



Phage Therapy

Joana Azeredo^{[a][i]}, Jean-Paul Pirnay^[b], Diana Pires^[c], Mzia Kutateladze^[d], Krystyna Dabrowska^[e], Rob Lavigne^[f], Bob G Blasdel^[g]

Abstract

Phage therapy refers to the use of bacteriophages (phages - bacterial viruses) as therapeutic agents against infectious bacterial diseases. This therapeutic approach emerged in the beginning of the 20th century but was progressively replaced by the use of antibiotics in most parts of the world after the second world war. More recently however, the alarming rise of multidrug-resistant bacteria and the consequent need for antibiotic alternatives has renewed interest in phages as antimicrobial agents. Several scientific, technological and regulatory advances have supported the credibility of a second revolution in phage therapy. Nevertheless, phage therapy still faces many challenges that include: i) the need to increase phage collections from reference phage banks; ii) the development of efficient phage screening methods for the fast identification of the therapeutic phage(s); iii) the establishment of efficient phage therapy strategies to tackle infectious biofilms; iv) the validation of feasible phage production protocols that assure quality and safety of phage preparations; and (v) the guarantee of stability of phage preparations during manufacturing, storage and transport. Moreover, current maladapted regulatory structures represent a significant hurdle for potential commercialization of phage therapeutics. This article describes the past and current status of phage therapy and presents the most recent advances in this domain.

History of Phage Therapy

Origins of Phage Therapy

In 1915, Frederick Twort, a medically trained bacteriologist from England, reported a bacteriolytic phenomenon and advanced the hypothesis that it may be due to a virus.^[1] However, excitement with the possibilities of bacteriophage can be said to have begun six months before when Félix d'Hérelle, a microbiologist at the Pasteur Institute, was sent 50 miles from the Western Front to Maisons-Laffitte to investigate an outbreak of dysentery among 10 French mounted infantrymen. Returning with samples he described a soon eponymous novel bacillus.^[2] However, in his investigations of this bacteria over the next 18 months, he found that some seemingly

sterile Chamberland filtrates of it were capable of effecting the killing (lysis) of another dysentery bacillus (likely *Shigella*). In one of the great scientific works of the twentieth century, d'Herelle described in two short pages the experiments that he performed demonstrating that this lytic property could be serially passaged from one culture to the next by transferring 10⁻⁶ dilutions to new cultures fifty times.^[3] Any toxin would be too diluted after fifty passages to have a biological effect. Similarly, he showed that no dilution of these lysed cultures would produce partial growth inhibition when added over a lawn of bacteria like an antibacterial toxin would, but instead would display a number of clear glassy holes (called "plaques") equal to the concentration that would lyse a liquid culture. From these observations, d'Hérelle radically intuited that he had discovered "*un microbe invisible antagoniste des bacilles dysentériques*" described it as "*un bactériophage obligatoire*" ("an invisible microbe antagonistic to dysentery bacilli"), suggesting that his other bacteria would also be infected by these pathogens of pathogens, and (perhaps too radically) posited that these bacteriophage were the true awiki journal gent of natural immunity.

^[a] Centre of Biological Engineering, University of Minho, Braga, Portugal

^[b] Queen Astrid Military Hospital, Brussels, Belgium

^[c] Centre of Biological Engineering, University of Minho, Braga, Portugal

^[d] G. Eliava Institute of Bacteriophages, Microbiology

^[e] Polish Academy of Sciences, Wrocław, Poland

^[f] Division of Gene Technology, KU Leuven, Heverlee, Belgium

^[g] Vésale Bioscience, Vésale Pharma, Noville-Sur-Mehaigne, Belgium

^[i] Author correspondence: jazeredo@deb.uminho.pt

ORCID: [0000-0002-5180-7133]



Phage Therapy in the West

Soon after this seminal publication, d'Hérelle and others began experimenting with the use of phage as an antimicrobial therapeutic for infections, beginning with the treatment of chicken typhoid.^[4] In 1919, d'Hérelle used phages to successfully treat four children dysentery at the "Hôpital des Enfants-Malades" in Paris. These were probably the first clinical applications of phages in humans. However, the results of his experiments were not published at that time, and so the first published use of phages to treat bacterial infections in humans was reported in 1921 by [Richard Bruynoghe](#) and Joseph Maisin.^[5] They had used phages to treat a staphylococcal infection in surgical lesions and were able to report a regression of the infections within 24 to 48 hours. The contemporary paucity of effective antimicrobial treatments and the exciting promise of these early results produced an enthusiastic 'early period' of phage therapy.^[6] However, sober hindsight was provided by a deeply critical and widely read report by two physicians, Eaton and Bayne-Jones, commissioned by the American Medical Association in 1934.^[7] This report demonstrated clearly that this period was largely characterized by inconsistent results, unrealistic claims, and unreliable companies. In an era lacking even a basic understanding of the nature of bacteriophages, phage preparations were marketed as treatments of implausible ailments such as gallstones, herpes, kidney stones and various cancers. Commercial preparations, even from major pharmaceutical companies, were found to be devoid of phages active against the target pathogens.^[6] Sometimes these defects were due to practical considerations, like deleterious sterilization procedures or inactivation in storage. However, some phage preparations were targeted against the wrong pathogen or were restricted to only a limited set of strains of the right pathogen. Nevertheless, even decades after the 1934 Eaton/Bayne-Jones report, phage therapy was still in sporadic use in the West into the 1950s and 1960s, and only ended in France in the 1990s. However, phages continued to be studied as tools to uncover life processes. Phages were also instrumental in the advent of genetic engineering, cancer biology and the discovery of [CRISPR](#).

Phage Therapy in the East and Central Europe

Around 1934, d'Hérelle was invited by a Georgian microbiologist George Eliava to help expand a scientific institute in Tbilisi, in what is now the Republic of Georgia, for the production of both vaccines and phage prepara-

tions. This institute, now called the [George Eliava Institute of Bacteriophage, Microbiology and Virology](#), along with others across the Soviet Union, were tasked with providing the Red Army, public health officials, and the general public with preparations that could be used to prevent and treat intestinal and purulent infections. The institute began rapidly isolating, and then industrially producing phage preparations for a variety of military and civilian purposes, with bacteriophages used as part of the standard of care for a wide variety of diseases. Although just three years later Eliava and his wife were accused of implausible crimes against the state and executed after a show trial, the institute thrived and expanded under the leadership of the primarily female scientists who both men trained.

The structure of the Soviet healthcare system and the distinct intellectual framing of infectious disease by Soviet scientists^[8] provided several particular advantages in exploring phage therapy compared with the West. Indeed, the centralised control of the Soviet healthcare system allowed for the creation of comprehensive centralised banks of bacteria from infected patients from the USSR. This allowed phage scientists to maintain libraries of phages that would be active against the most current pathogens in a particularly tailored way. At the same time the way that Soviet microbiologists precociously framed bacterial infection as, in part, an ecological problem,^[8] made the ecological solution offered by phages particularly natural. Also, importantly, the ideological blinders demanded of Soviet researchers very effectively insulated phage scientists from the criticism that was dominating Western discussions about phage therapy. Moreover, antibiotics (particularly specialised antibiotics) were not available in quantities that were considered necessary for a functioning Western medical system, leading to a need for alternatives.

At its peak in the 1980s, Soviet phage production reached 2 tons per week, primarily as formulated tablets against intestinal indications, for the Red Army as well as Central Asian Republics. Notably, the concept was widely considered to be conclusively demonstrated in the 60s after extensive testing,^[6] although most of the early phage therapy related scientific work was published in Russian, Georgian or Polish and thus not easily available in the West. Today, researchers from across the Former Soviet Union including the [Eliava Institute](#) publish their results and clinical experience in English, but these do not yet include RCTs (Randomized Clinical Trials) that would be considered necessary by [EU](#) and [US authorities for medicines](#) for marketing authorization. In 2012, a book was published in English that comprehensively reviews the publications on phage therapy that were found in the library of the Eliava Institute.^[9]

Although the design and quality of old Soviet clinical trials and scientific publications do not conform with current international standards, they often contain valuable information that should not be neglected by current phage therapy stakeholders. One of the largest and most imaginative studies was conducted in Tbilisi, Georgia, during 1963 and 1964.^[10] In this study, more than 30,000 children, segregated by residency on opposite sides of streets were given either *Shigella* phages, or a placebo. The incidence of dysentery based on symptoms was found to be 3.8-fold higher in the placebo group than in the phage-treated group. Based on the culture-confirmed cases, the incidence of dysentery was 2.6-fold higher in the placebo group.

The [Ludwik Hirsfeld Institute of Immunology and Experimental Therapy](#) in Wrocław, Poland, continues the tradition of phage therapy in Poland, which originated in the 1920s. A detailed retrospective analysis of the safety and efficacy of phage therapy in 153 patients, admitted between 2008 and 2010, with a wide range of infections resistant to antibiotic therapy, was published in 2012.^[11] In general, results suggested that phage therapy could provide good clinical results in a significant cohort of patients with otherwise untreatable chronic bacterial infections and without significant adverse events.

The Underlying Biology of Phage Therapy

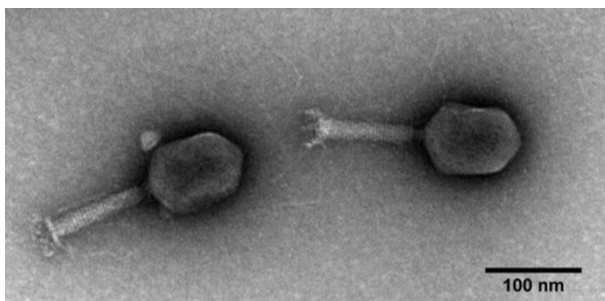


Figure 1 | Bacteriophages; image obtained by Transmission Electron Microscopy

What are bacteriophages?

The word, "phage" is derived from the ancient Greek verb '*phagein*', meaning to eat. Felix d'Hérelle appended the word "bacteria" with this suffix to form the construct bacteriophage, which suggests that entity he discovered "eats" bacteria. It has since been found that phages are similar in nature to Eukaryotic viruses, with

their infectious particles consisting of DNA or RNA enclosed within a protein capsid. Phages are the most abundant biological entities on the earth (the number of phage particles on the planet has been speculatively estimated to be approximately 10^{31} ^{[12][13]}).

Many types of phages are known but the phages used for therapeutics belong to the order Caudovirales, by far the most abundant type. These phages have double-stranded DNA; the size of the genomic DNA (gDNA) ranges from ~ 15 kb to >700 kb (kb = 1000 base pairs, or about one typical gene). Caudovirales means "tailed viruses", because the gDNA is encapsidated in a protein "head" with a tail (Figure 1) that, in general, serves as an apparatus for recognizing a feature, or "receptor", on the surface of a particular bacterial host. After the virion binds to this receptor, the gDNA is ejected through the tail apparatus into the cytoplasm of the bacterial cell. The viral genes are then expressed by the host machinery, leading to a program of DNA replication, morphogenesis of progeny virions, and, eventually, lysis of the cell leading to release of the progeny. As an example, typical phages of *E. coli* release 100 to 300 progeny virions after an infection cycle of 15 to 45 minutes. This rate of multiplication, otherwise unmatched in biology, means that, if sufficient host cells and nutrients are present, one phage particle can generate billions in just a few hours. In addition to this lytic, vegetative pathway, some phages, designated as "*temperate*", have the capacity to shut off the infection cycle and assume a dormant form. This form, called a prophage, can integrate into the host chromosomal DNA or form a self-replicating plasmid. The host cell carrying a prophage, called a "lysogen", has the advantage that it is immune to infections of the same phage. However, although the lysogenic state can last indefinitely, the prophage can also activate spontaneously or after stress applied to the bacterial host. This activation, known as "lysogenic induction" results in viral replication, virion morphogenesis and lysis of the host, just as in the normal lytic cycle.

Bacteriophage application in Phage Therapy

The ability of phages to replicate in host bacteria, lyse them and form new phage progeny raises the potential of using these bacterial viruses to combat infectious bacterial diseases. The natural activity of phages harnessed and directed against specific bacterial infections in humans, animals and plants could augment an organism's own immune system or replace/support antibiotics, particularly those that have become ineffective due to [antimicrobial resistance](#).



In principle, phages can be used for the treatment of different acute or chronic bacterial infections, including antibiotic resistant infections. The first stage of phage treatment typically includes the identification of the **pathogen** – the causative agent of the infection – from clinical samples taken from infected material (mucus, blood, stool, urine, tissue swabs, sputum, etc) of a patient. It is possible to give to a patient a multicomponent phage mixture (often called a cocktail) that contains phages specific for multiple bacterial species before performing a bacteriological analysis. In this case, if the bacterial pathogen is not sensitive to the phages in the mixture, it will most probably not cause any serious adverse reaction to the patient and will be easily cleared from the body by the reticuloendothelial system, primarily the liver and the spleen. However, preliminary bacteriological analysis is recommended.

After identification of the pathogen (infectious agent), the bacteria should be checked **in vitro** against a library of phages to select the most effective phage for therapeutic application. The successful use of therapeutic phages in modern clinical settings depends on having a capable diagnostic laboratory. It is essential to have a set of well-characterized phages available covering a broad range of bacterial pathogens to compose the most effective phage preparation.^[14]

In case the library of phages does not include a bacterial virus with the desired activity, an "active" phage (lytic to the specific pathogenic bacterial strain) often can be isolated from different sources. Phages are natural entities and are also natural predators of bacteria; however, only a few of these bacterial viruses have proven to be effective as therapeutic agents. Phages that are used for therapy should exhibit a strictly lytic infection cycle during propagation on the host bacteria^[15] (temperate phages are usually not recommended for therapeutic use, as there is a possibility of transferring undesired genes, coding for virulence or antibiotic resistance, through lysogeny or **transduction**, which should not be allowed to occur). After the treatment course is completed, the phage, in the absence of host bacterial cells, are typically eliminated from the body without disturbing any cells or organs.

Phages also can be used as a mono-phage preparation or in combination with other phages in a cocktail. Based on the site of the infection, the diagnosed disease, and specific indications, phage preparations can be administered orally, locally, subcutaneously, or intravenously, among other routes. Additionally, phages have been proposed for prophylactic (prevention) purposes against bacterial diseases, mainly in cases where a high incidence of infection, or an epidemic spread of infections is expected. The best examples of phage usage for

prophylaxis include applications against intestinal problems such as diarrheal diseases caused by *Salmonella*, *Shigella*, and enteropathogenic *E. coli*.

Advantages of Phage Therapy

There are numerous advantages of phages over other antibacterial agents, namely:

1. **Universality:** Specific phages can be either isolated or adapted to target almost any pathogenic and conditionally-pathogenic bacterial strain, including those that are **multidrug resistant**. Exceptions include several infections where the bacterial pathogen is **intracellular** or is difficult to propagate in a laboratory environment.
2. **Specificity:** The range of host bacteria that are targeted by a specific phage is much narrower than that treated by convention antibiotics or most other antibacterial agents. Indeed, phages recognize and attach to features on the surface of the bacteria that are specific to their hosts. Most phages can only target a single bacterial species, although some phages reveal non-specific lysis of bacteria of broader categories of bacteria, but still within the same family (e.g. **mycobacteriophages**). Therapeutic phages can thus be selected to mainly target bacterial species, or even relevant subgroups of species, that cause infectious disease while sparing the patient's beneficial bacteria (e.g. the gut, skin or oral commensal bacterial communities). Most routinely employed **antibiotics**, in contrast, have a broad spectrum of activity, which can cause "collateral damage" to the patient's **microbiome**, which in turn can result in adverse effects such as the selection of antibiotic resistant bacteria (e.g. **Clostridioides**) or antibiotic-associated diarrhea.^[16]
3. **Self-dosing and self-limiting ability:** In contrast to antibiotics, phages are able to replicate at the site of infection, having the ability to multiply on their host bacterial cells and thus making them self-propagating in the presence of problematic bacteria. Once the infection is cleared, applied phage do not have any new targets to attack and their concentration declines, making them in theory self-limiting in the system. In contrast, the concentration of an antibiotic introduced into the human organism decreases only with time (through the natural drug clearance mechanism from the



body), whereas phages will continue to multiply exponentially, and decrease in numbers only as the target bacterial cells are eliminated or become inaccessible.

4. *Local activity*: Phages only replicate at the sites of infection.
5. *Non-toxicity*: Phages are part of the [natural human virome](#) and therefore, they are unlikely to be harmful to human cells. No significant adverse reactions have been observed in several reported clinical trials (described below).
6. *Anti-biofilm activity*: Phages can effectively penetrate and destroy bacterial biofilms, which make them a useful therapeutic option for the patients with deep wound infections, secondary infections in cystic fibrosis patients and many others (detailed below).
7. *Easy production and formulation*: phages can be rapidly isolated, can potentially have relatively low production costs, and can be versatile in formulation and application. It is possible to formulate therapeutic phage preparations in different medicinal forms such as liquids, tablets (pills), cream, tampons, droplets, ointments, suppositories, as aerosols, intrapleural applications, intradermal, subcutaneous, badder washes, and intravenous applications. Phages are compatible most other pharmaceuticals, such as antibiotics, vaccines, enzymes, probiotics, etc, and have no known drug interaction complications.
8. *Synergistic effect with antibiotics*: the synergistic effect between phages and antibiotics has been shown for several bacterial species (described below).

Current challenges of Phage Therapy

Phage therapy also faces some challenges that should be considered during the preparation of therapeutic phage formulations, as well as during the actual therapy:

1. *High specificity*: The narrow host range of phages can be a complication when the patient is affected by multiple pathogenic bacteria. This challenge may be addressed by using several phages in a single mixture (cocktail) that are active against different bacterial pathogens; or by using a combined antibiotic-phage approach. At best, a single phage may infect a considerable fraction of strains from one single

bacterial species, but generally target only a small number of strains within one species.

2. *Storage conditions*: Bacteriophages are typically stored and preserved at 4-5°C, which can create some challenges for long transportation or storage in the developing world. While their stability varies, commercially viable phages are selected to remain stable under optimal conditions for over a year. Lyophilization and other forms of drying are being explored for the long-term storage of phages.^[17] Therapeutic phages can thus have a significant shelf life (1.5–2 years).
3. *Lack of fast and routine screening methods*: As a result of the high specificity of phages, finding a phage for a particular strain might require the screening of large phage collections. In a clinical context, the rapid identification of the therapeutic agent(s) is crucial for the success of the therapy and so, high-throughput and fast screening methods are highly needed. Although multiple methods have been developed, most of them are too slow and require too much labor to be applied in routine clinical settings.
4. *Resistance to phages*: As is the case with some antimicrobials, phages can select for spontaneous resistance mutants. In contrast to chemical antimicrobials, phages sometimes have the capacity to overcome this resistance through the application of directed evolution techniques. Bacterial resistance to phages can be overcome by developing a second generation phage cocktail that targets the bacterial mutant.
5. *Inconsistent activity against biofilms*: The biofilm structure and composition (polysaccharides, lipids, etc.) might impair phage infection in several ways. First, the biofilm matrix can limit phage access and docking to the receptors of bacterial cells by forming a physical barrier for phage diffusion. Second, the presence of dormant persister cells in the deeper layers of the biofilm also constitute an obstacle to phage infection as most phages are unable to replicate in metabolically inactive cells.
6. *Lack of standardized protocols*: One challenge associated with the treatment process is the lack of standardized protocols regarding dosing, frequency of dosage, etc., and which vary for phage therapy for different infectious diseases approved by the international health



community and legal authorities. Current protocols are variably based on the long-term experience of physicians and researchers from the Former Soviet Union or by individual doctors working on their own.

7. *Phage bank*: A key condition for phage therapy is an eligible phage library (phage bank) of well characterized phages that needs to be constantly updated. The phage banks established so far are small and the information in most of the existing collections is incomplete. This challenge can conceivably be overcome with the development of a large, ever-expanding phage library that maps on to a repository of bacterial pathogens.^[18]
8. *Public perception*: In general, phage therapy is often poorly understood by the public, with its limited foundational knowledge of phages. Indeed, there is the potential for irrational but understandable fear by the public of being treated by viruses. Additionally, unrealistically high or uninformed expectations for phage treatment are widespread, leading patients to seek to use phages incorrectly against a non-diagnosed bacterial infection, or even attempting to treat another kind of infection (e.g., fungal or viral).
9. *Limited expertise*: the selection and preparation of therapeutic phages and cocktails requires skilled personnel with significant knowledge and experience, specific laboratories, and existing phage collections. Isolation and selection of active phages against specific host bacteria need to follow several preliminary steps, for example, selection of good sources for phage isolation (environmental samples, human or animal samples, soil samples, etc.), and following specific protocols for isolation.

Phage Therapy approaches

"One size fits all" medicine vs. precision medicine

When the intent is to use phages for therapeutic purposes, i.e. using them to control bacterial pathogens, then two of their characteristic properties are particularly of interest. First, as already mentioned, phages are highly specific for their target [bacteria](#). Secondly, bacteria and phages are engaged in an antagonistic host-

parasite relationship, which drives a continuous evolution of phage infectivity and bacterial phage resistance traits.

When it comes to phage therapy, two distinct approaches have been proposed.^[19] In what could be called the *one-size-fits-all* or mass produced phage therapy approach, defined broad spectrum phage cocktails, which are intended to target the majority of bacteria that are implicated in certain infectious diseases, would be industrially produced and widely distributed, in a manner similar to conventional antibiotics or other small molecule drugs. In *personalized* or *precision* phage therapy concepts, one or more phages are selected from a phage bank, or from the environment, and possibly adapted (i.e. the *in vitro* selection of phage mutants that exhibit an increased infectivity and a delayed emergence of phage resistance) to infect bacteria that have been isolated from the patient (as indicated by *in vitro* test, i.e. a so-called phagogram) are produced on a small scale, and then administered to that patient.

The predefined phage cocktails could in principle be developed, produced and marketed within current regulatory and economic frameworks, which have evolved to regulate defined drug ingredients such as vaccines or small molecule-type antibiotics. However, truly 'broad spectrum' phage cocktails, active against most Gram-positive and/or Gram-negative bacteria commonly encountered in infectious diseases, would need to contain hundreds of phages and would be very difficult to develop considering the current state of the field. In the future, synthetic biology approaches (e.g. structure-guided design) could generate phages with more predictable and extended host ranges.^[20] Today, it could be feasible to use narrower spectrum phage cocktails that are active against one or a few bacterial species, to be used in certain indications, assuming that pre-treatment bacteriology has been performed prior to the initiation of therapy. For some (clonal) bacterial species, such as *Staphylococcus aureus*, phages have been isolated and characterized that show an exceptionally broad host range, such as phage ISP of the Eliava Institute, which was shown to be active against 86% of *S. aureus* strains.^[21] However, even then one needs to keep in mind that these cocktails will not always be efficient due to the greater biodiversity outside of the laboratory and due to the pre-existing resistance to the chosen phages. In the [PhagoBurn](#) project, a randomized, controlled, double-blind phase I/II trial, assessing the efficacy and tolerability of a cocktail of phages to treat burn wounds infected *Pseudomonas aeruginosa*, the success in achieving sustained reduction in bacterial burden was linked to initial (i.e. day 0) *P. aeruginosa* sus-



ceptibility to the phage cocktail, highlighting the importance of preliminary phage-susceptibility testing.^[22] The susceptibility of bacterial strains isolated at day 0 was tested in 10 participants in the phage group. Three of the 10 participants (30%) were shown to harbor pre-existing *P. aeruginosa* strains resistant to the phage cocktail, which consisted of no less than 12 natural lytic anti-*P. aeruginosa* phages. It is thus likely that for the time being, most so-called broad-spectrum approaches will greatly depend on rapid pre-treatment identification of the bacterial pathogen(s).

In addition, phage cocktails that are initially suspected to be efficient, based on the sensitivity of the target pathogen(s) to the phages, will likely need to be regularly updated (e.g. supplemented with new phages) in response to the anticipated emergence of phage resistance or the involvement of geographically divergent or newly emerging clinical strains. There are indications, from *in vitro* experiments, that bacterial resistance to phages, even to cocktails containing multiple phages, will inevitably occur, albeit later than if one single phage is used.^[23] Pre-adapting phages to a pathogen was shown to lead, *in vitro*, to increased pathogen clearance and slower resistance evolution.^[24]

Precision phage therapy approaches tend to be more laborious, but are potentially more sustainable as they are suspected to apply less selection pressure towards bacterial phage resistance. These approaches are required the creation of phage banks consisting of a large assortment (dozens to hundreds) of characterized phages. From these banks, phages are selected, and – ideally – adapted to the causative bacterial strain. These precision phage products, which will be used in a limited number of patients, can be rapidly produced on a small scale. Georgian (Tbilisi) and Polish (Wroclaw) phage therapy centers set up and maintain large therapeutic phage banks, which are regularly updated with new phages, widening and adapting the host range of the bank to the ever changing bacterial populations, with regard to the emergence of new clinically relevant bacterial strains and species, as well as phage resistance. However, this concept is not very compatible with most (Western) medicinal product (drugs in the US) development and licensing pathways, which is usually very time consuming and expensive to complete, even when considering abbreviated pathways, and this for every phage in the bank. Moreover, it is very difficult to imagine how existing medicinal product pathways will be able to cater for the ad hoc adaptation of banked phages, or the application of newly acquired or isolated (from the environment) phages.

It might be wise to develop both options, defined broad spectrum phage medicines, produced at a large scale

and globally supplied for first line use, and a local small scale supply of precision phage products for use in personalized therapies or public health or medical emergencies such as the O104:H4 (hybrid EaggEC STEC/VTEC pathotype) *Escherichia coli* outbreak, which caused the death of more than 50 patients in Germany in 2011.^[25] At the Eliava Institute in Tbilisi, historically, scientists verify their commercial phage cocktails at least annually against the pathogen isolates that were recently isolated and then add phages and/or adapt the phage preparations to keep pathogen coverage ranges high. In addition, a personalized "autophage" approach was set up for patients harboring bacterial strains that are not targeted by the "broad spectrum" phage cocktails. These strains are then screened against the phages present in the phage bank of the Eliava Institute, which comprises of hundreds of therapeutically promising phages. If a suitable phage is found, it is produced as quickly as possible for use in that individual patient. A growing number phage therapy centers, companies and programs in North America now operate using a similar approach (e.g. Center for Innovative Phage Applications and Therapeutics).

Phage Therapy against infectious biofilms

Bacterial **biofilms** can be defined as microbial communities surrounded by a complex extracellular matrix, also known as EPS (extracellular polymeric substances). These complex structures constitute an important bacterial survival strategy in adverse environments.^[26] Biofilm can form spontaneously on both inert and living systems and are frequently implicated in many chronic infections as consequence of their inherent tolerance to antibiotics and disinfectants.^[26] To deal with this problem, several alternative strategies to antibiotics have been proposed, including phages.

Many researchers have been studying the potential of phages for biofilm control or prevention. In fact, significant reductions of single or dual-species biofilms have been reported when a phage treatment was applied.^{[27][28][29]} However, most studies have been performed using *in vitro* models of biofilm formation and it remains unclear whether such models resemble the biofilms found in real situations.^[27] Although *in vitro* biofilm studies are relevant for an initial screening of the best phages for this application, they cannot replace the *in vivo* studies for an accurate evaluation of the outcome of infection.

Despite the fact that some *in vivo* models of biofilm infection have been described, there is still a limited knowledge regarding the therapeutic application of



phages on real biofilms. Rodent models of wound infection are the most commonly used *in vivo* model to study the therapeutic potential of phages against bacterial biofilms. Most of these studies were focused on *P. aeruginosa* and *S. aureus*, two of the most common pathogens present in wound infections, and have demonstrated that phages can indeed be a valuable alternative or complementary approach for biofilm treatment.^[30] There are now a number of case reports where phage therapy was successfully used to treat biofilm-associated infections.^{[31][32][33]}

Nonetheless, despite all the promising results achieved, the total eradication of biofilms is difficult to achieve.^[27] The biofilm structure and composition can indeed pose some limitations to the success of phage infection. First, the access of phages to the cell surface receptors can be impaired by the biofilm matrix surrounding the cells. Additionally, the heterogeneity and reduced metabolic activity of the biofilm cells and the fast proliferation of phage-insensitive mutants (BIMs) within the biofilm, constitute some of the major obstacles to the success of phage infection.^[27] To deal with these limitations several strategies have been proposed, namely: i) the use of phage cocktails; ii) combined therapies of phages with antibiotics, antiseptics or enzymes; iii) mechanical debridement of biofilm prior phage application; or iii) genetic manipulation of phage genomes to encode genes that would enhance their anti-biofilm activity.^[27]

Phage-antibiotic combinations

To improve the outcome of the treatment, phages can be combined with other antimicrobial agents such as antibiotics. This type of combined treatment has been widely studied by the scientific community and it has been reported that subinhibitory concentrations of antibiotics can indeed enhance the activity of virulent phages, a phenomenon that was named as phage-antibiotic synergy (PAS).^{[34][35]} This synergy has been shown for several bacterial species grown as suspended cultures or biofilms.^{[34][36]} For instance, it has been reported that sublethal concentrations of antibiotics can foster the replication of virulent phages and increase the size of phage plaques.^{[34][37]} Additionally, some combinations of phages and antibiotics have been shown *in vitro* to almost eradicate bacterial biofilms, depending on the antibiotic concentration and the order of application.^[36] Synergism occurs because phage associated bacterial lysis releases nutrients that will reactivate the metabolic activity of the growth-arrested cells, which become sensitive to antibiotics. Lysis also causes a dispersion of the biofilm matrix enhancing the diffusion of the antibiotic to the inner matrix layers,

whereas the oxygen availability increases the drug activity.^[34] Besides the synergistic effect, it has been further reported that the combination of phages and antibiotics can significantly arrest the emergence of resistant variants, which is a very important feature.^[38]

However, this combination has not always resulted in synergistic effects as, in some cases, additional or antagonistic effects were reported.^{[34][39]} It is important to highlight that the success of the combined therapies is very dependent of key factors such as the dosage levels, order of administration, frequency or time points. Furthermore, potential drawbacks associated with combined therapies can be anticipated such as the development of double-resistant variants or the selection for antibiotic-resistant subpopulations in case that phages preferentially target the antibiotic-sensitive ones.^[39] In addition to antibiotics, the synergistic effect between phages and antiseptics or other antimicrobial compounds has also been assessed and revealed encouraging results.^[27]

Biotechnological advances in Phage Therapy

The success of phage therapy is highly dependent on the safety of phage preparations, which raises manufacturing, formulation and delivery challenges. Although phage therapy must comply with the strict regulations applied for pharmaceutical products, clear guidelines have yet to be developed specifically for phages, which limits their broad therapeutic application.^[40] In addition, robust manufacturing processes that will avoid variability as well as production under GMP will probably be required to use phages as a first-line treatment.

The phage research community has begun to put forward some quality and safety requirements for sustainable phage products. One of them is that phages capable of lysogeny, encoding virulence factors or antibiotic resistance should not be considered suitable for therapy.^[41] However, no strictly virulent phages have been found for some bacterial species such as *C. difficile*,^[42] which might limit the use of phage therapy with natural phages in some fastidious bacteria. The presence of impurities in phage preparations, such as endotoxin (LPS), should also be avoided, and several purifications methods have been developed and optimized to ensure phages' safety.^{[43][44]}

Another important feature is the stability of phage preparations since the efficacy of the treatment is highly dependent on the phage concentration delivered to the site of infection.^[45] The reduction of phage titers due to their interaction with host antibodies or other



clearance mechanisms might reduce the efficacy of the therapy. Therefore, strategies to enhance phages' stability to pH and increase their circulation time at infective doses in human body are being developed. One of these strategies is the encapsulation of phages in different matrices such as liposomes, alginate, cellulose or other polymers.^[45] Indeed, *in vivo* studies have demonstrated that liposome-encapsulated phages are able to persist for longer periods in the stomach and also adhere to the intestinal membrane.^[46]

The stability of phages can also be enhanced by genetic manipulation of phage genomes. In fact, several phage-engineering tools have been developed and opened a window of opportunities to shape phages with better antibacterial properties.^[47] Using such strategies, it was already possible to expand phages' host ranges, improve their antibacterial and anti-biofilm activities, reduce their immunogenicity or enhance the bactericidal activity of antibiotics.^[47] The tremendous potential of phage engineering was already proved in clinical practice as engineered phages were successfully used to treat a 15-year-old patient with a disseminated drug-resistant *Mycobacterium abscessus* infection.^[48] This study clearly indicates that engineered phages may be an important weapon in the future for the treatment of bacterial infections.

Pre-clinical evaluation

A significant number of pre-clinical studies using animal models has been performed to study the efficacy and safety of phage therapy against several types of infections (reviewed in ^[49]). These studies are the major source of information on the most efficient routes of phage administration for controlling bacterial diseases and the most effective phage dosing. The first obvious conclusion drawn from all animal trials is that phage therapy is safe, as no harmful effects of phage therapy were identified in any of the reported studies. In terms of efficacy, the results are quite variable and very dependent on the type of infection and therapeutic schedule, including moment of the first application, phage dose, and route of administration.

In most cases, stage of infection when a phage was first applied was important, with the tendency for early administrations to be more effective, and more difficult treatments in case of established infections. In general, chronic infections are generally more difficult to tackle than acute infections. Chronic infections are often characterized by the presence of biofilms, which are intrinsically tolerant to antimicrobials. Nevertheless, pre-clinical trials demonstrated successful outcomes with

topical application of phages usually associated with prior debridement and using high phages titres. Phage concentration seems to be a key factor for the outcome of phage therapy, since *in vivo* trials demonstrated better therapeutic efficacy when high phage titres were applied, usually at an MOI of 10 and concentrations greater than 10^7 phage active particles per treatment (10^7 - 10^9 PFU). In many cases, local/topical administration allows for efficient delivery of phage to the site of infection (e.g., burn wounds and diabetic foot ulcers), while in other cases, systemic delivery should be considered (e.g., disseminated infections and bacterial endocarditis). In systemic deliveries, achieving effective concentration of phage depends on the route of administration; the most effective are all types of injections (intramuscular, intravenous, subcutaneous, others) and the least effective is oral delivery with usually very limited spread of phage amount in the body, as revealed by systematic review of phage pharmacokinetics.^[50]

The administration of phage cocktails has usually resulted in a better outcome than single phage applications. Other treatment variables such as the optimal schedule of administration or duration of treatment are difficult to generalize based on pre-clinical studies.

Clinical experience and randomized controlled trials

The competent authorities for medicines in the EU and in the US classified therapeutic phages as "medicinal products" and "drugs" respectively, and decided that they cannot be recommended for (marketing) authorization before their efficacy and safety have been proven in new and appropriately designed controlled trials.^[51] Historical data and clinical experience are difficult to assess rigorously.

Several Randomized controlled trials (RCTs) have been conducted in Europe, but none of them clearly demonstrated the efficacy of phage medicines. Note that, as a result of the medicinal product or drug classification, most of these clinical trials evaluated static phage medicines using conventional clinical trial designs, but did not evaluate personalized phage therapy approaches, which use regularly updated phage preparations. Several phase I, I/II and III clinical trials did demonstrate the safety of certain phage medicines, which is consistent with the safety data provided by numerous preclinical animal studies.

To date, two European RCTs showing some phage treatment efficacy have been reported in the scientific literature. In the first one, a small, but well-designed,



randomized, double-blind, placebo-controlled phase I/II clinical trial, phage therapy against chronic *P. aeruginosa* otitis was investigated.^[52] A defined cocktail of 6 phages was shown to be successful, as the bacterial burden was found to be significantly lower in 12 phage-treated patients as compared to 12 placebo-treated patients, and no adverse effects were observed. The second one is a small randomized, controlled, double-blind phase I/II clinical trial, designed to evaluate the treatment of *P. aeruginosa* infected burn wounds using a defined phage cocktail.^[22] This study did not demonstrate clear efficacy of phage treatment but was also beset by multiple unanticipated technical, logistical and regulatory issues.^[53] One batch of the investigational phage cocktail, containing 12 phages, took 20 months and most of the study budget to manufacture in compliance with [Good Manufacturing Practices](#) (GMP). Phage specificity issues hampered the recruitment of patients, resulting in only 27 patients enrolled and randomly assigned to receive either phage therapy (n=13) or standard of care (n=14). Standard of care consisted of 1% [sulfadiazine silver emulsion](#) cream, an antiseptic that had never been subjected to an RCT before.^[54] Due to product stability issues, only a very small number of phages (10-100 PFU/ml instead of the anticipated 10⁶ PFU/ml) were applied. Bacterial burden was shown to decrease over time in the phage-treated patient group, albeit at a rate slower than that of the standard of care. These results are difficult to interpret due to the above-mentioned technical issues, but phage treatment as applied here was clearly not superior to the standard of care. This study is still considered an important milestone as physicians and scientists learn how to bring phage therapy into practice under an EU regulatory framework.

In Bangladesh, an RCT of phage therapy with two different coliphage cocktails was performed in children with *E. coli* diarrhea, between June 2009 and September 2011.^[55] Oral coliphage were shown to reach the children's intestines, but they did not achieve treatment effects over placebo, consisting of reduced osmolarity [oral rehydration solution](#) (ORS) supplemented with zinc, the standard treatment for uncomplicated watery [diarrhea](#) at the clinical trial center. As a possible reason for this disappointing result, the authors stated that microbiota analysis had revealed a marked outgrowth of fecal streptococci during the acute phase of diarrhea with *E. coli* titers close to the replication threshold of the coliphages.

In Australia, a phase I, first-in-humans, open-label clinical trial was conducted to assess the safety, tolerability, and preliminary efficacy of ascending multiple intranasal doses of an investigational phage cocktail, containing three *S. aureus* phages, in patients with recalcitrant

chronic rhinosinusitis due to *S. aureus*.^[56] Intranasal irrigation with phage cocktail doses up to 3 × 10⁹ PFU for 14 days were shown to be safe and well tolerated. Preliminary efficacy observations were promising with patients showing clinical and microbiological evidence of eradication of infection.

In [Georgia](#), the feasibility, tolerability, safety, and clinical/microbiological outcomes of adapted phages in the treatment of [urinary tract infections](#) were evaluated in a case series as a pilot for a double-blind RCT.^[57] Nine patients that had undergone transurethral resection of the prostate (TURP) and had positive urinary cultures with uropathogens sensitive to the adapted Pyo bacteriophage (one of the commercial phage preparations produced by the Eliava Biopreparations), were selected for phage therapy. Bacterial burden decreased in six of the nine patients (67%) and no phage-associated adverse events were detected. Currently, the RCT has been completed and the results are being published. The safety of oral exposure to the Eliava Pyophage and to a *S. aureus* monophage, as compared to a placebo, was assessed in healthy human carriers of *S. aureus*.^[58] No adverse effects were observed in any of the treatment groups, which supports the clinical safety of *S. aureus* phages administered either as a single phage or as part of a phage cocktail.

Another commercial preparation of the Eliava Institute, the anti-staphylococcal phage Sb-1, was used in the treatment of a series of diabetic toe ulcers conducted as a prelude to full-scale controlled clinical trials.^[59] Nine patients with diabetes with poorly perfused toe ulcers infected (culture-proven) with *S. aureus* and who had responded poorly to recommended antibiotic therapy, were treated with Sb-1 in combination with infected bone and soft tissue debridement. The ulcers healed in an average of seven weeks and one ulcer with very poor vascularity required 18 weeks of treatment.

The effect of a commercial *E. coli* phage cocktail on gut microbiota and markers of [intestinal](#) and [systemic inflammation](#) in a healthy human population was examined in a double-blinded, placebo-controlled crossover trial.^[60] Reductions in fecal *E. coli* loads were observed, without global disruption of the gut microbiota. Specific populations were altered in response to the phage treatment, including increases in members of the butyrate-producing genera [Eubacterium](#) and a decreased proportion of taxa most closely related to [Clostridium perfringens](#), which demonstrated the potential of phages to selectively reduce target organisms without gut dysbiosis.



In addition to the above-mentioned clinical trials, an increasing number of (small series of) case studies, involving different clinical indications and infecting bacterial species, have been reported to show promising results.^{[48][61][62][63][64][65][66][67][68][69][70][71][72]} The big pharmaceutical industry has so far not invested in phage therapy, and RCTs to evaluate and compare safety and efficacy might be too costly for smaller biotechnology companies. Hence, there is a relatively low number of completed phage-related RCTs so far. If medicinal product or drug classification is obtained, a coordinated private-public effort might be necessary to forward the phage therapy field.^[73]

It should be highlighted that in 2018, based on experience with a growing number of successful patients treated with phage therapy, the first dedicated phage therapy center was launched in the US.^[33] Additionally, a phage therapy center has been operating in Belgium.^[74]

Development of resistance to phages

One of the major concerns usually associated with phage therapy is the emergence of phage-insensitive mutants (BIMs) that could hinder the success of this therapy. In fact, several *in vitro* studies have reported a fast emergence of BIMs within a short period of time after phage treatment.^{[75][76][77]} The emergence of BIMs has also been observed *in vivo* using different animal models, although usually occurs later than *in vitro* (reviewed in ^[78]). This fast adaptation of bacteria to phage attack is usually caused by mutations on genes encoding phage receptors,^{[76][79]} which include lipopolysaccharides (LPS), outer [membrane proteins](#), [capsules](#), [flagella](#), [pili](#), among others.^[80] However, some studies suggested that when phage resistance is caused by mutations in phage receptors, this might result in fitness costs to the resistance bacterium, which will ultimately become less virulent.^{[78][81]} Moreover, it has been shown that the evolution of bacterial resistance to phage attack changes the efflux pump mechanism, causing increased sensitivity to drugs from several antibiotic classes.^[82] Therefore it is conceivable to think that phage therapy that uses phages that exert selection for MDR bacteria to become antibiotic sensitive could potentially reduce the incidence of antibiotic resistant infections.

Besides the prevention of phage adsorption by loss or modification of bacterial receptors, phage-insensitivity can be caused by: (i) prevention of phage DNA entry by superinfection exclusion systems; (ii) degradation of

phage DNA by [restriction-modification systems](#) or by [CRISPR-Cas systems](#); and (iii) use of abortive infection systems that block phage replication, transcription or translation, usually in conjugation with suicide of the host cell.^[83] Altogether, these mechanisms promote a quick adaptation of bacteria to phage attack and therefore, the emergence of phage-resistance mutants is frequent and unavoidable.

It is still unclear whether the wide use of phages would cause resistance similar to what has been observed for antibiotics. In theory this is not very likely to occur, since phages are very specific and therefore their selective pressure would affect a very narrow group of bacteria. However, we should also consider the fact that many phage resistance systems are mounted on mobile genetic elements, including prophages and plasmids, and thus may spread quite rapidly even without direct selection. Nevertheless, in contrast to antibiotics, phage preparations for therapeutic applications are expected to be developed in a personalized way because of the high specificity of phages. In addition, strategies have been proposed to counter the problem of phage resistance. One of the strategies is use of phage cocktails with complementary host ranges (different host ranges, which - when combined - result in an overall broader host range) and targeting different bacterial receptors. Another strategy is the combination of phages with other antimicrobials such as antibiotics, [disinfectants](#) or [enzymes](#) that could enhance their antibacterial activity. The genetic manipulation of phage genomes can also be a strategy to circumvent phage resistance.

Safety aspects

Bacteriophages are bacterial viruses, evolved to infect bacterial cells; to do that, phages must use characteristic structures at cell surfaces (receptors), and to propagate they need appropriate molecular tools inside the cells. Bacteria are [Prokaryotes](#) and their cells differ substantially from [Eukaryotes](#) including humans or animals. For this reason phages meet the major safety requirement: they do not infect treated individuals. Even engineered phages and induced artificial internalization of phage into mammalian cells did not result in phage propagation. Internalization can be induced e.g. by adding adenovirus penton base protein on the phage surface, it allows for the attachment of engineered phages to integrin receptors and for endocytosis. These mimic adenoviral infection, but no resulting propagation of phage nor any cell damage were observed.^[84] Natural transcytosis of unmodified phages, that is: uptake and internal transport to the other side of a cell, which was observed in human epithelial cells, did not



result in phage propagation or cell damage.^[85] Recently, however, it was reported that filamentous temperate phages of *P. aeruginosa* can be endocytosed into human and murine leukocytes, resulting in transcription of the phage DNA. In turn, the product RNA triggers maladaptive innate viral pattern-recognition responses and thus inhibits the immune clearance of the bacteria.^[86] Whether this also applies to dsDNA phages like the Caudovirales has not yet been established; this is obviously an important question to be addressed as it may affect the overall safety of phage therapy.

Due to many experimental treatments in human patients conducted in past decades, and to already existing RCTs (see section: Clinical experience and randomized controlled trials), phage safety can be assessed directly. The first safety trial in healthy human volunteers for a phage was conducted by Bruttin and Brüssow in 2005;^[87] they investigated the oral administration of *Escherichia coli* phage T4 and they found no adverse effects of the treatment. Historical record shows that phages are safe, with mild side effects if any. The most frequent (though still rare) adverse reactions to phage preparations found in patients were symptoms from the digestive tract, local reactions at the site of administration of a phage preparation, superinfections, and a rise in body temperature.^{[11][88][89]} Notably, these reactions may have been (i) due to the liberation of endotoxins from bacteria lysed in vivo by the phages, since such effects also can be observed when antibiotics are used,^[90] or (ii) caused by bacterial debris that accompanied phage in cases where unpurified lysates were used.

Bacteriophages must be produced in bacteria that are lysed (i.e. fragmented) during phage propagation. As such, phage lysates contain bacterial debris that may affect the human organism even when the phage itself is harmless. For these and other reasons, purification of bacteriophages is considered important and phage preparations need to be assessed for their safety as the whole, particularly when phages are to be administered intravenously. This is consistent with general procedures for other drug candidates. In 2015, a group of phage therapy experts summarized Quality and Safety Requirements for Sustainable Phage Therapy.^[41]

Phage effects on [human microbiome](#) also contribute to the safety issues in phage therapy. It is important to note that many phages, especially temperate phages, carry genes that can affect the pathogenicity of the host. Even the famous lambda, a temperate phage of the *E. coli* K-12 laboratory strain, carries two genes that provide potential virulence benefits to the lysogenic host, one that increases intestinal adherence and the other that confers resistance to complement killing in

the blood. For this reason, temperate phages are generally to be avoided as candidates for phage therapy, although in some cases, the lack of lytic phage candidates and emergency conditions may make such considerations moot.^[48] Another potential problem is generalized transduction, a term for the ability of some phages to transfer bacterial DNA from one host to another. This occurs because the systems for packaging of the phage DNA into capsids can mistakenly package host DNA instead. Indeed, with some well-characterized phages, up to 5% of the virus particles contain only bacterial DNA; thus in a typical lysate, the entire genome of the propagating host is present in more than a million copies in every milliliter. For these reasons, it is imperative that any phage to be considered for therapeutic useage should be subjected to thorough genomic analysis and tested for the capacity for generalized transduction.

As antibacterials, phages may also affect the composition of microbiomes, by infecting and killing phage-sensitive strains of bacteria. However, a major advantage of bacteriophages over antibiotics is the high specificity of bacteriophages; this specificity limits antibacterial activity to a sub-species level, typically a phage kills only selected bacterial strains. For this reason phages are much less likely (than antibiotics) to disturb the composition of natural microbiome or to induce [dysbiosis](#). This was demonstrated in experimental studies where microbiome composition was assessed by [next-generation sequencing \(NGS\)](#) that revealed no important changes correlated with phage treatment in phage human treatments.^{[55][58][91][92][93][94]}

Regulatory aspects

Since its inception in 1923, and until the 1980s, the [Eliava Institute](#) in Georgia has produced tons of therapeutic phage preparations, for the [Red Army](#) as well as for the civil sector, which led to the registration of phages for oral and topical applications as an over-the-counter product in pharmacies in several Member States of the former Soviet Union. Today, the phage production center and the pharmacy of the Eliava Institute manufacture and deliver several commercial phage preparations under a license from the Georgian government. The Ludwik Hirsfeld Institute of Immunology and Experimental Therapy in Wrocław has produced numerous phage formulations for phage therapy in different hospitals in Poland, and for many decades.

In France, phage therapy disappeared officially with the withdrawal of the [Vidal dictionary](#) (France's official drug directory) in 1978. The last phage preparation, produced by l'Institut du Bactériophage, was an ointment against skin infections. Phage therapy research ceased at about the same time in France, with the closure of the bacteriophage department at the [Pasteur Institute](#). However, Professor J.-F. Vieu had collected several hundreds of phages potentially usable against staphylococci and digestive infections and the Pasteur Institutes of Paris and Lyon continued to provide these phages for medical use. As such, some hospital physicians continued to practice compassionate phage therapy until the 1990s when production eventually died out.^[95]

On their rediscovery, at the end the 1990s, phage preparations were logically classified as medicines, i.e. "medicinal products" in the EU or "drugs" in the US.^[96] However, the pharmaceutical legislation that had been implemented since their disappearance from Western medicine was mainly designed to cater for industrially-made pharmaceuticals, devoid of any customization and intended for large-scale distribution,^[97] and it was not deemed necessary to provide phage-specific requirements or concessions. Today's phage therapy products need to comply with the entire battery of medicinal product licensing requirements: manufacturing according to [GMP](#), preclinical studies, phase I, II and III [clinical trials](#) and [marketing authorization](#). Technically, industrially produced predefined phage preparations could make it through the conventional pharmaceutical processes, minding some adaptations. However, phage specificity and resistance issues are likely to cause that these defined preparations will have a relatively short useful lifespan.^[98] In addition, it appeared that the pharmaceutical industry, the stakeholder which is foreseen to develop and market industrially-made medicines, is currently not considering phage therapy products. Yet, a handful of [small and medium-sized enterprises](#) (SMEs) picked up the gauntlet, with the help of risk capital and/or public funding. The reality today is that decades after the renewed interest in the Western world, not one defined therapeutic phage product has made it to the EU or US markets, despite the fact that clinicians are under increasing pressure to use phages in the emergency treatment of [multidrug-resistant](#) bacterial infections.

According to some, therapeutic phages should be prepared individually and kept in large phage banks, ready to be used, upon testing for effectiveness against the patient's bacterial pathogen(s). Intermediary or combined (industrially-made as well as precision phage preparations) approaches could be appropriate.^[98]

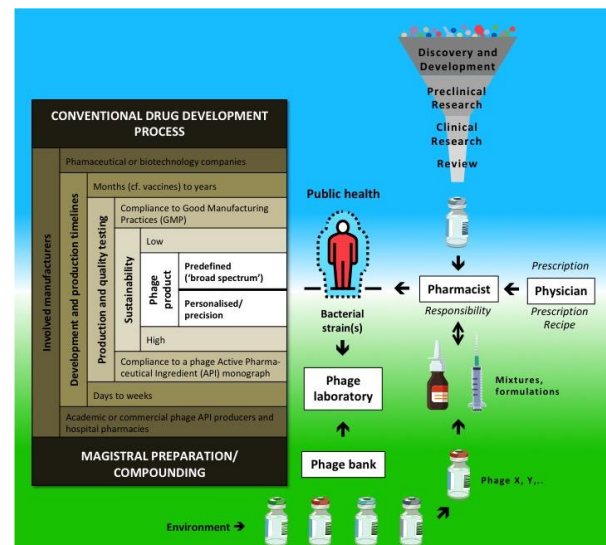


Figure 2 | Conventional drug development process vs. Magistral preparation

However, it turns out to be difficult to reconcile the classical phage therapy concepts, which are based on the timely adaptation of phage preparations, with the current Western pharmaceutical R&D and marketing models. The repeated calls for a specific regulatory framework have not been heeded by the European policy-makers, who appear to be resistant to change in this regard.^[97] A phage therapy framework based on the [Biological Master File](#) (BMF) concept has been proposed as a (European) solution to the regulatory issues, but the European regulation did not allow for an extension of this concept to biologically active substances such as phages.^[99]

Meanwhile, responsible representatives from the medical, academic and regulatory communities have established some (temporary) national solutions. For instance, phage applications have been performed in Europe under the umbrella of Article 37 (Unproven Interventions in Clinical Practice) of the [Helsinki Declaration](#). To enable the application of phage therapy after Poland had joined the EU in 2004, the Ludwik Hirszfeld Institute of Immunology and Experimental Therapy in Wrocław opened its own Phage Therapy Unit (PTU). Phage therapy performed at the PTU is considered an "Experimental Treatment", covered by the adapted Act of 5 December 1996 on the Medical Profession (Polish Law Gazette, 2011, No. 277 item 1634) and Article 37 of the Declaration of Helsinki.^[73] Similarly, in the last few years, a number of phage therapy interventions have been performed in the US under the FDA's emergency Investigational New Drug (eIND) protocol.^[100]



Some patients have been treated with phages under the umbrella of "compassionate use", which is a treatment option that allows a physician to use a not yet authorized medicine in desperate cases. Under strict conditions, medicines under development can be made available for use in patients for whom no satisfactory authorized therapies are available, and who cannot participate in clinical trials. In principle, this approach can only be applied to products for which earlier study results have demonstrated efficacy and safety, but have not yet been approved. Much like Article 37 of the Helsinki Declaration, the compassionate use treatment option can only be applied when the phages are expected to help in life-threatening or chronic and/or seriously debilitating diseases that are not treatable with formally approved products.

In France, l'Agence Nationale de Sécurité du Médicament et des produits de santé (ANSM), the French medicine agency, has organized a specific committee "Comité Scientifique Spécialisé Temporaire (CSST)" for phage therapy, which consists of experts in various fields. Their task is to evaluate and guide each phage therapy requests that ends up at the ANSM. Phage therapy requests are discussed together with the treating physicians and a consensus advice is sent to the ANSM, which will grant permission or not. Between 2006 and 2018, 15 patients have been treated in France (11 healed) using this pathway.^[66]

In Belgium, in 2016 and in response to a number of parliamentary questions, the Minister of Social Affairs and Health acknowledged that it is indeed not evident to treat phages as industrially-made drugs and therefore she proposed to investigate if the magistral preparation pathway could offer a solution.^[98] Magistral preparations (compounding pharmacies in the US) are not subjected to certain constraints such as GMP compliance and marketing authorization. As the "magistral preparation framework" was created to allow for adapted patient treatments and/or to use medicines for which there is no commercial interest, it seemed a suitable framework for precision phage therapy concepts. Magistral preparations are medicines prepared in a pharmacy in accordance with a medical prescription for an individual patient. They are made by a pharmacist (or under his/her supervision) from their constituent ingredients, according to the technical and scientific standards of pharmaceutical technology. Phage **Active Pharmaceutical Ingredients** (APIs) to be included in magistral preparations must meet the requirements of a monograph, which describes their production and quality control testing. They must be accompanied by a certificate of analysis, issued by a "Belgian Approved Laboratory (BAL)", which has been granted an accreditation to

perform batch release testing of medicinal products. Since 2019, phages are delivered in the form of magistral preparations to nominal patients in Belgium.

Dozens of patients have been treated thanks to the above-mentioned national solutions. No safety issues were reported and most targeted infections seemed to have been resolved, but the diversity of these "desperate" phage therapy cases, in terms of clinical indications, involved bacterial pathogens, phage products, and treatment and sampling protocols, make it impossible to unambiguously demonstrate that the positive clinical outcomes were due to phages and the lack of control populations.

It is time to find a broader solution to the phage therapy regulatory issues. Medicine agencies, such as the **European Medicines Agency** (EMA) and the **US Food and Drug Administration** (FDA) are urged to build on the initiatives that were developed by some national regulatory authorities.^[97] Policymakers need to be convinced to open the door for a broad and fast (interim) solution with reduced stringency until the present-day pharmaceutical requirements can be fulfilled, which may require many years.^[101] Phage banks containing large amounts of well-characterized (e.g. host range, annotated genome map) and safe phages need to be set up. Physicians must be aware of the existence and content of these banks.

Additional information

Acknowledgements

All authors are members of the International Society for Viruses of Microorganisms (<http://www.isvm.org/>).

JA, J-PP, KD, MK & RL are members of the FWO Vlaanderen funded "Phagebiotics" research community (WO.016.14).

The work of BB was supported by the Walloon Public Service, BIOWIN project: Inteliphages

Competing interests

JA, DPP, J-PP, KD, MK & RL declare no competing interests.

BB is employed by Vésale Biosciences, which is a company dedicated to commercially developing personalized phage therapy for human applications.

Ethics statement

None required



References

- Twort, F. W. (1915-12-04). "AN INVESTIGATION ON THE NATURE OF ULTRA-MICROSCOPIC VIRUSES". *The Lancet*. Originally published as Volume 2, Issue 4814 **186** (4814): 1241–1243. doi:10.1016/S0140-6736(01)20383-3. ISSN 0140-6736.
- d'Herelle, F. (1916). Sur un bacille dysentérique atypique. Ann. de l'Inst. Pasteur, 30, 145.
- D'Herelle, M. F. (1961-02). *Sur un microbe invisible antagoniste des bacilles dysentériques* (in fr). ISSN 0515-2909.
- d'HERELLE, F. (1919). Sur le rôle du microbe bactériophage dans la typhose aviaire. *CR Acad. Sci.*, 169, 932-934.
- Lavigne, Rob; Robben, Johan (2012-01-01). "Professor Dr. Richard Bruynoghe: A 1951 overview of his bacteriophage research spanning three decades". *Bacteriophage* **2** (1): 1–4. doi:10.4161/bact.20024. ISSN 2159-7073. PMID 22666651. PMC 3357380.
- Abedon, Stephen T.; Kuhl, Sarah J.; Blasdel, Bob G.; Kutter, Elizabeth Martin (2011-03-01). "Phage treatment of human infections". *Bacteriophage* **1** (2): 66–85. doi:10.4161/bact.1.2.15845. PMID 22334863. PMC PMC3278644.
- Eaton, Monroe D.; Bayne-Jones, Stanhope (1934-12-08). "BACTERIOPHAGE THERAPY: REVIEW OF THE PRINCIPLES AND RESULTS OF THE USE OF BACTERIOPHAGE IN THE TREATMENT OF INFECTIONS". *Journal of the American Medical Association* **103** (23): 1769–1776. doi:10.1001/jama.1934.72750490003007. ISSN 0002-9955.
- Myelnikov, Dmitriy (2018-10-01). "An Alternative Cure: The Adoption and Survival of Bacteriophage Therapy in the USSR, 1922–1955". *Journal of the History of Medicine and Allied Sciences* **73** (4): 385–411. doi:10.1093/jhmas/jry024. ISSN 0022-5045.
- Chanishvili, Nina (2012). *A Literature Review of the Practical Application of Bacteriophage Research* (in en). Nova Biomedical Books. ISBN 978-1-62100-851-4.
- Sulakvelidze, A.; Alavidze, Z.; Morris, J. G. (2001-03). "Bacteriophage therapy". *Antimicrobial Agents and Chemotherapy* **45** (3): 649–659. doi:10.1128/AAC.45.3.649-659.2001. ISSN 0066-4804. PMID 11181338.
- Międzybrodzki, Ryszard; Borysowski, Jan; Weber-Dąbrowska, Beata; Fortuna, Wojciech; Letkiewicz, Sławomir; Szufnarowski, Krzysztof; Pawełczyk, Zdzisław; Rogóż, Paweł et al. (2012). "Clinical aspects of phage therapy". *Advances in Virus Research* **83**: 73–121. doi:10.1016/B978-0-12-394438-2.00003-7. ISSN 1557-8399. PMID 22748809.
- Hendrix, R. W.; Smith, M. C. M.; Burns, R. N.; Ford, M. E.; Hatfull, G. F. (1999-03-02). "Evolutionary relationships among diverse bacteriophages and prophages: All the world's a phage". *Proceedings of the National Academy of Sciences* **96** (5): 2192–2197. doi:10.1073/pnas.96.5.2192. ISSN 0027-8424. PMID 10051617. PMC PMC26759.
- Mushegian, A. R. (2020-02-18). Margolin, William. ed. "Are There 10 31 Virus Particles on Earth, or More, or Fewer?". *Journal of Bacteriology* **202** (9): e00052–20. /jeb/202/9/JB.00052–20.atom. doi:10.1128/JB.00052-20. ISSN 0021-9193. PMID 32071093. PMC PMC7148134.
- Gill, Jason J.; Hyman, Paul (2010-01). "Phage choice, isolation, and preparation for phage therapy". *Current Pharmaceutical Biotechnology* **11** (1): 2–14. doi:10.2174/138920110790725311. ISSN 1873-4316. PMID 20214604.
- Goodridge, Lawrence D. (2010-01). "Designing phage therapeutics". *Current Pharmaceutical Biotechnology* **11** (1): 15–27. doi:10.2174/138920110790725348. ISSN 1873-4316. PMID 20214605.
- Jernberg, Cecilia; Löfmark, Sonja; Edlund, Charlotta; Jansson, Janet K. (2010-11). "Long-term impacts of antibiotic exposure on the human intestinal microbiota". *Microbiology (Reading, England)* **156** (Pt 11): 3216–3223. doi:10.1099/mic.0.040618-0. ISSN 1465-2080. PMID 20705661.
- Manohar, Prasanth; Ramesh, Nachimuthu (2019-12). "Improved lyophilization conditions for long-term storage of bacteriophages". *Scientific Reports* **9** (1): 15242. doi:10.1038/s41598-019-51742-4. ISSN 2045-2322. PMID 31645642. PMC PMC6811570.
- Wu, Nannan; Zhu, Tongyu (2021-01-28). "Potential of Therapeutic Bacteriophages in Nosocomial Infection Management". *Frontiers in Microbiology* **12**: 638094. doi:10.3389/fmicb.2021.638094. ISSN 1664-302X. PMID 33633717. PMC PMC7901949.
- Pirnay, Jean-Paul; De Vos, Daniel; Verbeken, Gilbert; Merabishvili, Maia; Chanishvili, Nina; Vaneechoutte, Mario; Zizi, Martin; Laire, Geert et al. (2011-04). "The phage therapy paradigm: prêt-à-porter or sur-mesure?". *Pharmaceutical Research* **28** (4): 934–937. doi:10.1007/s10955-010-0313-5. ISSN 1573-904X. PMID 21063753.
- Dunne, Matthew; Rupf, Beatrice; Tala, Marc; Qabrati, Xhem; Ernst, Patrick; Shen, Yang; Sumrall, Eric; Heeb, Laura et al. (2019-10-29). "Reprogramming Bacteriophage Host Range through Structure-Guided Design of Chimeric Receptor Binding Proteins". *Cell Reports* **29** (5): 1336–1350.e4. doi:10.1016/j.celrep.2019.09.062. ISSN 2211-1247. PMID 31665644.
- Vandersteegen, Katrien; Mattheus, Wesley; Ceyssens, Pieter-Jan; Bilocq, Florence; De Vos, Daniel; Pirnay, Jean-Paul; Noben, Jean-Paul; Merabishvili, Maia et al. (2011). "Microbiological and molecular assessment of bacteriophage ISP for the control of *Staphylococcus aureus*". *PLoS One* **6** (9): e24418. doi:10.1371/journal.pone.0024418. ISSN 1932-6203. PMID 21931710. PMC 3170307.
- Jault, Patrick; Leclerc, Thomas; Jennes, Serge; Pirnay, Jean Paul; Que, Yok-Ai; Resch, Gregory; Rousseau, Anne Françoise; Ravat, François et al. (01 2019). "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". *The Lancet. Infectious Diseases* **19** (1): 35–45. doi:10.1016/S1473-3099(18)30482-1. ISSN 1474-4457. PMID 30292481.
- Hall, Alex R.; De Vos, Daniel; Friman, Ville-Petri; Pirnay, Jean-Paul; Buckling, Angus (2012-08). "Effects of sequential and simultaneous applications of bacteriophages on populations of *Pseudomonas aeruginosa* in vitro and in wax moth larvae". *Applied and Environmental Microbiology* **78** (16): 5646–5652. doi:10.1128/AEM.00757-12. ISSN 1098-5336. PMID 22660719. PMC 3406105.
- Friman, V.-P.; Soanes-Brown, D.; Sierocinski, P.; Molin, S.; Johansen, H. K.; Merabishvili, M.; Pirnay, J.-P.; De Vos, D. et al. (01 2016). "Pre-adapting parasitic phages to a pathogen leads to increased pathogen clearance and lowered resistance evolution with *Pseudomonas aeruginosa* cystic fibrosis bacterial isolates". *Journal of Evolutionary Biology* **29** (1): 188–198. doi:10.1111/jeb.12774. ISSN 1420-9101. PMID 26476097.
- Muniesa, Maite; Hammerl, Jens A.; Hertwig, Stefan; Appel, Bernd; Brüssow, Harald (2012-06). "Shiga toxin-producing *Escherichia coli* O104:H4: a new challenge for microbiology". *Applied and Environmental Microbiology* **78** (12): 4065–4073. doi:10.1128/AEM.00217-12. ISSN 1098-5336. PMID 22504816. PMC 3370534.
- Azeredo, J.; Sutherland, I. W. (2008-08). "The use of phages for the removal of infectious biofilms". *Current Pharmaceutical Biotechnology* **9** (4): 261–266. doi:10.2174/138920108785161604. ISSN 1873-4316. PMID 18691087.
- Pires, D. P.; Melo, Ldr; Vilas Boas, D.; Sillankorva, S.; Azeredo, J. (2017-10). "Phage therapy as an alternative or complementary strategy to prevent and control biofilm-related infections". *Current Opinion in Microbiology* **39**: 48–56. doi:10.1016/j.mib.2017.09.004. ISSN 1879-0364. PMID 28964986.
- Chan, Benjamin K.; Abedon, Stephen T. (2015). "Bacteriophages and their enzymes in biofilm control". *Current Pharmaceutical Design* **21** (1): 85–99. doi:10.2174/1381612820666140905112311. ISSN 1873-4286. PMID 25189866.
- Gutiérrez, Diana; Rodríguez-Rubio, Lorena; Martínez, Beatriz; Rodríguez, Ana; García, Pilar (2016). "Bacteriophages as Weapons Against Bacterial Biofilms in the Food Industry". *Frontiers in Microbiology* **7**: 825. doi:10.3389/fmicb.2016.00825. ISSN 1664-302X. PMID 27375566. PMC 4897796.
- Melo, Luís D. R.; Pires, Diana Priscila; Monteiro, Rodrigo; Azeredo, Joana (2019). Górski, Andrzej. ed. *Phage Therapy: A Practical Approach* (in en). Cham: Springer International Publishing. pp. 295–313. doi:10.1007/978-3-030-26736-0_11. ISBN 978-3-030-26736-0.
- Duplessis, Christopher Anthony; Biswas, Biswajit (2020-07-04). "A Review of Topical Phage Therapy for Chronically Infected Wounds and Preparations for a Randomized Adaptive Clinical Trial Evaluating Topical Phage Therapy in Chronically Infected Diabetic Foot Ulcers". *Antibiotics* **9** (7): 377. doi:10.3390/antibiotics9070377. ISSN 2079-6382. PMID 32635429. PMC PMC7400337.
- Cano, Edison J.; Cafilisch, Katherine M; Bollyky, Paul L; Van Belleghem, Jonas D; Patel, Robin; Fackler, Joseph; Brownstein, Michael J; Horne, Bri'Anna et al. (2020-07-23). "Phage Therapy for Limb-threatening Prosthetic Knee *Klebsiella pneumoniae* Infection: Case Report and In Vitro Characterization of Anti-biofilm Activity". *Clinical Infectious Diseases: ciae705*. doi:10.1093/cid/ciae705. ISSN 1058-4838.
- Aslam, Saima; Lampley, Elizabeth; Wooten, Darcy; Karris, Maile; Benson, Constance; Strathdee, Steffanie; Schooley, Robert T (2020-09-01). "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 Infectious Diseases* **7** (9): ofaa389. doi:10.1093/ofid/ofaa389. ISSN 2328-8957. PMID 33005701. PMC PMC7519779.
- Tagliaferri, Thaysa Leite; Jansen, Mathias; Horz, Hans-Peter (2019). "Fighting Pathogenic Bacteria on Two Fronts: Phages and Antibiotics as Combined Strategy". *Frontiers in Cellular and Infection Microbiology* **9**: 22. doi:10.3389/fcimb.2019.00022. ISSN 2235-2988. PMID 30834237. PMC 6387922.



35. Segall, Anca M; Roach, Dwayne R; Strathdee, Steffanie A (2019-10). "Stronger together? Perspectives on phage-antibiotic synergy in clinical applications of phage therapy". *Current Opinion in Microbiology* **51**: 46–50. doi:10.1016/j.mib.2019.03.005.
36. Akturk, Ergun; Oliveira, Hugo; Santos, Sílvia B.; Costa, Susana; Kuyumcu, Suleyman; Melo, Luís D. R.; Azeredo, Joana (2019-07-25). "Synergistic Action of Phage and Antibiotics: Parameters to Enhance the Killing Efficacy Against Mono and Dual-Species Biofilms". *Antibiotics (Basel, Switzerland)* **8** (3). doi:10.3390/antibiotics8030103. ISSN 2079-6382. PMID 31349628. PMC 6783858.
37. Comeau, André M.; Tétart, Françoise; Trojet, Sabrina N.; Prère, Marie-Françoise; Krisch, H. M. (2007-08-29). "Phage-Antibiotic Synergy (PAS): beta-lactam and quinolone antibiotics stimulate virulent phage growth". *PLoS One* **2** (8): e799. doi:10.1371/journal.pone.0000799. ISSN 1932-6203. PMID 17726529. PMC 1949050.
38. Verma, Vivek; Harjai, Kusum; Chhibber, Sanjay (2009-12). "Restricting ciprofloxacin-induced resistant variant formation in biofilm of *Klebsiella pneumoniae* B5055 by complementary bacteriophage treatment". *The Journal of Antimicrobial Chemotherapy* **64** (6): 1212–1218. doi:10.1093/jac/dkp360. ISSN 1460-2091. PMID 19808232.
39. Torres-Barceló, Clara; Hochberg, Michael E. (2016-04). "Evolutionary Rationale for Phages as Complements of Antibiotics". *Trends in Microbiology* **24** (4): 249–256. doi:10.1016/j.tim.2015.12.011. ISSN 1878-4380. PMID 26786863.
40. Mutti, Michele; Corsini, Lorenzo (2019). "Robust Approaches for the Production of Active Ingredient and Drug Product for Human Phage Therapy". *Frontiers in Microbiology* **10**: 2289. doi:10.3389/fmicb.2019.02289. ISSN 1664-302X. PMID 31649636. PMC 6791927.
41. Pirnay, Jean-Paul; Blasdel, Bob G.; Breteau, Laurent; Buckling, Angus; Chanishvili, Nina; Clark, Jason R.; Corte-Real, Sofia; Debarbieux, Laurent et al. (2015-07). "Quality and safety requirements for sustainable phage therapy products". *Pharmaceutical Research* **32** (7): 2173–2179. doi:10.1007/s11095-014-1617-7. ISSN 1573-904X. PMID 25585954. PMC 4452253.
42. Hargreaves, Katherine R.; Clokie, Martha R. J. (2014). "Clostridium difficile phages: still difficult?". *Frontiers in Microbiology* **5**: 184. doi:10.3389/fmicb.2014.00184. ISSN 1664-302X. PMID 24808893. PMC 4009436.
43. Hietala, Ville; Horsma-Heikkinen, Jenni; Carron, Annelie; Skurnik, Mikael; Kiljunen, Saija (2019). "The Removal of Endo- and Enterotoxins From Bacteriophage Preparations". *Frontiers in Microbiology* **10**: 1674. doi:10.3389/fmicb.2019.01674. ISSN 1664-302X. PMID 31396188. PMC 6664067.
44. Luong, Tiffany; Salabarria, Ann-Charlott; Edwards, Robert A.; Roach, Dwayne R. (2020-09). "Standardized bacteriophage purification for personalized phage therapy". *Nature Protocols* **15** (9): 2867–2890. doi:10.1038/s41596-020-0346-0. ISSN 1754-2189.
45. Malik, Danish J.; Sokolov, Ilya J.; Vinner, Gurinder K.; Mancuso, Francesco; Cinquerrui, Salvatore; Vladislavjevic, Goran T.; Clokie, Martha R. J.; Garton, Natalie J. et al. (2017-11). "Formulation, stabilisation and encapsulation of bacteriophage for phage therapy". *Advances in Colloid and Interface Science* **249**: 100–133. doi:10.1016/j.cis.2017.05.014. ISSN 1873-3727. PMID 28688779.
46. Otero, Jennifer; García-Rodríguez, Alba; Cano-Sarabia, Mary; Maspocho, Daniel; Marcos, Ricard; Cortés, Pilar; Llagostera, Montserrat (2019). "Biodistribution of Liposome-Encapsulated Bacteriophages and Their Transcytosis During Oral Phage Therapy". *Frontiers in Microbiology* **10**: 689. doi:10.3389/fmicb.2019.00689. ISSN 1664-302X. PMID 31019499. PMC 6458305.
47. Pires, Diana P.; Cleto, Sara; Sillankorva, Sanna; Azeredo, Joana; Lu, Timothy K. (09 2016). "Genetically Engineered Phages: a Review of Advances over the Last Decade". *Microbiology and molecular biology reviews: MMBR* **80** (3): 523–543. doi:10.1128/MMBR.00069-15. ISSN 1098-5557. PMID 27250768. PMC 4981678.
48. Dedrick, Rebekah M.; Guerrero-Bustamante, Carlos A.; Garlena, Rebecca A.; Russell, Daniel A.; Ford, Katrina; Harris, Kathryn; Gilmour, Kimberly C.; Soothill, James et al. (05 2019). "Engineered bacteriophages for treatment of a patient with a disseminated drug-resistant *Mycobacterium abscessus*". *Nature Medicine* **25** (5): 730–733. doi:10.1038/s41591-019-0437-z. ISSN 1546-170X. PMID 31068712. PMC 6557439.
49. Melo, Luís D. R.; Oliveira, Hugo; Pires, Diana P.; Dabrowska, Krystyna; Azeredo, Joana (2020-02). "Phage therapy efficacy: a review of the last 10 years of preclinical studies". *Critical Reviews in Microbiology* **46** (1): 78–99. doi:10.1080/1040841X.2020.1729695. ISSN 1549-7828. PMID 32091280.
50. Dąbrowska, Krystyna (09 2019). "Phage therapy: What factors shape phage pharmacokinetics and bioavailability? Systematic and critical review". *Medicinal Research Reviews* **39** (5): 2000–2025. doi:10.1002/med.21572. ISSN 1098-1128. PMID 30887551. PMC 6767042.
51. Debarbieux, Laurent; Pirnay, Jean-Paul; Verbeken, Gilbert; De Vos, Daniel; Merabishvili, Maia; Huys, Isabelle; Patey, Olivier; Schoonjans, Dirk et al. (2016-01). "A bacteriophage journey at the European Medicines Agency". *FEMS microbiology letters* **363** (2): fnv225. doi:10.1093/femsle/fnv225. ISSN 1574-6968. PMID 26656541. PMC 5812529.
52. Wright, A.; Hawkins, C. H.; Anggård, E. E.; Harper, D. R. (2009-08). "A controlled clinical trial of a therapeutic bacteriophage preparation in chronic otitis due to antibiotic-resistant *Pseudomonas aeruginosa*; a preliminary report of efficacy". *Clinical otolaryngology: official journal of ENT-UK; official journal of Netherlands Society for Oto-Rhino-Laryngology & Cervico-Facial Surgery* **34** (4): 349–357. doi:10.1111/j.1749-4486.2009.01973.x. ISSN 1749-4486. PMID 19673983.
53. Servick, Kelly (2016-06-24). "DRUG DEVELOPMENT. Beleaguered phage therapy trial presses on". *Science (New York, N.Y.)* **352** (6293): 1506. doi:10.1126/science.352.6293.1506. ISSN 1095-9203. PMID 27339963.
54. Glesinger, Ronen; Cohen, Arnon D.; Bogdanov-Berezovsky, Alex; Krieger, Yuval; Rosenberg, Lior (2004-04). "A Randomized Controlled Trial of Silver Sulfadiazine, Biafine, and Saline-soaked Gauze in the Treatment of Superficial Partial-thickness Burn Wounds in Pigs". *Academic Emergency Medicine* **11** (4): 339–342. doi:10.1197/j.aem.2003.11.015.
55. Sarker, Shafiqul Alam; Berger, Bernard; Deng, Ying; Kieser, Silas; Foata, Francis; Moine, Deborah; Descombes, Patrick; Sultana, Shamima et al. (01 2017). "Oral application of *Escherichia coli* bacteriophage: safety tests in healthy and diarrheal children from Bangladesh". *Environmental Microbiology* **19** (1): 237–250. doi:10.1111/1462-2920.13574. ISSN 1462-2920. PMID 27750388.
56. Ooi, Mian Li; Drilling, Amanda Jane; Morales, Sandra; Fong, Stephanie; Moraitis, Sophia; Macias-Valle, Luis; Vreugde, Sarah; Psaltis, Alkis James et al. (2019-06-20). "Safety and Tolerability of Bacteriophage Therapy for Chronic Rhinosinusitis Due to *Staphylococcus aureus*". *JAMA otolaryngology-- head & neck surgery*. doi:10.1001/jamaoto.2019.1191. ISSN 2168-619X. PMID 31219531. PMC 6587246.
57. Ujmajuridze, Aleksandre; Chanishvili, Nina; Goderdzishvili, Marina; Leitner, Lorenz; Mehnert, Ulrich; Chkhotua, Archil; Kessler, Thomas M.; Sybesma, Wilbert (2018). "Adapted Bacteriophages for Treating Urinary Tract Infections". *Frontiers in Microbiology* **9**: 1832. doi:10.3389/fmicb.2018.01832. ISSN 1664-302X. PMID 30131795. PMC 6090023.
58. McCallin, Shawna; Sarker, Shafiqul A.; Sultana, Shamima; Oechslein, Frank; Brüssow, Harald (09 2018). "Metagenome analysis of Russian and Georgian Pyophage cocktails and a placebo-controlled safety trial of single phage versus phage cocktail in healthy *Staphylococcus aureus* carriers". *Environmental Microbiology* **20** (9): 3278–3293. doi:10.1111/1462-2920.14310. ISSN 1462-2920. PMID 30051571.
59. Fish, Randolph; Kutter, Elizabeth; Wheat, Gordon; Blasdel, Bob; Kutateladze, Mzia; Kuhl, Sarah (2018). "Compassionate Use of Bacteriophage Therapy for Foot Ulcer Treatment as an Effective Step for Moving Toward Clinical Trials". *Methods in Molecular Biology (Clifton, N.J.)* **1693**: 159–170. doi:10.1007/978-1-4939-7395-8_14. ISSN 1940-6029. PMID 29119440.
60. Febvre, Hallie P.; Rao, Sangeeta; Gindin, Melinda; Goodwin, Natalie D. M.; Finer, Elijah; Vivanco, Jorge S.; Lu, Shen; Manter, Daniel K. et al. (2019-03-20). "PHAGE Study: Effects of Supplemental Bacteriophage Intake on Inflammation and Gut Microbiota in Healthy Adults". *Nutrients* **11** (3). doi:10.3390/nu11030666. ISSN 2072-6643. PMID 30897686. PMC 6471193.
61. Letkiewicz, S.; Miedzybrodzki, R.; Fortuna, W.; Weber-Dąbrowska, B.; Górski, A. (2009-09). "Eradication of *Enterococcus faecalis* by phage therapy in chronic bacterial prostatitis—case report". *Folia Microbiologica* **54** (5): 457–461. doi:10.1007/s12223-009-0064-z. ISSN 1874-9356. PMID 19937220.
62. Jennes, Serge; Merabishvili, Maia; Soentjens, Patrick; Pang, Kim Win; Rose, Thomas; Keersebilck, Elkana; Soete, Olivier; François, Pierre-Michel et al. (2017-06-04). "Use of bacteriophages in the treatment of colistin-only-sensitive *Pseudomonas aeruginosa* septicemia in a patient with acute kidney injury—a case report". *Critical Care (London, England)* **21** (1): 129. doi:10.1186/s13054-017-1709-y. ISSN 1466-609X. PMID 28583189. PMC 5460490.
63. Schooley, Robert T.; Biswas, Biswajit; Gill, Jason J.; Hernandez-Morales, Adriana; Lancaster, Jacob; Lessor, Lauren; Barr, Jeremy J.; Reed, Sharon L. et al. (10 2017). "Development and Use of Personalized Bacteriophage-Based Therapeutic Cocktails To Treat a Patient with a Disseminated Resistant *Acinetobacter baumannii* Infection". *Antimicrobial Agents and Chemotherapy* **61** (10). doi:10.1128/AAC.00954-17. ISSN 1098-6596. PMID 28807909. PMC 5610518.



64. Zhvania, Pikria; Hoyle, Naomi Sulinger; Nadareishvili, Lia; Nizharadze, Dea; Kutateladze, Mzia (2017). "Phage Therapy in a 16-Year-Old Boy with Netherton Syndrome". *Frontiers in Medicine* **4**: 94. doi:10.3389/fmed.2017.00094. ISSN 2296-858X. PMID 28717637. PMC 5494523.
65. Hoyle, N.; Zhvaniya, P.; Balarjishvili, N.; Bolkvadze, D.; Nadareishvili, L.; Nizharadze, D.; Wittmann, J.; Rohde, C. et al. (2018-11). "Phage therapy against *Achromobacter xylosoxidans* lung infection in a patient with cystic fibrosis: a case report". *Research in Microbiology* **169** (9): 540–542. doi:10.1016/j.resmic.2018.05.001. ISSN 1769-7123. PMID 29777836.
66. Patey, Olivier; McCallin, Shawna; Mazure, Hubert; Liddle, Max; Smithyman, Anthony; Dublanchet, Alain (12 28, 2018). "Clinical Indications and Compassionate Use of Phage Therapy: Personal Experience and Literature Review with a Focus on Osteoarticular Infections". *Viruses* **11** (1). doi:10.3390/v11010018. ISSN 1999-4915. PMID 30597868. PMC 6356659.
67. Chan, Benjamin K.; Turner, Paul E.; Kim, Samuel; Mojibian, Hamid R.; Eleftheriades, John A.; Narayan, Deepak (2018). "Phage treatment of an aortic graft infected with *Pseudomonas aeruginosa*". *Evolution, Medicine, and Public Health* **2018** (1): 60–66. doi:10.1093/emph/eoy005. ISSN 2050-6201. PMID 29588855. PMC 5842392.
68. Onsea, Jolien; Soentjens, Patrick; Djebara, Sarah; Merabishvili, Maia; Depypere, Melissa; Spriet, Isabel; De Munter, Paul; Debaveye, Yves et al. (09 23, 2019). "Bacteriophage Application for Difficult-to-treat Musculoskeletal Infections: Development of a Standardized Multidisciplinary Treatment Protocol". *Viruses* **11** (10). doi:10.3390/v11100891. ISSN 1999-4915. PMID 31548497. PMC 6832313.
69. Corbellino, Mario; Kieffer, Nicolas; Kutateladze, Mzia; Balarjishvili, Nana; Leshkasheli, Lika; Askilashvili, Lia; Tsertsvadze, George; Rimoldi, Sara Giordana et al. (2020-04-15). "Eradication of a Multidrug-Resistant, Carbapenemase-Producing *Klebsiella pneumoniae* Isolate Following Oral and Intra-rectal Therapy With a Custom Made, Lytic Bacteriophage Preparation". *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America* **70** (9): 1998–2001. doi:10.1093/cid/ciz782. ISSN 1537-6591. PMID 31414123.
70. Law, Nancy; Logan, Cathy; Yung, Gordon; Furr, Carrie-Lynn Langlais; Lehman, Susan M.; Morales, Sandra; Rosas, Francisco; Gaidamaka, Alexander et al. (2019-08). "Successful adjunctive use of bacteriophage therapy for treatment of multidrug-resistant *Pseudomonas aeruginosa* infection in a cystic fibrosis patient". *Infection* **47** (4): 665–668. doi:10.1007/s15010-019-01319-0. ISSN 1439-0973. PMID 31102236.
71. Aslam, Saima; Courtwright, Andrew M.; Koval, Christine; Lehman, Susan M.; Morales, Sandra; Furr, Carrie-Lynn Langlais; Rosas, Francisco; Brownstein, Michael J. et al. (09 2019). "Early clinical experience of bacteriophage therapy in 3 lung transplant recipients". *American Journal of Transplantation: Official Journal of the American Society of Transplantation and the American Society of Transplant Surgeons* **19** (9): 2631–2639. doi:10.1111/ajt.15503. ISSN 1600-6143. PMID 31207123. PMC 6711787.
72. Maddocks, Susan; Fabijan, Aleksandra Petrovic; Ho, Josephine; Lin, Ruby C. Y.; Ben Zakour, Nouri L.; Dugan, Chris; Kliman, Ivana; Branston, Steven et al. (11 01, 2019). "Bacteriophage Therapy of Ventilator-associated Pneumonia and Empyema Caused by *Pseudomonas aeruginosa*". *American Journal of Respiratory and Critical Care Medicine* **200** (9): 1179–1181. doi:10.1164/rccm.201904-0839LE. ISSN 1535-4970. PMID 31437402.
73. Henein, Alexandra (2013-04-01). "What are the limitations on the wider therapeutic use of phage?". *Bacteriophage* **3** (2): e24872. doi:10.4161/bact.24872. ISSN 2159-7073. PMID 24228220. PMC 3821673.
74. Djebara, Sarah; Maussen, Christiane; De Vos, Daniel; Merabishvili, Maya; Damanet, Benjamin; Pang, Kim; De Leenheer, Peggy; Strachinaru, Isabella et al. (2019-03-17). "Processing Phage Therapy Requests in a Brussels Military Hospital: Lessons Identified". *Viruses* **11** (3): 265. doi:10.3390/v11030265. ISSN 1999-4915. PMID 30884879. PMC 6466067.
75. Fu, Weiling; Forster, Terri; Mayer, Oren; Curtin, John J.; Lehman, Susan M.; Donlan, Rodney M. (2010-01). "Bacteriophage cocktail for the prevention of biofilm formation by *Pseudomonas aeruginosa* on catheters in an in vitro model system". *Antimicrobial Agents and Chemotherapy* **54** (1): 397–404. doi:10.1128/AAC.00669-09. ISSN 1098-6596. PMID 19822702. PMC 2798481.
76. Pires, Diana P.; Dötsch, Andreas; Anderson, Erin M.; Hao, Youai; Khursigara, Cezar M.; Lam, Joseph S.; Sillankorva, Sanna; Azeredo, Joana (2017). "A Genotypic Analysis of Five *P. aeruginosa* Strains after Biofilm Infection by Phages Targeting Different Cell Surface Receptors". *Frontiers in Microbiology* **8**: 1229. doi:10.3389/fmicb.2017.01229. ISSN 1664-302X. PMID 28713356. PMC 5492357.
77. Le, Shuai; Yao, Xinyue; Lu, Shuguang; Tan, Yinling; Rao, Xiancai; Li, Ming; Jin, Xiaolin; Wang, Jing et al. (2014-04-28). "Chromosomal DNA deletion confers phage resistance to *Pseudomonas aeruginosa*". *Scientific Reports* **4**: 4738. doi:10.1038/srep04738. ISSN 2045-2322. PMID 24770387. PMC 4001099.
78. Oechslin, Frank (06 30, 2018). "Resistance Development to Bacteriophages Occurring during Bacteriophage Therapy". *Viruses* **10** (7). doi:10.3390/v10070351. ISSN 1999-4915. PMID 29966329. PMC 6070868.
79. Oechslin, Frank; Piccardi, Philippe; Mancini, Stefano; Gabard, Jérôme; Moreillon, Philippe; Entenza, José M.; Resch, Gregory; Que, Yok-Ai (03 01, 2017). "Synergistic Interaction Between Phage Therapy and Antibiotics Clears *Pseudomonas aeruginosa* Infection in Endocarditis and Reduces Virulence". *The Journal of Infectious Diseases* **215** (5): 703–712. doi:10.1093/infdis/jiw632. ISSN 1537-6613. PMID 28007922. PMC 5388299.
80. Bertozzi Silva, Juliana; Storms, Zachary; Sauvageau, Dominic (2016-02). "Host receptors for bacteriophage adsorption". *FEMS microbiology letters* **363** (4). doi:10.1093/femsle/fnw002. ISSN 1574-6968. PMID 26755501.
81. León, Marcela; Bastías, Roberto (2015). "Virulence reduction in bacteriophage resistant bacteria". *Frontiers in Microbiology* **6**. doi:10.3389/fmicb.2015.00343. ISSN 1664-302X.
82. Chan, Benjamin K.; Siström, Mark; Wertz, John E.; Kortright, Kaitlyn E.; Narayan, Deepak; Turner, Paul E. (2016-07). "Phage selection restores antibiotic sensitivity in MDR *Pseudomonas aeruginosa*". *Scientific Reports* **6** (1): 26717. doi:10.1038/srep26717. ISSN 2045-2322. PMID 27225966. PMC 64880932.
83. Labrie, Simon J.; Samson, Julie E.; Moineau, Sylvain (2010-05). "Bacteriophage resistance mechanisms". *Nature Reviews. Microbiology* **8** (5): 317–327. doi:10.1038/nrmicro2315. ISSN 1740-1534. PMID 20348932.
84. Di Giovine, M.; Salone, B.; Martina, Y.; Amati, V.; Zamburo, G.; Cundari, E.; Failla, C. M.; Saggio, I. (2001-03-30). "Binding properties, cell delivery, and gene transfer of adenoviral penton base displaying bacteriophage". *Virology* **282** (1): 102–112. doi:10.1006/viro.2000.0809. ISSN 0042-6822. PMID 11259194.
85. Nguyen, Sophie; Baker, Kristi; Padman, Benjamin S.; Patwa, Ruzeen; Dunstan, Rhys A.; Weston, Thomas A.; Schlosser, Kyle; Bailey, Barbara et al. (11 21, 2017). "Bacteriophage Transcytosis Provides a Mechanism To Cross Epithelial Cell Layers". *mBio* **8** (6). doi:10.1128/mBio.01874-17. ISSN 2150-7511. PMID 29162715. PMC 5698557.
86. Sweere, Johanna M.; Van Belleghem, Jonas D.; Ishak, Heather; Bach, Michelle S.; Popescu, Medeea; Sunkari, Vivekananda; Kaber, Gernot; Manasherob, Robert et al. (2019-03-29). "Bacteriophage trigger antiviral immunity and prevent clearance of bacterial infection". *Science* **363** (6434): eaat9691. doi:10.1126/science.aat9691. ISSN 0036-8075. PMID 30923196. PMC 6656896.
87. Bruttin, Anne; Brüssow, Harald (2005-07). "Human volunteers receiving *Escherichia coli* phage T4 orally: a safety test of phage therapy". *Antimicrobial Agents and Chemotherapy* **49** (7): 2874–2878. doi:10.1128/AAC.49.7.2874-2878.2005. ISSN 0066-4804. PMID 15980363. PMC 1168693.
88. Kutter, Elizabeth; De Vos, Daniel; Gvasalia, Guram; Alavidze, Zemphira; Gogokhia, Lasha; Kuhl, Sarah; Abedon, Stephen T. (2010-01). "Phage therapy in clinical practice: treatment of human infections". *Current Pharmaceutical Biotechnology* **11** (1): 69–86. doi:10.2174/138920110790725401. ISSN 1873-4316. PMID 20214609.
89. Speck, Peter; Smithyman, Anthony (2016-02). "Safety and efficacy of phage therapy via the intravenous route". *FEMS microbiology letters* **363** (3). doi:10.1093/femsle/fnv242. ISSN 1574-6968. PMID 26691737.
90. Prins, J. M.; van Deventer, S. J.; Kuijper, E. J.; Speelman, P. (1994-06). "Clinical relevance of antibiotic-induced endotoxin release". *Antimicrobial Agents and Chemotherapy* **38** (6): 1211–1218. doi:10.1128/aac.38.6.1211. ISSN 0066-4804. PMID 8092816.
91. Sarker, Shafiqul Alam; McCallin, Shawna; Barretto, Caroline; Berger, Bernard; Pittet, Anne-Cécile; Sultana, Shamima; Krause, Lutz; Huq, Sayeda et al. (2012-12-20). "Oral T4-like phage cocktail application to healthy adult volunteers from Bangladesh". *Virology* **434** (2): 222–232. doi:10.1016/j.virol.2012.09.002. ISSN 1096-0341. PMID 23102968.
92. Sarker, Shafiqul Alam; Sultana, Shamima; Reuteler, Gloria; Moine, Deborah; Descombes, Patrick; Charton, Florence; Bourdin, Gilles; McCallin, Shawna et al. (2016-02). "Oral Phage Therapy of Acute Bacterial Diarrhea With Two Coliphage Preparations: A Randomized Trial in Children From Bangladesh". *EBioMedicine* **4**: 124–137. doi:10.1016/j.ebiom.2015.12.023. ISSN 2352-3964. PMID 26981577. PMC 4776075.
93. Galtier, Matthieu; De Sordi, Luisa; Maura, Damien; Arachchi, Harindra; Volant, Stevann; Dillies, Marie-Agnès; Debarbieux, Laurent (07 2016). "Bacteriophages to reduce gut carriage of antibiotic resistant uropathogens with low impact on microbiota composition". *Environmental*



- Microbiology* **18** (7): 2237–2245. doi:10.1111/1462-2920.13284. ISSN 1462-2920. PMID 26971586.
94. Majewska, Joanna; Kaźmierczak, Zuzanna; Lahutta, Karolina; Lecion, Dorota; Szymczak, Aleksander; Miernikiewicz, Paulina; Drapała, Jarosław; Harhala, Marek *et al.* (2019). "Induction of Phage-Specific Antibodies by Two Therapeutic Staphylococcal Bacteriophages Administered per os". *Frontiers in Immunology* **10**: 2607. doi:10.3389/fimmu.2019.02607. ISSN 1664-3224. PMID 31803179. PMC 6871536.
95. Dublanchet, A.; Fruciano, E. (2008-08). "[A short history of phage therapy]". *Medecine Et Maladies Infectieuses* **38** (8): 415–420. doi:10.1016/j.medmal.2008.06.016. ISSN 0399-077X. PMID 18692974.
96. Verbeken, Gilbert; Pirnay, Jean-Paul; Lavigne, Rob; Jennes, Serge; De Vos, Daniel; Casteels, Minne; Huys, Isabelle (2014-04). "Call for a dedicated European legal framework for bacteriophage therapy". *Archivum Immunologiae Et Therapiae Experimentalis* **62** (2): 117–129. doi:10.1007/s00005-014-0269-y. ISSN 1661-4917. PMID 24500660. PMC 3950567.
97. Fauconnier, Alan (04 17, 2019). "Phage Therapy Regulation: From Night to Dawn". *Viruses* **11** (4). doi:10.3390/v11040352. ISSN 1999-4915. PMID 30999559. PMC 6521264.
98. Pirnay, Jean-Paul; Verbeken, Gilbert; Ceysens, Pieter-Jan; Huys, Isabelle; De Vos, Daniel; Ameloot, Charlotte; Fauconnier, Alan (02 06, 2018). "The Magistral Phage". *Viruses* **10** (2). doi:10.3390/v10020064. ISSN 1999-4915. PMID 29415431. PMC 5850371.
99. Fauconnier, Alan (02 2017). "Regulating phage therapy: The biological master file concept could help to overcome regulatory challenge of personalized medicines". *EMBO reports* **18** (2): 198–200. doi:10.15252/embr.201643250. ISSN 1469-3178. PMID 28082313. PMC 5286392.
100. McCallin; Sacher; Zheng; Chan (2019-04-12). "Current State of Compassionate Phage Therapy". *Viruses* **11** (4): 343. doi:10.3390/v11040343. ISSN 1999-4915. PMID 31013833. PMC PMC6521059.
101. Moelling, Karin; Broecker, Felix; Willy, Christian (12 05, 2018). "A Wake-Up Call: We Need Phage Therapy Now". *Viruses* **10** (12). doi:10.3390/v10120688. ISSN 1999-4915. PMID 30563034. PMC 6316858.