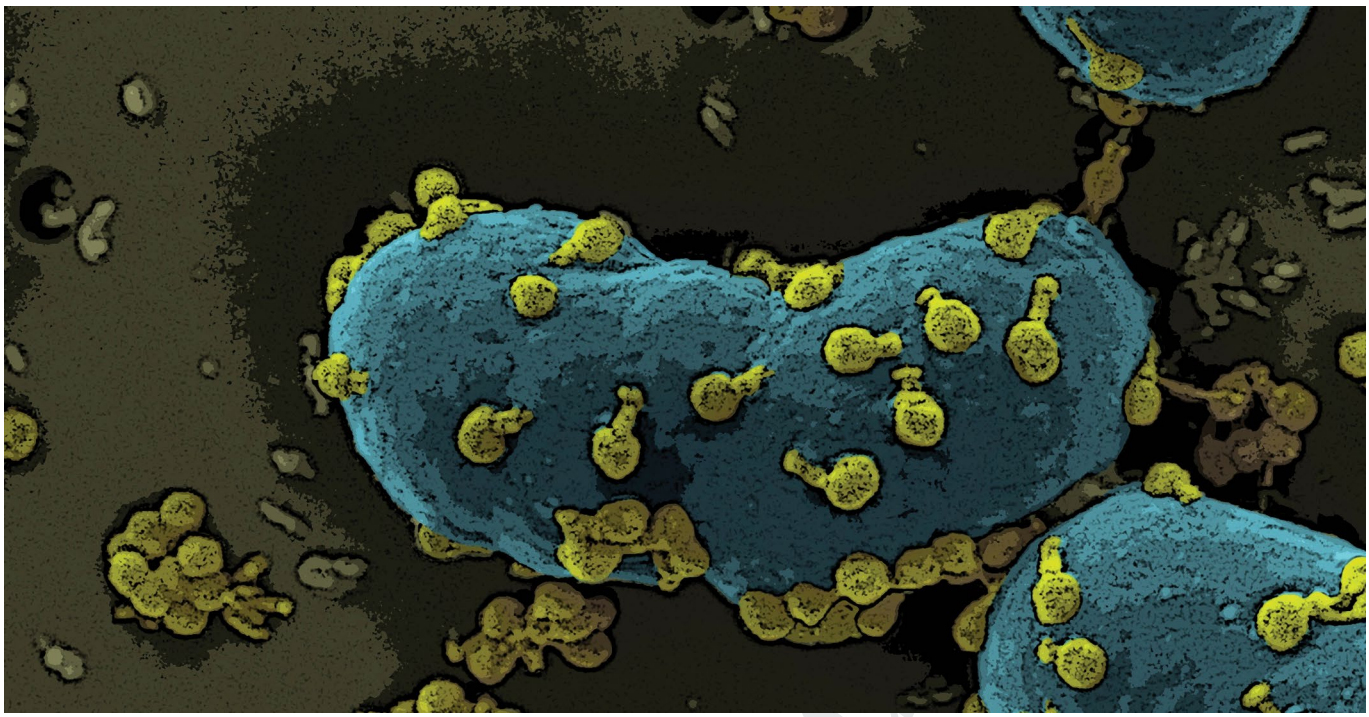


Credit: Dr. Robert Pope, National Biodefense Analysis & Countermeasures Center

Phage therapy's latest makeover

As issues of product consistency, standardization and specificity are being tackled, can phage therapeutics—long oversold and overhyped—finally realize their antibacterial potential? Charles Schmidt investigates.

Charles Schmidt

In May of 2018, an international team of researchers and clinicians reported they successfully treated a seriously ill teenager with cystic fibrosis who had disseminated infection by *Mycobacterium abscessus* with a cocktail of genetically engineered phage[1]. According to the University of Pittsburgh's Graham Hatfull, who led the research team, this accomplishment represents a number of firsts: the first genetically engineered phage treatment—in this case, to convert a lysogenic phage to a lytic variety—and the first treatment of a mycobacterium. It also bodes well for a therapy that has long been dismissed by Western practitioners, as well as for the future of synthetic-biology approaches to the vexing problem of antibiotic-resistant bacteria.

This news follows last year's launch of a phage translational research center at the University of California, San Diego (UCSD), another sign of optimism in this old but controversial approach for treating bacterial infections. Supported with a three-year, $1.2 million grant from the UCSD chancellor, the new Center for Innovative Phage Applications and Therapeutics (IPATH) is applying "the same principles of clinical evaluation and development to phage therapy that would be applied to any other therapeutic entity," says center co-director Robert Schooley, a physician and infectious disease specialist at UCSD.

A worsening crisis of multi-drug-resistant (MDR) infections, along with advanced technologies for characterizing viruses and their host interactions, is prompting a re-evaluation of phage therapy. And pharma, which has steered clear of antibiotics, let alone phage-derived ones, may be taking notice. Johnson & Johnson struck two deals centered on phage in January: one with Locus Biosciences, worth upwards of $818 million, to develop CRISPR phages (Box 1), and the other with the Israeli company BiomX, which is applying phage therapy to dysbiosis of the microbiome.

Still, previous experience, mostly in the context of compassionate-use phage treatments, has shown the approach to be hit-and-miss, time-consuming and expensive. To turn bacteriophage from a laboratory tool into an efficacious therapeutic for broader markets, companies are seeking to scale up production and deliver potent phage products under good manufacturing practices (GMP) quickly and reliably. Can companies deliver on expectations? We may get the answer soon as several companies developing phage therapies—AmpliPhi Biosciences, Adaptive Phage Therapeutics and Intralytix— move toward the clinic this year.

100 years of interlude

First tested as a prophylactic against avian typhosis in rural France in 1919, cocktails of phages were used therapeutically in Europe and the United States during the pre-antibiotic era, and they are still prevalent in Russia and Central and Eastern Europe

Box 1 | Turning bacterial defenses on themselves

There is yet another way researchers are turning phage into therapy: by loading them up with CRISPR–Cas systems that target host genes—that is, using the very system that bacteria evolved to eliminate external threats, like a phage, against itself.

Locus Biosciences is using Cas3, which differs from the more widely used Cas9 in that it degrades its target via an exonucleolytic activity, essentially destroying its target gene, rather than introducing double-strand breaks, which prepare a template for repair or replacement. Cofounder and researcher at North Carolina State University Rodolphe Barrangou and colleagues published a foundational paper in 2013 in which they show that Cas systems targeting specific bacterial sequences can distinguish among closely related species and can control the number of individual strains in a mixture[9]. According to senior VP Joseph Nixon, CRISPR–Cas3 efficiently kills target cells regardless of the function of the targeted sequence, and multiplexing is possible, which, like phage cocktails, forestalls the development of resistance. Their first trial, which is in the planning stages, could be among the first controlled, randomized trials of bacteriophage therapy and the first using engineered phage.

Eligo Bioscience in Paris is developing a non-replicative phage platform that carries Cas9 targeted to bacterial virulence or resistance genes. Cofounders Timothy Lu and Xavier Duportet (now Eligo's CEO) showed that they could knock out a virulent strain of *S. aureus* while leaving an avirulent strain intact. Likewise, they could knock out antibiotic resistance genes from plasmids, preventing the spread of resistance[10,11]. They are positioning themselves in the microbiome space, with their lead indication being a gut pathogenic bacteria causing a rare (undisclosed) disease.

Finally, Cambridge, UK, start-up Nemesis Biosciences is developing a platform of so-called 'transmids' that borrows elements of phage biology to deliver RNA-guided nucleases to knock out antimicrobial resistance genes. Transmids carry only a short signal phage sequence that is needed for packaging into a phage capsid. They are grown in a helper bacterial host that harbors a defective phage particle. After being purified from the host bacteria, the transmids invade the pathogenic bacteria in what CSO Conrad Lichtenstein calls a "hit and run" attack: transmid DNA enters the host cell, replicates and expresses the nuclease, inactivating resistance genes. In this way, the strategy avoids waves of phage infection against which the patient might mount an immune response, and, since it avoids killing directly, the bacterial pathogens are not under direct selection to evolve resistance, Lichtenstein says.

today, for wound infections, gastroenteritis, sepsis and other ailments. In the West, phage therapy was abandoned after broad-spectrum antibiotics came on the scene. Later, during the 1940s, the California Institute of Technology's Seymour Benzer pioneered work with bacteriophage T4 as an experimental genetic tool, leading to the deciphering of the central dogma of molecular biology and spurring its rise as a field. However, for a variety of reasons—the preponderance of phage work on laboratory rather than pathogenic bacteria (which often are already naturally resistant to bacteriophages) and a lack of peer-reviewed published data showing efficacy in animal models, not to mention political bias against a science associated with the Soviet Union and Nazi Germany—meant that the therapeutic utility of these viruses remained largely unexplored; research funding in the field dropped off during the 1970s and remained flat for decades.

Then in the early 2000s the field sputtered back into life. The rise of modern sequencing technology began to revive interest in phage biology and suggested that molecular engineering and characterization could start to address such issues as product consistency (phage cocktails often contain more than ten phage strains), poor tissue distribution, pharmacodynamics and immunogenicity issues. Despite the difficulty of gaining intellectual property protection (the technology has been around for nearly a century), the skepticism of traditional pharma and venture investors, and the uncertainties surrounding clinical trials and regulatory oversight, a clutch of companies sprang up around the concept by the mid-2000s, including Biophage Pharma, Enzobiotics, Exponential Biotherapies, GangaGen, Hexal Gentech, Intralytix, MicroStealth Technologies, Phage Biotech, Phage Therapeutic, PhageGen, PhageTech, Phage Therapy and Phico Therapeutics[2]. But funding remained scarce and validated evidence of reproducible clinical efficacy remained elusive, so many companies went out of business. Today, of that cadre of companies, only GangaGen in Bangalore, India; Baltimore-based Intralytix; and Phage Biotech of Rehovot, Israel, are in business. Intralytix and Phage Biotech survived by deploying phage in agriculture to control plant diseases, detect pathogens and assess food safety (Table 1).

A center of excellence

In 2010, Texas A&M University launched the Center of Phage Technology (CPT), with Ry Young, professor of biochemistry, biophysics and biology, at the helm. Like many of the companies, Young says CPT's initial strategy was to focus on agriculture and animal husbandry, "while avoiding human involvement because of high regulatory loads." Now that's changing. Like other facilities that work in this area, the CPT is overwhelmed with requests for phages as treatment of last resort for patients with MDR infections. Indeed, the recent resurgence in human interventions and emergency investigational new drug (eIND) applications "has come out of nowhere as a spontaneous reaction to the MDR problem," Young says.

The CPT supplied phages for a highly publicized eIND treatment in 2016. Tom Patterson, a UCSD professor of psychiatry, had picked up an MDR *Acinetobacter baumannii* infection during a trip to Egypt and was near death from organ failure by the time his therapy was delivered. Patterson's wife, Steffanie Strathdee, an epidemiologist at UCSD, helped coordinate the phage treatments that ultimately saved her husband's life[3]. Contributed by three separate entities—the CPT; AmpliPhi Biosciences; and the US Naval Medical Research Center (NMRC), which has been researching phage since 2011 for possible battlefield use—the phages were given daily for two months through a catheter into Patterson's abdomen, as well as through an intravenous line. Within days of beginning treatment, Patterson awoke from a coma, and after three months he was cleared of infection. He remains in good health. Meanwhile, Strathdee, who is the associate dean of global health science at UCSD, and Schooley, who was Patterson's treating physician, joined forces to launch IPATH.

Aside from one-off treatments like Patterson's, the hope is that phages will find broad use as precision antimicrobials that target specific pathogens while sparing the healthy members of the human microbiome. The treatments can also be

Table 1 | Selected companies developing phage therapies

Company	Founded	Funding	Platform	Target	Status
Adaptive Phage Therapeutics	2016	$5 million seed	Individualized phage therapy, PhageBank, Host Range Quick Test	Infectious disease, urinary tract infections	Preclinical
AmpliPhi Biosciences	2002	$8.23 million market capitalization	Phage combinations for bacteria	Infectious disease	Phase 1 cocktail of three phages, skin safety test; individual access for *S. aureus* and *P. aeruginosa*
BiomX (Ness Ziona, Israel)	2015	$24 million	Customized phage cocktails	Irritable bowel disease	Preclinical
C3J Therapeutics	2005	$136 million	Antimicrobial peptides and engineered phages	Infectious disease, microbiome	Preclinical
Eligo Bioscience (Paris)	2014	$20.2 million series A (Khosla, Seventure)	CRISPR engineered phage	infectious disease	Preclinical
EnBiotix	2012	Not disclosed	Engineered phage	Joint, skin, wound, cystic fibrosis, prosthetic joint infections	Preclinical
Intralytix	1998	$17.5 million	Phage cocktail against adherent, invasive *E. coli*	Crohn's disease	Phase 1/2
Locus Biosciences	2015	$26 million	CRISPR engineered phage	Infectious disease, microbiome	Preclinical
Nemesis Biosciences (Cambridge, UK)	2014	$2.3 million	Transmid	Extended spectrum β-lactamase-producing bacteria	Preclinical
Pherecydes Pharma (Romainville, France)	2006	$12.3 million	Individualized phage therapy	Infectious disease	Phase 2 (burns)

used in combination with antibiotics. Indeed, evidence shows that surviving bacteria following phage treatment are in some cases resensitized to antibiotics and incur additional fitness costs that render them susceptible to immune cells. MDR *A. baumannii* cells, for instance, are shielded in a capsule that protects them from antibiotics. The surviving bacteria during Patterson's treatment lacked this capsule and succumbed to antibiotic treatment.

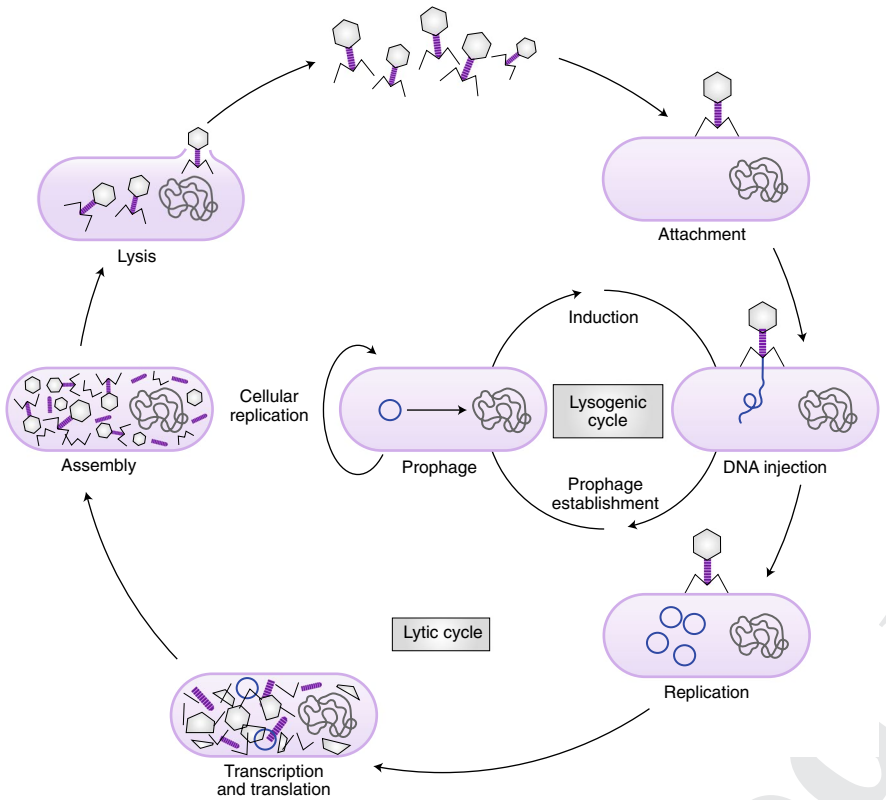
But despite the fact that phage have been a subject of research for nearly a century, very little is known about them, outside of the few research workhorse species. Even for these comparatively simple organisms, for many phage species the function of as much as 90% of the genome remains unknown function. "So you can get a readout of the genes, but unfortunately most of the genes are annotated as hypothetical. No idea what they are doing," says Karen Maxwell, a biochemist and phage specialist at the University of Toronto. Maxwell cautions that phage, which are notoriously promiscuous and can swap genes among phage species as well as hosts, could be carrying virulence genes or genes for bacterial toxins or antibiotic resistance.

Phage hunts

Phage therapy has been benefitting from technical advances on multiple fronts. As Rotem Sorek, of the Weizmann Institute of Science in Rehovot, Israel and one of the founders of BiomX, puts it, where researchers once lacked the means to characterize phages rapidly, it's now possible to "sequence a phage, assemble its genome and analyze the results in a matter of days." Next-generation sequencing has expanded the numbers of viral sequences deposited in databases. Since the 1990s, the University of Pittsburgh's Hatfull has been collecting actinobacteriophages as part of his long-standing interest in mycobacteria (a genus of Actinobacteria). It was slow going at first—the first genome sequence took a year. But the pace has quickened, and the database, PhagesDB, which is nourished by an annual phage hunt, has collected over 15,000 isolates, of which over nearly 3,000 have been sequenced completely. In fact, it was from this cache of phage that the three-phage cocktail used to treat the patient with cystic fibrosis was derived. Hatfull says finding and preparing the phage was not trivial: after finding isolates that infected *M. abscessus*, they used a phage-based recombineering technique developed in his lab ten years ago[4] to extend the host range of one isolate and convert another from lysogenic to lytic.

Recently, researchers at the University of California, Berkeley have received internal funding from the university's Innovative Genomics Institute to create aphage foundry. Here the goal is to identify genes involved in phage–host interactions, using loss-of-function mutational analysis[5] and gain-of-function gene dosage effects to identify the bacterial genes that enable particular phages to bind or resist binding by others. These insights would enable engineering of phages and phage-like systems to target specific groups of microbes and deliver designed function, and conversely, engineering of possible hosts for particular resistance and sensitivity phenotypes. According to joint principal investigator Vivek Mutalik, the focus initially will be on eliminating plant and human pathogens, and later efforts will involve rationally engineering microbial communities involved in plant health and productivity, those that remediate heavy-metal contaminants at complex watersheds, and those populating the human gut.

Identifying phage target receptors is key to moving phage into the clinic, as well as to preventing the host from developing



Phage can infect host bacteria in two distinct pathways: lytic cycle, in which new phage are created and dispersed, and lysogenic, whereby the phage and host form a stable association. Reprinted with permission from ref. [8], Springer Nature.

resistance, notes Young. By combining phages into cocktails, each targeting a different viral receptor, it's possible to prevent the onset of resistance, he says. Benjamin Chan, at the Department of Ecology & Evolutionary Biology at Yale University, adds that deep sequencing can allow clinicians to monitor in real time whether treatment is reducing the target bacterial population, and annotation tools can identify sequences for undesirable genes, such as integrases, that enable phage to integrate within bacterial genomes and remain dormant. Chan curates a growing phage library housed in the laboratory of Yale professor Paul Turner. By using these tools to assess patient samples during the course of therapy, "we can see changes in bacterial populations at the genomic level, check for bacterial resistance patterns, and determine how phages adapt to them," he says. With over 500 phages for over a dozen human pathogens, the library has already been mined for phage to be applied in upcoming clinical trials. They are planning a clinical trial at Yale New Haven Hospital to treat MDR *Pseudomonas aeruginosa* infections associated with cystic fibrosis. "Our objectives are to reduce bacterial burden and potentially resensitize bacteria to chemical antibiotics and reduce the expression of extracellular virulence factors," says Chan.

At the same time, Yale's phage library, in addition to those at other institutions, is amassing collections of characterized, purified phages for clinical use—each of them targeted at known bacterial receptors—which can then be pulled off the shelf and made available for treatment[6]. That capacity marks progress over the ad hoc experience of treating Patterson's infection, during which "people worked round the clock for three weeks to identify phages that matched his isolate and then grow and purify them," according to Strathdee. And that wasn't the end of it. Patterson developed resistance to his initial treatment after two weeks, forcing researchers to hunt for new therapeutic candidates in sewage samples, which then had to be characterized and amplified in sufficient quantities for dosing. The saga of Patterson and Strathdee has been documented in a forthcoming book, *The Perfect Predator* (https://bioengineeringcommunity.nature.com/).

Meeting the clinical benchmark

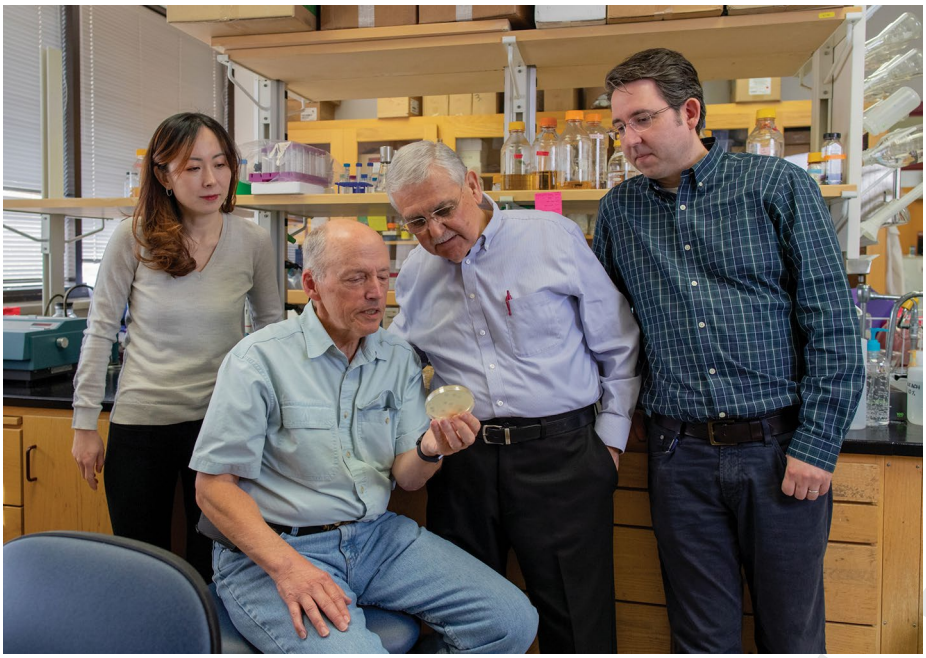
Accompanying these technical advances are encouraging signals from regulators at the US Food and Drug Administration (FDA). During an FDA-sponsored workshop on phage therapy held in 2017, Scott Stibitz, a lab chief in the agency's Center for Biologics Evaluation and Research, said that after many years of largely anecdotal experience, the field must now "initiate scientifically rigorous programs that include adequate and well-controlled clinical trials that support licensure of phage therapy products," a process that, he said, "FDA is committed to facilitating." The principal concerns for FDA for phage preparations are that they are safe, pure, potent, non-lysogenic, non-transducing, free of undesirable genes, and low in bacterial endotoxins, which can contaminate phage lysates extracted from bacterial hosts.

As Stibitz made those comments, the first clinical trial with GMP-compliant phage therapy was already underway. Launched in 2013 by Pherecydes Pharma, a biotech company in Romainville, France, the multicenter PhagoBurn trial tested a cocktail of 12 phages in burn patients who had developed MDR *P. aeruginosa* infections. Unfortunately, the results, which were published last October, were both lackluster and disappointing for phage proponents[7].

The patients had been randomized to either phage therapy or a control antibiotic, sulfadiazine silver, both given in topical emulsions. Although the phages showed activity—bacterial loads fell substantially in the treated patients—reduction were not as fast as in the control group, so the trial was terminated prematurely. Jérôme Gabard, the chief operating officer at Pherecydes, says the treatment wasn't as effective as hoped because the number of phages in the cocktail was too high. The aim had been to create an off-the-shelf cocktail with enough different phages to fully address *P. aeruginosa*'s intra-species diversity and its assortment of receptors. Each of 12 phages was individually stable, but when mixed together, they reacted in ways that Gabard says aren't well understood.

The phages were combined months before the patients were treated, during which time phage titers in the mixture fell by up to five orders of magnitude, resulting in delivered doses that were far lower than intended. The titers were reduced further still after Pherecydes diluted its cocktail with saline to address a growing problem with endotoxin contamination.

According to Young, the PhagoBurn team made another critical error: they never identified the target receptors for each phage



Phage researchers Mei Liu, Ry Young, Carlos Gonzalez and Jason Gill from the Center for Phage Technology at Texas A&M take the measure of the phage. Since 2010, the center has been working on phage therapeutics for humans, animals and plants. Credit: Mark Guerrero, Division of Marketing & Communications, Texas A&M University

before the viruses were combined. Adding two phages targeted against the same receptor wouldn't make sense, he says, "since resistance to one automatically confers resistance to the other." The appropriate number and combination of phages varies with the genetic diversity of the target pathogen. Bacterial species with low genetic diversity and a correspondingly limited suite of phage receptors—*Staphylococcus aureus*, for instance—can be treated with just a few phages. As the genetic diversity of the target pathogen increases, however, so too does the diversity of phages needed for effective therapy. *P. aeruginosa* is intermediate in diversity, whereas *A. baumannii* varies so widely from strain to strain that dozens of different phages are needed to treat it successfully.

Differing testing strategies

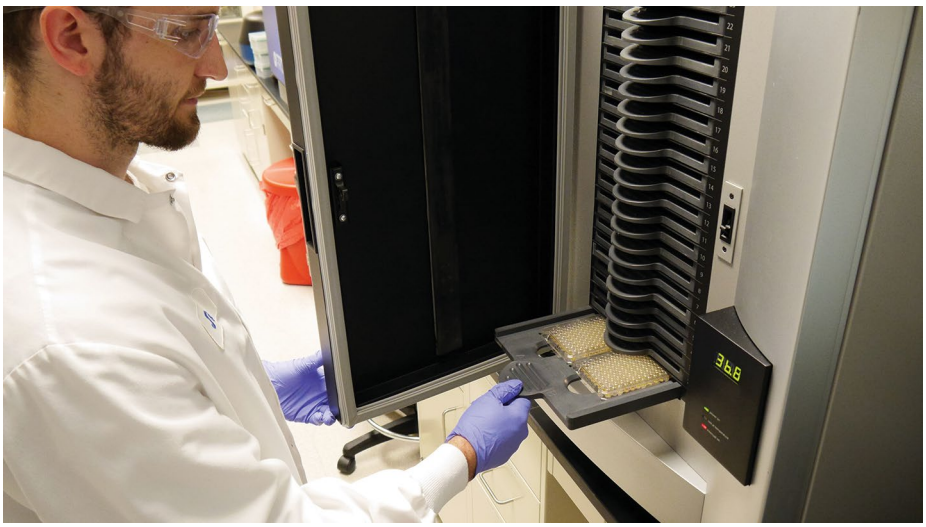
The link between bacterial diversity and phage combinations is fundamental to the strategies that companies are exploring as they enter the clinic. One strategy employs fixed cocktails with a minimal number of phages (Young recommends no more than three or four) targeting low-diversity bacteria. The phages in this case can be pulled out of a refrigerator and used for treatment, much like any other therapeutic agent.

Another strategy is personalized and geared for patients whose infectious pathogens are more genetically diverse. As occurred during Patterson's treatment, infectious isolates in these cases have to be monitored continually for resistance. And when a given phage, either in isolation or combined with other phages in a cocktail, stops working, a new one targeted at a different receptor can be added in its place.

Clinical trials planned by two companies this year illustrate the two approaches. In collaboration with IPATH, AmpliPhi has plans to test a fixed cocktail of three lytic phages targeted against *S. aureus* infections located within ventricular assist devices, infections that are hard to treat. Paul Grint, the company's CEO, says the fixed cocktail is more amenable to a conventional development program, and it's also "more consistent with our goals for a product that's stable in a refrigerator and addresses the many issues FDA has with quality and reproducibility." The company tested over 100 phages before selecting three candidates with a 95% kill rate against a panel of *S. aureus* strains. According to Grint, the phages induce the production of enzymes that disrupt bacterial biofilms, which tend to grow on prosthetic implants and shield pathogens from antibiotics. Grint says phages could therefore expose bacteria to antibiotic treatment, and thereby potentiate their action.

"It's not about phages *or* antibiotics," Grint says of the company's therapeutic approach. "It's about phages *and* antibiotics." Should FDA approval follow, the company intends to market an off-the-shelf product prepared at its GMP-certified facility in Slovenia and then monitor for resistance with post-market surveillance that screens the phages against isolates sampled from different countries over time. Grint expects that, should resistance be detected, they will address it by tweaking the mixture and periodically bringing revised products to market, not unlike the way new flu vaccine are made available every year. The company's three-phage cocktail targeting *S. aureus* has undergone two phase 1 trials for topical application, and this cocktail, as well as one for *P. aeruginosa*, is available for expanded access for patients with life-threatening infections. In October, they reported clinical case series data obtained from 13 Australian patients with MDR *S. aureus* sepsis and/or bacteremia. Ten of the patients also had infective endocarditis, and in half of them, the infection has moved into prosthetic valves. Collectively, over 290 intravenous phage doses were administered, and 83% of the intent-to-treat population (10 of 12 patients) were either symptom-free or significantly improved by the end of the treatment. The company's planned trials for later this year will test cocktails targeting two different indications: MDR *S. aureus* infections in implanted vascular assist devices and *P. aeruginosa* associated with cystic fibrosis.

An upcoming clinical trial from Adaptive Phage Therapeutics (APT), meanwhile, will test a more personalized strategy for urinary tract infections. The company's cofounder and chief science officer, Carl Merril, spent over four decades trying to bring phage therapy forward while working as a lab chief at the US National Institutes of Health. Rebuffed with repeated cuts to his funding, Merril retired in frustration from the NIH in 2005, while his postdoc, Biswajit Biswas, persevered with phage research, first in the private sector and then at the NMRC, at Fort Dietrick, Maryland, where he is now chief of bacteriophage sciences. Since arriving at the NMRC, Biswas has been building up a library of phages collected from around the world, now called PhageBank, which was inspired initially by the need for better therapy against MDR *A. baumannii* infections that occur commonly among injured military personnel returning from the Middle East. To speed the phage screening process, he developed a high-throughput 96-well system, the Host Range Quick Test (HRQT), which uses a colorimetric assay to assess the effects of phage on bacterial growth and survival. Strathdee had enlisted NRMC's help during



Q5 Adaptive Phage Therapeutics was founded to move technology developed at the US Navy's Biological Defense Research Directorate phage research from compassionate use into commercially available therapy. Credit: Carl Merril, Adaptive Phage Therapeutics

her husband's ordeal in 2016. Galvanized by his successful recovery, the NMRC began looking into commercial opportunities with the PhageBank and HRQT, and APT was subsequently formed by Merril and his son Gregory Merril (now the company's CEO) for that purpose.

APT entered into a multi-year cooperative research and development agreement with the NMRC in 2016 and was awarded exclusive license to the HRQT and the PhageBank the following year. The PhageBank is split between the company's facility in Gaithersburg and the NMRC. Infectious isolates delivered to APT by the treating hospital can be screened against the contents of the PhageBank to search for effective candidates.

"Say the patient has a *Klebsiella* infection," Biswas says. "There are hundreds of phages targeting that pathogen in the PhageBank, and you can screen them all." As resistance emerges during treatment, phages can simply be swapped out, explains Mike Stockelman, deputy director for infectious diseases at the NMRC. "The whole premise behind this approach is that a cocktail works for as long as it does, and when it stops, you go back to the assay and run it again—we can do that in eight hours—and develop a second cocktail. And you continue doing for as long as it takes to knock down an infection to the degree that the patient's immune system can take over."

As the NMRC's commercial partner, APT is negotiating the regulatory processes involved in turning the PhageBank into an FDA-approved product. Merril says the long-range goal is to set up APT kiosks in major hospitals, each stocked with single-use phage vials manufactured under GMP.

Phage engineers

AmpliPhi and APT are working with natural phages, but several other companies have recently sprung up that are engineering the viruses to deliver enhanced therapeutic payloads. C3J Therapeutics (which in January announced plans to merge with AmpliPhi) acquired the proprietary phage platform of Synthetic Genomics in February 2018, along with what Brian Varnum, C3J's chief development officer, says is a lead program targeting *P. aeruginosa* that he anticipates will reach the clinic in 2019. C3J's strategy is to hunt for natural phages with broad host ranges and then engineer them for desired attributes, such as improved pharmacology, greater depth of kill and greater access to biofilms. The company plans to develop fixed cocktails. Though Varnum acknowledges that fixed cocktails likely won't work against highly diverse pathogens such as *A. baumannii*, he proposes that with synthetic biology it's possible to engineer phages that can overcome host-range limitations.

Also working on engineered phages, as one of a number of strategies for combatting antibiotic resistance, is EnBiotix, in Boston, deploying technology developed by James Collins, who is now at the Massachusetts Institute of Technology. The company acquires phages from its global network of academic laboratories and engineers them for a range different payloads, including antimicrobial peptides, antitoxins, and genes for reversing phage resistance. EnBiotix's lead product, sponsored initially by a grant from Mayo Clinic Ventures, is engineered to accelerate biofilm degradation[7] while at the same time targeting *S. aureus*–induced prosthetic joint infections. Jeff Wager, the company's CEO, says this fixed product is about two years away from the clinic.

Finally, phage are also being coopted as delivery vehicles for other therapeutic modalities. This has spurred a cadre of companies exploiting molecular tricks to turn bacteria's own CRISPR–Cas endonuclease immune systems on themselves (Box 1).

Au naturel or modified?

Engineered phage may have certain advantages over natural ones, particularly from a standpoint of commercial development. Collins points out that "engineered modifications" are "eminently patentable and natural phages likely not." Even so, Biswas questions the wisdom of spending years on patenting genetic modifications to which bacteria might easily develop resistance. And Greg Merril points out that while they're unable to patent viral sequences, companies working with natural phages can pursue other forms of intellectual property. APT, for instance, has a portfolio of pending patents related to banking phage, high-throughput bacterial-phage matching, and in silico phage matching with artificial intelligence. Similarly, AmpliPhi has patents on "properties, selection, and combination of natural phages," Grint says.

But even as companies move toward clinical trials, they're confronting entrenched biases against phage therapy by physicians inclined to view it as an old Soviet technology that was never backed by reliable evidence. Phages may offer the promise of selective anti-infective activity, but using them requires that physicians first identify the species, which in a typical hospital setting might take 24–48 h, if not more. "What you really need to be successful in this space is phage plus a rapid diagnostic kit," says Aleks Radovic-Moreno, vice president of PureTech Health. Though sequencing technologies can identify bacteria to species within hours, they haven't been widely adopted in clinical settings due to their high cost. "Most of the time, you can give antibiotics and there isn't a problem," Radovic-Moreno says. "So the urgency isn't there—you ask doctors and they'll tell you 'Yes, MDR bacteria are an issue we need to deal with,' but ask them to buy a $500,000 box and take a chance on phage for one out of every 100 patients, and it's going to be difficult." Until there's a pull from the clinical side, Radovic-Moreno predicts "phage companies [that don't provide diagnostics] are going to be pushing the boulder up a very steep hill for a long time."

Yet Schooley strikes a more optimistic tone, and says that he and others at IPATH view themselves as "filling a black hole that's been plaguing phage therapy for years." IPATH has a full plate, he says, working with the Antibacterial Resistance Leadership Group of the US National Institute of Allergy of Infectious Diseases and with several biotech companies on what's shaping up to be the first wave of randomized clinical and translational studies focused on phage therapeutics in the United States. The first of these studies—its collaboration with AmpliPhi targeting S. *aureus*–infected vascular assist devices—was announced in January.

Schooley says phage therapy is better positioned now to make broad advances than it ever was before: screening is faster and cheaper, he says, and scientists have made key strides in their understanding of phage pharmacology. At the same time, GMP production costs pose a major hurdle, and Young says scientists still have a long way to go in terms of characterizing basic phage biology and its interactions with pathogenic bacteria.

"Phages are viruses and they will do what they want to do and not necessarily what we want them to," he says. "So ultimately when phage are widely used—and I think they will be—it has to be on firm scientific footing." ❐

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References

1. Dedrick, R. M. et al. *Nat. Med.* https://doi.org/10.1038/s41591-019-0437-z (2019).
2. Thiel, K. *Nat. Biotechnol.* **22**, 31–36 (2004).
3. Schooley, R. T. et al. *Antimicrob. Agents Chemother.* **61**, e00954–17 (2017).
4. van Kessel, J. C. & Hatfull, G. F. *Nat. Methods* **4**, 147–152 (2007).
5. Price, M. N. et al. *Nature* **557**, 503–509 (2018).
6. Chan, B. K. et al. *Evol. Med. Public Health* **2018**, 60–66 (2018).
7. Jault, P. et al. *Lancet Infect. Dis.* **19**, 35–45 (2019).
8. Salmond, G. P. & Fineran, P. C. *Nat. Rev. Microbiol.* **13**, 777–786 (2015).
9. Gomaa, A. A. *MBio* https://doi.org/10.1128/mBio.00928-13 (2014).
10. Citorik, R. J., Mimee, M. & Lu, T. K. *Nat. Biotechnol.* **32**, 1141–1145 (2014).
11. Citorik, R. J. et al. *Nat. Biotechnol.* **32**, 1141–1145 (2014).

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