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Review article

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Phage therapy: from basic biology to clinical application

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Abstract

Bacteriophages have been applied therapeutically for more than a century for the management of bacterial infections in humans. Although their potential as a safe alternative or adjunct to antibiotics is well established, clinical success is difficult to predict from in vitro testing. There is currently no consensus on the optimal way to use them, nor are there any simple extrapolations from the use paradigms of conventional antibiotics. A rational approach to phage therapy requires informed phage selection and a comprehensive understanding of bacterial–phage–human host relationships and the management of bacterial and phage adaptations that occur in vivo. Phages may be used in ways that antibiotics are not and are a powerful approach for addressing the problem of antimicrobial resistance, not least because their optimal use requires a more thoughtful approach. In this Review, we explore key aspects of phage biology that underpin the effective use of phages for therapy. We outline clinical strategies for applying phages against a range of infections, highlighting their advantages and limitations. We then address challenges in production, scalability and regulation of phage preparations, identifying areas requiring further development. Finally, we discuss interactions between phages and the human immune system, and their importance for therapeutic success.

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Introduction

Antimicrobial-resistant infection is arguably the greatest threat to global health in the twenty-first century. Predicted to increase by more than 70% over the next generation with massive impacts on health systems and the global economy, it is already responsible for at least 5 million deaths per year and is expected to be the leading cause of global mortality by 2050 (refs. 1,2). Complex surgical and medical advances that we currently take for granted are all now at risk and there is an urgent need for new solutions.

The natural viral predators of bacteria, the bacteriophages (from *phagein*, φαγεῖν, to eat), were first deployed therapeutically more than a century ago but decades passed before they were visualized in one of the earliest applications of electron microscopy[3–5]. Non-motile and lacking an energy source, bacteriophages (phages)[3] are fundamentally opportunistic and their archetype is that of a lethal obligate parasite. In nature, phages exist in a fine balance with their bacterial hosts. They act as microbial control systems, with waves of adaptation and counter-adaptations sweeping through natural populations[6]. They are the most numerous and diverse biological entities on earth, shaping ecosystems in our soils, oceans, plants and all the animals that live here[7,8].

Restricted host bacterial specificity means that phages were difficult to use at first (without modern diagnostics) and often missed the target. Additionally, administered crude bacterial lysates frequently caused inflammatory reactions. Sulfonamides and penicillins emerged soon after scepticism about the therapeutic value of phages began to rise[9] and phage therapy was quickly eclipsed by mass-produced antibiotics in booming post-war Western economies. Now, modern diagnostics and genomics combined with efficient scalable biomanufacturing has enabled improved purity and precision in phage therapeutics, to build on a rich history of phage therapy that continues to this day in countries such as Poland[10] and Georgia[11].

Although the optimal clinical use of these 'living antibiotics'[12] is not yet settled, there are increasingly well-documented descriptions of phage applications and examples of new opportunities for genetic engineering and the role of modern diagnostics[13–15]. A highly cited cure of a severe and highly resistant *Acinetobacter baumannii* infection illustrates the diagnostic power of genomics to guide the use of multiple phages over successive weeks[16]. An inflammatory response during curative short-course phage therapy for limb-threatening *Pseudomonas aeruginosa* infection in a child was elucidated by the correlation of quantitative PCR and human transcriptomics with clinical findings, to point to the causative role of marked bacterial lysis[14]. Genetically modified phages are used to manage otherwise untreatable infections caused by *Mycobacterium abscessus*[15], and phage components are being developed as small-molecule therapeutics[17]. Standardized treatment and monitoring protocols, as well as randomized clinical trials, are building on a strong safety profile, and convincing signals of efficacy are emerging[18].

However, phages differ fundamentally from antibacterial drugs in being self-amplifying ('auto-dosing') and highly adaptive in vivo. Their high specificity (reduced collateral ecosystem damage compared with broad-spectrum antibiotics), utility as delivery vectors (of antibiotics, small peptides, diagnostic markers or engineered payloads[19]), synergy with antibiotics and potency in the face of antibacterial drug resistance are key therapeutic strengths. In this Review, we will discuss the most relevant aspects of phage biology for the successful implementation of phage therapy. Although derived primarily from human studies, these insights could inform therapy in other animals and uses beyond the purely clinical.

Phage biology relevant to phage therapy

An ideal therapeutic phage introduces no unwanted genetic material, is innocuous to the host presenting the bacterial infection, is easy to purify, store and administer, and hunts its prey to extinction. Not all these attributes are necessarily advantageous in nature but they may be engineered or selected in vitro.

Phage life cycles in nature and in human infection

The archetypal phage has a double-stranded DNA genome packed tightly into an icosahedral capsid 'head', and a 'tail' (often contractile, hence myovirus) with slender fibres that give it the distinctive 'lunar lander' appearance. Common phage morphotypes are shown in Box 1. For the taxonomist, these have mostly historic value[20] as it has long been known that similar morphologies do not predict genetic relatedness or common ancestry[21].

Understanding phage behaviour across different growth states is essential to their effective use as anti-infectives. A typical predating phage uses its tail fibres to attach to one or more bacterial cell surface receptors, injecting genetic material from the viral capsid through the cylindrical tail into the cell, where it hijacks host machinery to replicate. New phage structural proteins are synthesized in the host cytoplasm and assembled into capsid heads that are filled with new copies of phage genomes. These are then attached to their tails before the bacterium is lysed from within, with the help of phage-encoded proteins such as holins (which disrupt the bacteria inner cell membrane) and endolysins (which digest the cell wall), releasing a 'burst' of virus progeny (Fig. 1).

This process of lysis is commonly deferred in nature in the interests of both host bacterial populations and infecting phages[22]. Bacteria benefit by avoiding population extinction as well as unchecked population growth, while phages maintain prey availability and opportunities for rapid expansion. Some abundant human gut phages (for example, CrAssphage) can persist as extrachromosomal phage–plasmids, with similarities to classic episomal plasmids[23]. Both horizontal (phage-like) transmission and vertical (plasmid-like) transmission mechanisms may have an important role in genetic recombination and gene transfer in these phage–plasmids (phagemids)[24]. Avoidance of bacterial host destruction is also a feature of small filamentous phages (Tubulavirales, especially Inoviridae), which generate chronic productive infection from an episomal form in normally growing bacteria[25].

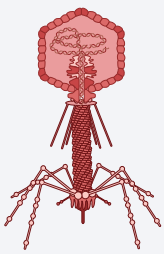
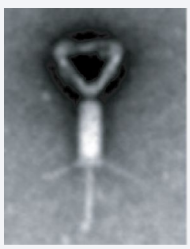
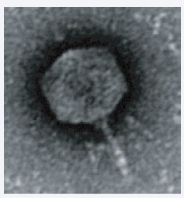
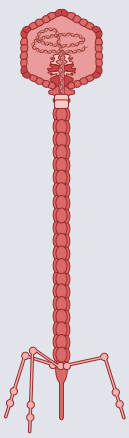
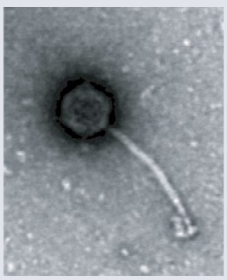
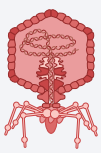
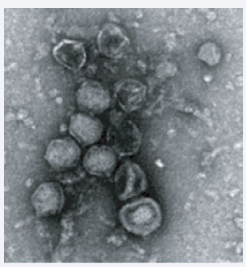
The best-known mechanism of deferred lysis includes the use of phage-encoded integrases to integrate ('temperate') phages into host bacterial chromosomes to replicate passively[26] (Fig. 1). These integrated 'prophages' intercommunicate and monitor bacterial cell signalling through quorum-sensing systems akin to those in bacteria, as well as through peptide-based communications such as the arbitrium system[27,28].

This process of passive (pro)phage reproduction with deferred lysis ('lysogeny') can switch quickly to explosive phage production when bacterial populations are stressed, including by antibiotics (for example, quinolones[29]); in fact, prophage-mediated lysis may be an important mediator of bacterial death upon antibiotic exposure[30]. In the meantime, host bacteria benefit from virulence attributes encoded by integrated phages as well as from the immunity that prophages provide against other predating phages[31].

This carefully tuned lifestyle makes most sense in highly competitive environments where bacterial specialization is important, phage and bacterial population densities are high and interactions are frequent (for example, in the mammalian gut)[32], as opposed to a 'hit and run' obligate lytic lifestyle when sparse opportunities in dispersed ecosystems must be immediately exploited (for example, the marine environment)[33,34].

Box 1 | Phage morphology, taxonomy and receptor interactions

Most naturally occurring therapeutic phages are tailed double-stranded DNA (dsDNA) viruses with genomes of less than 200 kb and icosahedral capsids with diameters of 45–185 nm. The myovirus, siphovirus and podovirus morphotypes, which dominated phage taxonomy for decades, are shown in the table[45,46]. Some key proteins (for example, tape measure and distal tail proteins, and tail-associated lysozyme in siphoviruses) are important structural determinants but there is evidence of modular and convergent evolution, and limited genetic relatedness within morphotypes[188]. The now-abolished order Caudovirales (dsDNA) included five families: Myoviridae (for example, T4, with a contractile sheath around the tail, along with the distinct Ackermannviridae and Herelleviridae), the Siphoviridae (for example, T5, with a long non-contractile tail) and the Podoviridae (for example, T7, with a short tail)[189]. The class Caudoviricetes now contains more than 70 families of dsDNA viruses of bacteria and archaea with icosahedral capsids[190]. Original proposals for reclassification[20,190] have had to accommodate some genetic mosaicism[7,191] and the International Committee on Taxonomy of Viruses maintain the definitive phylogenetic taxonomic reference on their website.

Myovirus morphotype 	 	Long contractile tail with sheath For example, T4 phage, approximately 160 kb dsDNA	Common features: irreversible blinding by long tail fibres (for instance, to outer membrane proteins) typically precedes definitive receptor binding (for example, to inner core oligosaccharides of *Escherichia coli* by 'T-even' phages), which triggers infection[48]
Siphovirus morphotype 		Long non-contractile tail For example, T5 phage, approximately 120 kb dsDNA	Long flexible tails may facilitate direct recognition of definitive receptors (for example, T5 phage and FhuA; λ-phage and LamB)[192]
Podovirus morphotype 		Short non-contractile tail; fibres assembled on capsid head For example, T7, T3 and P22, approximately 40 kb dsDNA; Φ29 (approximately 19 kb)	Tail spike proteins or enzymes initiate infection, oriented by phage tail fibres (for example, T7 binding *E. coli* lipopolysaccharide; Φ29 binding *Bacillus subtilis* teichoic acids[193,194])

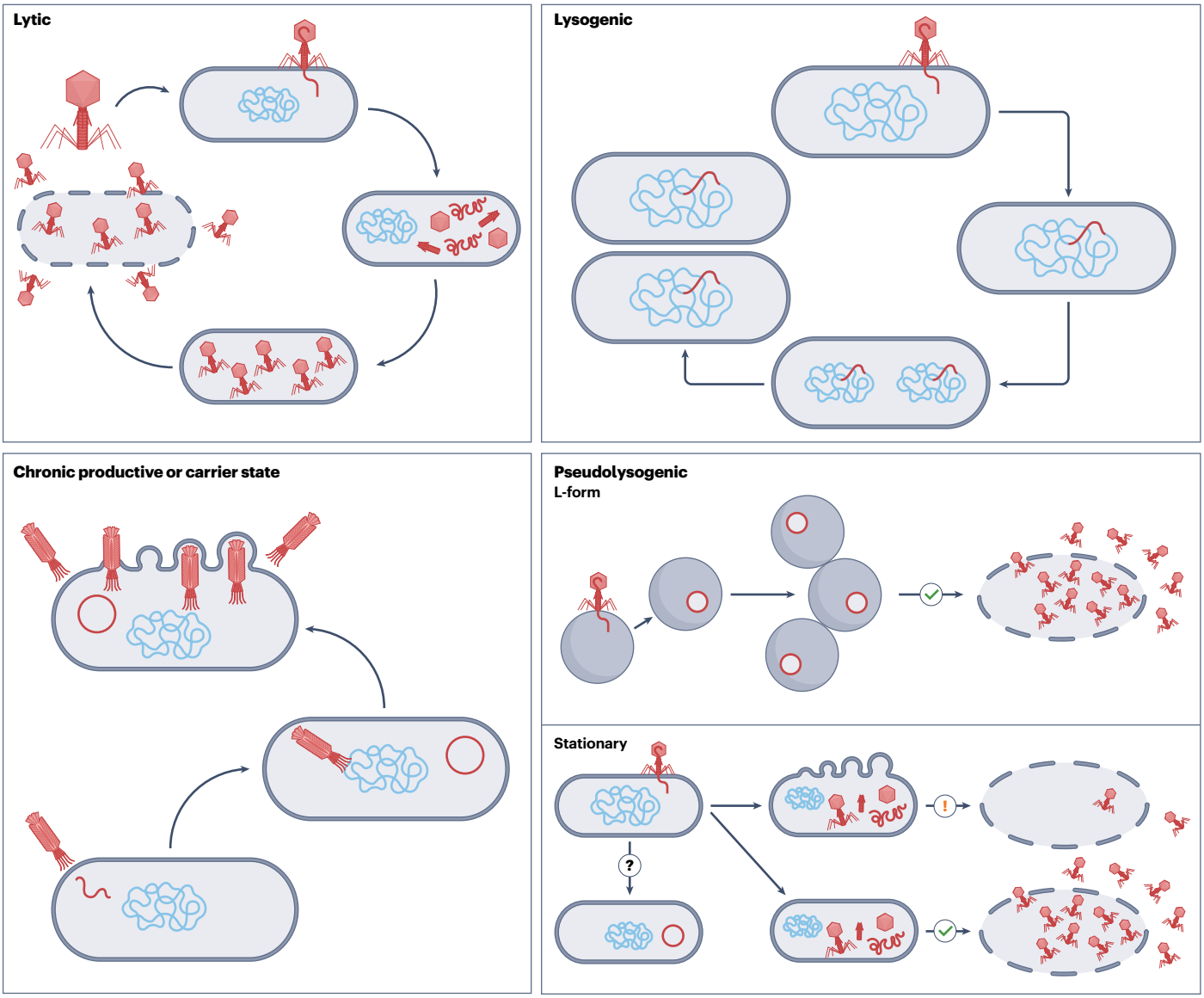


Fig. 1 | Bacteriophage lifestyles. Four life cycle models are commonly described. A lytic life cycle ends in destruction of the host population but is deferred in lysogeny (chromosomally integrated 'prophages') and in pseudolysogeny, whereas filamentous phages generate chronic productive infection. Potential contrasting pathways are included in the 'pseudolysogenic' panel. Pseudolysogenized L-forms grow slowly and support passive proliferation of (episomal) phages. In pseudolysogenized stationary phase bacteria, bacterial growth and phage lytic cycle are suspended. A scavenger phase may produce small numbers of virus progeny if stringent conditions persist (exclamation mark), for which an episomal phase is not defined (question mark). Phage lytic cycles resume with optimal bacterial growth.

The lysis cycle of apparently obligate lytic ('virulent') phages may also be paused when the bacterial host faces existential threat (for example, from nutrient stress[29]) or when other stress responses are activated[30], as well as in slow-growth bacterial lifestyles commonly encountered in human infections (for instance, in stationary phase growth and in L-forms, which lack a normal cell wall[35,36]). This 'pseudolysogeny' prevents extinction of bacterial populations that are already sub-lethally stressed[37]. Pseudolysogenized bacteria generally proceed to lysis when vigorous bacterial growth resumes[29,30].

Slow-growing and L-form states are expected in chronic urinary tract infections and device-related infections with biofilms, and (for L-forms) when using β-lactam and carbapenem antibiotics[35], in the presence of which L-forms continue to grow steadily[35,36,38], possibly also with phages in episomal forms[38]. The osmotically fragile L-forms often go unnoticed, as they are not detected in normal (isotonic) laboratory media and require special handling for isolation and testing[37].

The therapeutic corollary is that important antibiotics such as carbapenems may induce or promote an in vivo bacterial growth state in which many commonly used phages may not proceed to complete

the lytic cycle. This occurs despite any demonstrable antibacterial synergy with those antibiotics in standard laboratory growth conditions in vitro. These phage lifestyles are therefore important to consider when planning phage therapy.

Lysogenic conversion and the therapeutic choice

The delivery of extraneous genetic material to an infected bacterium ('lysogenic conversion') by integrating temperate (pro)phages is responsible for virulence in the causative agents of cholera[39], diphtheria[25] and botulism[40], among others. Inadvertent packaging of non-phage DNA is also an important consideration in lysogenic (temperate) phages. It is associated with a mechanism, favoured by larger phage genomes, which packs DNA tightly into the capsid head until filled ('headful' packaging, as opposed to more precise cohesive end site (cos) mechanisms)[23,24]. Random chromosomal integration, aberrant excision mechanisms (specialized transduction) or false packaging initiation recognition (pseudo-pac) sites may result in accidental packaging of bacterial DNA (reviewed elsewhere[41]).

For all these reasons, temperate (lysogenic, chromosomally integrating) phages are generally not preferred for therapy. Nevertheless, faecal microbial transplantation, rich in temperate phages, is widely accepted as safe even in critically ill individuals[42] and temperate phages may still provide an option when others are limited[43]. Virulent (obligate lytic) phages more reliably control rapidly expanding bacterial populations but often also defer lysis to preserve equilibrium between predator and prey, resident and host. Phages that are most successful in nature are likely to include those in sustainable dynamic equilibrium with their host. Conversely, virulent phages that regularly lyse stressed bacterial populations (for example, stationary phase or L-forms) might be expected to be less commonly found in nature and/or in therapeutic phage repositories.

Host specificity and receptor binding

Bacteria commonly deny phage attachment to prevent phage infection and this may be regarded as a first defence. Initial binding (adsorption) is typically of lower affinity (often reversible) and followed by higher-affinity binding to a second receptor that triggers DNA injection.

In Gram-positive bacteria such as *Staphylococcus aureus*, cell wall teichoic acids are common targets, and their relatively limited diversity is reflected in that of their commonly occurring obligate lytic phages[44]. In Gram-negative bacteria such as *Escherichia coli* or *P. aeruginosa*, hydrophobic lipopolysaccharide (LPS)-rich outer membranes and exopolysaccharide capsules provide highly diverse receptors that provide an effective defence but also act as physical barriers[45] against which many phages deploy depolymerases to improve outer membrane access. The lipid A core itself is often a definitive receptor, along with outer membrane proteins that may serve as initial (adsorption) receptors[46] (Box 1). Surface structures such as pili or flagellae are occasional receptors[47] and can be modified or downregulated to avoid predation, often at the expense of important corresponding virulence attributes such as adhesion or motility. The common T4-like phage uses its long, contractile tail to bind LPS and/or major outer membrane proteins such as OmpC in *E. coli*, with its long tail fibres adjusting orientation[48]. The T7-like phages and others that share 'podovirus' structural features such as short non-contractile tails can also bind LPS core and lipid A and, similarly to many phages, have receptor specificities that can be 'trained' to accommodate common bacterial variations[49,50].

Bacterial phage resistance and internal defence systems

Once attached, a phage must deal with the intracellular defences that are used by bacterial populations to build fitness and preserve specific 'memory' and to deal with sudden opportunistic phage infections[8,51]. Recent studies have greatly expanded the known repertoire of bacterial defence mechanisms and interested readers are referred to comprehensive reviews[52–54], but note that this is a rapidly growing literature as modern techniques allow for the discovery of previously unknown anti-phage defence systems[55]. The most important post-attachment defences appear to be restriction modification and adaptive CRISPR systems (named for the clustered regularly interspaced short palindromic repeats that define them) and abortive infection (abi) or cell 'suicide' systems (in which self-destruction mediated through toxin–antitoxin modules can abort phage infection)[56] (Fig. 2).

These internal anti-phage defences may be particularly important for regulating prophage lysogeny within bacterial populations, whereas phage receptor usage, rather than specialized post-attachment defence mechanisms, may play a greater role in defining the host range of lytic phages used therapeutically[57–60]. An important corollary of this is that occasional bacteria will resist large sets of phages that would normally be tested on the basis that they can infect the target bacteria's close relatives. Such pan-phage-resistant bacteria are more likely to be those with potent post-attachment defences, in which phage training[61] (to bypass receptor-level defences) may be less feasible. Nevertheless, phage adsorption (attachment) may still occur (and is easily tested) and an antibacterial payload attached to the phage may therefore still be delivered[62].

Bacterial adaptation and phage counter-adaptation

Like the Red Queen[63], phages and bacteria run fast just to stay in the same place, rapidly adapting and counter-adapting in vivo. It is intuitive that adaptations are easiest when bacteria and viruses have only each other to contend with, and thus bacterial mutations easily demonstrated in vitro during phage infection in optimal growth media may be much less common in vivo, where the adaptive landscape is more restrictive. Essentially unchanged bacteria are commonly recovered after treatment failure, even with intravenous administration of high doses of phages during high-density bacteraemia in vivo[64,65]. This is unsurprising, as bacteria can survive phage attack by modulating receptor expression – a strategy that sometimes entails trading off growth and/or virulence attributes – and/or various other inherent mechanisms, including after phage attachment.

It follows that inherently more plastic[66] or highly diversified bacterial populations beyond the reach of host immune defences and antibiotics[16] should be most tolerant of the trade-offs required and most able to generate successful mutants. Bacterial biofilms, which commonly include stressed populations, present challenges, yet some phages have developed strategies to overcome them[67–69], including direct interference with bacterial population-level regulation via quorum sensing[70,71]. In such situations, bacterial populations with the greatest adaptive possibilities are likely to be advantaged[72]; for example, highly mutation-prone mismatch repair mutants are common in long-term *P. aeruginosa* lung infections[73]. Failure to recognize and anticipate bacterial target population diversity and plasticity promotes the emergence of phage resistance and the risk of therapeutic failure[16]. Multiple and periodic sampling should therefore be considered as bacteria and phage populations co-adapt in these situations, including before, during and after phage administration.

It is also worth emphasizing that bacterial adaptation to phages differs in many ways from adaptation to antibiotic drugs. Gene

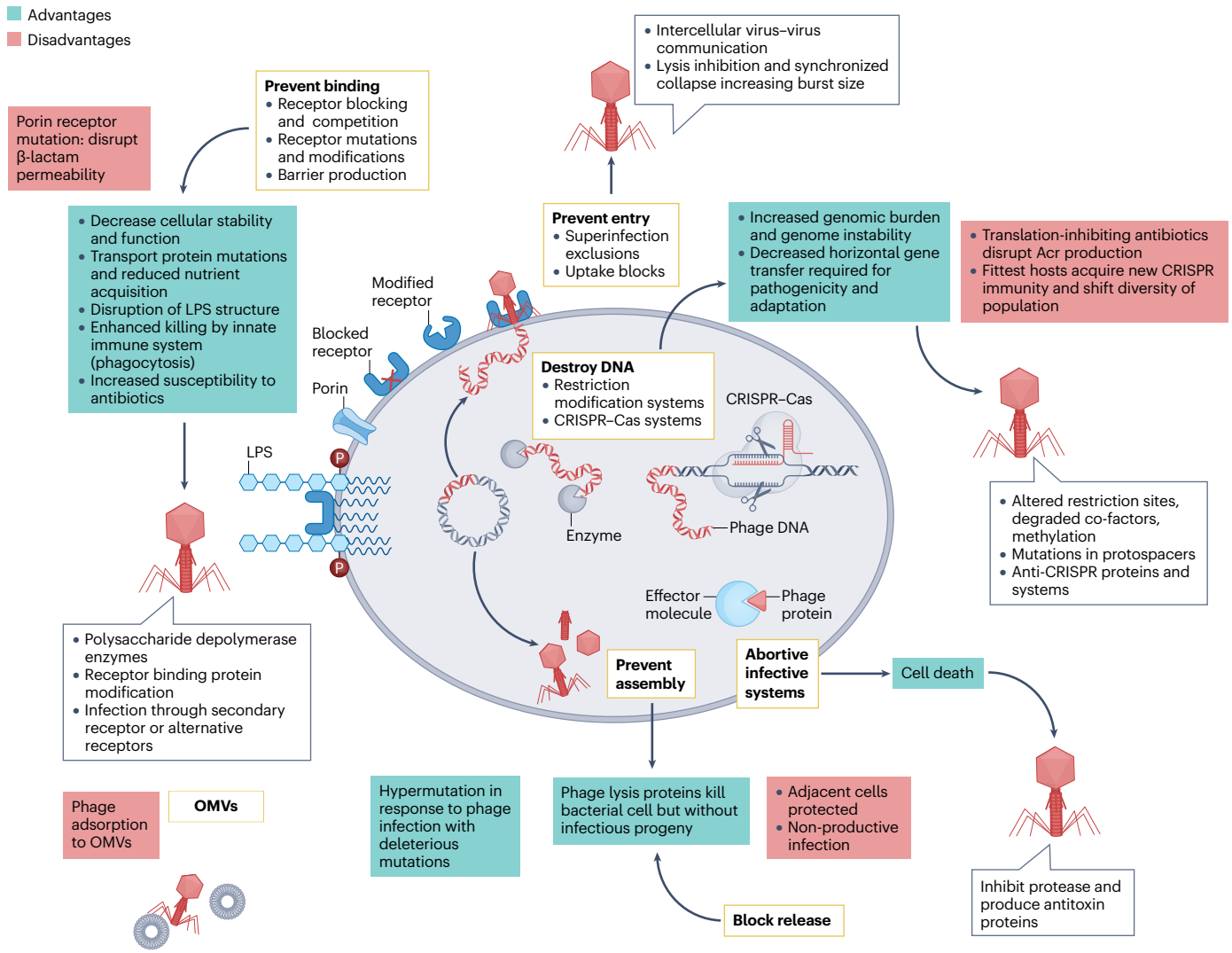


Fig. 2 | Anti-phage defence, counter-phage responses and strategies for phage therapy. Common bacterial anti-phage defence mechanisms (yellow-outlined boxes), phage counter-defence mechanisms (callout boxes) and possible implications from bacterial trade-offs in phage therapy (green boxes, advantages; red boxes, disadvantages). Strategies for phage therapeutics encompass a range of approaches. These include synergistic interactions between phages and antibiotics, and combinations of phage with biofilm-active agents. Lytic phages self-propagate (auto-dosing) and evolve; although repeated dosing may encourage adaptive evolution in bacteria. The use of phages capable of evading CRISPR–Cas, as well as phage training (pre-adaptation), natural selection or engineering, can help select the most successful predatory phages. Monitoring during and after therapy for development of resistance, bacterial and phage genomics, and amplification responses can help understand the contribution of phage therapy to clinical outcomes. LPS, lipopolysaccharide; OMVs, outer membrane vesicles.

acquisition, deletion and mutation feature strongly in the latter whereas bacterial adaptations to phages, fine-tuned over aeons, are generally less dependent on the acquisition of new mechanisms in vivo. Phages might become useful in situations where antibacterial drugs are avoided or highly restricted in an effort to prevent antibiotic resistance.

Clinical experience with phage therapy

Despite early reports of success[74,75], phage therapy failed to meet expectations. Filtered lysates of varying potency and purity were often used too late in the course of infection. Reactions were common and phages were even sometimes deliberately injected rapidly to produce a shock reaction[75]. More than 70 years on, mixed reports continue and only a minority of published clinical trials meet the stringent criteria required for therapeutic registration (Table 1); however, increasing numbers of registered clinical trials up to phase III are beginning to provide data (Supplementary Table 1). A small but well-conducted study of locally administered phages for *P. aeruginosa* chronic otitis in 2009 is an example, being so successful that the trial was stopped early[76]. However, phage stability (shelf-life) and diagnostic targeting remain important risks to manage in trial design[77–79]. Orally administered phages targeting *E. coli* showed no benefit in children with acute bacterial diarrhoea; however, only 60% of participants had proven

Table 1 | Published clinical phage trials

Infection type and organism	Bacteriophage	Study design	Outcomes	Notes	Ref.
Randomized, placebo-controlled					
Chronic otitis *Pseudomonas aeruginosa*	Phage cocktail	24 patients Cocktail phage 1×10^5 PFU once into ear versus placebo	Clinical and microbiological benefit Phage amplification in vivo	Stopped early due to efficacy	76
Acute bacterial diarrhoea	Coliphages (1.4×10^9 PFU) or coliphage product (3.6×10^8 PFU)	120 children Oral coliphages or coliphage product for 4 days versus placebo	No benefit over standard of care No in vivo amplification	Not powered for efficacy Phage mismatch >40%	79
Cholera	Polyvalent cocktail (1×10^8 to 1×10^9 PFU)	Phase I: 30 adults, 6 children; oral 3 doses (24–48 h after first dose) cocktail versus placebo Phase II: 20 adults, 10 children; i.m. once, then oral 24 and 48 h versus tetracycline	No clinical or microbiological benefit; tetracycline superior	El Tor strains non-susceptible	176
UTI after TURP	Polyvalent cocktail (1×10^5 to 1×10^6 PFU)	113 patients; cocktail versus placebo, twice-daily bladder irrigation	No difference Simple irrigation performed better than phage administration	In vitro phage matching done Low doses	78
Chronic diabetic foot or pressure ulcers	Polyvalent cocktail 1×10^9 PFU ml^{-1}	60 patients Phage cocktail 1×10^9 PFU ml^{-1} topically on gauze, 3 times a day (alternate days), weekly for 4 weeks, every 2 weeks for 3 months	Phage 46.7% sterile at 1 month versus 0% placebo 93.3% sterile and healed at 3 months versus 13.3% placebo	Limited information on phages and susceptibilities; no power calculation	177
Burns *P. aeruginosa*	Polyvalent cocktail (12 phages)	25 patients Cocktail versus standard of care, topical 7 days	No clinical efficacy Microbiological benefit for correctly targeted organisms	1×10^4 to 1×10^5 PFU loss of titre in transit Low final dose (10–100 PFU ml^{-1})	[77](PhagoBurn study)
Randomized, non-placebo					
Secondary bacterial pneumonia in COVID-19	Polyvalent cocktail (>1×10^{12} PFU)	60 patients 10 ml (1×10^{12} PFU ml^{-1}) nebulized 12-hourly for 7 days	Clinical and microbiological efficacy	Phages not fully characterized No phage susceptibility testing of isolates	178
Uncomplicated UTI *Escherichia coli*	Polyvalent cocktail	LBP-EC01 with oral TMP-SMX Intraurethral plus dose finding intravenously	Clinical and microbiological response at day 10 in 88%	Small study No placebo or antibiotic-only controls Regimen finding	179
Comparative					
Acute tonsilitis	Polyvalent cocktail	128 children Pyobacteriophage complex nebulized versus standard of care	Clinical response: 1.4 times faster improvement in symptoms	No phage susceptibility testing of isolates at enrolment No power calculation	180
Periprosthetic hip infections *Staphylococcus aureus*	Staphylococcal bacteriophage solution (at least 1×10^5 PFU ml^{-1})	45 patients Comparative control Phage >1×10^5 PFU ml^{-1} in bone cement with >1×10^6 PFU into joint at day 10 and antibiotics	Clinical and microbiological response; relapse at 12 months 4.5% versus 36.4% matched comparators	Small sample size but reached power calculation versus matched comparators	181
Chronic venous leg ulcers *P. aeruginosa* *S. aureus* *E. coli*	Polyvalent cocktail	42 patients 12 weeks of topical phage versus saline	Safety trial, not designed to show efficacy No significant clinical or microbiological response	No microbiology or phage susceptibility testing	182
Non-comparative					
Chronic rhinosinusitis *S. aureus*	Polyvalent cocktail	9 patients Phage twice-daily intranasal irrigations AB-SA02 3 phages 1×10^9 PFU for 7 days (cohort 1), 3 at 1×10^8 PFU 14 days (cohort 2), 3 at 1×10^4 PFU 14 days (cohort 3)	Safety trial, not designed to show efficacy Reduction in growth observed Negative cultures with evident 'cure' 2/9	Safety and tolerability demonstrated	183

Table 1 (continued) | Published clinical phage trials

Infection type and organism	Bacteriophage	Study design	Outcomes	Notes	Ref.
Non-comparative (continued)					
Burn wounds *P. aeruginosa*	Polyvalent cocktail	30 patients Phage 1×10^{10} PFU ml^{-1} topical 5–17 days	Clinical and microbiological response: eradication 60%; clinical improvement 80% Improved healing more than 50%	–	184
Chronic venous ulcers	Polyvalent cocktail PhageBioDerm pyophage cocktail (biodegradable polymer impregnated with ciprofloxacin and phage) for *P. aeruginosa*, *E. coli*, *S. aureus*, *Streptococcus* spp., *Proteus* spp. (1×10^{6} PFU cm^{-2})	96 patients Cocktail (1×10^{6} PFU cm^{-2}) variable duration	Healed 70% (6 days–15 months) Improved 25% Nil response 5%	Study of topical antibiotic plus phage in combination	185
Healthcare-associated infection (ventilated) *Klebsiella pneumoniae* *Acinetobacter baumannii* *P. aeruginosa* *S. aureus*	Polyvalent cocktail Phagebiotics in treatment and prophylaxis in healthcare-associated infection Phage cocktail (1×10^{8} PFU ml^{-1})	*K. pneumoniae*: 13 patients ETA–urine, 5 ml mouth–ETT once *A. baumannii*, *K. pneumoniae*, *P. aeruginosa*, *S. aureus*: 14 patients colonization; 20 ml cocktail (1×10^{8} PFU ml^{-1}) intragastric for 3 days	Microbiological response: reduced colonization	Phage detected in urine and faeces	186
S. aureus sepsis and endocarditis	Polyvalent cocktail	13 patients AB-SA01 i.v. twice a day for 14 days (3 phages at 1×10^{9} PFU)	Safety and tolerability; pharmacokinetics	Detailed host kinetics	65
Compassionate cases					
Mycobacterium spp. infections	Varied mycobacterial phages (n=1 or 2 phages per treatment course); matched in vitro	20 compassionate cases Non-comparative Mostly i.v. twice a day 1×10^{9} PFU ml^{-1}, nebulized or both	Clinical or microbiological response 11/20	Non-comparative, retrospective Detailed microbiological, phage, monitoring analysis, neutralizing antibodies	81
Difficult-to-treat infections	Personalized phages	100 patients (consecutive) Retrospective, observational Varied schedules, infections, follow-up	Clinical improvement 77.2% (at least one symptom) Eradication 70% (culture or physician assessment)	Non-comparative retrospective, heterogenous group, subjective outcomes Detailed analyses; proof of feasibility	82
Case series					
Periprosthetic joint infections	Personalized phages (1×10^{10} PFU ml^{-1})	10 patients Case series Protocol 1: intraoperative phage then i.v. for 5 days Protocol 2: intraoperative phage plus intraarticular daily for 4 days plus i.v. daily for 5 days	No recurrence in all cases	Non-comparative study	187

ETA, endotracheal aspirate; ETT, endotracheal tube; i.m., intramuscular; i.v., intravenous; PFU, plaque-forming units; TMP-SMX, trimethoprim–sulfamethoxazole; UTI after TURP, urinary tract infection after transurethral resection of the prostate.

E. coli infections[79]. The PhagoBurn trial suffered from loss of phage potency in transit but showed microbiological efficacy in those with phage-susceptible isolates[77]. Bladder instillation of a phage cocktail for urinary infections in patients undergoing transurethral resection of the prostate showed no benefit; however, the formulation failed to target all relevant pathogens and provided a relatively low dose even against pathogens that were phage-susceptible[78].

Studies of adjunctive phage therapy conducted within adaptive platform trials allow multiple intervention arms[80] and pragmatic comparisons with standard-of-care therapies, but much of the current evidence still derives from uncontrolled case series. Publication bias against reporting of negative outcomes is a concern, but is mitigated by the fact that most reported experience is of 'compassionate use', in situations where all other therapeutic options have failed and the

clinical response to any intervention is unlikely. More than half of 20 patients with refractory non-tuberculous mycobacterial infection receiving 'compassionate use' mycobacteriophages exhibited favourable responses, some with complete resolution[81]. In a cohort of 100 consecutive patients receiving phage therapy for diverse indications (mostly topical and nebulized, and mostly for *S. aureus* and *P. aeruginosa* infections), approximately three-quarters of those who reported back described subjective improvement[82]. Patients receiving concomitant antibiotics showed better outcomes, and overall rates of clinical improvement (77%) and bacterial eradication (>60%) are similar to other published cases, with adverse events generally reported at rates similar to or below those of control groups[18].

Phage selection and therapeutic strategies

Most demands for therapy can be met by naturally occurring phages: those that attack gut pathogens (for example, *E. coli*) are found in sewage; and phages that attack versatile opportunistic pathogens in water and soils (for example, *Pseudomonas* and *Acinetobacter* spp.) are readily found with them in such locations. Enhanced infectivity[83], expanded host range[84] and increased capacity to overcome bacterial evasion strategies[85] may be derived by forced evolution in vitro — 'training' the phage onto a target organism with[85] or without[86] bacterial co-evolution — or by deliberate engineering[15,87,88]. All these are important tools for harnessing or enhancing the efficacy of modern phage therapeutics.

Predicting therapeutic efficacy

The precision of phage therapy means that phage host specificity (host range) and potency should ideally be defined in vitro before use and ideally under the same conditions used for antimicrobial drugs, to allow direct comparisons[89]. Phage potency is usually measured as bacterial growth suppression in liquid medium and/or a zone of clearing (plaque) on a bacterial lawn[89,90]. Phages and bacteria are combined at defined ratios (a phage to bacteria multiplicity of infection (MOI) that ranges over several logs but is commonly 0.1–10 for susceptibility testing)[89]. Although that sort of growth suppression is easy to compare with antibiotic effects, the plaque of absent bacterial growth on double-layer agar is quite different from the growth inhibition zone created by diffusion of antibiotics from a central disc. The hazy edge of an antibiotic diffusion zone usually indicates resistant subpopulations, whereas the hazy edge of a phage plaque might signal polymerase activity or a zone of lysis inhibition associated with enhanced virus yield[91].

The viable phage count (determined by serial dilution and expressed as plaque-forming units (PFU) per millilitre) is commonly compared with a reference host as 'efficiency of plating' and expressed as a decimal or percentage (see review[89]), but is not perfectly concordant with growth kinetics in broth microdilution and, similar to antibiotic testing, may vary with growth media[90]. Although growth inhibition in liquid media may appear similar, and therefore appeal as a correlate to the antibiotic drug minimum inhibitory concentration (MIC), especially in a synergy assay, it may also reflect different trade-offs to those in vivo or even in solid media and may not be highly reproducible[89,90] (Box 2). Concordance of susceptibility testing varies[92] and standardized assay conditions are important to establish. For now, it is reasonable to generalize that a phage unable to inhibit bacterial growth in vitro is a poor therapeutic choice in vivo. In silico binding site predictions used to guide in vitro testing[93] are not yet a substitute, being generally most useful at or above the genus level[94]. Predictive models are expected to improve with experimentally determined phage–bacterial interaction data, such as those for common coliphages[46,58,95].

Selecting phage combinations

An altered (for example, broader) host range can be achieved naturally, by manipulation, or by combination with antibiotics or with other phages in 'cocktails'[96]. Phages combined for an improved host range, by targeting bacterial subtypes most represented in surveillance programmes, provide confidence that any given member of a target species will be treated. This is similar to vaccine development approaches that update regularly according to epidemiological shifts and increases both general utility and commercial value. Synergistic phage combinations that precisely target a particular isolate may offer greater certainty of success but require specific diagnostics and the benefit (over a single phage) is unproven: it is still the case that phage cocktails are often assumed to be, but not confirmed as, the superior approach[97]. Competition for intracellular resources[98], accelerated resistance adaptations[99] and increased human immune responses might devalue a cocktail approach[15] over, for instance, sequential therapy. A counter-argument is that sequential adaptations may be less taxing to bacterial adaptive capacity than simultaneous demands from multiple phages. An ideal phage prescription generally aims to force competing bacterial adaptations[100] (for example, simultaneous OmpK35 and OmpK36 porin targeting in *Klebsiella pneumoniae*[101,102]) or simultaneous distinct high-cost trade-offs (for example, pilin and LPS targeting in *P. aeruginosa*), with defensive adaptations coming at the cost of reduced overall virulence or ability to withstand antibiotics and other common (oxidative, nutritional) stresses.

Box 2 | Measuring bacterial killing in vitro

Optimization of dosing with specific attention to the 'minimum inhibitory concentration' (MIC) of antibiotics predicts outcomes in serious infections and is used to set 'breakpoints' which define an organism as 'susceptible' or 'resistant'[195] according to whether a therapeutic concentration can be achieved in the tissues. Some antibiotics have concentration-dependent killing (more is better), for example, aminoglycosides, which bind the 16S ribosomal subunit and inhibit protein synthesis[196]; for others, time above the MIC is important (longer is better), for instance, β-lactams such as penicillins, which bind and inactivate cross-linking transpeptidases in the cell wall[197]. It remains unclear whether achieving a higher multiplicity of infection (MOI) to overcome the 'inundation threshold'[115] is preferred (more is better) over maintained phage pressure to mitigate natural tolerance (longer is better), or whether this varies with phage–bacteria pairs[198]. For antibacterial drugs, bacterial growth inhibition, as defined by the MIC, and bacterial killing, as defined by the minimum bactericidal combination (MBC), have different relationships for each bacteria–drug pairing, and growth inhibition rather than killing (that is, MIC rather than MBC) is the key predictor of clinical outcomes. If this was applied to the phage–bacteria interaction, then bacterial growth inhibition is most important. However, these principles are not yet clinically tested, and phage MOI and antibiotic MIC concepts are not the same. None of the many methods used to determine in vitro susceptibility to phages has established clinical correlations[199], and laboratory growth conditions that do not favour expression of a receptor that is essential in vivo will make an otherwise potent phage therapeutic seem ineffective in vitro[200,201].

Sensible guides to cocktail design are widely available[59,100,103–105] but phages are also invariably used with antibiotics. Phage–antibiotic synergy or antagonism (reviewed elsewhere[106]) is "dictated by the primary target of the antibiotic and the cellular processes required for phage replication"[107] and determined in vitro at sub-MIC antibiotic doses and low MOI[107,108]. Co-administration of multiple synergistic phages and antibiotics that impose intolerable bacterial trade-offs should be considered when designing phage therapy protocols, including subinhibitory (subtherapeutic) antibiotic concentrations in tissue sites (for example, reduced local bioavailability due to restricted tissue perfusion) and difficult growth states such as biofilm-mediated infections[109,110].

Dosing strategies

Phage dosing for human infections ranges widely (typically 1×10^7 to 1×10^{10} PFU per dose), as does the frequency and duration[82]. A dose must survive innate immune inactivation[111] and, especially with intravenous therapy, reticuloendothelial (mononuclear phagocyte system) clearance[112,113] in order to reach target bacterial populations at an optimal MOI for successful infection. The passive 'inundation threshold' at which the bacterial population stops growing and the active 'proliferation (or replication) threshold', beyond which phage replication drives ongoing bacterial population decline (more comparable with the MIC), are hard to predict a priori[114,115], and the actual MOI at the site even more so. Higher doses or higher 'burst sizes' (more phages per lytic cycle) and short lag phases (between infection and lysis) seem desirable, but some processes (for example, lysis inhibition) that increase viral proliferation overall and are successful traits in some phage types (including the common T4-like phages) are associated with delayed lysis and smaller in vitro plaque sizes, complicating the choice of phages from in vitro performance[116].

Therapeutic monitoring

Quantitative PCR is a well-established tool for managing viral infections that offers precise quantification and strong concordance with culture-based methods[117], including phage detection and monitoring in vitro[118]. Quantitative PCR enables detection of phages in patient serum where residual antibiotics or in vivo co-evolution of both predator phage and prey bacteria may render plaque assays unhelpful.

Quantification of virus in tissues (or its spillover into blood) is a simple and robust indicator of phage proliferation[14,119]: intravenous doses of 1×10^9 PFU are mostly cleared from the bloodstream within 1 hour[14,16,65] and samples taken prior to repeat dosing provide useful proof of successful phage amplification (auto-dosing) that is usually most evident within the first week[14,65].

Production, scalability and regulatory challenges

Phage preparations with <350 endotoxin units (EU) per 1×10^9 PFU (defined by limulus lysate assay and/or monocyte activation testing or similar, and generally only a problem in lysates of Gram-negative bacteria) are easy to achieve with modern chromatographic methods, and many have been issued 'Generally Recognized As Safe' notices by the US Food and Drug Administration[120]. Clinical authorities require 'bacterial endotoxin' to be administered below the widely accepted human pyrogenic threshold of <5 EU kg^{-1} h^{-1}. In addition, when assessing preparation safety of such preparations (reviewed elsewhere[121]), pH, sterility and genomic data should be considered, with the latter requiring a specialized genomic and bioinformatics workflow (Fig. 3). Published guidelines from the European Union[122], Belgium[123], the USA[124] and the UK[125] provide minimum acceptable standards. Phage production is sensitive to economies of scale. Utilization of at least 10–15% of a batch within its shelf-life ensures some economies[126], meaning

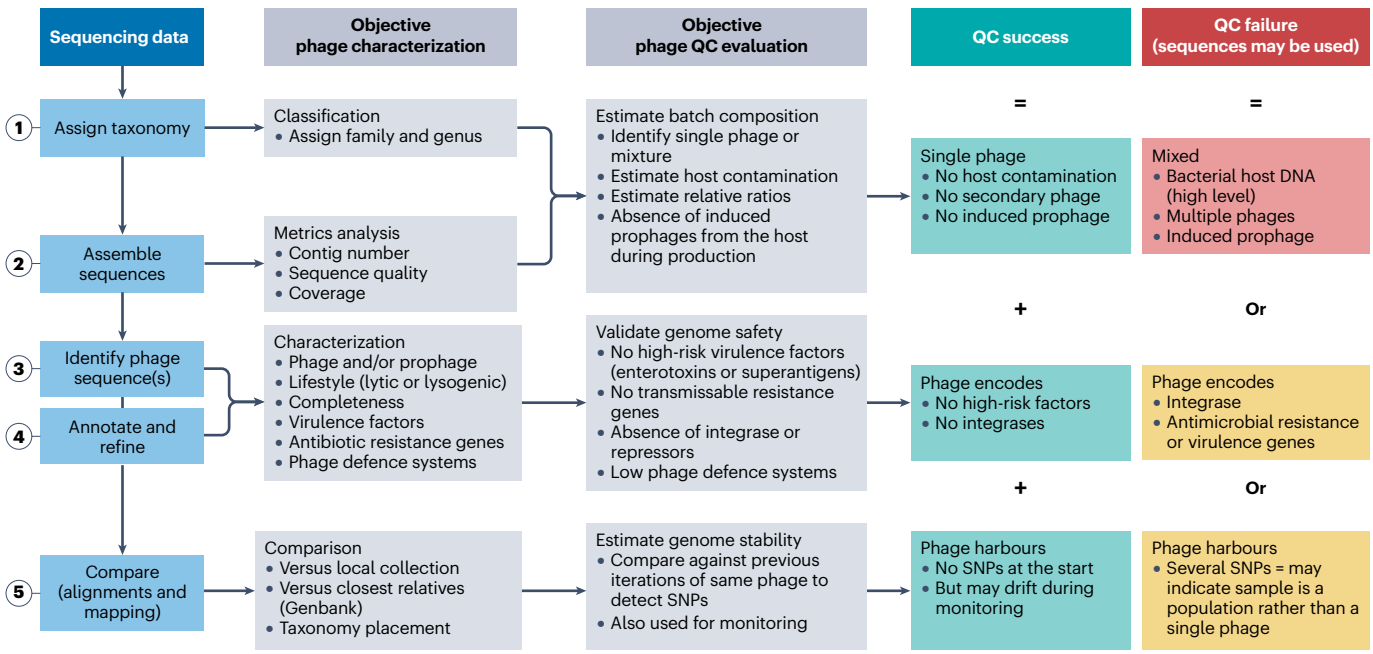


Fig. 3 | Genomic workflow for therapeutic phage characterization and quality control. A bioinformatic workflow should accommodate phage characterization (including novel phages), and quality control (QC) evaluation (phage verification and purity). DNA modifications and unique end structures of linear DNA may complicate assemblies. Short read (for example, 150 bp paired end) at 50-fold to 100-fold coverage allows basic phage characterization; population-level assessment in production batches (evolved phage variants at <5% representation) may require 400-fold to 1,000-fold coverage, with minimum 5-fold coverage for detection of contaminating host DNA (prophages). SNPs, single-nucleotide polymorphisms.

that stability (shelf-life) is an important determinant of cost-effective batch utilization[127,128]. The cost of goods, market access and investment appetite are all shaped by the regulatory environment in which production occurs. Phage cocktails that accommodate the greatest diversity of causative pathogens may be based on a syndrome or, more simply, a common bacterial cause of serious infection. Clinical experts highlight bone and joint infections (for example, with *S. aureus*) and antibiotic-resistant infections (for example, *P. aeruginosa*, *A. baumannii* and other Gram-negative bacteria, as well as mycobacteria such as *M. abscessus*) as principal targets[129,130]. Detailed foreknowledge of likely pathogens (from surveillance and/or published data) maximizes the likelihood of a successful 'hit' and therefore efficient development of a phage library and usage of a batch[126]. In fact, a two-stage approach ('best guess', then diagnostically targeted) is how most antibiotics are prescribed for severe infection[131], simply due to clinical urgency[132]. A 'best guess' preparation might need to sacrifice some synergy or potency for breadth of coverage but is least dependent on diagnostics and best suited to large-scale production.

Human–phage interactions and immune responses

It is to be expected that phages, the most diverse and numerous life forms on earth[34], would themselves develop a sophisticated relationship with adaptive immune systems[133]. Human–phage interactions engage both innate and adaptive immune pathways, with phages capable of modulating inflammation, influencing microbiome composition and eliciting humoral and cellular responses that shape therapeutic outcomes. In humans, low-level inflammatory and immune responses are a natural and necessary counter-measure to the presence of potentially pathogenic organisms, including viruses[112,113], and there is increasing evidence that mammalian cells internalize phages as part of normal homeostasis[134,135]. Pro-inflammatory and even anti-inflammatory effects should be considered during phage therapy.

Pro-inflammatory responses

Human gene signatures during phage therapy suggest direct modulation of pro-inflammatory as well as innate and adaptive immune response regulatory pathways[14,65]. Both in vitro and animal models indicate crosstalk between hub regulatory gene networks including Toll-like receptors (TLRs)[136], Janus kinase–signal transducers and activators of transcription (JAK–STAT)[137] and nucleotide-binding oligomerization domain-like receptor (NLR) signalling pathways[138], as well as autophagy[139] and mitogen-activated protein kinase (MAPK) signalling pathways[140] in the presence of bacterial and/or viral infection (Fig. 4a), with downstream reduction in anti-inflammatory modulators and upregulation of pro-inflammatory cytokines[141,142]. Phages binding macrophage surface receptors trigger production of inflammatory cytokines and reactive oxygen species[143], and this macrophage 'priming' may in turn alter the antibody response[144].

Injection of 10^9 PFU into a human blood volume of 5 l results in transient viraemia of approximately 10^5 PFU ml^{-1}. The elicited innate immune response may differ between phages, and the macrophage priming effect that sometimes accompanies it[145,146] may vary from one phage–bacterial–mammalian interaction to another. In addition, bacterial lysis by phages generates bacterial breakdown products[147] such as lipid A endotoxin and nucleic acids[148]. These potent inflammatory stimuli may boost immune responses against target bacteria in immunologically privileged sites[149] and phages may partner with the mammalian immune response in this way to clear infections[150,151]. Phage self-amplification ('auto-dosing') is frequently evident in accessible tissues (for example, in the bloodstream) in the days after dosing, indicating phage replication[14,65]. Its onset is sometimes marked by an inflammatory response to bacterial lysis products[14] akin to the Jarisch–Herxheimer reaction[152], which may be the most common and important inflammatory effect that occurs with the administration of properly prepared (clean) phage preparations. Allergic or anaphylactoid reactions to naturally occurring bacteria and viruses are unlikely and any such reaction should prompt careful review of the preparation and especially of any co-administered drugs.

Anti-inflammatory effects

The filamentous Pf phages of *P. aeruginosa* trigger antiviral responses that antagonize bacterial clearance by macrophages, an act of phage self-preservation that is associated with worse outcomes in lung infections and wound healing[153,154]. Indeed, the relationship between phages and bacterial debris may be an important determinant of the immune response to phages[148]. Although much of the anti-inflammatory effect of phage therapy can be attributed to suppression of bacterial infection, some phages appear to also directly downregulate inflammatory responses to bacterial components in mononuclear cells[142] and macrophages[153,155]. Specific downregulation of pro-inflammatory genes and upregulation of anti-inflammatory genes are reported in vitro and in vivo (reviewed elsewhere[154]) and suggested in patients undergoing phage therapy[14,65].

As is the case for pro-inflammatory effects, both the phage type and the infection environment are important. Different *E. coli*-targeting phages induced opposing spectrums of interleukin-6 (IL-6), IL-10 and tumour necrosis factor (TNF) expression in human colorectal adenocarcinoma cells[156]. Similarly, *P. aeruginosa* phages are reported to induce strong (pro-inflammatory) IL-6 and TNF expression in human alveolar epithelial and monocyte cell lines, but not in lung co-culture enhanced microenvironment models[157,158] (Fig. 4b).

Adaptive immune responses

Antibody development within a few weeks is common following virus exposures, including intravenous phage administration[159], but this response is not invariable[81,112,113]. The clinical impact of neutralizing antibodies is uncertain as it is unclear whether prolonged courses are necessary[76] or even whether naturally induced antibodies in most individuals are sufficient to neutralize an intravenous dose of 10^9 PFU. Indeed, phage-immunized mice experienced no drop in phage titre and no change in infection outcomes compared with non-immunized controls[144] and therapeutic success has been reported in the presence of anti-phage antibodies[160–162]. When present, specific antibodies may limit the efficacy of prolonged intravenous courses for slow-growing organisms[163] but this may not be important if most of the benefit is in the first week[164] or even after a single dose[76].

Although immune ablation (for example, in individuals who are immunocompromised) may explain some 'non-responders', it also appears that some phages are less immunogenic than others (reviewed elsewhere[165]). This may be influenced by the formulation[166] and/or by some of the factors referred to above, or simply by whether they are more likely to have been seen by the host immune system before, for example, anti-staphylococcal kayvirus phages compared with diverse anti-pseudomonal T4-like phages. The mammalian host immunogenotype (for example, variation in major histocompatibility complex molecules) plays a significant role in response to infection and therapeutic phages[14,65]. Finally, the classical complement pathway inhibits

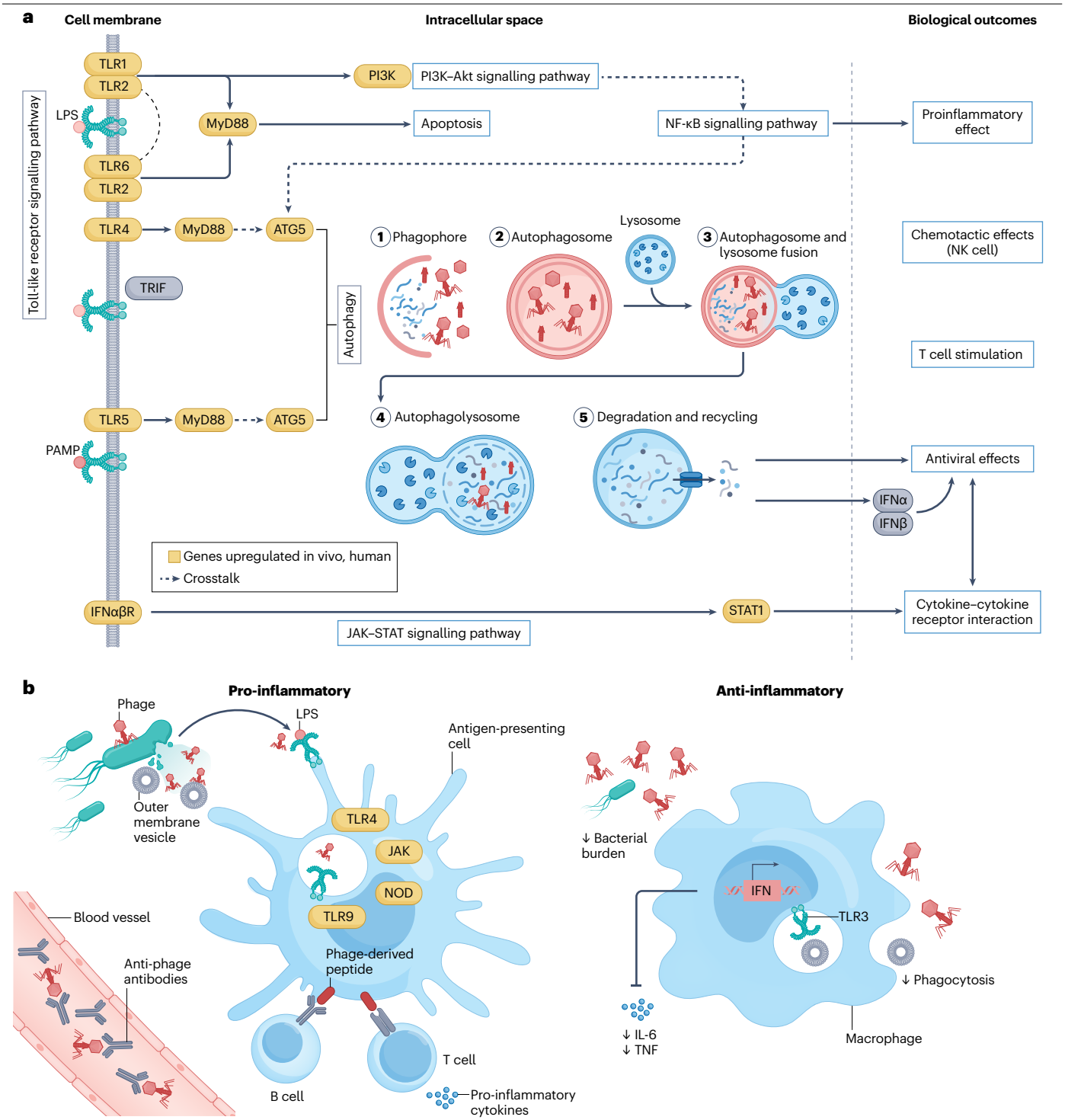


Fig. 4 | Inflammatory responses to phage therapy. a, Possible phage-induced responses. Phages exert pro-inflammatory and anti-inflammatory effects but elimination of bacteria reduces inflammation. **b**, Interactions may vary with different phages. Phages trigger pro-inflammatory Toll-like receptor 9 (TLR9), TLR4, TLR3, nucleotide-binding oligomerization domain (NOD) and Janus kinase–signal transducers and activators of transcription (JAK–STAT) pathways, often attributed to bacterial debris associated with phage lysis. Phage-derived peptides presented by antigen-presenting cells to T cells and B cells drive production of pro-inflammatory cytokines and anti-phage antibodies, respectively. IL-6, interleukin-6; LPS, lipopolysaccharide; NK, natural killer; TNF, tumour necrosis factor.

some phages[111,159] but this is not universal[162,167] and it may be possible to engineer phages to limit complement-mediated clearance.

Phages for intracellular bacterial infections

Effective treatment of intracellular bacterial pathogens may be a special case. The mechanisms of phage entry into mammalian cells are many and varied, and directional traffic of phages through cells is well described[145]. Macropinocytosis, more than receptor-mediated endocytosis, appears to be a predominant pathway for intracellular uptake and concentration of phages in mammalian cells (for example, dendritic cells, macrophages and epithelial cells). This may increase antigen presentation but also have distinct therapeutic advantages, such as the opportunity for 'trojan horse' delivery of capsid-bound payloads[134].

Even if the pathogen becomes free in the mammalian cell cytoplasm, as in the case of *Shigella* spp., the phage must negotiate the different intracellular vacuoles, often in different stages of maturation arrest, that are characteristic of such infections[168]. Phage-mediated lysis of phagocytosed bacteria within macrophages is not easily predicted without testing, but synergy between phage and antibiotic inside the cell is an ideal outcome and certain drugs (for example, quinolones and macrolides) have long been understood to concentrate in intracellular locations better than others (for instance, aminoglycosides)[169]. Neutrophil activation, acting synergistically with other phages, antibiotics and the host immune system, can enhance elimination of both phage-susceptible and phage-resistant mutant strains[150,151].

Conclusions

It seems clear that phages are safe to use therapeutically when properly prepared. The preference for 'obligate lytic' phages is mindful of phage-encoded virulence traits in notorious 'lysogenized' pathogens, but the dichotomous lytic versus lysogenic life cycle divide is an oversimplification. Apparently obligate lytic ('virulent') phages commonly pause the lytic cycle in certain growth states, whereas temperate (integrating) phages may be powerful biocontrol agents[170,171].

Similarly, the exclusion of production hosts with known virulence and/or antimicrobial resistance traits can be managed by employing alternative, even engineered, hosts. Bacterial toxins, prophages and DNA can also be removed during purification in production, but it should be noted that these are invariably more abundant in the infection that phage preparations are designed to treat. This is most important to consider when the specific target strain must serve as the production host. Indeed, training (adapting) 'onto' the target is an important part of preparing therapeutic phages[82], and the training effect of moving the phage onto a different production host may compromise activity against the primary target. Regulatory frameworks must accommodate diversity in phages, as well as their production hosts and growth requirements, if phage therapy is to reach its full potential as a precision therapeutic.

Commercial imperatives favour high-volume production of a limited number of therapeutic combinations (cocktails) most likely to be effective without requiring sophisticated diagnostics, particularly for high-demand pathogens such as *S. aureus* and *P. aeruginosa*. These cocktails may not achieve the synergistic effects of different phages or phage–antibiotic combinations in customized therapy, which are thought to increase the likelihood of success[172].

As phage therapy finds its place in mainstream clinical practice as a precision treatment for bacterial infection in humans, the importance of education and training becomes paramount. Phages are more difficult to prescribe than antibiotics — more target-specific with more complex dosing — but of great potential value as adjunctive or synergistic therapy and generally unaffected by antibiotic resistance. Phages may be used in circumstances where antimicrobial stewardship principles prevent use of antibiotic drugs. The auto-dosing phenomenon and the development of antibodies means that intravenous courses are potentially self-limiting and the optimal duration may be similar to that of most antibiotics (for example, a week or two for most fast-growing bacteria, or possibly much less). The detection of auto-dosing in clinical monitoring (therapeutic phage monitoring)[119] may be especially useful as we explore phage therapy pharmacokinetics and pharmacodynamics, but this is still an open question as even therapeutic (antibiotic) drug monitoring has yet to find its place[173].

As susceptibility testing is standardized, it will need to be correlated with clinical outcomes for validation; however, there is no clear equivalent to the MIC concept that has proven so useful for antibiotics. The choice of phage–phage and phage–antibiotic combinations, which demand high-cost adaptive bacterial trade-offs, is a logical preference but in vitro trade-offs may not be relevant in vivo. Optimal design can be inferred from knowledge of bacterial pathogenesis and is ideally also evident in vitro (so as to be detectable in laboratory tests). Large bacterial populations that are already highly diversified and/or beyond the reach of antibiotics and host defences are the most likely to successfully counter-adapt to therapeutic phage administration and to support the emergence of resistance in vivo. However, even these can be managed effectively when monitored closely[16].

In some scenarios, bacterial suppression is more realistic than bacterial eradication; however, there are rational arguments for aiming to extinguish large, rapidly growing populations[174] and these should be considered on a case-by-case basis.

True allergy is expected to be very rare but inflammatory responses occasionally occur in response to sudden release of bacterial lysis products[14], and the converse, phage-mediated downregulation of certain inflammatory responses must also be further explored for its therapeutic potential[175]. Direct mammalian host immunomodulation is complex and monitoring this is not a part of routine clinical management but may be an important characteristic of certain phages. Clarity is expected to emerge as we accumulate systematic data from human therapy.

The power and accessibility of modern genomics and computational analysis places well-designed phage therapeutics within easy reach for the first time in a century, promising a new therapeutic partner to antibiotics and an answer to the inexorable rise of drug-adapted bacterial pathogens. Although many scientific questions remain, the safety signal is strong and the accumulated data show that phages are life-saving when properly deployed. The challenge for those who would use phages therapeutically is to understand them well enough to do so.

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References

1. Naghavi, M. et al. Global burden of bacterial antimicrobial resistance 1990–2021: a systematic analysis with forecasts to 2050. *Lancet* **404**, 1199–1226 (2024).
2. O'Neill, J. *Tackling Drug-Resistant Infections Globally: Final Report and Recommendations* (Wellcome Trust, 2016).
3. Ruska, H. Die Sichtbarmachung der bakteriophagen Lyse im Übermikroskop [German]. *Naturwissenschaften* **28**, 45–46 (1940).
4. d'Herelle, F. Sur le rôle du microbe filtrant bactériophage dans la dysentérie bacillaire [French]. *C. R. Acad. Sci.* **167**, 970–972 (1918).
5. d'Hérelle, F. *Le bactériophage : Son rôle dans l'immunité* [French] (Masson et Cie, 1921).
6. Angermeyer, A. et al. Evolutionary sweeps of subviral parasites and their phage host bring unique parasite variants and disappearance of a phage CRISPR–Cas system. *mBio* **13**, e0308821 (2021).
7. Dion, M. B., Oechslin, F. & Moineau, S. Phage diversity, genomics and phylogeny. *Nat. Rev. Microbiol.* **18**, 125–138 (2020).

8. Piel, D. et al. Phage–host coevolution in natural populations. *Nat. Microbiol.* **7**, 1075–1086 (2022).
9. Eaton, M. D. & Bayne-Jones, S. Bacteriophage therapy: review of the principles and results of the use of bacteriophage in the treatment of infections. *JAMA* **103**, 1769–1776 (1934).
10. Żaczek, M., Weber-Dąbrowska, B., Międzybrodzki, R., Łusiak-Szelachowska, M. & Górski, A. Phage therapy in Poland—a centennial journey to the first ethically approved treatment facility in Europe. *Front. Microbiol.* **11**, 1056 (2020).
11. Dadiani, M., Nadareishvili, L., Nakaidze, N. & Nizharadze, D. Experience of Eliava Phage Therapy Center in the treatment of respiratory infections. *Eur. Respir. J.* **64**, PA5099 (2024).
12. Schooley, R. T. & Strathdee, S. Treat phage like living antibiotics. *Nat. Microbiol.* **5**, 391–392 (2020).
13. Koncz, M. et al. Genomic surveillance as a scalable framework for precision phage therapy against antibiotic-resistant pathogens. *Cell* **187**, 5901–5918.e28 (2024).
14. Khatami, A. et al. Bacterial lysis, autophagy and innate immune responses during adjunctive phage therapy in a child. *EMBO Mol. Med.* **13**, e13936 (2021).
15. Dedrick, R. M. et al. Engineered bacteriophages for treatment of a patient with a disseminated drug-resistant *Mycobacterium abscessus*. *Nat. Med.* **25**, 730–733 (2019).
16. Schooley, R. T. et al. Development and use of personalized bacteriophage-based therapeutic cocktails to treat a patient with a disseminated resistant *Acinetobacter baumannii* infection. *Antimicrob. Agents Chemother.* **61**, 00954-17 https://doi.org/10.1128/AAC.00954-17 (2017).
17. Ferry, T. et al. Past and future of phage therapy and phage-derived proteins in patients with bone and joint infection. *Viruses* **13**, 2414 (2021).
18. Uyttebroek, S. et al. Safety and efficacy of phage therapy in difficult-to-treat infections: a systematic review. *Lancet Infect. Dis.* **22**, e208–e220 (2022).
19. Bernard, J. M. L. & Francis, M. B. Chemical strategies for the covalent modification of filamentous phage. *Front. Microbiol.* **5**, 734 (2014).
20. Turner, D., Kropinski, A. M. & Adriaenssens, E. M. A roadmap for genome-based phage taxonomy. *Viruses* **13**, 506 https://doi.org/10.3390/v13030506 (2021).
21. Schwarz, H., Riede, I., Sonntag, I. & Henning, U. Degrees of relatedness of T-even type *E. coli* phages using different or the same receptors and topology of serologically cross-reacting sites. *EMBO J.* **2**, 375–380 (1983).
22. Mäntynen, S., Laanto, E., Oksanen, H. M., Poranen, M. M. & Díaz-Muñoz, S. L. Black box of phage–bacterium interactions: exploring alternative phage infection strategies. *Open. Biol.* **11**, 210188 (2021).
23. Rao, V. B. & Feiss, M. Mechanisms of DNA packaging by large double-stranded DNA viruses. *Annu. Rev. Virol.* **2**, 351–378 (2015).
24. Casjens, S. R. The DNA-packaging nanomotor of tailed bacteriophages. *Nat. Rev. Microbiol.* **9**, 647–657 (2011).
25. Freeman, V. J. Studies on the virulence of bacteriophage-infected strains of *Corynebacterium diphtheriae*. *J. Bacteriol.* **61**, 675–688 (1951).
26. Lwoff, A. Lysogeny. *Bacteriol. Rev.* **17**, 269–337 (1953).
27. Bassler, B. L. Small talk: cell-to-cell communication in bacteria. *Cell* **109**, 421–424 (2002).
28. Aframian, N. et al. Dormant phages communicate via arbitrium to control exit from lysogeny. *Nat. Microbiol.* **7**, 145–153 (2022).
29. Bryan, D., El-Shibiny, A., Hobbs, Z., Porter, J. & Kutter, E. M. Bacteriophage T4 infection of stationary phase *E. coli*: life after log from a phage perspective. *Front. Microbiol.* **7**, 1391 (2016).
30. Maffei, E. et al. Phage Paride can kill dormant, antibiotic-tolerant cells of *Pseudomonas aeruginosa* by direct lytic replication. *Nat. Commun.* **15**, 175 (2024).
31. Bondy-Denomy, J. et al. Prophages mediate defense against phage infection through diverse mechanisms. *ISME J.* **10**, 2854–2866 (2016).
32. Sutcliffe, S. G., Reyes, A. & Maurice, C. F. Bacteriophages playing nice: lysogenic bacteriophage replication stable in the human gut microbiota. *iScience* **26**, 106007 (2023).
33. Brown, T. L., Charity, O. J. & Adriaenssens, E. M. Ecological and functional roles of bacteriophages in contrasting environments: marine, terrestrial and human gut. *Curr. Opin. Microbiol.* **70**, 102229 (2022).
34. Clokie, M. R., Millard, A. D., Letarov, A. V. & Heaphy, S. Phages in nature. *Bacteriophage* **1**, 31–45 (2011).
35. Errington, J., Mickiewicz, K., Kawai, Y. & Wu, L. J. L-form bacteria, chronic diseases and the origins of life. *Philos. Trans. R. Soc. Lond. B* **371**, 20150494 https://doi.org/10.1098/rstb.2015.0494 (2016).
36. Petrovic Fabijan, A. et al. L-form switching in *Escherichia coli* as a common β-lactam resistance mechanism. *Microbiol. Spectr.* **10**, e0241922 (2022).
37. Łoś, M. & Wegrzyn, G. Pseudolysogeny. *Adv. Virus Res.* **82**, 339–349 (2012).
38. Wohlfarth, J. C. et al. L-form conversion in Gram-positive bacteria enables escape from phage infection. *Nat. Microbiol.* **8**, 387–399 (2023).
39. Faruque, S. M. & Mekalanos, J. J. Phage–bacterial interactions in the evolution of toxigenic *Vibrio cholerae*. *Virulence* **3**, 556–565 (2012).
40. Fortier, L. C. The contribution of bacteriophages to the biology and virulence of pathogenic clostridia. *Adv. Appl. Microbiol.* **101**, 169–200 (2017).
41. Borodovich, T., Shkoporov, A., Ross, R. & Hill, C. Phage-mediated horizontal gene transfer and its implications for the human gut microbiome. *Gastroenterol. Rep.* **10**, goac012 https://doi.org/10.1093/gastro/goac012 (2022).
42. Zuo, T. et al. Bacteriophage transfer during faecal microbiota transplantation in *Clostridium difficile* infection is associated with treatment outcome. *Gut* **67**, 634–643 (2018).
43. Hargreaves, K. R. & Clokie, M. R. *Clostridium difficile* phages: still difficult? *Front. Microbiol.* **5**, 184 (2014).
44. Krusche, J. et al. Characterization and host range prediction of *Staphylococcus aureus* phages through receptor-binding protein analysis. *Cell Rep.* **44**, 115369 (2025).
45. Letarov, A. V. Bacterial virus forcing of bacterial O-antigen shields: lessons from coliphages. *Int. J. Mol. Sci.* **24**, 17390 https://doi.org/10.3390/ijms242417390 (2023).
46. Maffei, E. et al. Systematic exploration of *Escherichia coli* phage–host interactions with the BASEL phage collection. *PLoS Biol.* **19**, e3001424 (2021).
47. Esteves, N. C. & Scharf, B. E. Flagellotropic bacteriophages: opportunities and challenges for antimicrobial applications. *Int. J. Mol. Sci.* **23**, 7084 https://doi.org/10.3390/ijms23137084 (2022).
48. Chen, P. et al. LamB, OmpC, and the core lipopolysaccharide of *Escherichia coli* K-12 function as receptors of bacteriophage Bp7. *J. Virol.* **94**, 00325-20 https://doi.org/10.1128/JVI.00325-20 (2020).
49. Holtzman, T. et al. A continuous evolution system for contracting the host range of bacteriophage T7. *Sci. Rep.* **10**, 307 (2020).
50. Appelmans, R. Le dosage du bactériophage [French]. *C. R. Soc. Biol.* **85**, 1098 (1921).
51. Arias, C. F., Acosta, F. J., Bertocchini, F., Herrero, M. A. & Fernández-Arias, C. The coordination of anti-phage immunity mechanisms in bacterial cells. *Nat. Commun.* **13**, 7412 (2022).
52. Georjon, H. & Bernheim, A. The highly diverse antiphage defence systems of bacteria. *Nat. Rev. Microbiol.* **21**, 686–700 (2023).
53. Hyman, P. & Abedon, S. T. Bacteriophage host range and bacterial resistance. *Adv. Appl. Microbiol.* **70**, 217–248 (2010).
54. Labrie, S. J., Samson, J. E. & Moineau, S. Bacteriophage resistance mechanisms. *Nat. Rev. Microbiol.* **8**, 317–327 (2010).
55. Nagy, T. A., Gersabeck, G. W., Conte, A. N. & Whiteley, A. T. A phage protein screen identifies triggers of the bacterial innate immune system. *Nat. Microbiol.* **11**, 597–609 (2026).
56. Arber, W. & Linn, S. DNA modification and restriction. *Annu. Rev. Biochem.* **38**, 467–500 (1969).
57. Costa, A. R. et al. Accumulation of defense systems in phage-resistant strains of *Pseudomonas aeruginosa*. *Sci. Adv.* **10**, eadj0341 (2024).
58. Gaborieau, B. et al. Prediction of strain level phage–host interactions across the *Escherichia* genus using only genomic information. *Nat. Microbiol.* **9**, 2847–2861 (2024).
59. Kim, M. K. et al. A blueprint for broadly effective bacteriophage–antibiotic cocktails against bacterial infections. *Nat. Commun.* **15**, 9987 (2024).
60. Müller, D. M. et al. Bacterial receptors but not anti-phage defence mechanisms determine host range for a pair of *Pseudomonas aeruginosa* lytic phages. Preprint at *bioRxiv* https://doi.org/10.1101/2024.04.30.591980 (2026).
61. Torriero-Smith, N., Rogers, B. A., McDonald, M. J. & Barr, J. J. Harnessing 'phage training' to bolster the therapeutic potential of bacteriophages. *Curr. Opin. Microbiol.* **89**, 102696 (2026).
62. Wang, H. et al. Phage-based delivery systems: engineering, applications, and challenges in nanomedicines. *J. Nanobiotechnol.* **22**, 365 (2024).
63. Van Valen, L. A new evolutionary law. *Evol. Theory* **1**, 1–30 (1973).
64. Oechslin, F. et al. Synergistic interaction between phage therapy and antibiotics clears *Pseudomonas aeruginosa* infection in endocarditis and reduces virulence. *J. Infect. Dis.* **215**, 703–712 (2017).
65. Petrovic Fabijan, A. et al. Safety of bacteriophage therapy in severe *Staphylococcus aureus* infection. *Nat. Microbiol.* **5**, 465–472 (2020).
66. Simmons, E. L. et al. Biofilm structure promotes coexistence of phage-resistant and phage-susceptible bacteria. *mSystems* **5**, 00877-19 https://doi.org/10.1128/mSystems.00877-19 (2020).
67. Pires, D. P., Melo, L. D. R. & Azeredo, J. Understanding the complex phage–host interactions in biofilm communities. *Annu. Rev. Virol.* **8**, 73–94 (2021).
68. Visnapuu, A., Van der Gucht, M., Wagemans, J. & Lavigne, R. Deconstructing the phage–bacterial biofilm interaction as a basis to establish new antibiofilm strategies. *Viruses* **14**, 1057 https://doi.org/10.3390/v14051057 (2022).
69. Bowder, M., Kapoor, H., Carrell, S. J. & Danelishvili, L. Phage isolation and functional characterization reveal strong antibiofilm activity against *Pseudomonas aeruginosa* in a cystic fibrosis sputum model. *Front. Cell Infect. Microbiol.* **16**, 1753740 (2026).
70. Hendrix, H. et al. Metabolic reprogramming of *Pseudomonas aeruginosa* by phage-based quorum sensing modulation. *Cell Rep.* **38**, 110372 (2022).
71. Fernández-Garcia, L. et al. Phages and quorum sensing: findings to consider in phage therapy. *Eur. J. Clin. Microbiol. Infect. Dis.* **45**, 615–629 (2026).
72. Beardmore, R. E., Gudelj, I., Lipson, D. A. & Hurst, L. D. Metabolic trade-offs and the maintenance of the fittest and the flattest. *Nature* **472**, 342–346 (2011).
73. Oliver, A. & Mena, A. Bacterial hypermutation in cystic fibrosis, not only for antibiotic resistance. *Clin. Microbiol. Infect.* **16**, 798–808 (2010).
74. d, Hérelle, F. *Le bactériophage et ses applications thérapeutiques* [French] (Doin, 1933).
75. MacNeal, W. J. & Frisbee, F. C. One hundred patients with *Staphylococcus* septicemia receiving bacteriphage service. *Am. J. Med. Sci.* **191**, 179–195 (1936).
76. Wright, A., Hawkins, C. H., Anggard, E. E. & Harper, D. R. A controlled clinical trial of a therapeutic bacteriophage preparation in chronic otitis due to antibiotic-resistant *Pseudomonas aeruginosa*; a preliminary report of efficacy. *Clin. Otolaryngol.* **34**, 349–357 (2009).
77. Jault, P. et al. Efficacy and tolerability of a cocktail of bacteriophages to treat burn wounds infected by *Pseudomonas aeruginosa* (PhagoBurn): a randomised, controlled, double-blind phase 1/2 trial. *Lancet Infect. Dis.* **19**, 35–45 (2019).

78. Leitner, L. et al. Intravesical bacteriophages for treating urinary tract infections in patients undergoing transurethral resection of the prostate: a randomised, placebo-controlled, double-blind clinical trial. *Lancet Infect. Dis.* **21**, 427–436 (2021).
79. Sarker, S. A. et al. Oral phage therapy of acute bacterial diarrhea with two coliphage preparations: a randomized trial in children from Bangladesh. *eBioMedicine* **4**, 124–137 (2016).
80. Adaptive Platform Trials Coalition. Adaptive platform trials: definition, design, conduct and reporting considerations. *Nat. Rev. Drug. Discov.* **18**, 797–807 (2019).
81. Dedrick, R. M. et al. Phage therapy of mycobacterium infections: compassionate use of phages in 20 patients with drug-resistant mycobacterial disease. *Clin. Infect. Dis.* **76**, 103–112 (2023).
82. Pirnay, J. P. et al. Personalized bacteriophage therapy outcomes for 100 consecutive cases: a multicentre, multinational, retrospective observational study. *Nat. Microbiol.* **9**, 1434–1453 (2024).
83. Kok, D. N., Turnbull, J., Takeuchi, N., Tsourkas, P. K. & Hendrickson, H. L. In vitro evolution to increase the titers of difficult bacteriophages: RAMP-UP protocol. *Phage* **4**, 68–81 (2023).
84. Laanto, E., Mäkelä, K., Hoikkala, V., Ravantti, J. J. & Sundberg, L.-R. Adapting a phage to combat phage resistance. *Antibiotics* **9**, 291 https://doi.org/10.3390/antibiotics9060291 (2020).
85. Borin, J. M., Avrani, S., Barrick, J. E., Petrie, K. L. & Meyer, J. R. Coevolutionary phage training leads to greater bacterial suppression and delays the evolution of phage resistance. *Proc. Natl Acad. Sci. USA* **118**, e2104592118 https://doi.org/10.1073/pnas.2104592118 (2021).
86. Morello, E. et al. Pulmonary bacteriophage therapy on *Pseudomonas aeruginosa* cystic fibrosis strains: first steps towards treatment and prevention. *PLoS ONE* **6**, e16963 (2011).
87. Yehl, K. et al. Engineering phage host-range and suppressing bacterial resistance through phage tail fiber mutagenesis. *Cell* **179**, 459–469.e9 (2019).
88. Gencay, Y. E. et al. Engineered phage with antibacterial CRISPR–Cas selectively reduce *E. coli* burden in mice. *Nat. Biotechnol.* **42**, 265–274 (2024).
89. Daubie, V. et al. Determination of phage susceptibility as a clinical diagnostic tool: a routine perspective. *Front. Cell Infect. Microbiol.* **12**, 1000721 (2022).
90. Ramesh, N., Archana, L., Madurantakam Royam, M., Manohar, P. & Eniyan, K. Effect of various bacteriological media on the plaque morphology of *Staphylococcus* and *Vibrio* phages. *Access Microbiol.* **1**, e000036 (2019).
91. Krieger, I. V. et al. The structural basis of T4 phage lysis control: DNA as the signal for lysis inhibition. *J. Mol. Biol.* **432**, 4623–4636 (2020).
92. Parmar, K. et al. Interlaboratory comparison of *Pseudomonas aeruginosa* phage susceptibility testing. *J. Clin. Microbiol.* **61**, e0061423 (2023).
93. Boeckaerts, D. et al. Prediction of *Klebsiella* phage–host specificity at the strain level. *Nat. Commun.* **15**, 4355 (2024).
94. Aggarwal, S. et al. An ensemble method for prediction of phage-based therapy against bacterial infections. *Front. Microbiol.* **14**, 1148579 (2023).
95. Humolli, D. et al. Completing the BASEL phage collection to unlock hidden diversity for systematic exploration of phage–host interactions. *PLoS Biol.* **23**, e3003063 (2025).
96. Chan, B. K., Abedon, S. T. & Loc-Carrillo, C. Phage cocktails and the future of phage therapy. *Future Microbiol.* **8**, 769–783 (2013).
97. Chan, B. K. et al. Personalized inhaled bacteriophage therapy for treatment of multidrug-resistant *Pseudomonas aeruginosa* in cystic fibrosis. *Nat. Med.* **31**, 1494–1501 (2025).
98. Turner, P. E. & Chao, L. Prisoner's dilemma in an RNA virus. *Nature* **398**, 441–443 (1999).
99. van der Steen, B. A., Gralka, M. & Mulla, Y. Rapid resistance evolution against phage cocktails. *ISME J.* **20**, wrag067 (2026).
100. Burmeister, A. R. et al. Pleiotropy complicates a trade-off between phage resistance and antibiotic resistance. *Proc. Natl Acad. Sci. USA* **117**, 11207–11216 (2020).
101. Majkowska-Skrobek, G. et al. The evolutionary trade-offs in phage-resistant *Klebsiella pneumoniae* entail cross-phage sensitization and loss of multidrug resistance. *Environ. Microbiol.* **23**, 7723–7740 (2021).
102. Tsai, Y. K. et al. *Klebsiella pneumoniae* outer membrane porins OmpK35 and OmpK36 play roles in both antimicrobial resistance and virulence. *Antimicrob. Agents Chemother.* **55**, 1485–1493 (2011).
103. Abedon, S. T., Danis-Wlodarczyk, K. M. & Wozniak, D. J. Phage cocktail development for bacteriophage therapy: toward improving spectrum of activity breadth and depth. *Pharmaceuticals* **14**, 1019 https://doi.org/10.3390/ph14101019 (2021).
104. Gu, J. et al. A method for generation phage cocktail with great therapeutic potential. *PLoS ONE* **7**, e31698 (2012).
105. Molina, F. et al. A new pipeline for designing phage cocktails based on phage–bacteria infection networks. *Front. Microbiol.* **12**, 564532 (2021).
106. Abedon, S. T. Phage–antibiotic combination treatments: antagonistic impacts of antibiotics on the pharmacodynamics of phage therapy? *Antibiotics* **8**, 182 https://doi.org/10.3390/antibiotics8040182 (2019).
107. Liu, C. G. et al. Phage–antibiotic synergy is driven by a unique combination of antibacterial mechanism of action and stoichiometry. *mBio* **11**, 04162-20 https://doi.org/10.1128/mBio.01462-20 (2020).
108. Gordillo Altamirano, F. L. et al. Phage–antibiotic combination is a superior treatment against *Acinetobacter baumannii* in a preclinical study. *eBioMedicine* **80**, 104045 (2022).
109. Save, J. et al. Bacteriophages combined with subtherapeutic doses of flucloxacillin act synergistically against *Staphylococcus aureus* experimental infective endocarditis. *J. Am. Heart Assoc.* **11**, e023080 (2022).
110. Khatami, A. et al. Standardised treatment and monitoring protocol to assess safety and tolerability of bacteriophage therapy for adult and paediatric patients (STAMP study): protocol for an open-label, single-arm trial. *BMJ Open* **12**, e065401 (2022).
111. Egido, J. E. et al. Human complement inhibits myophages against *Pseudomonas aeruginosa*. *Viruses* **15**, 2211 https://doi.org/10.3390/v15112211 (2023).
112. Hodyra-Stefaniak, K. et al. Natural and induced antibodies against phages in humans: induction kinetics and immunogenicity for structural proteins of PB1-related phages. *Phage* **1**, 91–99 (2020).
113. Hodyra-Stefaniak, K. et al. Mammalian host-versus-phage immune response determines phage fate in vivo. *Sci. Rep.* **5**, 14802 (2015).
114. Cairns, B. J., Timms, A. R., Jansen, V. A. A., Connerton, I. F. & Payne, R. J. H. Quantitative models of in vitro bacteriophage–host dynamics and their application to phage therapy. *PLoS Pathog.* **5**, e1000253 (2009).
115. Payne, R. J. H. & Jansen, V. A. A. Evidence for a phage proliferation threshold? *J. Virol.* **76**, 13123–13124 (2002).
116. Hvid, U. & Mitarai, N. Competitive advantages of T-even phage lysis inhibition in response to secondary infection. *PLoS Comput. Biol.* **20**, e1012242 (2024).
117. Falsey, A. R., Formica, M. A., Treanor, J. J. & Walsh, E. E. Comparison of quantitative reverse transcription-PCR to viral culture for assessment of respiratory syncytial virus shedding. *J. Clin. Microbiol.* **41**, 4160–4165 (2003).
118. Liu, H., Niu, Y. D., Li, J., Stanford, K. & McAllister, T. A. Rapid and accurate detection of bacteriophage activity against *Escherichia coli* O157:H7 by propidium monoazide real-time PCR. *Biomed. Res. Int.* **2014**, 319351 (2014).
119. Bosco, K., Lynch, S., Sandaradura, I. & Khatami, A. Therapeutic phage monitoring: a review. *Clin. Infect. Dis.* **77**, S384–S394 (2023).
120. Kahn, L. H. et al. From farm management to bacteriophage therapy: strategies to reduce antibiotic use in animal agriculture. *Ann. N. Y. Acad. Sci.* **1441**, 31–39 (2019).
121. João, J., Lampreia, J., Prazeres, D. M. F. & Azevedo, A. M. Manufacturing of bacteriophages for therapeutic applications. *Biotechnol. Adv.* **49**, 107758 (2021).
122. Balasubramanian, G., Morampudi, S., Chhabra, P., Gowda, A. & Zomorodi, B. An overview of compassionate use programs in the European Union member states. *Intractable Rare Dis. Res.* **5**, 244–254 (2016).
123. Pirnay, J. P. & Verbeken, G. Magistral phage preparations: is this the model for everyone? *Clin. Infect. Dis.* **77**, S360–S369 (2023).
124. Suh, G. A. et al. Considerations for the use of phage therapy in clinical practice. *Antimicrob. Agents Chemother.* **66**, e0207121 (2022).
125. Science, Innovation and Technology Committee. The antimicrobial potential of bacteriophages. *UK Parliament* https://publications.parliament.uk/pa/cm5804/cmselect/cmsctech/328/report.html (2024).
126. Iredell, J. & Sacher, J. Investing in the future of phage therapy. *REVIVE* https://revive.gardp.org/investing-in-the-future-of-phage-therapy/ (2023).
127. Duyvejonck, H. et al. Evaluation of the stability of bacteriophages in different solutions suitable for the production of magistral preparations in Belgium. *Viruses* **13**, 865 https://doi.org/10.3390/v13050865 (2021).
128. Wdowiak, M., Paczesny, J. & Raza, S. Enhancing the stability of bacteriophages using physical, chemical, and nano-based approaches: a review. *Pharmaceutics* **14**, 1936 https://doi.org/10.3390/pharmaceutics14091936 (2022).
129. Lee, S., Lynch, S., Lin, R. C. Y., Myung, H. & Iredell, J. R. Phage therapy in Korea: a prescribers' survey of attitudes amongst Korean infectious diseases specialists towards phage therapy. *Infect. Chemother.* **56**, 57–65 (2024).
130. Plymoth, M. et al. Attitudes to phage therapy among Australian infectious diseases physicians. *Int. J. Antimicrob. Agents* **66**, 107579 (2025).
131. Harvey, K., Dartnell, J. & Hemming, M. Improving antibiotic use: 25 years of antibiotic guidelines and related initiatives. *Commun. Dis. Intell. Q. Rep.* **27** (Suppl), S9–S12 (2003).
132. Evans, L. et al. Surviving Sepsis Campaign: international guidelines for management of sepsis and septic shock 2021. *Intensive Care Med.* **47**, 1181–1247 (2021).
133. Flajnik, M. F. & Kasahara, M. Origin and evolution of the adaptive immune system: genetic events and selective pressures. *Nat. Rev. Genet.* **11**, 47–59 (2010).
134. Bichet, M. C. et al. Mammalian cells internalize bacteriophages and use them as a resource to enhance cellular growth and survival. *PLoS Biol.* **21**, e3002341 (2023).
135. Dabrowska, K., Switata-Jelen, K., Opolski, A., Weber-Dabrowska, B. & Gorski, A. Bacteriophage penetration in vertebrates. *J. Appl. Microbiol.* **98**, 7–13 (2005).
136. Bell, E. TLR4 signalling. *Nat. Rev. Immunol.* **8**, 241 (2008).
137. Shuai, K. & Liu, B. Regulation of JAK–STAT signalling in the immune system. *Nat. Rev. Immunol.* **3**, 900–911 (2003).
138. Platnich, J. M. & Muruve, D. A. NOD-like receptors and inflammasomes: a review of their canonical and non-canonical signaling pathways. *Arch. Biochem. Biophys.* **670**, 4–14 (2019).
139. He, C. & Klionsky, D. J. Regulation mechanisms and signaling pathways of autophagy. *Annu. Rev. Genet.* **43**, 67–93 (2009).
140. Liu, Y., Shepherd, E. G. & Nelin, L. D. MAPK phosphatases—regulating the immune response. *Nat. Rev. Immunol.* **7**, 202–212 (2007).
141. Eriksson, F. et al. Tumor-specific bacteriophages induce tumor destruction through activation of tumor-associated macrophages. *J. Immunol.* **182**, 3105–3111 (2009).
142. Van Belleghem, J. D., Clement, F., Merabishvili, M., Lavigne, R. & Vaneechoutte, M. Pro- and anti-inflammatory responses of peripheral blood mononuclear cells induced by *Staphylococcus aureus* and *Pseudomonas aeruginosa* phages. *Sci. Rep.* **7**, 8004 (2017).
143. Ueno, T., Yamamoto, Y. & Kawasaki, K. Phagocytosis of microparticles increases responsiveness of macrophage-like cell lines U937 and THP-1 to bacterial lipopolysaccharide and lipopeptide. *Sci. Rep.* **11**, 6782 (2021).

144. Berkson, J. D. et al. Phage-specific immunity impairs efficacy of bacteriophage targeting vancomycin resistant *Enterococcus* in a murine model. *Nat. Commun.* **15**, 2993 (2024).
145. Kan, L. & Barr, J. J. A mammalian cell's guide on how to process a bacteriophage. *Annu. Rev. Virol.* **10**, 183–198 (2023).
146. Hou, X. et al. Gut bacteriophages induce specific-reprogramming of macrophages to generate a protective innate immunity response to lipopolysaccharide exposure. *Gut Microbes* **17**, 2524540 (2025).
147. Reyes-Robles, T. et al. *Vibrio cholerae* outer membrane vesicles inhibit bacteriophage infection. *J. Bacteriol.* **200**, e00792-17 (2018).
148. Pennetzdorfer, N. et al. Bacterial outer membrane vesicles bound to bacteriophages modulate neutrophil responses to bacterial infection. *Front. Cell Infect. Microbiol.* **13**, 1250339 (2023).
149. Mohan, A. & Saxena, H. M. Effect of phage targeting therapy of brucellosis on host antibody response in cattle. *Phage* **1**, 223–229 (2020).
150. Bodner, K. et al. Engineered fluorescent *E. coli* lysogens allow live-cell imaging of functional prophage induction triggered inside macrophages. *Cell Syst.* **10**, 254–264.e9 (2020).
151. Roach, D. R. et al. Synergy between the host immune system and bacteriophage is essential for successful phage therapy against an acute respiratory pathogen. *Cell Host Microbe* **22**, 38–47 e34 (2017).
152. Dhakal, A. & Sbar, E. *Jarisch–Herxheimer Reaction* (StatPearls, 2024).
153. Secor, P. R. et al. Pf bacteriophage and their impact on pseudomonas virulence, mammalian immunity, and chronic infections. *Front. Immunol.* **11**, 244 (2020).
154. de Souza, E. B., Pinto, A. R. & Fongaro, G. Bacteriophages as potential clinical immune modulators. *Microorganisms* **11**, 2222 https://doi.org/10.3390/microorganisms11092222 (2023).
155. Sweere, J. M. et al. Bacteriophage trigger antiviral immunity and prevent clearance of bacterial infection. *Science* **363**, eaat9691 https://doi.org/10.1126/science.aat9691 (2019).
156. Mirzaei, M. K. et al. Morphologically distinct *Escherichia coli* bacteriophages differ in their efficacy and ability to stimulate cytokine release in vitro. *Front. Microbiol.* **7**, 437 (2016).
157. Shiley, J. R., Comfort, K. K. & Robinson, J. B. Immunogenicity and antimicrobial effectiveness of *Pseudomonas aeruginosa* specific bacteriophage in a human lung in vitro model. *Appl. Microbiol. Biotechnol.* **101**, 7977–7985 (2017).
158. Nickerson, R., Thornton, C. S., Johnston, B., Lee, A. H. Y. & Cheng, Z. *Pseudomonas aeruginosa* in chronic lung disease: untangling the dysregulated host immune response. *Front. Immunol.* **15**, 2024 (2024).
159. Tan, X. et al. Evaluation of the impact of repeated intravenous phage doses on mammalian host–phage interactions. *J. Virol.* **98**, e0135923 (2024).
160. Dan, J. M. et al. Development of host immune response to bacteriophage in a lung transplant recipient on adjunctive phage therapy for a multidrug-resistant pneumonia. *J. Infect. Dis.* **227**, 311–316 (2023).
161. Łusiak-Szelachowska, M. et al. Antiphage activity of sera during phage therapy in relation to its outcome. *Future Microbiol.* **12**, 109–117 (2017).
162. Żaczek, M. et al. Antibody production in response to staphylococcal MS-1 phage cocktail in patients undergoing phage therapy. *Front. Microbiol.* **7**, 1681 (2016).
163. Dedrick, R. M. et al. Potent antibody-mediated neutralization limits bacteriophage treatment of a pulmonary *Mycobacterium abscessus* infection. *Nat. Med.* **27**, 1357–1361 (2021).
164. Maddocks, S. et al. Bacteriophage therapy of ventilator-associated pneumonia and empyema caused by *Pseudomonas aeruginosa*. *Am. J. Respir. Crit. Care Med.* **200**, 1179–1181 https://doi.org/10.1164/rccm.201904-0839LE (2019).
165. Champagne-Jorgensen, K., Luong, T., Darby, T. & Roach, D. R. Immunogenicity of bacteriophages. *Trends Microbiol.* **31**, 1058–1071 (2023).
166. Onsea, J. et al. Bacteriophage therapy for the prevention and treatment of fracture-related infection caused by *Staphylococcus aureus*: a preclinical study. *Microbiol. Spectr.* **9**, e0173621 (2021).
167. Kucharewicz-Krukowska, A. & Slopek, S. Immunogenic effect of bacteriophage in patients subjected to phage therapy. *Arch. Immunol. Ther. Exp.* **35**, 553–561 (1987).
168. Fajardo-Lubian, A. & Venturini, C. Use of bacteriophages to target intracellular pathogens. *Clin. Infect. Dis.* **77**, S423–S432 (2023).
169. Tulkens, P. M. Intracellular distribution and activity of antibiotics. *Eur. J. Clin. Microbiol. Infect. Dis.* **10**, 100–106 (1991).
170. Mardiana, M. et al. Characterization of a novel and active temperate phage vB_AbaM_ABMM1 with antibacterial activity against *Acinetobacter baumannii* infection. *Sci. Rep.* **13**, 11347 (2023).
171. Monteiro, R., Pires, D. P., Costa, A. R. & Azeredo, J. Phage therapy: going temperate? *Trends Microbiol.* **27**, 368–378 (2019).
172. Liu, Y. et al. Clinical application of customized and non-customized bacteriophage therapy in patients with refractory/resistant bacterial infections: a systematic review and meta-analysis. *Int. J. Antimicrob. Agents* **66**, 107570 (2025).
173. Luxton, T. N., King, N., Walti, C., Jeuken, L. J. C. & Sandoe, J. A. T. A systematic review of the effect of therapeutic drug monitoring on patient health outcomes during treatment with carbapenems. *Antibiotics* **11**, 1311 https://doi.org/10.3390/antibiotics11101311 (2022).
174. Tanaka, M. M. & Wahl, L. M. What drives bacterial extinction? The role of bacteriophages. *Theor. Popul. Biol.* **167**, 1–10 (2026).
175. Strzelec, M., Detka, J., Mieszczak, P., Sobocińska, M. K. & Majka, M. Immunomodulation — a general review of the current state-of-the-art and new therapeutic strategies for targeting the immune system. *Front. Immunol.* **14**, 1127704 (2023).
176. Marčuk, L. M. et al. Clinical studies of the use of bacteriophage in the treatment of cholera. *Bull. World Health Organ.* **45**, 77–83 (1971).
177. Karn, S. L. et al. A randomized, placebo-controlled, double-blind clinical trial of bacteriophage cocktails in chronic wound infections. *Int. J. Low. Extrem. Wounds* https://doi.org/10.1177/15347346231226342 (2024).
178. Samaee, H. R. et al. Inhalation phage therapy as a new approach to preventing secondary bacterial pneumonia in patients with moderate to severe COVID-19: a double-blind clinical trial study. *J. Drug. Deliv. Sci. Technol.* **84**, 104486 (2023).
179. Kim, P. et al. Safety, pharmacokinetics, and pharmacodynamics of LBP-EC01, a CRISPR–Cas3-enhanced bacteriophage cocktail, in uncomplicated urinary tract infections due to *Escherichia coli* (ELIMINATE): the randomised, open-label, first part of a two-part phase 2 trial. *Lancet Infect. Dis.* **24**, 1319–1332 (2024).
180. Turdieva, S. T. & Nasirova, G. R. Bacteriophagic therapy: an alternative to antibacterial therapy in acute tonsillitis. *Epidemiol. Int. J.* **5**, 000192 https://doi.org/10.23880/EIJ-16000192 (2021).
181. Fedorov, E. et al. Short-term outcomes of phage–antibiotic combination treatment in adult patients with periprosthetic hip joint infection. *Viruses* **15**, 499 https://doi.org/10.3390/v15020499 (2023).
182. Rhoads, D. D. et al. Bacteriophage therapy of venous leg ulcers in humans: results of a phase I safety trial. *J. Wound Care* **18**, 237–243 (2009).
183. Ooi, M. L. et al. Safety and tolerability of bacteriophage therapy for chronic rhinosinusitis due to *Staphylococcus aureus*. *JAMA Otolaryngol. Head Neck Surg.* **145**, 723–729 (2019).
184. Abul-Hassan, H., El-Tahan, A., Massoud, B. & Gomaa, R. Bacteriophage therapy of *Pseudomonas* burn wound sepsis. *Ann. Medit. Burns Club* **3**, 262–264 (1990).
185. Markoishvili, K. et al. A novel sustained-release matrix based on biodegradable poly(ester amide)s and impregnated with bacteriophages and an antibiotic shows promise in management of infected venous stasis ulcers and other poorly healing wounds. *Int. J. Dermatol.* **41**, 453–458 (2002).
186. Aleshkin, A. V. et al. Phagebiotics in treatment and prophylaxis of healthcare-associated infections. *Bacteriophage* **6**, e1251379 (2016).
187. Doub, J. B. et al. Experience using adjuvant bacteriophage therapy for the treatment of 10 recalcitrant periprosthetic joint infections: a case series. *Clin. Infect. Dis.* **76**, e1463–e1466 (2023).
188. Goulet, A., Spinelli, S., Mahony, J. & Cambillau, C. Conserved and diverse traits of adhesion devices from siphoviridae recognizing proteinaceous or saccharidic receptors. *Viruses* **12**, 512 (2020).
189. Ackermann, H. W. Phage classification and characterization. *Methods Mol. Biol.* **501**, 127–140 (2009).
190. Turner, D. et al. Abolishment of morphology-based taxa and change to binomial species names: 2022 taxonomy update of the ICTV bacterial viruses subcommittee. *Arch. Virol.* **168**, 74 (2023).
191. Bobay, L. M., Touchon, M. & Rocha, E. P. Manipulating or superseding host recombination functions: a dilemma that shapes phage evolvability. *PLoS Genet.* **9**, e1003825 (2013).
192. Ge, X. & Wang, J. Structural mechanism of bacteriophage lambda tail's interaction with the bacterial receptor. *Nat. Commun.* **15**, 4185 (2024).
193. Garcia-Doval, C. & van Raaij, M. J. Structure of the receptor-binding carboxy-terminal domain of bacteriophage T7 tail fibers. *Proc. Natl Acad. Sci. USA* **109**, 9390–9395 (2012).
194. Xiang, Y. et al. Crystallographic insights into the autocatalytic assembly mechanism of a bacteriophage tail spike. *Mol. Cell* **34**, 375–386 (2009).
195. European Committee on Antimicrobial Susceptibility Testing. Clinical breakpoint tables. *EUCAST* https://www.eucast.org/clinical_breakpoints (2023).
196. Kotra, L. P., Haddad, J. & Mobashery, S. Aminoglycosides: perspectives on mechanisms of action and resistance and strategies to counter resistance. *Antimicrob. Agents Chemother.* **44**, 3249–3256 (2000).
197. Fisher, J. F. & Mobashery, S. Constructing and deconstructing the bacterial cell wall. *Protein Sci.* **29**, 629–646 (2020).
198. Shabram, P. & Aguilar-Cordova, E. Multiplicity of infection/multiplicity of confusion. *Mol. Ther.* **2**, 420–421 (2000).
199. Yerushalmy, O. et al. Towards standardization of phage susceptibility testing: the Israeli Phage Therapy Center "clinical phage microbiology"—a pipeline proposal. *Clin. Infect. Dis.* **77**, S337–S351 (2023).
200. Kus, J. V., Tullis, E., Cvitkovitch, D. G. & Burrows, L. L. Significant differences in type IV pilin allele distribution among *Pseudomonas aeruginosa* isolates from cystic fibrosis (CF) versus non-CF patients. *Microbiology* **150**, 1315–1326 (2004).
201. Mahenthiralingam, E., Campbell, M. E. & Speert, D. P. Nonmotility and phagocytic resistance of *Pseudomonas aeruginosa* isolates from chronically colonized patients with cystic fibrosis. *Infect. Immun.* **62**, 596–605 (1994).

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Author contributions

J.R.I. and H.A.S. conceptualized the article. All authors researched data for the article. J.R.I., H.A.S., R.C.Y.L. and N.L.B.Z. wrote the article. All authors reviewed and edited the manuscript before submission. R.C.Y.L., H.A.S., N.L.B.Z. and P.L.B. contributed to figure visualization.

Competing interests

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