Phage therapy for pulmonary infections: lessons from clinical experiences and key considerations

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Abstract

Lower respiratory tract infections lead to significant morbidity and mortality. They are increasingly caused by multidrug-resistant pathogens, notably in individuals with cystic fibrosis, hospital-acquired pneumonia and lung transplantation. The use of bacteriophages (phages) to treat bacterial infections is gaining growing attention, with numerous published cases of compassionate treatment over the last few years. Although the use of phages appears safe, the lack of standardisation, the significant heterogeneity of published studies and the paucity of robust efficacy data, alongside regulatory hurdles arising from the existing pharmaceutical legislation, are just some of the challenges phage therapy has to overcome. In this review, we discuss the lessons learned from recent clinical experiences of phage therapy for the treatment of pulmonary infections. We review the key aspects, opportunities and challenges of phage therapy regarding formulations and administration routes, interactions with antibiotics and the immune system, and phage resistance. Building upon the current knowledge base, future pre-clinical studies using emerging technologies and carefully designed clinical trials are expected to enhance our understanding and explore the therapeutic potential of phage therapy.

Introduction

Lower respiratory tract infections carry the highest mortality amongst communicable diseases and represent the fourth leading cause of death according to a 2020 report by the World Health Organization [1]. Antibiotics remain the only currently approved therapy for the treatment of pulmonary infections. However, their extensive, and in some cases unregulated, use is driving the worsening crisis of antimicrobial resistance (AMR), which poses a relevant threat to global health. Among the first populations to face the hurdles of AMR are patients with cystic fibrosis (CF), ventilator-associated pneumonia (VAP) and lung transplant recipients suffering from multidrug resistant (MDR) infections.

Due to the spread of AMR and stalling in the development of novel antibiotics, the use of bacteriophages (phages) to treat infections, termed “phage therapy”, has attracted increasing attention in recent years. Phages are viruses that infect bacteria to complete their life cycle. After injection of their genetic material
inside their hosts, phages exploit bacterial metabolic functions to enter either a lysogenic state (lysogenic or temperate phages) or follow a strictly lytic life cycle (lytic phages). During the lysogenic life cycle, the phage genome is integrated into the bacterial chromosome (prophage) and is replicated alongside the bacterial host DNA. In this dormant state, i.e. without new virion production, prophages can nonetheless regulate gene expression and change cell physiology. Prophages can activate the lytic state either spontaneously or as a result of external factors [2], resulting in new virion production and contributing to transduction of genomic DNA between bacteria, including transfer of genes encoding antibiotic resistance or toxin production [3]. In contrast, strictly lytic phages do not enter a lysogenic cycle; instead, they infect and readily kill their hosts by producing new virions within minutes.

Following their discovery and broad early use in the twentieth century, phages were subsequently abandoned by Western countries after the advent of antibiotics. Substantial clinical use continued to occur in Eastern Europe, but it did not follow the typical modern pharmaceutical development pathway [4]. The lack of robust evidence stemming from randomised controlled trials (RCTs) [5–8] has hindered the establishment of phage therapy as an approved antimicrobial treatment, limiting its use, for now, to within a compassionate framework. On the other hand, this extensive clinical experience has confirmed the favourable safety profile of phage therapy.

Over the last few years, interest in phage therapy has re-emerged, backed by significant progress at a pre-clinical level in exploring phage pharmacology and novel formulations, phage antibiotic synergy, and its role in biofilm infections. Numerous case reports have been published and multiple clinical trials are currently underway [9–15], including three phase I/II clinical trials in CF patients with chronic Pseudomonas aeruginosa infection [10, 14, 15].

**Recent experiences in phage therapy for the management of respiratory infections**

Over the past 5 years, 20 cases of phage therapy for the treatment of respiratory infections have been reported, mostly by expert centres in the US and Europe. These include 12 case reports [16–25] and a case series [26, 27] describing 15 patients, and two clinical trials [28, 29] involving four participants with respiratory infections. The first trial is an open-label two-arm study evaluating the efficacy of inhaled phage therapy for secondary Acinetobacter baumannii infection in four critically ill coronavirus 2019 (COVID-19) patients [28]. The authors selected phage combinations based on in vitro predictions of the emergence of phage-resistant bacterial strains after phage therapy, a process known as "phage training" [30]. The second study is a single-arm noncomparative trial assessing the safety of a combination of three phages (AB-SA01) as adjunctive therapy for the treatment of severe Staphylococcus aureus infection [29]. One participant in this trial was treated for a methicillin-resistant S. aureus (MRSA) pulmonary infection and is reported here. A detailed overview of all published cases of phage therapy for respiratory infections since 2017 is presented in table 1.

The majority of patients (n=8, 40%) were treated for VAP [16, 21, 24, 28, 29] but a significant proportion suffered from chronic pulmonary infections following solid organ transplantation (n=6, 30%) [20, 22, 26, 27]. The remainder were patients with CF without lung transplantation (n=4, 20%) [17–19, 25], and cases with chronic empyema [27] and non-CF bronchiectasis [23]. The most common targeted pathogens were carbapenem-resistant A. baumannii (CRAB) (n=6, 30%), followed by P. aeruginosa (n=4, 20%) and Achromobacter spp. (n=3, 15%).

For all patients, phage therapy was proposed within a compassionate framework, following the failure of optimal anti-infective therapy to control the underlying infection. However, phages were always used as an adjunct to antibiotics. ASLAM et al. [26] reported their experience of compassionate use of phage therapy for the treatment of respiratory infections in three lung transplant recipients. Amongst them, a 57-year-old woman with a complicated post-lung transplant course for non-CF bronchiectasis [23]. The most common targeted pathogens were carbapenem-resistant A. baumannii (CRAB) (n=6, 30%), followed by P. aeruginosa (n=4, 20%) and Achromobacter spp. (n=3, 15%).

Most patients (n=15, 75%) were treated with a combination of two or more phages. Whether the use of a single phage was related to nonavailability of other active phages is not systematically reported. The most
| Author [ref.] | Indication | Phage(s) used | Treatment modalities | Duration of phage therapy | Concomitant antibiotics | Outcome |
|---------------|------------|--------------|----------------------|---------------------------|------------------------|---------|
| Maddocks et al. [16] | *P. aeruginosa* VAP | AB-PA01<sup>4</sup> | 1×10⁹ PFU·mL⁻¹ di uted NaCl 100 mL i.v. q12h, inh 4 mL (undiluted) q12h | 7 days | Gentamycin, ciprofloxacin, ceftolozane–tazobactam | Infection resolution, eradication |
| Law et al. [17] | *P. aeruginosa* chronic lung infection in CF patient | AB-PA01<sup>4</sup> | 4×10⁹ PFU·mL⁻¹ i.v. q8h | 8 weeks | Piperacillin–tazobactam, ciprofloxacin, doripenem | Infection resolution, no eradication |
| ASlam et al. [26] | *P. aeruginosa* chronic lung infection, post lung transplantation | AB-PA01<sup>4</sup> | 4×10⁹ PFU·mL⁻¹ i.v. q6h, inh 4× dose q12h | 4 weeks | Piperacillin–tazobactam, colistin | Active infection resolution, no eradication |
| ASlam et al. [26] | *P. aeruginosa* chronic lung infection, post lung transplantation | Navy phage 1<sup>4</sup> | 1×10⁹ PFU·mL⁻¹ i.v. q2h, inh 4× dose q12h | 8 weeks | Piperacillin–tazobactam, tobramycin, colistin inh | |
| ASlam et al. [26] | *B. dolosa* chronic lung infection in CF patient, post lung transplantation | BdPF16phi4281d+ | 5.3×10⁶–3.5×10⁷ PFU·mL⁻¹ i.v. | 12 weeks | Piperacillin–tazobactam, ceftazidime–avibactam, meropenem, minocycline, tobramycin inh | No response (patient died) |
| Petrovic Fabijan et al. [29] | MRSA pneumonia, septic shock | AB-SA01<sup>4</sup> | 3×10⁹ PFU·mL⁻¹ diluted 50–100 mL NaCl i.v. q12h | 3 days | Vancomycin, clindamycin, meropenem, azithromycin | No response (treatment withdrawn) |
| Rubalksi et al. [27] | *S. aureus* empyema | Staphylococcus phage CH1<sup>9</sup> | 1×10⁹ PFU·mL⁻¹ 20 mL intrapleural q12h | 7 days | Daptomycin | Infection resolution, no eradication |
| Rubalksi et al. [27] | *K. pneumoniae* VAP post heart transplantation | KPV811, KPV15<sup>9</sup> | 1×10⁸ PFU·mL⁻¹ 2 mL inh q24h, 18 mL via NGT q24h for 2 days, 2 mL inh q12h and 18 mL via NGT q12h | 4 days | Ceftazidime, linezolid, avibactam, meropenem, cotrimoxazole tobramycin, colistin inh | Infection resolution, eradication |
| Hoyle et al. [18] | *Achromobacter* spp. chronic lung infection in CF patient | Two *Achromobacter* phages<sup>1</sup> | 3×10⁹ PFU·mL⁻¹, 2 mL inh and p.o. q12h | 20 days, repeated at 1, 3, 6 and 12 months | Cotrimoxazole, ciprofloxacin, tobramycin inh, piperacillin–tazobactam | Infection control, no eradication |
| Gainey et al. [19] | *Achromobacter* spp. chronic lung infection in CF patient | AxCJ4562<sup>2</sup> | Dose NS, i.v. infusion over 1 h, q24h | 2 weeks | Meropenem–vaborbactam, cefiderocol | Infection control, no eradication<sup>***</sup> |
| Lebeaux et al. [20] | *Achromobacter* spp. chronic lung infection in CF patient post lung transplantation | APC 1.1, APC 2.1<sup>**</sup> | 4×10¹⁰ PFU·mL⁻¹; 5 mL of tenfold diluted (NaCl) inh q8h 5×10⁹ PFU·mL⁻¹; 30 mL tenfold diluted in each lobe (bronchoscopy), inh 5 mL q8h | 2 days | Imipenem | Active infection resolution, no eradication |
| Tan et al. [24] | Carbapenem-resistant *A. baumannii* VAP | Ab_SZ2<sup>2</sup> | Final dose: 5×10¹⁰ PFU·mL⁻¹, diluted to 5 mL, inh q12h | 16 days | Tigecycline, cefoperazone–sulbactam, polymyxin inh | Infection resolution, eradication |

**TABLE 1** Overview of published cases of phage therapy for the treatment of respiratory infections since 2017

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**EUROPEAN RESPIRATORY REVIEW PHAGE THERAPY | G. MITROPOULOU ET AL.**
**TABLE 1**

| Author [ref.] | Indication | Phage(s) used | Treatment modalities | Duration of phage therapy | Concomitant antibiotics | Outcome |
|---------------|------------|---------------|----------------------|--------------------------|-------------------------|---------|
| **Wu et al. [28]** | Carbapenem-resistant *A. baumannii* VAP in COVID-19 patients (n=4) | $\phi$Ab124, $\phi$Ab121 $^\#$ | 1×$10^8$ PFU·mL$^{-1}$ diluted in NaCl 10 mL, inh two doses | Two doses | Various $^5$ | Infection control in three patients, eradication in one patient, two patients died $^3$ |
| **Rao et al. [21]** | Carbapenem-resistant *A. baumannii* VAP | AbW4932a1, AbW4878a1 $^\#$ | 1×$10^9$ PFU·mL$^{-1}$, diluted in NaCl 50 mL infused over 30–60 min, i.v. q12h, 0.1×$10^8$ PFU·mL$^{-1}$ diluted in NaCl 10 mL inh q12h | 5 weeks i.v., 3 weeks inh | Cotrimoxazole, tigecycline cefepime, cefiderocol | Infection resolution, no eradication $^3$ |
| **Dedrick et al. [22]** | *M. abscessus* disseminated infection in CF patient, post lung transplantation | Muddy, BPs$\Delta$, ZoeJ$\Delta$ $^\#$ | 3×$10^9$ PFU·mL$^{-1}$ i.v. q12h, topical 10$^9$ PFU·mL$^{-1}$ | 32 weeks | Amikacin, imipenem, tigecycline, bedaquiline, clofazimine | Active infection resolution, no eradication |
| **Dedrick et al. [23]** | *M. abscessus* chronic lung disease in non-CF bronchiectasis | Muddy, BPs$\Delta$, ZoeJ$\Delta$ $^\#$ | 1×$10^9$ PFU·mL$^{-1}$ i.v. q12h | 6 months | Imipenem, ethambutol, azithromycin, clofazimine, omadacycline | No response (neutralising antibodies against phages) |
| **Nix et al. [25]** | *M. abscessus* chronic lung disease in CF patient | BPsD33HTHHRM10, D29_HRM6040$^\#$ | 10$^6$–10$^7$ PFU·mL$^{-1}$ i.v. q12h | >12 months (ongoing) | Omadacycline, bedaquiline, clofazimine, amikacin, tzediolidine | Infection resolution, eradication |

Eradication of the target bacterial pathogen is defined as negative cultures at 6 months of follow-up. q2/4/6/8/12/24h: every 2/4/6/8/12/24 h; A: *Acinetobacter*; B: *Burkholderia*; CF: cystic fibrosis; COVID-19: coronavirus disease 2019; inh: inhaled; i.v.: intravenous; K: *Klebsiella*; M: *Mycobacterium*; MRSA: methicillin-resistant *Staphylococcus aureus*; p.o.: per os; NGT: nasogastric tube; NS: not specified; P: *Pseudomonas*; PFU: plaque-forming units; S: *Staphylococcus*; spp.: species; ref.: reference; VAP: ventilator-associated pneumonia. $^6$: Phage combinations AB-PA01, AB-PA01-m1 and AB-SA01 were produced according to Good Manufacturing Practices (GMP) standards by AmpliPhi Biosciences Corporation, USA (now Armata Pharmaceuticals (AMP), USA). $^5$: Navy phages 1 and 2 were supplied by the Naval Medical Research Centre, Fort Detrick, USA. $^7$: Phage BdPF16phi4281d and phages AbW4932a1 and AbW4878a1 were produced by Adaptive Phage Therapeutics (APT), USA. $^8$: *Staphylococcus* phage CH1 and phages KPV811 and KPV15 were provided by the Gabrichevsky Institute, Russia. $^9$: The *Achromobacter* phage combination was prepared by the Elava Institute using phages provided under collaboration of the Elava Institute of Bacteriophages, Microbiology and Virology, Tbilisi, Georgia and the Leibniz Institute DSMZ–German Collection of Microorganisms and Cell Cultures, Germany. $^10$: Phage Ax2CJ45s2 was isolated by the Naval Medical Research Centre, USA and was supplied by APT, USA. $^11$: *Staphylococcus* phage CH1 and phages KPV811 and KPV15 were provided by the Gabrichevsky Institute, Russia. $^12$: The *Achromobacter* phage combination was prepared by the Elava Institute using phages provided under collaboration of the Elava Institute of Bacteriophages, Microbiology and Virology, Tbilisi, Georgia and the Leibniz Institute DSMZ–German Collection of Microorganisms and Cell Cultures, Germany. $^13$: Phage Ab$_{-}$S23 was isolated and prepared at the Shenzhen Institute of Advanced Technology, China. $^5$: Phages $\phi$Ab124 and $\phi$Ab121 were isolated from the local library and prepared in GMP facilities at the Zhongshan Hospital of Fudan University, Shanghai, China. $^14$: Screening for phages was performed using the Science Education Alliance Phage Hunters Advancing Genomics and Evolutionary Science (SEA-PHAGES) programme. Lytic derivatives were engineered using Bacteriophage Recombining of Electroporated DNA (BRED). $^15$: Negative culture at 4 months of follow-up. $^16$: One patient was discharged from the intensive care unit but died of respiratory failure a month later secondary to a carbapenem-resistant *K. pneumoniae* infection. A second patient died from a carbapenem-resistant *K. pneumoniae* infection after *A. baumannii* eradication. $^3$: Negative cultures at 6 days after the end of therapy. $^9$: Patient 1: cefoperazone–sulbactam; patient 2: cefoperazone–sulbactam, tigecycline, levofloxacin; patient 3: meropenem, cefoperazone–sulbactam; patient 4: tigecycline, imipenem, cefoperazone–sulbactam.
common route of administration was i.v. [16, 17, 19, 21–23, 25, 26, 29], either alone (n=7, 35%) or in combination with the inhaled route (n=3, 15%). Some patients received phage therapy exclusively via inhalation (n=5, 25%) [24, 28] and, in one case, inhaled therapy was coupled with direct phage administration via bronchoscopy [20]. Other topical routes included pleural administration via chest drain in a patient with chronic empyema [27] and cutaneous applications in a patient with disseminated *M. abscessus* infection [22]. The duration of phage therapy varied greatly, ranging from 2 days to more than 12 months (average duration was 28 days).

Sensitivity to phages prior to treatment initiation was tested in all but one patient and serial phage susceptibility testing during therapy was performed in most cases (n=15, 75%). Although the emergence of resistance to at least one of the phages used was detected in six patients (30%) during treatment, few required adaptations of phage therapy due to an absence of clinical response [26]. In the majority of reported cases (n=16, 80%), phage therapy was associated with a favourable clinical outcome, defined here as resolution of the underlying infection with [16, 24, 25, 27, 28] or without [17, 20, 22, 26] bacterial eradication (negative cultures at 6 months), or as partial control of the underlying infection but without complete resolution [18, 19, 21, 26]. An improved antibiotic susceptibility profile of the targeted pathogen following treatment with phages was observed in two patients treated for MDR *P. aeruginosa* [26] and pan-resistant *Klebsiella pneumoniae* [27].

No response to phage therapy occurred in the case of a 28-year-old lung transplant recipient with CF presenting *Burkholderia dolosa* pulmonary infection [26]. Although the 12-week i.v. phage therapy course in combination with antibiotics resulted in an initial clinical improvement, the patient died from progressive multi-organ failure related to *B. dolosa* sepsis, antimicrobial toxicity and surgical complications. The emergence of phage resistance could not be excluded in this patient, as phage susceptibility testing was not performed. In another patient with MRSA pneumonia treated with a pre-fixed phage combination (AB-SA01) [29], phage therapy was interrupted after evidence of pre-existing phage resistance in vitro. Dedrick *et al.* [23] used a combination of natural and genetically engineered phages for the treatment of refractory *M. abscessus* lung disease in an 81-year-old immunocompetent patient with non-CF bronchiectasis. *M. abscessus* load increased significantly after 1 month of i.v. phage therapy, attributed to the emergence of potent neutralising antibodies against all three phages. Of note, pre-existing immunoglobulin G antibodies against two out of three phages were detected in this patient; however, without neutralising activity.

**Lessons learned from clinical applications and key considerations**

**Indications for phage therapy**

Therapeutic phage preparations are currently only commercially available for clinical use in Georgia and Russia. In the rest of the world, their use is limited to compassionate treatment of individual patients, guided by the Article 37 of the Declaration of Helsinki [31], or to clinical trials. With regard to respiratory infections, phage therapy has been exclusively utilised following ineffective antibiotic treatment for the management of acute MDR or chronic resistant infections. Antibiotic intolerance, in the presence of allergies or severe complications related to antibiotics, can also be considered an appropriate indication for compassionate use of phage therapy. There is, however, a growing interest in the use of phage therapy outside a strictly compassionate context, such as for the prevention or eradication of MDR infections [32]. Some of the cases previously discussed resulted in eradication of the respiratory pathogen [16, 24, 25, 27, 28] but the benefit from the prophylactic use of phage therapy for the prevention of respiratory infections remains unknown.

Infection control and prevention of exacerbations remain major concerns for individuals with CF. The combination of elixacaftor, tezacaftor and ivacaftor has revolutionised CF care, resulting in significant improvements in lung function and a decreased rate of pulmonary exacerbations [33–36]. However, long-term data on rates of *P. aeruginosa* infections under such treatment are not yet available [36]. Furthermore, currently available modulators of the CF transmembrane protein may not restore its function to normal levels, are not available for all CF-associated genotypes and might not be sufficient to control chronic infection in patients with established bronchiectasis. Phage therapy could find a place as an adjunct to inhaled antibiotics for the control of chronic infections in this setting or even as an alternative for patients allergic or intolerant to antibiotics. Along these lines, the results of the ongoing phage therapy trials [10, 14, 15] in this field are eagerly awaited.

**Safety of phage therapy**

According to recently published data [37, 38], treatment with phages is well tolerated in most cases, independent of the route of administration. Adverse events are rare and, when reported, are mild,
self-resolving or resolving after dose reduction or treatment interruption. With regard to the treatment of respiratory infections, only one patient developed an adverse event attributable to phage therapy (cytokine storm with fever and increase in interleukin (IL)-6 and IL-8 levels), which occurred 4 h after inhaled Good Manufacturing Practice (GMP)-grade phage administration ($10^8$ plaque-forming units (PFU)·mL$^{-1}$, endotoxin level not reported) [28]. The authors report that symptoms regressed spontaneously and cytokine levels normalised after 24 h.

To ensure the safety of phage therapy, there is a need to stringently characterise and manufacture phage preparations produced with GMP standards. Phages intended for therapy should undergo genotyping with whole-genome sequencing to assert that they are free of relevant antibiotic resistance and virulence factors, and to avoid lysogenic phages. Nonetheless, in the absence of active lytic phages, modern genetic engineering methods can transform temperate phages into therapeutically useful candidates [22]. Phages should be propagated on well-characterised bacterial strains, devoid as much as possible of known prophages and undesired genes (encoding for toxins or antibiotic resistance determinants). Phage suspensions destined for administration should be sterile, free from contamination by nonphage products and have endotoxin levels lower than 5 endotoxin units (EU)·kg·h$^{-2}$ for i.v. injections, 0.5 EU·mL$^{-1}$ for subcutaneous injections, and 0.2 EU·kg$^{-1}$ for intrathecal injections, as defined by the Food and Drug Administration (FDA) [39]. Currently there are no defined limits for exotoxin levels, which should, nevertheless, be documented and maintained as low as possible.

**Interactions with commensal flora**

The favourable safety profile of phage therapy can be attributed to the high host-specificity of phages and the less profound perturbation of commensal flora [40] compared to nonselective antibiotics. On the other hand, cascading effects of phage administration on nontarget species have been observed in the gut [41]. In this experiment using gnotobiotic mice, targeted phage predation correlated with specific shifts in the metabolic products produced, suggesting that phages may enable a precise modulation of the gut metabolome [41]. Although still largely unexplored, a similar phage-dependent modulation of lung microbiota could present an opportunity of therapeutic benefit in patients with respiratory infections. The respiratory ecosystem of healthy lungs consists of diverse bacterial communities [42], themselves influenced mostly by the equilibrium between bacterial immigration and elimination [43]. In CF patients and lung transplant recipients, prolonged exposure to antibiotics and immunosuppression may shift a homeostatic lung microbiota to dysbiosis, characterised by low microbial diversity, high microbial burden and host inflammation [44, 45]. Phages could lead to the possibility of selective modulation to restore homeostasis by specific antibacterial activity [46].

**Efficacy of phage therapy**

The previously discussed individual reports suggest some clinical benefit of adding phages to standard-of-care (SoC) anti-infectives in the management of respiratory infections. However, the efficacy of phage therapy cannot be reliably estimated in the absence of evidence originating from RCTs. Moreover, phages have been employed mostly in conjunction with antibiotics, making it difficult to assess their individual therapeutic contribution. Previous RCTs assessing phage therapy versus SoC [5, 6, 8] failed to produce robust efficacy results of phages. On the other hand, these studies were marked by significant flaws in terms of phage product stability [5], distribution [8] and, ultimately, bactericidal activity [6].

Even though the limited size and heterogeneity of reported cases of phage therapy for respiratory infections mitigate the interpretability of the observed results, the absence of a clinical response was directly associated with the lack of lytic activity in one patient [29], while the emergence of phage resistance could not be excluded in two other unsuccessful cases [26, 28]. As for most cases treated with phages, it should be noted that these patients suffered from severe MDR infections, with two requiring extracorporeal membrane oxygenation [26, 28] and facing a poor prognosis [47, 48]. The use of a single phage (versus a combination) at a relatively low titre ($10^7$ PFU·mL$^{-1}$) was postulated as a factor potentially influencing efficacy by ASLAM et al. [26]. Although phages are often used in combinations (cocktails) to ensure activity over different strains or to prevent phage resistance selection, the clinical superiority of cocktails over single phages has not been proven. Phage–phage dynamics are complex and difficult to predict in vivo [49]. In vitro screening for phage competition and antagonistic interactions can potentially reduce the risk of using antagonistic phages [50].

**Immune response to phage therapy**

In the case reported by Dedrick et al. [23], the lack of efficacy of phage therapy was attributed to a neutralising immune response to all of the phages used. There is increasing evidence that phages interact
with eukaryotic cells and elicit innate and adaptive immune responses that have a significant impact on their clearance and distribution [23, 51–56].

Although the efficacy of phages for the management of respiratory infections has been previously demonstrated in neutropenic mouse lung models designed to exclude the influence of bacterial host cells [57–59], the interaction between phages and the immune system can also prove to be beneficial against infections [60]. In an MDR *P. aeruginosa* pneumonia mice model, ROACH et al. [61] compared the efficacy of intranasal phage administration in immunocompetent mice and mice with distinct types of immunodeficiencies (MyD88-deficient, lymphocyte-deficient and neutropenic). Phage–neutrophil synergy was shown to be crucial for infection control, as neutropenic mice were completely unresponsive to phage therapy. In this experiment, no evidence of immune-mediated phage neutralisation was observed after a single dose of phage therapy.

The immunogenicity of phages and the emergence of phage-neutralising antibodies seem to depend, at least partially, on the route of administration [62, 63], as parenteral application induces a stronger response compared to topical or enteral routes. Repeated parenteral administration of phages over a 6-week period in rabbits [64] resulted in complete neutralisation of phages 3–5 weeks after immunisation. Humoral response was possibly enhanced by repeated and prolonged phage administration. In this study, phage neutralisation was independent of the presence of pre-existing phage-specific antibodies. Pre-existing immunisation or cross-immunisation is indeed not uncommon [65, 66] given the ubiquity of phages, but its role on phage neutralisation is not yet clear.

The presence of phage-specific antibodies does not always correlate with a lack of therapeutic response [65, 67–69] but phage neutralisation has not been evaluated systematically in the clinical setting [22, 23, 70]. In view of these results, it is worth further considering the specific modalities of phage administration (i.v. versus topical) and duration (limited versus extended) with regard to their potential immunogenicity in immunocompetent versus immunocompromised hosts, as well as the presence of pre-existing immunisation against specific phages. A sequential administration of phages could potentially carry the advantage of avoiding the simultaneous neutralisation of all phages, circumvent potential adverse phage–phage interactions and allow for better prediction of genetic changes in the emerging resistant bacterial hosts [50, 71].

**An alternative or an adjunct to antibiotics?**

At an estimated number of \(10^{31}\), phages are the most diverse and abundant biological entities on our planet [72]. The process of phage infection and lysis of bacteria differs from the antibiotic mechanisms of action and resistance [73, 74], rendering phage therapy a promising addition to the existing armamentarium against AMR [75]. Rather than an alternative to antibiotics, phages have been used as an adjunct treatment for infections not responding to antibiotics alone [76–79]. The enhanced antimicrobial activity of phage–antibiotic combinations, also referred to as phage–antibiotic synergy (PAS), was first observed when the addition of sub-lethal concentrations of certain antibiotics substantially stimulated the bacterial cell’s production of a virulent phage [80]. Although the synergistic effect of phages and antibiotics has been observed in vitro [81–84] and in vivo [85, 86], the mechanisms underlying PAS are not completely understood, and most likely are phage- [82], strain- [87] and antibiotic-dependent [82, 88].

One aspect of synergistic antibacterial activity most relevant to respiratory infections is the role of PAS in biofilm. Pathogens commonly responsible for respiratory infections in CF, bronchiectasis and VAP produce biofilms, i.e. aggregates of microorganisms within a self-produced matrix of extracellular polymeric substances (EPS). Biofilm is capable of adhering to surfaces such as living tissue and medical devices, for instance endotracheal tubes, and acts as a “fortress”, growing rapidly, inhibiting antibiotic penetration and contributing to the emergence of antibiotic tolerance and resistance [89]. Phages can encode depolymerases that degrade EPS matrix components [90], enabling them to penetrate biofilm and replicate *in situ*. A study of the interaction between a three-phage cocktail and *P. aeruginosa* flow-cell biofilms showed that, although single treatment with phages significantly reduced bacterial load [91], repeated phage applications resulted in biofilm growth [91], possibly due to the selection of phage-resistant strains and upregulated expression of virulence factors [92]. The co-administration of phages and ciprofloxacin significantly reduced bacterial loads [91]. Results from other studies have underlined the importance of treatment sequence when combining phages and antibiotics: pre-treatment of biofilms with phages and subsequent application of antibiotics led to significant EPS degradation, bacterial growth inhibition and biofilm eradication [93–97]. When the treatment order was inverted, biofilm exposure to antibiotics prior to phage treatment resulted in antagonistic interactions for some combinations [97]. These results demonstrate that phages may act synergistically with antibiotics, possibly due to the ability of phages to
degrade biofilm, but treatment outcome is ultimately influenced by various factors, including treatment sequence, phage and antibiotic type, and concentration.

Phage–antibiotic interactions (synergistic or antagonistic) have not been systematically assessed in the clinical setting, and reliable methods for in vitro phage–antibiotic combination testing are currently lacking. Presently, the absence of robust data on the efficacy of phage therapy alone indicates that, outside the context of a clinical trial, phages should be used as an adjunct to antibiotics. Further studies aiming to improve our understanding of the molecular mechanisms underlying phage–antibiotic interactions will allow for the prediction and selection of in vivo synergistic combinations associated with improved therapeutic outcomes [98].

**Treatment modalities**

Until now, phage therapy has been largely empirical [99]. As shown in table 1, amongst the 20 reported cases, various dosing protocols have been applied, with great disparity regarding the use of a single phage versus phage combinations, the type, combination and sequence of routes of administration, phage dose and endotoxin level, dosing frequency and duration, and concomitant or sequential antibiotic administration.

Phage therapy can be delivered *via* different routes (including i.v., inhaled, direct topical and oral) according to the underlying infection. *I.v.* administration results in the systemic distribution of phages to other organs, however at reduced titres [100], due to their rapid clearance by the reticulo–endothelial system and inactivation by innate and adaptive immune defences [62, 101].

Pre-clinical data suggest a variable penetration of phages in the lung following systemic administration, compared to more direct delivery through inhalation or intranasal instillation [101–103].

Formal pharmacokinetic studies are lacking in humans, but high titres of active phages have been detected in patients’ serum within a few minutes and up to 12 h after *i.v.* administration [26, 29, 68, 104].

Evidence of lung distribution following *i.v.* phage treatment was provided by ASLAM et al. [26]; active phages were detected in the bronchoalveolar lavage (BAL) of a patient 4 days after *i.v.* therapy with cocktail AB-PA01.

For the management of respiratory infections, given the favourable safety profile of phage therapy based on published data, *i.v.* administration can be proposed for the treatment of severe cases of respiratory sepsis with or without suspected bacteraemia. Direct pulmonary administration carries the advantages of a higher quantity of phages in the lung and immediate contact with the target pathogen [100, 105]. Pulmonary delivery using direct instillation or nebulisation of liquid formulations [59, 100, 105] and dry powder inhalation [106] has been shown to be effective *in vivo*, but limitations include the risk of loss of phage viability during the nebulisation or lyophilisation process and the potentially uneven lung deposition of phage particles [107]. In humans, nebulisation of liquid phage formulations is becoming established and incorporated into clinical trials, proposed mostly for the management of chronic recalcitrant respiratory infections. Pharmacokinetic data in human respiratory samples are scarce [24]. A study of serial BALs demonstrated a progressive increase in lung phage titres following treatment with incremental doses of inhaled phage therapy for CRAB pneumonia but did not assess the distribution of phages across the respiratory tract after inhalation.

In practice, thorough testing of the stability of the phage preparation with the nebuliser intended for use is highly recommended due to the risk of phage inactivation during the nebulisation process, influenced by the specific morphology of each phage and the composition of the liquid suspension, temperature, humidity and nebuliser type [108, 109]. The concept of dry powder phage delivery is an attractive option as it can provide long-term storage stability without requiring a cold supply chain [110]. This potentially low cost and scalable process would be particularly useful in resource-poor settings but has not been tested in human subjects yet.

In conclusion, the optimal dosing regimens remain unknown and most likely depend on several factors, such as the phage product(s) used, the concomitant antibiotic therapy, the route of administration and the underlying infection, to name a few. Dedicated pharmacokinetic studies and efforts to systematically collect and publish such information, notably in unsuccessful cases, are required to improve our understanding of these parameters and design future clinical trials. The key considerations regarding clinical applications of phage therapy are summarised in table 2.
Pre-defined versus personalised phage combinations

So far, clinical trials in phage therapy have employed pre-defined combinations of phages [5, 6, 8, 29]. Pre-defined combinations are readily available “off-the-shelf”, often with a broad host range, offering a significant advantage for severe infections when rapid access to effective treatment is essential. However, the utility of pre-defined phage products will eventually be limited by the very nature of phages and their narrow host specificity, as resistance to phages is essentially inevitable. Pre-existing or de novo phage resistance was discovered in a significant number of patients in previous “unsuccessful” clinical trials using pre-defined phage cocktails [5–8, 29]. Meanwhile, a post hoc analysis of the negative PHAGOBURN trial [5] demonstrated a clinical benefit in the subset of patients with P. aeruginosa strains susceptible to the phages used. A personalised approach therefore seems more appropriate for phage therapy, where phages are selected on demand and before administration, based on in vitro efficacy against the isolated bacterial strain. This strategy and the monitoring of bacterial phage susceptibility during treatment allows for adjustment of phage cocktail compositions, if necessary, i.e. if resistance develops to one or more of the phages present in the cocktail used.

Phage bactericidal activity is assessed in vitro (phage susceptibility testing), commonly using the double-layer agar method, which involves measuring the efficiency of plating [111] and the turbidity assay [112]. It is important to note, however, that these methods are not yet standardised nor routinely incorporated in microbiology laboratories. Well-defined interpretation criteria of in vitro phage activity against the targeted pathogen are lacking and correlation between in vitro activity and clinical outcome is not validated. The emergence of phage-resistant isolates during or after phage therapy has not always been accompanied by treatment failure [17, 20], potentially because of associated beneficial genetic trade-offs [113]. Conversely, evidence of in vitro bactericidal activity does not always translate to clinical benefit. The development of rapid, reliable and widely available phage susceptibility testing is a prerequisite for the establishment of personalised phage therapy.

Although the benefits of personalised phage therapy are clear, on-demand phage product manufacturing is a time- and resource-intensive procedure, financially problematic on a larger scale, and probably more attractive for academic institutions focused on small-scale production than larger volume manufacturing by biopharmaceutical companies [114, 115]. One of the main drawbacks of personalised therapies is that

### Table 2: Key considerations regarding clinical applications of phage therapy

| Phages destined for clinical application | Effective against target pathogen |
|-----------------------------------------|----------------------------------|
|                                         | Well characterised (WGS), without antibiotic resistance genes and virulence factors |
|                                         | Non-lysogenic/transducing |
|                                         | Propagated on well-characterised bacterial strains |

| Phage preparations | Ideally produced according to GMP standards |
|--------------------|----------------------------------|
|                    | Sterile and free from nonproduct phages |
|                    | Endotoxin level <5 EU·kg·h⁻² for i.v. injections |
|                    | Ensured stability along production line |

| Candidate patient selection | Bacterial infections refractory to antibiotics |
|-----------------------------|----------------------------------|
|                            | Allergy/intolerance to antibiotics |
|                            | Progressive loss of function of involved organ |
|                            | Discussion with the patient regarding the experimental nature of phage therapy and the potential risks and benefits |

| Route of administration | Type and severity of infection |
|-------------------------|--------------------------------|
|                         | Inhaled administration: stability testing with nebuliser |

| Phage concentration | Highest safe and tolerated dose below endotoxin limits |
|--------------------|--------------------------------------------------------|

| Duration of therapy | Usually guided by clinical course |
|--------------------|----------------------------------|

| Antibiotic therapy | Usually maintained in compassionate use |
|--------------------|----------------------------------|
|                    | Consider testing for phage-antibiotic synergy/antagonism |

| Monitoring | Close clinical and laboratory monitoring |
|-----------|----------------------------------|
|           | Systematic documentation of adverse events |
|           | Serial phage susceptibility testing of bacterial isolates (emergence of phage resistance) |
|           | Consider phage neutralisation assay in case of no improvement |
|           | Consider phage pharmacokinetics to guide dosing |

EU: endotoxin units; GMP: Good Manufacturing Practices; i.v.: intravenous; WGS: whole-genome sequencing.
existing pharmaceutical legislation is insufficiently adapted to the regulation of dynamic personalised therapies with varying compositions.

Notwithstanding their “living” nature, phages are currently classified as “medicinal products” by numerous regulatory authorities. As such, they must be manufactured in accordance with GMP guidelines, which ensure that medicinal products are produced and controlled according to quality standards appropriate for their intended use and do not put patients at risk due to insufficient quality or efficacy [116]. In the US, for each case of phage therapy, a complex single-use investigational new drug application needs to be filed and approved by the FDA, in a process that can last several weeks to months. Similarly, France established a specific scientific committee for phage therapy within the national regulatory authority (French National Agency for Medicines and Health Products Safety) that examines and authorises individual phage therapy requests. The process appears more straightforward in Belgium, where legislation allows for “magistral” phage preparations, manufactured by a pharmacist in a certified laboratory according to a pre-specified monograph approved by the Pharmacopeia Commission. Phage therapy is subsequently administered under the direct responsibility of physicians and pharmacists (see supplementary table S1).

An alternative approach could be the regulatory approval of a phage product pipeline, encompassing all procedures related to phage isolation, identification, banking, amplification, purification and preparation of the final phage product, manufactured according to GMP standards. Such a process could be adopted by academic institutions and private partners, allowing the continuous enrichment of phage collections, provided that adherence to the approved pipeline is guaranteed, and would significantly shorten the delays associated with the approval of single-use applications for individual patients that, not infrequently, require urgent treatment.

**Resistance to phages**

Phage resistance can be innate in clones harbouring anti-phage defence systems, such as restriction-modification enzymes [117], clustered regularly interspaced palindromic repeat-associated protein (CRISPR)–Cas systems [118], the recently discovered widespread bacterial exclusion system (BREX) [119] and defence island systems associated with restriction–modification [120]. Besides obvious reasons such as the lack of genes coding phage receptors on the bacterial genome, these systems are examples of bacterial genetic factors that determine phage specificity and partly explain why a given phage is systematically active on a limited number of strains of the bacterial species it targets. A personalised approach in which the phage susceptibility of the pathogenic strain is tested before treatment enables the initial avoidance of innate resistance by selecting only active phages, for instance phages that have evolved their own strategies to overcome these bacterial defence systems, such as anti-CRISPR [121] and anti-BREX phage mimic proteins [122].

However, phage resistance can be acquired de novo during phage treatment [123] by point mutations, insertion of mobile genetic elements and genomic deletion. Although such phage-resistant clones seem to emerge rapidly in vitro, they are often associated with reduced infectivity [124] and inconsistently present in vivo [125]. Ultimately, even a personalised approach with serial testing of bacterial susceptibility will most likely not escape the emergence of bacterial resistance to phages during treatment.

TORRES-BARCELÒ et al. [126] recently proposed two complementary approaches to mitigate the selection of bacterial phage resistance: First, evolved phage resistance is minimised using elevated doses of highly efficient lytic phages to rapidly reduce bacterial numbers. Second, phage resistance towards therapeutically beneficial outcomes is directly developed through phage-steering, by employing multiple phage mixtures designed to act synergistically and target systems that are essential for bacterial survival and/or infectivity. The utilisation of experimentally evolved pre-adapted phages, recovered after in vitro co-evolution with their bacterial hosts through so-called phage training, enables the suppression of bacterial growth and delays the development of phage resistance [28, 127].

Phage-steering strategies take advantage of the selective evolutionary pressures imposed by phages on bacterial hosts, leading to beneficial genetic adjustments towards reduced virulence [128], antibiotic re-sensitisation [77, 113, 129, 130] or increased susceptibility to other phages [131]. CHAN et al. [113] demonstrated that OMKO1, a lytic phage of *P. aeruginosa*, forces a genetic trade-off between phage resistance and antibiotic sensitivity by using the outer membrane porin M of the multidrug efflux systems MexAB-OprM and MexXY-OprM as a receptor-binding site. Infection with OMKO1 induced mutations of the gene encoding OprM, which caused a deficiency of the drug efflux pumps and loss of the ability to eliminate antibiotics. As a result, a single dose of phage OMKO1 and ceftazidime effectively treated a chronic aortic graft infection with *P. aeruginosa* with no evidence of recurrence [77]. Recently,
Rodriguez-Gonzalez et al. [132] simulated phage and antibiotic treatments of *P. aeruginosa* infection with a population dynamics model. The authors observed that combination therapy with phage OMKO1 and antibiotics against *P. aeruginosa* resistant to either treatment alone led to an increased therapeutic efficacy that was further enhanced in the presence of an innate immune response [132]. Although the phage-steering approach is potentially attractive, it requires an in-depth understanding of phage–bacteria–host dynamics before it can be used as a therapeutic tool. Mathematical modelling and the use of artificial intelligence may prove helpful in exploring such complex relationships.

### Points for clinical practice and questions for future research

- Phage therapy is a promising addition to the existing armamentarium against AMR.
- Clinical experience suggests a satisfactory safety profile, across all routes of administration, but data on efficacy based on controlled trials are lacking.
- Translational studies are required to explore the complex interactions between phages, bacteria, antibiotic resistance, the immune system and the microbiota.
- Future clinical trials need to be purposefully designed with appropriate endpoints to fully explore the potential of phage therapy.

### Conclusion

Recent evidence from clinical studies corroborates the potential of phage therapy for treating pulmonary infections with bacteria extensively resistant to antibiotics. Although an increasing body of clinical experience reinforces the safety of phage therapy, it remains an experimental treatment until future RCTs unambiguously demonstrate its efficacy. In addition, further pre-clinical studies are required to improve our understanding of how phages influence antibiotic resistance, interact with the immune system and modulate the microbiota. Furthermore, detailed pharmacokinetic–pharmacodynamic studies of different routes of administration are necessary to develop future treatment protocols. Future clinical trials will need to be purposefully designed with appropriate end-points that are tailored towards a personalised and dynamic “living” therapy. Such conditions are the key to unfold the full potential of phage therapy for the treatment of antibiotic-resistant bacterial infections.

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