PK–PD and Dose

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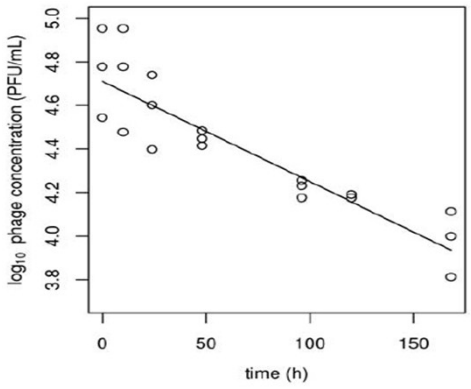
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Predictive Modeling, Improved Outcomes

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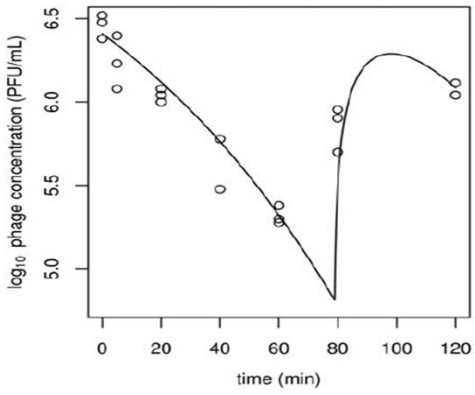
How much drug over time?

No bacterial host



Exposure driven by host elimination

In the presence of susceptible bacteria



Exposure driven by both clearance and bacterial burden

Cairns et al. Plos Pathogens, Jan. 2009

2

The key question is: when the drug replicates: will the agent establish, amplify, and clear the infection ?

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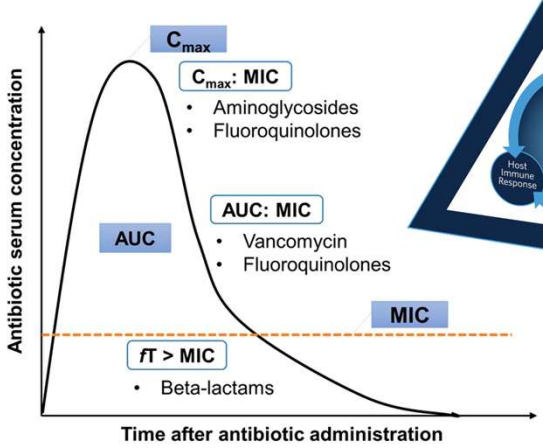
The antibiotic PK-PD paradigm

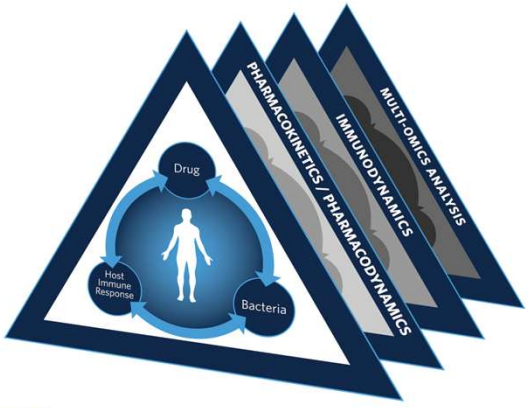
Factors influencing antimicrobial dosing

Patient factor
- Age
- Obesity
- Comorbidities
- Physiological changes
- Host immune response

Antimicrobial factor
- Solubility
- Volume of distribution
- Protein binding
- Drug clearance
- Antimicrobial effect

Microorganism factor
- MIC values
- Heteroresistance
- Laboratory variability





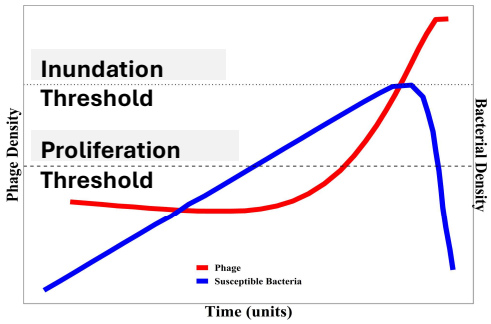
Garcia E, Ly N, Diep JK, Rao GG. Clin Pharmacol Ther. 2021 Nov;110(5):1196-1206.

Yow H-Y, et al. (2022) Front. Pharmacol. 13:915355.

4

Phage break the traditional paradigm

- Bidirectional: both bacteria and phage are growing
- **Proliferation threshold**: Bacteria density threshold necessary in order for phage to proliferate
- **Inundation threshold:** Minimum phage concentration that results in decline of bacteria burden
- Passive Therapy: Occurs when initial concentration of phage exceeds the bacterial concentration at the site of infection
- Active Therapy: Requires ongoing replication of phage to control bacteria concentration



Payne RJ, Jansen VA. J Theor Biol. 2001 Jan 7;208(1):37-48

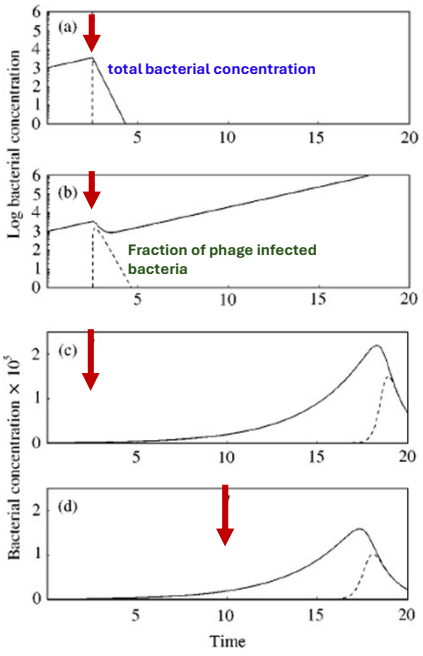
Schmalstig & Freidy. (2021) CPT

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Treatment Outcome Depends on Phage-Bacteria Dynamics, Not Just Dose

- **High phage doses** can eliminate bacterial populations through direct killing, even in the absence of significant phage replication (**a**).
- **Moderately lower initial phage doses** may produce only a temporary reduction in bacterial burden, resulting in a transient inundation effect without sustained control (**b**).
- **Low phage doses** can still achieve complete bacterial clearance when conditions support active phage replication, enabling amplification of the therapeutic effect over time (**c**).
- **Timing of administration is critical:** delaying phage inoculation can, in some cases, improve treatment efficacy. Comparison of **c** and **d** shows that identical doses and parameters lead to different outcomes solely due to the timing of phage delivery.



Payne RJ, Jansen VA. J Theor Biol. 2001 Jan 7;208(1):37-48

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The MIC problem: what do we even measure?

Measuring Phage Activity: A Fundamental Challenge

- **No direct equivalent to the MIC used for antibiotics**
  - Unlike antibiotics, phages cannot be characterized by a single susceptibility threshold because their effectiveness depends on biological interactions with the target bacteria.
- **Phage activity depends on multiple factors**
  - **Host range** (which bacterial strains can be infected) and **replication kinetics** (how efficiently the phage amplifies within the infection environment)
  - Testing in the **relevant biological matrix** (e.g., blood, sputum, wound fluid, biofilm)
- **Several complementary metrics are being explored**
  - **Efficiency of plating (EOP):** infectious titer of the phage suspension on the bacterial strain of interest/ infectious titer of the same phage suspension on a reference strain (usually the phage production strain)
  - **Killing/lysis curves:** measure the rate and extent of bacterial reduction over time
  - **Virulence index:** integrates infection dynamics into a single measure of phage potency
  - **Host-range matrices:** map susceptibility across diverse bacterial isolates to guide phage selection
- **Standardized susceptibility testing remains unresolved**
  - There is currently no universally accepted **"phagogram"** analogous to the antibiotic antibiogram
  - Variability in testing methods limits comparability between studies and treatment centers.

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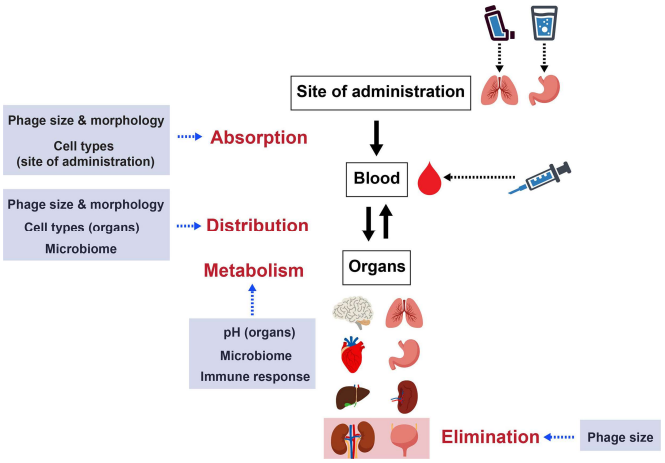
Reliable phage susceptibility testing is essential for:

- Selecting the most appropriate phage(s) for individual patients
- Defining rational dosing strategies
- Establishing eligibility criteria for clinical trials
- Comparing outcomes across studies and regulatory submissions

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What animal models can and cannot tell us

- Get insights into
  - Biodistribution
  - Comparison based on route of administration
  - Immune clearance, proof of density-dependence.
- Limited for: human immune dynamics, chronic/biofilm infections, strain-specific host range.
- Route matters enormously: IV vs inhaled vs topical vs oral give different exposure at the target site.

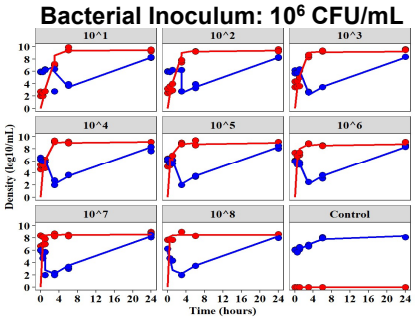


Sue C. Nang, Yu-Wei Lin, Aleksandra Petrovic Fabijan, Rachel Y.K. Chang, Gauri G. Rao, Jonathan Iredell, Hak-Kim Chan, Jian Li CMI, June 2023.

9

Is there a "dose" for the label ?

- **MOI is a laboratory construct**
  - Not a reliable clinical dosing metric
  - Bacterial burden at the infection site is unknown and changes over time.
- **Effective therapy appears to depend on delivering sufficient active phage titer** at the infection site
  - Potentially with repeated dosing to maintain activity above the proliferation threshold.
- **PhagoBurn used a 12-phage cocktail (PP1131)** designed to cover >90% of prescreened *P. aeruginosa* isolates
  - Need for broad strain coverage in cocktail design



- **Insufficient active phage titer at treatment initiation was a major contributor to treatment failure**
  - Dose reaching patients must match the intended therapeutic dose.
- Follow-up analyses identified **bacterial adaptation, phage evolution, and non-canonical phage-bacterium interactions** as additional factors that may influence clinical outcomes.
- **Polyclonal infections should be considered during phage selection**
  - Different bacterial subpopulations within the same wound may vary in phage susceptibility.

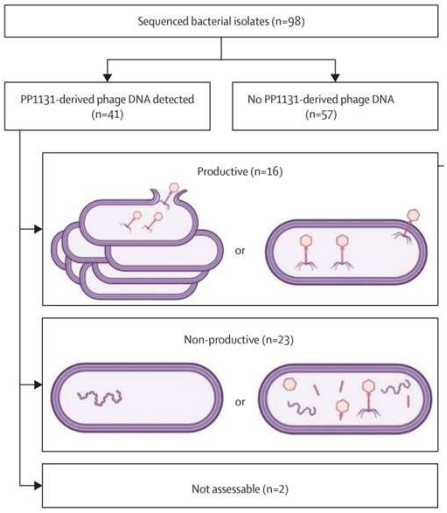
Jault P. et al. (PhagoBurn). Lancet Infect Dis. 2019 Jan;19(1):35-45.

Camille Boross, et al., The Lancet Microbe, 2026

10

Key Lessons from PhagoBurn for Phage Therapy Development

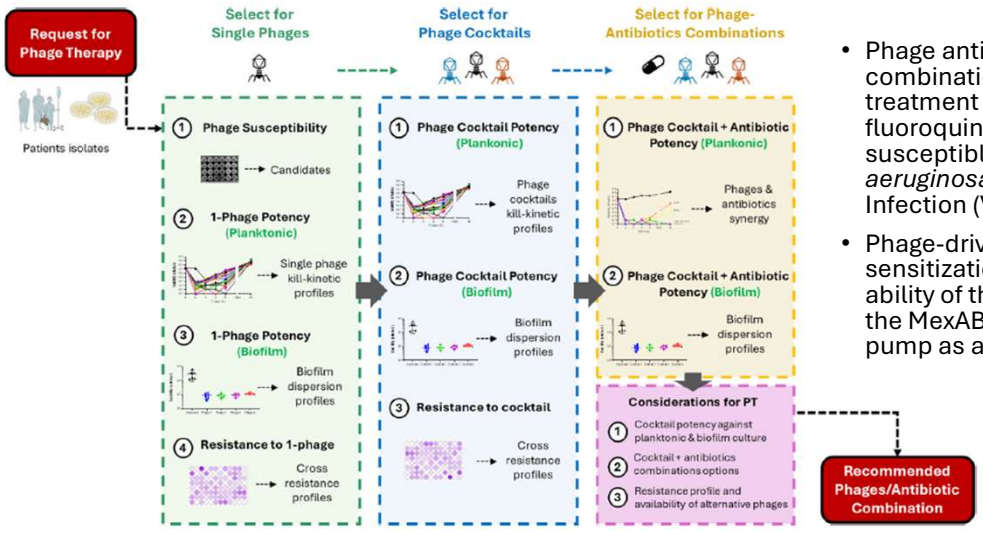
- **Baseline susceptibility of the infecting strain(s) is critical**; intermediate susceptibility or resistance can significantly reduce clinical efficacy even when an appropriate cocktail is used.
- **Phage cocktails require ongoing verification of composition and potency,** including monitoring for changes in phage populations that could affect activity.
- Follow-up analyses identified **bacterial adaptation, phage evolution, and non-canonical phage-bacterium interactions** as additional factors that may influence clinical outcomes.
- **Future trials should prioritize active titer, strain coverage, robust susceptibility testing, and monitoring of cocktail integrity** to maximize the likelihood of clinical success.



Jault P. et al. (PhagoBurn). Lancet Infect Dis. 2019 Jan;19(1):35-45.
Camille Boross, et al., The Lancet Microbe, 2026

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Phage Therapy Workflow for Phage Antibiotic Combination



- Phage antibiotic combination for the treatment of a refractory, fluoroquinolone non-susceptible *Pseudomonas aeruginosa* Vascular Graft Infection (VGI)
- Phage-driven antibiotic sensitization was due to the ability of their phages to use the MexAB-OprM efflux pump as a receptor.

Chung SJ, et al. Nat Commun. Jan 9;17(1):1385 (2026).

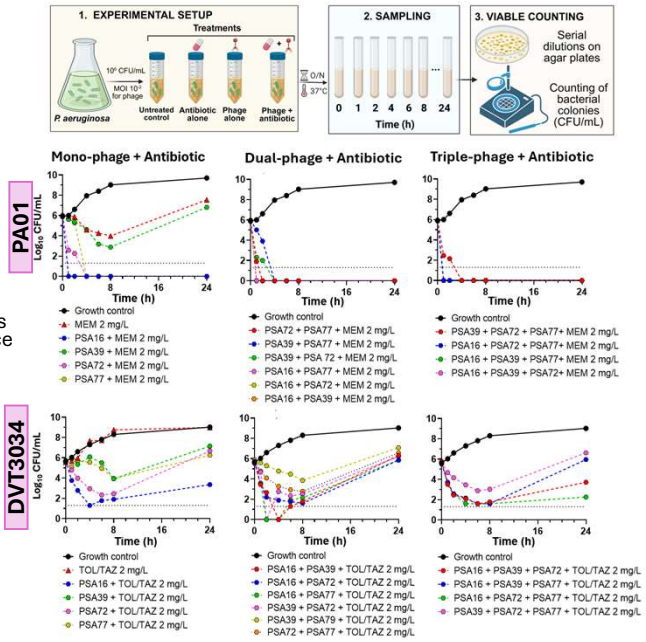
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Phage/Antibiotic Combination Strategies

- **Phage-antibiotic synergy (PAS):** Sub-MIC antibiotic exposure can enhance phage replication, bacterial killing, and biofilm disruption.
- **Treatment sequencing matters:** The timing and order of phage-antibiotic administration can influence outcomes; inappropriate sequencing may result in antagonism.
- **Evolutionary steering:** Phage-resistant mutants often exhibit reduced virulence or regain susceptibility to conventional antibiotics.
- **Combination therapy offers dual pressure:** Simultaneous targeting by phages and antibiotics can suppress resistance emergence and improve bacterial clearance.

Clinical Evidence: diSArm Trial (AP-SA02 + Best Available Therapy, BAT)

- **Faster infection resolution:** 2.7 vs. 9.3 days for placebo + BAT.
- **Shorter hospital stay:** 11.7 vs. 19.2 days.
- **Greater reduction in inflammation:** Day 12 CRP 50.2 vs. 97.3 mg/L.
- Supports the potential of phage-antibiotic combination therapy to improve outcomes in *S. aureus* bacteremia.

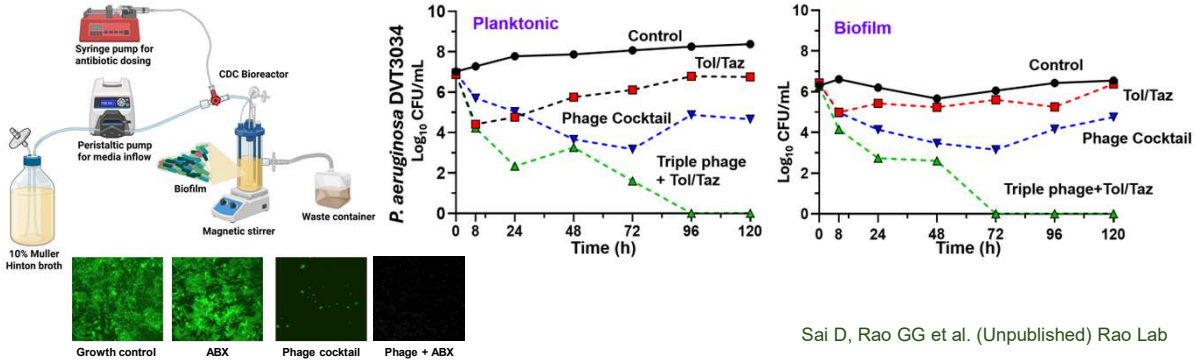


Sharma R et al. (Unpublished) Rao Lab
https://www.clinicaltrialsarena.com/analyst-comment/idweek-2025-ap-sa02-s-aureus-bacteremia/?cf-view

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Getting active phage to the target

- *A high titer that never reaches the target is not a dose.*
- Penetration to lung, bone, prosthetic surfaces and biofilm is the practical bottleneck.
- Biofilm and stationery-phase bacteria alter replication and access.

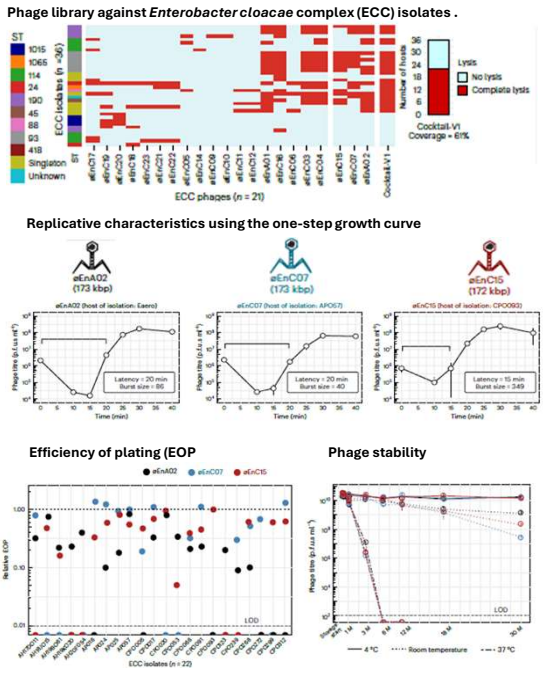


Sai D, Rao GG et al. (Unpublished) Rao Lab

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Resistance is expected — design for it

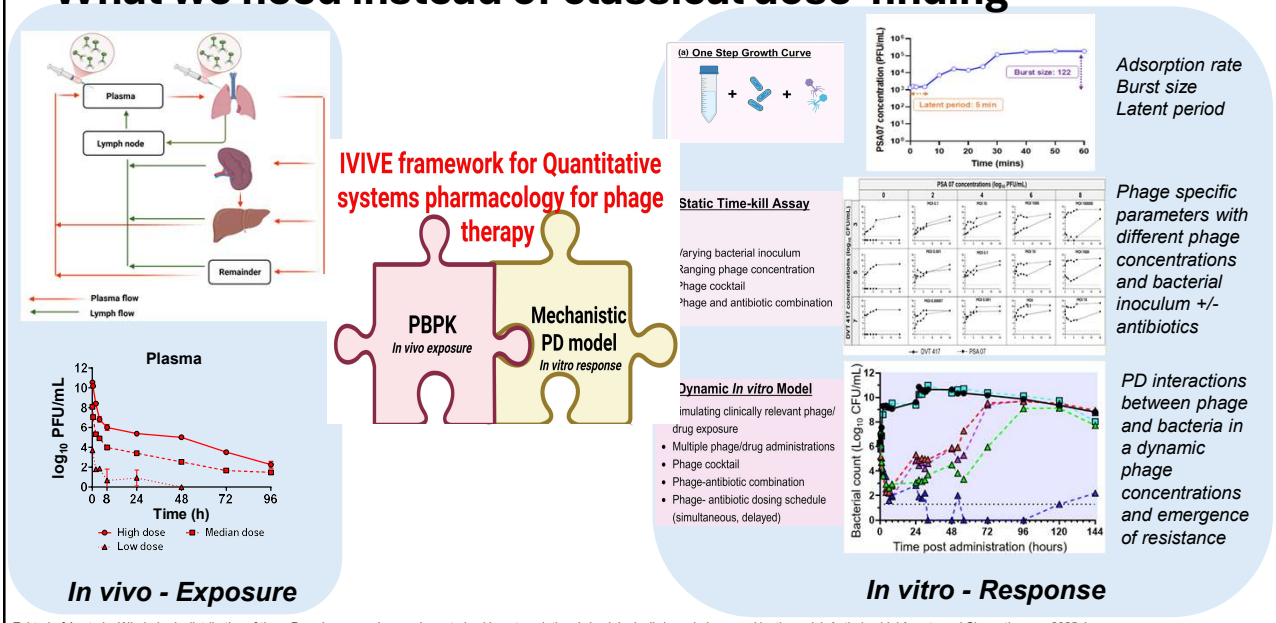
- **Maximize strain coverage** by combining phages with complementary host ranges.
- **Promote resistance suppression** by targeting diverse bacterial receptors and leveraging phage combinations.
- **Ensure safety** through sequencing and genome analyses to ensure that genes known to allow lysogeny , including integrases, recombinases, mobile genetic elements or genes encoding antibiotic resistance or toxicity are excluded.
- **Prioritize fitness and efficacy** based on replication kinetics, lytic activity, and antimicrobial performance.
- **Ensure developability** by selecting phages with robust stability during storage and handling.
- **Enhance potency through phage training** by exploiting the ability of phages to evolve and adapt to bacterial hosts.



Subedi, D., *et al. Nat Microbiol* **10**, 2702–2719 (2025).

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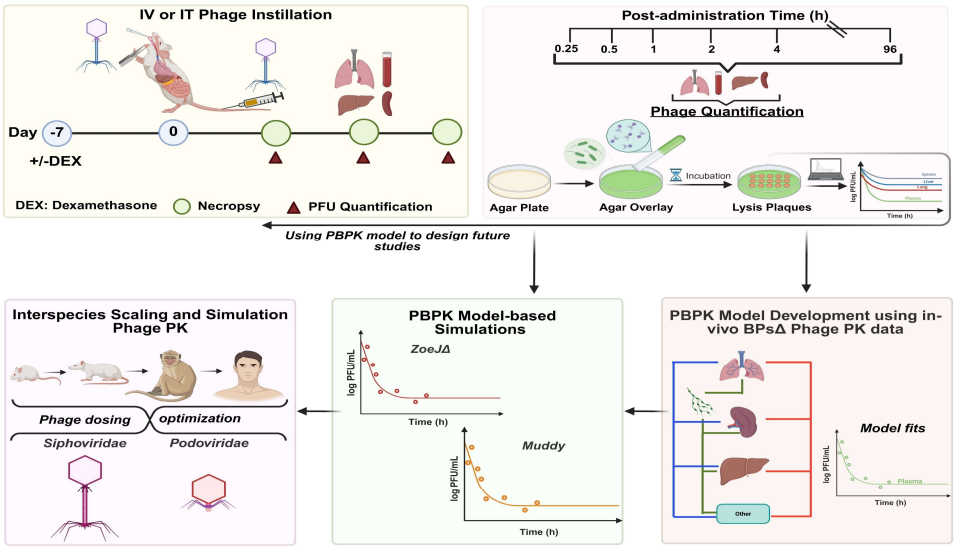
What we need instead of classical dose-finding



Echterhof A, et.al.,. Whole-body distribution of three Pseudomonas phages characterized by a translational physiologically based pharmacokinetic model. Antimicrobial Agents and Chemotherapy. 2025 Jan.
Sharma R, et.al.,. Pharmacokinetics and Physiologically Based Pharmacokinetic Modeling of Mycobacteriophages: Insights into Pulmonary Distribution and Clearance. bioRxiv. 2026:2026-01.
Rao GG, et.al.,. Crossing the chasm: how to approach translational pharmacokinetic–pharmacodynamic modeling of phage dosing. Clinical Pharmacology & Therapeutics. 2025 Jan;117(1):94-105.
Rostami-Hodjegan A. Physiologically based pharmacokinetics joined with in vitro–in vivo extrapolation of ADME: a marriage under the arch of systems pharmacology. Clinical Pharmacology & Therapeutics. 2012 Jul;92(1):50-61.

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In vivo PK/PD studies –Model Development & Validation





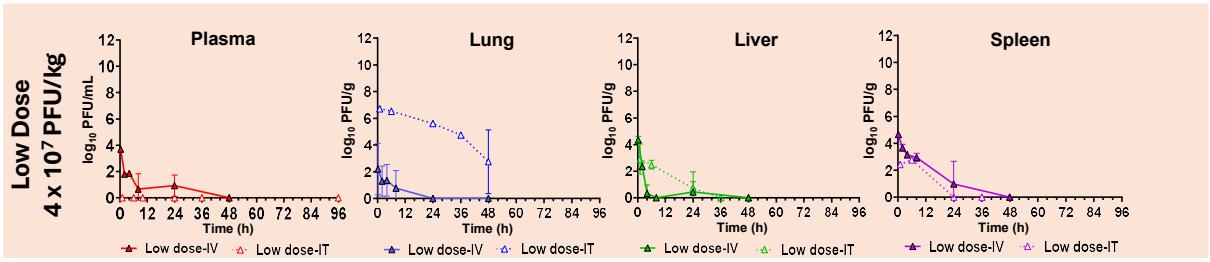


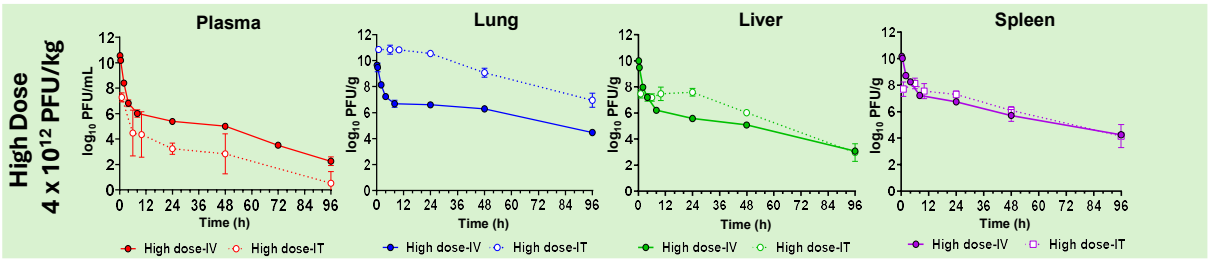


Sharma R, et.al.,. Pharmacokinetics and Physiologically Based Pharmacokinetic Modeling of Mycobacteriophages: Insights into Pulmonary Distribution and Clearance

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In vivo PK of single dose of BPsΔ (*Siphoviridae*) phage via IV and IT routes

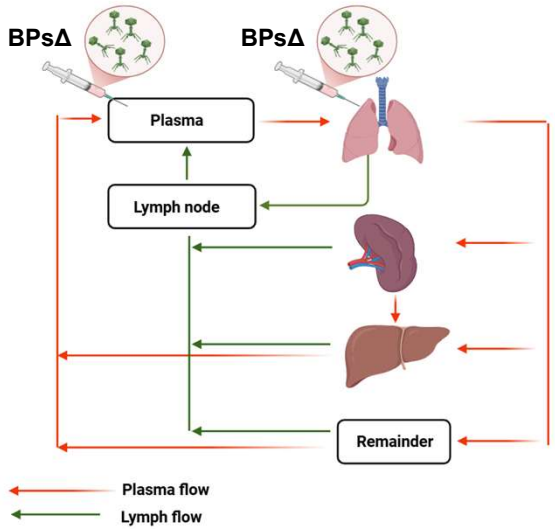




Sharma R, et.al.,. Pharmacokinetics and Physiologically Based Pharmacokinetic Modeling of Mycobacteriophages: Insights into Pulmonary Distribution and Clearance

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Modeling Phage Pharmacokinetics (PK)

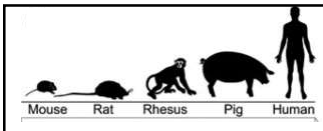


Physiologically based PK (PBPK) Model

- The PBPK model structure includes compartments representing organs relevant to phage distribution, connected by blood (plasma) flow.
- Each compartment is assigned a physiological organ volume.
- Organ volumes and plasma flow rates for mice were obtained from published literature.
- Phage levels in lymph nodes were quantified – supporting the potential role of lymphatic system in phage distribution.

Sharma R, et.al.,. Pharmacokinetics and Physiologically Based Pharmacokinetic Modeling of Mycobacteriophages: Insights into Pulmonary Distribution and Clearance. bioRxiv. 2026:2026-01.

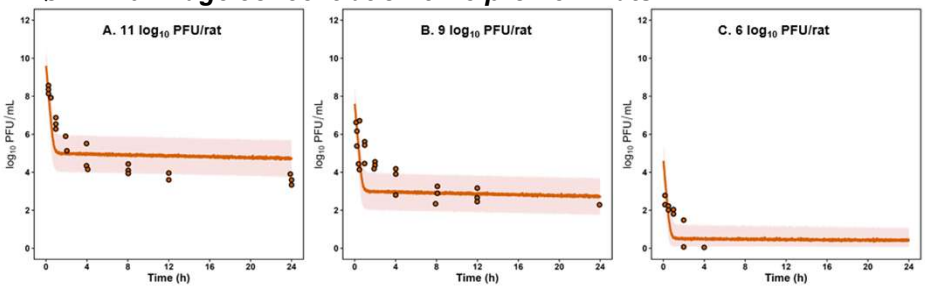
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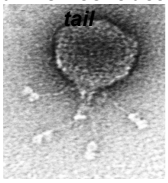
Allometric scaling to other species

- Species specific physiological parameters
- Allometric scaling of plasma clearance (Cl_P).
- Accounted phage family specific parameter optimization.

øPEV20 Phage concentration-time profile in rats



Podoviridae
small non-contractile tail



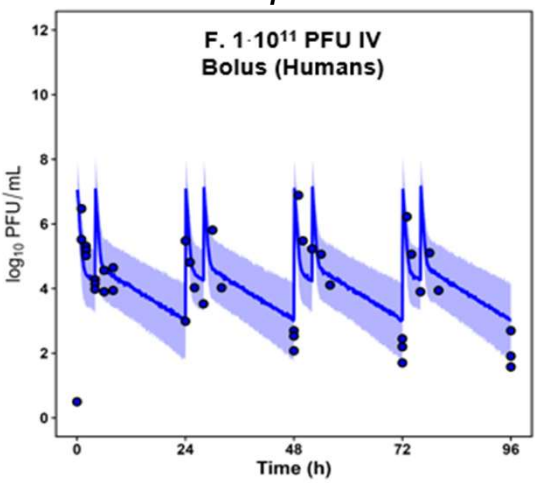
- Podoviridae: associated with higher cellular uptake (i.e, intracellular concentrations)
- Faster plasma clearance compared to siphophages.

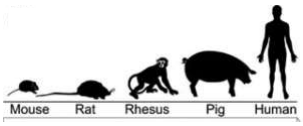
Lin, Y. *et al. Clinical Microbiology and Infection* **26**, 1229-1235 (2020)
Sharma R, et.al.,. Pharmacokinetics and Physiologically Based Pharmacokinetic Modeling of Mycobacteriophages: Insights into Pulmonary Distribution and Clearance. bioRxiv. 2026:2026-01.
Phage images are representative examples of each family from the literature and are not the specific phages investigated in this work.

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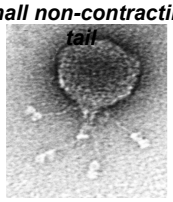
Allometric scaling: Characterizing Phage PK in Humans

LBP-EC01 Phage concentration versus time profile





Podoviridae
small non-contractile tail



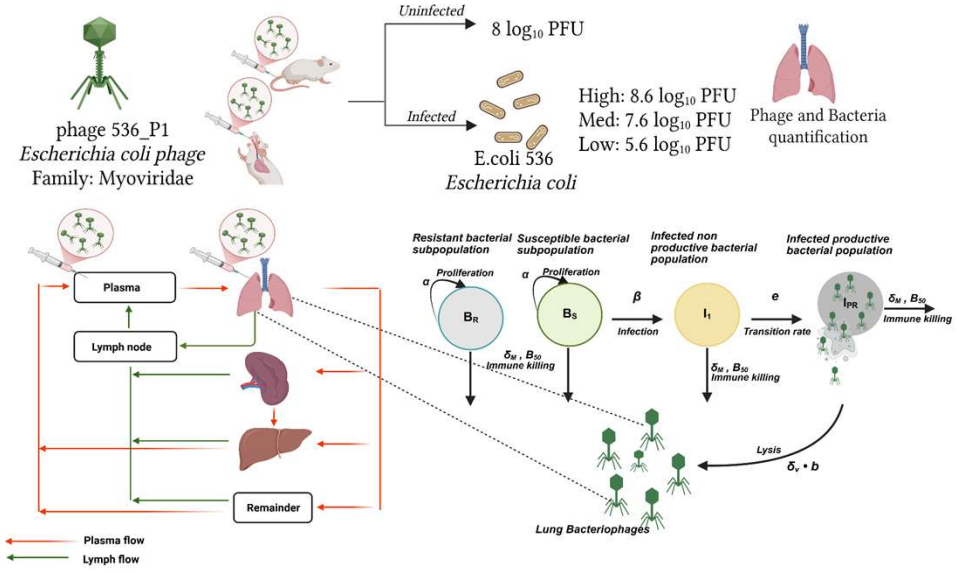
Kim, P. *et al. The Lancet Infectious Diseases* **24**, 1319-1332 (2024).

Sharma R, et.al.,. Pharmacokinetics and Physiologically Based Pharmacokinetic Modeling of Mycobacteriophages: Insights into Pulmonary Distribution and Clearance. bioRxiv. 2026:2026-01.

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21

Utility of the PBPK-PD Model Framework

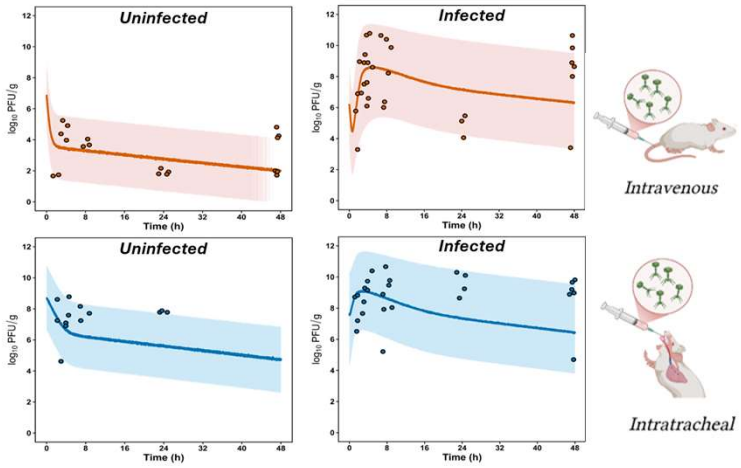


Delattre R, et.al.. Combination of in vivo phage therapy data with in silico model highlights key parameters for pneumonia treatment efficacy. Cell Reports. 2022 May 17;39(7).

Mahadevan R, et.al., In Vitro–In Vivo Extrapolation (IVIVE) of Phage–Bacteria Dynamics Using an Integrated PBPK–PD Modeling Framework . Under review

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Predictive Performance of the PK-PD Model



Delattre R, et.al.. Combination of in vivo phage therapy data with in silico model highlights key parameters for pneumonia treatment efficacy. Cell Reports. 2022 May 17;39(7).
Mahadevan R, et.al., In Vitro–In Vivo Extrapolation (IVIVE) of Phage–Bacteria Dynamics Using an Integrated PBPK–PD Modeling Framework . Under review

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Thank You



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Sara Maloney, RTI Inc.
Jian Li, Monash University
Andrea Kwa, Singapore General Hospital

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