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Phage Therapy in the Twenty-First Century: Facing the Decline of the Antibiotic Era; Is It Finally Time for the Age of the Phage?

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Abstract

Burgeoning problems of antimicrobial resistance dictate that new solutions be developed to combat old foes. Use of lytic bacteriophages (phages) for the treatment of drug-resistant bacterial infections is one approach that has gained significant traction in recent years. Fueled by reports of experimental phage therapy cases with very positive patient outcomes, several early-stage clinical trials of therapeutic phage products have been launched in the United States. Eventual licensure enabling widespread access to phages is the goal; however, new paths to regulatory approval and mass-market distribution, distinct from those of small-molecule antibiotics, must be forged first. This review highlights unique aspects related to the clinical use of phages, including advantages to be reaped as well as challenges to be overcome.

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INTRODUCTION

Now over a century after the discovery of the first antibiotics, it is safe to say that the ability to quell infections that elude the body's innate defenses marked a monumental leap forward for humankind. Antibiotics and their mass production revolutionized the medical field, in many ways doing for medicine and pharmacology what the cotton gin did for manufacturing. Early widespread use of penicillin produced remarkable gains, subduing the widespread scourge that was syphilis in the early twentieth century and curtailing military casualties due to secondary infection (22, 41). Less conspicuously but no less significantly, antibiotics underpin the success of many modern-day treatment modalities: organ transplantation, stem cell transplantation, and cancer chemotherapy, in particular. The ability to bridge patients from myeloablation to immune reconstitution with broad-spectrum antibiotics (in conjunction with infection control measures) is a testament to their potent utility.

The problem of antibiotic resistance is therefore a prodigious one. Besides starting to erode the success of advanced medical therapies, it threatens to return all of humankind to a much more precarious existence. It is an existence that, as we are all hyperacutely aware, is intimately and inextricably linked to the coexistence of multitudes of bacteria and other microorganisms. That, on occasion, a rogue bacterium should find itself in a hospitable environment beyond the confines of its usual domain and afflict perfectly healthy individuals with a nontrivial infection is to be expected. In a practical sense, antibiotics are our insurance policy in such circumstances, mitigating the consequences of these unhappy accidents and averting numerous fatalities. Maintaining a supply of effective antibiotics is in the interest of all of society.

The idea of using bacteriophages (aka phages) as a stopgap measure for cases of antibiotic-treatment failure has been gaining momentum in recent years, spurred on by reports of experimental phage therapy cases with exceptionally positive patient outcomes (7, 72). While phages and their therapeutic applications are undergoing a revival in the Western world, it is notable that for parts of Eastern Europe and the former Soviet Union, phage therapy has been a constant reality. A number of purified phage preparations have been and continue to be produced for clinical use by the Eliava Institute in Tbilisi, Georgia (30). Assigned names like Intestiphage or Pyophage, these

preparations are prescribed for a variety of indications and administered via oral and/or topical routes. Substantial documentation has been amassed attesting to the safety and efficacy of these and other phage-based remedies over the years (31, 48). Unfortunately, despite general acceptance of the literature's credibility, it has not yet produced evidence capable of satisfying international standards of rigor. The world is still awaiting its first large-scale, well-controlled, transparently executed clinical trial to assess the efficacy of phages for the treatment or prophylaxis of bacterial infection in humans.

With greater interest in phage therapy come more questions from interested parties about the nature of bacteriophages and the most pressing challenges facing this still-emergent field. This review addresses both in turn, focusing on aspects relevant to the US experience where applicable. The therapeutic potential of phage-derived products such as lysins and depolymerases, though no less worthy of review, is not included here. Our goal is to present a well-balanced overview of phage therapy (referring to the use of whole bacteriophages as antibacterial agents) in order to facilitate recognition of its promise, as well as deficiencies, as it exists in its present state.

BACTERIOPHAGES: NATURE'S MOST ABUNDANT ANTIBIOTIC

Bacteriophages are simply viruses that infect bacteria. They are ubiquitous in nature and have been shown to outnumber bacteria 10:1 in aquatic environments (10). Their replication is inextricably tied to a bacterial host, leading to complex evolutionary relationships between bacteria and phages that have matured over millennia (47, 55). For example, as opposed to lytic phages that rely on repeated cycles of bacterial infection in order to sustain their replication, lysogenic phages are master evolutionary bet-hedgers. With recourse to two distinct life cycles, a lysogenic phage may integrate into the bacterial genome and replicate with the host (lysogenic cycle) or may direct host cell machinery to produce phage progeny and replicate at the expense of the host (lytic cycle). Activation of the appropriate genetic program to enable either lysis or lysogeny is driven by input received by the phage regarding the condition of the host. Obviously, this makes it imperative that therapeutic phages be strictly lytic.

With a better understanding of phage biology comes a deeper appreciation for the ways in which phage therapy deviates from the traditional antibiotic mold (**Figure 1**). Each point of dissimilarity presents a double-edged sword. To what extent phage-specific advantages can be maximized and phage-specific disadvantages minimized remains to be seen. Phage engineering is still in its infancy, and its long-term potential looms large. Therefore, any discussion of differences between phages and traditional antibiotics is perpetually subject to revision, the following included.

Phage Specificity

Phages attach to bacteria via interactions between their tail fibers and compatible cell surface structures in a process called adsorption. Adsorption signals to the phage that it has docked on what is likely to be a suitable host, triggering the irreversible process of DNA (or RNA) injection. Often these bacterial structures, also referred to as phage receptors, are highly variable and therefore highly specific. It is not uncommon for a phage host range to be limited to a handful of strains within a bacterial species. This specificity renders phages very microbiome-friendly, but it also creates a logistical nightmare when considering how phage therapy might be adapted to provide broad coverage of clinical strains.

There are a variety of ways to expand the host range of phages either biologically or as a therapeutic preparation. To accomplish the former, one of several clever genetic techniques may

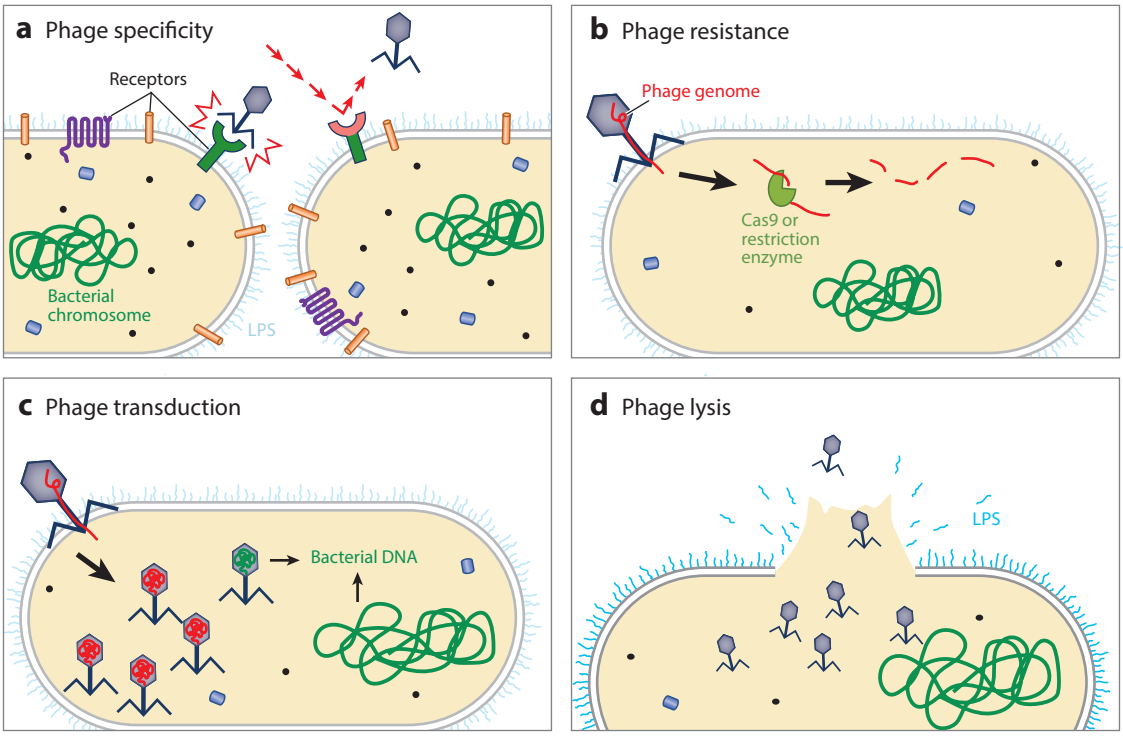

Figure 1

Biological challenges for phage therapy. (*a*) High phage receptor specificity confers a narrow host range for individual phages. (*b*) Evolved bacterial defenses act to subvert phage infection. (*c*) Incidental packing of bacterial DNA into a phage capsid can lead to transfer of virulence genes to another bacterial cell. (*d*) Lysis of the bacterial host releases phage progeny as well as cellular components, potentially exacerbating inflammation in the human body.

be employed to facilitate tail fiber interchangeability between distinct phages (1, 43, 74). This approach has its constraints, though, as phage tails are not universally compatible. Also lacking is the ability to dictate that tail fiber expression follow a set proportion; i.e., if a phage genome contains three distinct tail fiber genes and the phage baseplate has a six-tail-fiber capacity, there is currently no means to ensure that a given phage will display two tail fibers of each moiety.

A number of phages have naturally evolved tail fiber polyvalency, making screening phage from the environment a possible alternative to genetic engineering. These phages tend to be large, earning the designations jumbo phage and giant phage for a genome size exceeding 200 kilobase pairs (89). One jumbo phage capable of infecting multiple capsular types of *Klebsiella pneumoniae* has been observed to display nine unique tail fiber proteins consistently within a single virion (52). This phage encodes an assortment of genes to direct its intracellular replication, including two RNA polymerase sigma factors, which raises an interesting, albeit unrelated, point: Jumbo phages challenge the traditional paradigm in which bacteria and other self-replicating microorganisms are regarded as inherently more complex than phages. The largest characterized phage genome, that of *Bacillus megaterium* phage G, contains almost 700 protein-coding genes—easily more than the protein-coding content of the smallest bacterial genomes (16, 29).

Of course, the simplest way to expand phage host range is to add more phages. Phage cocktails, referring to combinations of phages in one preparation, can accommodate a number of unique

phages and still maintain high per-phage concentrations. The practical cost to demonstrate clinical safety and efficacy for each phage within a cocktail is more problematic (more on this topic to come). Limiting the number of unique phages in a cocktail becomes even more challenging when the number of phages added to expand host range must be balanced with the number added to suppress phage resistance.

Phage Resistance

Nothing in life is certain excepting death, taxes, and the ability of bacteria to evolve survival-enabling adaptations. Bacteria have amassed numerous mechanisms to counter phage infection, including phage receptor masking or alteration, restriction-modification systems, and CRISPR-Cas (32). Assembling cocktails of phages capable of infecting the same host via adsorption to different receptors may prevent, or at least delay, the emergence of resistant mutants. Like combination antiretroviral therapy, phage cocktails nonlinearly increase the genetic barrier to resistance (64, 86).

The addition of same-host-targeting phages not only decreases the genetic probability of a panresistant bacterial mutant but also increases the likelihood that such a mutant will have a fitness defect. By requiring that any surviving bacterial population adopt modifications that natural selection has not already made present at high frequency, growth impairment and/or loss of accessory (i.e., antibiotic-resistance) genes is often a necessary trade-off (8, 36, 69). This makes clinically relevant phage resistance difficult to predict, as phage-resistant bacteria that emerge readily under laboratory conditions may never appear under physiologic conditions, where accumulated fitness defects may lead to immune destruction, antibiotic resensitization, or simply failure to thrive.

Presumably resistance will be a perpetual threat to any antimicrobial agent. Phages, as replicative entities subject to evolutionary forces, may be better equipped to deal with this than most. Millions of years of coevolution have endowed phages with an array of antibacterial counterdefenses, including genomic methylation to evade recognition by restriction enzymes, depolymerization of bacterial capsule to gain access to its receptor, and genomically encoded antitoxin molecules to subvert bacterial abortive-infection (Abi) systems (68). The ingenuity of existing phage defense mechanisms and the longevity of phage over millennia support the notion that phage resistance cannot be sustained indefinitely so long as bacteria remain sufficiently prevalent.

Also beneficial is the fact that phage populations evolve rapidly, resulting in bacteria-phage coevolution occurring on the same timescale. In a seminal *Science* paper, Lenski and colleagues dissected out deceptively complex coevolutionary relationships between a strictly lytic derivative of phage λ and its host, *Escherichia coli* (47). They showed that in all 96 replicate populations of λ-sensitive *E. coli*, a mutation downregulating the expression of LamB, the sole receptor of phage λ, became fixed in the population within 8 days. Continued coculture of bacteria and phages allowed phage λ to evolve the ability to adsorb to a new receptor, outer membrane porin F (OmpF), thereby restoring its infectivity in 24 replicate cultures by a median of 12 days.

The ability of phages to keep pace with bacterial evolution confers some practical advantages. For one, the evolution of phages with novel host specificities can be carried out in the laboratory, offering a third source of phages in addition to engineering or foraging from the environment (18, 50). Second is the potential for phages to adapt to resistant bacterial populations in vivo, provided the site is conducive to phage retention and survival. In this way phage therapy may reload and cycle through several iterations without requiring readministration. Undoubtedly this occurs in at least some cases of chronic infection where phages are present; however, the challenge would be to establish the phenomenon consistently in a clinical model.

Phage Transduction

In the absence of nuclear compartmentalization, the genetic material of bacteria and phages are free to intermingle—and intermingle they do. Transduction occurs when genetic material from the host is packaged into the phage capsid during replication and then is injected into another bacterium as that phage initiates a new infection cycle. Generalized transduction, the type capable of transferring literally any part of the bacterial chromosome, occurs with variable frequency depending on the specific DNA-packing mechanism employed by the phage to fill its procapsids (57). One generic estimate of phage-mediated transduction quotes a frequency on the order of one in every 10,000 phage progeny (23). Though not a high rate relative to conservative estimates of burst sizes (10–100 phages per bacterium), it becomes a mathematical inevitability when large bacterial burdens of infection are factored into the equation (78).

Transduced genetic material may include discrete genes, mobile genetic elements such as plasmids and transposons, and, depending on capsid size, even large tracts of DNA encompassing genomic islands (57, 62, 91). The potential for virulence factors to make up part of that genetic payload is a legitimate safety concern. Certainly, transduction has been established as an effective means by which bacteria acquire survival-enabling and growth-enhancing genes, including those conferring antibiotic resistance (4, 5, 71). However, the extent to which therapeutic phage administration will facilitate injurious transduction events in vivo is unclear, as are the projected epidemiological consequences of such events.

Incidentally, it is often said that phage-capsid-packing mechanisms are error prone or that transduction occurs when host DNA is mistaken for the phage genome. This is a subtle but significant mischaracterization. Phages are not incentivized to lyse bacteria to the point of eradication; but quite the opposite, they are invested in the long-term reproductive success of their host(s). By engineering a low error rate into the capsid-packing process, phages can funnel genetic tools to their future hosts without compromising their own replication efficiency. Appreciation of this and other evolutionary drivers of bacteria-phage interactions is critical, since natural phages cannot be expected to behave in a manner that conflicts with evolutionary selection pressure.

Phage Lysis

Phage lysis, deconstructed, is a marvel. Penetration through two to three structural layers (the inner membrane, the peptidoglycan layer, and, in gram-negative bacteria, the outer membrane) designed to keep the cell protected and intact is no small feat. To do that in a regionally controlled, temporally regulated fashion is all the more impressive (87).

The clinical concern is that phage lysis may liberate more endotoxin and other pathogen-associated molecular patterns (PAMPs) than antibiotic-mediated cell death, which may trigger harmful inflammation. This certainly warrants concern as humans are exquisitely sensitive to endotoxin. An intravenous dose of 4 ng/kg has been shown to induce hemodynamic instability in healthy volunteers (79). The physiologic impact of progressively larger endotoxin doses, though ethically untestable, would be expected to culminate in death (as acute cardiovascular insufficiency is wont to do).

Yet if years of sepsis research have taught us anything, it is that the relationship between levels of measured endotoxin, levels of immune mediators, and individual patient outcomes is anything but straightforward (25, 49). Evaluating the physiologic impact of phage lysis is further complicated by the fact that analogous determinations have not been established for bactericidal antibiotics. While there are measurable differences in endotoxin release between approved antibiotics, there is no conclusive evidence that this either is or is not clinically relevant (38, 76). Therefore, the potential for toxicity from phage lysis is highly speculative but at the same time too risky to ignore.

Frederick Twort | Félix d'Herelle | Jules Bordet

Figure 2

Key figures in the early bacteriophage field. Sources: (*left*) Reference 63, (*middle*) Service Photo, Institut Pasteur, Photothèque, and (*right*) A. B. Lagrelius & Westphal, Stockholm.

PHAGE THERAPY IN THE UNITED STATES: A BRIEF HISTORY

Despite the caveats raised in the preceding section, phage therapy has been administered sporadically to patients in the United States since the 1920s. Review of this early experience with phages, insofar as it can be reconstructed from historical texts, not only is far more riveting than it sounds but also provides lessons relevant to modern-day development. If phage therapy is to be successfully launched in the twenty-first century, it is critical to understand why it failed to do so in the previous one.

The Early Years: Uncertainty, Inconsistency, and Animosity

Soon after the first descriptions of transmissible bacteriolysis by Frederick Twort (84) and Félix d'Herelle (13) (**Figure 2**) in 1915 and 1917, reports of clinical phage use began cropping up. d'Herelle focused his first human phage therapy trials on bacillary dysentery, building on work he had done in the laboratory with *Shigella dysenteriae*–infected rabbits (80). Others broadened this scope significantly, applying phages to a wide range of infections including furunculosis, osteomyelitis, meningitis, puerperal sepsis, typhoid, and hordeolum—with mixed results (28, 44, 67, 70, 83). Cases heralded as stunning successes were offset by those branded as obvious failures. The vast majority of cases, however, fell into a much more ambiguous category. Clinical research methods were rudimentary at this point, making it difficult for the medical community to reach consensus about optimal treatments or the effectiveness of new therapeutics (81). In retrospect, if the randomized, double-blind, placebo-controlled trial design had been precociously conceived, the discourse surrounding phage therapy in the 1920s and 1930s might have been much less prejudiced, and consequently, clinical applications of phages might be much further along in development today.

On the other hand, clinical trial design was not the only impediment to a fair evaluation of phage therapy. Phages themselves were so poorly understood at the time that it is no wonder they were frequently and egregiously misapplied (88). For over 20 years after d'Herelle first described "an invisible microbe antagonistic toward dysenteric bacilli," the very nature of phages was a point of bitter contention (13). A vocal opposing camp with two Nobel laureates among its ranks, Jules

Bordet and John Northrup, maintained that the "transmissible glassy transformation" first reported by Twort reflected the activity of a lytic enzyme, not a virus (81, p. 131). It was not until the advent of electron microscopy years later that the debate was finally settled. In the meantime, phage therapy lost some legitimacy in the eyes of the scientific establishment and became more fringe, like its founder.

For a revolutionary new idea to be received with skepticism by established authority figures is to be expected. Yet in the case of d'Herelle and Bordet, professional differences of opinion devolved into fiery disputes stoked by personal rancor. Summers (81), a physician and historian of science and medicine, chronicled the series of events that shaped this relationship and, by extension, the early bacteriophage field. It is a fascinating read. The details are not recapitulated here, but suffice it to say it has all the makings of a hit Broadway musical—the scientific version of *Hamilton*. Among the shared features are a brash and brilliant young upstart, an equally ambitious but more orthodox rival, a discipline/nation on the verge of exponential growth, and, yes, even a formal challenge to duel. (Thankfully in the case of d'Herelle and Bordet, the publicly issued invitation to duel specified that the grounds would be scientific.) But all joking aside, the narrative of d'Herelle and Bordet and the birth of phage therapy underscore the importance of separating scientific judgement from ambition, egos, and personal antagonism.

Early clinical phage studies suffered from another critical shortcoming—one that made the virus versus enzyme debate irrelevant; that is, phage preparations were not routinely verified for activity against the causative agent of infection. Consequently, it was not uncommon for phages to be administered without any curative potential. A number of factors were to blame, including an underappreciation for phage specificity and insufficient knowledge of proper phage propagation. It has been observed that phages with gram-positive hosts display broader host range than their gram-negative-bacteria-targeting compatriots, likely secondary to the less variable nature of the outer peptidoglycan layer (26, 53). This may account for the condemnation by Eaton & Bayne-Jones (17, p. 1939) in their 1934 *JAMA* review on phage therapy that, "Only in the treatment of local staphylococcic infections… has evidence at all convincing been presented."

Obtaining confirmation that phage preparations contained viable phages, not to mention in the quantities and with the specificities they claimed, was simply not feasible for nonmicrobiologists. In an era of scant regulation of pharmaceuticals (prior to the Kefauver Harris Amendment of 1962), manufacturers were not required to provide evidence of therapeutic efficacy or to restrict their advertising claims to statements of fact. As a result, many peddled phage preparations were completely devoid of active phages, the reasons for which ranged from the misguided use of preservatives to considerably less honorable explanations (80). Unbeholden to scientific accuracy, purveyors of phages seized upon the very marketable imagery of phages as voracious bacteria-eaters that would relentlessly roam the body in pursuit of their prey. This malignant combination of poor-quality product and overblown advertising did not go unnoticed by the medical community. A 1933 editorial in *JAMA* denounced the "premature commercial exploitation of 'bacteriophage'" and the "suppression of scientific fact in the promotion of bacteriophage preparations," which had "induced expenditure of considerable sums for therapeutically inert preparations" (11, pp. 1603–4). More prophetically, the editorial lamented that these practices were fueling a "rapidly growing resentment and distrust of the whole bacteriophage promotion which certainly will delay final clinical evaluation" (11, p. 1604).

Lacking a solid scientific knowledge base and a reliable source of product, the field of phage therapy did indeed succumb to growing doubt and frustration by the 1940s. By this time d'Herelle had left his post at Yale University to cofound a center for phage therapy with Giorgi Eliava in Tbilisi, Georgia (30). It is known today as the Eliava Institute, and at the time of its establishment it was within Soviet borders. Dovetailing with World War II and the early mass production of

penicillin, phage therapy research in the United States essentially ground to a halt. Gunther Stent, author of the canonical book *Molecular Biology of Bacterial Viruses* published in 1963, hammered the last nail in the coffin with his ringing proclamation, "the strange bacteriophage therapy chapter of the history of medicine may now be fairly considered as closed" (77, p. 9).

Seeds of Revival

After a long period of dormancy, US phage therapy cases are springing up once again (7, 33, 72). It is a resurgence driven not by nostalgia but by necessity. And this time, armed with a more sophisticated understanding of molecular biology, clinical medicine, and everything in-between, phage therapy version 2.0 may be poised to fulfill part of d'Herelle's vision that has hitherto gone unrealized on this side of the Atlantic.

Among the cases in this new, second-generation phage therapy cohort is one that deserves special attention. Colloquially known as the Tom Patterson case, it is distinctive for a variety of reasons, but first and foremost for an astounding clinical outcome following therapeutic phage administration (72). Because this case is regarded by many as a proof of concept, its details bear careful scrutiny and thus are presented here.

Tom Patterson, a 68-year-old professor with diabetes, became acutely ill with necrotizing pancreatitis (locally destructive inflammation of the pancreas) in late 2015. As a secondary complication, a pancreatic pseudocyst (walled-off intra-abdominal fluid collection) formed. Once formed, this stagnant fluid becomes prone to infection, and once infected, it becomes difficult to clear with antibiotics, even when the organism is antibiotic sensitive (3). Therefore, it signaled a serious turn of events when Patterson's pancreatic pseudocyst became infected with an extremely drug-resistant *Acinetobacter baumanii*.

In the acute stage of infection, Patterson required treatment in the intensive care unit. His condition stabilized on a regimen of last-resort antibiotics and percutaneous drainage, although the infection was far from eradicated. While still an inpatient at the University of California, San Diego (UCSD), Patterson suffered an acute decompensation when bacteria festering within the protected confines of his intra-abdominal fluid collection seeded his bloodstream and precipitated septic shock. *A. baumanii* was cultured from his blood, but now it was resistant to the last-resort antibiotics that had aided his first partial recovery. Source control, the mainstay of treatment for large, infected collections, had not been achieved with percutaneous drainage. A more aggressive means of obtaining source control, i.e., surgical, was no longer an option, as Patterson's condition made surgery excessively risky. Thus, suboptimal antibiotics were continued with the rationale that at least they were better than nothing, but without much expectation for cure.

By the time phages entered the discussion, Patterson's condition had gone from bad to worse. He had been intubated and minimally responsive for weeks. A heavy burden of resistant *A. baumanii* had been cultured from respiratory secretions in addition to intra-abdominal drainage. His hemodynamics were extremely precarious, requiring the support of three pressors. His liver function and kidney function were caving under the pressures of systemic inflammation, drug toxicity, and poor perfusion. It is at this point that multiorgan dysfunction tends to become a vicious, self-fed cycle, and clinical trajectory a downhill slope. Even with correction of the original pathophysiological trigger, recovery is not always possible.

Against this backdrop, Patterson's wife, Steffanie Strathdee, reached out to phage groups at the Naval Medical Research Center (NMRC) and Texas A&M University. Patterson's *A. baumanii* isolate was shipped to both institutions and used to screen phage libraries, leading to the development of two personalized phage cocktails. Notably, NMRC made use of an automated screening system that measures bacterial growth over time from independent bacteria-phage cocultures (24). This

enabled identification of the 4 most potently lytic phages from a collection of 98 *Acinetobacter*-specific phages within 18 hours. Phage selection was followed by amplification, purification, and compounding, which extended the preparation time to over a week, although these latter steps could have been performed in advance had circumstances been different.

In the meantime, Strathdee and colleagues at UCSD initiated the process of obtaining US Food and Drug Administration (FDA) and institutional approval for the use of experimental phage cocktails. On day 109 after initial infection, with all paperwork signed, sealed, and delivered, the first dose of phage was administered percutaneously via three indwelling abdominal drains. After 36 hours and multiple doses, Patterson's condition was largely unchanged, prompting escalation to intravenous (IV) administration. IV phage was well tolerated over the first 24 hours on a conservative dosing schedule, and phage levels in the blood were observed to decline rapidly over the first 2 hours postinfusion. Phage dosing frequency was quickly ramped up to every 6 hours, and broad-spectrum antibiotics were continued. Within 48 hours of IV phage initiation there were clear signs of improvement, though none more impressive than Patterson regaining consciousness and becoming communicative. His subsequent recovery was rocky and protracted (as to be expected given the length of his critical illness), but he was eventually discharged from the hospital on day 245, after receiving 59 days of phage therapy. Professor Patterson has since returned to work and has a healthy publication record to show for it (56, 59–61).

This extraordinary turn of events following a last-ditch phage therapy effort has made the Tom Patterson case among the most compelling in phage therapy history. Although the case is categorically unable to prove anything, suggestions of short-term safety and potent efficacy have been extracted from its analysis. Under these auspices and aided by strong advocacy on the part of both Strathdee and Patterson, the US phage therapy field has been steadily building momentum.

Yet for phage therapy to become an accessible, FDA-approved reality, there are clear problems of scale to be surmounted. The Tom Patterson case called for a heroic investment (or rather, donation) of skilled labor. Teams of people at UCSD, NMRC, Texas A&M, and San Diego State University had responded accordingly, setting aside sleep, normal work schedules, and less urgent demands on their time. Obviously, such efforts are to be celebrated, not prototyped. So, what are the key challenges facing the field of phage therapy as it contemplates mass-market distribution? The following section will highlight a few of them, as well as proposed solutions where applicable.

CHALLENGES TO MAINSTREAM COMMERCIALIZATION

Translational Hurdles

The efficiency of any drug development pipeline is only as good as the animal models used to screen preclinical candidates. No animal model is a perfect prediction tool, but models that have been refined and validated by years of clinical experience are worth their weight in gold. In the case of small-molecule antibiotics, such models exist. With data obtained from standardized laboratory and animal studies, pharmacokinetic/pharmacodynamic (PK/PD) indices and PK/PD targets for efficacy become matters of simple calculation (37). This information can then be used to streamline clinical trial design, making phase 1–3 studies safer for patients and less costly for drug developers (90).

Unfortunately, these hard-won tools are not readily adaptable to phage. The ability of phage to replicate adds a layer of complexity for which there is no existing pharmacologic model. Eventually phage-specific models may be defined, but currently there is insufficient data to even attempt to fit such a construct. In the Tom Patterson case, there was no spike in plasma phage titer to

indicate overwhelming replication in the blood. At local sites of infection where bacterial density was higher and phage clearance was likely lower, the contributions of progeny phage may have been more significant.

Putting the issue of phage replication aside for a moment, it is worth noting that the in vivo distribution of phage following high-titer administration (via any route) also suffers undercharacterization. To this day, the best description of the pharmacokinetic fate of phage in an animal model remains that of Merril and colleagues, published in 1973 (20). In germ-free mice administered bacteriophage λ via injection or gavage, infective phage titers were measured in various physiologic compartments over time. At the first time point after phage administration, high quantities of infective phages were recovered from both the spleen and liver. Over time, this number steeply declined in the liver (mirroring the observed decline in blood) while slowly dwindling in the spleen. It was concluded that the reticuloendothelial system, nowadays referred to as the mononuclear phagocyte system, consumed a significant percentage of circulating phages—through either Kupffer-cell-mediated phagocytosis or splenic sequestration.

An even earlier study by Schultz & Neva (73) can be invoked for corroboration. Following IV injection of high-titer bacteriophage T2 into mice, vanishingly small amounts of infective phage appeared in the urine. For doses containing less than 10^9 plaque-forming units, not a single urinary phage was detected by plaque assay. In a separate ex vivo assay, phage T2 was incubated with murine whole blood and observed to retain over 90% of its infective activity after 2 hours. Together, these results pointed away from renal excretion and blood/serum inactivation as causes for the relatively rapid decline in circulating phage titer observed in mice.

That said, rapid bloodstream clearance does not apply to all phages. In 1996, Adhya and colleagues demonstrated that long-circulating phage can be evolved by serial passage in mice (45). Starting with bacteriophage λ, they successfully selected for λ mutants that retained up to 16,000 times the lytic activity of their parental counterparts after 18 hours in circulation. They determined that phage persistence in the blood correlated with a mutation in the capsid E gene, which resulted in substitution of a basic amino acid for an acidic one. Phage engineered to harbor this mutation have since been shown to recapitulate the phenotype (85).

The wide phenotypic variability among phages, even closely related ones, is both a blessing and a curse. On one hand, an array of features adapted to different hosts and different environments should enable intelligent selection of phage to suit specific applications. On the other hand, the limited generalizability of phage-based PK/PD parameters can be expected to reduce efficiency in the product development pipeline, at least initially. Both considerations point to huge knowledge gaps in our understanding of phage behavior in vivo.

Bridging these gaps will be neither easy nor glamorous. There is a significant amount of translational grunt work to be done and increasingly fewer people and resources with which to do it. With Big Pharma backing away from antimicrobial R&D, the burden of preclinical innovation and development has been falling more heavily (too heavily!) on academia and small start-up companies (82). At least it can be said that for researchers in the phage therapy field, there will be no shortage of critical research questions for some time to come.

Regulatory Hurdles

While it is true that no human phage therapeutic has been licensed for use by the FDA, the same cannot be said for all phage-based products. In 2006, ListShield became the first FDA-approved phage-containing food additive (6). Serving as a spray-on disinfectant for ready-to-eat deli meats, this *Listeria*-specific phage cocktail averts problems engendered by a pathogenic species capable

of growth at refrigeration temperatures. The approval of ListShield has implications for phage therapy in that it led to a first-time GRAS (generally recognized as safe) designation for a phage product (2). This suggests that the preclinical toxicology data requirement for therapeutic phage may be more manageable than would be expected otherwise.

The other major component of the investigational new drug (IND) preclinical data requirement, pharmacology, has already been discussed. We move on, then, to the real crux of this section, which is the question of how the current regulatory framework may be made to accommodate the safe and efficient evaluation of therapeutic phage. Despite the unassuming (and frankly boring) tenor of the query, it is a question of immense pragmatic importance. There is simply no other issue that stands to invigorate (or stifle) the field more at present.

Phage cocktails target a much narrower range of bacteria than traditional antibiotics, and clinical trials will need to be adapted accordingly. Unfortunately, implementing these changes within the confines of preexisting regulatory paradigms would seem to jeopardize feasibility. Take, for example, Tom Patterson's *A. baumanii* phage cocktail: In order for it to become a licensed therapy, its efficacy as a fixed-dose combination drug would need to be established in a phase 3 clinical trial (42). This would necessitate that the incidence of bloodstream infections with *Acinetobacter* strains sufficiently similar to Patterson's be fairly high, in order to allow statistically significant effects to overcome the noise of patient-to-patient variability and random error.

Review of real-world epidemiology data suggests that the numbers just are not there. At a large teaching hospital in Taiwan, 252 patients with monomicrobial *A. baumanii* bacteremia were seen over an eight-year period (35). This number includes patients with antibiotic-susceptible strains as well as strains that presumably would not be recognized by the cocktail. Both subsets would need to be subtracted from the phage-eligible pool (the former for strictly ethical reasons), likely cutting that number by more than half. Subsequent application of the typical exclusion criteria designed to select for a reasonably homogeneous and treatable study population would reduce the number of patients eligible for trial enrollment even further, making statistical power difficult, or at least very costly, to come by.

And yet, even if evidence of phage efficacy were consistently wrought from small study populations, the war would not be won. (The battle, yes, but the war, no.) Antimicrobials yield notoriously low returns on investment as a rule, and there is nothing to suggest phage would be the exception (34). Quite the opposite, phage cocktails with their ultranarrow spectrum would be hard pressed just to recuperate the costs of product development and clinical trials (which are staggering!). Calculated out-of-pocket costs exceed $1.3 billion per new FDA-approved drug at present, making it no surprise that pharmaceutical companies have been loath to sponsor late-stage clinical trials for a high-risk, low-profit product like phages (15).

Although commercial prospects for fixed phage cocktails look bleak, developing phage as a personalized therapeutic could be a game changer. Customized cocktail assembly from a preapproved library of phages should alleviate problems with strain coverage/market size while also enhancing efficacy. The success of the Tom Patterson case may very well have been predicated on this process.

Phage isolation in order to assemble a library is neither difficult nor expensive (21). Preparing phage stocks that comply with current good manufacturing practices can be tedious, but new automated methods could streamline the process considerably (40). As mentioned previously, high-throughput phage screening is already a reality. Taking this all into account, a customized cocktail approach is technically feasible (with minor caveats), making the most significant obstacle to its actualization the regulatory standards that will govern its licensure. For the most part, phage-specific regulatory standards have yet to be clearly delineated (65).

Can in-human safety and efficacy be predicted on the basis of in vitro phage characterization and data from animal models? That may be a $1.3 billion question. There are definitely characteristics that would render phages unfit for therapeutic use, such as the capacity for lysogeny or harboring of encoded toxins (46). Many of these are genetically identifiable, which has led Hamilton and colleagues at NMRC to develop a preclinical screening rubric based on phage genome analysis (58). They have used this rubric to guide phage selection in emergency IND cases with apparent success (or rather, lack of obvious failure), lending support to the notion that full-scale clinical validation of each and every phage within a library may be an unjustifiably low-yield requirement.

The case for a streamlined phage approval process has been further strengthened by recent success in adapting phage to a modular design platform. Lu and his group at the Massachusetts Institute of Technology have pioneered a phage engineering approach that takes an extremely well-characterized phage like T7 and exploits it as a scaffold for an array of interchangeable tail fibers (1). The technique is simple: Overlapping PCR fragments constituting a complete phage genome (including tail fiber genes of a selected phage and excluding the tail fiber genes of the scaffolding phage) are cotransformed into yeast along with a linearized, compatible yeast artificial chromosome (YAC). The phage genome undergoes reassembly and integration into the YAC with the aid of gap-repair cloning. The YAC-phage DNA construct is then extracted and transformed into host bacteria, enabling the phage to be rebooted. The process culminates in bacterial lysis and the release of infective phages that have shed their YAC shuttle vector (**Figure 3**).

Though some loss of lytic efficiency is observed with hybrid phages generated in this manner, the potential regulatory advantages of this technique are compelling. Provided that tail fibers encompassing a wide host range can be fitted to a few phage scaffolds, phage libraries may

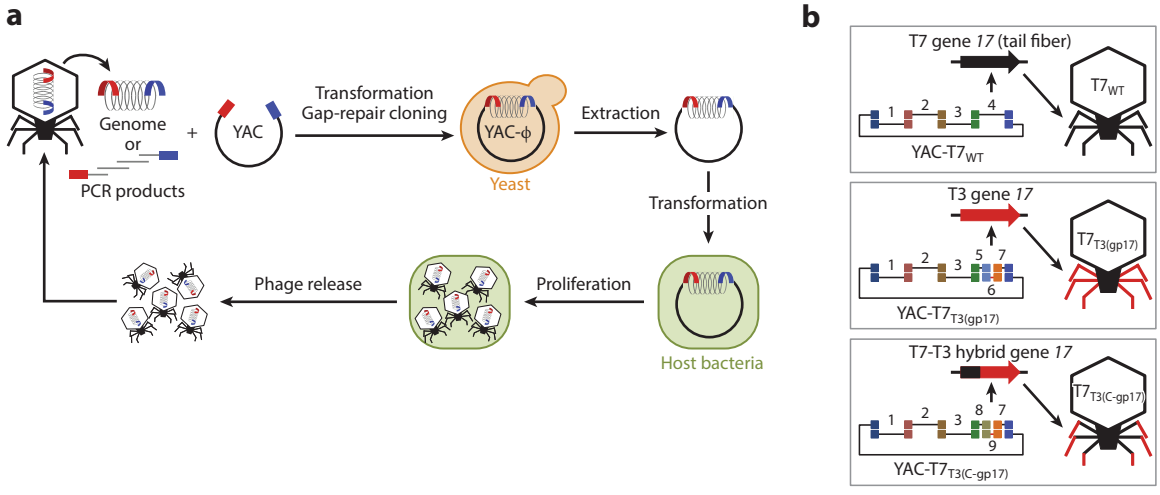

Figure 3

Modular engineering of phage host range. Hybrid phages consisting of the tail fibers from one phage assembled onto another phage's scaffold without tail fibers have the potential to confer a new host range while retaining significant structural similarity to the phage from which the scaffold was derived. (*a*) Phage engineering approach developed by Lu and colleagues (1) using a yeast platform. (*b*) Genetic blueprint for two variations of phage T7 tail fiber replacement, successfully executed by Lu and colleagues. Adapted from Reference 1 with permission.

be capable of achieving significant homogeneity without sacrificing strain coverage (9). The clinical safety evaluation of a few scaffold phages might then be reasonably expected to yield data applicable to other phages assembled from the same backbone. The extent to which these and other strategies can be leveraged to prevent phage therapy from arresting in the regulatory stage of development will ultimately determine the fate of the field.

Clinical Hurdles

What would phage therapy look like at the point of care? How might phage therapy be integrated into the clinical workflow at a busy hospital or clinic? These are questions not just for industry but for science as well. The biggest barrier to the clinical delivery of phages, a lack of methodology for the rapid identification of patient-specific phages, is a practical deficiency with a scientific question at its core: Is phage host range amenable to prediction?

It does not take a medical degree to appreciate that serious infections call for rapid initiation of treatment. In cases of septic shock, the time to administration of effective antibiotics is a critical determinant of morbidity and mortality (66). In cases of less acute illness, such as community-acquired pneumonia, less dramatic but still significant benefits have been observed with prompt antibiotic administration, which has led the Joint Commission of Accredited Healthcare Organizations (JCAHO) to designate it as a publicly reportable quality measure (75). Tom Patterson defied all odds by surviving the delay in treatment initiation while his phages were undergoing selection, amplification, and purification—just another aspect that makes his case truly exceptional.

With time being a precious commodity, determination of bacterial sensitivity to individual phages through coculture is a luxury not all patients can afford. Additionally, unless bacterial isolates are to be mailed for screening at a central phage repository, automated systems containing a full library complement would need to be on site at each health care facility. One way to circumvent this may be through development of a completely in silico process of phage selection (**Figure 4**).

Sequencing technologies are becoming faster, cheaper, and more accurate by the day, turning routine whole-genome sequencing of patient-specific pathogens into a soon-to-be-realized reality (14, 54). This information is expected to revolutionize clinical epidemiology and diagnostics, but the relevant question here is whether it can also be mined to predict phage susceptibility. Like most genotype-phenotype correlations, susceptibility to a specific phage is unlikely to be a monogenic determination. Analysis of a large, fully sequenced data set of bacterium-phage pairs that have been empirically tested for phage sensitivity may be the most efficient means for tackling this question. Identified patterns might then be developed into an algorithmic prediction tool and tested prospectively. To our knowledge, there has yet to be a group of sufficiently brave (and computationally oriented) souls to endeavor such a project.

The other critical uncertainty to address in this section is the question of whether induction of phage-specific immune responses will preclude the use of prolonged or repeated phage treatment. Most phages are fairly immunogenic, a trait that is exploited in the use of phage-based vaccines (12, 19). Antiphage antibody titers are present in unmanipulated animals and humans, no doubt secondary to intermittent low-level exposure to phages, and rise sharply after concentrated phage injection (27, 39). Higher antiphage antibody titers correlate with faster rates of phage inactivation in serum, which may affect treatment outcome (39). Yet overall, very little investigation has focused on phage interactions with the immune system, much less the implications for phage therapy, making most conjectures on the topic premature.

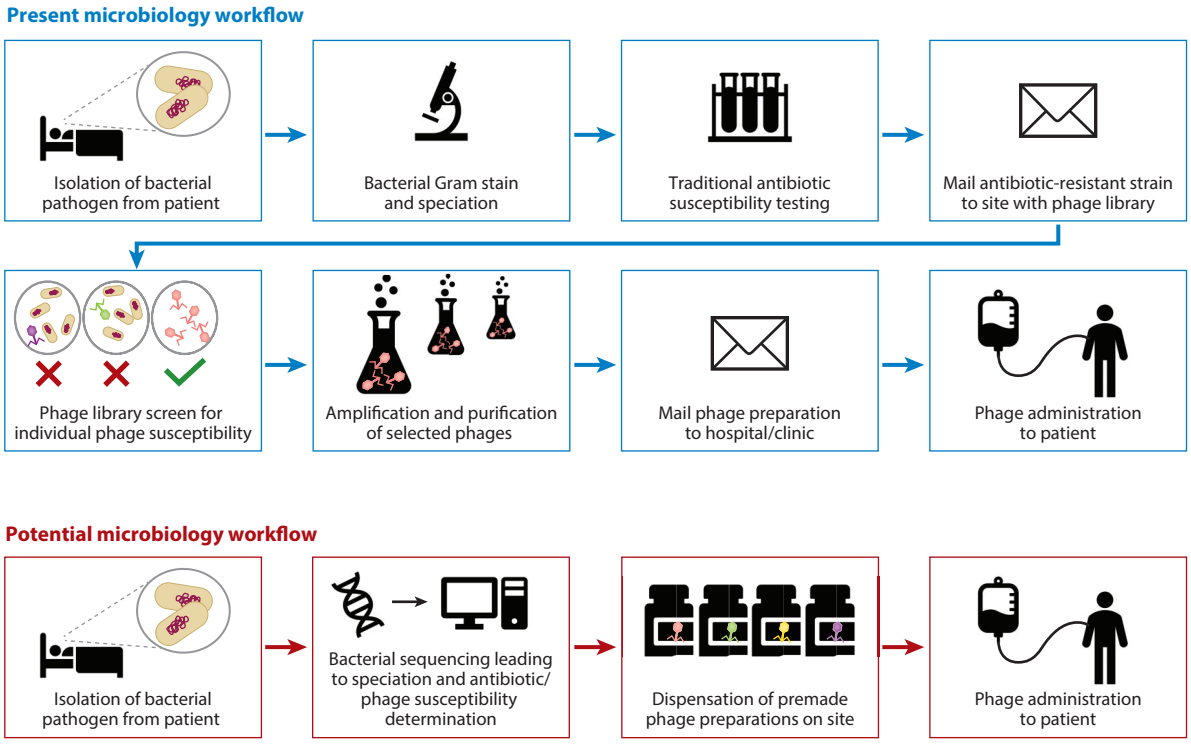

Figure 4

Toward a faster, more efficient process of clinical phage delivery. Every hour from the time a clinical bacterial isolate is first obtained to the time an appropriate phage cocktail can be made available to the patient may be crucial in cases of acute infection. Simplifying the microbiology workflow from the process exemplified in the Tom Patterson case (*top*) to a completely in silico process of phage selection (*bottom*) would be expected to improve phage therapy's therapeutic profile and cost-effectiveness.

CONCLUSION

> The report of my death has been grossly exaggerated.
>
> —Mark Twain (51)

The first one hundred years following the discovery of phages have been anything but dull. Over this period, the history of phage has intersected with the birth of molecular biology, the establishment of modern medicine, the institution of US federal drug regulations, and the Cold War. Phage therapy itself has cycled between being the subject of optimism, skepticism, disinterest, and now renewed curiosity. All of this has contributed to a sense that phages' long-envisioned therapeutic potential may soon become ripe for realization.

Significant challenges remain, as outlined above, and will require coordinated, multidisciplinary efforts to overcome. Realistically, not all of these obstacles will be surmountable (at least not completely and/or immediately), but this should not become cause for repeated abandonment of the field. Antimicrobial resistance is a thorny, ever-worsening problem for which there will be no one perfect solution. The prospects of any antibiotic alternative deserve to be judged with this in mind.

Ultimately, if phage therapy is ever to get its global launch, lessons from the past must be incorporated into prescriptions for the future. To the authors, this means the following:

1. Clinical applications of phage must be developed strategically, driven by a fundamental understanding of phage biology and not by hopes for what phages may or may not be able to achieve.
2. Clinical trials must be designed efficiently as well as rigorously, allowing for confident assessment of phage safety and efficacy.
3. Support from science, medicine, industry, and the public must be cultivated, but cultivated with care. The right blend of optimism married to realistic expectations will be crucial to sustain long-term commercial development and research productivity.

Stay tuned, because the next 100 years should be no less exciting than the first.

FUTURE ISSUES

1. Achieve broad coverage of clinical bacterial strains with a tractable number of phages.
2. Minimize and quantify phage-specific patient-safety risks.
3. Establish pharmacologic models for phage in animals and humans.
4. Characterize the host immune response to high-titer, repetitively-dosed phage.
5. Design rigorous and feasible clinical trials to assess the therapeutic efficacy of phage.
6. Streamline the clinical delivery of phage.

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