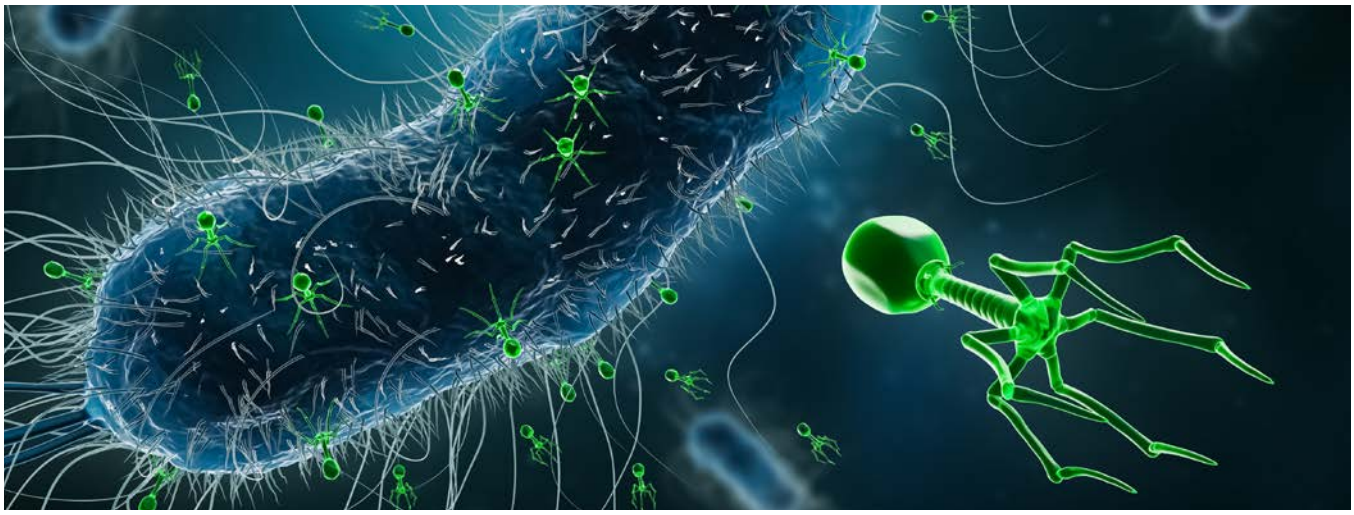

Recommendations and considerations for bacteriophage therapy usage in clinical settings

Phage therapy holds great promise as a new therapeutic option in the face of increasing antimicrobial resistance, but sound clinical data remains a stumbling block to its application. Here, Snehit Satish Mhatre from Eurofins Biopharma Product Testing outlines key considerations to address when designing clinical trials.

THE RISE IN antimicrobial resistance,[1] lack of significant antimicrobial discovery in recent years, and increasing instances of multidrug-resistant (MDR) microorganisms[2] have propelled the interest in bacteriophage (Phage) therapy as a potential new course of treatment. Patients with implantable devices are more prone to biofilm-mediated infections,[3] while other infections such as skin structure infections, chronic lung diseases resulting from respiratory infections, and urinary tract infections (UTIs) can have reoccurrences despite antibiotic treatments. Over the years, phage therapy has consistently evolved and been subjected to multiple clinical trials.[4,5] Phages selectively eliminate the target bacterial host population with no impact on human cells and a much smaller impact on the host commensal bacterial population, which otherwise would be affected by antibiotics. However, one of the most significant challenges concerning phage therapy is the regulations surrounding its clinical usage.

The need for validated and controlled clinical trials is the major hurdle in the clinical approval of phage therapy. Therefore, utmost care should be practiced with an experimental plane and design of phage clinical trials. The clinical trial design for phage therapy is similar to a standard drug clinical trial; however, several factors are unique to phages and thus require special consideration.[6] Given the severe lack of knowledge and solid clinical data, here we make an attempt to provide considerations that would help develop a clinical framework for the testing of phage therapy products.

Infections

Various infections such as respiratory tract infections, UTIs, endovascular infections, gastrointestinal tract infections, osteoarticular infections, and biofilm-born infections can be treated using phage therapy. In general, phage therapy is limited to treating bacterial infections like *Klebsiella pneumoniae, Enterococcus faecalis and faecium, Acinetobacter baumannii, Escherichia coli, Proteus mirabilis, Burkholderia* species, and nontuberculous mycobacteria.[7] However, data is also emerging for treating infections caused by *Aspergillus* species.[8,9]

Antibiotics

A synergistic effect of the phage-antibiotic combination is recommended for MDR- and biofilm-associated cases that do not resolve with antibiotic treatments alone.[10,11] The added benefit of this approach would be to reinstate antimicrobial susceptibility in the target bacterial population. One of the most recommended approaches is testing bacterial isolates before and after phage therapy treatment for antibiotic and phage susceptibility. One can also

perform clinical studies for antibiotics alone and antibiotic-phage treatment to understand the value of phage therapy over antibiotic treatment.

Patient safety

Phage therapy patients should be monitored weekly for renal and liver function and blood counts until robust data is established. Published data on phage therapy has shown no adverse reactions for phages administered orally, locally, or intravenously.[12,13] However, because there is still a dearth of knowledge concerning clinical trials, patients should be monitored closely during clinical trials.

Practical considerations

Two important parameters determine whether a patient is a potential phage candidate. First, knowledge of a qualified bacterial host for identifying phage with lytic capabilities against the isolate. Second, the clinical status of the patient is critical. A terminally ill patient needing therapy within hours or days will not be an ideal candidate for phage therapy. The ideal time from patient request to drug administration ranges between 28 and 386 days.[10]

Patient immunological parameters

Patient immunological response should be tested for phage neutralising antibodies, especially during prolonged phage administration. The complex interplay between the human immune system, phages and bacteria could affect phage activity.[14,15] The neutrophile and innate immune response was shown to affect the phage treatment of acute pneumonia in mice.[16] Similarly, a seven-year-old girl treated for *P.aeruginosa* septic arthritis and osteomyelitis showed upregulation of innate and adaptive immunity genes.[17] Several factors determine the level and class of antibodies produced against the phages, such as route of administration, phage dosage, and frequency of administration. However, despite decreased phage levels, phage therapy effectively reduced the target bacterial population.

Acceptable standard

The phages used for phage therapy should not encode any of the genes of concern, for example, antibiotic resistance genes or toxin genes, in their genome. Furthermore, the phages should also be incapable of undergoing lysogeny. Therefore, whole genome sequencing and annotation should be performed for both the phage and host bacterial strain used for its propagation. The host bacterial and phage genomes must be screened for antibiotic resistance genes, toxin genes, presence of prophage genomes, integrase genes, regulators for integrase genes, and integrase-like genomic elements using appropriate bioinformatics solutions.[18]

Phage quantification in clinical specimens

Phages should be quantified in the clinical specimen obtained from the infection site using accurate and reproducible methods. The rationale behind obtaining such data is to determine the optimal dose, dosage frequency, dose route administration and duration of treatment.[19] A mix of microbiological, microscopic and molecular biology methods can be used to assess the number of phages in your clinical specimen. »

Types of phage products

Phage products are available in different forms, eg, phages as monophage, cocktails of different phage strains, premanufactured phages, and phage products built as per patient needs in real-time. Among the range of phage products, cocktails may be the preferred choice to either increase the chances of targeting specific bacterial populations or multiple bacterial species, such as in biofilm-born infections.[20] Phage cocktails also optimise bacterial lysis over time, reducing the risk of developing resistance. A potential drawback of the phage cocktail, however, is the formation of agglomerate, which can be avoided by administrating phages sequentially. Premade phage mixes are prepared in advance and available as over-the-counter medicine.[21] Premade phage products can be for either empirical or personalised use, however, personalised phage products are developed on demand for patients with known isolates.[20]

Pharmacokinetic and pharmacodynamic considerations

The large size of phages, their protein content, and their self-replicating properties provide phages with unique pharmacokinetic properties compared to antibiotic molecules.[22,23] These properties therefore limit the concentration of phages at the site of infection as the mononuclear phagocytes rapidly remove them and are potentially also neutralised by antibodies. Therefore, factors such as the density of host bacterial cells and their growth rates, the number of phages and their infectivity rates, the phage latency period and burst size determine the killing of the bacterial population at the site of infection.

Frequency of phage dose administration

To ascertain the ideal dosing frequency of phage therapy for any route of administration or any form of infection, requires more data.[10,24] There are published cases where doses have been administered daily, twice daily, and every six to eight hours. Phage DNA was detected 12 hours after administration in the human bloodstream for staphylococcal infection. A handful of studies demonstrate phages in faeces and blood following oral administration.

Phage therapy has emerged as a strong contender to combat increasing cases of antimicrobial resistance. Over the last decade, many clinical trials have been carried out, with several uncontrolled case studies reporting successful clinical outcomes, but simultaneously many studies likely underreport clinical failures. Much remains unknown about the efficacy of phage therapy and the potential reason for failures, such as dosage, frequency of dosage, duration of therapy, routes of administration, interaction with antibiotics, and super host immune response. Therefore, more data is needed to draw further conclusions on building a solid regulatory case for therapeutic phage products. Although the field is making great strides towards therapeutics, we must refine regulatory processes from a novel phage-based perspective. The recommendations provided in this article are by no means comprehensive or final. However, we make an honest effort to provide critical points for clinicians to address while applying phage therapy in the future.

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References

1. O'neill J. 2014. Antimicrobial resistance: tackling a crisis for the health and wealth of nations. *Rev. Antimicrob. Resist.*
2. Bassetti M, Poulakou G, Ruppe E, *et al.* 2017. Antimicrobial resistance in the next 30 years, humankind, bugs and drugs: a visionary approach. *Intensive Care Med.* 43, 1464–1475.
3. Woitschach F, Kloss M, Schlodder K, *et al.* 2022. Bacterial adhesion and biofilm formation of Enterococcus faecalis on Zwitterionic Methylmethacrylat and Polysulfones. *Front. Cell. Infect. Microbiol.*, Volume 12 - 2022
4. Villarroel J, Larsen MV, Kilstrup M, Nielsen M. 2017. Metagenomic analysis of therapeutic PYO phage cocktails from 1997 to 2014. *Viruses* 9:E328.
5. Abedon ST. 2017. Information phage therapy research should report. *Pharmaceuticals* 10:E43.
6. Furfaro L, Payne M, Chang B. 2018. Bacteriophage Therapy: Clinical trials and regulatory hurdles. *Front Cell Infect Microbiol.*
7. Górski A, Bollyky PL, Przybylski M, *et al.* 2018. Perspectives of phage therapy in non-bacterial infections. *Front Microbiol* 9:3306.
8. van de Sande WWJ, Vonk AG. 2019. Mycovirus therapy for invasive pulmonary aspergillosis? *Med Mycol* 57:S179–S188.
9. Aslam S, Lampley E, Wooten D, *et al.* 2020. 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* 7: ofaa389..
10. Bao J, Wu N, Zeng Y, *et al.* 2020. Non-active antibiotic and bacteriophage synergism to successfully treat recurrent urinary tract infection caused by extensively drug-resistant Klebsiella pneumoniae. *Emerg Microbes Infect* 9:771–774.
11. Plaut S. 2019. Regulatory considerations for bacteriophage products, p 342. In Gorski AMR, Borysowski J (ed), Phage therapy: a practical approach. Springer, Germany.
12. Liu D, Van Belleghem JD, de Vries CR, *et al.* 2021. The safety and toxicity of phage therapy: a review of animal and clinical studies. *Viruses* 13:1268.
13. Popescu M, Van Belleghem JD, Khosravi A, Bollyky PL. 2021. Bacteriophages and the immune system. *Annu Rev Virol.*
14. Van Belleghem J, Dąbrowska K, Vaneechoutte M, *et al.* 2018. Interactions between bacteriophage, bacteria, and the mammalian immune system. *Viruses* 11:10.
15. Roach DR, Leung CY, Henry M, *et al.* 2017. Synergy between the host immune system and bacteriophage is essential for successful phage therapy against an acute respiratory pathogen. *Cell Host Microbe* 22:38–47.
16. Khatami A, Lin RCY, Petrovic-Fabijan A, *et al.* 2021. Bacterial lysis, autophagy and innate immune responses during adjunctive phage therapy in a child. *EMBO Mol Med* 13:e13936.
17. Philipson C, Voegtly L, Lueder M, *et al.* 2018. Characterizing Phage genomes for therapeutic applications. *Viruses* 2018, 10, 188.
18. Anderson B, Rashid MH, Carter C, *et al.* 2011. Enumeration of bacteriophage particles: comparative analysis of the traditional plaque assay and realtime qPCR- and nanosight-based assays. *Bacteriophage* 1:86–93.
19. Schooley RT, Biswas B, Gill JJ, *et al.* 2017. Development and use of personalized bacteriophage-based therapeutic cocktails to treat a patient with a disseminated resistant Acinetobacter baumannii infection. *Antimicrob Agents Chemother* 61:e00954-17.
20. Luong T, Salabarria AC, Roach DR. 2020. Phage therapy in the resistance era: where do we stand and where are we going? *Clin Ther* 42:1659–1680.
21. Dąbrowska K, Abedon ST. 2019. Pharmacologically aware phage therapy: pharmacodynamic and pharmacokinetic obstacles to phage antibacterial action in animal and human bodies. *Microbiol Mol Biol Rev* 83: e00012-19.
22. Dabrowska K. 2019. Phage therapy: what factors shape phage pharmacokinetics and bioavailability? Systematic and critical review. *Med Res Rev* 39:2000–2025.
23. Kuipers S, Ruth MM, Mientjes M, *et al.* 2019. A Dutch case report of successful treatment of chronic relapsing urinary tract infection with bacteriophages in a renal transplant patient. *Antimicrob Agents Chemother* 64:e01281-19.

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