Phage therapy in a lung transplant recipient with cystic fibrosis infected with multidrug-resistant *Burkholderia multivorans*

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Abstract

Background: There is increased interest in bacteriophage (phage) therapy to treat infections caused by antibiotic-resistant bacteria. A lung transplant recipient with cystic fibrosis and *Burkholderia multivorans* infection was treated with inhaled phage therapy for seven days before she died.

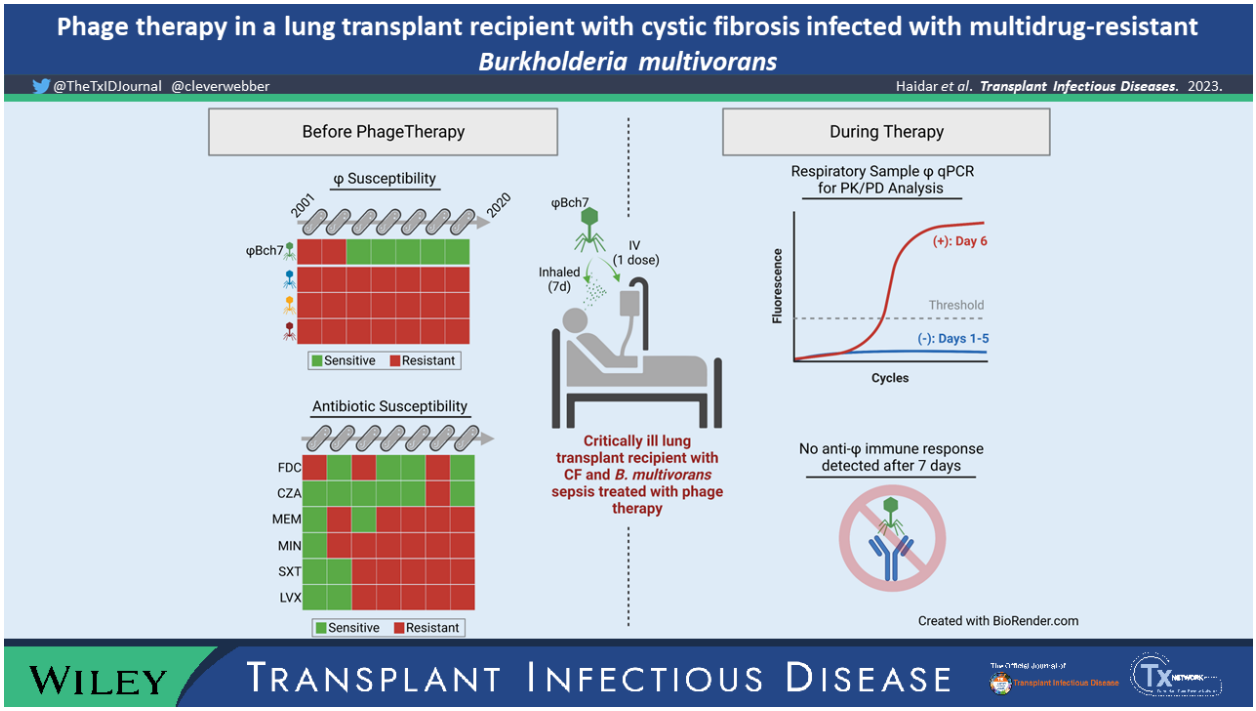
Methods: Phages were given via nebulization through the mechanical ventilation circuit. Remnant respiratory specimens and serum were collected. We quantified phage and bacterial DNA using quantitative PCR, and tested phage neutralization in the presence of patient serum. We performed whole genome sequencing and antibiotic and phage susceptibility testing on 15 *B. multivorans* isolates. Finally, we extracted lipopolysaccharide (LPS) from two isolates and visualized their LPS using gel electrophoresis.

Results: Phage therapy was temporally followed by a temporary improvement in leukocytosis and hemodynamics, followed by worsening leukocytosis on day five, deterioration on day seven, and death on day eight. We detected phage DNA in respiratory samples after six days of nebulized phage therapy. Bacterial DNA in respiratory samples decreased over time, and no serum neutralization was detected. Isolates collected between 2001 and 2020 were closely related but differed in their antibiotic and phage susceptibility profiles. Early isolates were not susceptible to the phage used for therapy, while later isolates, including two isolates collected during phage therapy, were susceptible. Susceptibility to the phage used for therapy was correlated with differences in O-antigen profiles of an early versus a late isolate.

Conclusions: This case of clinical failure of nebulized phage therapy highlights the limitations, unknowns, and challenges of phage therapy for resistant infections.

Graphical Abstract Text

A critically ill lung transplant recipient with CF and B. multivorans sepsis was treated with phage therapy for seven days before they died. We investigated phage and antibiotic susceptibility of serial B. multivorans isolates collected from the patient as well as phage pharmacokinetics and immune responses.



Abbreviations

DNA = deoxyribonucleic acid; PCR = polymerase chain reaction; LAL = Limulus amebocyte lysate; FDA = Food and Drug Administration

Introduction

Individuals with cystic fibrosis (CF) are susceptible to infections with multidrug-resistant (MDR) bacteria[1]. *Burkholderia* species are major pathogens in CF and can cause devastating infections after lung transplantation[2]. *Burkholderia* species can develop extensively drug-resistant phenotypes that require treatment with agents of last resort[3]. Bacteriophage (phage) therapy is a potentially promising treatment for individuals with *Burkholderia* infections[4]. Here, we describe our experience with inhaled phage therapy in a

person with CF and severe *Burkholderia multivorans* sepsis three years after lung transplantation. Although there was no objective evidence of a clinical or microbiologic response, we aimed to describe the patient's clinical trajectory, pharmacokinetics and pharmacodynamics of phage, impact of phage on *Burkholderia* abundance, humoral immune response to phage, the evolution of *B. multivorans* isolates recovered over time, and associated changes in bacterial susceptibility to phage and antibiotics.

Methods

Phage administration

The patient received phage therapy under a compassionate use protocol, with an emergency investigational new drug (eIND) application approval by the Food and Drug Administration and the University of Pittsburgh Internal Review Board (IRB) (eIND #26961; IRB protocol EA20100058). Because of the patient's clinical status, written informed consent was provided by the patient's family. Remnant clinical biological samples from the patient were obtained under an IRB-approved protocol at the University of Pittsburgh (STUDY19110005).

Laboratory experiments

Detailed methods can be found in the **Supplementary Materials**. Briefly, *Burkholderia* isolate 1187 (the most recent clinical isolate available at the time) was used for phage screening, isolation and propagation. A total of 10 phages were tested, one of which demonstrated lytic activity against the patient's *Burkholderia* isolates. Phage Bch7 (named for *B. cepacia* phage from screening well H7) was identified, plaque passaged, amplified, and formulated for therapy. Endotoxin concentration was determined by LAL assay (Hyglos, Bernried, Germany). Phage was formulated at 3.33×10^9 plaque-forming units (PFU), and each dose (total 3 mL per dose) contained 1543 endotoxin units (EU). The formulation was tested for sterility by overnight culture in brain heart infusion and tryptic soy broths, as well as USP71 sterility testing, which confirmed sterility. To determine the pharmacokinetics of phage and the impact of phage on *Burkholderia* abundance, Bch7 and *Burkholderia* DNA were quantified using

qPCR. To determine the host immune response to phage, serum samples collected before (day 0) and 7 days after phage therapy were tested for their ability to neutralize phage activity by mixing phage and serum and determining PFU/mL. Bch7 and 15 longitudinal *Burkholderia* isolates collected over the span of 20 years underwent whole-genome sequencing (Illumina on all isolates and Oxford Nanopore on three isolates). Antibiotic susceptibility testing was performed on bacterial isolates using standard methods established by the Clinical Laboratory Standards Institute (CLSI)[5]. Phage susceptibility testing was performed by determining the titer of Bch7 against each bacterial isolate. LPS was extracted and analyzed as previously described[6].

Statistical analysis

Differences in bacterial abundance and differences in phage neutralization were assessed with two-tailed t-tests. *P*-values < 0.05 denoted statistical significance.

Results

Clinical details

A 32-year-old female with CF and a history of respiratory and sinus tract *B. multivorans* infection since 2001 (**Supplementary Table 1**) underwent a double lung transplant in 2017. Sputum cultures obtained prior to transplant were persistently positive for *B. multivorans*. Her perioperative antimicrobial regimen consisted of levofloxacin, meropenem, minocycline, piperacillin-tazobactam, and trimethoprim-sulfamethoxazole. Explanted lung cultures grew pan-resistant *B. multivorans*, as did several post-transplant bronchoalveolar lavage fluid (BALF) cultures. After transplant, she received approximately four weeks of systemic ceftazidime-avibactam, levofloxacin, meropenem, minocycline, and trimethoprim-sulfamethoxazole, as well as inhaled meropenem. A BALF culture obtained three weeks after transplant did not grow any bacteria. The patient was subsequently discharged home off all systemic anti-*Burkholderia* therapy and only on inhaled meropenem, and in the intervening three years she did well. She was hospitalized three times for respiratory tract infections

caused by *B. multivorans*, all of which responded to 2-4 weeks of combination antimicrobial therapy including ceftazidime-avibactam, levofloxacin, meropenem, minocycline, and piperacillin-tazobactam.

In the summer of 2020, approximately 8 months after her last hospitalization, the patient was hospitalized with respiratory failure and multifocal pneumonia requiring mechanical ventilation. At the time, respiratory tract cultures grew *Pseudomonas aeruginosa, Stenotrophomonas maltophilia*, and *Aspergillus flavus*, but not *B. multivorans;* testing for SARS-CoV-2 and other respiratory viruses was negative. The patient was treated for all three of these pathogens, respiratory status improved, and she was extubated. She developed liver dysfunction, resulting in premature discontinuation of her antifungals. She also developed acute kidney injury as a result of acute tubular necrosis from hypotension and required dialysis. Additionally, she was profoundly debilitated and thus remained in the hospital while participating in rehabilitation. Nonetheless, she appeared to be recovering from her pneumonia.

Unfortunately, approximately 8 weeks after she was admitted (and during the same hospital stay), she developed a new fever to 38.3^{o}C, initially with otherwise stable vital signs and laboratory parameters (oxygen saturation of 100% with a nasal cannula at 1 L/min, heart rate of 103 beats/min, blood pressure of 182/107, and low-to-normal WBC count) **(Supplementary Figure 1**). This fever prompted blood cultures to be sent (collected day -33 relative to the start of phage therapy), which were positive for *Burkholderia multivorans* (**Figure 1A**). Antibiotics used to treat this bacteremia included combinations of trimethoprim-sulfamethoxazole, ceftazidime-avibactam, meropenem, minocycline, cefiderocol, levofloxacin, and inhaled colistin (**Figure 1A**). This treatment temporarily cleared the bacteremia, as blood cultured collected on days -29, -27, and -22 were negative. However, despite the temporary clearance of bacteremia, the patient continued to deteriorate, developing septic shock and requiring intubation on day -29 (**Figure 1A**).

Between this deterioration and the administration of the first phage dose, the patient was afebrile, mechanically ventilated via tracheostomy, and maintaining an oxygen saturation of 90-100% on 40-50% FiO_2. Her systolic and diastolic blood pressures were generally over 90 and 50 mmHg, respectively, and she was ultimately weaned off pressors by day -27. Additionally, between days -35 and -5 prior to initiation of phage therapy, her WBC count was within the normal range, except for a small and transient increase to 12 x 10^9 cells/L on days -8 and -7 (**Supplementary Figure 1**). Despite her stable but critical illness, she continued to have intractable bacteremia (no negative blood cultures between days -17 and -8, **Figure 1A**). Thus, during this time, a phage search was initiated (begun on day -10), after extensive discussions with the patient's family, primary ICU service, and transplant team. The rationale behind the phage search was the persistence of bacteremia despite various antibiotic combinations (**Figure 1A**). A phage with lytic activity was identified, formulated, and shipped from B.K.C.'s laboratory at Yale University to initiate phage therapy ten days after referral.

After a period of relative stability, the patient's leukocytosis began to increase starting day -3 (**Supplementary Figure 1**). On day -2 the patient acutely deteriorated. She remained afebrile but had an oxygen saturation in the low 90's on 60% FiO_2 and required up to 100% FiO_2 to maintain an oxygen saturation of 100%. She was restarted on pressors due to new-onset shock, with systolic and diastolic blood pressures around 80 and 30 mmHg, respectively. Between this deterioration and the first phage dose (day 0), the patient continued to require pressors, and her FiO_2 fluctuated between 70 and 100% to maintain a saturation of 100%.

The patient received inhaled phage Bch7 (day 0) under compassionate use at 3.33x10^9 plaque forming units (PFU) per dose (3mL per dose) three times daily (for a total of 1x10^{10} PFU/day i.e. 9 mL administered per day) via mechanical ventilation circuit using an AirLife jet nebulizer (Vyaire Medical, Mettawa, IL) per usual intensive care unit procedures, in addition to antibiotics. Due to the severity of the patient's illness, we were unable to first test the viability of the phages with the nebulizer used. Initial phage administration was well-tolerated, and no immediate side effects were observed. By day +1, the patient's oxygen

requirements decreased to around 40-50% FiO_2, she was weaned off pressors, and there appeared to be an improvement in her leukocytosis: her white blood cell count, which had become elevated 3 days prior to phage administration, declined from a pre-phage level of 20.7 $x10^9$/L approximately 4 hours before administration to 10.2 $x10^9$/L approximately 12 hours after phage administration, and remained within normal limits for 4 days. (**Supplementary Figure 1**). However, this improvement in ventilation, hemodynamics, and leukocytosis occurred despite no objective evidence of a sustained microbiologic response, as *B. multivorans*-positive respiratory cultures were collected on days +1, +3, +4, +5, and +6 and positive blood cultures were collected on days +1 and +5 (**Figure 1A**).

Unfortunately, on day +5, the patient's leukocytosis began to increase again (**Supplementary Figure 1)**, followed shortly thereafter (days +6-7) by progressively worsening shock requiring pressor support (for systolic/diastolic blood pressure of around 80/30 mmHg) and a renewed need for 100% FiO_2 to maintain and oxygen saturation in the 90s. As outlined in **Figure 1A**, this period of acute deterioration was marked by not only *Burkholderia* bacteremia (day +5) but also candidemia (day +7) and pulmonary aspergillosis (days +3 through +6, with growth of *Aspergillus fumigatus* from respiratory cultures and a serum galactomannan assay result of 1), for which antifungals were restarted. In the setting of refractory hypoxic respiratory failure, refractory shock, disseminated intravascular coagulation, lactic acidosis, and progressive multiorgan dysfunction with worsening liver failure, a meeting was held between the family and members of the care team (ICU, transplant, and transplant infectious diseases) to discuss goals of care.

After extensive counseling, a decision was made to administer a single intravenous dose of phage. Following discussion with the investigational drug pharmacy, 0.5 mL of phage diluted in 100 mL of normal saline was infused over a 3-hour period. Since the phage formulation contained 1543 EU per 3 mL dose, this dosing regimen resulted in the administration of 257.2 EU/hour. The patient's actual weight was 71.5 kg (resulting in an FDA "5 endotoxin unit (EU)/kg/hour" cap of 357.5 EU/hour), and her ideal body weight was 50.5 kg

(resulting in an FDA EU/kg/hour cap of 252.5 EU/hour). Thus, the administered endotoxin content was deemed to be acceptable. The family wished to proceed, and the patient received a single IV dose of phage on day +7. The patient's condition, which had been progressively worsening throughout the day, continued to worsen throughout the evening, with increasing pressor requirements, worsening multiorgan dysfunction and acidosis, and an inability to wean off 100% FiO_2. The family withdrew care around 10 hours after the IV phage dose due to futility, and the patient ultimately expired on day +8. Three sets of blood cultures obtained on day +7 after initiation of phage therapy (one day before death and before administration of the IV phage dose), were negative for *B. multivorans*.

Microbiologic and immune responses to phage therapy

The pharmacokinetics and pharmacodynamics of phage therapy are largely unknown. We collected remnant respiratory samples, including tracheal aspirate and bronchoalveolar lavage fluid, during phage therapy (**Supplementary Table 2**). We quantified Bch7 and *B. multivorans* abundance over time in these samples with quantitative real-time PCR (qPCR). Phage DNA was only detected in the final sample collected after six days (**Figure 1B**). We also found that bacterial abundance decreased following initiation of inhaled phage therapy ($P < 0.05$) (**Figure 1C**). We tested whether phage-neutralizing activity was present in remnant serum samples collected just prior to initiating phage therapy on Day 0 compared to Day 7 (**Figure 1D**). No significant decrease in phage activity was observed compared to the control condition, suggesting that after a week of phage therapy there was no evidence of phage-neutralizing activity in the patient's serum.

Comparative genomics and susceptibility testing

The genome of Bch7 was sequenced and compared with the genomes of other publicly available *Burkholderia* phages (**Figure 2A**). Bch7 was predicted to be a lytic phage due to the absence of identifiable phage integrase and repressor genes. The genome showed modest similarity to other *Myoviridae* phages targeting *Burkholderia* spp., with the *Burkholderia*

ambifaria phage BcepF1 being the closest match. Other phages showing weaker homology included the *Burkholderia gladioli* phage Maja and the *Burkholderia* spp. phage BCSR52. The fact that similar *Myoviridae* phages were repeatedly isolated on different *Burkholderia* species suggests that these phages may exhibit a broad host range, consistent with other *Burkholderia*-targeting phages[2].

We next sought to determine whether Bch7 was active against historic and contemporary *B. multivorans* isolates collected from the patient. We sequenced the genomes of 15 isolates collected over 20 years and compared them with one another (**Figure 2B, Supplementary Tables 1 and 3**). The high similarity of these genomes and their nested phylogenetic relationship confirmed that the patient was initially infected with a single *B. multivorans* strain that evolved over time. A total of 75 single nucleotide polymorphisms (SNPs) were identified (**Supplementary Table 3**). By measuring the accumulation of SNPs over time compared to the earliest isolate, we calculated a mutation rate of approximately 2.7 SNPs/year (**Supplementary Figure 2**). This rate was consistent with prior estimates of *B. multivorans* evolution during long-term infection in people with CF[7].

Susceptibility testing of the 15 sequenced isolates against levofloxacin, trimethoprim-sulfamethoxazole, minocycline, meropenem, ceftazidime-avibactam, and cefiderocol revealed that early isolates collected in 2001 and 2002 were largely antibiotic-susceptible, while isolates collected from 2013 onwards were generally more resistant (**Figure 2B, Supplementary Table 1**). Levofloxacin resistance was associated with a Ser83Arg mutation in DNA gyrase subunit A, while trimethoprim-sulfamethoxazole resistance was associated with a Phe158Ser mutation in dihydrofolate reductase (**Supplementary Table 3**). No single mutation was associated with changes in resistance to minocycline, meropenem, ceftazidime-avibactam, or cefiderocol, suggesting that the resistance mechanisms for these agents in *B. multivorans* are yet to be described.

We tested all 15 isolates for their susceptibility to Bch7 and observed that isolates collected in 2001 and 2002 were resistant to Bch7, while all subsequent isolates—including two isolates collected five days after initiation of phage therapy (isolates 1230 and 1310)—were susceptible (**Figure 2B, 2C**). We sought to identify a putative mechanism underlying this observation. A total of 36 SNPs distinguished the phage-susceptible isolates collected in 2013 and later from the phage-resistant isolates collected in 2001 and 2002. Among these were a Gly399Ser mutation in a D-alanyl-D-alanine carboxypeptidase (DacB) and an Arg187His mutation in a lipopolysaccharide (LPS) export system protein (LptA) (**Supplementary Table 3**). Suspecting that alterations in LPS might affect phage susceptibility[8], we extracted LPS from isolates 1213 (collected in 2001) and 1187 (collected in 2020) and observed differences in their LPS profiles (**Figure 2D**). These data suggest that mutations affecting cell wall and LPS biosynthesis that emerged between 2001 and 2013 may underlie susceptibility to Bch7, and that Bch7 may not use LPS as a receptor for cellular entry.

Discussion

We report the clinical and microbiological characteristics of a lung transplant recipient with CF who received phage therapy for *B. multivorans* sepsis, and in whom phages were not associated with clinical success. While most CF transplant recipients with *B. multivorans* prior to transplant have excellent outcomes[9], this case highlights the potential for late life-shortening recurrence. Despite the negative outcome, we made several noteworthy observations. First, phages were not detected in the patient's respiratory tract by qPCR until day six of administration, suggesting that there was either a lag between initiation of inhaled phage therapy and phage replication or a reduction in phage viability due to the nebulizer used[10] . However, we were unable to test for phage viability prior to administration. Second, even though the patient was infected with genetically related *B. multivorans* isolates for approximately 15 years, we observed a discordance between phage and antimicrobial susceptibilities over time, with older isolates being more antibiotic-susceptible and phage-resistant, and newer isolates being antibiotic-resistant and phage-susceptible. Third, although

initial administration of phage therapy was followed by improvements in the patient's white blood cell count and hemodynamics, we cannot make conclusions about the clinical effectiveness of phage therapy in a critically ill patient with multiple potential causes of fluctuating leukocytosis and hemodynamics. Finally, while the literature demonstrates that phages are generally safe and adverse events are extremely rare[11,12], it is possible that the patient's extreme leukocytosis beginning on day five of phage therapy and rapid decline on day seven may have been associated with phage administration.

To our knowledge this is the third documented case of phage therapy for refractory *Burkholderia* infection after lung transplantation[13,14]. Unfortunately, in all three cases phage therapy was not successful and all three patients died. Whether this was due to delayed administration of phage, the underlying status of the host, or a combination of these and other factors is unknown. In our patient, although there were no immediate adverse events related to phage therapy, we cannot exclude the possibility that clinical worsening and eventual death were related to phage administration, perhaps by facilitating cytokine release[15]. This is especially true for the single IV phage dose, which was given because the patient's clinical status was already at its most dire and had begun deteriorating further. However, since the phage preparation was diluted and administered intravenously over three hours, the endotoxin content was within the recommended range of 5 EU/kg/hour. This, combined with the overall clinical picture, lead us to conclude that the patient most likely expired because of progressive multiorgan failure and shock, and not as a result of the single IV phage dose given. Additionally, a search for lytic phages was only initiated once the patient was critically ill and had persistent *B. multivorans* bacteremia. An active phage was identified, formulated, and administered in ten days, which likely represents the "best case" scenario for providing customized phage treatments. Identifying and formulating individualized phage therapies requires time, and infecting strains can vary in their phage susceptibility profiles throughout the course of disease. Thus, medical centers caring for patients with known *Burkholderia*

colonization might consider routine phage screening of contemporary patient isolates, which could allow for prompt administration of phage therapy when needed.

Barriers to progress in the clinical development of phage therapy include a lack of data regarding the optimal dose and route of therapy as well as a limited understanding of how host immune responses affect clinical efficacy[4]. In our patient, the dose of phage selected was based on discussions with the FDA and to prioritize patient safety. Whether higher doses of phage would have been more efficacious yet still safe is unknown. Furthermore, whether inhaled phage is sufficient in patients with pneumonia, or whether systemic phage therapy is also needed, is unknown. A recent study of phage therapy for *Staphylococcus aureus* pneumonia in rats found that a combination of both intravenous and inhaled phages resulted 91% survival, compared to monotherapy with either route, which was successful in only 50% of animals[16]. Furthermore, a recent systematic review of phage therapy for difficult-to-treat infections identified 16 patients given phage therapy for pulmonary infections[11], who either received inhaled phages alone (n=7), intravenous phages alone (n=4), or combination therapy (n = 4). All cases experienced at least transient response to phages, regardless of route of administration. Another recent study of *P. aeruginosa* pulmonary infection in mice demonstrated that intratracheal phage administration resulted in detection of phages not only in the lung but also the blood, spleen, liver, and kidney[17]. Nonetheless, additional work is needed to define optimal phage dosing guidelines for critically ill patients.

Despite administration of inhaled phage therapy three times daily, we only found evidence of phage amplification in respiratory samples collected on day +6 after initiation of inhaled phage therapy. The reason for this observation remains unknown but may be related to reductions in phage viability during the aerosolization process, which have been documented after nebulization and vary by nebulizer types[4,10,18]. Indeed, the AirLife jet nebulizer we used has been shown to result in significantly reduced mycobacteriophage delivery compared to mesh nebulizers[10]. Because of the gravity of the patient's illness, we were unable to perform stability testing to ensure that the phages were not destroyed by the

nebulizer that was used. Whether use of a different nebulizer would have resulted in more effective phage delivery and thus a more favorable clinical response is not known. We also detected a modest decrease in bacterial abundance in respiratory samples collected during phage therapy, though it is important to note that the qPCR approach we employed likely detected DNA from both live and dead bacteria. Finally, we found no evidence of phage-neutralizing antibodies after one week of therapy. Given prior literature suggesting that a humoral immune response to phage does not develop until several weeks to months after phage administration[19-21], it is unlikely for host immunity to adversely impact phage therapy in the early days of treatment.

This study has several limitations. First, because our findings derive from a single patient who ultimate died, we cannot make any conclusions about the clinical effect of phages; indeed, any improvements, including changes in leukocytosis, oxygenation, and hemodynamics, may have been completely coincidental and unrelated to the administration of phages. Second, because of the gravity of the patient's illness, we were unable to perform a phage simulation experiment to determine the optimal aerosolization method of this phage. Although phages were detected in the airways on day +6, were we unable to test phage viability with the nebulizer that was used, and thus we are unable to make any conclusions about how effective this nebulizer was at delivering phages to the site of infection. Third, although we characterized the changes in phage and antibiotic susceptibility profiles in the patient's longitudinal *Burkholderia* isolates, we did not perform phage-antibiotic synergy testing and thus cannot make any conclusions about whether there were additive, synergistic, or antagonistic effects between the phages and antibiotics administered to the patient. Our future work will focus on exploring these questions further to facilitate the appropriate clinical application of phage therapy.

In conclusion, this study highlights the limitations, unknowns, and challenges associated with phage therapy in patients with critical illness due to *Burkholderia* infection. Taken together, our findings highlight that despite the promise of phage therapy in the

literature[4,11], not all cases will end with a positive clinical outcome. Nonetheless, this study provides a framework in which future "bench to bedside" phage investigations can be approached. Additional studies are needed to define the precise role of phages in patients with *Burkholderia* infections. Such studies would benefit from expanded *Burkholderia* phage libraries, increased understanding of the pharmacokinetic, pharmacodynamic, and immunogenic properties of phages, and more information regarding phage delivery to the site of infection.

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Conflicts of Interest

G.H. is the recipient of research grants from Allovir, Karius, and AstraZeneca. These grants are unrelated to the work described here. G.H. also serves on the scientific advisory boards of Karius and AstraZeneca. All other authors declare no relevant conflicts of interest.

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Figure Legends

Figure 1. Clinical course and microbiologic and immune responses to phage therapy. (A) Clinical course of antibiotic and phage therapy. Organisms that grew in respiratory and blood cultures following detection of initial *B. multivorans* bacteremia are indicated (BCC=*B. multivorans*, NEG=negative). Timing of antibiotic and phage therapy are noted below the timeline. # = Index *B.multivorans*-positive blood culture on Day -33. * = Patient was intubated on Day -29. (B) Bch7 phage abundance in respiratory samples collected after initiation of phage therapy, quantified by real-time PCR. (C) *B. multivorans* abundance in the same respiratory samples, quantified by real-time PCR. (D). Phage neutralization testing of serum collected before and after seven days of phage therapy. Neutralization was tested by incubating phage and diluted patient serum together and then titering the mixture on a

susceptible bacterial isolate. “No serum” = phage incubated in buffer.

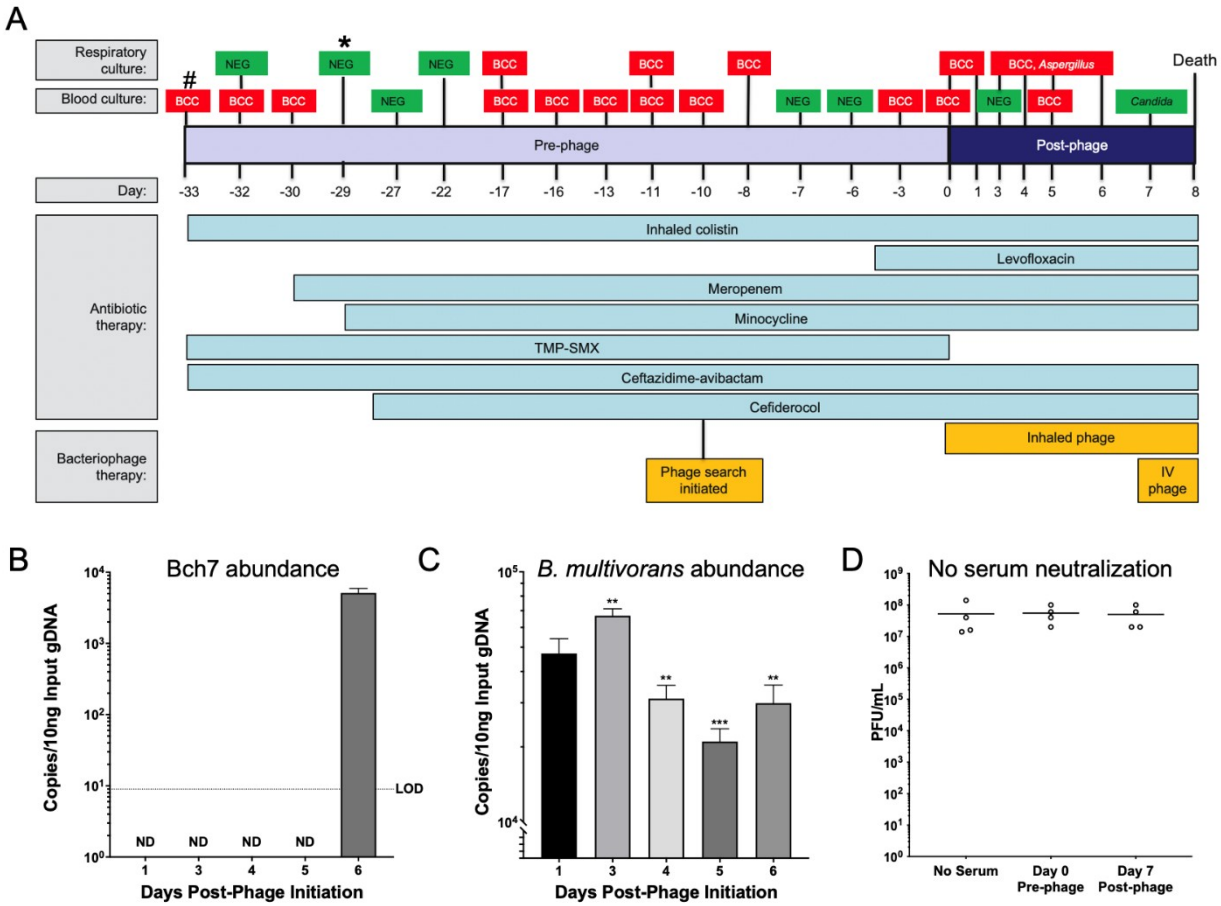

Figure 2. Comparative genomics and susceptibility testing. (A) Whole genome alignment of phage Bch7 with similar *Burkholderia* spp. phages. Red shading shows sequence identity, and genes are colored by functional category. (B) Core genome phylogeny and antimicrobial susceptibilities of *B. multivorans* isolates collected from the patient starting in 2001. The phylogenetic tree was made from a core genome alignment generated by snippy using RAxML with 100 iterations. LVX = levofloxacin, SXT = trimethoprim/sulfamethoxazole, MIN = minocycline, MEM = meropenem, CZA = ceftazidime-avibactam, FDC = cefiderocol, Bch7 = phage Bch7. Asterisks indicate isolates that were collected after initiation of inhaled phage therapy. (C) Phage susceptibility of isolates 1213 (isolated in 2001) and 1187 (isolated in 2020). Serial 10-fold dilutions of phage Bch7 were spotted onto top agar lawns of each bacterial isolate. (D) O-antigen profiles of isolates 1213 and 1187. Lipopolysaccharide (LPS) was extracted from each isolate, separated on a polyacrylamide gel, and then stained with