Phage2030 – Introducing phage therapy in Germany by 2030

Berlin, 2023-08-21

Christian Willy & Felix Broecker

Summary - Results and Conclusion

- Research landscape is characterized by numerous groups and companies with highly innovative approaches to increase the clinical efficacy of phage therapy necessary for broad application and approval by regulatory authorities
- An overarching Europe-wide coordination and focusing/prioritization of key research aspects is required to achieve a timely provision of highly active phage therapeutics to the population
- Coordination of research efforts includes the formation of highly interconnected international research clusters in which research groups are actively interacting and streamlining their activities to prevent redundant research and exploit synergies
- Identified research clusters require targeted funding of individual research groups and a high-level coordination to promote the necessary interactions between the clusters
- Key to the research efforts is the use of artificial intelligence/machine learning approaches that have a high potential to accelerate characterization and selection of phages for therapeutic use
- Personalized approaches are necessary at least for some pathogens of the ESKAPE spectrum due to the high strain specificity of phages
- Highly flexible small-scale production capacities to manufacture tailor-made phage cocktails for individual patients are required, ideally on-site in the treating healthcare facilities
- Conventional phage production methods are inefficient and will not be sufficient to provide phages to all ESKAPE pathogens
- Cell-free phage production is a key technology with various advantages over conventional production, especially for GMP-grade production
- A *Center for Phage Therapy* needs to be founded, which will address the major limitations of current phage therapy approaches in terms of phage identification, production, and therapeutic efficacy
- Funding required to support the translational research efforts amount to €18.3 Mio for 3 years (1st priority) plus an optional €7.1 Mio (2nd priority)
- In addition, 2-4 RCTs should be supported (€1.5 Mio per trial)

Table of contents

1. BACKGROUND 4

2. STATUS OF PHAGE THERAPY IN GERMANY, 2023 5

2.1. Legal and regulatory situation 5

2.2. Clinical application in Germany today 5

3. NECESSARY ACTIVITIES IN TRANSLATIONAL PHAGE RESEARCH 7

3.1. Cluster 1: AI-based phage diagnostics and therapy 9

3.2. Cluster 2: Development of a rapid test to identify phage-host pairs 11

3.3. Cluster 3: Phage-antibiotic combination/phage cocktail composition 12

3.4. Cluster 4: Resistance development of bacteria to phages 13

3.5. Cluster 5: Phages against biofilms 14

3.6. Cluster 6: Cell-free phage production 15

3.7. Cluster 7: Genetically modified phages 16

3.8. Cluster 8: Fully synthetic phages 17

3.9. Cluster 9: One Health 18

3.10. Additional research 19

4. NECESSARY ACTIVITIES IN THE FIELD OF PHAGE PRODUCTION 20

4.1. Most common bacterial pathogens in Germany 20

4.2. Frequency and development of MDR 21

4.3. Required phage quantities 23

4.4. Suitable healthcare facilities for phage therapy 24

4.5. Estimation of costs for phage production and treatment 25

4.6. Future phage production scenarios 27

5. ASPECTS OF A ONE HEALTH APPROACH 30

5.1. Urgent research questions in the veterinary field 30

5.2. Relevant research groups 30

5.3. Expected benefits of phage therapy for human health 31

5.4. The regulatory burden in the veterinary sector 31

6. REFERENCES 32

7. THE TEAM 33

Abbreviations

AI	Artificial intelligence
AMG	Arzneimittelgesetz (German Medicinal Products Act)
API	Active pharmaceutical ingredient
BfArM	Bundesinstitut für Arzneimittel und Medizinprodukte (German Federal Institute for Drugs and Medical Devices)
DZIF	Deutsches Zentrum für Infektionsforschung (German Center for Infectious Disease Research)
EEA	European Economic Area
EU	European Union
FTE	Full-time employee
GMO	Genetically modified organism
GMP	Good manufacturing practice
ML	Machine learning
MDR	Multidrug resistance
MRSA	Methicillin-resistant *Staphylococcus aureus*
MRSE	Methicillin-resistant *Staphylococcus epidermidis*
PFU	Plaque-forming unit
Ph.Eur.	European Pharmacopoeia
PTMP	Phage therapy medicinal products
RCT	Randomized-controlled clinical trial
RKI	Robert Koch Institute
RWE	Real-world evidence
SOP	Standard Operating Procedure
WHO	World Health Organization
ZIB	Zuse Institute Berlin

1. Background

First successes of phage therapy were already reported ~100 years ago.[1] Various factors, including the introduction of antibiotics in the 1940s, improper phage use, and problems with production led to the decline of phage therapy in the West. In the former Soviet Union, with institutions like the Eliava Institute (Tbilisi, Georgia) and the Hirszfeld Institute (Wroclaw, Poland), however, phage therapy continued. The Cold War climate contributed to Western skepticism and dwindling interest in the use of phages. Although phage research and use had continued in France into the 1980s, it was the search for expansion of antimicrobial strategies under the pressure of multidrug resistance (MDR) to antibiotics that led to renewed interest in phage therapy.

In recent years, phage therapy has been boosted by various reports of patients with MDR infections, in which phage administration led to a turning point that ensured their survival (=high evidence class 1c). Among those was a comatose patient with necrotizing pancreatitis, caused by *Acinetobacter baumannii* resistant to conventional therapy, who was finally cured by a personalized phage cocktail.[2] Another example is the successful treatment of a 15-year-old cystic fibrosis patient who, after receiving a lung transplant, suffered from an MDR *Mycobacterium* infection, and was only treated palliatively with antibiotics. In the end, the patient survived due to complete pathogen eradication after phage therapy.[3]

There are relatively few randomized-controlled clinical trials (RCT; evidence class 1b or 2a) to evaluate phage therapy (2009-2017).[4-7] These studies could only demonstrate significant benefits of phage therapy in one study and showed numerous methodological limitations, including:

- Too low pathogenic bacterial titers to sustain phage replication,
- Treatment of patients with too low a phage concentration (unexpected loss of active phages during the production process),
- No personalized approach (no testing if available phages are active for the patients' bacterial isolates),
- Phage cocktail not tailored to regionally relevant pathogen strains (phages ineffective),
- Oral application without antacid (leading to inactivation of the phages at low pH),
- Instability of the phage mixture.

Recently, however, positive interim results of a phase 1 clinical trial investigating orally administered genetically modified phages against pathogenic MDR *E. coli* were reported by the Danish company SNIPR Biome (press release of May 31st, 2023). The phages were well-tolerated and reduced intestinal *E. coli* levels. In addition, numerous new clinical trials have been initiated, and the World Economic Forum highlighted phage therapy as one of the Top 10 Emerging Technologies of 2023.[8]

In addition to these RCTs, recent clinical evidence and systematic reviews (analyses of mostly individual curative trials, case series; evidence class 3 and 4) now point to the effectiveness of phage therapy for a variety of infectious diseases.[9-11] For example, two systematic reviews from 2020 show, based on the treatment of 1,432 patients from 43 evaluated articles, that the use of phages resulted in a clinical cure in 80.8%, improvement in 10.7% and no improvement in 8.5% of cases.[12,13] Thus, it is becoming increasingly evident that phage therapy can contribute to the control of MDR infections in various clinical situations.

2. Status of phage therapy in Germany, 2023

2.1. Legal and regulatory situation

Currently, no therapeutic phage products are approved in Germany by the Federal Institute for Drugs and Medical Devices (BfArM). In the European Union (EU), phages for therapeutic use in humans are defined as biological medicinal products under Directive 2001/83/EC. This means that a marketing authorization is required for phage therapy medicinal products (PTMPs) to enter the market. Article 3 of Directive 2001/83/EC (and §21(2b) of the German Medicines Act, AMG) defines exceptions to the need for a marketing authorization, including prescription-only medicinal products prepared in a pharmacy for a specific patient ("magistral prescription"). According to §55(8) AMG, these products must be prepared according to the recognized pharmaceutical rules, e.g., the European Pharmacopoeia (Ph.Eur.). Recently (April 2023), a general chapter of the Ph.Eur. on PTMPs was published, providing harmonized quality standards. According to §13 AMG, a manufacturing authorization is required for commercially produced PTMPs to be placed on the market, with an exception, §13(2) AMG, covering manufacture in pharmacies ("in the course of normal pharmacy business") or by physicians ("under their direct professional responsibility for the purpose of personal application to a specific patient"). The manufacture of PTMPs must also be carried out in accordance with recognized pharmaceutical rules and is subject to the obligation to notify the competent supervisory authorities (§67(2) AMG).

The use of PTMPs is permitted in individual cases. Here, the treating physician decides under their responsibility within the framework of therapeutic freedom and with the patient's consent if there is an "unmet medical need" according to Article 37 of the Declaration of Helsinki of the World Medical Association, i.e., unproven interventions in clinical practice, and if a benefit of the phage use for the patient can be expected based on scientific findings. The focus of the individual treatment study is not to obtain research results, but to cure the patient.

A phase 1/2 clinical trial in Germany (*Phage4Cure*; https://phage4cure.de) has started in 2023, testing an inhaled phage cocktail against *Pseudomonas aeruginosa*-infected lungs of cystic fibrosis patients.

A revision of the AMG is planned to be completed in ~3-5 years, likely including a yet undefined solution for the authorization of phages. Nevertheless, high-quality RCTs are urgently required. A scientifically sound database (i.e., a registry), including data from individual therapeutic trials, is also needed, and would likely be considered as supporting evidence for approval (in general, real-world evidence (RWE) such as registry data is becoming increasingly considered in approval processes in many countries).

2.2. Clinical application in Germany today

The status of clinical phage application in Germany in 2023 has recently been described in detail by the authors.[1] However, the main aspects are described below.

Phages are used clinically according to Article 37 of the Declaration of Helsinki, i.e., in cases of failed conventional therapy, typically MDR infections (exception: the *Phage4Cure* clinical trial). The following lists the clinics in Germany that have in recent years applied phage therapy.

Berlin: In the Military Academic Hospital (*Bundeswehrkrankenhaus*) Berlin, 3 patients have been treated with phages from the Eliava Institute (Tbilisi, Georgia) since 2016. As part of the *PhagoFlow* research project (www.phagoflow.de), patients with *P. aeruginosa* and *S. aureus* infections are currently being treated on a larger scale with magistral phage preparations. Additionally, at the German Heart Institute Berlin, Germany, 6 patients were treated from 2018-2021, in collaboration with Charité—Universitätsmedizin Berlin, Germany, Center for Musculoskeletal Surgery, with phages acquired from the Belgian LabMCT and the Eliava Institute.

Hannover: Phage therapy is performed in the Department of Cardiac, Thoracic, Transplantation and Vascular Surgery of the Hannover Medical School. Out of 33 cases of personalized therapy since 2015 31 were successful. The clinical success rate of >90% is due to a combination of modern principles of permission-free preparation using mostly self-isolated phages, an interdisciplinary approach to administration of phages and an optimal, concomitant conventional treatment, including antibiotic therapy.

Regensburg: At the Department of Trauma Surgery University Hospital Regensburg, phage therapy has been performed since 2022. The first case was an infected non-union of the proximal femur with different MDR bacteria treated with a commercial phage cocktail produced by the Eliava Institute active against various strains of *Shigella*, *Salmonella*, *E. coli*, *Proteus*, *S. aureus*, *P. aeruginosa*, and *Enterococcus*. No local or systemic side effects were observed after a 3-month follow-up. Additionally, the department participates in the *PhagoDAIR* trial, a randomized, non-comparative, double-blinded phase 1/2 clinical study in patients with *S. aureus* periprosthetic joint infections (ClinicalTrials.gov ID: NCT05369104).

Rostock: Phage therapy is performed at the Clinic for General, Visceral, Thoracic, Vascular and Transplant Surgery, Rostock University Medical Centre. Indications in the field of vascular surgery are mainly patch- or bypass-associated infections in the groin area and life-threatening aortic prosthesis infections. In cardiac surgery, the most frequent indication is drive-line infection after insertion of left ventricular assist devices. In orthopedic surgery patients, periprosthetic-joint infections in the hip area dominate, increasingly also after implantations of endo-exo systems in transfemoral amputations. Patients have been treated a composite phage cocktail, SniPha 360 (Phage24, Austria), which to date has only been therapeutically successful in ~30% of cases. Individualized therapy with phages produced on-site is planned for 2023 and expected to significantly improve success rates.

3. Necessary activities in translational phage research

Based on literature review, discussions at scientific meetings and intensive exchange within the phage research community, it became apparent that translationally oriented research needs to be promoted to increase efficiency, reduce costs and facilitate clinical approval in the process.

A considerable need for optimization of phage therapy was identified, e.g., due to the currently small number of available phages, the slow and labor-intensive conventional susceptibility testing (phagogram), the preparation of phage cocktails according to the trial-and-error principle, the lack of knowledge on the interaction of phages and antibiotics, the failure to consider biofilm in septic situations and the repeatedly observed development of bacterial resistance to phages. Therefore, the key development steps that will allow for a timely increase in the effectiveness of phage therapy were defined.

To this end, we identified the major research groups in Europe that could help advance phage therapy into the clinics based on published literature (~4,150 references of the last 3 years were analyzed). We excluded those groups exclusively engaged in basic research unless a clear impact on translational aspects was identified. Abstracts from conferences and letters of interests submitted by various research groups were also considered. The most important research groups were identified in intensive exchange with the Brussels working group that has the most experience in Europe with phage production, research, and therapy (Jean-Paul Pirnay; Queen Astrid Military Hospital, Brussels, Belgium) to form translational, interdisciplinary research clusters that promise to answer the most urgent practice-relevant open research questions as quickly as possible [Fig. 1]. Emphasis was placed on the translational utility of the research for clinical implementation of phage therapy. The groups were also asked to provide an approximate cost estimate for the proposed projects (for a period of 3 years), so that the total costs for research activities could be calculated.

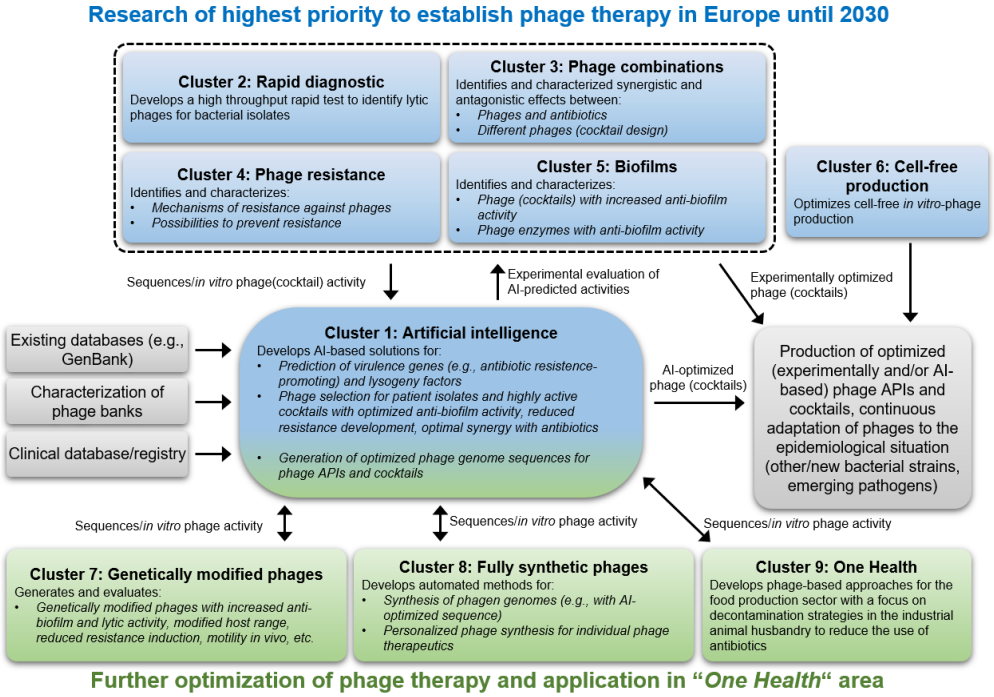

Figure 1: Overview of research clusters of *Phage2030*.

Central to the research activities is the cluster on phage-centric application of artificial intelligence (AI) (Cluster 1) that will become increasingly important over the course of the project as more data from the other research clusters defined here are incorporated. Thus, AI and machine learning (ML) approaches will be trained by means of continuous expansion and networking of well-characterized phage databases, a clinical registry for all patients treated with phages, and comprehensive data collection of phage characteristics (e.g., activity against biofilms, synergies with antibiotics, interactions of phages with each other) and, above all, genome sequence data of experimentally verified (induction of lysis) phage-host pairs. These data are extracted from existing databases such as GenBank, arise in the various research clusters, as well as through sequencing and characterization of existing phage collections that are continuously being expanded. Properties of phages such as synergy with antibiotics (Cluster 3), potential emergence of resistance in the host (Cluster 4), or anti-biofilm activity (Cluster 5) will also serve to train ML algorithms. Therefore, the clusters are interdependent and will evolve together (synergy). The development of a rapid test (Cluster 2) that identifies phage-host pairs in a few hours or minutes in high-throughput, replacing the labor-intensive and slow conventional phagogram, both supports clinical applications by rapidly identifying tailored phage therapeutics for patients and further accelerates training of AI/ML approaches so that predictions become more accurate.

Another highly important advance is the cell-free production of phages (Cluster 6), which offers numerous advantages over conventional production in a host bacterium (e.g., lower endotoxin content, higher flexibility, rapid switch in production from one phage to another possible, better scalability, and the possibility of using phage DNA banks instead of phage banks). Currently, cell-free phage production is not yet possible for all phages, so further development is needed for this technique. Through the expansion and complete characterization of the available phage banks (e.g., *Klebsiella* phage bank at the Institute of Microbiology of the German Armed Forces, Munich, and clinical phage banks at the University of Helsinki and in Pisa) and the establishment of phage production, the goal of establishing phage therapy against the most important pathogens (ESKAPE group) will be possible by 2030. However, only with the accompanying scientific work on the most urgent application-related questions (Clusters 1-6) phage therapy will have a realistic chance for broad implementation by 2030. Therefore, funding measures need to be initiated as soon as possible and should especially focus on these clusters (1st priority).

Further research into genetically modified phages (Cluster 7) and the complete synthesis (e.g. full genome synthesis and bioprinting) of phages for clinical application (Cluster 8), will serve to optimize phage therapy beyond the year 2030, whereby the still unclear regulatory framework for the use of genetically modified phages will have to be taken into account. For example, in Germany, genetically modified organisms (GMOs) for clinical use are not within responsibility of BfArM, but instead of the Paul-Ehrlich-Institut. Regulations vary substantially between countries and are typically more restrictive for GMOs (vs natural phages). However, GMO phages are already being tested clinically (e.g., SNIPR Biome using CRISPR-Cas armed phages). Clusters 7 & 8 are initially not essential for achieving the overall goal but will bring about further decisive improvements in clinical efficiency and simplified, automated production (therefore 2nd priority). Cluster 9 ("One Health") aims to develop phage application especially in industrial animal husbandry, livestock, and food industries. Here, too, there will be much potential for cooperation and interaction with the other clusters, as there are numerous clinically relevant issues (e.g., biofilms, resistance development) in the veterinary field as well.

AI-based approaches will be important to advance all research clusters. Most importantly, AI/ML algorithms will be able to predict phage-host pairs. Of note, due to the host specificity of phages,

at least initially (existence of still relatively small data sets), pathogen-focused training of AI approaches will be more promising than approaches that include all phages. The initial focus should be on clinically relevant pathogens for which there are no broadly effective natural phages, e.g., *K. pneumoniae* and *A. baumannii*. A critical milestone will be achieved when AI/ML approaches can accurately (e.g., >90%) predict effective lysis with (natural or genetically modified) phages for a given bacterial isolate. The following step will be algorithms that have "learned" from existing information to combine new, artificial genome sequences that generate tailored, replicating synthetic phages for specific patient isolates. The presumably necessary continuous adaptation of phages (APIs and cocktails) to currently circulating bacterial strains and the identification of phage (cocktails) against emerging pathogens could also take place in an AI-assisted manner.

In the following, the research clusters shown in Fig. 1 are briefly described (more details can be provided upon request).

3.1. Cluster 1: AI-based phage diagnostics and therapy

The goal of this cluster is to develop AI-based approaches that primarily identify/predict a phage (cocktail) that is optimally effective for bacterial isolates from patients. This will require phages characterized in terms of their genome sequence and various parameters such as experimentally determined host specificity, anti-biofilm activity, synergies/antagonisms with other phages or antibiotics, and resistance development in the host. Such data for training the AI algorithms will initially come from databases such as GenBank and well-characterized phage banks from the research community. Sufficient sequence data are already available for the initial establishment of AI-based phage-host prediction algorithms (personal communication by Thomas Sicheritz-Pontén). Additional data will be generated in the experimental research clusters (esp. Clusters 3-6) and collected in a database; they will further improve the AI/ML approaches for more accurate predictions. AI-based approaches also have great potential for cocktail design, as even a small collection of 20 phages against pathogen X would require over a million experiments for all possible combinations. Pre-selecting the most promising combinations by AI approaches would thus substantially reduce the experimental effort.

AI-identified phage-host pairs or the lytic activity of phage cocktails or phage-antibiotic combinations will be experimentally verified, further contributing to training/learning. In a further iteration, the AI should be able to generate genome sequences of completely new phages with optimized activity against specific bacterial isolates, which can then be generated using synthetic biology (Cluster 7) and again tested experimentally. Both iterations of the AI (identification of natural phages as well as generation of new phages) will be used to support and inform the treatment of patients whose antibiotic-resistant pathogens are not lysed by phage preparations produced on a large scale (no cocktail is 100% effective) or are not (yet) covered by any of these phage preparations (e.g., *K. pneumoniae* or rare pathogens for which large-scale composite phage production is not practical). Completely newly generated phages could also be used to combat pathogens for which no natural, effective lytic phages are known (e.g., *Clostridioides difficile*). AI could also be used to optimize (or adapt to changing epidemiological situations) the composition of phage preparations produced on a large scale and generate phage cocktails against emerging infectious diseases. The identified research groups for targeted funding are:

- **Marcus Weber** (Zuse Institute Berlin, ZIB):
  - Develops AI-based prediction of phage-host interactions and of the impact of the microbiome on phage therapy and *vice versa*.
  - Has extensive and long-standing expertise in research on molecular and biological processes such as protein folding as well as protein-protein and protein-ligand interactions.
  - Has access to necessary computing capabilities (supercomputers).
  - Is experienced in modeling complex interaction networks of the human microbiome.
  - In addition, ZIB could provide the IT infrastructure for a phage database.
- **Ralf Herwig** (Max Planck Institute for Molecular Genetics, Berlin):
  - Plans to develop ML approaches for phage classification, host and virulence prediction, and identification of the most effective phages for pathogens of patients.
  - Will initially use genome sequences of known phage-host relationships from the GenBank database (>14,000 phage genome sequences, 87% of which have a known host)
  - Will implement a "meta-learning framework" ("learning to learn") for these purposes.
  - High potential for synergies with ZIB.
- AI-based phage genome characterization and host prediction approaches are also being developed and applied by **Andrew Millard** and **Martha Clokie** (University of Leicester, UK), **Bob Blasdel** (Vésale Bioscience, Noville-sur-Mehaigne, Belgium) and **Thomas Sicheritz-Pontén** (GLOBE Institute, Copenhagen, Denmark) providing good opportunities for collaboration with the above groups.
- **Yves Briers** and **Bernard De Baets** (Ghent University, Belgium):
  - Develop AI algorithms to predict the host specificity of *Klebsiella* phages.
  - Develop a platform technology that equips a common phage scaffold with AI-identified receptor binding proteins to generate synthetic phages with desired host specificities.
  - High potential to significantly advance personalized phage therapy for *K. pneumoniae*.
- **Christian Cambillau** and **Adeline Goulet** (Aix-Marseille University, France):
  - Have expertise in sequence-based prediction of the 3D structure of phage receptor binding proteins with AlphaFold2 and other algorithms.
  - Important to predict the first step of phage infection (host surface receptor recognition).
  - Will develop AI-based approaches to targeted modification of phage proteins to modulate host specificities.
  - Cooperations with groups of **Gemma Atkinson** (Lund University, Sweden), **Bas Dutilh** (Utrecht University, The Netherlands), **Burkard Rost** (TU Munich, Germany) and **Rachael Wilkinson** (Swansea University, UK) working on similar approaches will create synergies.
- **Felipe Molina** (University of Extremadura, Badajoz, Spain):
  - Develops computational approaches to design and optimize phage cocktails, considering various parameters of the phages.
  - Extensive experience in computational design of phage cocktails and computational analysis of phage-bacteria interaction networks and phage-phage interactions.
- **Mariagrazia Di Luca** (University of Pisa, Italy):
  - Develops AI-based algorithms to predict the host specificity of phages, optimized phage cocktails and phage-antibiotic synergies.
  - Can already predict an optimized cocktail within 20 minutes using genome sequences of the target bacterium and phages from a proprietary phage bank.
  - Phage bank currently comprises ~200 phages against various clinically relevant pathogens.
  - Aims to expand the phage bank to 1,000 phages in the coming years (personal communication by Di Luca) to allow for further training of the AI approaches.

- **Nina Chanishvili** (Eliava Institute, Tbilisi, Georgia):
  - Develops text mining approaches to access the Georgian and Russian literature on phage therapy.

Conclusions: Use of AI will substantially advance the clinical development of phage therapy, particularly by predicting effective phage-host pairs. Predicting the host specificity of therapeutic phages is disruptive especially for personalized therapy in that it can limit the need for time-consuming and labor-intensive experimental susceptibility testing. AI-based-pre-selection of phages will result in a significant time advantage from diagnosis to therapy. Current published phage-host prediction algorithms are still imprecise due to the relatively low number of phage sequences used for training. The research efforts described here will generate a large number of sequenced phage-host pairs (and integrate existing data) so that the potential of AI can be exploited far more effectively than in the small networks that already exist. Overall, each of the relevant research topics will benefit significantly from a targeted use of AI-assisted data analysis. This cluster will generate data in a magnitude that has not existed before to enable a wide range of analyses.

3.2. Cluster 2: Development of a rapid test to identify phage-host pairs

The conventional method to identify phages that lyse a patient isolate (phagogram) requires the patient's bacterial isolate in pure culture, is labor-intensive, slow (at least 2-3 days), low throughput and difficult to automate. A rapid test that can identify suitable phages within hours or minutes will not only revolutionize clinical applications but will also facilitate screening of large numbers of phages, e.g., from phage banks, significantly advancing characterization and generating data to train AI/ML algorithms (Cluster 1). The goal of this cluster is therefore to develop a method to rapidly screen phage-host pairs in high throughput, which replaces the conventional phagogram in research and clinical settings. The identified research groups for targeted funding are:

- Collaboration between **Joachim Bugert** (Institute of Microbiology of the German Armed Forces, Munich, Germany), **Jens-André Hammerl** (Federal Institute for Risk Assessment, Berlin, Germany) and **Hesham Yosef** (microphotonX, Tutzing, Germany):
  - Develop a spectroscopy-based approach enabling the detection of matching phages in under an hour (~25 min, depending on the phage).
  - Obtained proof-of-concept with *Klebsiella pneumoniae* and matching phages (specific lysis detected within ~25 min).
  - Miniaturized approach reduces the amount of sample required.
  - Possibility to detect phage-antibiotic synergies.
  - Fully automated detection will be developed.
  - No need for a pure culture of the patient's isolate, further reducing time from diagnosis to treatment (important for patients in life-threatening conditions such as sepsis).
  - High potential to substantially advance the possibilities and efficacy of personalized phage treatment.
- **Saija Kiljunen** (University of Helsinki, Finland):
  - Develops methods to immobilize and stabilize phages using specific matrices (hydrogels) to enable refrigerated storage of ready-to-use phage tests.

  - The immobilization methods will help to develop ready-to-use assays for other approaches (e.g., spectroscopy approach mentioned above).
- **Benedict Diederich** (Leibniz Institute for Photonic Technologies Jena, Germany):
  - Develops detection of phage-mediated lysis by light microscopy through the "disappearance" of individual bacteria in a microtiter plate.
  - Image analysis will be automated (cloud-based) with the aid of ML approaches.
  - Light microscopes required for this approach are significantly cheaper than microplate readers or spectroscopes; therefore, it is promising for low- and middle-income countries.

Conclusions: The Raman spectroscopy approach is highly promising for clinical use in microbiological diagnostics due to the above-described advantages over conventional microbiological diagnostics and the phagogram. Targeted support of the R&D activities of the already initiated collaboration has thereby great potential not only to develop a fast, high-throughput alternative to the phagogram, but also to revolutionize the entire microbiological diagnostics and the definition of personalized antiseptic (combination) therapies. To this end - after the already obtained proof-of-concept - robustness will be optimized with a larger number of isolates, clinical samples will be tested in direct comparison with the conventional phagogram (method validation), miniaturization and automation will be further developed, among other things. Phage immobilization/stabilizing approaches will assist in these developments. A light microscopy-based approach will help to develop a cost-effective alternative for fast, AI-assisted phage-host identification, especially for low- and middle-income countries.

3.3. Cluster 3: Phage-antibiotic combination/phage cocktail composition

Phages and antibiotics as well as phages together (in a cocktail) can exhibit additive, synergistic, but also antagonistic/inhibitory effects. Currently, treatment with phages is usually performed in the form of cocktails (usually 2-4 phages) and together with antibiotics, but often without precise knowledge about possible combination effects. A better understanding will help to exploit synergies and prevent antagonisms to enhance treatment success. The goal of this cluster is to uncover synergies and antagonisms between phages and antibiotics and between phages. The identified research groups for targeted funding are:

- **Jean-Paul Pirnay** (Queen Astrid Military Hospital, Brussels, Belgium) and **Rob Lavigne** (KU Leuven, Belgium):
  - Leading in Western Europe in the clinical application of phage therapy, including investigation of phage cocktails and phage-antibiotic synergies.
  - Outstanding experience with treating patients.
- **Martin Witzenrath** (Charité Berlin, Germany):
  - Investigates antagonistic behavior between phages in a cocktail (phage-phage competition).
  - Experienced in treating patients as part of the *Phage4Cure* clinical trial (treatment of *Pseudomonas aeruginosa* infected cystic fibrosis patients with phages).
  - Possible collaboration with **Julia Frunzke** (Forschungszentrum Jülich, Germany), who is also investigating antagonisms between phages and antibiotics.

- **Hans-Peter Horz** (RWTH Aachen, Germany):
  - Investigates synergistic effects between phages and antibiotics against methicillin-resistant *Staphylococcus aureus*, *Klebsiella pneumoniae* and other pathogens.
- **Bartłomiej Grygorcewicz** (University of Szczecin, Poland):
  - Studies the effect of phage cocktails and antibiotics (individually or combined) in biofilm models of relevant pathogens such as *S. aureus* and *Acinetobacter baumannii*.
  - Works on algorithms to characterize phage-antibiotic synergies, which provides good opportunities for collaboration with Cluster 1.
- **Frédéric Laurent** (Croix-Rousse University Hospital, Lyon, France):
  - Investigates phage-antibiotic synergies against *S. aureus* biofilms *in vitro* and *in vivo*.
  - Member of the *PHAGEinLYON* consortium of scientists and clinicians, advancing the identification of therapeutically active phages, their production, quality control, and application to patients, with a focus on bone and joint infections.
- **Martha Clokie** (University of Leicester, UK):
  - Experienced in optimizing phage cocktails, e.g., against *Clostridioides difficile* or *Salmonella*, and evaluating them *in vitro* and *in vivo* (e.g., wax moth model).
- **Li Deng** (TU Munich/Helmholtz, Germany):
  - Develops numerous *in silico* models to predict phage-phage and phage-antibiotic interactions (good opportunities for cooperation with Cluster 1).

Conclusions: Phage-phage and phage-antibiotic interactions are the most essential application-relevant topics besides resistance development. A coordinated research effort on these interactions has the potential to improve the therapeutic efficacy of phage therapy substantially. Several active groups with partly overlapping research questions are working on this topic in Europe. Despite the similarity of the research questions, there is currently no overarching coordination. An association of these groups in a coordinated cluster with a focus on ESKAPE pathogens, combined with targeted funding, will on the one hand avoid redundancies and on the other hand make targeted use of the strengths of the individual groups.

3.4. Cluster 4: Resistance development of bacteria to phages

Just as bacteria can develop antibiotic resistance, they can become resistant to phages. To minimize this, phage cocktails are often used for treatment, containing phages that ideally recognize different bacterial receptors. Resistance can, for example, develop *via* mutations in these receptors, or through adaptive CRISPR-Cas immunity. However, many other mechanisms of resistance development are still poorly understood. A better knowledge of these will help design phage cocktails that are less susceptible to resistance development. This will improve therapeutic success and, potentially reduce the number of phages in cocktails, which in turn will reduce the cost of treatment. The identified research groups for targeted funding are:

- **Frederic Bertels** (Max Planck Institute for Evolutionary Biology, Plön, Germany):
  - Develops phages with broad spectrum of activity against *Escherichia coli* by targeted *in vitro* evolution, to overcome resistance formation.
  - Studies the influence of antibiotics on resistance development in *E. coli*.
  - Initial findings: resistance development is slowed down even at sub-inhibitory antibiotic concentrations.

  - Consequences for clinical application: Should a poorly effective antibiotic be administered during phage therapy, as this could slow down resistance development to the phages?
- **Martin Witzenrath** (Charité Berlin, Germany):
  - Plans to determine the genotypic and phenotypic characteristics of bacterial phage resistance (focus on *P. aeruginosa*).
  - Recently found that phage-infected *P. aeruginosa* enter a transient phenotypically tolerant state to withstand stress before a subpopulation acquires a resistance-mediating gene mutation.
  - Blocking transient phage tolerance mechanisms could not only enhance phage therapy but also halt development of genetic phage resistance.
- **María Tomás** (University of A Coruña, Spain):
  - Identifies new resistance mechanisms *via* proteomic analyses.
  - Approach is complementary to those of Witzenrath (resistance development at the DNA/RNA levels) and Sorek (see below), who identifies bacterial immune systems and phage avoidance strategies at the genetic level.
- **Michael Brockhurst** (University of York, UK):
  - Investigates phage-bacteria coevolution to identify characteristics of phages that, when combined as a cocktail, minimize resistance development in the target bacterium.
- **Rotem Sorek** (Weizmann Institute of Science, Rehovot, Israel):
  - World leader in the discovery of resistance mechanisms of bacteria to phages.
  - Discovered over 30 different bacterial immune systems.
  - Investigates the countermeasures of phages, which can be exploited to limit resistance formation.
- **Angus Buckling** (University of Exeter, Penryn, UK):
  - Investigates bacteria-phage coevolution and factors driving resistance development.
  - These findings help to derive strategies to suppress resistance.

Conclusions: The development of bacterial resistance to phages is an extremely application-relevant problem. A better understanding of the mechanisms and the corresponding avoidance strategies will make it possible to design cocktails that are efficiently in suppressing resistance. In addition, *in vitro* coevolution experiments can be used to broaden the spectrum of phage activity without generating a genetically modified organism (which could only be used with strong regulatory restrictions).

3.5. Cluster 5: Phages against biofilms

Goal of this cluster is to develop effective phage-based therapies against biofilms, which primarily involves the identification of natural or modified phage (cocktails) with anti-biofilm activity and the use of biofilm-degrading enzymes. Biofilms are a major clinical problem as they protect bacteria from antibiotics and phages. Bacterial biofilms occur in infections of wounds, catheters, and implants, among others, and frequently in the lungs of patients with cystic fibrosis, who are often infected with *P. aeruginosa*. Thus, the topic is highly clinically relevant. The identified research groups for targeted funding are:

- **Saija Kiljunen** (University of Helsinki, Finland):
  - Has many years of experience in translational phage research and access to a large phage bank (currently >500 phages focusing on the ESKAPE pathogens).

  - The goal is to expand the collection to 2,000-3,000 phages for clinical use.
  - The phages are well characterized for host specificity and suitability for therapeutic purposes (stability, growth/production characteristics); ~60% are sequenced.
  - Establishes different biofilm models for ESKAPE pathogens to screen phages from the collection for anti-biofilm activity.
- **Ana Rodríguez** and **Pilar García** (Instituto de Productos Lácteos de Asturias, Villaviciosa, Spain):
  - Develop combination therapies of phages and recombinant biofilm-degrading enzymes.
  - Such enzymes could increase the therapeutic success, especially in topical applications (e.g., infected wounds) and could be incorporated into genetically engineered phages.
  - Plan to use a proprietary rapid test to identify combinations of phages with enzymes and antibiotics effective against biofilms of *S. aureus* and related pathogens.
- **Joana Azeredo** (University of Minho, Braga, Portugal):
  - Has extensive experience in analyzing the anti-biofilm activity of phages and biofilm-degrading enzymes.
  - Uses complex *in vitro* models such as multispecies biofilms that simulate human infections.
  - Generated phages with reduced genomes that did not affect bactericidal activity.
  - As genome length is limited by the phage's packaging process, downsizing phage genomes can "free up space" to insert transgenes, e.g., encoding biofilm-degrading enzymes.
- Collaboration between **Christian Hackenberger** (Leibniz Institute of Molecular Pharmacology, Berlin, Germany), **Daniel Lauster** (Freie Universität Berlin, Germany), **Alexandro Rodríguez-Rochas** (University of Veterinary Medicine Vienna, Austria) and **Marius Hittinger** of PharmBioTec (Saarbrücken, Germany):
  - Plan to generate phages with enhanced anti-biofilm activity, focusing on *Pseudomonas aeruginosa* and using two approaches:
  - 1. Equip phages with non-canonical amino acids that serve as attachment points for chemical or enzymatic attachment of biofilm-penetrating peptides to the phage capsid.
  - 2. Generate genetically engineered phages that produce biofilm-degrading enzymes from the infected bacteria.
  - Subsequently, the modified phages will be tested in organoid lung models and in mouse models of *P. aeruginosa*-infected cystic fibrosis.
- **Knut Drescher** (University of Basel, Switzerland):
  - Studies the mechanisms of resistance of biofilms to phages.
  - Such information points to new potential routes to improved anti-biofilm activity.

Conclusions: Three approaches to the use of phages against biofilms were identified, (i) high-throughput screening of natural phages (cocktails) for anti-biofilm activity, (ii) the use of recombinant biofilm-degrading enzymes in combination with phages, and (iii) genetic modification of phages with the aim of increased anti-biofilm activity. For all three approaches we identified strong research groups that through coordination and targeted funding can exploit synergies and significantly enhance phage therapy effectiveness against biofilms.

3.6. Cluster 6: Cell-free phage production

A highly critical aspect for the expansion of phage therapy by 2030 concerns production. Especially for personalized phage therapy against pathogens such as *K. pneumoniae* (each patient needs their own optimized phage cocktail), conventional phage production by propagation in the

host bacterium has disadvantages and limitations. On the one hand, conventional production of *Klebsiella* phages requires numerous host bacteria due to their high specificity (and for GMP conform production, a large master cell bank). On the other hand, compliance with the legally required limit for endotoxin content is problematic because, for example, phages bind endotoxin to varying extents and different host bacteria produce varying amounts of endotoxin. The logistical and quality control efforts would thus be disproportionately high, especially for clinics using permission-free production. Furthermore, if used according to the Belgian model (magistral preparation in a pharmacy), the necessary conventional GMP-compliant production would be disproportionately expensive (currently ~500,000 EUR for a 3-phage cocktail to treat one patient, personal communication from Frenk Smrekar, CEO of Jafral, Slovenia).

A promising alternative to conventional production, especially for personalized phage cocktails, is cell-free synthesis by *in vitro* translation. To this end, the company Invitris (Garching) is developing approaches to produce phages on a small scale relatively inexpensively, e.g., suitable for permission-free production. In a proof-of-concept experiment an *E. coli* phage was successfully produced within ~6h (and 5 min hands-on experimental time) at the Military Hospital Berlin, which in principle could be used for treatment within the scope of permission-free production. The main advantages are:

- Absence of variable endotoxin contaminations (endotoxin content is always below the legally required limits for i.v. injection due to dilution steps),
- No necessity for storing bacterial production strains (GMP: no master cell bank required, which greatly reduces logistic effort and cost),
- No necessity for storing phages; instead, phage DNA is stored, which is more stable,
- Appropriate for small-scale on-site production for tailor-made cocktails (good scalability),
- Easy switch from one phage to another.

However, the cell-free production approach still requires optimization; to date, not all phages can be produced. It has been developed for *E. coli* phages and needs to be optimized for other pathogens to cover the ESKAPE spectrum. This can be achieved by 2026 by tweaking the reaction conditions (personal communication by Patrick Grossmann, CEO of Invitris).

Cell-free synthesis is intended to take place initially at the Military Hospital Berlin for permission-free production according to the German Medicinal Products Act. The hospital also has a pharmacy capable of manufacturing magistral preparations and a microbiology department experienced with phages.

Conclusions: Highly personalized phage cocktails for infections with pathogens such as *K. pneumoniae* and *A. baumannii* cannot be produced by conventional means, as time requirements, cost and effort would be unreasonably high. Cell-free production provides a viable and highly attractive alternative to conventional phage production in host bacteria. Manufacturing of phages against the ESKAPE spectrum of pathogens will be achieved by 2026 if the necessary funding is provided.

3.7. Cluster 7: Genetically modified phages

Aim of this cluster is to generate phages with improved therapeutic efficacy *via* genetic alterations. Due to the clinical importance, such phages with enhanced anti-biofilm activity have already been

included in Cluster 5 above. Other modifications that extend or change the host range or "arm" phages for increased bactericidal activity also have the potential to enhance clinical efficacy. Of note, genetically modified phages (armed with CRISPR-Cas) have recently been successfully tested in a phase 1 clinical trial by SNIPR Biome (Copenhagen, Denmark), providing proof that such phages are safe and efficacious in humans. The identified research groups for targeted funding are:

- Collaboration between **Yves Briers** (Ghent University, Belgium), **Zuzanna Drulis-Kawa** (Kraków University, Poland), **Stan Brouns** (TU Delft, Netherlands) and **Kilian Vogele** (Invitris, Munich, Germany):
  - Develop an approach to change the host range of *Klebsiella* phages by means of inserting different receptor binding proteins into a common scaffold.
- **Tamás Fehér** (Szeged University, Hungary):
  - Investigates host range modulation of *E. coli* phages by altering the receptor binding protein
  - Investigates the possibility of removing repressor genes to convert lysogenic (not useful for therapy) to lytic phages (which can be used therapeutically).
- **Martin Loessner** (ETH Zurich, Switzerland):
  - Develops a method to modify the host range of phages by targeted structure-based modification of receptor binding proteins.
- **Heather Fairhead** (Phico Therapeutics, Bourn, UK):
  - Develops armed phages that inactivate the hosts genome by encoding a transgene for a DNA-binding protein.

Conclusions: Targeted genetic modification has great potential to increase the clinical efficacy of phages. This includes enhanced anti-biofilm activity and improved bactericidal activity. Moreover, *via* modifying or "transplanting" receptor binding proteins, phages with new specificities can be generated, for example, against strains or bacterial species that are not lysed by any known natural phage. The possibility of converting lysogenic to lytic phages by genetically inactivating repressor genes will greatly increase the number of clinically effective phages.

3.8. Cluster 8: Fully synthetic phages

Especially at a point where AI algorithms can generate newly combined, non-natural phage genomes it will be necessary to synthesize phages using synthetic biology approaches. The goal is to develop a fully automated phage synthesis device that generates tailor-made therapeutic phages for highly individualized treatment, using AI-generated optimized phage genome sequences as input. It is noteworthy that double-stranded DNA molecules up to 1.5 Mio basepairs can already be synthesized today using a combination of solid-phase synthesis and enzymatic assembly (the typical phage genome sizes ranges between ~30,000-170,000 basepairs). Approaches to generate fully synthetic phage particles are also being developed (see below), so that it appears realistic that generating fully synthetic phages can be achieved by 2030. The identified research groups for targeted funding are:

- **Sylvestre Marillonnet** (Leibniz Institute for Plant Biochemistry, Halle/Saale, Germany):
  - Develops methods for standardized assembly of large DNA molecules that could be suitable to synthesize phage genomes.

- **Joachim Spatz** (Max Planck Institute for Medical Research):
  - Develops fully synthetic phage particles based on lipid vesicles that, loaded with a phage genome, could initiate the phage's replication cycle.
- **Hendrik Dietz** (TU Munich, Germany):
  - Develops DNA nanostructures ("DNA origami") that could mimic structural phage proteins such as receptor binding proteins.

Conclusions: Fully synthetic phages require two components: 1. a synthesized DNA genome and 2. a synthetic particle with phage-like properties. DNA strands of the required length can already be synthesized; further development of DNA assembly strategies will help to achieve automated, fast, and cheap synthesis of phage genomes. Several approaches for synthetic phage particles are being developed that could pave the way for synthetic, tailor-made phages for clinical applications.

3.9. Cluster 9: One Health

The application of phages in animal husbandry and food production is a promising approach to limit the development and spread of antibiotic resistance. This will limit the transmission of antibiotic-resistant zoonotic pathogens, providing a direct benefit to human health as well. The identified research groups for targeted funding are:

- **Alicja Wegrzyn** (Polish Academy of Sciences, Gdánsk, Poland):
  - Develops a phage collection against *Salmonella* and other pathogens relevant in the poultry industry.
  - Develops AI-based algorithms to identify the most efficacious phage cocktails for use in the poultry industry.
- **Sophie Kittler** (TiHo Hannover, Germany):
  - Has many years of experience in using phages in animal husbandry and food production.
  - Has a large collection of well-characterized phages against relevant pathogens.
- **Jens-André Hammerl** (Federal Institute for Risk Assessment, Berlin, Germany):
  - Has a large collection of phages against relevant pathogens.
- **Martin Loessner** (ETH Zurich, Switzerland):
  - Has long-standing expertise in using phages for food safety.
- **Martha Clokie** (University of Leicester, UK):
  - Has many years of experience in using phages in veterinary medicine (e.g., *Salmonella* phages in pig breeding).

Conclusions: Especially in industrial livestock production the application of phages is a highly promising means to limit the use of antibiotics and thereby the spread of antibiotic resistance genes, including in various zoonotic pathogens. Thereby, there will be a benefit both to animals and humans. Developing large phage banks for relevant pathogens as well as innovative methods to identify highly effective phage cocktails are key to implementing phage therapy successfully in the livestock/food production sectors.

3.10. Additional research

Additional research not covered by the above clusters include various safety aspects of phage therapy. The identified research groups for targeted funding are:

- **Saija Kiljunen** (University of Helsinki, Finland):
  - Develops methods for endotoxin removal in conventionally produced phage preparations.
- **Martin Witzenrath** (Charité Berlin, Germany):
  - Studies the immunogenicity of phages that could negatively affect treatment success.
- **Maria Vehreschild** (University Clinic Cologne, Germany) and **Dennis Sandris Nielsen** (University of Copenhagen, Denmark):
  - Study the impact of phage therapy on the microbiota.

4. Necessary activities in the field of phage production

Considerations are based on the expected number of patients, the technical production possibilities for small and large scale, the necessity for personalized treatments, the host range of the respective phages, their availability, the capabilities of hospitals, microbiological institutes, and pharmacies to carry out the necessary preparation steps, a possible reimbursement of costs and regulatory requirements.

4.1. Most common bacterial pathogens in Germany

Of the ~13.7 Mio infection-related global deaths in 2019, 7.7 Mio were due to 33 relevant bacterial pathogens, both MDR and sensitive to antibiotics. These pathogens account for 13.6% of all deaths worldwide. *Staphylococcus aureus*, *E. coli*, *Streptococcus pneumoniae*, *K. pneumoniae*, and *P. aeruginosa* caused 54.9% of deaths among the bacteria studied.[17]

In Germany, *Streptococcus pneumoniae* plays a comparatively minor role. The pathogen statistics of the Robert Koch Institute (RKI) were used to record the situation in Germany. For the health care system in Germany, the overall view of the two sectors (outpatient and inpatient) for the number of pathogens results in the order shown in Table 1.

Table 1: Ranking of bacterial pathogens in the outpatient and inpatient sectors in 2021 calculated by summarizing the numbers published by the RKI for 2021.

Rank	Pathogen	Number of records	Share of total	Cumulative share of total
	Total	3,100,892	100%	
1	*Escherichia coli*	720,290	23.2%	23.2%
2	*Staphylococcus aureus*	250,300	8.1%	31.3%
3	*Enterococcus faecalis*	206,159	6.6%	37.9%
4	*Klebsiella pneumoniae*	137,588	4.4%	42.4%
5	*Proteus mirabilis*	128,458	4.1%	46.5%
6	*Staphylococcus epidermidis*	123,184	4%	50.5%
7	*Pseudomonas aeruginosa*	119,795	3.9%	54.4%
8	Streptococcus group B / *S. agalactiae*	107,072	3.5%	57.8%
9	Staphylococci Coagulase-negative	84,633	2.7%	60.5%
10	*Enterococcus* spp.	67,255	2.2%	62.7%

The top ten pathogens are responsible for >60% of all bacterial infections (non-MDR and MDR, nosocomial and community-acquired). Regarding the four most common pathogens, *E. coli*, *S. aureus*, *E. faecalis* and *K. pneumoniae*, there tends to be agreement with the data available for the EU and the European Economic Area (EEA).[18]

The most reported bacterial species (2021) in the EU and EEA was *E. coli* (39.4% of all cases), followed by *S. aureus* (22.1%), *K. pneumoniae* (11.9%), *E. faecalis* (8.8%), *E. faecium* (6.2%), *P. aeruginosa* (6.1%), *Acinetobacter* spp. (3%) and *S. pneumoniae* (2.5%).

4.2. Frequency and development of MDR

The absolute number of infections does not determine the need for phage products, assuming that initial focus will be on MDR infections. Therefore, the frequency of MDR was investigated.

Global: In 2019, ~4.95 Mio deaths related to MDR were observed, including 1.27 Mio directly attributable to MDR.[19] Death rates were highest in western sub-Saharan Africa and lowest in Australasia. The top six pathogens, *E. coli*, *S. aureus*, *K. pneumoniae*, *S. pneumoniae*, *A. baumannii* and *P. aeruginosa*, accounted for 929,000 MDR-attributed deaths and 3.57 Mio MDR-related deaths. In high-income regions, about half of the fatal MDR burden was associated with two pathogens, *S. aureus* and *E. coli*. The six leading pathogens contributing to the MDR burden have been identified as priority pathogens by the WHO, amongst others. However, only one of these pathogens, *S. pneumoniae*, has been the focus of a major global health intervention program, primarily through vaccination.

Europe: From the European Antimicrobial Resistance Network (EARS-Net) data, ~670,000 infections with selected MDR bacteria, *K. pneumoniae*, *Acinetobacter* spp., *E. coli*, *E. faecium*, *E. faecalis*, *P. aeruginosa*, *S. pneumoniae*, and *S. aureus*, with various antibiotic resistances, occurred in 2015, of which 63.5% were nosocomial. These were responsible for ~33,000 directly attributable deaths.[20] Relating the "European" incidence figures to Germany (population: 84 Mio) would result in ~110,000 infections with selected antibiotic-resistant bacteria.

Germany: The proportion of patients infected in hospitals is ~3.6%, corresponding to ~500,000 nosocomial infections per year, as estimated for 2006.[21] According to the RKI ~6% or ~36,000 of these were caused by MDR pathogens. The five most important MDR pathogens cause ~29,000 nosocomial infections (methicillin-resistant *S. aureus* (MRSA), vancomycin-resistant enterococci (*E. faecalis* and *E. faecium*), MDR *E. coli*, MDR *K. pneumoniae* and MDR *P. aeruginosa*). Of these, ~1,500 cases (0.3%) are caused by isolates resistant to almost all classes of antibiotics.[21-24] An analysis of data from 2015 found that ~54,500 people in Germany were infected by MDR pathogens.[20,25] Data from the Federal Statistical Office from 2019 provide information on the distribution of 4MRGN pathogens (resistant to four classes of antibiotics). A total of ~12,000 cases were registered, of which ~1,500 were "only" carriers (no active infection).[26] Bacteria of the *Enterobacterales* genus as well as *E. coli* and *K. pneumoniae* were the most relevant pathogens. The frequency of high-grade resistance and the associated mortality (cumulative 90-day mortality of 34.3%) underline the urgent need for effective therapies.[26] The proportions of MRSA in hospitals (2008-2010) and in outpatient care were 19.2 % and 10.6 %, respectively.[27] More recent (2021) data from the RKI indicate an incidence of up to 8.5% MRSA (oxacillin resistance).[28]

In the context of the present question, it must be considered that in Gram-negative bacteria, the *quality* of MDR can vary greatly in infections caused by the same species. Thus, the extent of MDR (how many antibiotics, which ones) significantly determines the necessary isolation measures, therapeutic success and, if necessary, the initialization of phage therapy.

The following resistance situations are seen as indications for the use of phage products:

- Colistin-, carbapenem-, or third generation cephalosporin-resistant *Escherichia coli*
- Colistin-, carbapenem-, or third generation cephalosporin-resistant *Klebsiella pneumoniae*
- Colistin-, carbapenem-, or multidrug-resistant *Pseudomonas aeruginosa*
- Colistin-, carbapenem-, or multidrug-resistant *Acinetobacter* spp.

- Vancomycin-resistant *Enterococcus faecalis*
- Vancomycin-resistant *Enterococcus faecium*
- Methicillin-resistant *Staphylococcus aureus* (MRSA)
- Methicillin-resistant *Staphylococcus epidermidis* (MRSE)

In Europe, the burden of carbapenem-resistant *K. pneumoniae* increased most substantially from 2007-2015 (6.2-fold), as measured by the number of associated deaths, followed by carbapenem-resistant *E. coli* and *K. pneumoniae* resistant to third generation cephalosporins. The number of deaths attributable to third-generation cephalosporin-resistant *E. coli* increased 4.1-fold from 2007-2015 to ~8,750.[20]

Within one year (2020-2021) the number of reported cases for all MDR pathogens has increased in Europe; most strongly for *Acinetobacter* spp. (+43%; in EU/EEA), *E. faecium* (+21%), *E. faecalis* (+14%), *S. aureus* (+9.4%), *P. aeruginosa* (+8.2%), *K. pneumoniae* (+8.1%), *S. pneumoniae* (+4.3%) and *E. coli* (+2.8%). For *K. pneumoniae*, carbapenem resistance is rising sharply (2018-2019: +8 %, 2020 +31%, 2021 again by 20% (56). Overall, MDR is undergoing a highly dynamic development, with double-digit yearly growth rates.

The burden of MDR was estimated based on 0.8-1.2 Mio nosocomial infections per year, as assumed by the German Society for Hospital Hygiene, which, at an MDR rate of ~6%, include 48,000-72,000 infections with MDR bacteria annually (excluding community-acquired infections).[29] The estimated numbers for the individual relevant pathogens are shown in Table 2.

Table 2: Estimated annual numbers of infections with multidrug resistant (MDR) bacteria (most relevant pathogens) in the outpatient and inpatient sectors.

Pathogen	Number of MDR infections per year
Colistin-, carbapenem-, or third generation cephalosporin-resistant *Escherichia coli*	15,000
Colistin-, carbapenem-, or third generation cephalosporin-resistant *Klebsiella pneumoniae.*	7,500
Colistin-, carbapenem-, or multidrug-resistant *Pseudomonas aeruginosa*	10,000
Colistin-, carbapenem-, or multidrug-resistant *Acinetobacter* spp.	1,500
Vancomycin-resistant *Enterococcus faecalis*	500
Vancomycin-resistant *Enterococcus faecium*	7,500
Methicillin-resistant *Staphylococcus aureus* (MRSA)	18,000
Methicillin-resistant *Staphylococcus epidermidis* (MRSE)	2,000
Total	**62,000**

It must be considered that the increasing geopolitical challenges leading to migration (war, poverty, climate change) and the care of war wounds (the incidence of MDR in infected wounds of Ukrainian soldiers is >50%) will considerably influence the dynamics of MDR in the coming years. Due to a lack of antibiotic stewardship and extensive antibiotic use, patients from the Middle East and North Africa (Palestine, Libya, Syria) and Ukraine treated since 2010 have shown

a relatively high proportion of MDR, according to our analyses. Thus, any current and future geographical population shifts for Germany will likely result in an increase of MDR.

In numerous non-MDR infection situations (e.g., chronic infection knee joint prosthesis, multiple implant changes, elderly, multimorbid patients) treating physicians do not necessarily have their "back against the wall" microbiologically. However, they may be forced, due to the lack of success with conventional treatment, to extend therapy to all available and reasonable measures. It is assumed that in these non-MDR cases the necessity for phage therapy will be increasingly realized - as is already evident today by numerous enquiries from clinics. The extent of this patient population is difficult to estimate.

Overall, the numbers provided in Table 2 are a very conservative estimate. They do not include recent geographical shifts of populations with high MDR rates, the foreseeable expansion to include non-MDR infections or possible prophylactic uses.

4.3. Required phage quantities

Estimating the required amount of phages per patient served as the basis to calculate the demand for the entire German population to cure difficult to treat infections (e.g., WHO critical pathogens, ESKAPE group). Consequently, the required production capacities for the various phages could be estimated. All data were collected *via* the PubMed database. Application modes of 395 patients from 69 publications were included in the analysis (151 patients from RCTs) [Table 3].

Table 3: Overview of the modes of application of phage therapy stratified by clinical indications. The total number of 422 results from the fact that of the 395 patients included, 26 were treated with several modes of application, including one patient with three different ones.

	Implant-associated infection	Respir-atory infection	Skin/ wound infection	Urogenital infection	Bacter-emia	Diarrhea	Skeletal infection	Other	Several	**Total**
Topical	23	23	126	32	1	-	4	17	-	**226**
Oral	3	3	1	4	-	79	-	1	43	**134**
Intraven-ously	10	7	-	1	16	-	2	5	-	**41**
Inhalative	-	14	-	-	-	-	-	-	-	**14**
Rectal	-	-	-	5	-	-	-	-	1	**6**
Vaginal	-	-	-	1	-	-	-	-	-	**1**
Total	**36**	**47**	**127**	**43**	**17**	**79**	**6**	**23**	**44**	**422**

The application characteristics varied markedly among studies. Thus, only an approximate estimate of the phage quantity per therapy of a single patient was possible. Table 4 shows the phage quantity per individual patient treatment stratified by application mode.

Table 4: Required phage quantities for the most relevant application modes. Estimates are according to literature analysis adapted to practicability (practicable sizes and conditions, e.g., container size, sensible application frequencies, tolerable swallowing volumes, etc.). Scientific studies were included that disclosed all information on dose, concentration, application frequency and duration. For treatments with several phages simultaneously (cocktails), amounts were multiplied by the number of phages used.

Application mode	Single dose (mL)	Phage concentration (PFU/mL) of the applied fluid	Applications per day (frequency)	Duration (days)	Total amount of phage fluid in mL	Total phage count (PFU)
Topical	30	10^8	1.5	14	630	6.3×10^{10}
Oral	15	10^8	2	20	600	6×10^{10}
Intravenously	30	10^9	2	20	1,200	1.2×10^{12}
Inhalative	6	10^9	2	20	240	2.4×10^{11}

The cocktails described in the literature are extremely variable in the number of phages administered at the same time (2-32 phages). Yet in most cases 2-4 phages were used against one bacterial species. This number was confirmed by leading phage experts in Germany who suggested combining 3 phages (in some situations preferably 4) in a cocktail (to expand host range and limit resistance formation).

It is, however, very difficult to predict how many phages should be included in the phage product for each pathogen. The host range is a critical feature that varies substantially between individual phages and the biology of phage-host interactions. Ideally, phages with a broad host range (most isolates of a pathogenic bacterial species) are used, however, these are not available for all clinically relevant pathogens. Personalized approaches (individual selection of only effective phages) usually require fewer phages than ready-to-use cocktails (personal communication from Mzia Kutateladze, Director of the Eliava Institute, Tbilisi, Georgia). Overall, however, a phage number of 3 per application of a phage product appears to be a realistic basis to calculate the demand within the framework of the *Phage2030* project.

To estimate the amount of phage needed for all patients, it must be considered that different modes of application require different phage doses, volumes, frequencies, and therapy durations. Application modes are infection-specific (e.g., endocarditis, urinary tract infection, sepsis, etc.) and each pathogen is responsible for different types of infections to varying degrees. The identity/identities of the MDR pathogen(s), type and severity of the infection also determines the extent to which phage products are used in single or combined modes of application (e.g., severe bone infection in orthopedics: topical AND intravenous administration).

4.4. Suitable healthcare facilities for phage therapy

In the future, it is conceivable that every physician will be able to apply therapeutic phages. In the (current) absence of approved phage products, personalized phage therapy using magistral preparations is preferred. The required infrastructure, however, is not available in all healthcare facilities:

- Availability of a microbiological laboratory (bacterial species identification and phage susceptibility measurement = phagogram),

- Availability of a (hospital) pharmacy (magistral preparation of the phage solution to be applied),
- Comprehensive therapy options and experience in treating the respective clinical picture to ensure the indication for phage therapy (e.g., intensive care medicine for pneumonia; trauma surgery/orthopedic reconstructive and plastic surgery for septic defect wounds, expertise in the treatment of chronic complex urinary tract infections and sinusitis),
- Implemented antibiotic stewardship,
- Possibility of participating in (register) studies, networking with academic structures to ensure the correct evaluation of the experience gained in phage therapy.

It is assumed that (initially) phages will be applied in specialized hospitals (e.g., *BG-Kliniken* that specialize in physical trauma from accidents and hospitals belonging to the Armed Forces). In the long term (2028+), we estimate that a maximum of 250 of the 1,887 German hospitals will be able to apply phage therapy in a clinically and qualitatively adequate manner. This means, however, that the ~62,000 MDR infections would be distributed arithmetically with 248 annual cases per hospital. This imposes a considerable demand on the respective hospital pharmacies and microbiological laboratories (personnel, training and further education, costs for the hospital operator) – which, however, are considered feasible.

4.5. Estimation of costs for phage production and treatment

The financial outlay for phage production depends on various factors:

- Need to maintain own biobank with phages and/or the pure phage DNA (when using cell-free production).
- Need for continuous adaptation and expansion of the biobank.
- Size of the biobank.
- Number of bacterial species against which phages must be produced.
- Amount of phages to be produced.
- The method of production or the type of technique used to produce the phages (conventional and/or cell-free production).
- Considered standard for manufacturing (GMP or restricted GMP).
- Legal framework (according to German drug law (AMG; use of approved phage therapies) or §13(2b) AMG (i.e., manufacture of medicinal products without a license)).

Sources of supply for which the approximate costs of phage treatment are estimated (based on experience and/or published figures):

- Purchase from abroad: e.g., from the Eliava Institute, non-GMP, no information on endotoxin content or phage concentration, 15-day topical treatment (the same applies to a purchase via phage24.com of phages produced in the Ukraine).

  Costs per patient: €1,500

- Production without permission in the treating hospital on a very small scale: minimal staffing (50% position of a non-scientific employee and a junior group leader/phage expert), without considering costs for rooms, consumables, phagogram preparation in the microbiology department or magistral cocktail production in the pharmacy, initial equipment investment of ~€360,000 (clean room 1 and 2; number of patients ~25 per year).

Costs per patient: €6,000

- Permission-free production in the treating hospital on a larger scale 2-3 clean rooms, 3-4 personnel (head, scientist, 2 non-scientific staff); initial equipment investment of ~€675,000, number of patients initially ~50, later ~250 per year; not considering consumables, expenditures of the microbiology department or of the pharmacy.

Costs per patient: €1,400-8,500

- Production of phages in a research institute with existing infrastructure and expertise, such as the Fraunhofer Institute for Toxicology and Experimental Medicine, Braunschweig (ITEM), as part of the *PhagoFlow* project (financed by Innovation Fund, G-BA, 15-day topical treatment).

Costs per patient: €25,000-50,000

- Large-scale approach, production by a company, centralized (since no approval of phage therapy has been granted so far, supply as a GMP-grade API, combination *via* magistral production in a pharmacy, phagogram in the microbiology department in the hospital).

Costs per patient: €3,000-50,000 (depending on the number of patients, 3-phage cocktail).

Of note, the use of cell-free production of phages can substantially reduce costs. Especially for highly individualized therapies necessary for many infections (e.g., *K. pneumoniae*) and when production needs to be changed frequently to different pathogens, cell-free synthesis has several fundamental advantages such as its host independence (no master cell bank required), the absence of endotoxin contaminations (thus simplifying downstream processing and increasing safety), excellent scalability and flexibility. The system does not require functional phages as input, but only their DNA (which is less costly to store and more stable). Switching from one phage to another (e.g., from phages against *E. coli* to those against *K. pneumoniae*) on a daily basis is impossible with conventional production technology (ITEM, Jafral), as the latter requires extensive decontamination/sterilization procedures to prevent contamination with the previous production strain. Such cleaning steps are not necessary for cell-free production, as host bacteria are not needed, and reactions can take place in inexpensive disposable sterile containers. Another advantage is that cell-free produced phages are virtually free of endotoxins and there are no prophages that pass on resistance genes, so that these products also ensure greater patient safety.

Calculation of the total financial volume for phages required to treat all MDR infections in Germany is based on a total number of 62,000 patients annually [Table 2]. The need for production of ~7.5 Mio vials per year is expected [Table 5]. With unit prices per vial of ~€20-30, this would correspond to phage purchasing costs of €150-225 Mio to supply all of Germany. This number does not consider the increased costs for the clinics carrying out the phage therapy:

- Training, implementation, and documentation effort for the medical and non-medical clinical staff within the scope of the application,
- Training, diagnostic (susceptibility testing/phagogram), production and documentation costs for microbiology staff (medical and non-medical),
- Training, diagnostic (phage identity), manufacturing, and documentation effort for the pharmacy (pharmaceutical and technical-assistance staff).

Table 5: Estimated numbers of phages required against the individual MDR pathogens (for the number of infections, see Table 2). Numbers were calculated assuming 62,000 MDR infections, the distribution of this number among the different bacterial species and types of infection, and the corresponding application modes. The number of phages required to identify an effective 3-phage cocktail against the respective species was also estimated. The calculation did not consider that the approach of selecting effective phages from small biobanks is not feasible for the species *Klebsiella pneumoniae* and *Acinetobacter baumannii* due to the very narrow specificity of their phages.

MDR species	Size of biobank (number of relevant phages as basis for selection of effective 3-phage cocktail)	Number of 1 mL vials per individual phage of the biobank / year (concentration)	Sum of the vials of a phage (both concentrations)	Number of vials FOR ALL phages of the biobank / year
Staphylococcus aureus	5	267,624 (10^9 PFU/mL) 161,827 (10^{10} PFU/mL)	429,451	2,147,255
Pseudomonas aeruginosa	7	121,494 (10^9 PFU/mL) 101,914 (10^{10} PFU/mL)	223,408	1,563,856
Escherichia coli	10	94,028 (10^9 PFU/mL) 53,730 (10^{10} PFU/mL)	147,758	1,477,580
Klebsiella pneumoniae	10	54,158 (10^9 PFU/mL) 40,860 (10^{10} PFU/mL)	95,018	950,175
Enterococcus faecium	10	46,862 (10^9 PFU/mL) 45,180 (10^{10} PFU/mL)	92,042	920,419
Staphylococcus epidermidis	5	22,752 (10^9 PFU/mL) 24,509 (10^{10} PFU/mL)	47,261	236,305
Acinetobacter spp.	10	10,436 (10^9 PFU/mL) 9,576 (10^{10} PFU/mL)	20,012	200,115
Enterococcus faecalis	10	3,133 (10^9 PFU/mL) 2,170 (10^{10} PFU/mL)	5,303	53,026
Total				**7,548,731**

It must be considered that besides costs for phage application, cost savings can also be expected:

- Savings in hospital days due to faster treatment of infections with fewer complications (shorter hospital stays),
- Saving high-care intensive care capacity,
- Reduced number of revision surgeries (fewer amputations),
- Saving cost-intensive reserve antibiotics,
- Saving the follow-up costs of amputations,
- Overall health-protective effect of reduced antibiotic use.

4.6. Future phage production scenarios

At present (2023) only ~50-100 patients per year are treated with phages in Germany. However, an increasing number of clinics are currently interested in producing phages. Assuming at least 62,000 patients per year, the current treatment capacity can only supply a maximum of 0.16% of the patients in need for phage therapy.

Potential production scenarios are:

- Centralized production (e.g., of all phages, Germany-wide supply of phages),
- Decentralized production of the phages required in the respective area of a “Supraregional Infection Treatment Center” or the production center,

- Combination of both approaches.

Centralized production: GMP grade phages are produced at one location (requirement ~7.5 Mio vials per year) and distributed from there. With conventional technology, this includes the creation of master cell banks of the host bacteria, multiplication of the individual phages that are stored in a biobank (the required number of phages depends on the phage biological properties), downstream processing, testing of endotoxin content, bioburden, and identity by means of whole genome sequencing, fill & finish under sterile conditions and distribution logistics.

In addition to a sufficient production capacity that does not yet exist worldwide, an optimized selection – 5-10, depending on the pathogen – of effective phages, which would need constant updates (due to changing epidemiological situations), would be required. The phages would need to be continuously tested for efficacy against currently circulating bacterial strains. Furthermore, phages could be adapted to newly circulating strains through *in vitro* evolution approaches. In Germany, there are several phage banks containing one to several hundred phages against the relevant bacterial species. It can be assumed that the prerequisite of continuously re-identifying enough effective phages can be fulfilled in principle.

This scenario, however, does not consider that conventional phage selection from a biobank of 5-10 phages per bacterial species is not feasible for the important species *K. pneumoniae* and *A. baumannii* due to narrow host specificity of these phages. These species require dozens or even hundreds of phages and a comparable number of host bacteria in the master cell bank, which would impose an insurmountable burden for production equipment, logistics and record-keeping.

For the remaining ESKAPE species, from today's perspective, the necessary quantities, 20,000-500,000 vials at 10^9 PFU/mL for ~70 phages can be produced in the short-term using either conventional or cell-free technology.

Decentralized production: Here, phage APIs would be produced in the hospitals for their "own" patients or for the respective catchment area of the production center. Production in an infection treatment center could follow the regulatory path of permission-free production (§13(2) AMG, which allows production in pharmacies ("within the framework of normal pharmacy operations") by physicians ("under their direct professional responsibility for the purpose of personal application to a specific patient"). This mode is already established at the MHH Medical School in Hannover, to date the only hospital in Germany that has produced phages (sporadically) for years.

The decentralized production of composite phage products (ready-made cocktails, not personalized) is conceivable in principle, but would also require larger production capacities.

In a decentralized setting, the use of cell-free production systems is feasible. This was carried out successfully as a proof of concept using *E. coli* and *K. pneumoniae* phages in the Military Hospital Berlin (microbiology) under normal laboratory conditions.

Of note, decentralized production alone (irrespective of the regulatory framework) could only generate a sufficient supply if ~250 facilities participate. However, production for 250 phage therapies per year in each facility would necessitate additional personnel in the clinical, pharmaceutical, and microbiological areas (e.g., at least 4 full-time employees (FTEs) x 250 facilities = 1,000 FTEs). A centralized facility with ~30-40 FTEs could produce the same amount of phages for Germany. However, the personnel costs could be borne by the hospital operator, as offering phage therapy would lead to savings (less complicated infection treatment, shorter

hospital stays, savings in intensive care capacity, lower number of revision operations, savings in cost-intensive reserve antibiotics) and would also appear attractive in the hospital landscape (complete spectrum of modern medicine on offer).

5. Aspects of a One Health approach

Human medicine can benefit from phage therapy in veterinary medicine and food production (e.g., prevention or treatment of diseases of livestock). The One Health approach calls for interdisciplinary cooperation between human and veterinary medicine and, in order to combat health threats such as zoonoses or infections caused by antibiotic-resistant bacteria, focuses on the interfaces between humans and animals and between the ecosystems in which they live.[30]

5.1. Urgent research questions in the veterinary field

- To date, phages have only been for few veterinary pathogens. Meta-analyses of the studies conducted are only available for pigs and poultry.[31-34] Phage collections for veterinary medicine should be expanded and clinical research should be developed.
- Appropriate dosage forms for administration need to be identified (convenient and animal-friendly administration of phages, especially for the treatment of lung infections, gastrointestinal infections, and skin infections).
- Large volumes of phages are needed for their broad application in veterinary medicine, especially in food-producing animals. As there is usually no cooling capacity available in stables for larger quantities of medicines, storage forms of phages, e.g., lyophilizates, are also needed to ensure simple logistics, delivery, and storage.
- Need to monitor bacterial properties during routine phage treatment to observe evolution of resistance and virulence.
- Influencing the microbial community in large-scale phage applications may have undesirable side effects (release of DNA and emergence of new pathovars, emergence of phage-resistant subpopulations and changes in evolutionary dynamics, etc.).
- Potential significance of phages for pathogen change and for the transmission of resistance determinants in the food sector need to be observed.
- Optimal timing (which production steps) and form of application need to be investigated. There may be a need for targeted inactivation of bacteriophages to remove them from the production process.

5.2. Relevant research groups

At the **University of Veterinary Medicine Foundation Hannover, Germany**, the Phage Technology Working Group and the Institute for Food Quality and Safety have had experience in the investigation and application of phages in livestock farming and food production for more than a decade (including application in commercial poultry houses and phage collection for the specialist areas of food, poultry, small animals, horses, reproductive medicine, reptiles, and pigs). In the **Department of Veterinary Medicine at the FU Berlin, Germany**, the Institute of Pharmacology and Toxicology is working on phage use in small animal dermatology and the Institute of Food Hygiene on phages in the field of food production. In the food sector, the **Federal Institute for Risk Assessment, Berlin, Germany,** and the Swiss **Federal Institute of Technology Zurich** also have many years of experience in the use of phages.

5.3. Expected benefits of phage therapy for human health

Experience gained in the field of human medicine regarding forms of application and method standardization as well as standardized production could be transferred to veterinary medicine. At the same time, the antibiotic resistance situation in human medicine could be significantly improved using phages in veterinary medicine and there are expected savings in antibiotic treatments, especially for treatment of zoonotic infectious agents.

5.4. The regulatory burden in the veterinary sector

Regulation (EC) No 2019/6 of the European Parliament and of the Council introduces for the first time in European legislation on veterinary medicinal products the category of advanced therapy veterinary medicinal products in Articles 4, 43.[35] Phages are also listed in this category. Requirements for a marketing authorization of a veterinary medicinal product specifically developed for phage therapy are set out in Annex II (V.1.5.4.) in accordance with Delegated Regulation (EU) No 2021/805:

- There is a need to select appropriate phages on a case-by-case basis for each disease outbreak.
- Phages should be continuously adapted to the epidemiological situation and therefore a stock of phages and host bacteria should be established and maintained.
- Propagation of phages should preferably be carried out on well-typed master cell systems, and it must be confirmed that phages used are lytic and that the master cell systems do not contain resistance or virulence genes.
- On 28-Jan-2022 the European Medicines Agency published a "Concept paper on quality, safety and efficacy of bacteriophages as veterinary medicines" for public consultation. A draft guideline for phage production was published beginning of 2023. In part, these guidelines set out specific requirements for the safety assessment of phage products.
- Approval will be required outside of treatment trials to treat animals with phages.
- Little progress has been made in the food sector. Extensive findings and studies are already available, especially on the use of phages to reduce *Listeria* and *Salmonella* on various foods (fish, cheese, meat) as well as *Campylobacter* in chicken farming. The use of phages for the food sector is considered politically highly controversial.
- Commercial phage cocktails for the food sector (against *Listeria*, *Salmonella*, *E. coli*; produced by Intralytix, Micreos and others) are already in use in many countries (e.g., FDA approval in the USA). The limited to non-existent use in Germany and Europe is not only due to regulation, but also to the reluctance to introduce phages (viruses) into food.
- It is necessary to establish a group of experts to deal extensively with this topic and to focus on corresponding applications in the food sector.

6. References

1. Willy C, et al. Viruses 2023, 15:288, doi: 10.3390/v15020588
2. Schooley RT, et al. Antimicrob Agents Chemother 2017, 61:e00954-17, doi: 10.1128/AAC.00954-17
3. Dedrick RM, et al. Nat Med 2019, 25:730-733, doi: 10.1038/s41591-019-0437-z
4. Jault P, et al. Lancet Infect Dis 2019, 19:35-45, doi: 10.1016/S1473-3099(18)30482-1
5. Leitner L, et al. Lancet Infect Dis 2021, 21:427-436, doi: 10.1016/S1473-3099(20)30330-3
6. Sarker SA, et al. EBioMedicine 2016, 4:124-137, doi: 10.1016/j.ebiom.2015.12.023
7. Wright A, et al. Clin Otolaryngol 2009, 34:349-357, doi: 10.1111/j.1749-4486.2009.01973.x
8. https://www3.weforum.org/docs/WEF_Top_10_Emerging_Technologies_of_2023.pdf (accessed 2023-08-17)
9. Onsea J, et al. Eur Cell Mater 2020, 39:193-210, doi: 10.22203/eCM.v039a13
10. Onsea J, et al. Viruses 2019, 11:891, doi: 10.3390/v11100891
11. Patey O, et al. Viruses 2018, 11:18, doi: 10.3390/v11010018
12. Clarke AL, et al. Antibiotics (Basel) 2020, 9:795, doi: 10.3390/antibiotics9110795
13. Steele A, et al. Antibiotics (Basel) 2020, 9:754, doi: 10.3390/antibiotics9110754
14. Wu JW, et al. J Biomed Sci 2023, 30:31, doi: 10.1186/s12929-023-00928-0
15. Eckstein S, et al. BMC Microbiol 2021, 21:186, doi: 10.1186/s12866-021-02251-w
16. https://ec.europa.eu/commission/presscorner/detail/en/ip_22_6951 (accessed 2023-08-17)
17. GBD 2019 Antimicrobial Resistance Collaborators. Lancet 2022, 400:2221-2248, doi: 10.1016/S0140-6736(22)02185-7
18. https://www.ecdc.europa.eu/sites/default/files/documents/AER-EARS-Net-2021_2022-final.pdf (accessed 2023-08-17)
19. Antimicrobial Resistance Collaborators. Lancet 2022, 399:629-655, doi: 10.1016/S0140-6736(21)02724-0
20. Cassini A, et al. Lancet Infect Dis 2019, 19:56-66, doi: 10.1016/S1473-3099(18)30605-4
21. Gastmeier P & Geffers C. Dtsch Med Wochenschr 2008, 133:1111-1115, doi: 10.1055/s-2008-1077224
22. Behnke M, et al. Dtsch Arztebl Int 2017, 114:851-857, doi: 10.3238/arztebl.2017.0851
23. Gastmeier P & Fätkenheuer G. Dtsch Arztebl 2015, 112:A-674 / B-576 / C-559
24. https://www.bundesgesundheitsministerium.de/fileadmin/Dateien/5_Publikationen/Gesundheit/Berichte/Abschlussbericht_Deutsche_Nationale_Punkt-Praevalenzstudie__zu_nosokomialen_Infektionen_und_Antibiotika-Anwendung_2011.pdf (accessed 2023-08-21)
25. Köck R, et al. Int J Environ Res Public Health 2020, 17:2337, 10.3390/ijerph17072337
26. Wilke MH, et al. Infection 2022, 1535-1542, doi: 10.1007/s15010-022-01843-6
27. Schweickert B, et al. Eur J Clin Microbiol Infect Dis 2012, 31:1855-1865, doi: 10.1007/s10096-011-1511-8
28. https://ars.rki.de/Content/Database/ResistanceOverview.aspx (accessed 2023-08-21)
29. Walger P, et al. Hyg Med 2013, 38:329-338
30. Network WEHC. Local-level policy recommendations: operationalizing a One Health approach. Annual business meeting and technical conference 2022. 2022;WHO/EURO:2023-7060-46826-68259 by World Health Organization.
31. Dec M, et al. Anim Health Res Rev 2020, 21:69-83, doi: 10.1017/S1466252319000161
32. Ferriol-González C, et al. Antibiotics (Basel) 2021, 10:559, doi: 10.3390/antibiotics10050559
33. Liu D, et al. Viruses 2021, 13:1268, doi: 10.3390/v13071268
34. Laponte R, et al. Antibiotics (Basel) 2021, 10:421, doi: 10.3390/antibiotics10040421
35. https://eur-lex.europa.eu/legal-content/DE/TXT/?uri=CELEX:32019R0006 (accessed 2023-08-21)
36. https://health.ec.europa.eu/system/files/2020-01/amr_2017_factsheet_0.pdf (accessed 2023-08-21)

7. The Team

Prof. Dr. med. Christian Willy

- Clinical Director Clinic for Trauma Surgery and Orthopedics, Septic Reconstructive Surgery, Research and Treatment Center Reconstruction of Septic Defect Wounds, Bundeswehr-krankenhaus Berlin
- General surgeon, trauma surgeon, orthopedist with a focus on endoprosthetics, infected wounds, wound healing disorders
- Since 2016 involved in the topic of phage therapy (among other things consortional guidance of PhagoFlow)

Phone: +49 (0) 179 940 81 33
Email: christian_willy@hotmail.com

Dr. rer. nat. Felix Broecker

- Laboratory Head Idorsia (Berlin) Pharmaceuticals: vaccine development for multidrug resistant pathogens (incl. *K. pneumoniae*)
- Experienced in virology: University of Zurich (2009-2011) and Mount Sinai, New York (2017-2019)
- Involved in phage therapy since 2013: pioneering work in characterization of intestinal phage populations and transplantation of phages, "Phage Therapy Award" 2016, 4th World Congress on Infectious Diseases, Paris

Phone: +49 (0) 173 7867 326
Email: felixbroecker@gmx.net