Developing Phages into Medicines for Europe

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1 Introduction

Considering the context of the growing threat of bacterial antimicrobial resistance (AMR) with potentially far-reaching health and economic impacts, bacteriophage therapy has been proposed as one novel strategy in countering this prospect (Czaplewski et al. 2016). Indeed, various historical data sources might suggest that bacteriophages could be safe and efficacious in treating both Gram-positive and Gram-negative bacterial infections, including multi-drug resistant (MDR) organisms (Sulakvelidze et al. 2001). This notion is further supported by more recent experience, such as reports on systemic phage administration in severely ill patients, suffering a difficult-to-treat infection (Jennes et al. 2017; Schooley et al. 2017). Nevertheless, comparative data remain sparse (Rhoads et al. 2009; Wright et al. 2009; Sarker et al. 2016), and convincing evidence from well-designed and rigorously conducted clinical trials is awaited to support introduction of bacteriophages into clinical practice.

Bacteriophages are classified by regulatory authorities as biological substances and fall within the scope of the pharmaceutical legislation (Pelfrene et al. 2016; Reindel and Fiore 2017). Mainly, whole-phage broad cocktails manufactured on an industrial scale may target a single or even multiple species, or conversely, a patient-specific cocktail can be selected from a local phage library (Pirnay et al. 2011). However, the phage concoctions do not easily align with the conventional concept of a medicinal product. Over the past few years, discussions between product developers

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and regulatory authorities have intensified on how the pharmaceutical regulation could offer flexibility in outlining the appropriate tests and studies prior to routine acceptance of bacteriophage intervention. In this light, the present contribution reviews the European regulatory requirements for bacteriophage therapeutics, reflects on some of the scientific hurdles and provides thoughts on how the licensing authority could support the specificities of phage therapeutic development.

2 EU Regulatory Framework

Bacteriophages meant for therapeutic administration are governed by the European regulatory framework on medicinal products and more specifically resort as biological products (Directive 2001/83/EC). This framework is applicable to whole phage products, either natural or recombinant, as well as phage-derived products, such as endolysins. It stipulates that for medicinal products "either prepared industrially or manufactured by a method involving an industrial process", a Marketing Authorisation (MA) constitutes a prerequisite. Hence, prior to placing the product on the market, it would be required that besides proving to be safe and efficacious for a given indication, quality can be assured with a manufacturing under current Good Manufacturing Processes (cGMP). The legislation calls also for predetermined "Qualitative and quantitative particulars of all the constituents of the medicinal product", and in case of differences, a separate authorisation may be necessary. Within the current context, it is however appreciated that this would be easier to suit phage-derived proteins and commercially prepared whole phage cocktails, rather than patient-specific concoctions consisting of lytic bacteriophages selected from a local phage library (i.e. local stock containing the active substances).

In absence of a specific quality guideline on bacteriophages, it is advised that the existing guidance pertaining to biotechnology and biological products broadly be followed: bacteriophages (as drug substances) and the final preparation (as drug product) need to be appropriately characterised and include a well-validated production process. Without being exhaustive on these requirements, the following principles apply (Parracho et al. 2012; Pelfrene et al. 2016):

1. Bacterial cell banking systems (cell substrates) should be devoid of prophages and lack antibiotic resistance.
2. Individual bacteriophages should display a lytic activity (i.e. not involving temperate phages) and specifically infect the bacterial isolate.
3. Preparations need to be controlled for impurities (endotoxins, pyrogenic exotoxins, host cell proteins and DNA and residual reagents).
4. Phages need testing for potency and purity (absence of adventitious phages and plasmids, bioburden and sterility).

As limitation to the aforementioned, it is acknowledged that identification and quantification of each individual phage in the Drug Product could prove to be demanding.

Apart from the above, the European Union legislation allows a few exceptions on the requirement to obtain a product licence; e.g. this applies to the magistral formula and the officinal formula, under Article 3 of the Directive (Directive 2001/83/EC), and as well for any advanced therapy medicinal product (ATMP) (EU-Regulation (EC) No 1394/2007), if prepared on a "non-routine" basis according to specific quality standards and meant as a custom-made product within the same EU Member State in a hospital under the exclusive professional responsibility of a medical practitioner. As such, if an applicant would develop a phage product that expresses a recombinant nucleic acid, classification as an ATMP could be sought, with the possibility to obtain such a "Hospital Exemption". Otherwise, specific authorisation requirements could anyway apply for recombinant phage products recognised as ATMP products, including the possibility of a risk-based approach to determine the amount of quality, preclinical and clinical data required in obtaining a MA. However, additionally, recombinant phage therapy would need to conform to the environmental regulation governing the deliberate release of genetically modified organisms (GMO) (Directive 2001/18/EC).

The exemption from EU licensing requirements are foreseen also under Article 83 of Regulation (EC) 726/2004 of the European Parliament and of the Council concerning "compassionate use" (EU-Regulation (EC) 726/2004). Although under remit of the Member States, this allows an unauthorised product to be made available to a group of patients who cannot satisfactorily be treated with currently licensed options and who cannot be enrolled in ongoing clinical trials. Eligibility to take part in such a programme is only possible when the medicinal candidate undergoes clinical trials or a submission has been made by the sponsor to obtain a product licence. As such, it transpires that compassionate use cannot be regarded as a permanent satisfactory regulatory option for phage therapy.

3 Preclinical and Clinical Development

In developing phage therapy, preclinical tests would provide an important contribution to establish proof-of-concept in support of the intended clinical use, including the route of administration, type of infection, whether as adjunctive therapy, concomitantly or sequentially, or for prevention. Pharmacodynamic studies would also contribute to dose selection and to characterise the potential for emergence of resistance. Additional tests to be conducted would mainly cover toxicity and immunogenicity potential. It is however remarked that no standardised methods for in vitro activity and susceptibility are currently available. Hence, as previously advocated by others, an international standard for preclinical effect would be desirable and provide opportunity to establish comparative data (Cooper et al. 2016).

In a classical trial setting, clinical development through stages aims to gather evidence that the therapeutic is safe and efficacious for its intended use in a well-defined patient population. It is however accepted that early phase tests conducted in healthy volunteers will not capture potential outcomes specific to the bacteriophage-bacterial host interaction. Further on, product development would need to establish

the appropriate exposure-response relationship of the therapeutic intervention, explore the role of the immune system in phage removal (including the influence of pre-existing antibodies generated in response to the abundant environmental exposure) and test with an appropriate dosing regimen for safety and efficacy in a large enough group of patients suffering a specified bacterial infection caused by specified species or strains of bacteria. Late stage clinical trials might as well be challenging in avoiding inclusion of a heterogeneous study population (e.g. due to differences in bacterial burden and host immune factors) and the limited host range of bacteriophages may necessitate broad enough cocktails to be effective. With therapeutic use, it is expected that the phage resistance profile of bacteria will evolve, necessitating adjustments to such cocktail composition. Thus, the therapeutic development will need to take account of some unique circumstances. Moreover, the ultimate goal of therapy might be different from increased cure rates as traditionally investigated with standard antibacterial agents and thus could cover other clinical benefits such as time-to-cure, relapse rates or else.

3.1 Clinical Pharmacology

Unlike conventional antimicrobials, whole bacteriophages are large size particles with poor diffusion capacity in nonaqueous media; only a small dose can be administered, with the antibacterial activity fully dependent on generating a "productive infection", i.e. new bacteriophages emerging upon lysis of the host bacteria, leading to their exponential amplification (Marza et al. 2006). This outcome can only hold true if enough bacteriophages can reach the bacterial target in first place, hitting it in a rather direct fashion. As such, they lend themselves ideal to be locally applied at the infection source, although for systemic use, virulent phages (fast producing a great number of progeny) might largely overcome such a limitation (Nilsson 2014). Thus, upon parenteral administration, resultant phage blood concentrations cannot be fully reflective of the activity at the site of infection. Following IV administration, whole bacteriophages are rapidly diluted and cleared from the bloodstream by combined action of the innate and adapted immune system. Although direct removal by the reticuloendothelial system (phagocytes) seems the most important mechanism involved, the potential for generating neutralising antibodies is well recognised (Hodyra-Stefaniak et al. 2015). In this regard, higher anti-phage antibody responses have been observed with the use of cocktails (viz. monovalent therapy) and also with longer treatment duration (Górski et al. 2018). A recently published analysis indicates that a good clinical outcome can nevertheless be expected in those developing high antibody titres during therapy involving oral or local phage administration (Łusiak-Szelachowska et al. 2017). With intravenous use, however, sufficient data are lacking, and a potential detrimental effect caused by high antibody levels cannot be dismissed.

For whole phage therapy, the composition of the cocktail will be critical to its success, and in all likelihood not each individual phage will successfully infect the bacterial target. The aim however is that component strains (ideally infecting via

different receptors) will achieve synergy, resulting in a fast reduction of bacterial density and residual clearance of the bacterial infection by host immunity. Other factors could be considered as well in deciding the composition of the cocktail, such as the capacity to evade phage resistance systems harboured in the host bacteria (Nilsson 2014).

Phage lysins are expected to behave in a more conventional way. To date, research mainly targets elimination of Gram-positive organisms, both systemically and from mucosal surfaces and biofilms (O'Flaherty et al. 2009; Schuch et al. 2017). In this regard, a multicentre, double-blinded, randomised, phase 2 trial has recently commenced in patients with MRSA bacteraemia (including endocarditis) and receiving anti-staphylococcal lysin or placebo, added to standard-of-care antibiotics [ClinTrial.gov NCT03163446] (Fischetti 2018). The development of engineered lysins able to penetrate the outer membrane of Gram-negative bacteria (Briers et al. 2014) may further broaden the appeal for their use as antibacterial products in difficult-to-treat infections.

3.2 Resistance

It has been hypothesised that bacteriophages characterised by fast adsorption rates and large burst size—so ensuring a quick reduction of the bacterial population—would minimise the emergence of resistance during treatment (Nilsson 2014). However, further data are warranted as the emergence of resistance following therapeutic use of phages seems inevitable and accordingly, cocktails will need adaptation at a certain time. Under the current paradigm, the resultant changes in qualitative and quantitative composition of the constituents of the industrial phage cocktail would trigger a rather time-consuming regulatory procedure and, depending on the extent of changes, may even require a new authorisation. As such, it might be desirable to accommodate this requirement for cocktail adaptation by granting a composition change in an expedited manner. Certain precedents exist in the EU pharmaceutical legislation, e.g. for influenza vaccines, for which changes in viral strain antigens are dealt with in an abbreviated fashion (EU-Regulation (EC) No 1234/2008; EMA-Guideline on influenza vaccines 2015). However, in case of bacteriophage therapies, it is still necessary to identify the exact nature of changes that would be required in practice and achieve a scientific consensus on the type and extent of data needed to assure that such changes would not adversely affect the efficacy or safety of the product.

3.3 Indications

Criteria for selecting suitable diseases depend on characteristics of infection, bacteria involved and nature of the bacteriophages themselves (Harper 2018). Use of obligatory lytic bacteriophages with broad strain coverage, limited number of bacterial species causing the pathology and sufficient accessibility of the infection site might increase the likelihood for a successful trial outcome. Hence, topical treatment seems

a most attractive proposition for this technology, e.g. in treatment of otitis media (Wright et al. 2009), diabetic foot ulcer infections (Fish et al. 2016) and as bladder instillation in treatment of urinary tract infection (Leitner et al. 2017). The ability for bacteriophages to disrupt biofilms also counts as an interesting feature to be exploited (Harper et al. 2014; Chan and Abedon 2015). In this sense, Chan et al. (2018) reported on successful phage treatment of a *Pseudomonas aeruginosa* chronically infected aortic graft with associated aorto-cutaneous fistula (Chan et al. 2018). Interestingly, for the case management, the authors indicate leveraging phage-resistance versus antibiotic-sensitivity trade-off, emphasising that understanding of evolutionary biology may help inform future phage therapy strategies. For oral administration—although previously demonstrated as innocuous in healthy volunteers (Bruttin and Brüssow 2005; Sarker et al. 2012; McCallin et al. 2013) and patients alike (Sarker et al. 2016)—a recent murine experiment cautions that orally provided bacteriophages may lead to increased gut permeability and systemic inflammation (Tetz and Tetz 2016). Others however could not confirm such finding in mice and pigs (Hong et al. 2016); moreover Górski's group recently commented upon the role of phages in maintenance of gut immune homeostasis and their capacity to downregulate activation of immune responses, providing an avenue for their therapeutic potential in inflammatory bowel disease and other conditions thought to be influenced by gut microbiota dysbiosis (Górski et al. 2017). This will require further validation through well-designed translational and confirmatory clinical research. Separately, with parenteral administration, concern has been expressed on the potential to invoke a cytokine-mediated inflammatory cascade upon rapid lysis of Gram-negative bacteria (Wittebole et al. 2014), although, in this respect, recently gathered anecdotal evidence suggests no untoward effects (Jennes et al. 2017; Schooley et al. 2017). Neither did comparative in vitro data on endotoxin release in *Escherichia coli* strains subjected to ß-lactam antibiotics, amikacin or bacteriophages cause reason for concern, but the in vivo relevance of these findings remains limited (Dufour et al. 2017). In a mini-review by Speck and Smithyman (2016), the authors comment on previously accumulated experience with intravenous phages administration and regard their use as safe in this manner (Speck and Smithyman 2016).

In summary, site of infection and route of phage administration could prove to be important determinants for efficacy, immune responses and the potential for adverse events. Ultimately, for proposed indications, appropriate evidence on safe and effective use would need to be demonstrated in well-conducted randomised clinical trials. Whether bacteriophages will be given as standalone treatment or as "add-on" to standard antibacterial therapy, will have implication on the study design and the hypotheses to be tested. Importantly, the clinical endpoints in the studies will have to reflect the expected clinical benefit to patients. Additionally, in relation to personalised phage therapy (with specific strains selected upon the infecting bacterial susceptibility), the extent of the safety and efficacy data obtained in such individual patients that would allow for broad generalisation remains to be discussed.

4 Could We Have Phage Products Available as a Standard Healthcare Measure?

As a stage-defined process, development aiming for marketing approval is time-consuming and costly. To date, however, the limited evidence generated to contemporary regulatory norms on safety, tolerability and effective administration of bacteriophages remains a fundamental limitation to its acceptance by the medical community. Thus, in order to propel development of phage treatment, "proof-of-concept" studies, assessing clinical benefit conform to stringent regulatory standards, are foremost required. Encouraging results obtained in small-scale controlled trial setting would certainly help in fostering confidence, inform late-stage trial design and facilitate the discussion on the most suitable regulatory framework for authorisation of bacteriophage products and their variants.

Broad phage cocktails intended for large-scale production could more easily comply with the current legislative requirements (Directive 2001/83/EC), and hence licensing these for treatment use might be a realistic prospect, although the need for cocktail adjustments still require a lengthy variation procedure. Likewise, it is expected that the regulatory framework could readily support the development of phage-derived proteins. However, the EU framework is less conducive in relation to personalised therapy. For the latter, the finished product is comparable to a magistral formula, although the phage library adheres to the concept of an industrial process. In this regard, a "hybrid approach" has been advocated, by which licensing of the active ingredient is deemed paramount, obviating the requirement to grant authorisation to the finished product (Fauconnier 2017). According to such a modus operandi, patient-specific phage therapy would be selected from a local "pre-authorised" phage library (i.e. approval of a European "Biological Master File", presently non-existent). In the advent of evolving bacterial host resistance during the treatment course, a polyphage concoction could then easily be adjusted in a timely and flexible manner by adding newly selected bacteriophages from the prequalified phage stock. The proposition has been advanced that granting an authorisation in such a manner could meet both societal expectations for quality, safety and efficacy and also the practitioners' and patients' needs for customised personalised medicines (Fauconnier 2017). However, this would not obviate the need to develop the standards for approval of individual bacteriophages and depending on the eventual data requirements, it remains to be seen if such approach of assessment at an active substance level (rather than the medicinal product) could bring important benefits and overcome the difficulties related to the need for authorisation of a large number of different bacteriophages.

Further on, initiatives have been taken at national level to support bacteriophage technology; e.g. Belgium recently created a "magistral phage medicines" framework—with bacteriophages conforming to provisions of internal phage monograph for active pharmaceutical ingredients (APIs)—as a pragmatic approach allowing regulatory-compliant use of such treatment for individual patients within its territory (Pirnay et al. 2018). Any developments of the EU-wide regulatory framework for personalised medicinal treatment might in the future result in new approaches in general, and more specifically, for the use of bacteriophages.

Separately, in undertaking the necessary studies, the use of adaptive licensing for phage therapy authorisation has been proposed (Cooper et al. 2016). According to this concept, the need for timely access is balanced with the importance of providing adequate, evolving information on medicines' benefits and risks (Eichler et al. 2015). As such, an initial authorisation of a phage therapy would be granted on the basis of the demonstration of a positive benefit-risk balance in a defined patient population (possibly on basis of surrogate endpoints, e.g. reduction of bacterial load and beneficial effect on inflammatory parameters, with explorative analyses for clinical endpoints, such as symptoms/signs resolution). This would be followed by iterative phases of evidence gathering, including real-world data, and the adaptation of the MA to extend the access of the therapeutic to broader patient populations while gradually refining the knowledge of the benefit-risk balance during the post-authorisation phase. It has been argued by Cooper et al. (2016) that not only pre-made cocktails, but also patient-customised therapy could be eligible for adaptive pathway trials. Use of pre-characterised libraries in formulating the custom cocktail would allow evidence gathering with bacteriophages targeting a specific bacterial pathogen in a defined condition. However, this proposal raises several regulatory concerns and would clash with the first and foremost need for robust clinical evidence on safety and efficacy of phage therapy.

5 Conclusions

Overall, it has become clear that in order to maximise the potential for phage therapy, a pro-active engagement between developers and regulatory authorities is deemed crucial. Informal exchanges on legislative requirements and subsequent formal guidance via established regulatory processes on study design and appropriate tests to be conducted, might provide the best chances to introduce treatment with bacteriophages, if indeed so proven safe and effective for intended indications.

It is recognised that with the threat posed by MDR bacterial pathogens, novel approaches will become necessary. Amongst others, the use of bacteriophages (and derived proteins) is regarded as promising. Spurred by latest state-of-art scientific developments, the regulatory environment for phage therapy is attempting to accommodate this potential treatment option within the parameters of strict safeguards. The regulators eagerly await availability of further data that would facilitate the discussion on how these safeguards can be ensured via appropriate authorisation requirements.

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