DEFENSIE
LA DÉFENSE

Phage therapy in Brussels (QAMH)

IABS congress (19-20 nov 24)

Dr Djebara Sarah, MD
Internal medicine physician
Phage therapy coordination
QAMH, Brussels

Belgian magistral phage medicine concept

PHAGE BANK

Seed lot

- Characterized phages
- Stored using a (tiered) banking system

CONTROLLED ENVIRONMENT

Active Pharmaceutical Ingredient (API)

- A phage API (single phage) is produced using a suitable host
- Produced according to a monograph
- External quality testing based on a monograph, performed by a “Belgian Approved Laboratory”

HOSPITAL PHARMACY

Magistral preparation

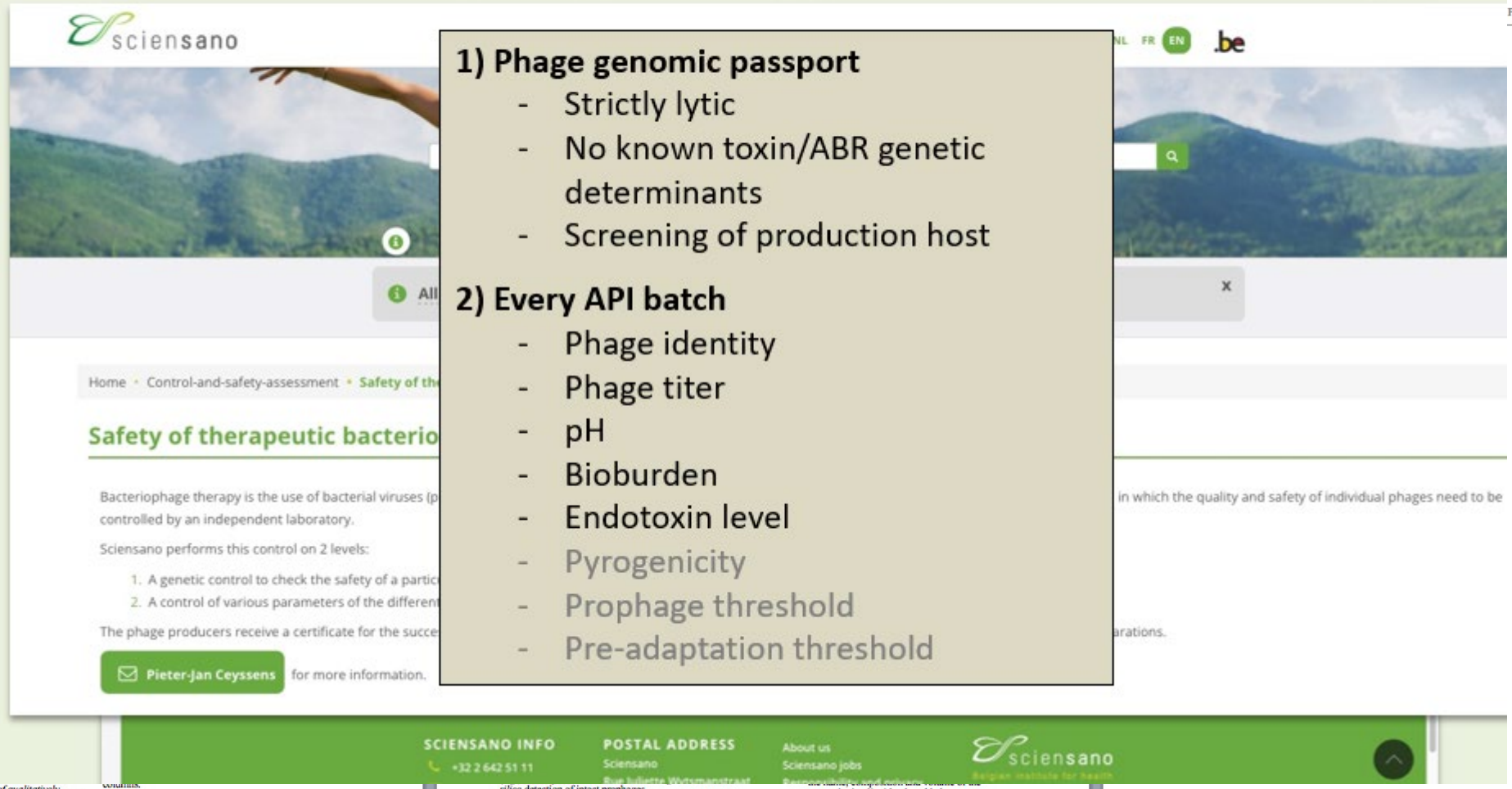
- Upon prescription
- One or more phage APIs are selected (phagogram)
- Phage APIs are mixed with a carrier (e.g. a hydrogel)

Patient

Pirnay et al. The Magistral Phage. Viruses. 2018;10(2). pii: E64.

Phage Active Pharmaceutical Ingredient (API) Monograph

Sciensano (Belgian Approved Laboratory)



1) Phage genomic passport

- Strictly lytic
- No known toxin/ABR genetic determinants
- Screening of production host

2) Every API batch

- Phage identity
- Phage titer
- pH
- Bioburden
- Endotoxin level
- Pyrogenicity
- Prophage threshold
- Pre-adaptation threshold

GENERAL MONOGRAPH – VERSI

PHAGE ACTIVE PHARM
INGREDIENT

DEFINITION

Phage active pharmaceutical ingredient
pharmaceutical preparations containing
bacteriophages (phages in short), whic
bacteria. Phages are composed of prote
DNA or RNA genome, and may have
elaborate structures. Phages replicate w
following the injection of their genome
Phages are among the most abundant a
in the biosphere. In general, phages are
nature.

Phage APIs are intended for use as acti
phage magistral preparations for *in viv*
infections (phage therapy).

Phage APIs are available as suspension
phages, in physiological solutions (e.g.,
solutions) that may contain a buffer or
dried phage suspensions. As active ingr
preparations, they are intended to be di
and/or combined with the necessary ing
pharmacy officina, immediately before
patient basis. Inactive ingredients may
ointments, liquids, capsules, etc. These
allow the required phage activity during
application period.

Phage APIs may contain one or more
various phage APIs may be combined
preparation to broaden the spectrum of
medicine.

*The magistral preparation of phage the
practical way for medical doctors to pe
antibacterial treatments.*

*This monograph does not apply to gene
phages and to phage derived products
endolysins. It does not necessarily appl
for veterinary use or for decontaminati*

*In addition to the requirements specifie
monograph, specific requirements for
testing and release testing might be inc
monographs.*

PRODUCTION

MANUFACTURING PROCESS

Phage APIs are generally obtained by
bacterial strains and are purified using
shown to preserve the biological prope
Phage APIs are manufactured under co
minimise microbial contamination and

Purification procedures need to be desi
content of harmful bacterial or culture
(e.g., bacterial endotoxins and animal p

*The manufacturing process must be de
(equipment, materials, culture media, a
conditions, purification steps,...) in stan
procedures (SOPs) and must be valida
process can reliably output phage APIs
standard.*

*The following manufacturing process h
suitable for the small-scale production of qualitatively
acceptable and safe phage APIs. It is* ***indicative*** *and based on
the state of the art and available knowledge from peer
reviewed scientific literature.*

The manufacturing process comprises various stages.

PRODUCTION ENVIRONMENT

The manufacturing of phage APIs from phage seed lots takes place in an environment with specified air quality and

Phage active pharmaceutical ingredients

silico detection of intact prophages.

PHAGE SEED LOTS

Phage identification. State of the art DNA or RNA

— the period of time within which the preparation is to be used after reconstitution;
— instructions for reporting serious adverse reactions and/or events;

EUROPEAN PHARMACOPOEIA

Phage therapy medicinal products

Published in accordance with the Convention on the Elaboration of a European Pharmacopoeia (European Treaty Series No. 50)

Council of Europe
Strasbourg

5.31. PHAGE THERAPY MEDICINAL PRODUCTS

This general chapter is published for information.

It offers a framework of requirements for phage therapy active substances and medicinal products for human and veterinary use and their production and control.

The provisions of the chapter do not exclude the use of alternative production and control methods that are acceptable to the competent authority.

1. DEFINITION

Bacteriophages (phages) are viruses that infect bacteria and depend on their bacterial host for replication. Phages consist of a genome comprised of single or double stranded DNA or RNA, encapsulated in a protein capsid.

Phage therapy medicinal products (PTMPs) are preparations of naturally occurring or genetically modified phages used to treat or prevent human or veterinary bacterial infections.

A PTMP can contain one phage, i.e. a single phage therapy active substance, or a mixture of phages, combined with excipients. PTMPs can be administered by various routes and are available in different dosage forms.

2. PRODUCTION

2-1. *GENERAL PROVISIONS*

Phages are obtained by propagation in bacterial host strains and are purified using suitable methods.

The production process yields a PTMP of consistent quality and stability. Appropriate in-process testing is implemented at relevant time points and/or key intermediate stages of the process.

Production of PTMPs is based on a well-characterised

of the bacterial strain, subsequent manipulations and the tests used to characterise the strain. This must include determination of its antibiotic susceptibility profile and of the nucleotide sequences of its chromosome(s) and plasmids. The use of bacterial strains whose genome contains sequences coding for detrimental factors (e.g. prophages, antibiotic resistance determinants, toxins) is avoided, unless otherwise justified and authorised.

Bacterial host cells used for PTMP production are derived from a well characterised bacterial master cell bank (MCB) that is of clonal origin and complies with the following requirements:

Identification. The identity is confirmed using a suitable method.

Microbial purity. The absence of microbial contaminants is determined by plating or any other suitable method.

Viability. The number of viable cells is determined by a plate count or any other suitable viable cell count method.

Phage sensitivity. The susceptibility of the strain to the phage therapy active substance is demonstrated using a plaque assay or any other suitable method.

Absence of detrimental phages. The absence of phage particles that could be detrimental to the quality of PTMPs is confirmed.

If a working cell bank (WCB) is used for production, it is a clonal derivative of the MCB and complies with the requirements for MCB.

2-3. *PHAGE SEED LOTS*

Phage seed lots used in PTMP production are derived from a single phage clone and must be characterised in detail. Information on the phage source, nucleotide sequence and susceptible bacterial species and/or strains is to be provided. Other parameters such as plaque morphology or phage morphology are determined, if relevant.

Phages whose genome contains sequences coding for known or potential detrimental genetic factors, e.g. antibiotic resistance determinants, toxins or lysogeny modules, are avoided, unless otherwise justified and authorised. For genetically or chemically modified phages, the modifications must be described and their effects characterised.

A phage master seed lot complies with the following requirements:

Identification. The phage seed lot is identified by a suitable method.

Microbial purity. The absence of microbial contaminants is demonstrated by a suitable method.

Phage purity. The absence of extrinsic phage contaminants is confirmed by a suitable method; however, as intrinsic phages may be unavoidable when using clinical isolates for production, their presence may in this case be justified and authorised when controlled by a suitable method.

Potency. The infectious phage titre is determined by a plaque

Several single harvests of the same phage clone may be pooled before the purification process.

Phages are purified by suitable techniques.

Only a purified harvest containing a single phage therapy active substance that complies with the following requirements may be used in the preparation of the final lot:

Identification. The identity of the phage is confirmed using a suitable method.

Potency. The infectious phage titre is determined by a plaque assay (expressed in PFU/mL or PFU/mg) or any other suitable method.

Microbiological examination (*2.6.12*). The purified harvest complies with the established specification.

Residual reagents. Based on risk analysis, tests for residues of reagents used during production and posing safety concerns are carried out on the purified harvest.

Host-cell impurities and contaminants. Contaminants and other potentially toxic substances derived from the host cells (e.g. endo- and exotoxins, host-cell proteins, host-cell DNA, temperate phages) are absent or within the established specifications.

2-5. *FINAL LOT*

The final lot can be administered by various routes and may be available in different dosage forms. Additional tests are required, depending on the dosage form and on the route of administration.

When it is not practical, for unlicensed pharmaceutical preparations, to carry out the tests (e.g. batch size, time restraints), other suitable methods are implemented to ensure that the appropriate quality is achieved in accordance with the risk assessment carried out and any local guidance or legal requirements.

A final lot complies with the following requirements:

Appearance. It complies with the established specification.

Identification. The identity of each phage is verified using a suitable method.

Potency. The infectious phage titre of each phage is determined by a plaque assay (expressed in PFU/mL or PFU/mg) or other suitable method and complies with the established specification for the particular preparation.

Microbiological quality. Sterile PTMPs comply with the test for sterility (*2.6.1*). For non-sterile PTMPs, the microbiological quality is determined using a suitable method and complies with the established specification for the particular preparation.

Pyrogenicity. If applicable, the final lot complies with a suitable test for pyrogenicity and with the limit approved for the particular product.

Water content (*2.5.12* or *2.5.32*). Solid PTMPs comply with the limit approved for the particular product.

pH (*2.2.3*). Liquid PTMPs comply with the limit approved for the particular product.

2-6. *ADAPTED PRODUCT*

Phage adaptation (training) is the process by which phages can be directed to evolve in order to increase their potency against (a) clinical isolate(s).

When the adapted PTMP is used in the individual patient that was the source of the clinical isolate, phage adaptation starts with a phage or mixture of phages, each complying with the provisions of section 2-3. The final lot complies with the provisions of section 2-5, unless otherwise justified and authorised. The increased potency of the final lot of the adapted PTMP against the target clinical isolate is confirmed, serving also as an appropriate substitute for the identification test.

3. LABELLING

The labelling requirements outlined in relevant supranational and national regulations apply.

Phage API Monograph 2.0?
New quality controls
Pyrogenicity
Immunogenicity of PTMPs

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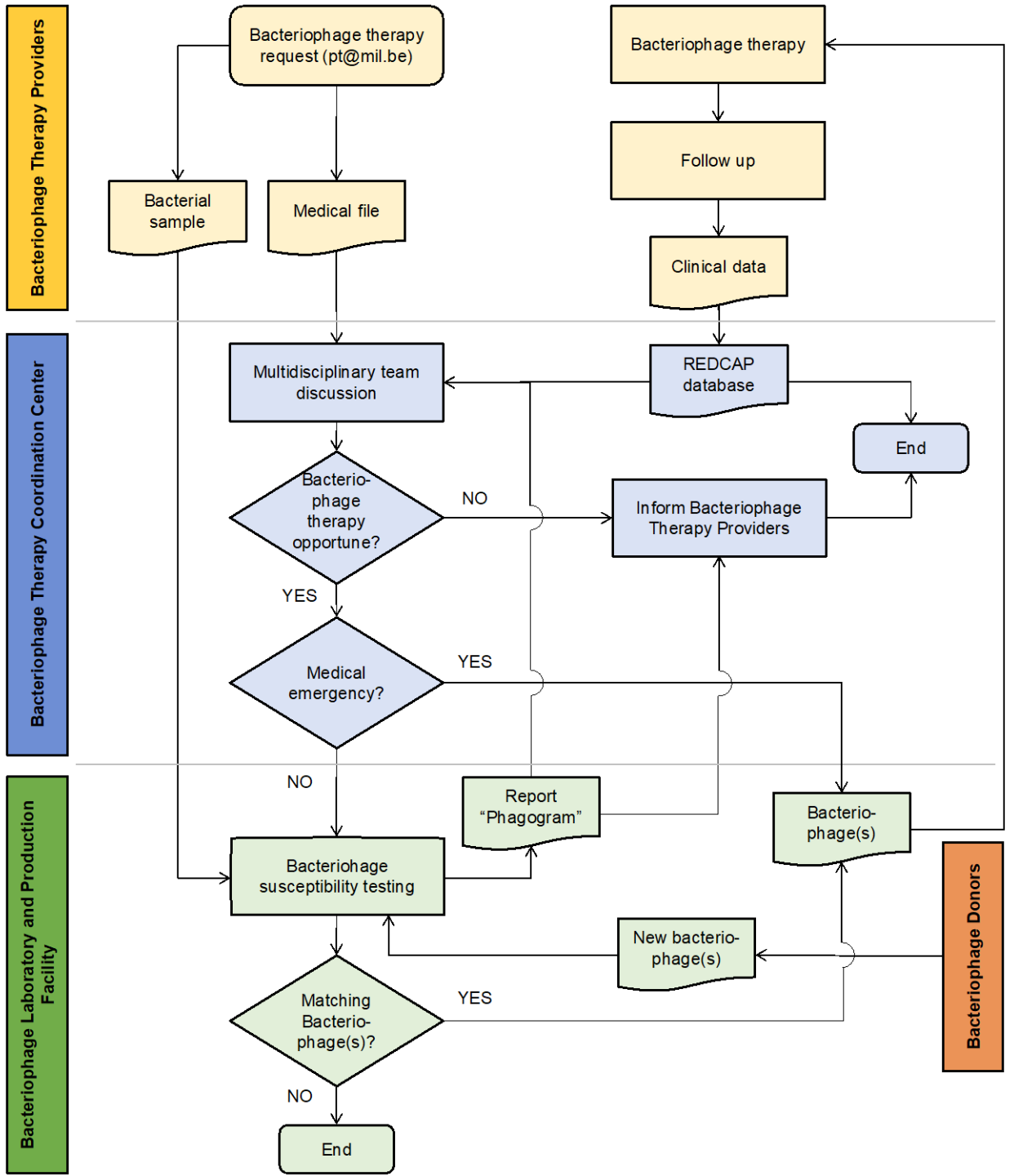
Sarah Djebara
Patrick Soentjens

170 BT cases

Multidisciplinary patient selection process for bacteriophage therapy



nature microbiology

Article

https://doi.org/10.1038/s41564-024-01705-x

Personalized bacteriophage therapy outcomes for 100 consecutive cases: a multicentre, multinational, retrospective observational study

", the development and application of phage therapy represent not only a promising but also a mandatory alternative approach to tackle this major public health threat"

https://doi.org/10.1038/s41564-024-01733-7

Advocating for phage therapy

Check for updates

As antibiotic-resistant infections continue to rise globally, the development and application of phage therapy represents not only a promising but also a mandatory alternative approach to tackle this major public-health threat.

by phage therapy include *Escherichia coli*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. Most of these bacteria belong to the ESKAPE (*Enterococcus faecium*, *S. aureus*, *K. pneumoniae*, *Acinetobacter baumannii*, *P. aeruginosa* and *Enterobacter* sp.) group of hospital-acquired pathogens that are show-

News & Views article by Jonathan Iredell, Holly Sinclair and Ameneh Khatami.

Although personalized phage therapy is less likely to induce bacterial phage resistance compared with pre-defined phage cocktails (as it does not carry a surplus of ineffective phages, which puts less selection pressure towards bacterial phage resistance), it is very

Pirnay J.- P., Djebara S. et al., Nat. Microbiol. 2024 Jun;9(6):1434-1453.

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Phage therapy

https://doi.org/10.1038/s41564-024-01712-y

Personalized bacteriophage therapy for difficult-to-treat infections

35 hospitals

29 cities

12 countries

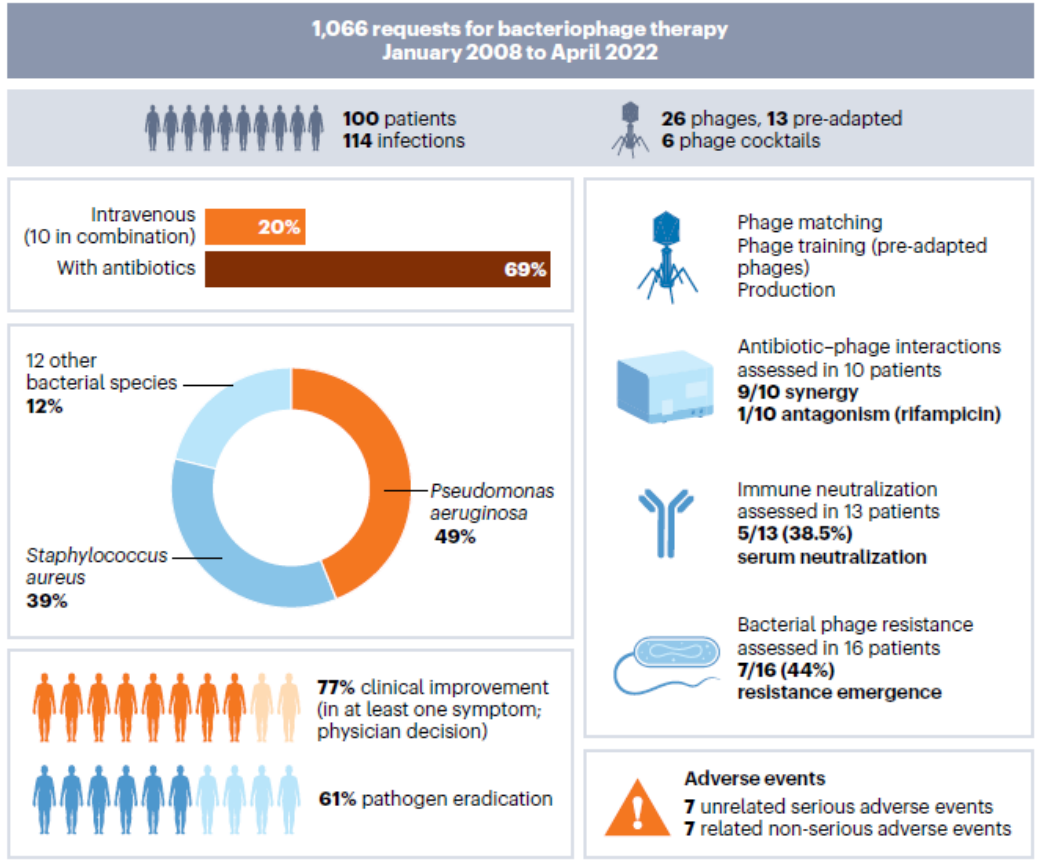
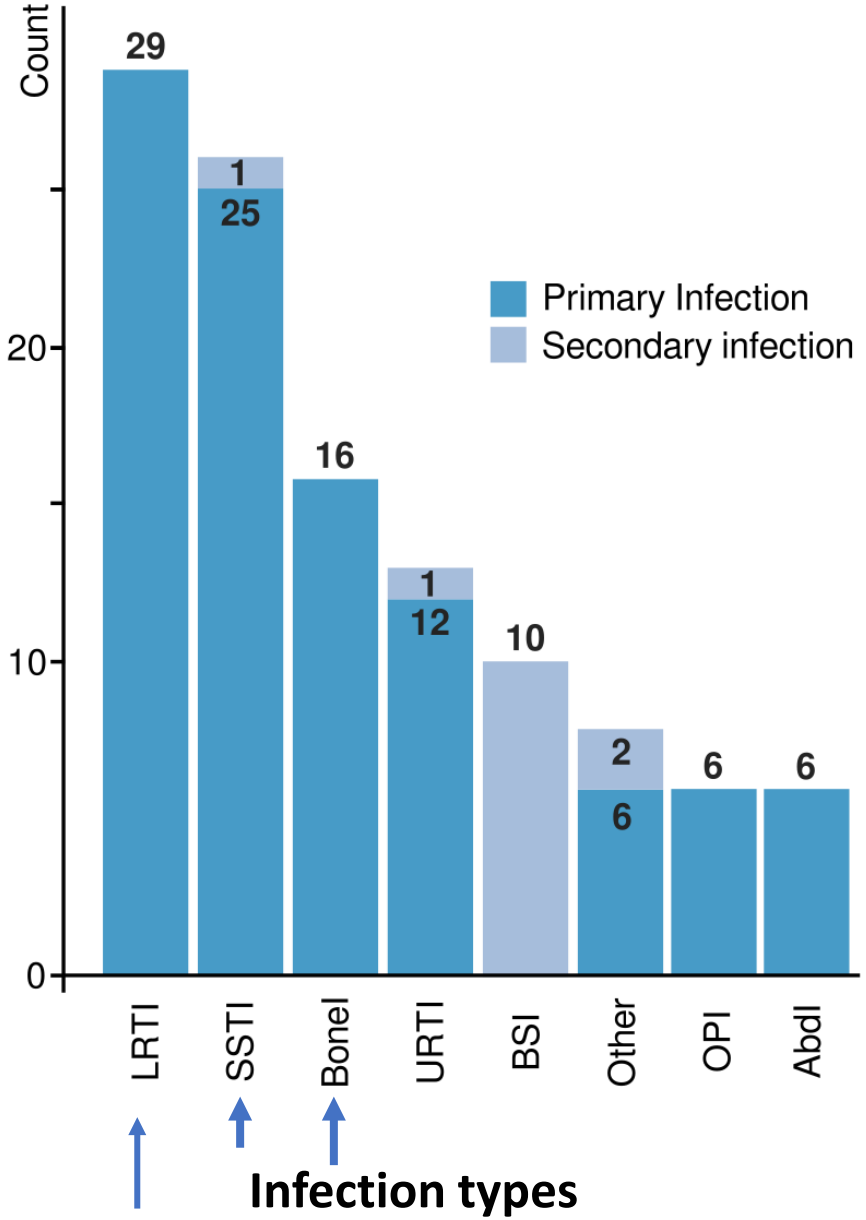


Fig. 1 | One hundred cases of individualized bacteriophage therapy. A summary of major pathogens and overall outcomes, along with laboratory data available for small opportunistic subgroups.

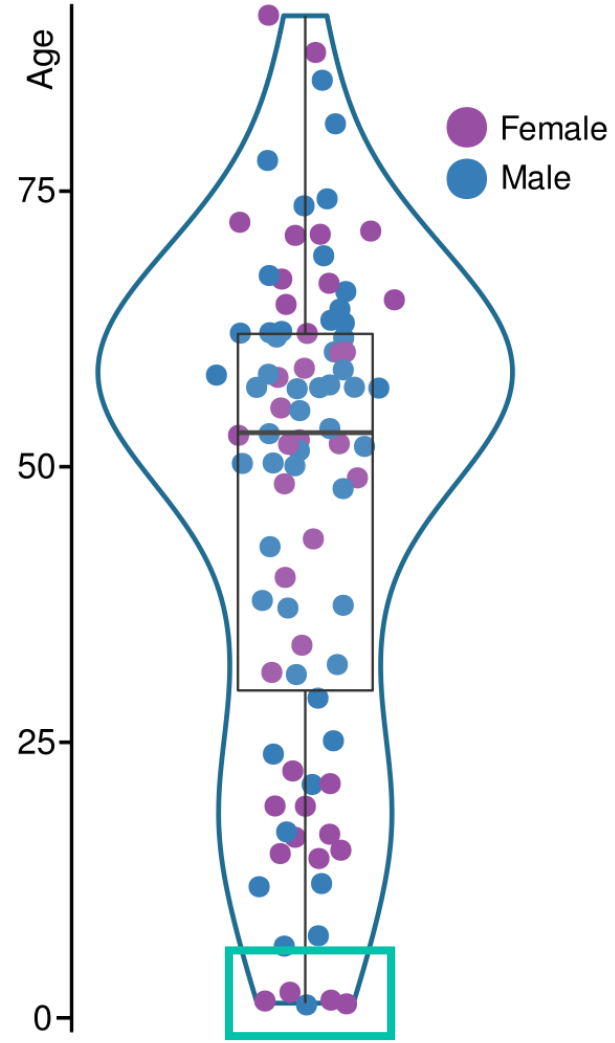
.be

Intrinsic Limitations

- Different indications and routes of administrations
- No control, blinding or randomization
- Evaluation of efficacy and safety not based on pre-defined standardized test but on subjective judgement of physicians
- Sample allowing supportive tests in **only 21 patients**



Infection types



Age and gender distribution

Pirnay J.- P., Djebara S. et al., Nat. Microbiol. 2024 Jun;9(6):1434-1453.

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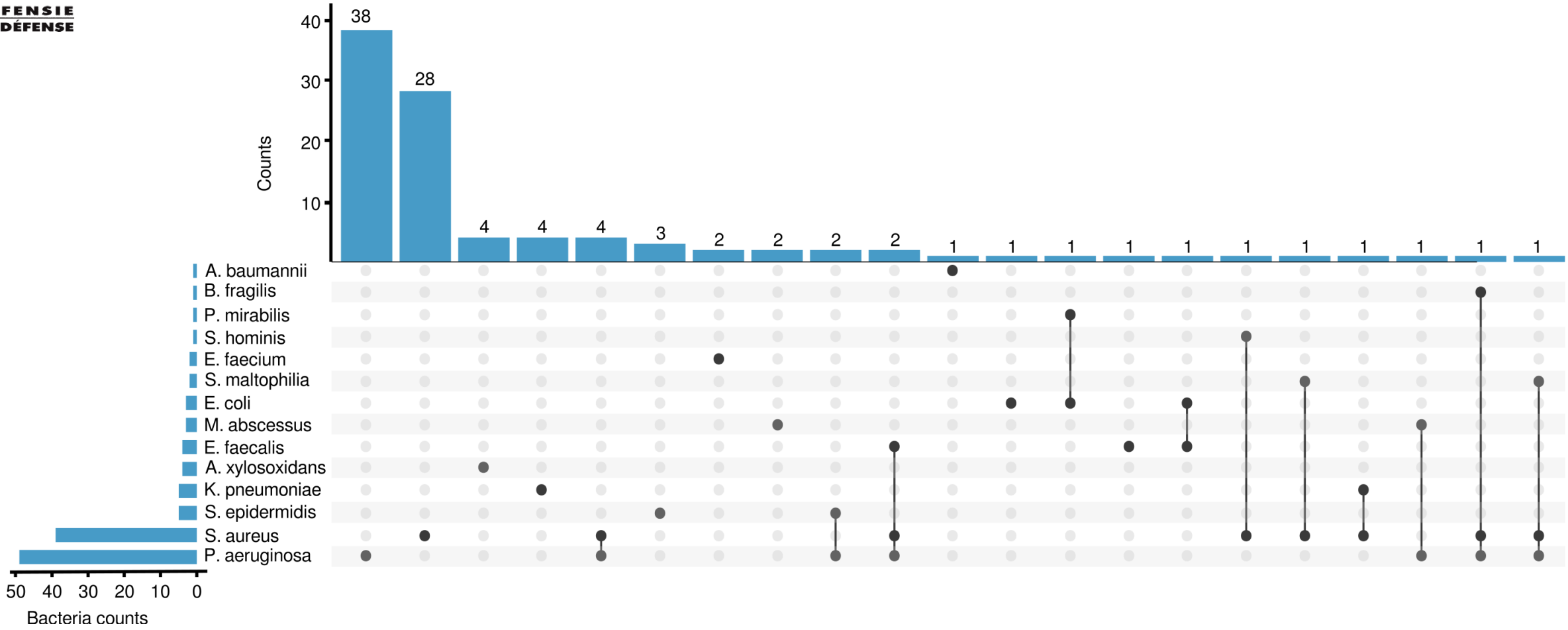

Targeted bacterial species

Pirnay J.- P., Djebara S. et al., Nat. Microbiol. 2024 Jun;9(6):1434-1453.

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Type of primary infection	# patients	# Concom. AB	% Concom. AB	# Clin. Improv.	% Clin. Improv.	# Eradication	% Eradication
Abdominal Infection	6	5	83,33%	5	83,33%	4	66,67%
Bone Infection	16	15	93,75%	13	81,25%	11	68,75%
Lower Respiratory Tract Infection	29	17	58,62%	20	68,97%	14	48,28%
Orthopedic Prostheses Infection	6	4	66,67%	5	83,33%	5	83,33%
Skin and Soft Tissue Infection	25	19	76,00%	21	84,00%	11	44,00%
Upper Respiratory Tract Infection	12	1	8,33%	8	66,67%	4	33,33%
Other	6	6	100,00%	5	83,33%	4	66,67%
	7		**67,00%**	**77**	**77,00%**	**53**	53,00%

P.aeruginosa: 11
S.aureus: 11
S.epidermidis: 5
E. Faecalis: 5
M. Morganii:2
E.coli: 1
K.pneumoniae: 1
P.mirabilis:1
Hungatella Hatheaway:1
S. agalactiae:1
S.maltophilia:1
Citrobacter Koseri:1

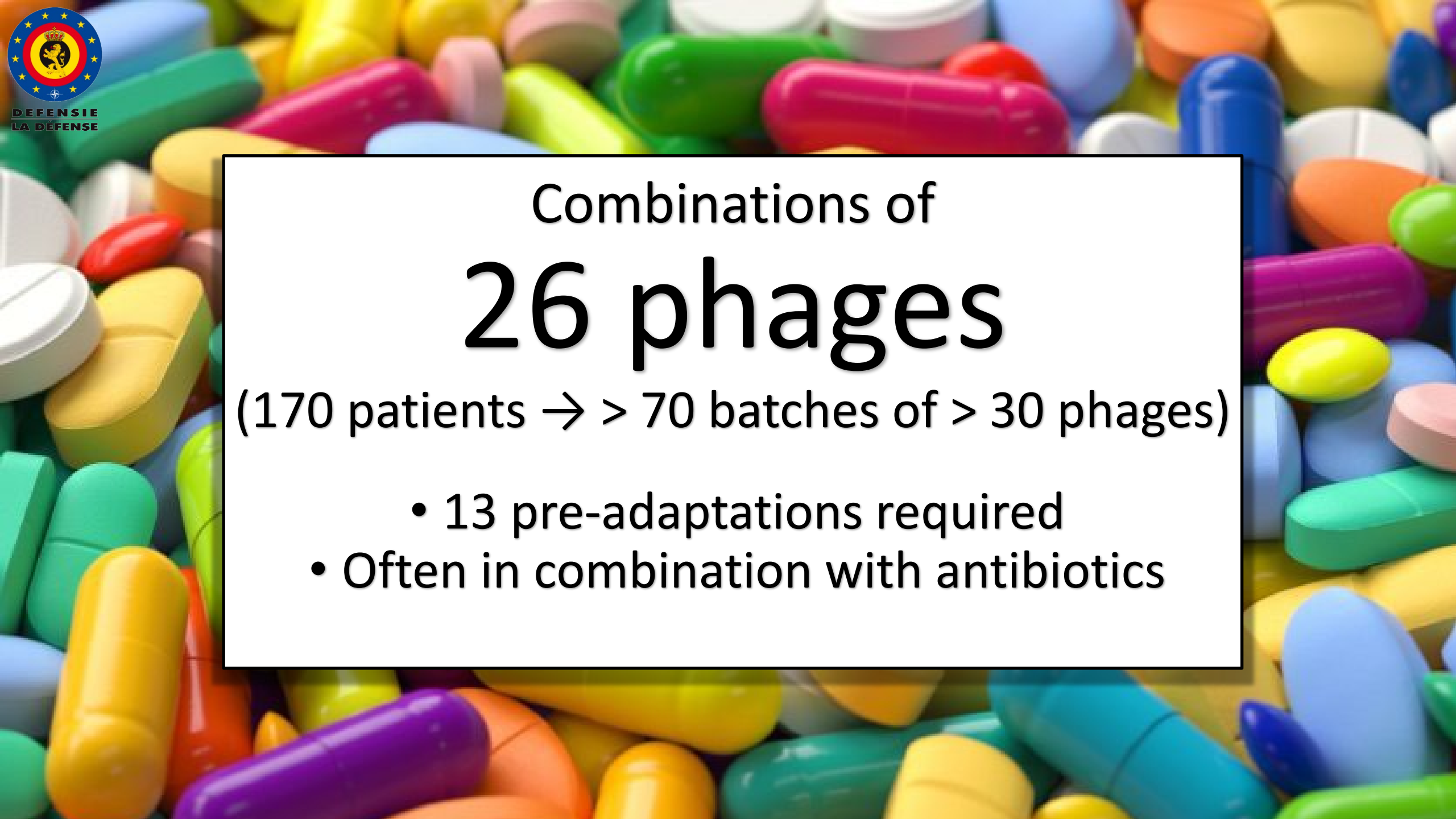
12 bacteria species

1 we->2 we of treatment

P.aeruginosa:21
M.abcessus:3
K.pneumoniae:2
S.aureus:2
A.xylosoxidans:2
E.coli:1
P.mirabilis:1
S.maltophilia

8 bacteria sepcies

1we-> 6we of treatment



Combinations of

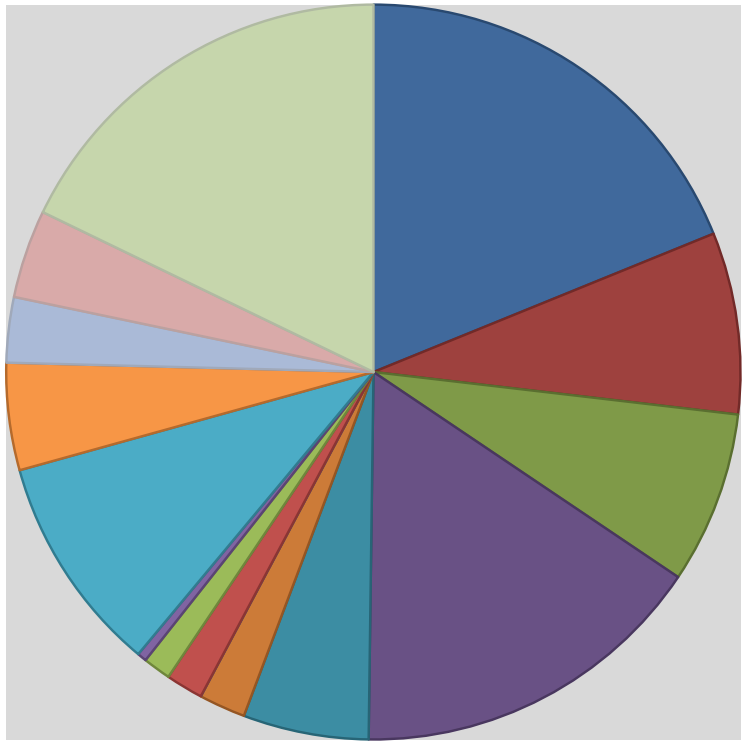
26 phages

(170 patients → > 70 batches of > 30 phages)

- 13 pre-adaptations required
- Often in combination with antibiotics

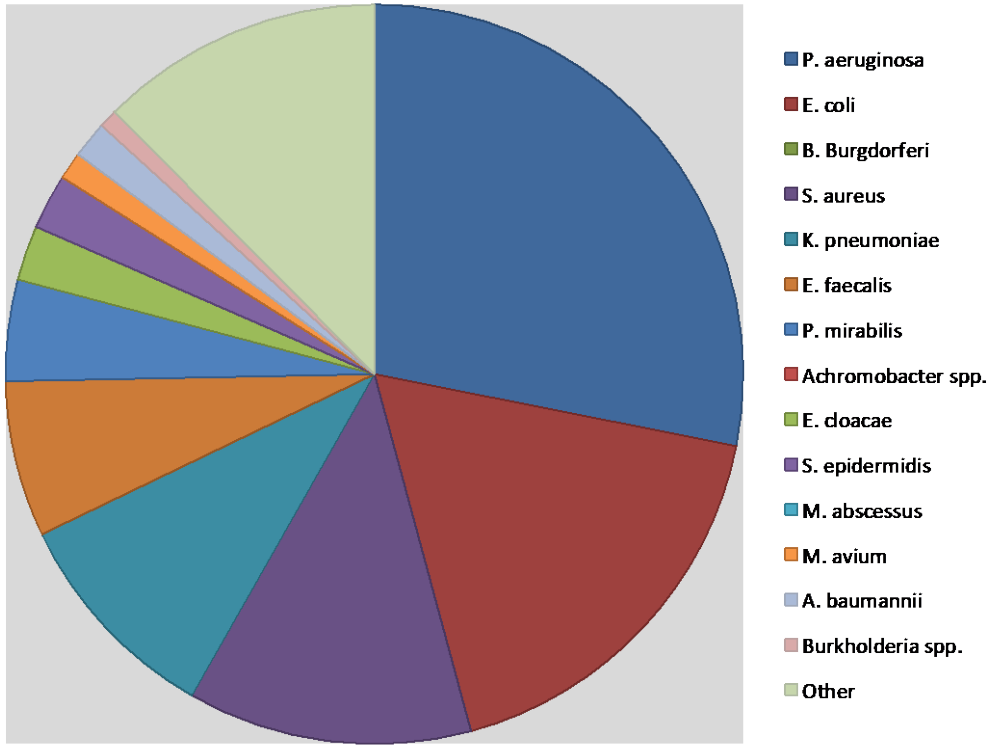
Analysis of PT requests

San Diego: 35 species/488 requests



Aslam S. et al. Open Forum Infect Dis. 2020; 7(9):ofaa389.

Brussels: 31 species/249 requests



Djebara S. et al. Viruses. 2019;11(3):265.

- **> 30 bacterial species**
- Geographical variations
- Variations over time

Hundreds of phages!

Overview of rnadomized controlled trials (RCTs) of phage therapeutics (status on 23 February 2024)

Trial identifier	Design	Phase	Target clinical indication	Targeted bacterial species	Phage product	Status	Sponsor (location)
EudraCT 2004-001691-39	R, C, B	1/2	Otitis externa	*Pseudomonas aeruginosa*	Biophage-PA, a cocktail with 6 phages	Completed	Biocontrol, Ltd (UK)
NCT00937274	R, C, B	NA	Pediatric diarrhea	*Escherichia coli*	T4 phage cocktail with 11 phages and Microgen ColiProteus cocktail with 18 phages	Completed	Société des Produits Nestlé (Switzerland)
NCT02116010	R, C, OL	1/2	Burn wound infection	*P. aeruginosa*	PP1131, a cocktail of 12 phages	Completed	Pherecydes Pharma, SA (France)
NCT03140085	R, C, B	2/3	Urinary tract infections	*Enterococcus* spp., *E. coli, Proteus mirabilis, P. aeruginosa , Staphylococcus* spp., and *Streptococcus* spp.	PYO phage, a complex cocktail with an unknown number of phages	Completed	Balgrist University Hospital (Switzerland)
NCT04596319	R, C, B	1b/2a	Cystic fibrosis and chronic pulmonary infection	*P. aeruginosa*	AP-PA02, a cocktail with an undiscosed number of phages	Completed	Armata Pharmaceuticals, Inc (USA)
NCT04737876	R, C, B	1	Healthy adults	*Klebsiella pneumoniae*	BX002-A, a cocktail with an undiscosed number of phages	Completed	BiomX, Inc (Israel)
NCT04325685, SGDC-VAP-1	R, C, B	INP	Decolonization of the oropharynx	INP	Sextaphag, a sterile phage lysate containing an undisclosed number of phages	Completed	Northern State Medical University (Russian Federation)
NCT05453578	R, C, B	1/2	Cystic fibrosis lung infection	*P. aeruginosa*	WRAIR-PAM-CF1, a cocktail with 4 phages	Recruiting	National Institute of Allergy and Infectious Diseases (USA)
NCT05177107	R, C, B	2a	Diabetic foot osteomyelitis	*Staphylococcus aureus*	Personalised phage therapy dependent on *in vitro* phage susceptibility testing	Recruiting	Adaptive Phage Therapeutics, Inc (USA)
NCT05184764	R, C, B	1b/2a	Bacteremia	*S. aureus*	AP-SA02, a cocktail with an undiscosed number of phages	Recruiting	Armata Pharmaceuticals, Inc (USA)
NCT05616221	R, C, B	2	Non-cystic fibrosis bronchiectasis and chronic pulmonary infection	*P. aeruginosa*	AP-PA02, a cocktail with an undiscosed number of phages	Recruiting	Armata Pharmaceuticals, Inc (USA)
NCT03808103	R, C, B	1/2a	Intestinal Adherent Invasive *E. coli* (AIEC) in patients with inactive Crohn's disease	Adherent Invasive *E. coli* (AIEC)	EcoActive, a cocktail with an undiscosed number of phages	Recruiting	Intralytix, Inc (USA)
NCT05369104	R, C, B	2	Prosthetic joint infection, at the end of DAIR	*S. aureus*	Phage cocktails PP1493 and/or PP1815 with an undisclosed number of phages	Recruiting	Pherecydes Pharma, SA (France)
EudraCT 2021-004469-11	R, C, B	2	Prosthetic joint infection with the indication of SAIR and suppressive antibiotic therapy	*S. aureus*	Phage cocktails PP1493 and/or PP1815 with an undisclosed number of phages	Ongoing	Pherecydes Pharma, SA (France)
NCT05182749	R, C, B	1/2a	Healthy adults and healthy adults after a challenge with *Shigella*	*Shigella* spp.	ShigActive, a cocktail with an undisclosed number of phages	Recruiting	Intralytix, Inc (USA)
NCT05948592	R, C, B	2b	Diabetic foot infection	*S. aureus , P. aeruginosa , Acinetobacter baumannii*	TP-102, a cocktail with an undisclosed number of phages	Recruiting	Technophage, SA (Portugal)
NCT05715619	R, C, B	1/2a	Decolonization of the gastrointestinal tract	Vancomycin-resistant *Enterococcus* (VRE)	VRELysin, a cocktail with an undisclosed number of phages	Recruiting	Intralytix, Inc (USA)
NCT05010577, EudraCT 2022-003810-35	R, C, B	1/2	Cystic fibrosis lung infection	*P. aeruginosa*	BX004-A, a cocktail with an undiscosed number of phages	Active, not recruiting	BiomX, Inc (Israel)
NCT02664740	R, C, B	1/2	Infected diabetic foot ulcer	*S. aureus*	Sterile dressing impregnated with a cocktail with an undisclosed number of phages	Not yet recruiting	CHU de Nîmes (France) with Pherecydes Pharma, SA (France)
NCT05240300	R, C, B	1b/2a	Atopic dermatitis	*S. aureus*	BX005-A, a gel containing an undiscosed number of phages	Unknown	BiomX, Inc (Israel)
NCT04323475	R, C, OL	1	Burn wound infection	*S. aureus , P. aeruginosa , K. pneumoniae*	Phage Cocktail-SPK spray with an undisclosed number of phages	Unknown	Precisio Biotix Therapeutics, Inc (USA)
NCT04815798	R, C, B	1/2	Colonised pressure ulcer	*S. aureus , P. aeruginosa , K. pneumoniae*	BACTELIDE, a spray of microcapsules loaded with an undisclosed number of phages	Unknown	Precisio Biotix Therapeutics, Inc (USA)
NCT04684641	R, C, B	1/2	Cystic fibrosis lung infection	*P. aeruginosa*	Yale Phage Therapy (YPT) 01, a cocktail with an undiscosed number of phages	Ended after opening of OL extension for the placebo group	Yale University (USA)
NCT04287478	R, C, OL	1/2	Urinary tract Infection	*E. coli , K. pneumoniae*	Personalised phage therapy dependent on *in vitro* phage susceptibility testing	Terminated due to a change in development strategy	Adaptive Phage Therapeutics, Inc (USA)
NCT05269134	R, C, B	2	Prosthetic joint infection	*Staphylococcus* spp., *Enterococcus* spp., *Streptococcus* spp., *E. coli , P. aeruginosa , K. pneumoniae*	Personalised phage therapy dependent on *in vitro* phage susceptibility testing	Withdrawn (sponsor decision)	Adaptive Phage Therapeutics, Inc (USA)

C, controlled; DAIR, debridement, antibiotics, and implant retention; OL, open label; R, randomized

- **ClinicalTrials.gov, EudraCT,..**
- **25 RCTs**
  - 15 active/recuiting
  - 7 completed
  - 3 terminated
- **24 phase 1 and/or 2**
- **1 phase 2/3 (unsuccessful)**
- **9 bacterial targets**
  1. *P. aeruginosa*: 12
  2. *S. aureus*: 11
  3. *K. pneumoniae*: 5
  4. *E. coli*: 5
  5. *Enterococcus* spp.: 3
  6. *Streptococcus* spp.: 2
  7. *A. baumannii*: 1
  8. *Shigella*: 1
  9. *Proteus mirabilis*: 1

  80%
- **Limited number of indications**

frontiers | Frontiers in Cellular and Infection Microbiology

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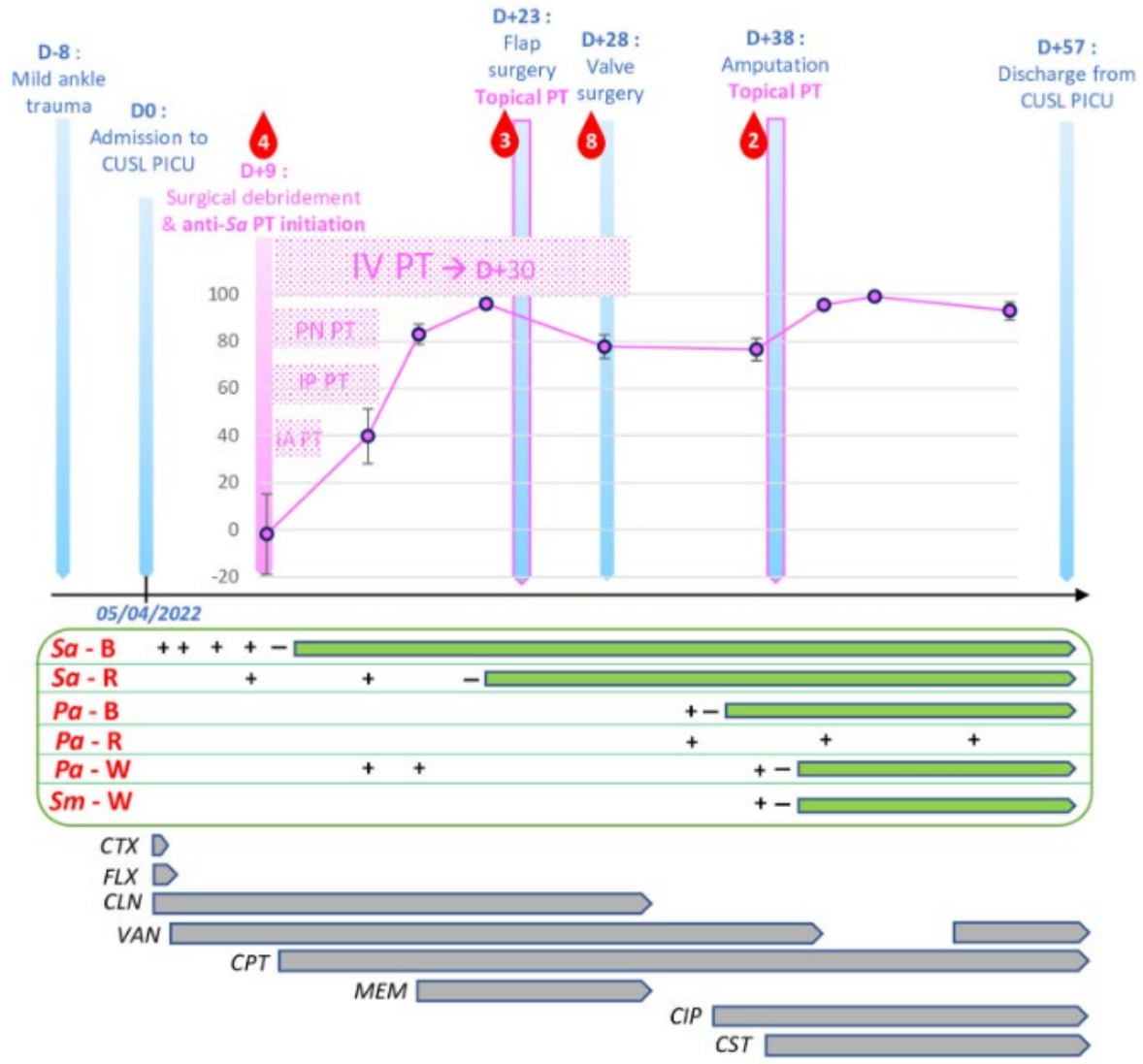
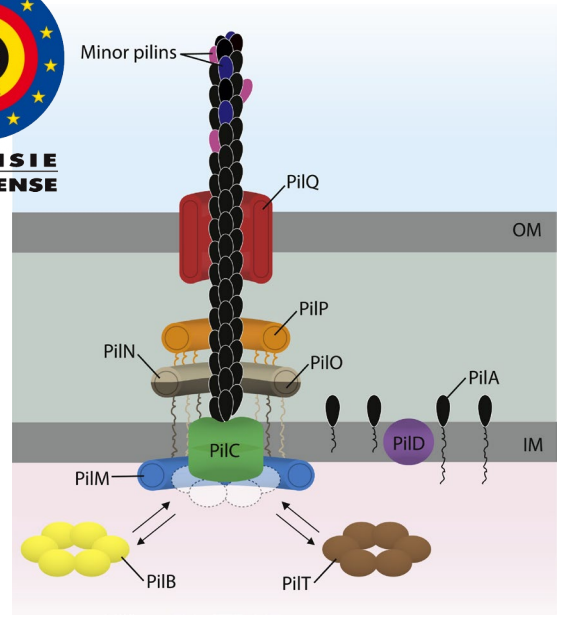
Case report: Personali phage-antibiotic com therapy to rescue nec fasciitis caused by Par Valentine leukocidin-p MRSA in a 12-year-old

Brieuc Van Nieuwenhuyse[1*†], Mathilde Bal Astrid Haenecour[2], Emilien Derycke[2], Thie Stéphan Clément de Cléty[2], Cécile Boular Leïla Belkhir[6,7], Jean-Cyr Yombi[6], Julien D Olivier Cornu[8,9], Pierre-Louis Docquier[8,9], Renaud Menten[11], Hector Rodriguez-Villal Alexia Verroken[12,13], Sarah Djebara[14], Maya Johann Griselain[15], Jean-Paul Pirnay[15], La and Dimitri Van der Linden[1,3‡]



Van Nieuwenhuyse B. *et al: Front Cell Infect Microbiol sept 24 17:14:1354681*

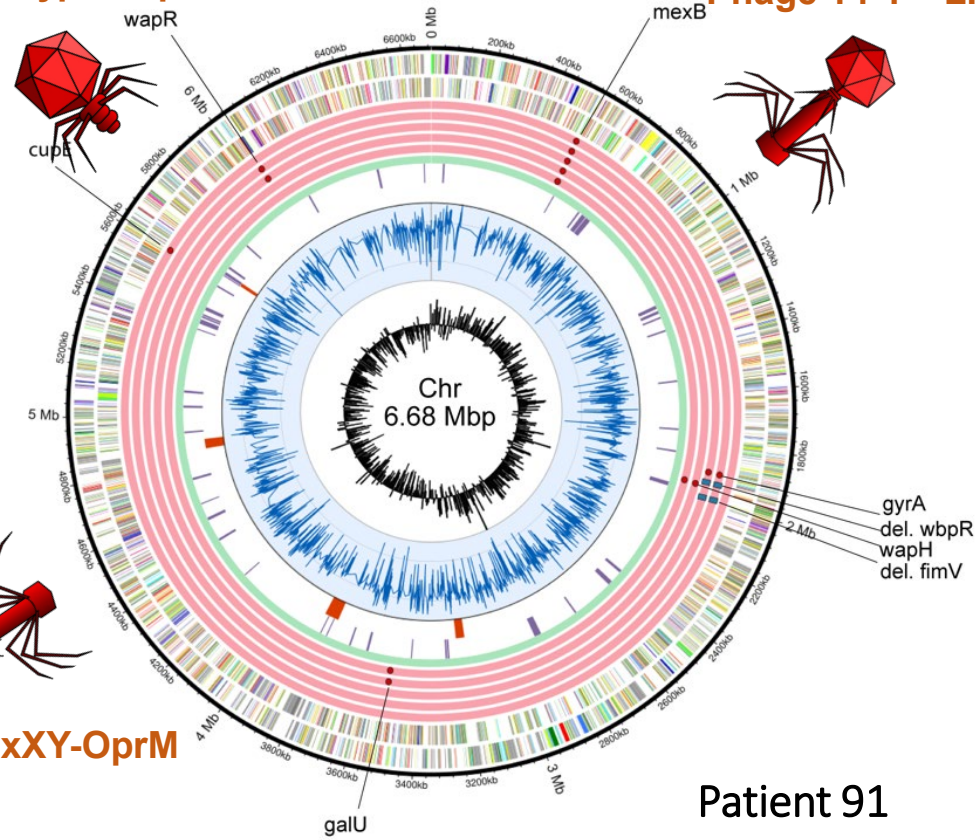
Resistance mutations trade-offs



165 AA missing from N terminal
FimV assembly protein

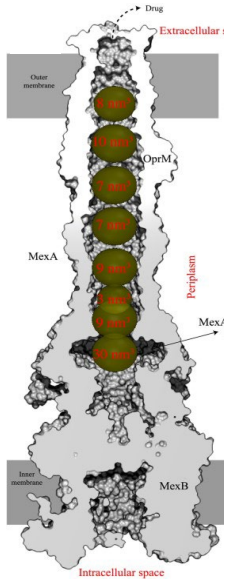
PNM – Type IV pili

Phage 14-1 – LPS

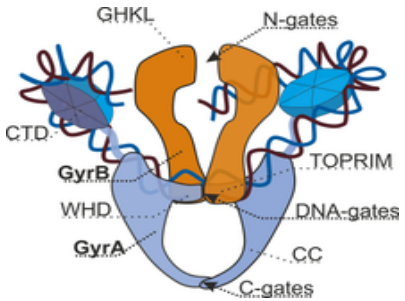


Phage PT07 – MexXY-OprM

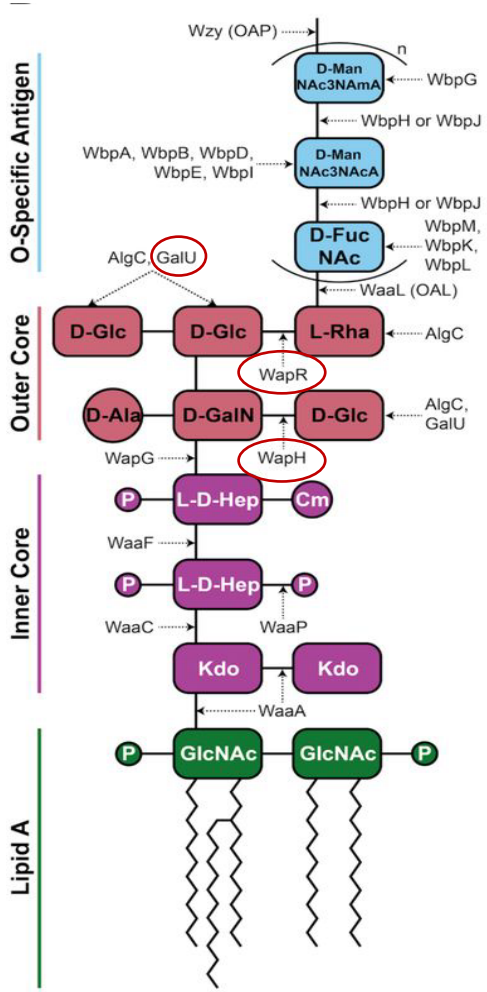
Patient 91



R994G in mexB



H87D in GyrA quinolone-resistance determining region (QRDR)



GalU: loss 41 AA
WapR: L162P
WapH: loss of 168 AA

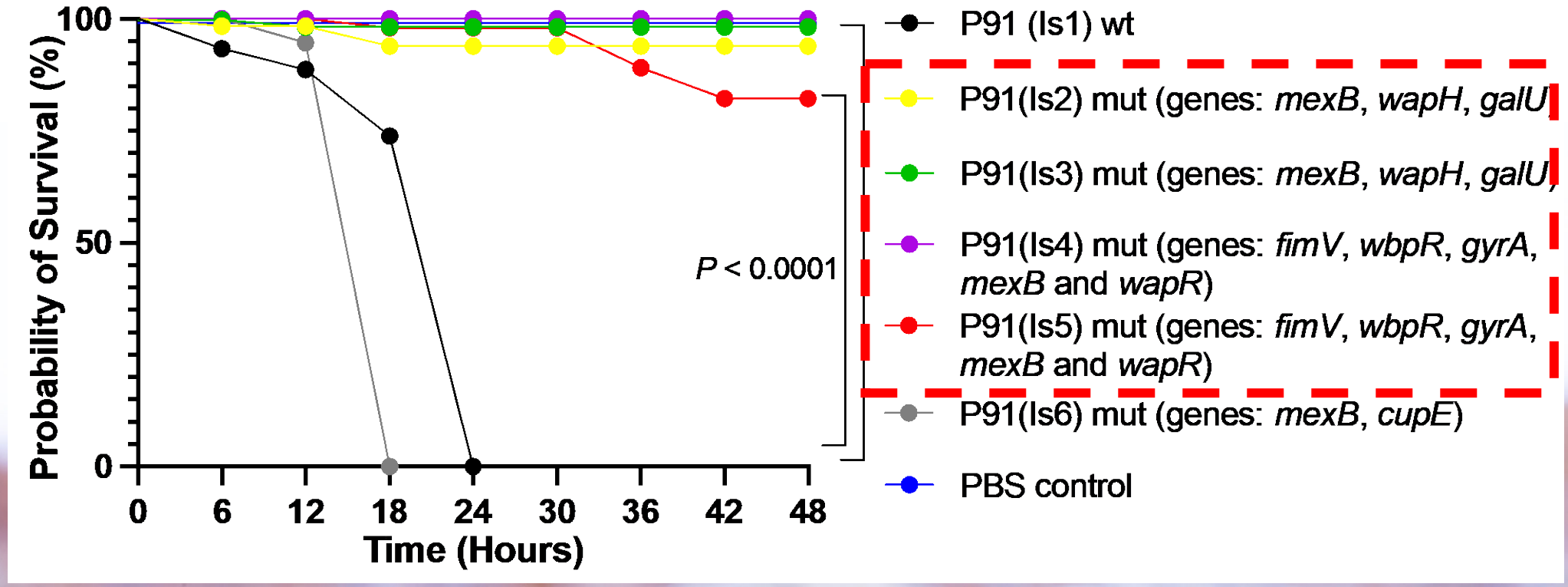
Re-sensitization to fluoroquinolones

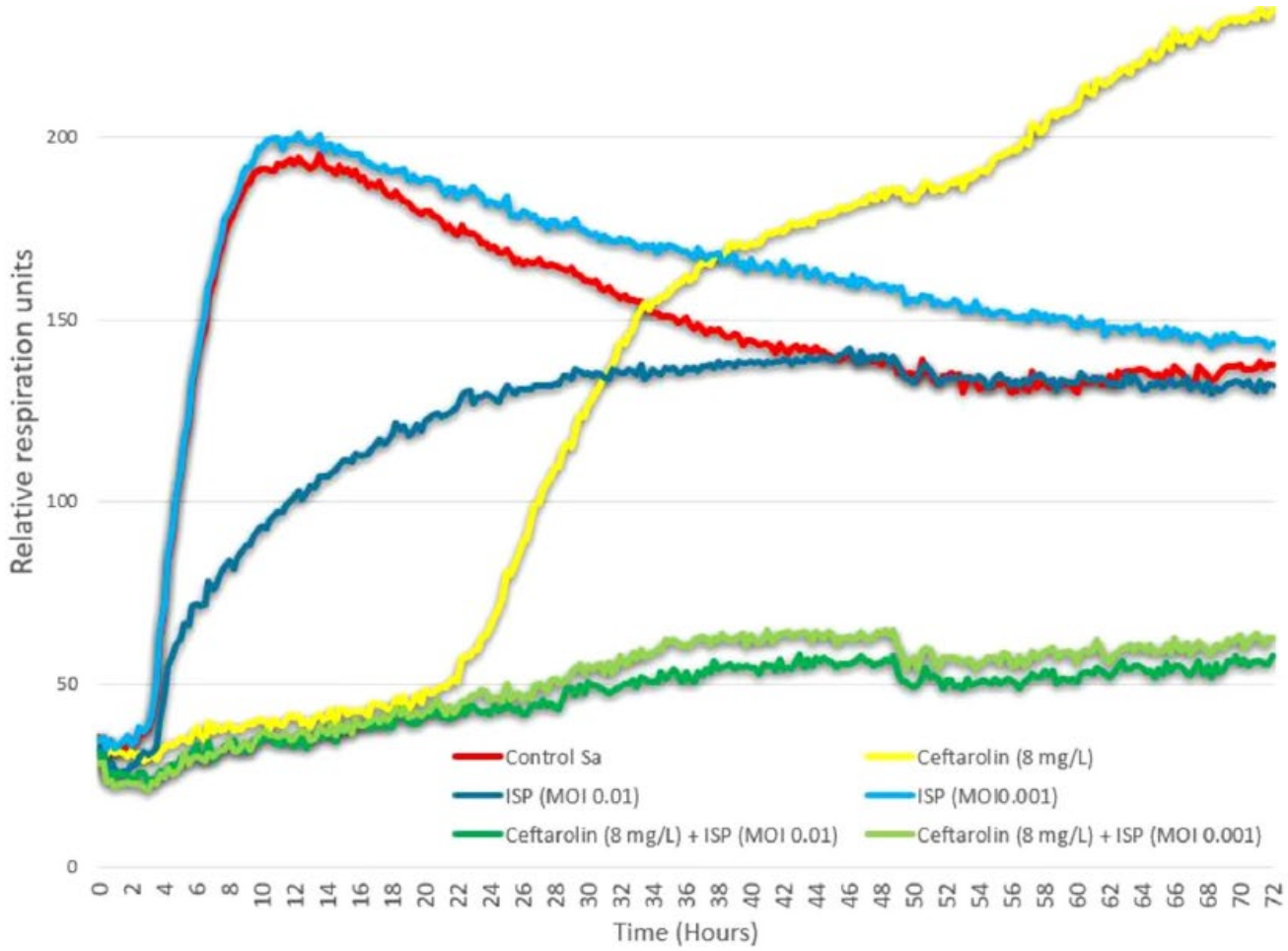
Substantial decrease in MIC for ciprofloxacin and levofloxacin

Pirnay J.- P., Djebara S. et al., Nat. Microbiol. 2024 Jun;9(6):1434-1453.

Reduced virulence

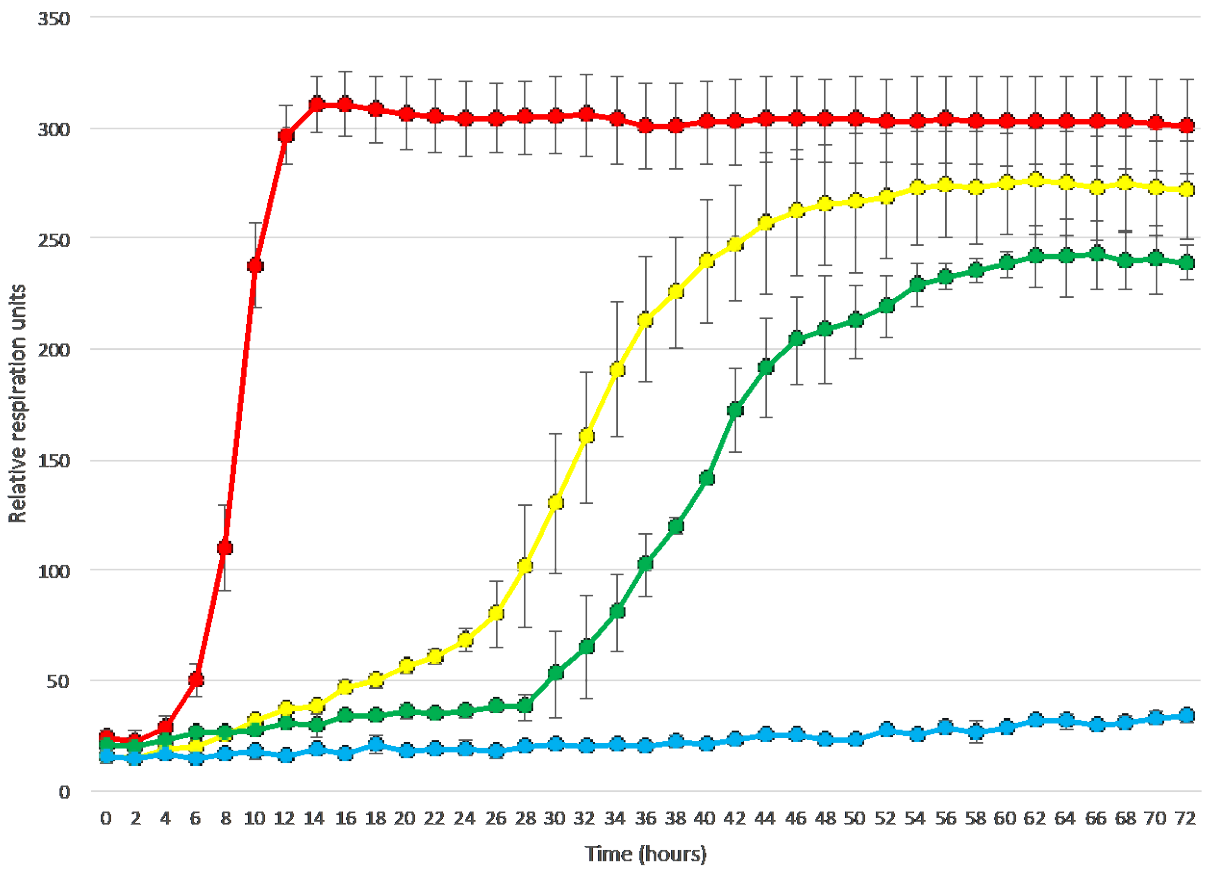
***Galleria mellonella* larvae model**





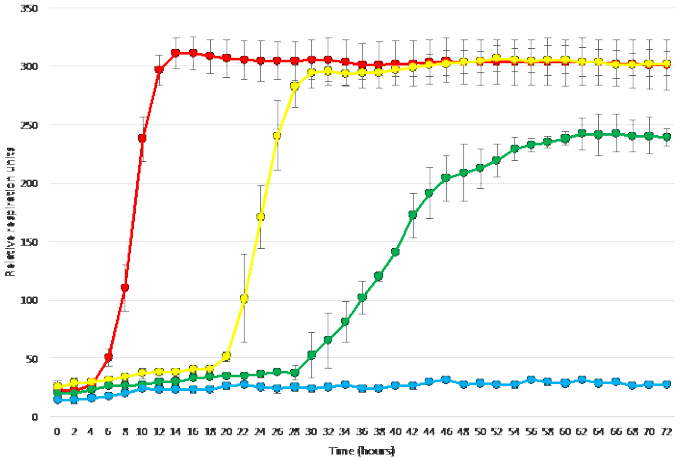
Van Nieuwenhuyse B. *et al: Front Cell Infect Microbiol sept 24 17:14:1354681*

Phage - antibiotic synergy

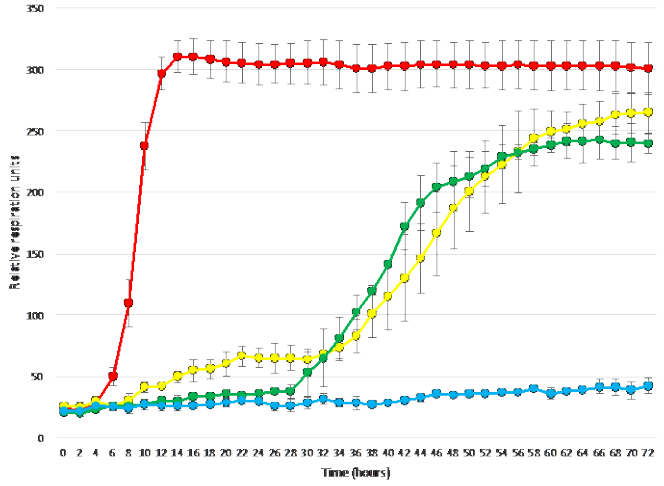


Aztreonam + PNM

- Control S1
- Aztreonam (8 mg/L)
- PNM (MOI 1.0)
- Aztreonam (8 mg/L) +PNM (MOI 1.0)



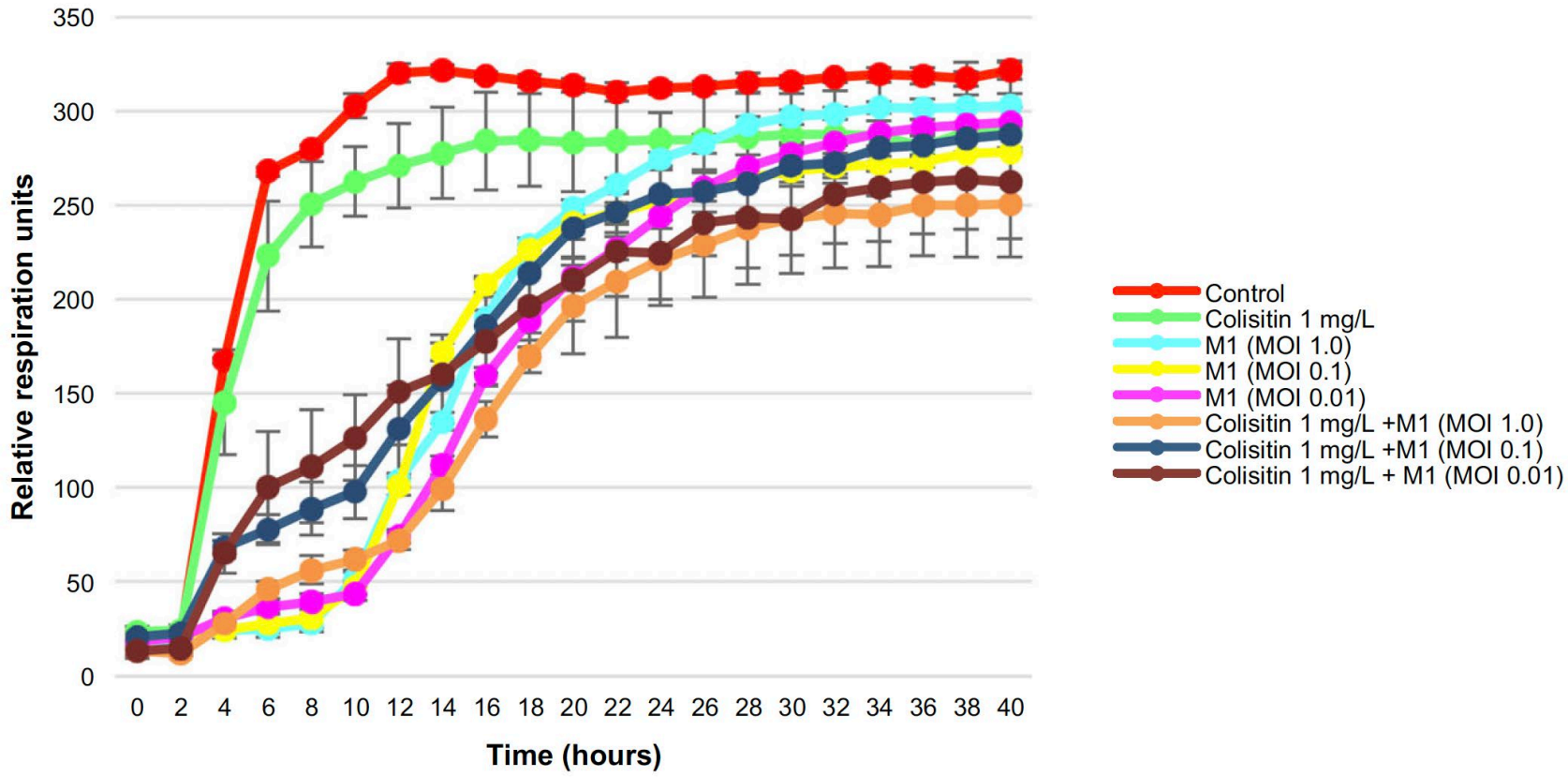
Colistin + PNM



Gentamycin + PNM

Van Nieuwenhuyse B. *et al.* Nat Commun. 2022 Sep 29;13(1):5725.

Not always…

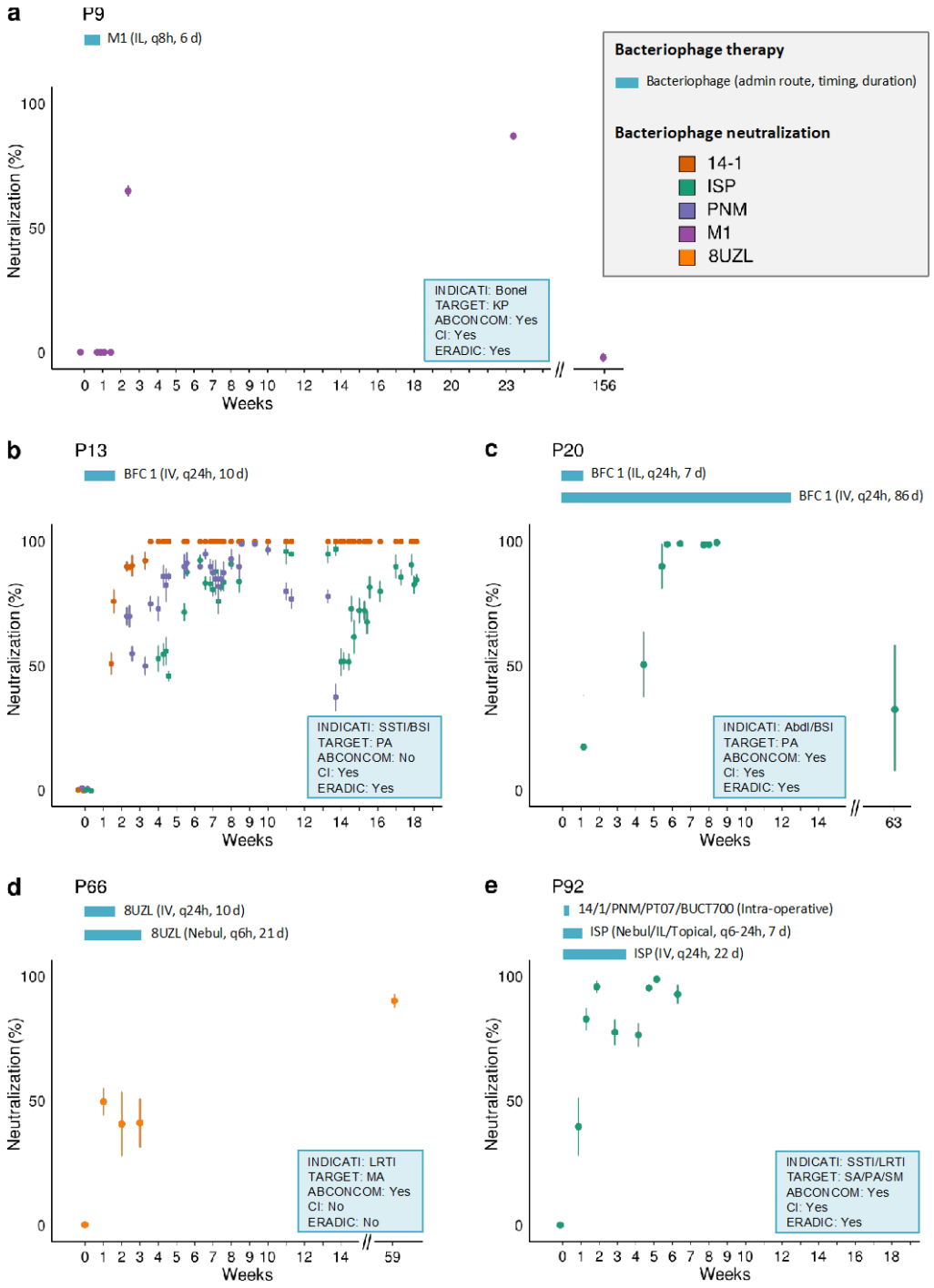


Colistin
+
M1

Depends on Phage – Bacterium – Antibiotic

Eskenazi et al. Nat. Commun. 2022 Jan 18;13(1):302.

Phage immune neutralisation



Occurred 6-35 days after initiation of **invasive** PT

Depends on Phage – Patient

Pirnay J.- P., Djebara S. et al., Nat. Microbiol. 2024 Jun;9(6):1434-1453.

Phage “training”

DEFENSIE
LA DÉFENSE

Combination of pre-adapted bacteriophage therapy and antibiotics for treatment of fracture-related infection due to pandrug-resistant *Klebsiella pneumoniae*

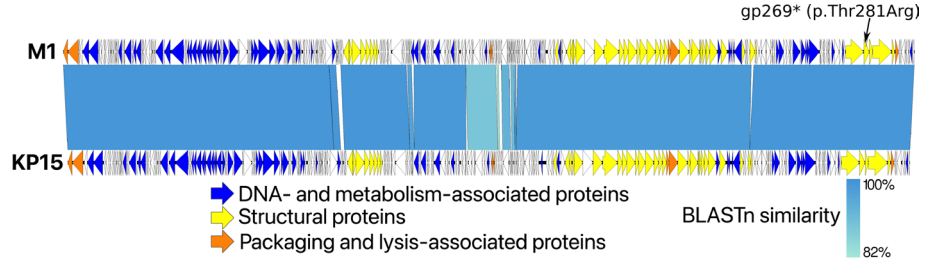
Anaïs Eskenazi[1,9✉], Cédric Lood[2,3], Julia Wubbolts[4], Maya Hites[1], Nana Balarjishvili[5], Lika Leshkasheli[5], Lia Askilashvili[5], Leila Kvachadze[5], Vera van Noort[3,6], Jeroen Wagemans[2], Marc Jayankura[7], Nina Chanishvili[5], Mark de Boer[4], Peter Nibbering[4], Mzia Kutateladze[5], Rob Lavigne[2], Maya Merabishvili[8] & Jean-Paul Pirnay[8]

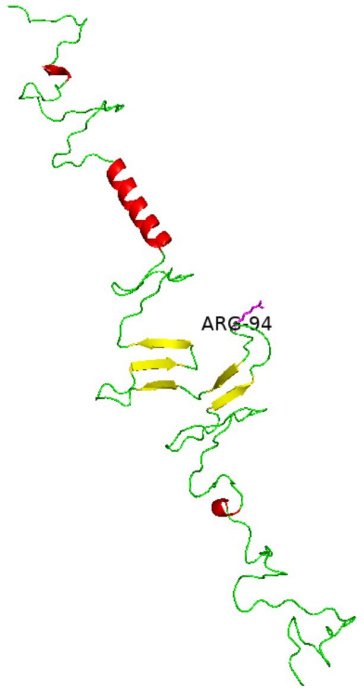
JOURNAL OF Evolutionary Biology eseb

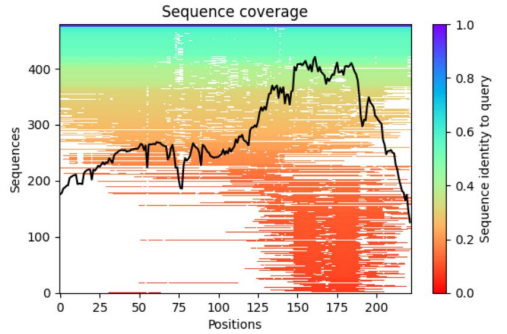
doi: 10.1111/jeb.12774

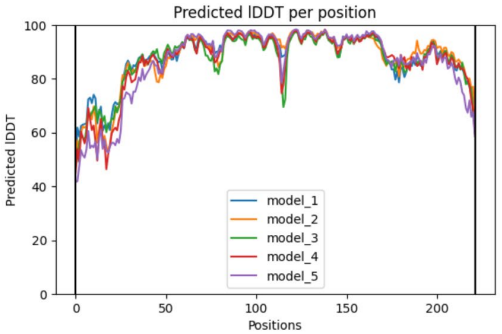
- **Increased pathogen clearance**
- **Lowered resistance evolution**

Friman *et al*. Evol Biol. 2016; 29(1):188-98.









Missense mutation in the loop region of the hinge connector of the distal tail fiber protein.

Eskenazi et al. Nat Commun. 2022 Jan 18;13(1):302.

DEFENSIE
LA DÉFENSE

- Patient with *Staphylococcus epidermidis* infection
- *Staphylococcus aureus* phage **ISP**

- Pre-adaptation (8 rounds) on the patient's strain

➔ **ISP$_{epi}$**

- Pre-adaptation resulted in 4 missense mutations
- **Trade-off: Reduction of host range (3/14 ➔ 1/14)**

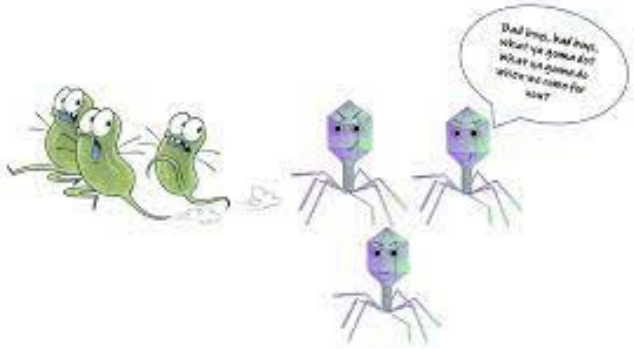
CLINICAL ISOLATE	ISP	ISPepi
STEP_UZL/TRH_1		
STEP_UZL/TRH_2A		
STEP_UZL/TRH_2B		
STEP_UZL/TRH_3		
STEP_UZL/TRH_4		
STEP_UZL/TRH_5		
Patient strain		
STEP_UZL/TRH_7		
STEP_UZL/TRH_8		
STEP_UZL/TRH_9		
STEP_UZL/TRH_10		
STEP_UZL/TRH_11A		
STEP_UZL/TRH_11B		
STEP_UZL/TRH_11C		

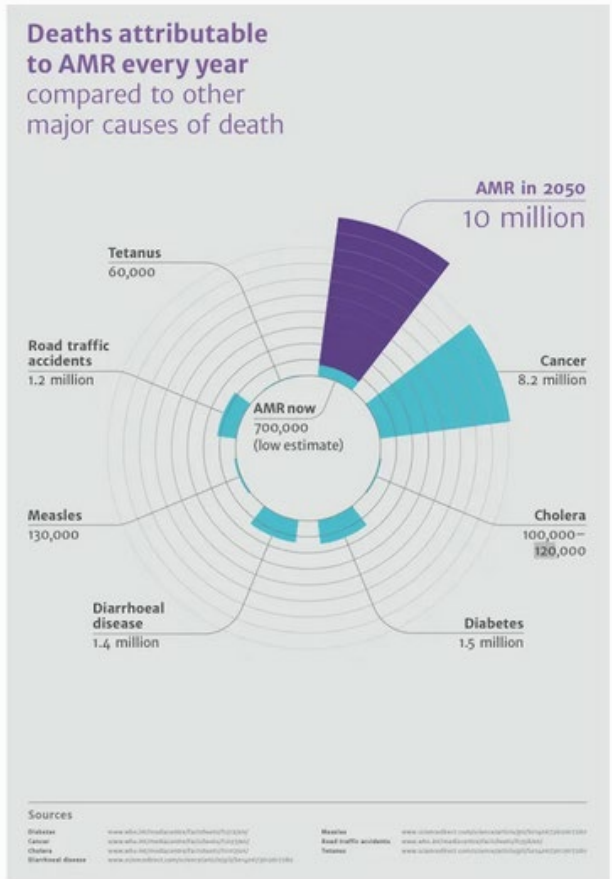
Conclusions

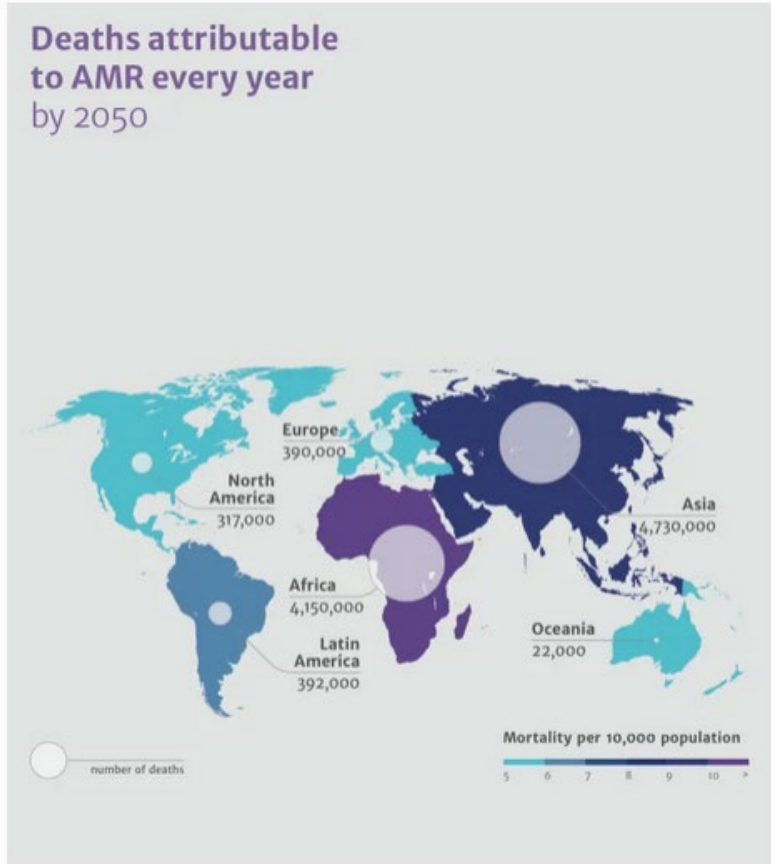
- Specific framework for phage therapy
- Standardization of clinical guidelines in a « learn as we go process »
- Clinicians,pharmacists working groups and phage hubs negociated with public helath authorities.
- API Monograph 2.0
- Phagistry implementation
- Unlicenced phage preparation more efficient and less prompt to spread bacterial phage resistance than phage cocktails. (risk of APDS spread)
- Merchandising drug concept and “Big Pharma” has contributed to the marginalization of phage therapy.
  - a return on investment seems impossible with a personalized and constantly evolving therapy.

Acknowledgements

- QAMH
- Lab-MCT (Maia Merabishvili, Jean-Paul Pirnay, Johann Griselain, Christelle Cochez, Griet Steurs, Gilbert Verbeken, Jean-Pierre Draye, Daniel De Vos, Tea Glonti
- All the academics and hospitals collaborators
- Patients







AMR

One Health

Health Policy

Challenges and opportunities for incentivising antibiotic research and development in Europe

Michael Anderson,[a,b,*] Dimitra Panteli,[b] Robin van Kessel,[a,c] Gunnar Ljungqvist,[a] Francesca Colombo,[d] and Elias Mossialos[a,b]

[a]Department of Health Policy, Lond
[b]European Observatory on Health Sy
[c]Faculty of Health, Medicine and Lif
Primary Care), Maastricht University,
[d]Health Division, Organisation for Economic Co-operation and Development, Paris, France

« Almost none of new antibiotics on the market have innovative characteristics »

Summary

Antimicrobial, and particularly antibiotic resistance are one of the world's biggest challenges today, and urgent action is needed to reinvigorate the antib
Swedish Presidency of the Counc
by the European Commission, an
relation to antibiotic research and
recent years, almost none have i
action. We consider four incentive options to incentivise research and development of new antibiotics, including subscription payments, market entry rewards, transferable exclusivity extensions, and milestone payments. While each option has advantages and drawbacks, a combination of incentives may be required and continued investment is needed by the EU in push incentives, such as direct funding and grants, to incentivise drug discovery and preclinical stages of development. The EU must also coordinate with international initiatives and support access to new and pre-existing antibiotics in LMICs through platforms such as the WHO, and G7 and G20 group of countries.

« A combination of incentives may be required and continued investment is needed by the EU in push incentives »

The Lancet Regional Health - Europe
705
e 26 July
g/10.
.2023.

It'more and more obvious that public authorities and private sector, but also civil society and philanthropic foundations will become the financing configurations which, in the very short term, will replace the binary "private-public" couple. Enough to further strengthen the weight of private interests in the implementation of ambitious public health policy without concrete results on AMR threat

Clinical Infectious Diseases

SUPPLEMENT ARTICLE

IDSA Infectious Diseases Society of America

hivma hiv medicine association

OXFORD

Magistral Phage Preparations: Is This the Model for Everyone?

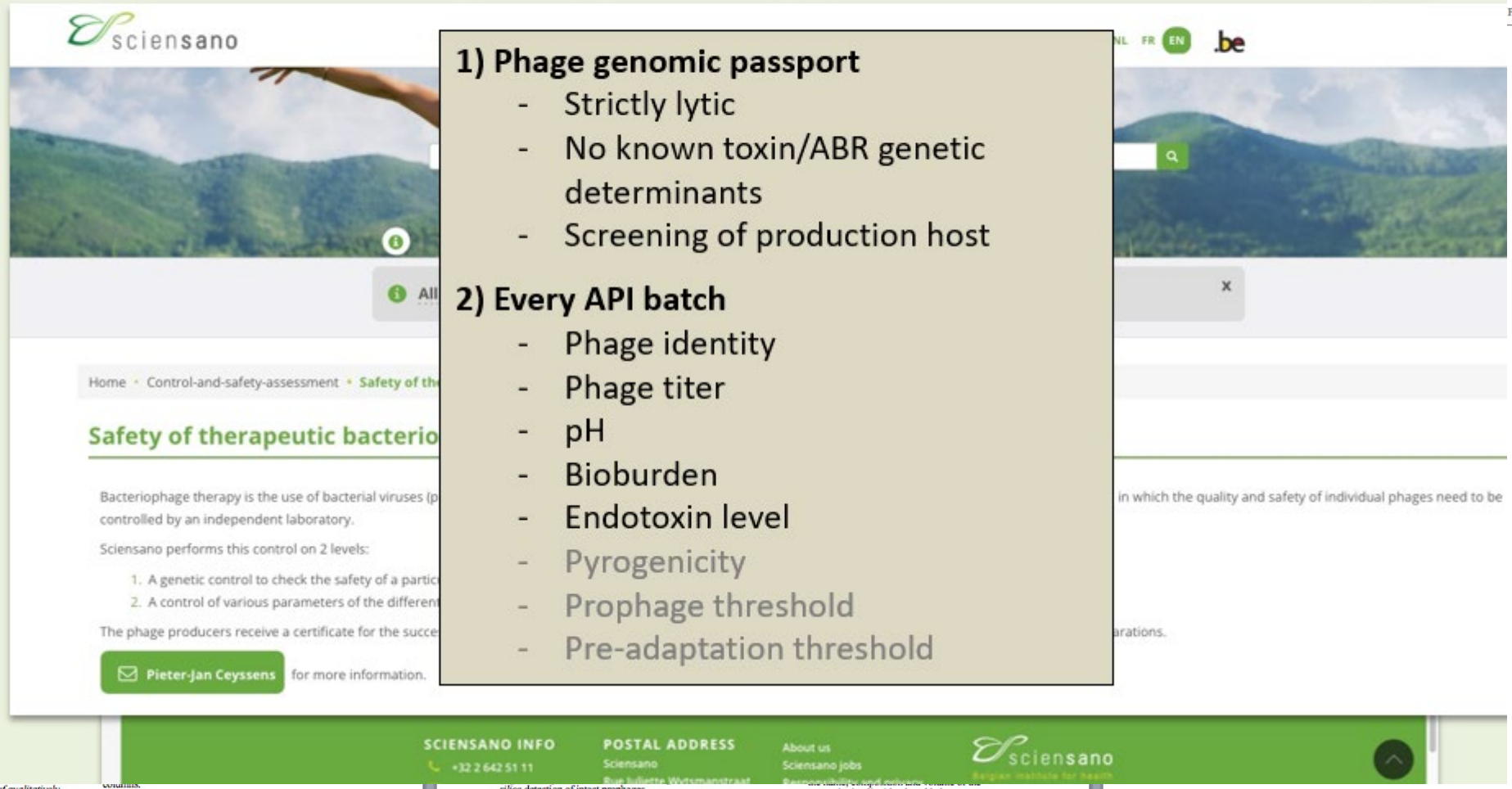
Jean-Paul Pirnay[1,2] and Gilbert Verbeken[1]

[1]Laboratory for Molecular and Cellular Technology, Queen Astrid Military Hospital, Brussels, Belgium; and [2]European Society of Clinical Microbiology and Infectious Diseases (ESCMID) Study Group for Non-traditional Antibacterial Therapy (ESGNTA), Basel, Switzerland

Phage therapy is increasingly put forward as a promising additional tool to help curb the global antimicrobial resistance crisis. However, industrially manufactured phage medicinal products are currently not available on the European Union and United States markets. In addition, it is expected that the business purpose–driven phage products that are supposed to be marketed in the future would mainly target commercially viable bacterial species and clinical indications, using fixed phage cocktails. hospitals or phage therapy centers aiming to help all patients with difficult-to-treat infections urgently need adequate phage preparations. We believe that national solutions based on the magistral preparation of personalized (preadapted) phage products by hospital and academic facilities could bring an immediate solution and could complement future industrially manufactured products. Moreover, these unlicensed phage preparations are presumed to be more efficient and to elicit less bacterial phage resistance issues than fixed phage cocktails, claims that need to be scientifically substantiated as soon as possible. Just like Belgium, other (European) countries could develop a magistral phage preparation framework that would exist next to the conventional medicinal product development and licensing pathways. However, it is important that the current producers of personalized phage products are provided with pragmatic quality and safety assurance requirements, which are preferably standardized (at least at the European level), and are tiered based on benefit–risk assessments at the individual patient level. Pro bono phage therapy providers should be supported and not stopped by the imposition of industry standards such as Good Manufacturing Practice requirements.

Phage Active Pharmaceutical Ingredient (API) Monograph

Sciensano (Belgian Approved Laboratory)



1) Phage genomic passport

- Strictly lytic
- No known toxin/ABR genetic determinants
- Screening of production host

2) Every API batch

- Phage identity
- Phage titer
- pH
- Bioburden
- Endotoxin level
- Pyrogenicity
- Prophage threshold
- Pre-adaptation threshold

GENERAL MONOGRAPH – VERSI

PHAGE ACTIVE PHARM
INGREDIENT

DEFINITION

Phage active pharmaceutical ingredient
pharmaceutical preparations containing
bacteriophages (phages in short), whic
bacteria. Phages are composed of prote
DNA or RNA genome, and may have
elaborate structures. Phages replicate w
following the injection of their genome
Phages are among the most abundant a
in the biosphere. In general, phages are
nature.

Phage APIs are intended for use as acti
phage magistral preparations for *in viv*
infections (phage therapy).

Phage APIs are available as suspension
phages, in physiological solutions (e.g.,
solutions) that may contain a buffer or
dried phage suspensions. As active ingr
preparations, they are intended to be di
and/or combined with the necessary ing
pharmacy officina, immediately before
patient basis. Inactive ingredients may
ointments, liquids, capsules, etc. These
allow the required phage activity during
application period.

Phage APIs may contain one or more
various phage APIs may be combined
preparation to broaden the spectrum of
medicine.

*The magistral preparation of phage the
practical way for medical doctors to pe
antibacterial treatments.*

*This monograph does not apply to gene
phages and to phage derived products
endolysins. It does not necessarily appl
for veterinary use or for decontaminati*

*In addition to the requirements specifie
monograph, specific requirements for
testing and release testing might be inc
monographs.*

PRODUCTION

MANUFACTURING PROCESS

Phage APIs are generally obtained by
bacterial strains and are purified using
shown to preserve the biological prope
Phage APIs are manufactured under co
minimise microbial contamination and

Purification procedures need to be desi
content of harmful bacterial or culture
(e.g., bacterial endotoxins and animal p

*The manufacturing process must be de
(equipment, materials, culture media, a
conditions, purification steps,...) in stan
procedures (SOPs) and must be valida
process can reliably output phage APIs
standard.*

*The following manufacturing process h
suitable for the small-scale production of qualitatively
acceptable and safe phage APIs. It is* ***indicative*** *and based on
the state of the art and available knowledge from peer
reviewed scientific literature.*

The manufacturing process comprises various stages.

PRODUCTION ENVIRONMENT

The manufacturing of phage APIs from phage seed lots takes place in an environment with specified air quality and

Phage active pharmaceutical ingredients

silico detection of intact prophages.

PHAGE SEED LOTS

Phage identification. State of the art DNA or RNA

— the period of time within which the preparation is to be used after reconstitution;
— instructions for reporting serious adverse reactions and/or events;

EUROPEAN PHARMACOPOEIA

Phage therapy medicinal products

Published in accordance with the Convention on the Elaboration of a European Pharmacopoeia (European Treaty Series No. 50)

Council of Europe
Strasbourg

5.31. PHAGE THERAPY MEDICINAL PRODUCTS

This general chapter is published for information.

It offers a framework of requirements for phage therapy active substances and medicinal products for human and veterinary use and their production and control.

The provisions of the chapter do not exclude the use of alternative production and control methods that are acceptable to the competent authority.

1. DEFINITION

Bacteriophages (phages) are viruses that infect bacteria and depend on their bacterial host for replication. Phages consist of a genome comprised of single or double stranded DNA or RNA, encapsulated in a protein capsid.

Phage therapy medicinal products (PTMPs) are preparations of naturally occurring or genetically modified phages used to treat or prevent human or veterinary bacterial infections.

A PTMP can contain one phage, i.e. a single phage therapy active substance, or a mixture of phages, combined with excipients. PTMPs can be administered by various routes and are available in different dosage forms.

2. PRODUCTION

2-1. *GENERAL PROVISIONS*

Phages are obtained by propagation in bacterial host strains and are purified using suitable methods.

The production process yields a PTMP of consistent quality and stability. Appropriate in-process testing is implemented at relevant time points and/or key intermediate stages of the process.

Production of PTMPs is based on a well-characterised

of the bacterial strain, subsequent manipulations and the tests used to characterise the strain. This must include determination of its antibiotic susceptibility profile and of the nucleotide sequences of its chromosome(s) and plasmids. The use of bacterial strains whose genome contains sequences coding for detrimental factors (e.g. prophages, antibiotic resistance determinants, toxins) is avoided, unless otherwise justified and authorised.

Bacterial host cells used for PTMP production are derived from a well characterised bacterial master cell bank (MCB) that is of clonal origin and complies with the following requirements:

Identification. The identity is confirmed using a suitable method.

Microbial purity. The absence of microbial contaminants is determined by plating or any other suitable method.

Viability. The number of viable cells is determined by a plate count or any other suitable viable cell count method.

Phage sensitivity. The susceptibility of the strain to the phage therapy active substance is demonstrated using a plaque assay or any other suitable method.

Absence of detrimental phages. The absence of phage particles that could be detrimental to the quality of PTMPs is confirmed.

If a working cell bank (WCB) is used for production, it is a clonal derivative of the MCB and complies with the requirements for MCB.

2-3. *PHAGE SEED LOTS*

Phage seed lots used in PTMP production are derived from a single phage clone and must be characterised in detail. Information on the phage source, nucleotide sequence and susceptible bacterial species and/or strains is to be provided. Other parameters such as plaque morphology or phage morphology are determined, if relevant.

Phages whose genome contains sequences coding for known or potential detrimental genetic factors, e.g. antibiotic resistance determinants, toxins or lysogeny modules, are avoided, unless otherwise justified and authorised. For genetically or chemically modified phages, the modifications must be described and their effects characterised.

A phage master seed lot complies with the following requirements:

Identification. The phage seed lot is identified by a suitable method.

Microbial purity. The absence of microbial contaminants is demonstrated by a suitable method.

Phage purity. The absence of extrinsic phage contaminants is confirmed by a suitable method; however, as intrinsic phages may be unavoidable when using clinical isolates for production, their presence may in this case be justified and authorised when controlled by a suitable method.

Potency. The infectious phage titre is determined by a plaque

Several single harvests of the same phage clone may be pooled before the purification process.

Phages are purified by suitable techniques.

Only a purified harvest containing a single phage therapy active substance that complies with the following requirements may be used in the preparation of the final lot:

Identification. The identity of the phage is confirmed using a suitable method.

Potency. The infectious phage titre is determined by a plaque assay (expressed in PFU/mL or PFU/mg) or any other suitable method.

Microbiological examination (*2.6.12*). The purified harvest complies with the established specification.

Residual reagents. Based on risk analysis, tests for residues of reagents used during production and posing safety concerns are carried out on the purified harvest.

Host-cell impurities and contaminants. Contaminants and other potentially toxic substances derived from the host cells (e.g. endo- and exotoxins, host-cell proteins, host-cell DNA, temperate phages) are absent or within the established specifications.

2-5. *FINAL LOT*

The final lot can be administered by various routes and may be available in different dosage forms. Additional tests are required, depending on the dosage form and on the route of administration.

When it is not practical, for unlicensed pharmaceutical preparations, to carry out the tests (e.g. batch size, time restraints), other suitable methods are implemented to ensure that the appropriate quality is achieved in accordance with the risk assessment carried out and any local guidance or legal requirements.

A final lot complies with the following requirements:

Appearance. It complies with the established specification.

Identification. The identity of each phage is verified using a suitable method.

Potency. The infectious phage titre of each phage is determined by a plaque assay (expressed in PFU/mL or PFU/mg) or other suitable method and complies with the established specification for the particular preparation.

Microbiological quality. Sterile PTMPs comply with the test for sterility (*2.6.1*). For non-sterile PTMPs, the microbiological quality is determined using a suitable method and complies with the established specification for the particular preparation.

Pyrogenicity. If applicable, the final lot complies with a suitable test for pyrogenicity and with the limit approved for the particular product.

Water content (*2.5.12* or *2.5.32*). Solid PTMPs comply with the limit approved for the particular product.

pH (*2.2.3*). Liquid PTMPs comply with the limit approved for the particular product.

2-6. *ADAPTED PRODUCT*

Phage adaptation (training) is the process by which phages can be directed to evolve in order to increase their potency against (a) clinical isolate(s).

When the adapted PTMP is used in the individual patient that was the source of the clinical isolate, phage adaptation starts with a phage or mixture of phages, each complying with the provisions of section 2-3. The final lot complies with the provisions of section 2-5, unless otherwise justified and authorised. The increased potency of the final lot of the adapted PTMP against the target clinical isolate is confirmed, serving also as an appropriate substitute for the identification test.

3. LABELLING

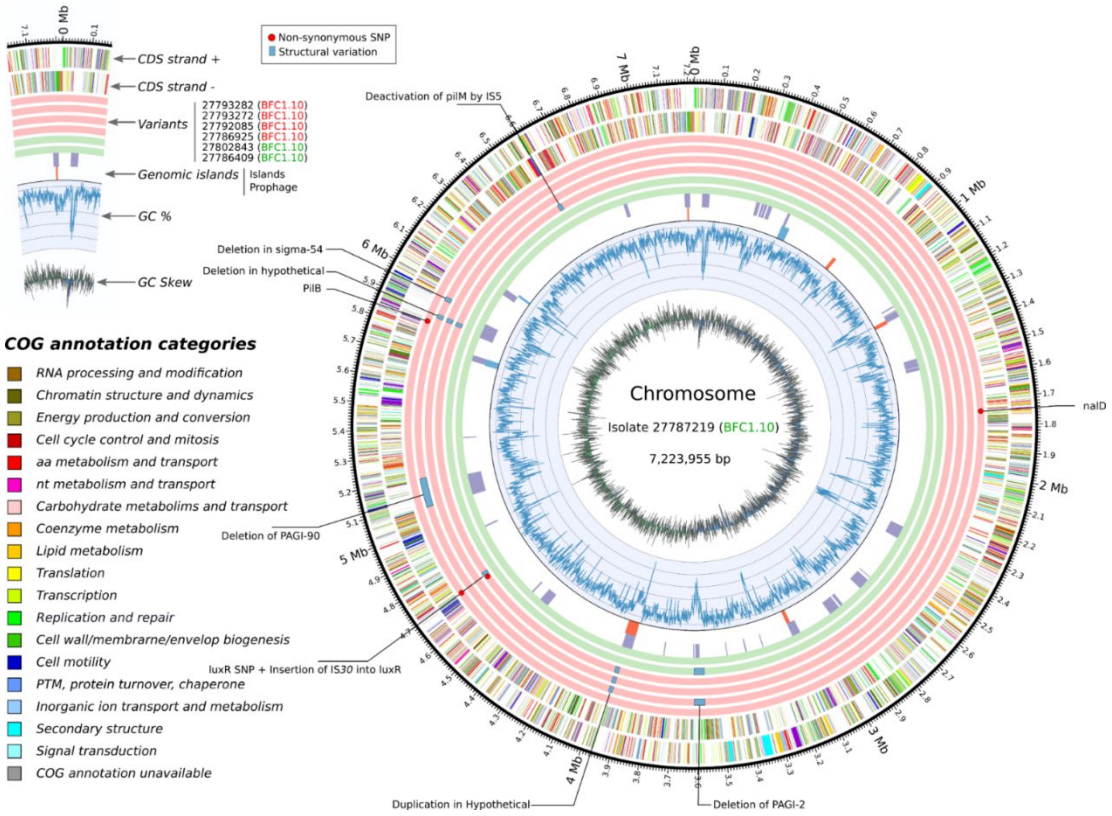
The labelling requirements outlined in relevant supranational and national regulations apply.

Phage API Monograph 2.0?
New quality controls
Pyrogenicity
Immunogenicity of PTMPs

In vivo emergence of BPR

1-yr-old patient with XDR *Pseudomonas aeruginosa* septicemia, post-liver transplantation.

➔ 86 days of intravenous phage therapy with phage PNM.

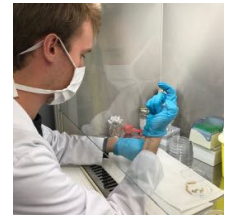


WGS showed that four isolates (red circles) emerged that expressed BPR. They possessed genetic alterations affecting the Type IV pili complex (PNM receptor).

Phage insensitive mutants: No reduced virulence in *Galleria mellonella.*

Liver transplant baby saved by "trained" virus at Saint-Luc hospital

Wednesday, 22 May 2019



Brieuc Van Nieuwenhuyse

Sabrina Green

Cédric Lood

Jeroen Wagemans

Rob Lavigne

Van Nieuwenhuyse B. et al. Nat Commun. 2022 Sep 29;13(1):5725.

Strain: *P. aeurginosa* 2409230420

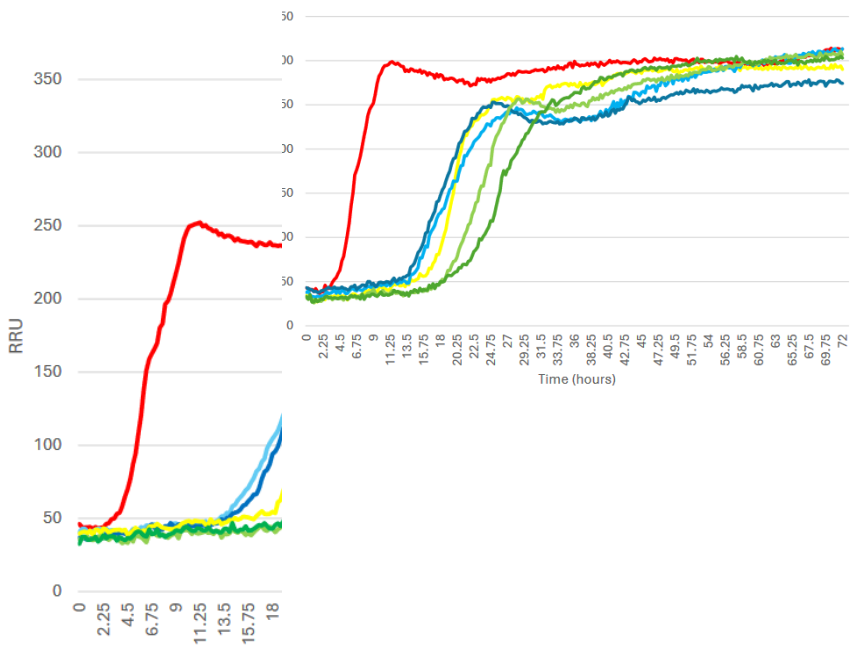
Phages(EOP): 14/1(0.001), NP3S (0.06), PNM (0.001)
MOI tested: 1.0; 0.1 & 0.01
All three phages inhibit growth of the strain for 13 h on average at MOI 1.0
Monitoring time: 72 h

Sensitivity & MIC v

Omnilog® experime
Each test is done at
*Bacterial growth is
Concentrations of

Abbreviations:
EOP_Efficiency of
MIC_Minimal Inhib
MOI_Multiplicity of
RRU_Relative Respiratory Units

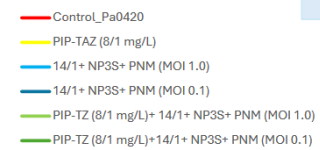
Phages at MOI 1.0



Conclusion:
Synergistic effect expressed at one concentration

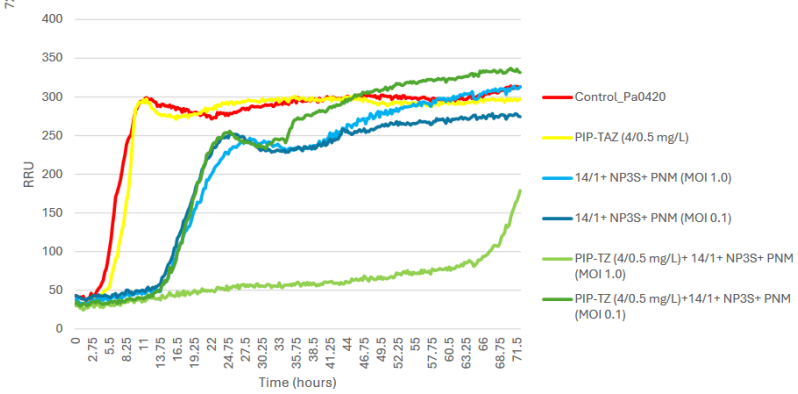
t one

TZP + 14/1+NP3S+PNM



Conclusion:
Neutral and synergistic effect expressed at two concentrations of 8/1-4/0.5 mg/L

TZP + 14/1+NP3S+PNM

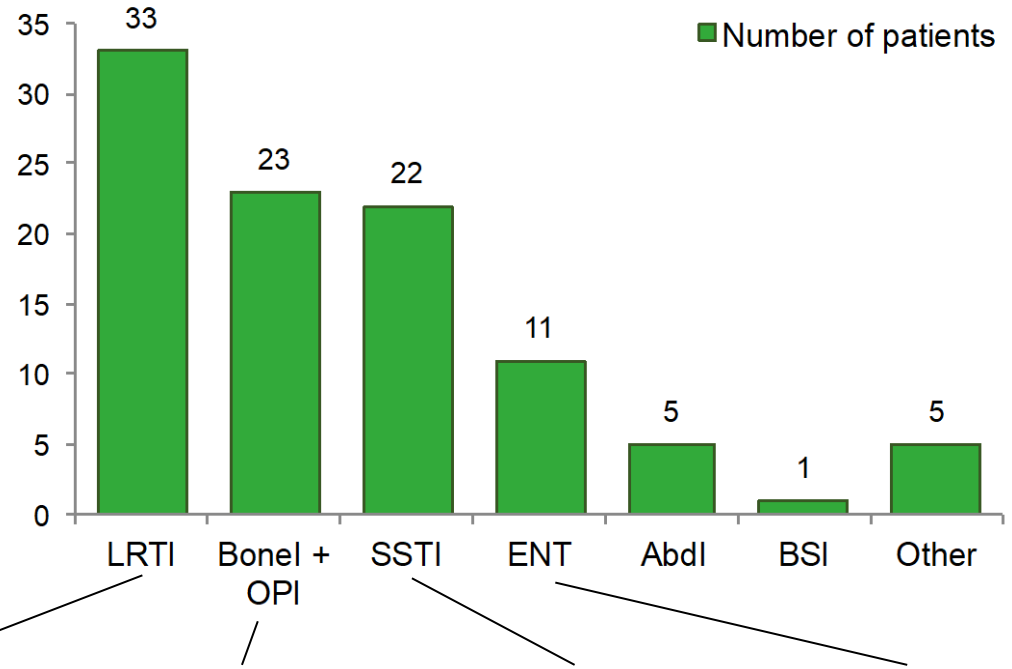


Results

- Clinical improvement reported in 77% of cases
- Eradication of targeted bacteria in 61% of cases
- 70% less probability of eradication without antibiotics
- Several interesting findings

Pirnay J.- P., Djebara S. et al., Nat. Microbiol. 2024 Jun;9(6):1434-1453.

Infection types



Nebulisation	Intralesional	Topical	Nasal spray	Intravenous (n=18)
2-4 ml	2-70 ml	In excess	1 – 15 ml	50 – 100 ml (6h infusion)
10^7 – 10^8 pfu/lm	10^7 – 10^8 pfu/lm	10^7 – 10^9 pfu/lm	10^7 pfu/lm	10^6 – 10^7 pfu/ml
q6h	q24h	q24h	q8h	q24h
5 days – 6 weeks	5 days – 3 weeks	5 days – 3 weeks	1 – 3 weeks	5-10 days

Table 2 | Results of the supportive tests performed for 21 of the present 100 consecutive bacteriophage therapy cases

Patient number	Infection type	Targeted bacterial species	Applied bacterio-phage(s)	Bacteriophage administration route(s)	In vivo selection of bacteriophage resistance—possible underlying mechanism(s)	In vitro bacteriophage–antibiotic interactions	Bacteriophage immune neutralization	Clinical improve-ment	Eradication of targeted bacteria	Reference
9	Fracture-related infection	*Klebsiella pneumoniae*	M1	Intralesional (catheter)	Not observed	M1 synergy with ceftazidime/avibactam and meropenem	M1 neutralization emerged between days 8 and 18 after BT initiation	Yes	Yes	Ref. [6]
13	Wound and bloodstream infection	*Pseudomonas aeruginosa*	14-1, PNM and ISP (BFC 1)	Topical and intravenous	Not observed	No concomitant antibiotics	14-1 neutralization emerged 10 days after BT initiation	Yes	Yes	Ref. [15]
16	Cystic fibrosis lung transplant infection	*Achromobacter xylosoxidans*	JWAlpha, JWDelta, JWT and 2-1 (APC 1.1 and APC 2.1)	Nebulization	Yes—p.Tyr601X MS Mut in colicin I receptor Cir	No concomitant antibiotics	NSA	Yes	Yes	Ref. [16]
20	Liver transplant and bloodstream infection	*P. aeruginosa*	14-1, PNM and ISP (BFC 1)	Intralesional (infusions) and intravenous	Yes—p.Asp388Ala MS Mut in PilB and deactivation of PilM by insertion of IS5 transposase, both involved in Type IV pili biosynthesis, without impact on virulence	PNM synergy with colistin, aztreonam and gentamycin	ISP neutralization emerged 5 weeks after BT initiation. No neutralization of 14-1 or PNM	Yes	Yes	Ref. [17]
21	Bone allograft infection	*Staphylococcus aureus*	14-1, PNM and ISP (BFC 1)	Intralesional (catheter) and intravenous	Not observed	ISP synergy with clindamycin, additive effect of ISP and ciprofloxacin, moderate ISP antagonism with rifampicin	NSA	Yes	Yes	Ref. [18]
22	Chronic osteomyelitis of the pelvis	*P. aeruginosa* and *S. epidermidis*	14-1, PNM, ISP (BFC 1)	Intralesional (catheter)	NSA	NA	1 month after BT initiation, no bacteriophage neutralization could be detected	Yes	Yes	Ref. [19]
23	Chronic osteomyelitis of the femur	*S. aureus*	14-1, PNM, ISP (BFC 1)	Intralesional (catheter)	NSA	NA	1 month after BT initiation, no bacteriophage neutralization could be detected	Yes	Yes	Ref. [19]
24	Chronic osteomyelitis of the femur	*P. aeruginosa* and *S. epidermidis*	14-1, PNM, ISP (BFC 1)	Intralesional (catheter)	NSA	NA	1 month after BT initiation, no bacteriophage neutralization could be detected	Yes	Yes	Ref. [19]
26	Spinal infection	*P. aeruginosa*	4029, 4032 and 4034	Local and intravenous	Not observed	NA	NSA	Yes	Yes	Ref. [20]
27	Orthopaedic infection	*P. aeruginosa*	14-1, PNM and ISP (BFC 1)	Local	Not observed	Additive effect of the bacteriophage cocktail with ceftazidime/avibactam	NSA	Yes	Yes	Ref. [21]
30	Chronic sinusitis	*P. aeruginosa* and *S. aureus*	14-1, PNM and ISP (BFC 1)	Nasal spray	Yes—p.Ala154 Pro MS Mut in PilC, involved in Type IV pili biosynthesis	No concomitant antibiotics	NSA	No	No	Unpublished
42	Chronic osteomyelitis of the femur	*Enterococcus faecalis*	PyoPhage	Intralesional (catheter)	NSA	NA	1 month after BT initiation, no bacteriophage neutralization could be detected	Yes	Yes	Ref. [19]
43	Liver transplant infection	*Enterococcus faecium*	EfgrKN and EfgrNG	Intravenous	Not observed	Synergy of EfgrKN with vancomycin, loss of vancomycin resistance	49 days after BT initiation, no bacteriophage neutralization could be detected	Yes	No	Ref. [23]
54	Ventilator-associated pneumonia	*P. aeruginosa*	14-1, PNM and PT07	Nebulization	Yes—p.Thr230Proline MS Mut in PilR, involved in Type IV pili biosynthesis	No clear interaction between PNM or 14-1 and colistin	2 months after BT initiation, no bacteriophage neutralization could be detected	Yes	No	Unpublished
55	Musculoskeletal infection	*S. epidermidis*	ISP and BE06	Intralesional and intravenous	Not observed	No concomitant antibiotics	NSA	No	No	Unpublished
64	Anal fistula	*P. aeruginosa*	14-1, PNM and PT07	Intralesional	Yes—selection of another strain, which is not a host for bacteriophages 14-1, PNM or PT07	No concomitant antibiotics	4 and 7 months after BT initiation, no bacteriophage neutralization could be detected	Yes	Yes	Unpublished
66	Cystic fibrosis lung infection	*M. abscessus*	8UZL	Nebulization and intravenous	NSA	NA	8UZL neutralization emerged 7 days after BT initiation	No	No	Unpublished
71	Lung infection	*P. aeruginosa*	PT07	Nebulization	Not observed	Additive effect of PT07 and ceftazidime	NSA	Yes	Yes	Unpublished
82	Cystic fibrosis lung infection	*P. aeruginosa*	4P and DP1	Nebulization	Yes—selection of another strain, which is not a host for bacteriophages 4P and DP1	Synergy of 4P and DP1 with levofloxacin, no clear interaction between 4P or DP1 and tobramycin	NSA	Yes	Yes	Unpublished
91	Lung infection	*P. aeruginosa*	14-1, PNM and PT07	Nebulization and intravenous	Yes— **LPS biosynthesis:** (Is 2 and 3) p.Trp139X NS Mut in WapH, (Is 2 and 3) p.Gln239X NS Mut in GalU, (Is 4 and 5) p.Leu162Pro MS Mut in WapR, (Is 4 and 5) p.Leu60_Leu63del in WbpR **Type IV pili biosynthesis:** (Is 4 and 5) p.Arg120fsX in FimV, missing the first 165 aa **Other:** (Is 6) p.Gly406Ser MS Mut in CupE5 fimbrae assembly protein, (Is 2, 3, 4, 5 and 6) p.Arg994Gly MS Mut in MexB of MexAB-OprM, (Is 4 and 5) p.His87Asp MS Mut in GyrA	PT07 synergy with colistin and meropenem	2 weeks after BT initiation, no bacteriophage neutralization could be detected	Yes	No	Unpublished
92	Generalized necrotizing fasciitis, empyema, bacteremia	*S. aureus*, *P. aeruginosa* and *Stenotro-phomonas maltophilia*	ISP, 14-1, PNM, PT07 and BUCT700	ISP: intravenous, intrapleural, intraperitoneal and nebulization; All: topical	Not observed	ISP synergy with vancomycin, ceftarolin and clindamycin	ISP neutralization emerged 6 days after BT initiation	Yes	Yes	Unpublished

aa, amino acids; del, deletion; fs, frameshift; Is, isolate; MS Mut, missense mutation; NA, not analysed; NS Mut, nonsense mutation; X, stop.

Overview of rnadomized controlled trials (RCTs) of phage therapeutics (status on 23 February 2024)

Trial identifier	Design	Phase	Target clinical indication	Targeted bacterial species	Phage product	Status	Sponsor (location)
EudraCT 2004-001691-39	R, C, B	1/2	Otitis externa	*Pseudomonas aeruginosa*	Biophage-PA, a cocktail with 6 phages	Completed	Biocontrol, Ltd (UK)
NCT00937274	R, C, B	NA	Pediatric diarrhea	*Escherichia coli*	T4 phage cocktail with 11 phages and Microgen ColiProteus cocktail with 18 phages	Completed	Société des Produits Nestlé (Switzerland)
NCT02116010	R, C, OL	1/2	Burn wound infection	*P. aeruginosa*	PP1131, a cocktail of 12 phages	Completed	Pherecydes Pharma, SA (France)
NCT03140085	R, C, B	2/3	Urinary tract infections	*Enterococcus* spp., *E. coli, Proteus mirabilis, P. aeruginosa , Staphylococcus* spp., and *Streptococcus* spp.	PYO phage, a complex cocktail with an unknown number of phages	Completed	Balgrist University Hospital (Switzerland)
NCT04596319	R, C, B	1b/2a	Cystic fibrosis and chronic pulmonary infection	*P. aeruginosa*	AP-PA02, a cocktail with an undiscosed number of phages	Completed	Armata Pharmaceuticals, Inc (USA)
NCT04737876	R, C, B	1	Healthy adults	*Klebsiella pneumoniae*	BX002-A, a cocktail with an undiscosed number of phages	Completed	BiomX, Inc (Israel)
NCT04325685, SGDC-VAP-1	R, C, B	INP	Decolonization of the oropharynx	INP	Sextaphag, a sterile phage lysate containing an undisclosed number of phages	Completed	Northern State Medical University (Russian Federation)
NCT05453578	R, C, B	1/2	Cystic fibrosis lung infection	*P. aeruginosa*	WRAIR-PAM-CF1, a cocktail with 4 phages	Recruiting	National Institute of Allergy and Infectious Diseases (USA)
NCT05177107	R, C, B	2a	Diabetic foot osteomyelitis	*Staphylococcus aureus*	Personalised phage therapy dependent on *in vitro* phage susceptibility testing	Recruiting	Adaptive Phage Therapeutics, Inc (USA)
NCT05184764	R, C, B	1b/2a	Bacteremia	*S. aureus*	AP-SA02, a cocktail with an undiscosed number of phages	Recruiting	Armata Pharmaceuticals, Inc (USA)
NCT05616221	R, C, B	2	Non-cystic fibrosis bronchiectasis and chronic pulmonary infection	*P. aeruginosa*	AP-PA02, a cocktail with an undiscosed number of phages	Recruiting	Armata Pharmaceuticals, Inc (USA)
NCT03808103	R, C, B	1/2a	Intestinal Adherent Invasive *E. coli* (AIEC) in patients with inactive Crohn's disease	Adherent Invasive *E. coli* (AIEC)	EcoActive, a cocktail with an undiscosed number of phages	Recruiting	Intralytix, Inc (USA)
NCT05369104	R, C, B	2	Prosthetic joint infection, at the end of DAIR	*S. aureus*	Phage cocktails PP1493 and/or PP1815 with an undisclosed number of phages	Recruiting	Pherecydes Pharma, SA (France)
EudraCT 2021-004469-11	R, C, B	2	Prosthetic joint infection with the indication of SAIR and suppressive antibiotic therapy	*S. aureus*	Phage cocktails PP1493 and/or PP1815 with an undisclosed number of phages	Ongoing	Pherecydes Pharma, SA (France)
NCT05182749	R, C, B	1/2a	Healthy adults and healthy adults after a challenge with *Shigella*	*Shigella* spp.	ShigActive, a cocktail with an undisclosed number of phages	Recruiting	Intralytix, Inc (USA)
NCT05948592	R, C, B	2b	Diabetic foot infection	*S. aureus , P. aeruginosa , Acinetobacter baumannii*	TP-102, a cocktail with an undisclosed number of phages	Recruiting	Technophage, SA (Portugal)
NCT05715619	R, C, B	1/2a	Decolonization of the gastrointestinal tract	Vancomycin-resistant *Enterococcus* (VRE)	VRELysin, a cocktail with an undisclosed number of phages	Recruiting	Intralytix, Inc (USA)
NCT05010577, EudraCT 2022-003810-35	R, C, B	1/2	Cystic fibrosis lung infection	*P. aeruginosa*	BX004-A, a cocktail with an undiscosed number of phages	Active, not recruiting	BiomX, Inc (Israel)
NCT02664740	R, C, B	1/2	Infected diabetic foot ulcer	*S. aureus*	Sterile dressing impregnated with a cocktail with an undisclosed number of phages	Not yet recruiting	CHU de Nîmes (France) with Pherecydes Pharma, SA (France)
NCT05240300	R, C, B	1b/2a	Atopic dermatitis	*S. aureus*	BX005-A, a gel containing an undiscosed number of phages	Unknown	BiomX, Inc (Israel)
NCT04323475	R, C, OL	1	Burn wound infection	*S. aureus , P. aeruginosa , K. pneumoniae*	Phage Cocktail-SPK spray with an undisclosed number of phages	Unknown	Precisio Biotix Therapeutics, Inc (USA)
NCT04815798	R, C, B	1/2	Colonised pressure ulcer	*S. aureus , P. aeruginosa , K. pneumoniae*	BACTELIDE, a spray of microcapsules loaded with an undisclosed number of phages	Unknown	Precisio Biotix Therapeutics, Inc (USA)
NCT04684641	R, C, B	1/2	Cystic fibrosis lung infection	*P. aeruginosa*	Yale Phage Therapy (YPT) 01, a cocktail with an undiscosed number of phages	Ended after opening of OL extension for the placebo group	Yale University (USA)
NCT04287478	R, C, OL	1/2	Urinary tract Infection	*E. coli , K. pneumoniae*	Personalised phage therapy dependent on *in vitro* phage susceptibility testing	Terminated due to a change in development strategy	Adaptive Phage Therapeutics, Inc (USA)
NCT05269134	R, C, B	2	Prosthetic joint infection	*Staphylococcus* spp., *Enterococcus* spp., *Streptococcus* spp., *E. coli , P. aeruginosa , K. pneumoniae*	Personalised phage therapy dependent on *in vitro* phage susceptibility testing	Withdrawn (sponsor decision)	Adaptive Phage Therapeutics, Inc (USA)

C, controlled; DAIR, debridement, antibiotics, and implant retention; OL, open label; R, randomized

- **ClinicalTrials.gov, EudraCT,..**
- **25 RCTs**
  - 15 active/recuiting
  - 7 completed
  - 3 terminated
- **24 phase 1 and/or 2**
- **1 phase 2/3 (unsuccessful)**
- **9 bacterial targets**
  1. *P. aeruginosa*: 12
  2. *S. aureus*: 11
  3. *K. pneumoniae*: 5
  4. *E. coli*: 5
  5. *Enterococcus* spp.: 3
  6. *Streptococcus* spp.: 2
  7. *A. baumannii*: 1
  8. *Shigella*: 1
  9. *Proteus mirabilis*: 1

  80%
- **Limited number of indications**

Type of primary infection	# patients	# Concom. AB	% Concom. AB	# Clin. Improv.	% Clin. Improv.	# Eradication	% Eradication
Abdominal Infection	6	5	83,33%	5	83,33%	4	66,67%
Bone Infection	16	15	93,75%	13	81,25%	11	68,75%
Lower Respiratory Tract Infection	29	17	58,62%	20	68,97%	14	48,28%
Orthopedic Prostheses Infection	6	4	66,67%	5	83,33%	5	83,33%
Skin and Soft Tissue Infection	25	19	76,00%	21	84,00%	11	44,00%
Upper Respiratory Tract Infection	12	1	8,33%	8	66,67%	4	33,33%
Other	6	6	100,00%	5	83,33%	4	66,67%
TOTAL	**100**	**67**	**67,00%**	**77**	**77,00%**	**53**	53,00%

Antibiotics resistance profile	# patients	# Concom. AB	% Concom. AB	# Clin. Improv.	% Clin. Improv.	# Eradication	% Eradication
UDR	49	27	55,10%	38	77,55%	19	38,78%
MDR	51	40	78,43%	39	76,47%	34	66,67%
TOTAL	**100**	**67**	67,00%	**77**	77,00%	**53**	53,00%

Type of infection	# patients	# Concom. AB	% Concom. AB	# Clin. Improv.	% Clin. Improv.	# Eradication	% Eradication
Acute	30	27	90,00%	21	70,00%	19	63,33%
Chronic	70	40	57,14%	56	80,00%	34	48,57%
TOTAL	**100**	**67**	67,00%	**77**	77,00%	**53**	53,00%