Review
Future Virology

Introducing yesterday's phage therapy in today's medicine

Jean-Paul Pirnay*[1], Gilbert Verbeken[1], Thomas Rose[1], Serge Jennes[2], Martin Zizi[3,4], Isabelle Huys[5,6], Rob Lavigne[7], Maia Merabishvili[1,8,9], Mario Vaneechoutte[8], Angus Buckling[10] & Daniel De Vos[1]

[1]Laboratory for Molecular & Cellular Technology, Burn Wound Centre, Queen Astrid Military Hospital, Brussels, Belgium
[2]Burn Wound Centre, Queen Astrid Military Hospital, Brussels, Belgium
[3]Well Being Department, Queen Astrid Military Hospital, Brussels, Belgium
[4]Department of Physiology, Free University Brussels, Brussels, Belgium
[5]Department of Pharmaceutical & Pharmacological Sciences, Centre for Pharmaceutical Care & Pharmacoeconomics, KU Leuven, Leuven, Belgium
[6]Center for Intellectual Property Rights, KU Leuven, Leuven, Belgium
[7]Laboratory of Gene Technology, KU Leuven, Leuven, Belgium
[8]Laboratory of Bacteriology Research, Faculty of Medicine & Health Sciences, Ghent University, Ghent, Belgium
[9]Eliava Institute of Bacteriophage, Microbiology, & Virology, Tbilisi, Georgia
[10]Biosciences, University of Exeter, Cornwall Campus, Penryn, UK
*Author for correspondence: Tel.: +32 2 264 4844 ■ Fax: +32 2 264 4907 ■ jean-paul.pirnay@mil.be

The worldwide emergence of 'superbugs' and a dry antibiotic pipeline threaten modern society with a return to the preantibiotic era. Phages – the viruses of bacteria – could help fight antibiotic-resistant bacteria. Phage therapy was first attempted in 1919 by Felix d'Herelle and was commercially developed in the 1930s before being replaced by antibiotics in most of the western world. The current antibiotic crisis fueled a worldwide renaissance of phage therapy. The inherent potential of phages as natural biological bacterium controllers can only be put to use if the potential of the coevolutionary aspect of the couplet phage–bacterium is fully acknowledged and understood, including potential negative consequences. We must learn from past mistakes and set up credible studies to gather the urgently required data with regard to the efficacy of phage therapy and the evolutionary consequences of its (unlimited) use. Unfortunately, our current pharmaceutical economic model, implying costly and time-consuming medicinal product development and marketing, and requiring strong intellectual property protection, is not compatible with traditional sustainable phage therapy. A specific framework with realistic production and documentation requirements, which allows a timely (rapid) supply of safe, tailor-made, natural bacteriophages to patients, should be developed. Ultimately, economic models should be radically reshaped to cater for more sustainable approaches such as phage therapy. This is one of the biggest challenges faced by modern medicine and society as a whole.

Spreading antibiotic resistance: a universal threat

The worldwide emergence of 'superbugs' and a dry antibiotic pipeline threaten modern society with a return to the preantibiotic era, when bacterial infections were the primary cause of morbidity and mortality [1]. A recent estimate indicates that 400,000 people in Europe were infected with multidrug-resistant (MDR) bacteria during 2007, with 25,000 attributable deaths [2]. In hospitals in both the developed and the developing world, the majority of nosocomial outbreaks are caused by a small group of pathogens (i.e., *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* species, hereafter referred to as the 'ESKAPE bugs') [3]. These ESKAPE bugs are increasingly prevalent and resistant to most of our antimicrobial agents, threatening patients' lives and confronting society with huge socioeconomic costs. To date, MDR pathogens, such as highly drug-resistant *A. baumannii* (often associated with military operations in the Middle East [4]), NDM-1-producing *Enterobacteriaceae* [5], pan-resistant *P. aeruginosa* clones [6] and methicillin-resistant *S. aureus* (MRSA) [7], have been mostly associated with hospital outbreaks.

In addition, community-associated MRSA infections and specific *Escherichia coli* outbreaks demonstrate that the community as a whole is increasingly threatened by virulent antibiotic-resistant pathogens. Community-associated MRSA infections arise in otherwise healthy individuals and are more virulent and transmissible than are traditional

Keywords
■ antibiotic ■ bacteriophage ■ bacterium ■ coevolution ■ drug ■ infection ■ medicinal product ■ multidrug-resistant ■ patent ■ phage ■ resistance ■ therapy

Future Medicine part of fsg

10.2217/FVL.12.24

hospital-associated MRSA strains [8], and the recent outbreak of enteroaggregative Shiga toxin/verotoxin-producing *E. coli* strain O104:H4 in Germany [9] caused over 4000 cases of diarrhea – 3167 without hemolytic–uremic syndrome (16 deaths) and 908 with hemolytic–uremic syndrome (34 deaths) [10]. These cases demonstrate that infectious agents are not confined to hospitalized patients, but are actually deeply settled in our environment.

For rapidly evolving, genetically versatile bacteria such as *Pseudomonas*, it has turned out to be quite easy to develop mechanisms to avoid the toxicity of antibiotics, which have remained more or less 'static' for the last decade. More reflection on the biological role of antibiotics in nature as secondary metabolites would have revealed that resistance evolution was inevitable. Also, in nature, bacteria are constantly outsmarting toxins produced by competitors. However, the difference is that these natural competitors in turn react by selection towards adjusted toxins. The biological phenomenon of antibiotic resistance is typically an emergent characteristic of a dynamic, highly complex and self-organizing system that evolves at the edge of chaos [11,12]. Moreover, the rate of resistance evolution has been exacerbated by the overuse and misuse of antimicrobial agents in both clinical and agricultural contexts [13–15].

Due to the complexity of the antibiotic resistance issue and the immense research and development costs and time-frames of developing new antibiotics, for which resistance will inevitably occur, the pharmaceutical industry is not keen to continue with the development of new molecules. Moreover, even if pharmaceutical companies succeed in developing and marketing highly active antibiotics, authorities, sensitized by past experiences concerning the rapid emergence of resistance, are likely to withhold these new antibiotics as third-line last-rescue drugs, thereby limiting the market and consequently the commercial interest of the pharmaceutical companies. As the industry antibiotic pipeline is virtually dry and infectious diseases – major causes of morbidity and mortality – are steadily on the increase, new initiatives are urgently needed.

Phage therapy

Could (bacterio)phages, the viruses of bacteria, help fight antibiotic-resistant bacteria [16–18]? A virus is a natural biological entity, consisting in essence of a molecular assemblage of nucleic acids (the genome) surrounded by proteins, that behaves as a genetic replicative parasite. Lytic phages attach to receptors on the surface of bacteria, inject their genetic material through the bacterial membrane and take over the bacterium's transcription and translation machinery to synthesize new phages. Finally, the bacterial cell wall is destroyed (lysed), releasing the newly assembled virions to the environment, where they can invade new bacteria. Importantly, phages are able to infect bacteria regardless of their susceptibility to antibiotics. Wherever bacteria are present, there are bound to be phages, generally in an order of magnitude higher than bacteria. With an estimated unit number of 10^{31}, phages are the most abundant biological lifelike constituents of our biosphere [19–21]. In fact, one could say that we live in an ocean of phages. But this does not automatically mean that all phages are safe at therapeutic concentrations. No phage-related nucleic acid sequence can be found in our genome, unlike the huge amount of human endogenous retroviral sequences, which make up 8–10% of the human genome [22,23]. Some phage-related polymerase gene sequences were identified in human mitochondrial DNA. It is common knowledge that mitochondria originated from *Rickettsia*-like ancestor bacteria that started a symbiotic relationship with prototype eukaryotic cells [24]. Phage DNA was likely introduced in the bacterial phase of the mitochondrion, at the time when the evolutionary split occurred between the prokaryotes and eukaryotes (endosymbiotic era), and does not constitute evidence for recent DNA exchange. Moreover, recent work suggests that even the eukaryotic nucleus itself is a viral import [25]. It is possible that phage sequences did enter the human genome, but were lost over time. In addition, entry into our germline may be irrelevant to the potential for causing harm, and we do not know how often phage DNA integrated into human somatic cells. One must also consider that the potential adverse effects of phages might not be caused by them acting as viruses. Researchers from the Hirszfeld Institute of Immunology and Experimental Therapy in Poland found phages to be constantly present in human and animal bodies [26], where they were shown to modulate immune functions [27] and interact with cancer cells [28]. It is virtually impossible for a phage to enter directly into a eukaryotic cell system and subsequently multiply since it requires prokaryotic-specific cell wall receptors and biochemical machinery for its attachment and replication (e.g., prokaryotic polymerases and tRNAs). However, we should also consider indirect ways for phages

to enter eukaryotes, no matter how far-fetched they may be. For example, theoretically, a phage could integrate into a plasmid, which could then transfer from a bacterium to a eukaryote.

According to most supporters, however, phage therapy has been proven safe through the massive application of lytic bacteriophages in humans in the past. We conclude that, although there are indications that phages are not harmful for eukaryotic organisms, more research is needed.

Today, a few laboratories and small and medium enterprises are developing phage cocktails or phage-based products for the treatment of bacterial infection [29]. This antibacterial therapeutic approach was first proposed by Felix d'Herelle almost a century ago. The first therapeutic application of phages probably occurred as early as 1919 in Paris, where d'Herelle used phages to treat patients suffering from bacterial dysentery [30]. Later, he founded the Laboratoire du Bactériophage in Paris, which produced five phage preparations for commercial use. They were marketed by the French company Robert et Carrière, which later was acquired by L'Oréal [31]. In the USA in the 1930s, pharmaceutical giants like Eli Lilly, Squibb & Sons (today Bristol-Myers Squibb) and the Swan–Myers division of Abbott Laboratories started marketing several phage preparations. Scientific uncertainties and the discovery and widespread marketing of antibiotics, however, relegated phage therapy to the history books in the western world. As such, the current 'knowledge' of the therapeutic effect of phages is mainly based on theoretical grounds, basic laboratory observations, animal models [32–37], safety studies in healthy humans [38,39] and decades of empirical medical experience [31,40–43]. These empirical data were mainly accumulated in the former Soviet Union and its eastern European satellite states, with an important role for the Eliava Institute of Bacteriophage, Microbiology and Virology in Tbilisi (Georgia), several institutes in Russia and the Hirszfeld Institute in Wroclaw (Poland). Phage therapy remained a valid therapeutic component in France until the early 1990s [44]. Unfortunately, the historical clinical data are not taken into account by regulators because it has not been validated according to current western regulatory standards. The emergence of MDR bacteria has caused a renewed interest in phage therapy in western Europe and the USA, as illustrated by an exponential increase in phage therapy-related papers in the medical literature (Figure 1).

Phages: not your regular medicinal products

Phages can be seen as bacteria's natural infectious agents. Up to 50% of bacterial mortality is thought to be due to phage-induced lysis [45]; hence, phages impose strong selection for bacteria resistance. However, lytic phages can only propagate by infecting and lysing bacteria, hence there is strong selection to overcome this resistance. This interaction leads to antagonistic coevolution, consisting of the repeated emergence of new phage infectivity and bacterial defense mutations [46–50]. Typically, coevolution results in continual increases in bacteria resistance and phage infectivity ranges, although recent work, including a study following real-time coevolution in soil [51], suggests that high costs associated with resistance may instead result in different, rather than greater, resistance mechanisms being selected through time [48,52]. In principle, coevolution between bacteria and phages could therefore allow the continual production of highly infectious phages that can overcome common bacterial defense mechanisms. However, it is important to emphasize that not all phages are lytic. Many integrate into bacterial genomes, and are propagated via bacterial reproduction [53]. Such lysogenic phages will themselves coevolve with each other [54], with bacteria and with other lytic phages, and the consequences of this for phage therapy are currently unclear. A recent study showed that *in vitro* coinfection of *Pseudomonas fluorescens* with multiple phages had no net effect of accelerating or slowing down

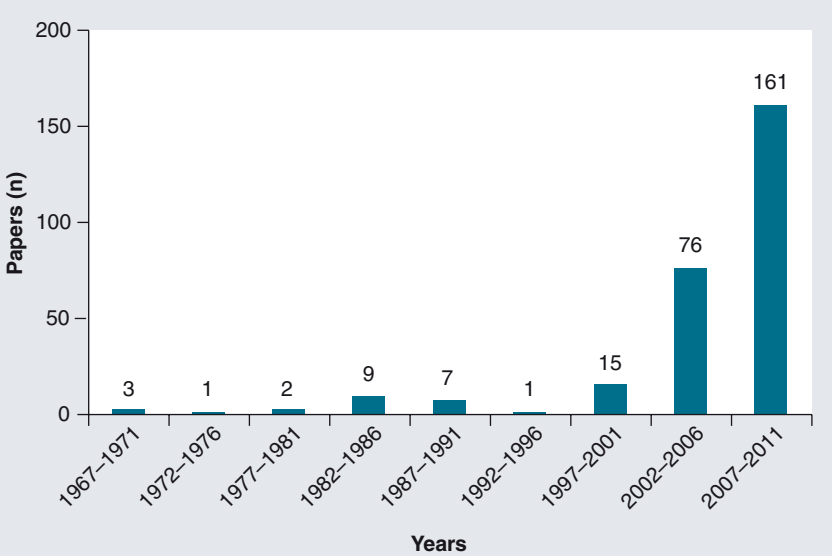

Figure 1. PubMed search results for 'phage therapy' or 'bacteriophage therapy' across time periods.

adaptation to the host through between-parasite conflict in the system [55]. It is thus tempting to speculate that phages act as 'evolving antibiotics' during real-time coevolution between therapeutic phages and infecting bacteria within patients. However, while real-time coevolution between bacteria and phages results in continual suppression of bacterial densities to some extent [51,56], the clinical significance of these relatively modest density directions is still unclear [57]. Phages do, however, play a major role in controlling bacterial densities in natural populations, and it is reasonable to assume that coevolution plays a role in this. For example, phages appear to be key players in ending cholera epidemics. Faruque *et al.* observed that seasonal epidemics of cholera inversely correlated with the prevalence of environmental cholera phages [58]. The removal of phages by conditions such as severe flooding might contribute to rendering water more conducive to human-to-human transfer of *Vibrio cholerae*. Phage amplification in cholera patients during a cholera epidemic likely contributed to increased environmental phage abundance, decreased load of environmental *V. cholerae* and, hence, the collapse of the epidemic. *In vivo* phage amplification in patients and subsequent phage infection in the environment could thus explain the self-limiting nature of seasonal cholera epidemics in Bangladesh [59].

It is clear that therapeutic phages are very different from classical (chemical, molecular) medicinal products such as antibiotics. Instead, they are natural biological entities that play an important role in maintaining equilibrium in bacterial populations of ecological environments, including humans. Hence, we should not see them as conventional stable medicinal products, but more as interactive and evolving antibacterial products, which could also be used in combination (synergy) with antibiotics [60]. The coevolutive aspect of the phage–bacterium couplet, which is essential for sustainable phage therapy, is often neglected.

However, there are potential negative consequences of this coevolutionary potential. For example, coevolution has been shown to drive the evolution of bacterial mutation rates in laboratory populations of the bacterium *P. fluorescens*. A quarter of the bacterial populations coevolving with phages had rapidly (i.e., in less than 200 generations) acquired mutations that resulted in ten- to 100-fold increases in mutation rates, whereas no significant change in mutation rates was observed in the absence of phages [61]. Given the increase in evolvability of mutator bacteria (e.g., elevated rates of resistance evolution to antibiotics), evolvable phages may have unknown net consequences on disease severity. Phage therapy should not be implemented widely and without limitation, without first determining these consequences through real-time experimental evolution studies. In the end, natural phages could prove useful, but maybe only in specific (niche) clinical contexts and under certain conditions (e.g., dosage).

Phage therapy fits well in the emerging field of Darwinian medicine (in contrast to a classical mechanistic – man as a machine – view) [62,63], whereby the insights into evolution are fully taken into account, but it is less compatible with our actual western drug development and marketing model.

Hurdles in the current medicinal product development & marketing model

This section discusses the problems encountered when trying to reintroduce traditional phage therapy in modern medicine.

An analysis of the current European regulatory framework [64] and multiple discussions with experts and the relevant competent authorities revealed that, although the development and marketing of phage medicinal products (including good manufacturing practice production, preclinical and Phase I, II and III clinical trials and centralized marketing authorization) is technically possible, in practice it is not compatible with traditional (sustainable) phage therapy [65].

The cost of conventional medicinal product development & marketing (millions of Euros) necessitates strong intellectual property protection, but today, for natural phages, this protection is fragile

Recently, the ruling in a US court in a case between the Association of Molecular Pathology and the US Patent and Trademark Office invalidated seven patents claiming genes and genetic diagnostic methods held by Myriad Genetics [66]. Although related to genes, this decision opens the discussion about the ability to patent naturally occurring organisms such as phages.

In patent law, an invention is considered to be new if it is not part of the state of the art. This means that a phage or a phage cocktail claimed in a patent should never have been isolated or produced before. The literature with respect to phages as natural entities to treat human bacterial infections is enormous. In addition, clinical studies using phages performed in

the eastern part of Europe have recently been translated into English (e.g., [40]). Therefore, many natural phages and their uses have been disclosed over the past century. European law allows the patenting of known substances, such as natural phages, for use in a medical method, provided that such use is new, meaning that such use may not be comprised in the state of the art. In the USA, several patents for phages used in the food sector were granted, such as US7507571 (food additive), claiming "an isolated bacteriophage of a bacteriophage strain selected from a [specific] group, [somewhere] deposited under a [specific] accession number, together with variants thereof, wherein said variants retain the phenotypic characteristics of said deposited bacteriophages and wherein said bacteriophages, and variants thereof, have lytic activity against *Listeria monocytogenes* strains" [67]. More important for therapeutic use is the US patent 7459272 of Intralytix, Inc., claiming "a method for reducing the risk of bacterial infection or sepsis in a person colonized with pathogenic bacteria comprising treating the colonized person with a pharmaceutical composition containing bacteriophage of one or more strains which produce lytic infections in said pathogenic bacteria." In 2001, a European patent application (EP1250143 A2) was filed, claiming "a method for reducing the risk of bacterial infection or sepsis in a susceptible patient by treating the susceptible patient with a pharmaceutical composition containing bacteriophages of one or more strains which produce lytic infections in pathogenic bacteria," but this application was withdrawn in 2004. Only recently, "a method for production of compositions of bacteriophages" was claimed in the USA by Phage Biopharm, LLC (US7588929). No European counterpart has been published yet. Another interesting patent is the US patent 7758856 (Biocontrol, Ltd) claiming "a composition for treating a bacterial biofilm," as well as "a method for treating a biofilm infection." A similar patent owned by the UK Health Protection Agency has been granted in Europe (EP1587520 B1).

Diverging views between Europe and the USA exist on the patenting of biological material. Next to the requirements of novelty, inventive steps and industrial applicability (which are the same for Europe and the USA), in order to be patentable in Europe, a certain technical intervention is needed to isolate the phage from its natural environment, and the isolated phage needs to be properly characterized. However, this 'technical intervention' has basically been known since the 1920s, and the requirement that the phage 'needs to be well characterized' seems obvious and is technically not particularly hard to meet [68]. In the USA, phages claimed in a patent need to have markedly different characteristics from their counterparts found in nature. But, for natural exclusively lytic phages – our object of concern here – they simply are the ones found in nature. It seems as if only genetically modified phages can agree with the US statement. While 'manipulated' or engineered phages certainly have potential applications (which are patentable), given the growing public concern and awareness over the potential health and environmental risks of genetically modified organisms, they are unlikely to obtain licensing approval in the near future.

Phage-encoded proteins such as cell wall-degrading endolysins [69] will be marketed a few years from now in the food industry, the veterinary field and possibly in medicine. They will select resistance, but presumably and hopefully at a slower pace than antibiotics. Of course, these phage-derived products are not capable of self-replicating and evolving in the infectious site.

In this paper, we focus on natural phages simply because of their natural intrinsic bacterial coevolutionary aspect making them suitable for flexible therapeutic applications. Patents claiming natural phages are fragile, and 'inventing around' (making an invention that accomplishes the same thing as the original patented invention but does not infringe the patented invention) also seems to be very difficult [70].

These intellectual property (IP) issues do not stimulate investment (of venture capital), for the actual paradigm is 'no IP protection, no investment'. However, the renewed interest in natural phages as therapeutic agents might trigger scientists' and entrepreneurs' creativity in defining the contours of appropriate patent claims for phages or, even better, because there are good reasons for not patenting certain natural substances, considering a new kind of IP instrument. New ideas on IP protection should not be based on the existing classical model, but on a broader 'new' philosophy in relation to sustainable economic and industrial development, as advocated by Petrella and Sachs [71,72]. Petrella states that, today, "being competitive" is no longer a tool for increased development, but an aim in itself [71]. This increasingly implies that the possession of patents, often as strategic weapons, is more important (in the short term)

than owning a truly functional innovative technology. This kind of attitude tends to block the development of new approaches such as phage therapy. The patent tragedy is indeed exemplified by the millions of AIDS victims who died while drug treatments existed and raises deep questions about global IP rights. How can the benefits of a global patent system that provides incentives for innovation and continuous development be combined with an assurance that the targeted people (rich and poor) gain access to the medical care they need and have rights to [73]?

Therefore, the Group of Lisbon, led by Petrella, proposed an evolution to world cooperative governance, which is based on a global contract that requires that each decision should be linked to the fact that each person should have access to basic livelihoods [71], including health access, which is actually often blocked by our outdated economic model. As such, phage therapy could be developed under the umbrella of, for example, the WHO. The WHO recognizes the importance of the worldwide antibiotic resistance issues [101] and is discussing new incentives to push the pharmaceutical industry to launch new research and development projects. Could phage therapy be one of them?

The time frames for conventional medicinal product development & marketing (years) are not compatible with a flexible, tailor-made & sustainable phage therapy concept

Phage therapy depends upon safe and well-defined phages, but is it really necessary to produce and market them in the same way as conventional medicinal products?

In 2009, a phage cocktail, BFC-1, which targeted the most prevalent MDR *P. aeruginosa* and MRSA bacteria in the burn wound center of the Queen Astrid Military Hospital in Brussels (Belgium), was produced. The cocktail consisted of two phages against *P. aeruginosa* and one against *S. aureus*. It was produced on a small scale and in concordance with certain relevant quality and safety standards (e.g., sterility, apyrogenicity, pH, adequate shelf life and stability). In addition, the phages were shown to be exclusively lytic and were characterized at the genomic and proteomic level. This specific production process was published in 2009 by Merabishvili *et al.* [68]. As the authors did not consider phages to be conventional medicinal products, the phage cocktail was not produced in concordance with the requirements of the EU medicinal product regulation. After approval by a leading Medical Ethical Committee (of the Free University of Brussels), phage cocktail BFC-1 was applied in a small pilot study in the burn unit of the Queen Astrid Military Hospital in Brussels. This small trial was discussed in a recent review by Kutter and colleagues [43]. No adverse events or side effects were observed.

However, the European Commission stated recently that EU's legislation on medicinal products does not define specific requirements related to bacteriophage therapy or medicines composed of bacteriophages because it considers that the existing regulatory framework is adequate for bacteriophage therapy. There is thus no need for a specific set of documentation for bacteriophage therapy [74]. We do not share this opinion for the reasons discussed below.

To exploit the main advantage of phages over classical 'static' drugs such as antibiotics, and more specifically their capacity to rapidly (in a matter of days to weeks) evolve to target emerging (phage-resistant) pathogenic bacterial strains, phage cocktails should not be submitted to the conventional long medicinal product development and licensing pathway. Even if the EMA would eventually adapt its rules in a similar manner to what they did for updated seasonal influenza vaccines, which are annually licensed [75], development times of many months are still much too long in view of the enormous challenges related to rapidly progressing bacterial resistance. The real power of phage therapy lies in the fact that the search for a potent natural phage and the preparation of a classic galenic preparation (e.g., physiological water or a basic ointment) containing phages is practically feasible in the time frame of days to weeks. In traditional phage therapy, new therapeutic phages are usually selected from environmental sources such as raw sewage water or isolated from clinical specimens from infected patients (Figure 2). Georgian and Polish phage therapy centers are keeping extensive therapeutic phage collections, which are regularly enriched with new phages, thus widening the host range of the collection. Ineffective phages can be 'trained', a term indicating the *in vitro* selection of phage mutants that exhibit an increased infectivity range. As such, it is possible to obtain potent lytic phages against problematic enteroaggregative *E. coli* strains [76] in a matter of days, for example. Theoretically, they could thus have been used to

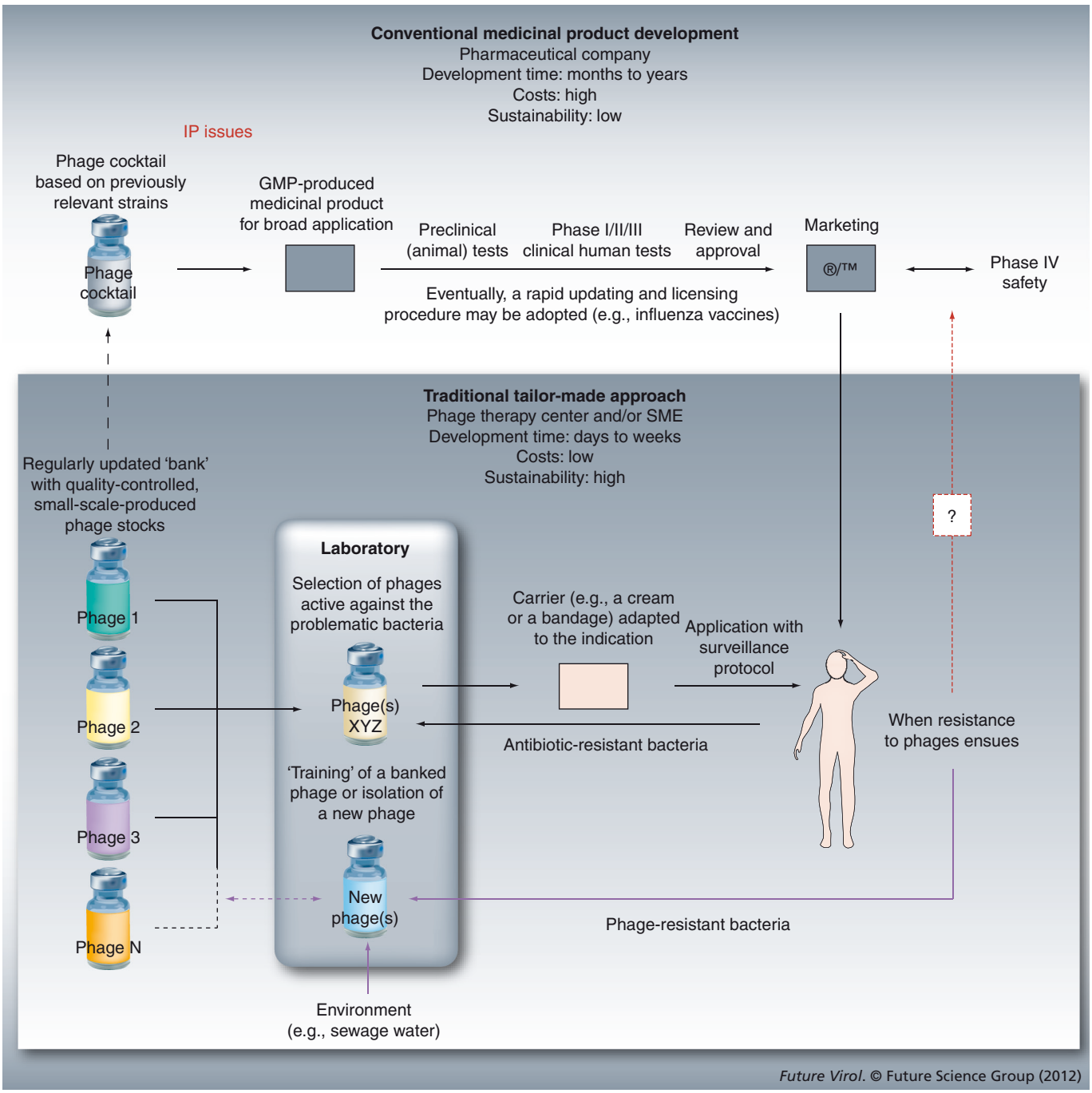

Figure 2. Two phage therapy concepts. IP issues may hamper pharmaceutical companies in the worldwide marketing of generic phage preparations. The long and expensive regulatory pathways form insurmountable obstacles for eventual nonprofit phage therapy centers or SMEs, which opt for a tailor-made concept, and for institutions that would like to use inexpensive phages for commercially unattractive applications (e.g., in developing countries) [65].
GMP: Good manufacturing practice; IP: Intellectual property; SME: Small and medium enterprise.

help control the O104:H4 outbreak that caused the death of 50 patients in Germany [9,10]. In this context, an O104:H4 phage preparation that takes months to years to develop, produce and register is ineffective. As phages are species- and often even strain-specific, it is very likely that current O104:H4-specific phage preparations will not be active against future epidemic enteroaggregative *E. coli* strains. Provided that future problematic bacteria are broadly known, some 'broad-spectrum' cocktails could be developed in advance and used as the first-line answer to acute healthcare problems (e.g., bioweapons). Some cocktails will inevitably fail due to the greater biodiversity outside of the laboratory, and the ones that initially work will need to

be regularly updated due to the emergence of resistance. In a recent study, it was shown that *P. aeruginosa* challenged *in vitro* with a cocktail of four potent phages swiftly developed resistance to all four phages [Hall AR, De Vos D, Friman VP, Pirnay JP, Buckling A. Effects of sequential and simultaneous application of bacteriophages on populations of *Pseudomonas aeruginosa in vitro* and in waxmoth larvae (2012), Submitted]. We are currently discussing our viewpoint with EMA's Innovation Task Force (ITF). The ITF has the competence to facilitate the informal exchange of information and the provision of guidance early in the development process of medicinal products. Our objectives are to develop a specific framework (e.g., realistic production and documentation requirements) that allows a timely (rapid) supply of tailor-made productions of natural bacteriophages to patients.

Responsible & sustainable phage therapy is not compatible with current pharmacoeconomic models

Acceptable IP protection and development and licensing procedures were available for antibiotics. They did not prevent the overuses and misuses that gave rise to the current antibiotic resistance crisis. Solving the aforementioned IP and development issues will thus not necessarily lead to rational and sustainable phage therapy. The question is, how can responsible and limited use be promoted? It is very doubtful that this will be compatible with actual economic incentives. Even world cooperative governance will provide no guarantees, as the primary goal of organizations such as the WHO is to limit infections, not to support sustainable approaches.

It is our opinion that, ultimately, economic models will need to be radically reshaped in order to cater for more sustainable approaches such as phage therapy.

Current state

The tailor-made approach and sustainable nature of traditional phage therapy and IP issues may hamper pharmaceutical companies in the worldwide marketing of generic phage preparations. Nonprofit/public institutions such as (university) hospitals that would like to develop flexible and sustainable tailor-made (i.e., to an outbreak) phage therapy and are not necessarily disheartened by the IP issues and the subsequent uncertainty of large profits are generally unable to generate the necessary funding. In addition, the prescribed medicinal product development and licensing pathways cancel the advantages of phage therapy over antibiotics. It is thus difficult to reconcile a flexible and sustainable phage therapy concept with the current (western) medical and pharmaceutical environment (**Figure 2**). As a result of this conundrum, only local and sporadic phage applications have been performed in the western world to date, often based on individual approval governed within the 'Declaration of Helsinki' framework [102]. In Poland, an EU member state, a specific national adaptive regulation, based on the Declaration of Helsinki, was issued to regulate phage therapy. A medical doctor is allowed to apply phage therapy where proven therapeutic methods do not exist or have been ineffective (e.g., in MDR infections) and provided that the patient or their legal representative gives informed consent. In France, Alain Dublanchet, a veteran of phage therapy, occasionally applies phages in hopeless osteomyelitis cases [65]. In Australia, phage therapy was recently applied under the umbrella of 'compassionate use' for the successful treatment of refractory *P. aeruginosa* urinary tract infection in a cancer patient [77].

Conclusion

Phages are not straightforward inanimate and stable substances, but evolvable and natural biological entities. Future sustainable phage therapy concepts should fully acknowledge the potential of the coevolutionary aspect of the phage–bacterium couplet. Only then can the inherent potential of phages as natural biological bacterium controllers be put to use. Indeed, bacteria will inevitably become resistant to phages, but due to the continuously ongoing arms race/competition between the two protagonists, specific phages that are able to infect the formerly resistant bacterial strains can be expected to quickly emerge. However, more experimental evolution studies are necessary to determine the potential negative evolutionary consequences of unlimited phage therapy.

The existing pharmaceutical regulatory framework and business models are not compatible with a dynamic and sustainable phage therapy concept. The actual economic models reduce pharmaceutical companies to 'common button' producers neglecting their main societal role: providing people with adequate products for better health. Therefore, a suitable environment for phage therapy should be developed. Fundamental changes of mentality in the medical and pharmaceutical environment (e.g.,

towards patentability and restrictive licensing) are essential for a successful introduction of phage therapy in modern (future) medicine. We need to radically reshape our (pharmaceutical) economic models to cater for more sustainable approaches that are beneficial for human survival.

Phage therapy fits well in the new emerging field of Darwinian medicine, where the insights of evolution are fully taken into account. Viruses, among which are phages, were involved in the origin of life itself and play a major role in biological evolution [78–82]. Hopefully, they will play a role in the future control of bacterial disease. We consider our plea for a more realistic approach to phage therapy, which takes into account the coevolutionary aspect of the bacterium and its phage, to be scientifically sound. We must learn from the errors that contributed to the rise of antibiotic resistance. We hope to foster this vision in collaboration with the competent authorities and responsible economic actors, as only a common effort will make it a (direly needed) reality.

Future perspective

In the short term, we predict the setting up of credible studies to gather the required data with regard to the efficacy and evolutionary consequences of phage therapy. These studies could be chaperoned by health protection agencies such as the European CDC.

In the medium term, we predict the development of a specific framework, in collaboration with the EMA's ITF (or with the US FDA), with realistic production and documentation requirements that allow a timely supply of safe, tailor-made natural bacteriophages.

In the long term, we predict the radical reshaping of our (pharmaceutical) economic models to cater for more sustainable approaches. Phage therapy could be developed under the umbrella of the WHO.

Executive summary

Spreading antibiotic resistance: a universal threat

- Overuse and misuse of antibiotics caused the emergence of organisms that are resistant to these medicinal products, leading to increased morbidity and mortality and increased healthcare costs.
- Because new antibiotics have become of limited use and are thus less profitable, pharmaceutical companies are reluctant to invest in the research and development of new antibiotics.

Phage therapy

- Phage therapy – the use of the viruses of bacteria to fight bacterial infection – was first advocated by Felix d'Herelle in 1919.
- Due to the advent of antibiotics and scientific controversies, phage therapy was abandoned in the western world.
- The current antibiotic resistance crisis has caused a renewed interest in phage therapy.

Phages: not your regular medicinal products

- Phages are very different from classical (chemical molecular) medicinal products.
- Phages are natural biological entities that coevolve with and control bacteria in the environment, including humans, which is the basis of sustainable phage therapy.
- There might also be potential negative consequences of bacterial phage coevolution.

Hurdles in the current medicinal product development & marketing model

- When trying to introduce traditional sustainable phage therapy in modern medicine, one is confronted with three issues:
  - The cost of conventional medicinal product development and marketing (millions of Euros) necessitates strong intellectual property protection, but today, for natural phages, this protection is fragile;
  - The time-frames for conventional medicinal product development and marketing (years) are not compatible with a flexible, tailor-made and sustainable phage therapy concept;
  - Responsible and sustainable phage therapy is not compatible with current pharmacoeconomic models.

Current status

- Only local and sporadic phage applications are performed in the western world, often based on individual approval governed within the 'Declaration of Helsinki' framework.

Conclusion

- Future sustainable phage therapy concepts should fully acknowledge the potential of the coevolutionary aspect of the phage–bacterium couplet.
- More research is needed to determine the potential negative coevolutionary consequences of unlimited phage therapy.
- Our (pharmaceutical) economic models need to be radically reshaped to cater for more sustainable approaches that are beneficial for human survival.

Financial & competing interests disclosure

The authors thank the FWO Vlaanderen ('PhageBiotics' research community grant WO.022.09) for its support. J-P Pirnay and M Merabishvili were supported by grant MED 12 of the Royal High Institute for Defence. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

References

Papers of special note have been highlighted as:
- ▪ of interest
- ▪▪ of considerable interest

1. Levy SB, Marshall B. Antibacterial resistance worldwide: causes, challenges and responses. *Nat. Med.* 10(12), S122–S129 (2004).
2. Bush K, Courvalin P, Dantas G *et al.* Tackling antibiotic resistance. *Nat. Rev. Microbiol.* 9(12), 894–896 (2011).

▪▪ **A group of 30 scientists from academia and industry gathered to explore how the problem of antibiotic resistance might best be addressed.**

3. Rice LB. Federal funding for the study of antimicrobial resistance in nosocomial pathogens: no ESKAPE. *J. Infect. Dis.* 197(8), 1079–1081 (2008).
4. Jones A, Morgan D, Walsh A *et al.* Importation of multidrug-resistant *Acinetobacter* spp infections with casualties from Iraq. *Lancet Infect. Dis.* 6(6), 317–318 (2006).
5. Kumarasamy K, Toleman MA, Walsh TR *et al.* Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. *Lancet Infect. Dis.* 10(9), 597–602 (2010).
6. Pirnay JP, Bilocq F, Pot B *et al. Pseudomonas aeruginosa* population structure revisited. *PLoS ONE* 4(11), E7740 (2009).
7. Michel M, Gutmann L. Methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant enterococci: therapeutic realities and possibilities. *Lancet* 349(9069), 1901–1906 (1997).
8. Deleo FR, Otto M, Kreiswirth BN, Chambers HF. Community-associated meticillin-resistant *Staphylococcus aureus*. *Lancet* 375(9725), 1557–1568 (2010).
9. Struelens MJ, Palm D, Takkinen J. Enteroaggregative, Shiga toxin-producing *Escherichia coli* O104:H4 outbreak: new microbiological findings boost coordinated investigations by European public health laboratories. *Euro Surveill.* 16(24), 1–3 (2011).
10. Rasko DA, Webster DR, Sahl JW *et al.* Origins of the *E. coli* strain causing an outbreak of haemolytic–uremic syndrome in Germany. *N. Engl. J. Med.* 365(8), 709–717 (2011).
11. Martinez JL, Baquero F. Interactions among strategies associated with bacterial infection: pathogenicity, epidemicity, and antibiotic resistance. *Clin. Microbiol. Rev.* 15(4), 647–679 (2002).
12. Baquero F, Coque TM, Canton R. Antibiotics, complexity and evolution. *ASM News* 69, 547–552 (2003).
13. Kümmerer K, Henninger A. Promoting resistance by the emission of antibiotics from hospitals and households into effluent. *Clin. Microbiol. Infect.* 9(12), 1203–1214 (2003).
14. Kümmerer K. Resistance in the environment. *J. Antimicrob. Chemother.* 54(2), 311–320 (2004).
15. Allen HK, Donato J, Wang HH, Cloud-Hansen KA, Davies J, Handelsman J. Call of the wild: antibiotic resistance genes in natural environments. *Nat. Rev. Microbiol.* 8(4), 251–259 (2010).
16. Maura D, Debarbieux L. Bacteriophages as twenty-first century antibacterial tools for food and medicine. *Appl. Microbiol. Biotechnol.* 90(3), 851–859 (2011).
17. Thiel K. Old dogma, new tricks – 21st century phage therapy. *Nat. Biotechnol.* 22(1), 31–36 (2004).

▪▪ **Provides an overview of how biotechnology is trying to develop phage therapy.**

18. Kutateladze M, Adamia R. Bacteriophages as potential new therapeutics to replace or supplement antibiotics. *Trends Biotechnol.* 28(12), 591–595 (2010).
19. Bergh O, Borsheim KY, Bratbak G, Heldal M. High abundance of viruses found in aquatic environments. *Nature* 340(6233), 476–468 (1989).
20. Fuhrman JA. Marine viruses and their biogeochemical and ecological effects. *Nature* 399(6736), 541–548 (1999).
21. Hendrix RW. Bacteriophages: evolution of the majority. *Theor. Popul. Biol.* 61(4), 471–480 (2002).
22. Lander ES, Linton LM, Birren B *et al.* Initial sequencing and analysis of the human genome. *Nature* 409(6822), 860–921 (2001).
23. Mi S, Lee X, Li X *et al.* Syncitin is a captive retroviral envelope protein involved in human placental morphogenesis. *Nature* 403(6771), 785–789 (2000).
24. Spelbrink JN, Li FY, Tiranti V *et al.* Human mitochondrial DNA deletions associated with mutations in the gene encoding Twinkle, a phage T7 gene 4-like protein localized in mitochondria. *Nat. Genet.* 28(3), 223–231 (2001).
25. Bell PJL. Viral eukaryogenesis: was the ancestor of the nucleus a complex DNA virus? *J. Mol. Evol.* 53(3), 251–256 (2001).
26. Górski A, Wazna E, Dabrowska BW, Dabrowska K, Switała-Jeleń K, Miedzybrodzki R. Bacteriophage translocation. *FEMS Immunol. Med. Microbiol.* 46(3), 313–319 (2006).
27. Gorski A, Dabrowska K, Switala-Jeleń K *et al.* New insights into the possible role of bacteriophages in host defense and disease. *Med. Immunol.* 2(1), 2 (2003).
28. Budynek P, Dabrowska K, Skaradziński G, Górski A. Bacteriophages and cancer. *Arch. Microbiol.* 192(5), 315–320 (2010).
29. Monk AB, Rees CD, Barrow P, Hagens S, Harper DR. Bacteriophage applications: where are we now? *Lett. Appl. Microbiol.* 51(4), 363–369 (2010).
30. d'Herelle F. Sur le rôle du microbe bactériophage dans la typhose aviaire. *C. R. Acad. Sci.* 169, 932–934 (1919).
31. Sulakhvelidze A, Alavidze Z, Morris JG. Bacteriophage therapy. *Antimicrob. Agents Chemother.* 45(3), 649–659 (2001).
32. Biswas B, Adhya S, Washart P *et al.* Bacteriophage therapy rescues mice bacteremic from a clinical isolate of vancomycin-resistant *Enterococcus faecium*. *Infect. Immunol.* 70(3), 204–210 (2002).
33. Chibani-Chenouffi S, Sidoti J, Brüttin A, Kutter E, Sarket S, Brüssow H. *In vitro* and *in vivo* bacteriolytic activities of *Escherichia coli* phages: implications for phage therapy. *Antimicrob. Agents Chemother.* 48(7), 2558–2569 (2004).
34. Wills QF, Kerrigan C, Soothill JS. Experimental bacteriophage protection against *Staphylococus aureus* abscess in a rabbit model. *Antimicrob. Agents Chemother.* 49(3), 1220–1221 (2005).
35. Marza JA, Soothill JS, Boydell P, Collyns TA. Multiplication of therapeutically administered bacteriophages in *Pseudomonas aeruginosa* infected patients. *Burns* 32(5), 644–646 (2006).
36. McVay CS, Velasquez M, Fralick JA. Phage therapy of *Pseudomonas aeruginosa* infection in a mouse burn wound model.

Antimicrob. Agents Chemother. 51(6), 1934–1938 (2007).

37. Debarbieux L, Leduc D, Maura D *et al.* Bacteriophages can treat and prevent *Pseudomonas aeruginosa* lung infections. *J. Infect. Dis.* 201(7), 1096–1104 (2010).
38. Brüttin A, Brüssow H. Human volunteers receiving *Escherichia coli* phage T4 orally: a safety test of phage therapy. *Antimicrob. Agents Chemother.* 49(7), 2874–2878 (2005).
39. Rhoads DD, Wolcott RD, Kuskowski MA, Wolcott BM, Ward LS, Sulakvelidze A. Bacteriophage therapy of venous leg ulcers in humans: results of a Phase I safety trial. *J. Wound Care* 18(6), 237–243 (2009).
40. Chanishvili N. *A Literature Review of the Practical Application of Bacteriophage Research*. Sharp R (Ed.). Eliava Institute, Georgia (2009).

▪ **Reports, in English, the results of screening for bacteriophage-related data in over 5000 scientific documents (papers, books and PhD theses) available from the library of the Eliava Institute in Tbilisi (Georgia).**

41. Dabrowska K, Switala-Jelen K, Opolski A, Weber-Dabrowska B, Gorski A. Bacteriophage penetration in vertebrates. *J. Appl. Microbiol.* 98(1), 7–13 (2003).
42. Gorski P, Miedzybrodski R, Borysowski J *et al.* Bacteriophage therapy for the treatment of infections. *Curr. Opin. Investig. Drugs* 10(8), 766–774 (2009).
43. Kutter E, De Vos D, Gvasalia G *et al.* Phage therapy in clinical practice: treatment of human infections. *Curr. Pharm. Biotechnol.* 11(1), 69–86 (2010).
44. Dublanchet A. *Des Virus Pour Combattre les Infections: La Phagothérapie: Renouveau d'un Traitement au Secours des Antibiotiques.* Favre SA (Ed.). Lausanne, Switzerland (2009).
45. Wommack KE, Colwell RR. Virioplankton: viruses in aquatic ecosystems. *Microbiol. Mol. Biol. Rev.* 64(1), 69–114 (2000).
46. Buckling A, Rainey PB. Antagonistic coevolution between a bacterium and a bacteriophage. *Proc. Biol. Sci.* 269(1494), 931–936 (2002).
47. Bohannan BJM, Lenski RE. Linking genetic change to community evolution: insights from studies of bacteria and bacteriophage. *Ecol. Lett.* 3, 362–377 (2000).
48. Hall A, Scanlan PD, Morgan AD, Buckling A. Host–parasite coevolutionary arms races give way to flcutuating selection. *Ecol. Lett.* 14, 635–642 (2011).
49. Mizoguchi K, Morita M, Fischer CR, Yoichi M, Tanji Y, Unno H. Coevolution of bacteriophage PP01 and *Escherichia coli* O157:H7 in continuous culture. *Appl. Environ. Microbiol.* 69(1), 170–176 (2003).
50. Stern A, Sorek R. The phage–host arms race: shaping the evolution of microbes. *Bioessays* 33(1), 43–51 (2011).
51. Gómez P, Buckling A. Bacteria–phage antagonistic coevolution in soil. *Science* 332(6025), 106–109 (2011).
52. Agrawal A, Lively CM. Infection genetics: gene-for-gene versus matching-alleles models and all points in between. *Evol. Ecol. Res.* 4(1), 79–90 (2002).
53. Calendar RL. *The Bacteriophages*. Oxford University Press, UK (2005).
54. Refardt D. Within-host competition determines reproductive success of temperate bacteriophages. *ISME J.* 5(9), 1451–1460 (2011).
55. Hall AR, Scanlan PD, Leggett HC, Buckling A. Multiplicity of infection does not accelerate infectivity evolution of viral parasites in laboratory microcosms. *J. Evol. Biol.* 25(2), 409–415 (2012).
56. Buckling A, Hodgson DJ. Short-term rates of parasite evolution predict the evolution of host diversity. *J. Evol. Biol.* 20(5), 1682–1688 (2009).
57. Levin BR, Bull JJ. Population and evolutionary dynamics of phage therapy. *Nat. Rev. Microbiol.* 2(2), 166–173 (2004).

▪ **Reviews the population and evolutionary dynamics of bacterial–phage interactions that are relevant to phage therapy and prophylaxis.**

58. Faruque SM, Naser IB, Islam MJ *et al.* Seasonal epidemics of cholera inversely correlate with the prevalence of environmental cholera phages. *Proc. Natl Acad. Sci. USA* 102(5), 1702–1707 (2005).

▪▪ **Good example of the major role that phages play in controlling bacterial densities in natural populations.**

59. Faruque SM, Islam MJ, Ahmad QS *et al.* Self-limiting nature of seasonal cholera epidemics: role of host-mediated amplification of phage. *Proc. Natl Acad. Sci. USA* 102(17), 6119–6124 (2005).
60. Comeau AM, Tétart F, Trojet SN, Prère MF, Krish HM. Phage–antibiotic synergy (PAS): beta-lactam and quinolone antibiotics stimulate virulent phage growth. *PLoS ONE* 2(8), E799 (2007).
61. Pal C, Maciá MD, Oliver A, Schachar I, Buckling A. Coevolution with viruses drives the evolution of bacterial mutation rates. *Nature* 450(7172), 1079–1081 (2007).

▪ **Provides evidence that, *in vitro*, phages can increase the evolvability of mutator bacteria, leading to unknown net consequences on disease severity.**

62. Williams GC, Nesse RM. The dawn of Darwinian medicine. *Q. Rev. Biol.* 66(1), 1–22 (1991).
63. Nesse RM. On the difficulty of defining disease: a Darwinian perspective. *Med. Health Care Philos.* 4(1), 37–46 (2000).
64. Verbeken G, De Vos D, Vaneechoutte M, Merabishvili M, Zizi M, Pirnay JP. European regulatory conundrum of phage therapy. *Future Microbiol.* 2(5), 485–491 (2007).
65. Pirnay JP, De Vos D, Verbeken G *et al.* The phage therapy paradigm: prêt-à-porter or sur-mesure? *Pharm. Res.* 28(4), 934–937 (2010).
66. Akst J. Key cancer patents killed. *The Scientist*, 30 March (2010).
67. FDA. FDA 21 CFR Part 172: food additives permitted for direct addition to food for human consumption; bacteriophage preparation. *Fed. Reg.* 71(160), 47729–47732 (2006).
68. Merabishvili M, Pirnay JP, Verbeken G *et al.* Quality-controlled small-scale production of a well-defined bacteriophage cocktail for use in human clinical trials. *PLoS ONE* 4(3), E4944 (2009).
69. Callewaert L, Walmagh M, Michiels CW, Lavigne R. Food applications of bacterial cell wall hydrolases. *Curr. Opin. Biotechnol.* 22(2), 164–171 (2011).
70. Van Overwalle G. Intellectual property. Turning patent swords into shares. *Science* 230(6011), 1630–1631 (2010).
71. The Group of Lisbon. *Limits to Competition*. MIT Press, MA, USA (1995).
72. Sachs JD. *Common Wealth: Economics for a Crowded Planet.* Penguin, UK (2008).

▪ **Provides an interesting roadmap to sustainable and equitable global prosperity.**

73. So AD, Gupta N, Brahmachari SK *et al.* Towards new business models for R&D for novel antibiotics. *Drug Resist. Updat.* 14(2), 88–94 (2011).
74. Verbeken G, Pirnay J-P, De Vos D *et al.* Optimizing the European regulatory frame for sustainable bacteriophage therapy in human medicine. *Arch. Immunol. Ther. Exp.* (2012) (In Press).
75. Wood JM, Levandowski RA. The influenza vaccine licensing process. *Vaccine* 21(16), 1786–1788 (2003).
76. Maura D, Morello E, du Merle L, Bomme P, Le Bouguénec C, Debarbieux L. Intestinal

colonization by enteroaggregative *Escherichia coli* supports long-term bacteriophage replication in mice. *Environ. Microbiol.* doi:10.1111/j.1462-2920.2011.02644.x (2011) (Epub ahead of print).

77. Khawaldeh A, Morales S, Dillon B *et al.* Bacteriophage therapy for refractory *Pseudomonas aeruginosa* urinary tract infection. *J. Med. Microbiol.* 60(11), 1697–1700 (2011).
78. Villarreal LP. Overall issues of viruses and host evolution. In: *Viruses and the Evolution of Life*. Villareal LP (Ed.). ASM Press, WA, USA, 1–28 (2005).
79. Villarreal LP, Witzany G. Viruses are essential agents within the roots and stem of the tree of life. *J. Theor. Biol.* 262(4), 698–710 (2010).
80. Bamford DH. Do viruses form lineages across different domains of life? *Res. Microbiol.* 154(4), 231–236 (2003).
81. Forterre P. Genomics and early cellular evolution, the origin of the DNA world. *C. R. Acad. Sci. III* 324(12), 1067–1076 (2001).
82. Forterre P. The origin of viruses and their possible role in major evolutionary transitions. *Virus Res.* 117(1), 5–16 (2006).

Websites

101. WHO. Policy Package to Combat Antimicrobial Resistance. www.who.int/world-health-day/2011/presskit/WHDIntrototobriefs.pdf?bcsi_scan_3c79e7817cdc4fd7=0&bcsi_scan_filename=WHDIntrototobriefs.pdf (Accessed January 2012)
102. WMA Declaration of Helsinki – Ethical Principles for Medical Research Involving Human Subjects. www.wma.net/en/30publications/10policies/b3/index.html (Accessed January 2012)

Copyright holders