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

Georgia Mitropoulou [1,2,10], Angela Koutsokera[1,2,10], Chantal Csajka [3,4,5], Sylvain Blanchon [2,6], Alain Sauty[2,7], Jean-Francois Brunet[2,8], Christophe von Garnier[1,2], Grégory Resch [2,3,4,11] and Benoit Guery[2,9,11]

[1]Division of Pulmonology, Department of Medicine, Lausanne University Hospital, Lausanne, Switzerland. [2]University of Lausanne, Lausanne, Switzerland. [3]Centre for Research and Innovation in Clinical Pharmaceutical Sciences, Lausanne University Hospital, Lausanne, Switzerland. [4]Institute of Pharmaceutical Sciences of Western Switzerland, University of Geneva, University of Lausanne, Geneva, Switzerland. [5]School of Pharmaceutical Sciences, University of Geneva, Geneva, Switzerland. [6]Paediatric Pulmonology and Cystic Fibrosis Unit, Division of Paediatrics, Department of Woman-Mother-Child, Lausanne University Hospital, Lausanne, Switzerland. [7]Division of Pulmonology, Neuchâtel Hospital Network, Neuchâtel, Switzerland. [8]Cell Production Centre, Dept of Interdisciplinary Centres, Lausanne University Hospital, Lausanne, Switzerland. [9]Service of Infectious Diseases, Department of Medicine, Lausanne University Hospital, Lausanne, Switzerland. [10]Shared first authorship. [11]Shared last authorship.

Corresponding author: Georgia Mitropoulou (geo.mitropoulou@gmail.com)

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 was treated with a 4-week intravenous (*i.v.*) course of AB-PA01 for MDR *P. aeruginosa* infection. The patient had developed progressive renal failure secondary to antibiotic toxicity after prolonged antimicrobial therapy. Phage therapy resulted in the absence of *P. aeruginosa* growth for 60 days after treatment completion. Although the targeted pathogen was not eradicated, subsequent strains presented improved antibiotic resistance profiles. Recently, NICK *et al.* [25] used a combination of phages that had been genetically engineered to render them lytic for the treatment of a CF patient with antibiotic-refractory *Mycobacterium abcessus* lung disease who had been declined for lung transplantation. Following year-long *i.v.* phage therapy, *M. abcessus* was eradicated and the patient underwent lung transplantation, with an uncomplicated post-transplant course.

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

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

Author [ref.]	Indication	Phage(s) used	Treatment modalities	Duration of phage therapy	Concomitant antibiotics	Outcome
Maddocks et al. [16]	P. aeruginosa VAP	AB-PA01[#]	1×10^9 PFU·mL^{-1} diluted NaCl 100 mL i.v. q12h, inh 4 mL (undiluted) q12h	7 days	Gentamycin, ciprofloxacine, ceftolozane–tazobactam	Infection resolution, eradication
Law et al. [17]	P. aeruginosa chronic lung infection in CF patient	AB-PA01[#]	4×10^9 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[#]	4×10^9 PFU·mL^{-1} i.v. q6h, inh 4× dose q12h	i.v. 2 weeks, inh 4 weeks	Piperacillin–tazobactam, colistin	Active infection resolution, no eradication
		AB-PA01-m1[#]	5×10^9 PFU·mL^{-1} i.v. q6h, inh 4× dose q12h	4 weeks	Piperacillin–tazobactam, tobramycin, colistin inh	
		Navy phage 1[¶]	1×10^9 PFU·mL^{-1} i.v. q2h, inh q4h	4 weeks		
		Navy phage 1[¶]	1×10^9 PFU·mL^{-1} i.v. q2h, inh q4h	8 weeks	Piperacillin–tazobactam, colistin inh	
		Navy phage 2[¶]	5×10^7 PFU·mL^{-1} i.v. q4h	8 weeks		
Aslam et al. [26]	P. aeruginosa chronic lung infection, post lung transplantation	AB-PA01[#]	4×10^9 PFU·mL^{-1} i.v. q12h	4 weeks	Colistin inh	Infection resolution, no eradication but improved antibiotic susceptibility profile
Aslam et al. [26]	B. dolosa chronic lung infection in CF patient, post lung transplantation	BdPF16phi4281d[+]	5.3×10^6 PFU·mL^{-1}–3.5×10^7 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[#]	3×10^9 PFU·mL^{-1} diluted 50–100 mL NaCl i.v. q12h	3 days	Vancomycin, clindamycin, meropenem, azithromycin	No response (treatment withdrawn)
Rubalskii et al. [27]	S. aureus empyema	Staphylococcus phage CH1[§]	1×10^9 PFU·mL^{-1} 20 mL intrapleural q12h	7 days	Daptomycin	Infection resolution, no eradication
Rubalskii et al. [27]	K. pneumoniae VAP post heart transplantation	KPV811, KPV15[§]	1×10^8 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[f]	3×10^8 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	Ax2CJ45φ2[##]	Dose NS, i.v. infusion over 1 h, q24h	2 weeks	Meropenem–vaborbactam, cefiderocol	Infection control, no eradication[###]
Lebeaux et al. [20]	Achromobacter spp. chronic lung infection in CF patient post lung transplantation	APC 1.1, APC 2.1[¶¶]	4×10^{10} PFU·mL^{-1}: 5 mL of tenfold diluted (NaCl) inh q8h	2 days	Imipenem	Active infection resolution, no eradication
		APC 2.1[¶¶]	5×10^9 PFU·mL^{-1}: 30 mL tenfold diluted in each lobe (bronchoscopy), inh 5 mL q8h	16 days		
Tan et al. [24]	Carbapenem-resistant A. baumannii VAP	Ab_SZ3^{++}	Final dose: 5×10^{10} PFU·mL^{-1}, diluted to 5 mL, inh q12h	16 days	Tigecycline, cefoperazone–sulbactam, polymyxin inh	Infection resolution, eradication

TABLE 1 Continued						
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)	φAb124, φAb121[§§]	1×10^8 PFU·mL^{-1} diluted in NaCl 10 mL, inh two doses	Two doses	Various[§§§]	Infection control in three patients, eradication in one patient, two patients died[¶¶]
Rao et al. [21]	Carbapenem-resistant A. baumannii VAP	AbW4932ø1, AbW4878ø1[+]	1×10^9 PFU·mL^{-1}, diluted in NaCl 50 mL infused over 30–60 min, i.v. q12h, 0.1×10^9 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[+++]
Dedrick et al. [22]	M. abcessus disseminated infection in CF patient, post lung transplantation	Muddy, BPsΔ, ZoeJΔ[ff]	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. abcessus chronic lung disease in non-CF bronchiectasis	Muddy, BPsΔ, ZoeJΔ[ff]	1×10^9 PFU·mL^{-1} i.v. q12h	6 months	Imipenem, ethambutol, azithromycin, clofazimine, omadacycline	No response (neutralising antibodies against phages)
Nick et al. [25]	M. abcessus chronic lung disease in CF patient	BPsD33HTH_HRM10, D29_HRM[GD40ff]	10^8–10^9 PFU·mL^{-1} i.v. q12h	>12 months (ongoing)	Omadacycline, bedaquiline, clofazimine, amikacin, tedizolid/linezolid	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. [#]: 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). [¶]: Navy phages 1 and 2 were supplied by the Naval Medical Research Centre, Fort Detrick, USA. [+]: Phage BdPF16phi4281d and phages AbW4932ø1 and AbW4878ø1 were produced by Adaptive Phage Therapeutics (APT), USA. [§]: Staphylococcus phage CH1 and phages KPV811 and KPV15 were provided by the Gabrichevsky Institute, Russia. [f]: The Achromobacter phage combination was prepared by the Eliava Institute using phages provided under collaboration of the Eliava Institute of Bacteriophages, Microbiology and Virology, Tbilisi, Georgia and the Leibniz Institute DSMZ–German Collection of Microorganisms and Cell Cultures, Germany. [##]: Phage Ax2CJ45φ2 was isolated by the Naval Medical Research Centre, USA and was supplied by APT, USA. [¶¶]: Phage combinations APC 1.1 and APC 1.2 were selected from the DSMZ–German Collection of Microorganisms and Cell Cultures, Germany and were prepared at the Laboratory for Bacteriology Research, Ghent University, Belgium and the Laboratory for Molecular and Cellular Technology, Queen Astrid Military Hospital, Brussels, Belgium. [++]: Phage Ab_SZ3 was isolated and prepared at the Shenzhen Institute of Advanced Technology, China. [§§]: Phages φAb124 and φAb121 were isolated from the local library and prepared in GMP facilities at the Zhongshan Hospital of Fudan University, Shanghai, China. [ff]: 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 Recombineering of Electroporated DNA (BRED). [###]: Negative culture at 4 months of follow-up. [¶¶¶]: 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. [+++]: Negative cultures at 6 days after the end of therapy. [§§§]: 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. abcessus* 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. abcessus* lung disease in an 81-year-old immunocompetent patient with non-CF bronchiectasis. *M. abcessus* 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 elexacaftor, 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. Notwithstanding this, 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 inversed, 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.

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.	

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

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-BARCELO *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.

References

1   World Health Organization, The top 10 causes of death. www.who.int/news-room/fact-sheets/detail/the-top-10-causes-of-death. Date last updated: 9 December 2020.
2   Howard-Varona C, Hargreaves KR, Abedon ST, *et al.* Lysogeny in nature: mechanisms, impact and ecology of temperate phages. *ISME J* 2017; 11: 1511–1520.
3   Clokie MR, Millard AD, Letarov AV, *et al.* Phages in nature. *Bacteriophage* 2011; 1: 31–45.
4   Abedon ST. Phage therapy of pulmonary infections. *Bacteriophage* 2015; 5: e1020260.
5   Jault P, Leclerc T, Jennes S, *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* 2019; 19: 35–45.
6   Sarker SA, Sultana S, Reuteler G, *et al.* Oral phage therapy of acute bacterial diarrhea with two coliphage preparations: a randomised trial in children from Bangladesh. *EBioMedicine* 2016; 4: 124–137.

7   Wright A, Hawkins CH, Anggard EE, *et al.* 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* 2009; 34: 349–357.

8   Leitner L, Ujmajuridze A, Chanishvili N, *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* 2021; 21: 427–436.

9   ClinicalTrials.gov. Bacteriophage therapy in patients with urinary tract infections. NCT04287478. https://clinicaltrials.gov/ct2/show/study/NCT04287478 Date last accessed: 27 June 2022. Date last updated: 7 December 2021.

10   ClinicalTrials.gov. Ph 1/2 study evaluating safety and tolerability of inhaled AP-PA02 in subjects with chronic *Pseudomonas aeruginosa* lung infections and cystic fibrosis (SWARM-Pa). NCT04596319. https://clinicaltrials.gov/ct2/show/NCT04596319 Date last accessed: 27 June 2022. Date last updated: 29 November 2021.

11   ClinicalTrials.gov. Bacteriophage therapy in patients with diabetic foot osteomyelitis. NCT05177107. https://clinicaltrials.gov/ct2/show/NCT05177107 Date last accessed: 27 June 2022. Date last updated: 1 August 2022.

12   ClinicalTrials.gov. Bacteriophage therapy TP-102 in diabetic foot ulcers (REVERSE). NCT04803708. https://clinicaltrials.gov/ct2/show/NCT04803708 Date last accessed: 27 June 2022. Date last updated: 21 June 2022.

13   ClinicalTrials.gov. Study evaluating safety, tolerability, and efficacy of intravenous AP-SA02 in subjects with *S. Aureus* bacteremia (diSArm). NCT05184764. https://clinicaltrials.gov/ct2/show/NCT05184764 Date last accessed: 27 June 2022. Date last updated: 17 May 2022.

14   ClinicalTrials.gov. CYstic fibrosis bacterioPHage study at Yale (CYPHY). NCT04684641. https://clinicaltrials.gov/ct2/show/NCT04684641;. Date last accessed: 27 June 2022. Date last updated: 8 October 2021.

15   ClinicalTrials.gov. Nebulized bacteriophage therapy in cystic fibrosis patients with chronic *Pseudomonas aeruginosa* pulmonary infection. NCT05010577. https://clinicaltrials.gov/ct2/show/NCT05010577 Date late accessed: 27 June 2022. Date last updated: 30 June 2022.

16   Maddocks S, Fabijan AP, Ho J, *et al.* Bacteriophage therapy of ventilator-associated pneumonia and empyema caused by *Pseudomonas aeruginosa*. *Am J Respir Crit Care Med* 2019; 200: 1179–1181.

17   Law N, Logan C, Yung G, *et al.* Successful adjunctive use of bacteriophage therapy for treatment of multidrug-resistant *Pseudomonas aeruginosa* infection in a cystic fibrosis patient. *Infection* 2019; 47: 665–668.

18   Hoyle N, Zhvaniya P, Balarjishvili N, *et al.* Phage therapy against *Achromobacter xylosoxidans* lung infection in a patient with cystic fibrosis: a case report. *Res Microbiol* 2018; 169: 540–542.

19   Gainey AB, Burch AK, Brownstein MJ, *et al.* Combining bacteriophages with cefiderocol and meropenem/vaborbactam to treat a pan-drug resistant *Achromobacter* species infection in a pediatric cystic fibrosis patient. *Pediatr Pulmonol* 2020; 55: 2990–2994.

20   Lebeaux D, Merabishvili M, Caudron E, *et al.* A case of phage therapy against pandrug-resistant *Achromobacter xylosoxidans* in a 12-year-old lung-transplanted cystic fibrosis patient. *Viruses* 2021; 13: 60.

21   Rao S, Betancourt-Garcia M, Kare-Opaneye YO, *et al.* Critically ill patient with multidrug-resistant *Acinetobacter baumannii* respiratory infection successfully treated with intravenous and nebulized bacteriophage therapy. *Antimicrob Agents Chemother* 2021; 66: AAC0082421.

22   Dedrick RM, Guerrero-Bustamante CA, Garlena RA, *et al.* Engineered bacteriophages for treatment of a patient with a disseminated drug-resistant *Mycobacterium abscessus*. *Nat Med* 2019; 25: 730–733.

23   Dedrick RM, Freeman KG, Nguyen JA, *et al.* Potent antibody-mediated neutralization limits bacteriophage treatment of a pulmonary *Mycobacterium abscessus* infection. *Nat Med* 2021; 27: 1357–1361.

24   Tan X, Chen H, Zhang M, *et al.* Clinical experience of personalized phage therapy against carbapenem-resistant *Acinetobacter baumannii* lung infection in a patient with chronic obstructive pulmonary disease. *Front Cell Infect Microbiol* 2021; 11: 631585.

25   Nick JA, Dedrick RM, Gray AL, *et al.* Host and pathogen response to bacteriophage engineered against *Mycobacterium abscessus* lung infection. *Cell* 2022; 185: 1860–1874.e12.

26   Aslam S, Courtwright AM, Koval C, *et al.* Early clinical experience of bacteriophage therapy in 3 lung transplant recipients. *Am J Transplant* 2019; 19: 2631–2639.

27   Rubalskii E, Ruemke S, Salmoukas C, *et al.* Bacteriophage therapy for critical infections related to cardiothoracic surgery. *Antibiotics* 2020; 9: 232.

28   Wu N, Dai J, Guo M, *et al.* Pre-optimized phage therapy on secondary *Acinetobacter baumannii* infection in four critical COVID-19 patients. *Emerg Microbes Infect* 2021; 10: 612–618.

29   Petrovic Fabijan A, Lin RCY, Ho J, *et al.* Safety of bacteriophage therapy in severe *Staphylococcus aureus* infection. *Nat Microbiol* 2020; 5: 465–472.

30   Rohde C, Resch G, Pirnay JP, *et al.* Expert opinion on three phage therapy related topics: bacterial phage resistance, phage training and prophages in bacterial production strains. *Viruses* 2018; 10: 178.

31   World Medical Association. WMA declaration of Helsinki – ethical principles for medical research involving human subjects. www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects Date last updated: 9 July 2018.

32 Corbellino M, Kieffer N, Kutateladze M, *et al.* Eradication of a multidrug-resistant, carbapenemase-producing *Klebsiella pneumoniae* isolate following oral and intra-rectal therapy with a custom made, lytic bacteriophage preparation. *Clin Infect Dis* 2020; 70: 1998–2001.

33 Heijerman HGM, McKone EF, Downey DG, *et al.* Efficacy and safety of the elexacaftor plus tezacaftor plus ivacaftor combination regimen in people with cystic fibrosis homozygous for the F508del mutation: a double-blind, randomised, phase 3 trial. *Lancet* 2019; 394: 1940–1948.

34 Middleton PG, Mall MA, Drevinek P, *et al.* Elexacaftor-tezacaftor-ivacaftor for cystic fibrosis with a single Phe508del allele. *N Engl J Med* 2019; 381: 1809–1819.

35 Keating D, Marigowda G, Burr L, *et al.* VX-445-tezacaftor-ivacaftor in patients with cystic fibrosis and one or two Phe508del alleles. *N Engl J Med* 2018; 379: 1612–16120.

36 Nichols DP, Paynter AC, Heltshe SL, *et al.* Clinical effectiveness of elexacaftor/tezacaftor/ivacaftor in people with cystic fibrosis: a clinical trial. *Am J Respir Crit Care Med* 2022; 205: 529–539.

37 Liu D, Van Belleghem JD, de Vries CR, *et al.* The safety and toxicity of phage therapy: a review of animal and clinical studies. *Viruses* 2021; 13: 1268.

38 Uyttebroek S, Chen B, Onsea J, *et al.* Safety and efficacy of phage therapy in difficult-to-treat infections: a systematic review. *Lancet Infect Dis* 2022; 22: e208–e220.

39 US Food and Drug Administration. Bacterial endotoxins/pyrogens. https://www.fda.gov/inspections-compliance-enforcement-and-criminal-investigations/inspection-technical-guides/bacterial-endotoxinspyrogens Date last updated: 17 November 2014.

40 Mu A, McDonald D, Jarmusch AK, *et al.* Assessment of the microbiome during bacteriophage therapy in combination with systemic antibiotics to treat a case of staphylococcal device infection. *Microbiome* 2021; 9: 92.

41 Hsu BB, Gibson TE, Yeliseyev V, *et al.* Dynamic modulation of the gut microbiota and metabolome by bacteriophages in a mouse model. *Cell Host Microbe* 2019; 25: 803–714.e5.

42 Dickson RP, Erb-Downward JR, Freeman CM, *et al.* Bacterial topography of the healthy human lower respiratory tract. *mBio* 2017; 8: e02287-16.

43 Dickson RP, Erb-Downward JR, Martinez FJ, *et al.* The microbiome and the respiratory tract. *Annu Rev Physiol* 2016; 78: 481–504.

44 Huang YJ, LiPuma JJ. The microbiome in cystic fibrosis. *Clin Chest Med* 2016; 37: 59–67.

45 Das S, Bernasconi E, Koutsokera A, *et al.* A prevalent and culturable microbiota links ecological balance to clinical stability of the human lung after transplantation. *Nat Commun* 2021; 12: 2126.

46 Federici S, Nobs SP, Elinav E. Phages and their potential to modulate the microbiome and immunity. *Cell Mol Immunol* 2021; 18: 889–904.

47 Myers LC, Lee C, Thompson BT, *et al.* Outcomes of adult patients with septic shock undergoing extracorporeal membrane oxygenation therapy. *Ann Thorac Surg* 2020; 110: 871–877.

48 Kalish LA, Waltz DA, Dovey M, *et al.* Impact of *Burkholderia dolosa* on lung function and survival in cystic fibrosis. *Am J Respir Crit Care Med* 2006; 173: 421–425.

49 Levin BR, Bull JJ. Population and evolutionary dynamics of phage therapy. *Nat Rev Microbiol* 2004; 2: 166–173.

50 Chan BK, Stanley G, Modak M, *et al.* Bacteriophage therapy for infections in CF. *Pediatr Pulmonol* 2021;56: Suppl. 1, S4–S9.

51 Van Belleghem JD, Clement F, Merabishvili M, *et al.* Pro- and anti-inflammatory responses of peripheral blood mononuclear cells induced by *Staphylococcus aureus* and *Pseudomonas aeruginosa* phages. *Sci Rep* 2017; 7: 8004.

52 Majewska J, Kazmierczak Z, Lahutta K, *et al.* Induction of phage-specific antibodies by two therapeutic staphylococcal bacteriophages administered *per os*. *Front Immunol* 2019; 10: 2607.

53 Kazmierczak Z, Majewska J, Miernikiewicz P, *et al.* Immune response to therapeutic staphylococcal bacteriophages in mammals: kinetics of induction, immunogenic structural proteins, natural and induced antibodies. *Front Immunol* 2021; 12: 639570.

54 Little JS, Dedrick RM, Freeman KG, *et al.* Bacteriophage treatment of disseminated cutaneous *Mycobacterium chelonae* infection. *Nat Commun* 2022; 13: 2313.

55 Van Belleghem JD, Dabrowska K, Vaneechoutte M, *et al.* Interactions between bacteriophage, bacteria, and the mammalian immune system. *Viruses* 2018; 11: 10.

56 Danis-Wlodarczyk K, Dabrowska K, Abedon ST. Phage therapy: the pharmacology of antibacterial viruses. *Curr Issues Mol Biol* 2021; 40: 81–164.

57 Chang RYK, Chen K, Wang J, *et al.* Proof-of-principle study in a murine lung infection model of antipseudomonal activity of phage PEV20 in a dry-powder formulation. *Antimicrob Agents Chemother* 2018; 62: e01714–e01717.

58 Lin Y, Quan D, Chang RYK, *et al.* Synergistic activity of phage PEV20-ciprofloxacin combination powder formulation-a proof-of-principle study in a *P. aeruginosa* lung infection model. *Eur J Pharm Biopharm* 2021; 158: 166–171.

59   Chang RYK, Chow MYT, Wang Y, *et al.* The effects of different doses of inhaled bacteriophage therapy for *Pseudomonas aeruginosa* pulmonary infections in mice. *Clin Microbiol Infect* 2022; 28: 983–989.

60   Tiwari BR, Kim S, Rahman M, *et al.* Antibacterial efficacy of lytic *Pseudomonas* bacteriophage in normal and neutropenic mice models. *J Microbiol* 2011; 49: 994–999.

61   Roach DR, Leung CY, Henry M, *et al.* Synergy between the host immune system and bacteriophage is essential for successful phage therapy against an acute respiratory pathogen. *Cell Host Microbe* 2017; 22: 38–47.e4.

62   Lusiak-Szelachowska M, Zaczek M, Weber-Dabrowska B, *et al.* Phage neutralization by sera of patients receiving phage therapy. *Viral Immunol* 2014; 27: 295–304.

63   Letkiewicz S, Lusiak-Szelachowska M, Miedzybrodzki R, *et al.* Low immunogenicity of intravesical phage therapy for urogenitary tract infections. *Antibiotics* 2021; 10: 627.

64   Archana A, Patel PS, Kumar R, *et al.* Neutralizing antibody response against subcutaneously injected bacteriophages in rabbit model. *Virusdisease* 2021; 32: 38–45.

65   Kucharewicz-Krukowska A, Slopek S. Immunogenic effect of bacteriophage in patients subjected to phage therapy. *Arch Immunol Ther Exp* 1987; 35: 553–561.

66   Dabrowska K, Miernikiewicz P, Piotrowicz A, *et al.* Immunogenicity studies of proteins forming the T4 phage head surface. *J Virol* 2014; 88: 12551–12557.

67   Zaczek M, Lusiak-Szelachowska M, Jonczyk-Matysiak E, *et al.* Antibody production in response to staphylococcal MS-1 phage cocktail in patients undergoing phage therapy. *Front Microbiol* 2016; 7: 1681.

68   Ramirez-Sanchez C, Gonzales F, Buckley M, *et al.* Successful treatment of *Staphylococcus aureus* prosthetic joint infection with bacteriophage therapy. *Viruses* 2021; 13: 1182.

69   Aslam S, Lampley E, Wooten D, *et al.* Lessons learned from the first 10 consecutive cases of intravenous bacteriophage therapy to treat multidrug-resistant bacterial infections at a single center in the United States. *Open Forum Infect Dis* 2020; 7: ofaa389.

70   Dedrick RM, Aull HG, Jacobs-Sera D, *et al.* The prophage and plasmid mobilome as a likely driver of *Mycobacterium abscessus* diversity. *mBio* 2021; 12: e03441-20.

71   Burmeister AR, Turner PE. Trading-off and trading-up in the world of bacteria–phage evolution. *Curr Biol* 2020; 30: R1120–R1124.

72   Weinbauer MG. Ecology of prokaryotic viruses. *FEMS Microbiol Rev* 2004; 28: 127–181.

73   Moghadam MT, Amirmozafari N, Shariati A, *et al.* How phages overcome the challenges of drug resistant bacteria in clinical infections. *Infect Drug Resist* 2020; 13: 45–61.

74   Mousavi SM, Babakhani S, Moradi L, *et al.* Bacteriophage as a novel therapeutic weapon for killing colistin-resistant multi-drug-resistant and extensively drug-resistant Gram-negative bacteria. *Curr Microbiol* 2021; 78: 4023–4036.

75   World Health Organization. 2020 Antibacterial Agents in Clinical and Preclinical Development: an Overview and Analysis. Geneva, World Health Organization, 2021.

76   Schooley RT, Biswas B, Gill JJ, *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* 2017; 61: e00954-17.

77   Chan BK, Turner PE, Kim S, *et al.* Phage treatment of an aortic graft infected with *Pseudomonas aeruginosa*. *Evol Med Public Health* 2018; 2018: 60–66.

78   Kuipers S, Ruth MM, Mientjes M, *et al.* A Dutch case report of successful treatment of chronic relapsing urinary tract infection with bacteriophages in a renal transplant patient. *Antimicrob Agents Chemother* 2019; 64: e01281-19.

79   Bao J, Wu N, Zeng Y, *et al.* Non-active antibiotic and bacteriophage synergism to successfully treat recurrent urinary tract infection caused by extensively drug-resistant *Klebsiella pneumoniae*. *Emerg Microbes Infect* 2020; 9: 771–774.

80   Comeau AM, Tetart F, Trojet SN, *et al.* Phage-antibiotic synergy (PAS): beta-lactam and quinolone antibiotics stimulate virulent phage growth. *PLoS One* 2007; 2: e799.

81   Kamal F, Dennis JJ. *Burkholderia cepacia* complex phage-antibiotic synergy (PAS): antibiotics stimulate lytic phage activity. *Appl Environ Microbiol* 2015; 81: 1132–1138.

82   Uchiyama J, Shigehisa R, Nasukawa T, *et al.* Piperacillin and ceftazidime produce the strongest synergistic phage-antibiotic effect in *Pseudomonas aeruginosa*. *Arch Virol* 2018; 163: 1941–1948.

83   Kim M, Jo Y, Hwang YJ, *et al.* Phage-antibiotic synergy *via* delayed lysis. *Appl Environ Microbiol* 2018; 84: e02085-18.

84   Luscher A, Simonin J, Falconnet L, *et al.* Combined bacteriophage and antibiotic treatment prevents *Pseudomonas aeruginosa* infection of wild type and *cftr*- epithelial cells. *Front Microbiol* 2020; 11: 1947.

85   Yilmaz C, Colak M, Yilmaz BC, *et al.* Bacteriophage therapy in implant-related infections: an experimental study. *J Bone Joint Surg Am* 2013; 95: 117–125.

86 Regeimbal JM, Jacobs AC, Corey BW, *et al.* Personalized therapeutic cocktail of wild environmental phages rescues mice from *Acinetobacter baumannii* wound infections. *Antimicrob Agents Chemother* 2016; 60: 5806–5816.

87 Tagliaferri TL, Jansen M, Horz HP. Fighting pathogenic bacteria on two fronts: phages and antibiotics as combined strategy. *Front Cell Infect Microbiol* 2019; 9: 22.

88 Lin Y, Chang RYK, Britton WJ, *et al.* Synergy of nebulized phage PEV20 and ciprofloxacin combination against *Pseudomonas aeruginosa*. *Int J Pharm* 2018; 551: 158–165.

89 Flemming HC, Wingender J, Szewzyk U, *et al.* Biofilms: an emergent form of bacterial life. *Nat Rev Microbiol* 2016; 14: 563–575.

90 Glonti T, Chanishvili N, Taylor PW. Bacteriophage-derived enzyme that depolymerizes the alginic acid capsule associated with cystic fibrosis isolates of *Pseudomonas aeruginosa*. *J Appl Microbiol* 2010; 108: 695–702.

91 Henriksen K, Rorbo N, Rybtke ML, *et al.* P. *aeruginosa* flow-cell biofilms are enhanced by repeated phage treatments but can be eradicated by phage-ciprofloxacin combination. *Pathog Dis* 2019; 77: 104834.

92 Oliveira VC, Steixner S, Nascimento CD, *et al.* Expression of virulence factors by *Pseudomonas aeruginosa* biofilm after bacteriophage infection. *Microb Pathog* 2021; 154: ftz011.

93 Chaudhry WN, Concepcion-Acevedo J, Park T, *et al.* Synergy and order effects of antibiotics and phages in killing *Pseudomonas aeruginosa* biofilms. *PLoS One* 2017; 12: e0168615.

94 Chang RYK, Das T, Manos J, *et al.* Bacteriophage PEV20 and ciprofloxacin combination treatment enhances removal of *Pseudomonas aeruginosa* biofilm isolated from cystic fibrosis and wound patients. *AAPS J* 2019; 21: 49.

95 Tkhilaishvili T, Lombardi L, Klatt AB, *et al.* Bacteriophage Sb-1 enhances antibiotic activity against biofilm, degrades exopolysaccharide matrix and targets persisters of *Staphylococcus aureus*. *Int J Antimicrob Agents* 2018; 52: 842–853.

96 Latka A, Drulis-Kawa Z. Advantages and limitations of microtiter biofilm assays in the model of antibiofilm activity of *Klebsiella* phage KP34 and its depolymerase. *Sci Rep* 2020; 10: 20338.

97 Kumaran D, Taha M, Yi Q, *et al.* Does treatment order matter? Investigating the ability of bacteriophage to augment antibiotic activity against *Staphylococcus aureus* biofilms. *Front Microbiol* 2018; 9: 127.

98 Valente LG, Federer L, Iten M, *et al.* Searching for synergy: combining systemic daptomycin treatment with localised phage therapy for the treatment of experimental pneumonia due to MRSA. *BMC Res Notes* 2021; 14: 381.

99 Suh GA, Lodise TP, Tamma PD, *et al.* Considerations for the use of phage therapy in clinical practice. *Antimicrob Agents Chemother* 2022; 66: e0207121.

100 Liu KY, Yang WH, Dong XK, *et al.* Inhalation study of mycobacteriophage D29 aerosol for mice by endotracheal route and nose-only exposure. *J Aerosol Med Pulm Drug Deliv* 2016; 29: 393–405.

101 Dabrowska K, Abedon ST. Pharmacologically aware phage therapy: pharmacodynamic and pharmacokinetic obstacles to phage antibacterial action in animal and human bodies. *Microbiol Mol Biol Rev* 2019; 83: e00012-19.

102 Prazak J, Valente L, Iten M, *et al.* Benefits of aerosolized phages for the treatment of pneumonia due to methicillin-resistant *Staphylococcus aureus* (MRSA): an experimental study in rats. *J Infect Dis* 2021; 225: 1452–1459.

103 Prazak J, Iten M, Cameron DR, *et al.* Bacteriophages improve outcomes in experimental *Staphylococcus aureus* ventilator-associated pneumonia. *Am J Respir Crit Care Med* 2019; 200: 1126–1133.

104 LaVergne S, Hamilton T, Biswas B, *et al.* Phage therapy for a multidrug-resistant *Acinetobacter baumannii* craniectomy site infection. *Open Forum Infect Dis* 2018; 5: ofy064.

105 Chow MYT, Chang RYK, Li M, *et al.* Pharmacokinetics and time-kill study of inhaled antipseudomonal bacteriophage therapy in mice. *Antimicrob Agents Chemother* 2020; 65: e01470-20.

106 Li M, Chang RYK, Lin Y, *et al.* Phage cocktail powder for *Pseudomonas aeruginosa* respiratory infections. *Int J Pharm* 2021; 596: 120200.

107 Wang X, Xie Z, Zhao J, *et al.* Prospects of inhaled phage therapy for combatting pulmonary infections. *Front Cell Infect Microbiol* 2021; 11: 758392.

108 Leung SSY, Carrigy NB, Vehring R, *et al.* Jet nebulization of bacteriophages with different tail morphologies – structural effects. *Int J Pharm* 2019; 554: 322–326.

109 Carrigy NB, Chang RY, Leung SSY, *et al.* Anti-tuberculosis bacteriophage D29 delivery with a vibrating mesh nebulizer, jet nebulizer, and soft mist inhaler. *Pharm Res* 2017; 34: 2084–2096.

110 Malik DJ. Bacteriophage encapsulation using spray drying for phage therapy. *Curr Issues Mol Biol* 2021; 40: 303–316.

111 Kutter E. Phage host range and efficiency of plating. *Methods Mol Biol* 2009; 501: 141–149.

112 Rajnovic D, Munoz-Berbel X, Mas J. Fast phage detection and quantification: an optical density-based approach. *PLoS One* 2019; 14: e0216292.

113   Chan BK, Sistrom M, Wertz JE, *et al.* Phage selection restores antibiotic sensitivity in MDR *Pseudomonas aeruginosa. Sci Rep* 2016; 6: 26717.

114   Pirnay JP, Ferry T, Resch G. Recent progress towards the implementation of phage therapy in Western medicine. *FEMS Microbiol Rev* 2021; 46: fuab040.

115   Wu N, Zhu T. Potential of therapeutic bacteriophages in nosocomial infection management. *Front Microbiol* 2021; 12: 638094.

116   European Commission. EudraLex – Volume 4 – Good Manufacturing Practice (GMP) guidelines. https://health.ec.europa.eu/medicinal-products/eudralex/eudralex-volume-4_en

117   Arber W. Host-controlled modification of bacteriophage. *Annu Rev Microbiol* 1965; 19: 365–378.

118   Ishino Y, Krupovic M, Forterre P. History of CRISPR-cas from encounter with a mysterious repeated sequence to genome editing technology. *J Bacteriol* 2018; 200: e00580-17.

119   Goldfarb T, Sberro H, Weinstock E, *et al.* BREX is a novel phage resistance system widespread in microbial genomes. *EMBO J* 2015; 34: 169–183.

120   Ofir G, Melamed S, Sberro H, *et al.* DISARM is a widespread bacterial defence system with broad anti-phage activities. *Nat Microbiol* 2018; 3: 90–98.

121   Maxwell KL. Phages fight back: inactivation of the CRISPR-cas bacterial immune system by anti-CRISPR proteins. *PLoS Pathog* 2016; 12: e1005282.

122   Isaev A, Drobiazko A, Sierro N, *et al.* Phage T7 DNA mimic protein Ocr is a potent inhibitor of BREX defence. *Nucleic Acids Res* 2020; 48: 5397–5406.

123   Castledine M, Padfield D, Sierocinski P, *et al.* Parallel evolution *of Pseudomonas aeruginosa* phage resistance and virulence loss in response to phage treatment *in vivo* and *in vitro. Elife* 2022; 11: e73679.

124   Torres-Barcelo C. Phage therapy faces evolutionary challenges. *Viruses* 2018; 10: 323.

125   Oechslin F, Piccardi P, Mancini S, *et al.* Synergistic interaction between phage therapy and antibiotics clears *Pseudomonas aeruginosa* infection in endocarditis and reduces virulence. *J Infect Dis* 2017; 215: 703–712.

126   Torres-Barcelo C, Turner PE, Buckling A. Mitigation of evolved bacterial resistance to phage therapy. *Curr Opin Virol* 2022; 53: 101201.

127   Borin JM, Avrani S, Barrick JE, *et al.* Coevolutionary phage training leads to greater bacterial suppression and delays the evolution of phage resistance. *Proc Natl Acad Sci USA* 2021; 118: e2104592118.

128   Sumrall ET, Shen Y, Keller AP, *et al.* Phage resistance at the cost of virulence: *Listeria monocytogenes* serovar 4b requires galactosylated teichoic acids for InlB-mediated invasion. *PLoS Pathog* 2019; 15: e1008032.

129   Wang S, Yin B, Yu L, *et al.* Overexpression of AmpC promotes bacteriophage lysis of ampicillin-resistant *Escherichia coli. Front Microbiol* 2019; 10: 2973.

130   Koderi Valappil S, Shetty P, Deim Z, *et al.* Survival comes at a cost: a coevolution of phage and its host leads to phage resistance and antibiotic sensitivity of pseudomonas aeruginosa multidrug resistant strains. *Front Microbiol* 2021; 12: 783722.

131   Shah M, Taylor VL, Bona D, *et al.* A phage-encoded anti-activator inhibits quorum sensing in *Pseudomonas aeruginosa. Mol Cell* 2021; 81: 571–583.e6.

132   Rodriguez-Gonzalez RA, Leung CY, Chan BK, *et al.* Quantitative models of phage-antibiotic combination therapy. *mSystems* 2020; 5: e00756-19.