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Silk route to the acceptance and re-implementation of bacteriophage therapy

Expert round table on acceptance and re-implementation of bacteriophage therapy.

Supporting information available online

This multidisciplinary expert panel opinion on bacteriophage therapy has been written in the context of a society that is confronted with an ever-increasing number of antibiotic resistant bacteria. To avoid the return to a pre-antibiotic era, alternative treatments are urgently needed. The authors aim to contribute to the opinion formation of relevant stakeholders on how to potentially develop an infrastructure and legislation that paves the way for the acceptance and re-implementation of bacteriophage therapy.

Rapid rise of antibiotic resistance has surged an increasing interest to develop alternative treatments to counter bacterial infections. The worldwide use of antibiotics has been also associated with reduced microbiome diversity, which in turn has been related to malnutrition and several types of other diseases [1]. To avoid the return to a pre-antibiotic era, alternative treatments are urgently needed. Bacteriophage therapy is accepted and practiced in parts of Eastern Europe, such as Russia, Georgia, and EU member state Poland. However, agreement has yet to be made in the rest of the world on a functional and practical legal frame-work that is flexible enough to exploit and further explore the specificity of bacteriophages (phages) as an antibacterial, while giving precedence to patient safety.

This paper arose through a series of workshops held during the conference "Phages as tools for therapy, prophylaxis and diagnostics" which took place in October 2015 in Tbilisi, Georgia. In order to evaluate the root cause of the actual delay of employing phage therapy in western countries, and to propose a solution, researchers, physicians, and industrial representatives discussed the necessary aspects of phage therapy to be incorporated into contemporary policy. Since this topic affects society as a whole, a stakeholder analysis was conducted by identifying and classifying all parties who are directly, or indirectly, involved in re-implementation of phage therapy. Next, elements in the present legal frame-work that the panel thought to be limiting, or even inhibiting, the re-introduction of phage therapy in western medicine were discussed, including topics such as intellectual property protection, clinical trial design, production methods, and composition of phage cocktails. The use of phage therapy for both curative and prophylactic purposes was also evaluated and related safety and quality norms were classified in three categories: essential, important and desirable. Subsequently, the expert panel concluded on a proposal that could be taken by different stakeholders and that also emphasizes the potential consequences for not implementing phage therapy applications.

Stakeholder analysis

Inspired by Mitchel et al. [2], a stakeholder analysis was conducted with the intention to position in a general context any group or individual who could affect or could be affected by the implementation of phage therapy (Supporting information, Table S1). The result of this analysis classifies national authorities and supra-national authorities as definitive stakeholders that can make significant influence on the implementation of phage therapy. Patients and patients' organizations alongside medical doctors, researchers, and small biotech companies are classified as dependent stakeholders, which means that although they are essential, removing the first hurdles for re-implementing the phage therapy is less dependent on these groups. Dormant stakeholders, like the established pharma industry, possess the power to impose their will, but their current involvement is limited by a lower degree of legitimacy or urgency. Dominant stakeholders, like politicians and health insurance companies, operate by a more formal mechanism to whether accept or not new treatments such as phage therapy. Finally, religious leaders and ethicists are classified as discretionary stakeholders whose insights can be considered as legitimate. However, the lack of power and urgency places these groups at a lower rank in the stakeholder hierarchy.

Root cause analysis and actions to take

The fundamental reasons limiting, or even inhibiting, the application of the phage therapy in the western world in the 21st century are summarized in Table 1. As key hurdles we identified previous study set-ups, phage cocktail production methods and composition in context of the current legal frame-work, limitations in intellectual property protection, and lack of awareness among (para-) medical staff and the general public. Table 1 also lists the measures that can be taken by the identified stakeholders to overcome these restraints. National

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Table 1. Top four root causes for delay in acceptation and application of phage therapy from scientific, legal, practical, financial, and educational points of view

Root cause	Description	Recommended solution	Main actor
Lack of well documented scientific evidence	Notwithstanding numerous case reports on the efficacy of phage therapy over 90 years of history, double blind and placebo and/or standard of care controlled randomized clinical trials in different fields of medical application are missing.	Set up more double blind and placebo and/or standard of care controlled randomized clinical trials, such as Swiss National Fund approved study using phage therapy for treating urinary tract infections (Kessler, T., personal communication) and Phagoburn (www.phagoburn.eu).	Universities, academic hospitals
Significant discrepancies between medicinal products legislation, GMP requirements, safety requirements and the nature of phages	The exploration and exploitation of the intrinsic strength of phages in terms of antagonistic evolution with their bacterial hosts, i.e. the continuous adjustment and adaptation of the composition of phage cocktails, is incompatible with current legislation and safety requirements concerning traditional static medicinal products.	Create a new EC Directive concerning phages and phage cocktails for human use, update the already existing Medicinal Products Directive 2001/83/EC with a specific amendment for phages and phage cocktails, or apply the Council Directive concerning medical devices (93/42/EEC) to phages and phage cocktails [14]. GMP requirements should be defined in the specific context of phages as natural entities. Safety risk assessments should be done in the context of alternative options. See also Table 2 that summarizes safety requirements. A monitoring system, much like that for antibiotic resistance, should be put into place at the initial implementation of phage therapy to collect data for prospective analyses, as well as to detect and follow the development of bacterial resistance to phages. Guidelines detailing the particular processes and services of phage therapy must be established. Accreditation systems would need to be created to train and inform clinicians and health care facilities on following these guidelines to ensure that phage therapy is used aptly.	(supra)National health authorities
Lack of awareness among medical staff and general public	In the curricula of (para-)medical trainees (MD's, nurses, pharmacists and biologists) there rarely is a basic course in evolutionary biology, including bacteria-phage antagonistic evolution. Despite the awareness on increased antibiotic resistance, there is no public awareness on phage therapy as potentially interesting and sustainable alternative/addition to antibiotics.	Educational programs must aim to remove the psychological stigma of phages as viruses and provide some basic microbiology knowledge in order to view phages as "good viruses". More communication by patient organizations on pro/cons of phages vs. antibiotics. Several of such initiatives have been taken at various places, e.g. in Lausanne, Switzerland, where a workshop on phages is available to graduate students, teachers (for collection of education credits), and the curious general public (http://wp.unil.ch/phageback/).	Academia, academic hospitals, national authorities, patients' organizations
IP legislation	As phages are ubiquitous natural organisms, which are in general relatively easy to isolate from the environment, and already in the public domain since the 1920s, the possibility for intellectual property protection are limited. In addition, it already occasionally happened that IP protection systems resulted in the inhibition of the advancement of science for society (e.g. the development of phage therapy).	Focus should be shifted from patenting the phages themselves to protecting downstream processes that transform them into therapeutic products with good quality and shelf life stability. Governments need to consider their stake in exploring and exploiting phage therapy as means to prevent further antibiotic resistance-associated costs. Private/government partnerships with patent pools under supra national governance should be managed through organizations such as the WHO, CDC, ECDC or UN [15].	(supra)National authorities

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phage therapy centers, operating under the supervision of public health authorities and in interaction with private stakeholders, could be a driving force behind the implementation of the proposed measures [3].

Despite the long history of use of phage therapy in eastern European countries and our conviction that it can add significant value around the globe, the production process of phage products must satisfy minimal quality and safety norms before they could be used in clinical applications. Table 2 details a priority path of safety and quality norms for the production of phage products and the application of phage therapy in terms of essential, important, and desirable. Our recommendation for safe production of phages deviates from current good manufacturing practices (GMP) requirements, which under the EU legislation require medicinal products including phage preparations to have a fixed, pre-defined composition. We reason that such legislation does not allow for the timely substitution of phage components and routine phage adaptation or phage "training", and actually limits the exploration of the natural strength of the phage concept. Adapted phages, as applied for instance at the Eliava Institute in Tbilisi, Georgia, are more effective in infecting relevant pathogens and elicit less bacterial resistance to phages [4, 5]. In our opinion we could combine a flexible production process with the highest degree of safety by applying a GMP approach and hazard analysis critical control points (HACCP) as is done in the food industry, where every step of the manufacture, storage and distribution of the product is scientifically analyzed for microbiological, physical and chemical hazards. Together with well-defined release criteria provided by modern analytical methods this approach would assure the absence of pathogenic host bacterial strains, virulence factors such as endotoxins and unintended phages lysed from the host strain used in the phage replication. Although phage therapy has already been applied for many years without reported adverse effects in countries mentioned before, we further propose the installation of monitoring systems at the initial implementation of phage therapy. This would allow the collection of meta-data for prospective analyses and the evaluation of the efficacy of continuously adapted phage cocktails in treating existing and emerging pathogenic bacterial strains.

In addition, we would like to promote the availability of large collections of well-characterized, therapeutically important phages and their bacterial host strains as an efficient way to design safe phage cocktails and rapidly adjust them with the needs of the patients. Such collections already exist at well-known phage therapy centers or repositories: Eliava Institute of Bacteriophages, Microbiology and Virology (IBMV, Georgia), Hirszfeld Institute of Immunology and Experimental Therapy (IIET, Poland), and the Félix d'Hérelle Reference Center for bacterial viruses of the University Laval (Canada). Recently, the Leibniz Institute DSMZ also launched the Therapeutic Phage Bank for deposition of phages with therapeutic interest.

Recommended targets for clinical trials

There is a clear need for well-conducted, randomized, double blind, placebo controlled clinical trials to settle the debate on the efficacy of phage therapy. Ideally, these trials should have the ability to convince the general public and alert decision makers about the potential benefits of phage therapy. We would recommend to set up a phage therapy study for the treatment of severe burn wounds infected with important nosocomial pathogens (ESKAPE bacteria: *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa*, and *Enterobacter* species). The proposed study set-up builds on the already running PhagoBurn FP7 clinical trial (http://www.phagoburn.eu/) that focuses on treating burn wound patients infected with *Escherichia coli* and *P. aeruginosa* bacteria. Since the general public and politicians are already aware of the consequences of hospital infections with antibiotic resistant "superbugs", we expect that multi-target phage therapy trials could serve as a springboard for further acceptance and re-implementation of phage therapy.

In addition to the aforementioned clinical study, we also recommend setting up trials that use phages for treating urinary tract and prostate infections. Although such targets are less attractive in terms of communication and public awareness, treating these infections with phage therapy is desirable from a scientific and technical perspective due to large number of patients, and because these patients are often infected only by one bacterial pathogen species allowing the use of single-phage preparations.

Based on common practice in eastern Europe, we further propose to investigate the treatment of infections by using a combination of phages and antibiotics, which are reported to have a synergistic effect in controlling bacterial infections [6].

Apart from the discussion on the application of phages therapeutically, the production and application of phage cocktails for decontaminating hospital environments is another opportunity that could be further explored: A previous study conducted by the Eliava Institute showed that phage decontamination of the hospital environment resulted in a lower incidence of nosocomial infections (Alavidze, Z., personal communication). Moreover and independent from our recommendations in Table 2, such specific prophylactic applications that do not involve direct contact between

Table 2. Minimal quality and safety requirements for phage therapy products

	Essential	Outcome	Important	Outcome	Desirable	Outcome
Phages	Free of potentially damaging genetic determinants (e.g. encoding for integrase, toxins and antibiotic-resistance)	Prevention of the spread of resistance and virulence genes	Characterization of phage morphology and classification of phages	Confirmation of phage identity (classification)	High barrier for host strain to develop resistance (e.g. pilus-dependent adsorption)	Maximal efficacy, additional benefit
	Activity against target strains as well as a broad host range at pathogen species level	High efficacy of final phage product	Low frequency of emergence of resistant bacterial mutants	Maximal efficacy of the final phage product	Immuno-modulatory effect on treated patient	
	Lytic only, non-transducing	Maximal exclusion of potential for horizontal gene transfer	High rate of clearance of target strains (physiological parameters, stability of lysis)		Compatibility with other anti-infectious treatments	Broader application
					Stable in various formulations (e.g. freeze-dried, solutions, gels)	
Production host bacterial strains	Non-virulent, non-toxin producing strain	Exclusion of toxicity of the final phage product	Stable strains (low mutation rate)	Prevention of resistance, exclusion of unpredictable mutations	Fast growing and easy to cultivate	Low cost of production
					Releasing as less temperate phages as possible (thresholds should be defined)	Negligible amount of temperate phages in final product. Negligible risk for horizontal gene transfer
Production process	Animal component free culture media and additives	Eliminate risk of transmitting zoonosis, including animal spongiform encephalopathy agents	Validated production process (published in peer-reviewed journals)	Exclusion of unpredictable results	–	
Final products	Non-pyrogenic	Exclusion of side effects (e.g. allergic reaction)	Sufficient coverage range for the targeted application	Maximal efficacy of the final phage product	Physiological pH	Limitation of side effects
	Sterile	Exclusion of microbial contaminants	Defined composition (by mass spectrometry)	Confirmation of expected composition	Minimize the amount of toxic preservatives (e.g. organomercury compounds)	Optimal dosage format
	Endotoxin levels within accepted levels for specific endotoxin definition	Reduction of side effects	Bacterial DNA residue-free (potentially damaging genetic determinants)	Exclusion of horizontal gene transfer		
			Long shelf-life (activity, pH, sterility)	Longevity of the product, lowered production costs		
Producer	Certified and frequently audited by authorized entity on compliance with essentials mentioned in this Table	To guarantee sustainable compliance with essential requirements	–		–	

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phages and patients are not expected to be hindered by regulatory and safety hurdles. As a part of future prophylactic studies, we would recommend to investigate the emergence and spread of bacterial phage resistance in the hospital environment and how it relates with the composition and number of phages used in the decontaminating phage cocktail. Such an approach is commonly practiced in the area of food safety [7].

Genetically engineered phages

Although phage therapy has been applied for more than 90 years by isolating and finding efficient phage species from environment, we now live in the era where we can directly edit phage genomes. For example, phages modified by the CRISPR-Cas genome editing tool have been demonstrated to be an effective mean in targeting and sensitizing antibiotic resistant bacteria by elimination of antibiotic resistance-conferring plasmids [8]. Genetic engineering could also be used to alter phage host range via reconstruction of the tail fibers [9]. Another option is the incorporation of a secondary payload into the phage genome, such as biofilm degrading enzymes or antimicrobial peptides, by replacement of nonessential phage genomic regions [10, 11]. Phages could also be engineered with antimicrobial agents that attack gene networks (e.g. SOS system) of antibiotic resistant bacteria. Such engineered phages could be further used alongside with antibiotic therapy to enhance the performance of antibiotics [12]. Apart from targeting phages, genetic engineering technology could be applied for the selective removal of virulence factors or prophages from the host strains, which could further increase the safety of phage products. This option could also be attractive in generating intellectual property rights.

In line with regulations surrounding genetic engineering in general, preventing genetically modified phages to escape into the environment is a major concern. To account this worry, phages could be modified to limit their replication upon infection of a host to ensure that once their task is completed, new virions are not released into the environment (*Phages in Interaction IV*, Leuven, BE 015).

Concluding remarks

Here we have given an opinion on the potential roles of several active and passive stakeholders involved in the debate surrounding the acceptance and re-implementation of the phage therapy. In many countries today, phage therapy falls under a legislation that does not recognize the specific nature of phages as self-amplifying and evolving anti-bacterials contrary to static chemical drugs. As a consequence, the application of phage therapy is still limited and its full potential has not been properly explored. The cost of delaying the implementation of phage therapy is enormous given the increasing prevalence of antibiotic resistant bacteria and its associated economic burden and public health consequences for the society.

We have also proposed a minimal list of safety and release requirements for phage cocktails. Patients' safety is not debatable and appropriate risk assessment of phage products and their subsequent application in phage therapy should be monitored continuously. In our opinion, such risk assessment should cover not only the potential adverse effects of the phage therapy but also the costs of not-applying phages as an alternative treatment when the antibiotics fail to treat resistant infections. For instance, the *E. coli* outbreak that caused the death of more than 50 patients in the Germany in 2011 [13] is a clearly higher cost compared to any potential risks phage therapy has ever been reported to cause.

Genetic engineering of phages could, on the one hand, offer vast opportunities for phage therapy. On the other hand, unintended release of genetically modified phages in the environment raises several concerns for the use of GM organisms. Thus at the time being, we propose that re-implementing phage therapy in its traditional form would already offer enough for creating new opportunities to treat bacterial infections.

We finally argue that new or adapted regulatory frame-works should acknowledge the inherent advantages of phages over antibiotics in terms of their sustainability (ever increasing phage infectivity via phage-bacteria coevolution) and their host specificity (no collateral damage to benign commensal flora). Recently, Verbeken et al. [14] evaluated possible regulatory pathways for the (re)introduction of phage therapy in a way that maintains its effectiveness, safety and as well its inherent advantages, evolvability and specificity. In addition to the proposal to classify phages under the medical devices frame-work, a more straightforward option would be to classify bacteriophage cocktails under the Medicinal Products Directive 2001/83/ EC that already comprises various pharmaceutical products and treatments which are different from the classical chemical molecules. Alternatively, a new Phage Directive could be created, e.g. "Directive Concerning the Therapeutic Use of Natural Phages" [14]. A new adapted regulatory frame-work should also include an accelerated time frame for developing phage preparations; while the regular timeline for a classical drug approval is often years, the development of a "new" natural phage product could take place within days or weeks.

This multidisciplinary expert panel opinion on bacteriophage therapy has been written in the context of a society that is confronted with an ever-increasing number of antibiotic resistant bacteria. This report aims to contribute to the opinion formation

of all mentioned stakeholders, especially (supra)national authorities in their role as definitive stakeholder, on how to develop an infrastructure and legislation that paves the way for the acceptance and re-implementation of bacteriophage therapy.

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