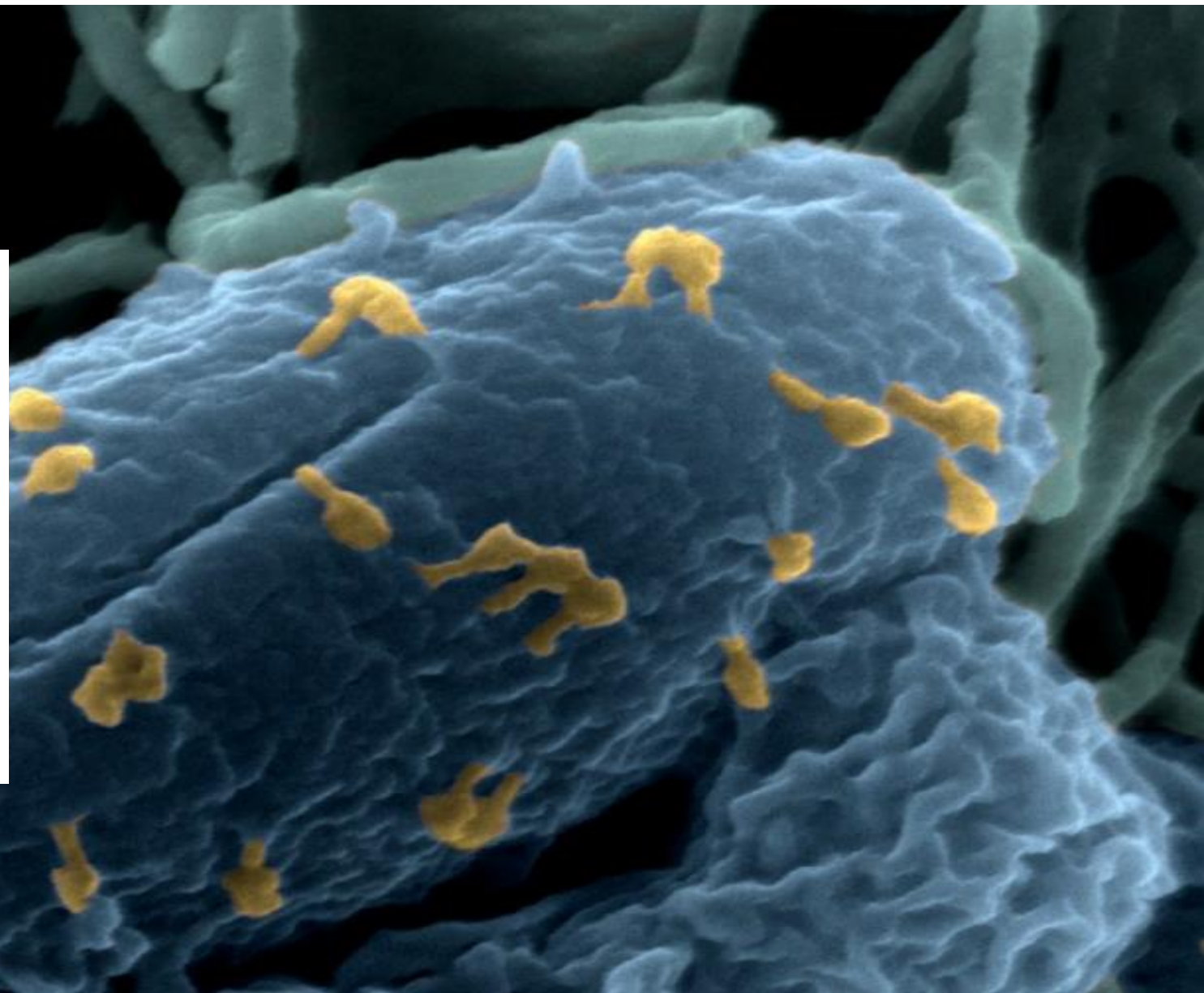


# Phagothérapie, Principes et Applications

**Laurent Debarbieux**

Bacteriophage, Bacterium, Host Laboratory





# What is Phage Therapy?

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## *The use of bacteriophages to kill pathogenic bacteria*

**Date:** 1920's

**Father:** Félix d'Herelle (1873-1949)



**Location:** Institut Pasteur, Paris, France

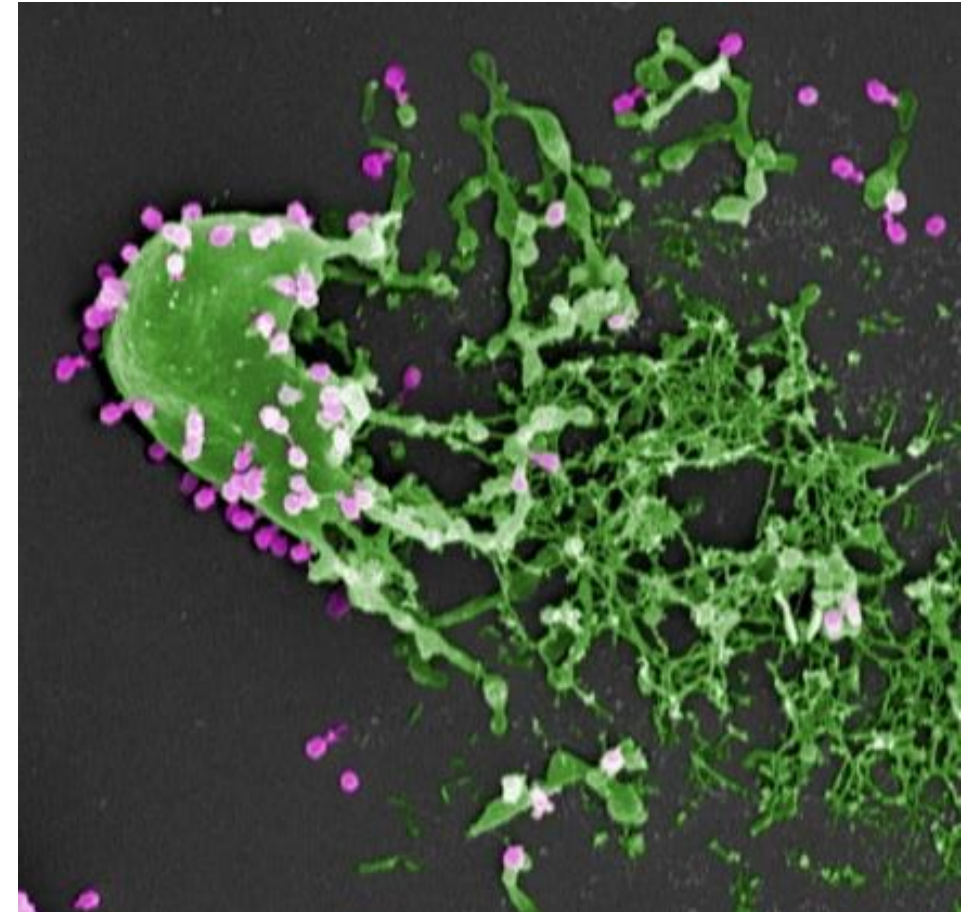
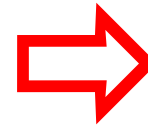
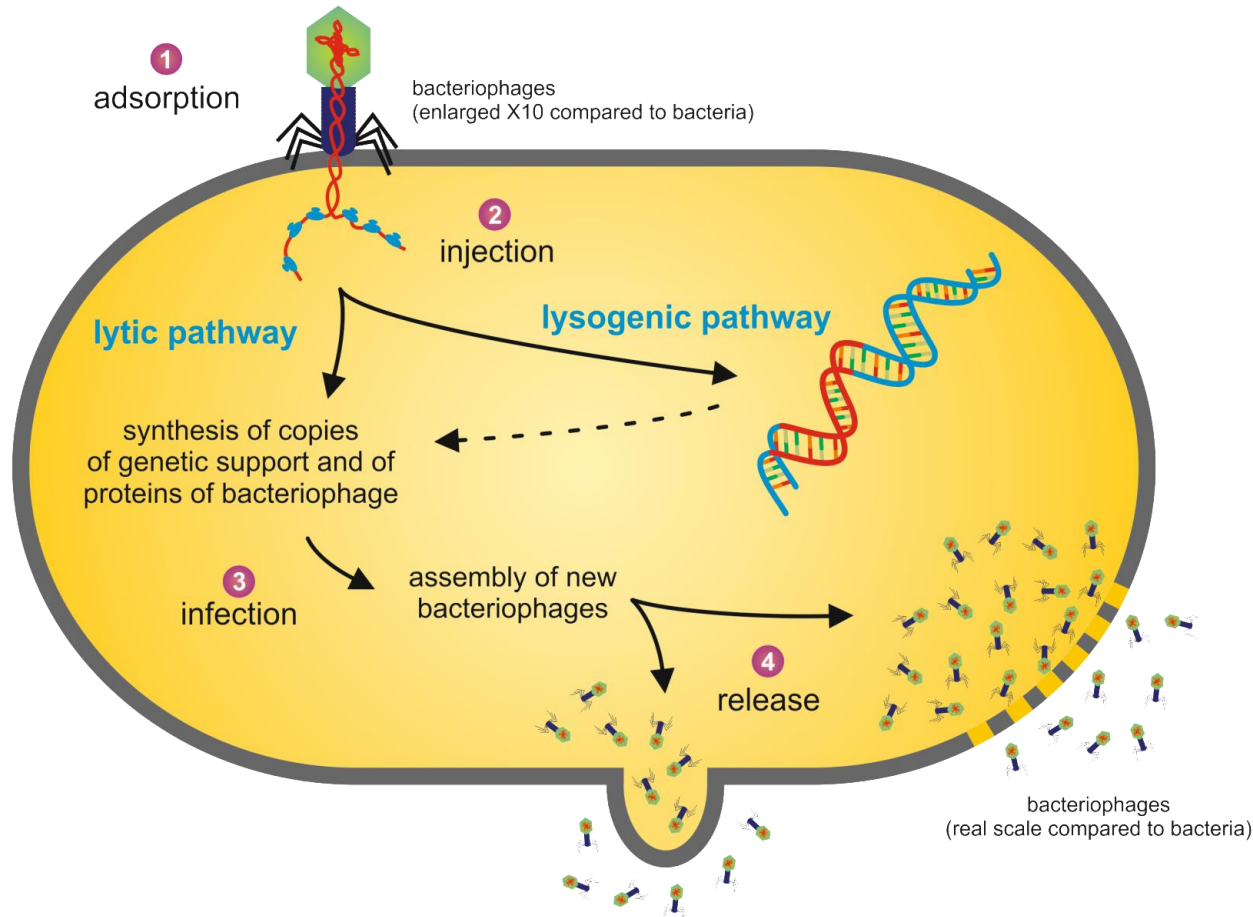
**Particular signs:** first specific antibacterial treatment

**1920's-1940's:** world expansion (Brazil, Egypt, Georgia,...)

**1950's-1990's:** world decline (except Eastern Europe),  
and paradoxically, resistance to antibiotics increased !



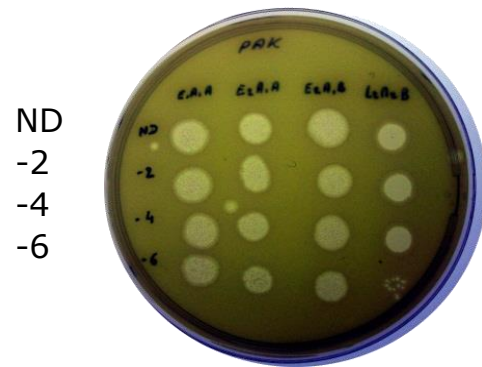
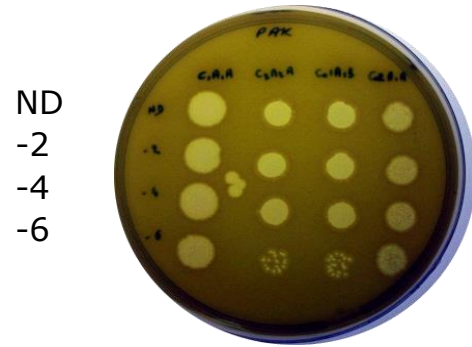
# Principle: the only antibacterial that self-amplifies



# Bacteriophages are highly specific

PAK strain

O:6



# Bacteriophages are highly specific

PAK strain

O:6

clinical isolates (serotypes)

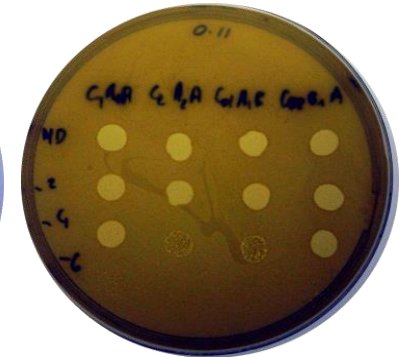
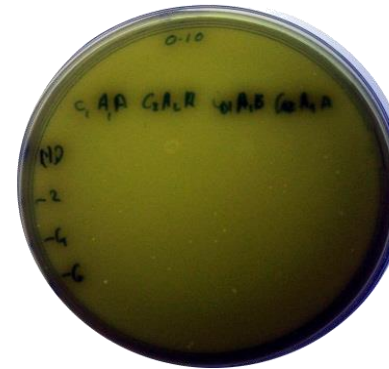
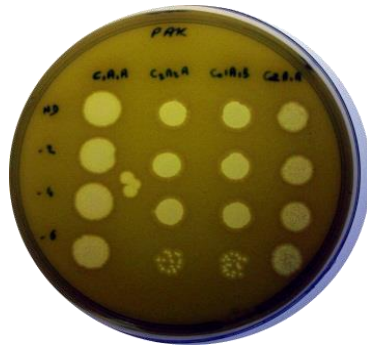
O:4

O:9

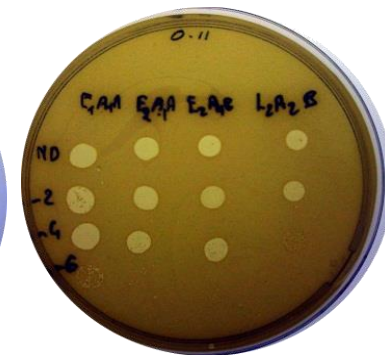
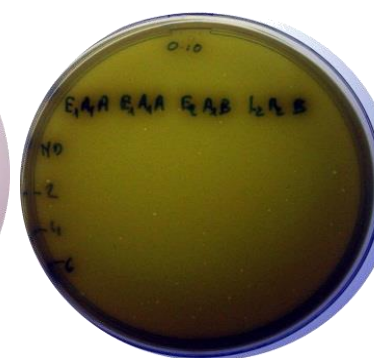
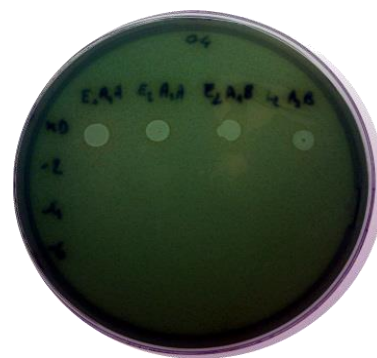
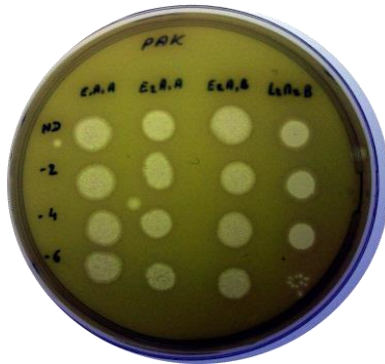
O:10

O:11

ND  
-2  
-4  
-6

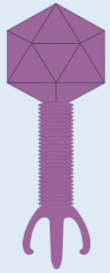


ND  
-2  
-4  
-6



# Simple concept but complex application

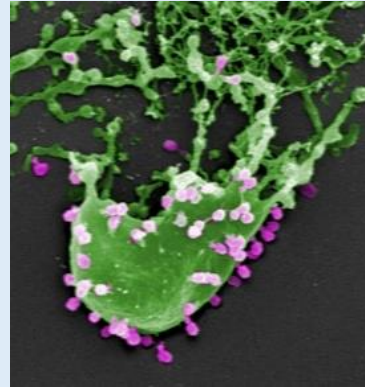
in the laboratory



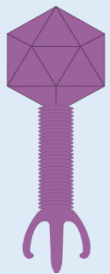
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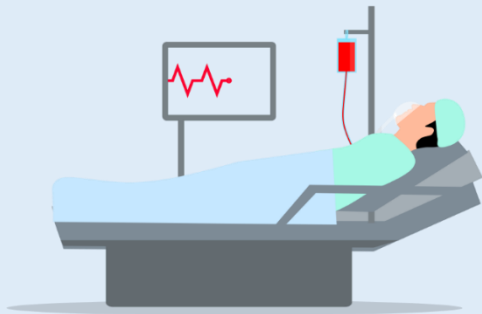
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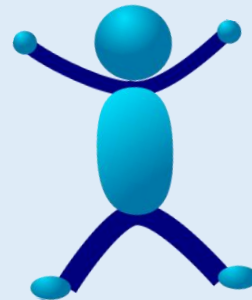
in the hospital



+



?



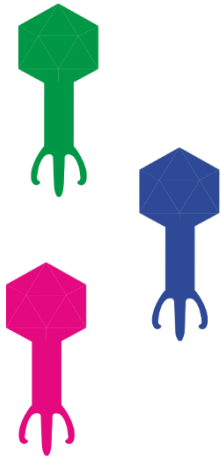
**Choice  
Combination  
Production  
Purification  
Dose  
Route  
and  
Regulations !!**

?



# Translation requires experimental models

**Bacteriophages**



+

**Bacteria**



*Pseudomonas aeruginosa*  
*Escherichia coli*

+

**Host**



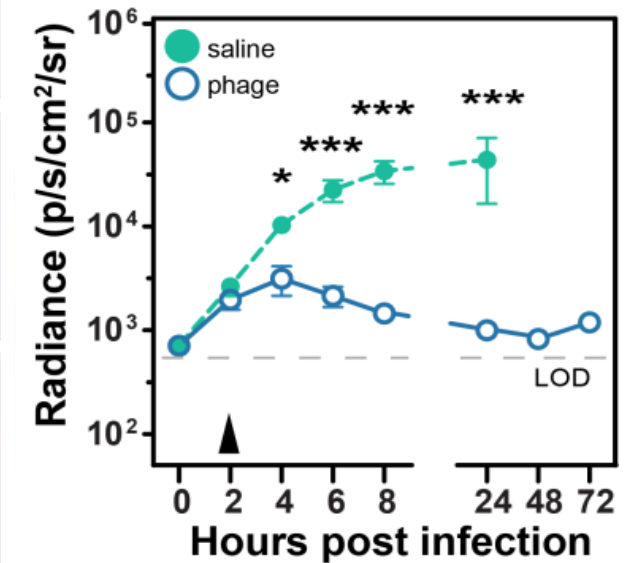
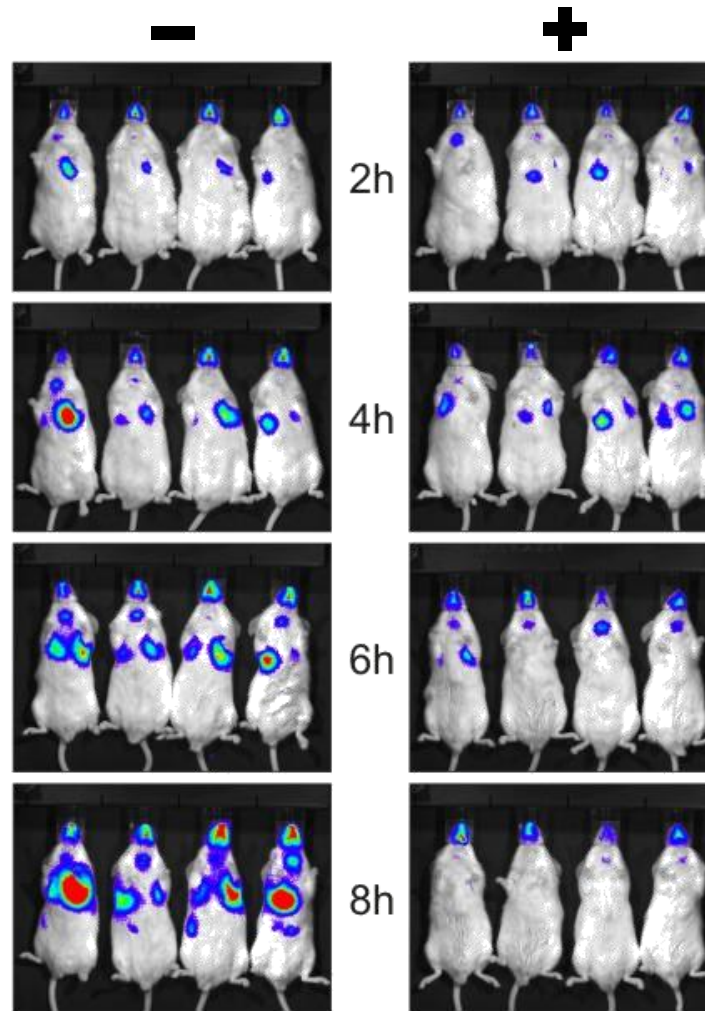
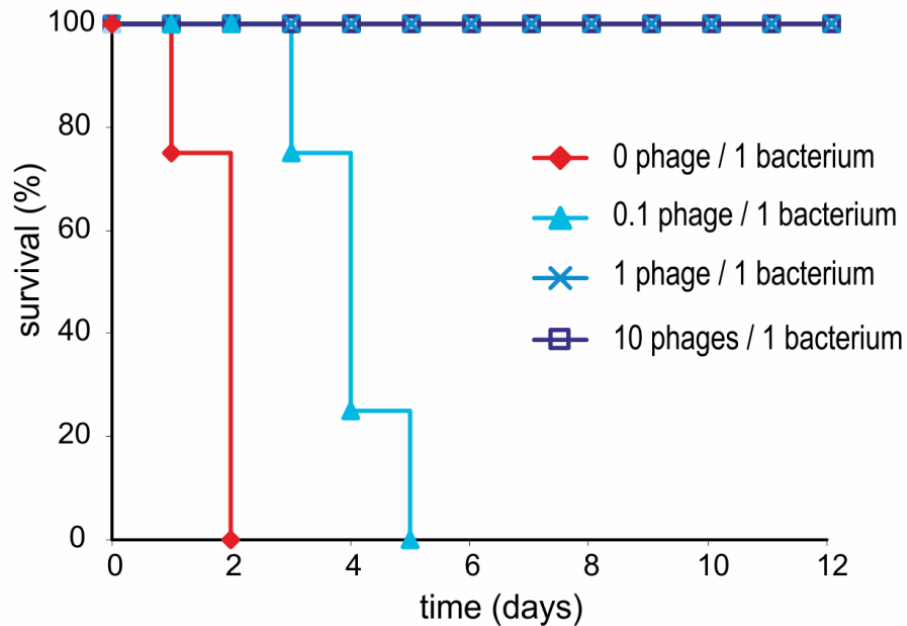
Our aim is to characterize **tripartite interactions**  
to identify factors that govern efficacy



# Assessment of the *in vivo* efficacy of bacteriophages

*Pseudomonas aeruginosa* strain **PAK** and its bacteriophage **PAK\_P1**.

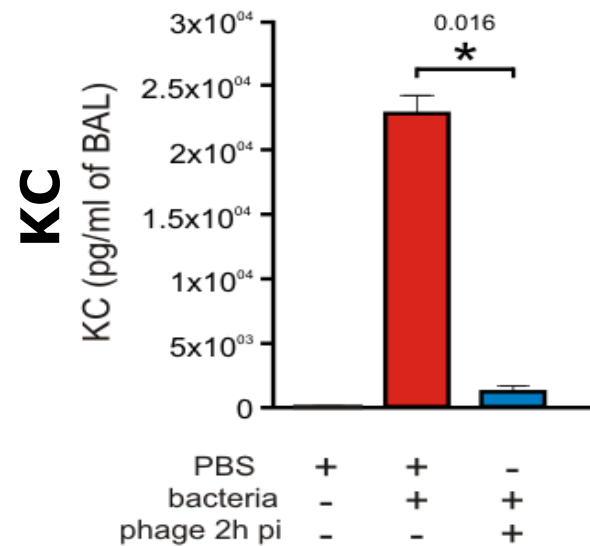
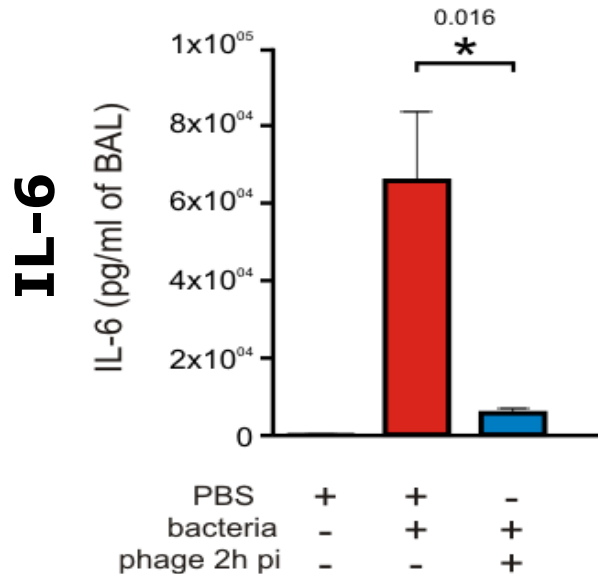
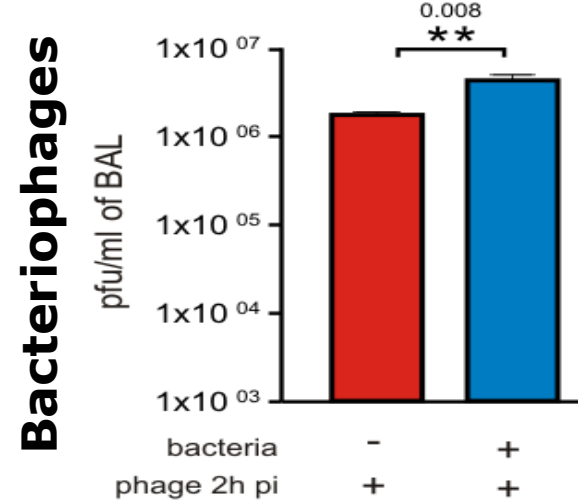
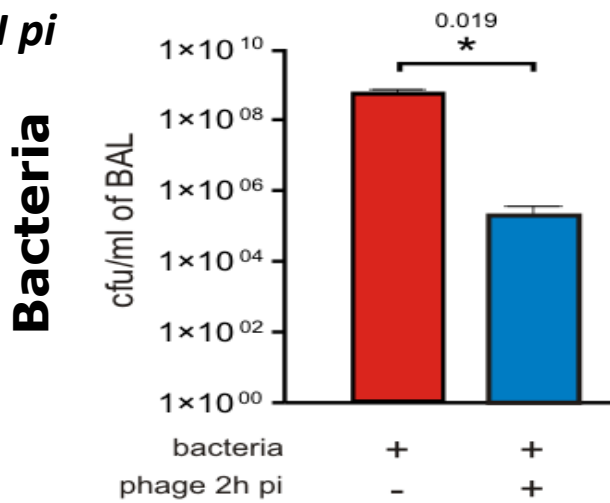
Infection at  $t=0$  and treatment at  $t=2h$



Debarbieux et al., *J. Infect. Dis.* 2010

# Quantitative data confirm indirect bioluminescent observations

Lung content at 20H pi



Bacteria ↘

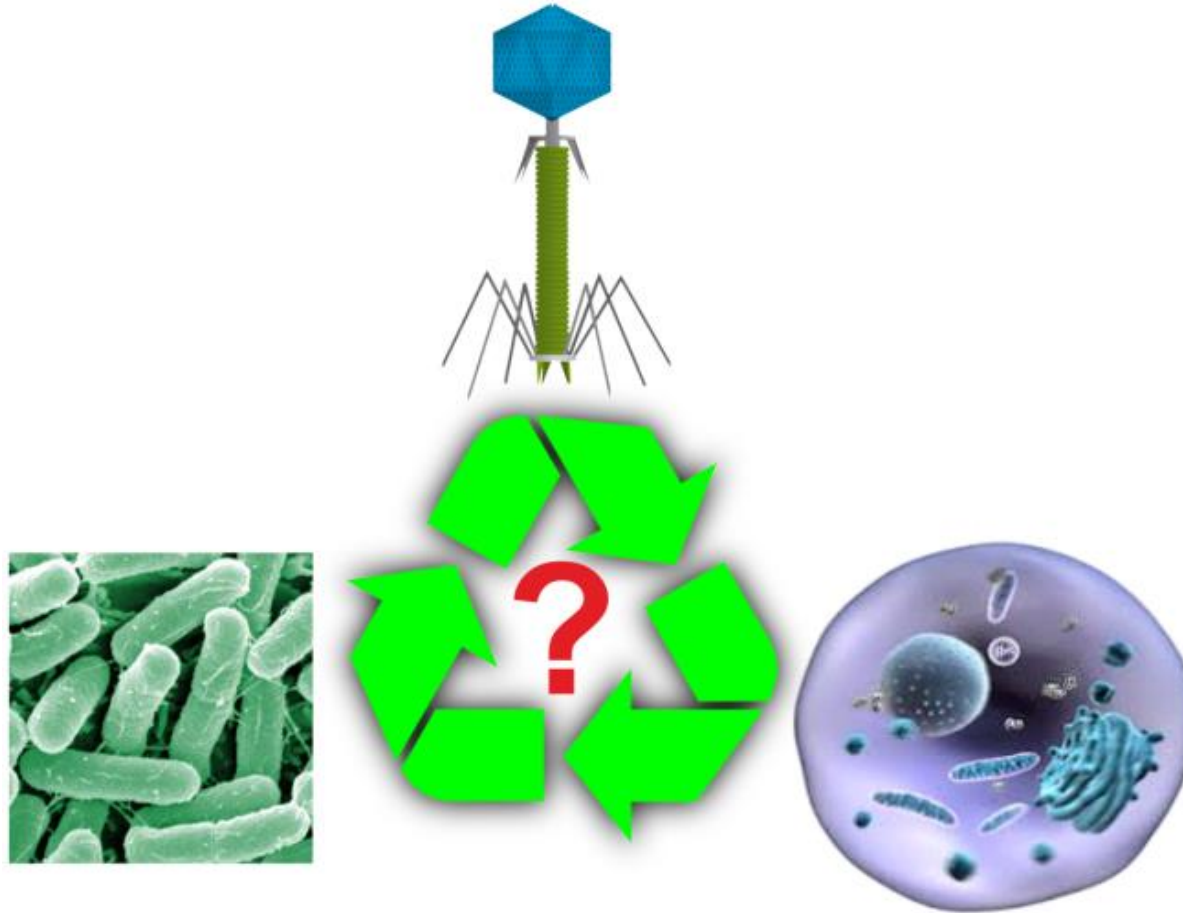
Bacteriophages ↗

Inflammation ↘

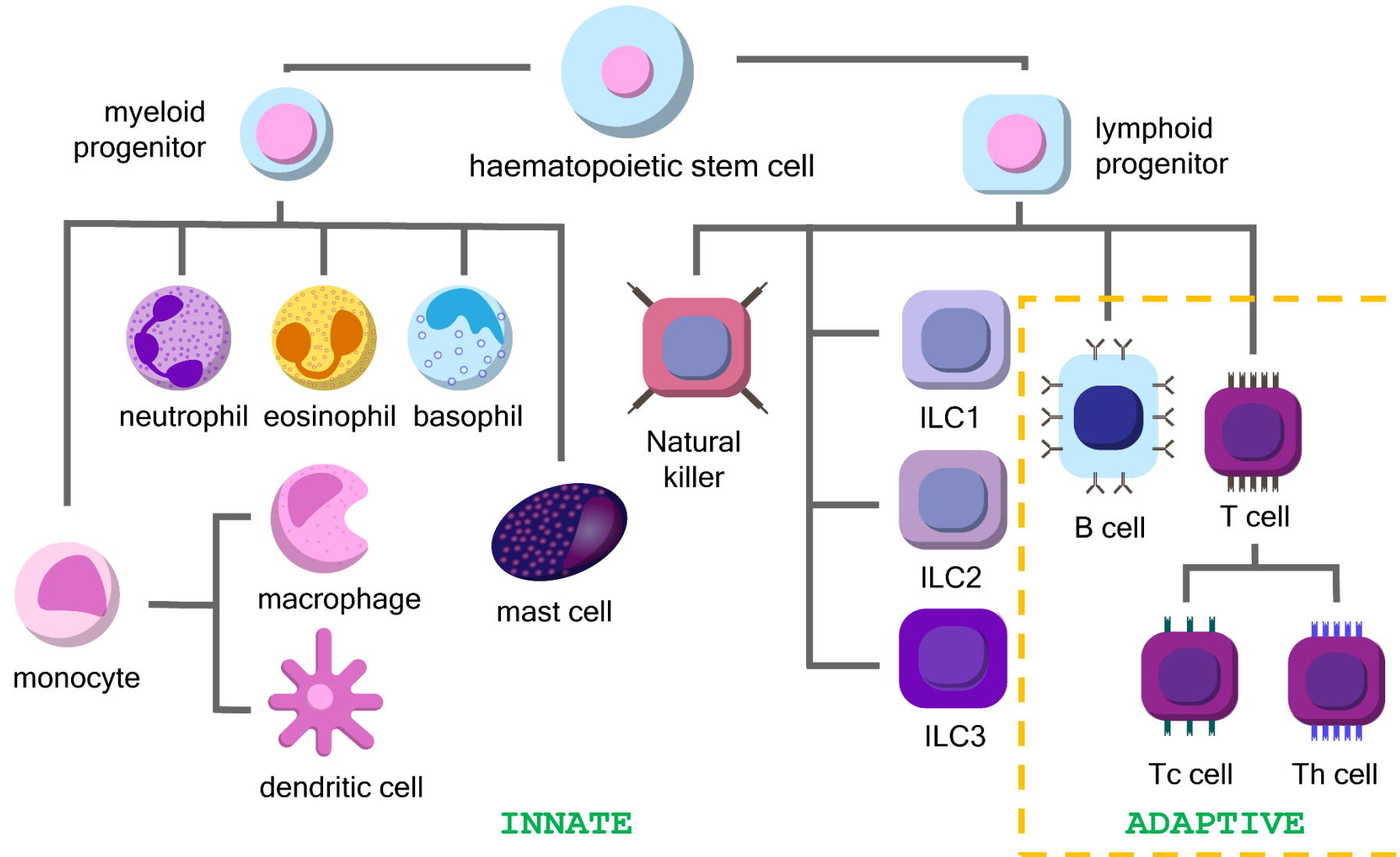
Survival ↗

# What is the role of the host ?

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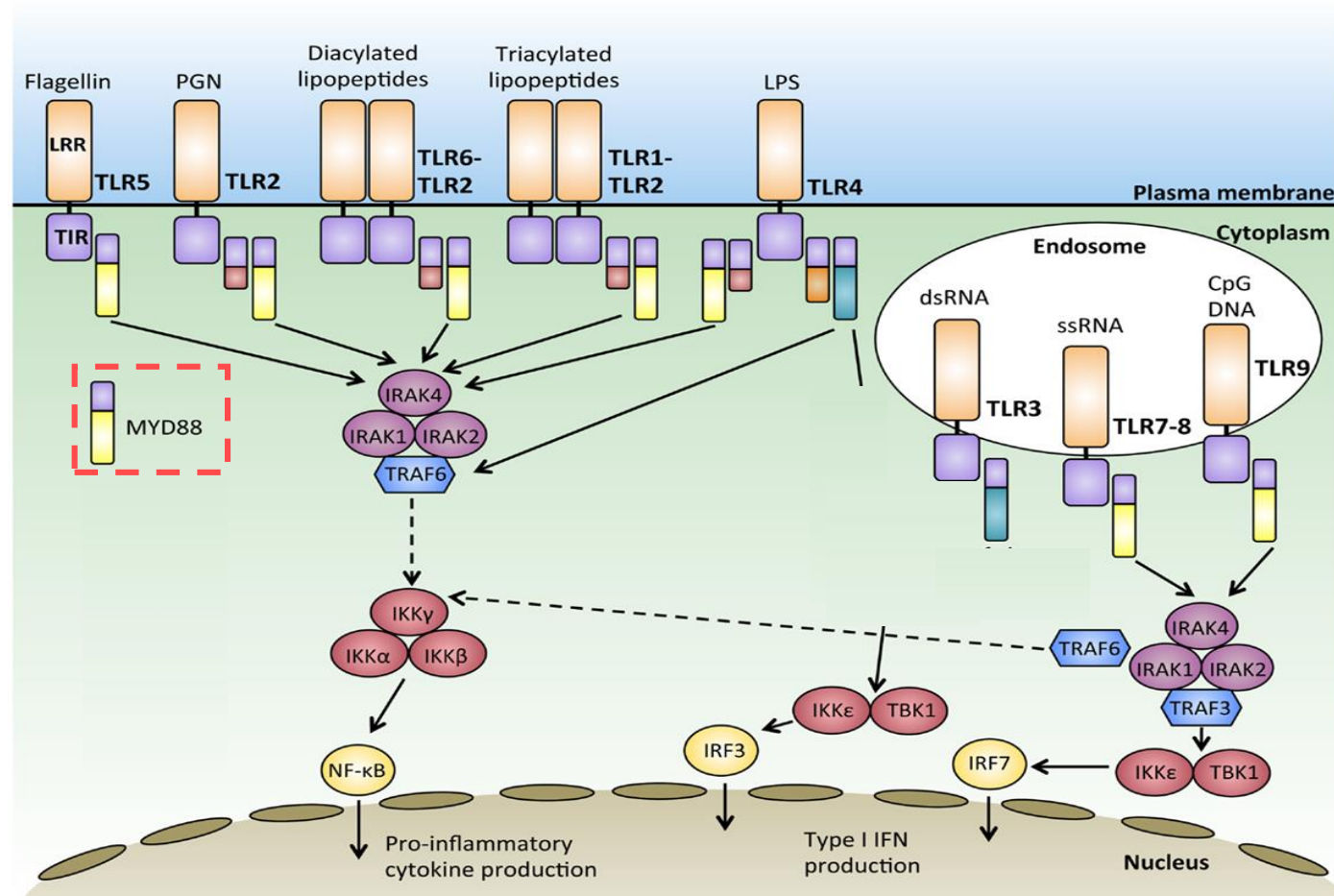


# Is the immune system involved in phage therapy efficacy ?

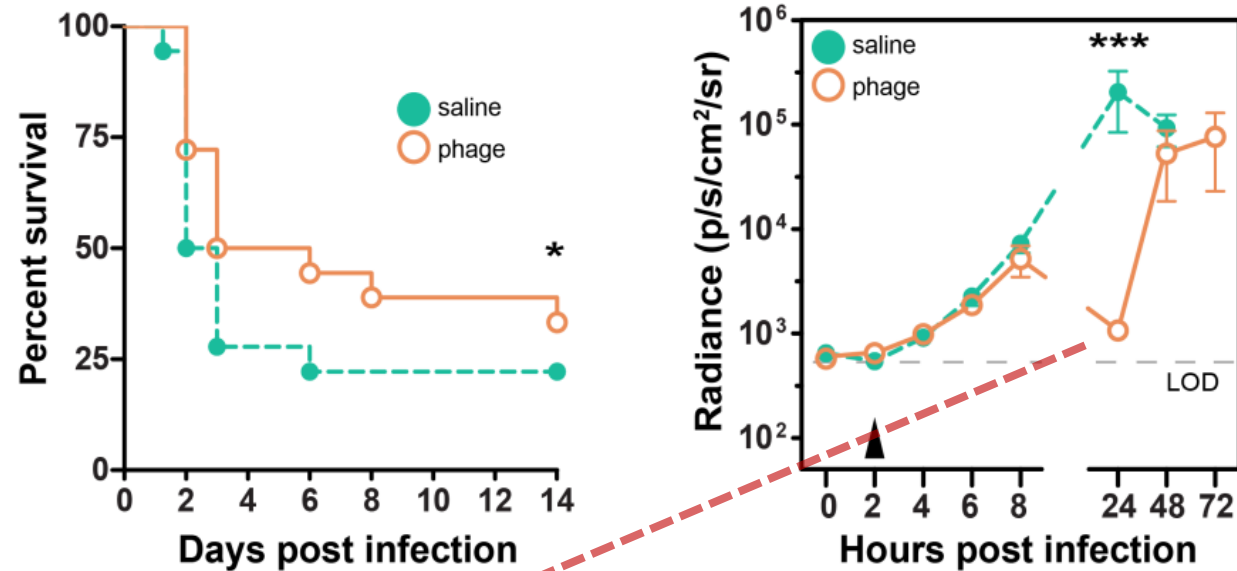


# MyD88 is an essential protein in signaling pathogens

MyD88 is a Toll-like receptor signaling molecule for innate immune cell activation and recruitment

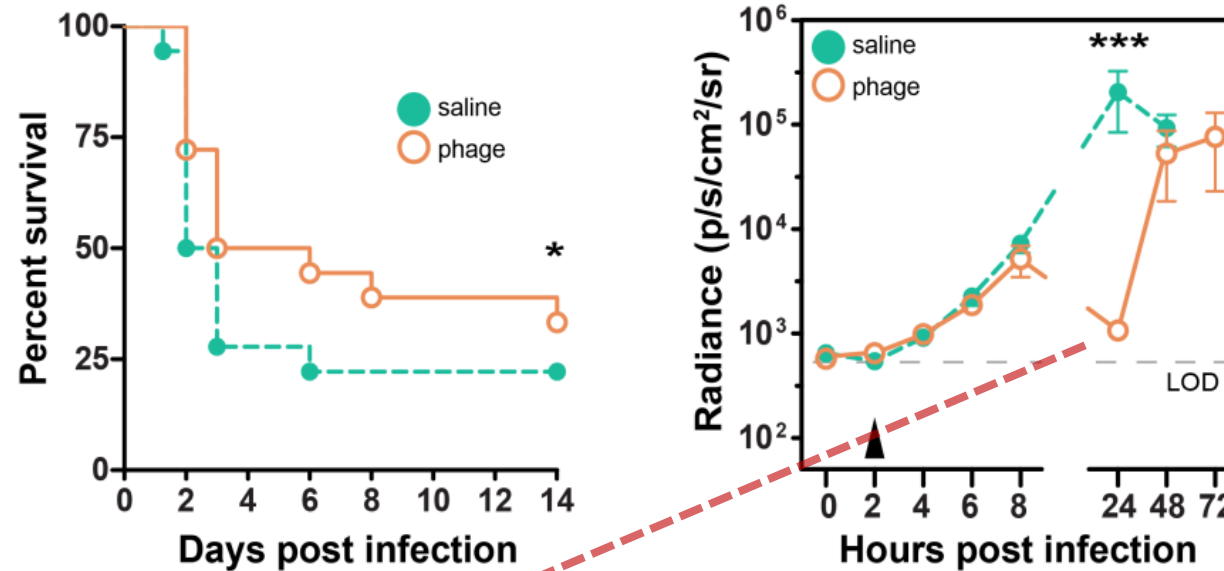


# Phage therapy efficacy in MyD88<sup>-/-</sup> mice ?



24 h p.i., reduction of bacteria load = **phages do their job!**

# Phage therapy efficacy in MyD88<sup>-/-</sup> mice ?



24 h p.i., reduction of bacteria load = **phages do their job!**

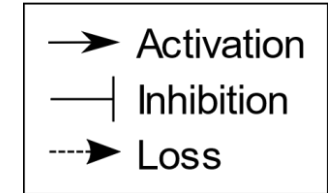
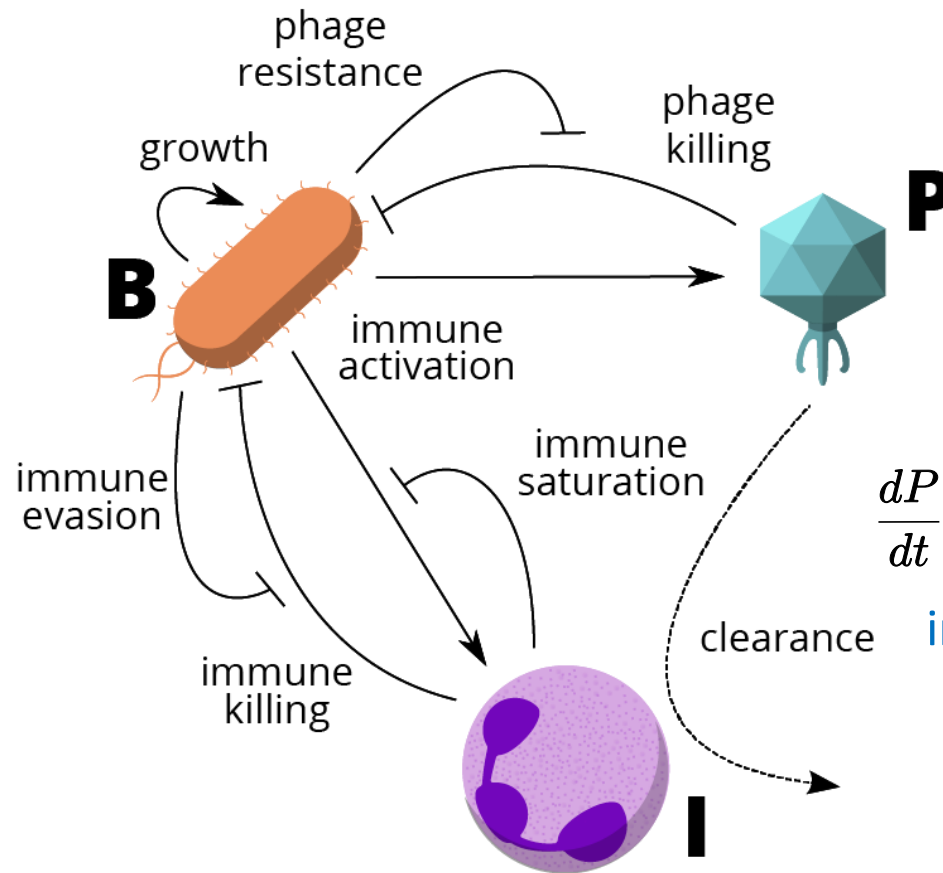
**72 h p.i., failure** = growth of phage-resistant bacteria

# Developing an *in silico* model of phage therapy

$$\frac{dB}{dt} = \overbrace{rB \left(1 - \frac{B}{K_C}\right)}^{\text{Growth}} - \overbrace{\frac{\epsilon IB}{1 + B/K_D}}^{\text{Immune killing}} - \overbrace{SF(P)}^{\text{Lysis}} + \overbrace{\mu rS \left(1 - \frac{S+R}{K_C}\right)}^{\text{Mutation}}$$

bacteria can develop resistance to phage

bacteria can evade the immune response



$$\frac{dI}{dt} = \overbrace{\alpha I \left(1 - \frac{I}{K_I}\right) \left(\frac{B}{B + K_N}\right)}^{\text{Immune stimulation}}$$

$$\frac{dP}{dt} = \overbrace{\beta \phi B P}^{\text{Viral release}} - \overbrace{\omega P}^{\text{Decay}}$$

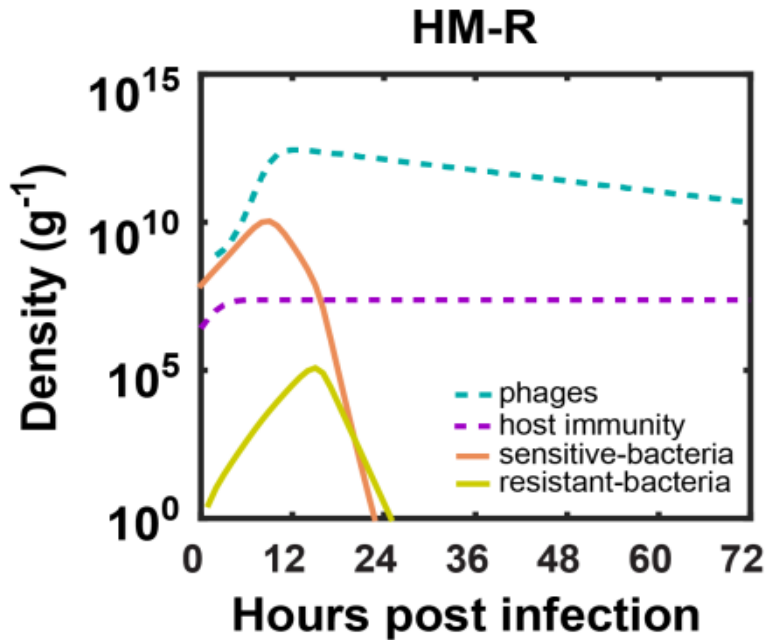
immune cell response to phages ??

Immune stimulation has a maximum "carrying capacity"

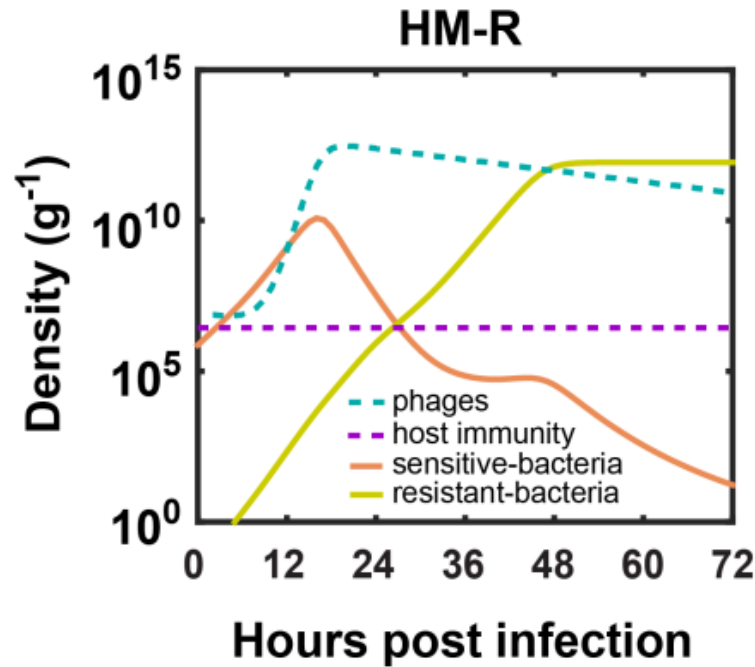


# Modelling phage therapy

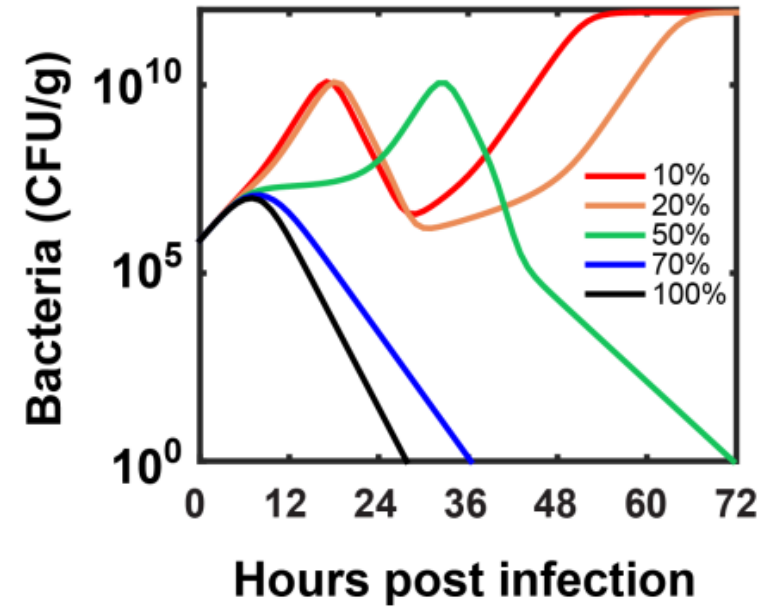
## WT mice



## MyD88<sup>-/-</sup> mice



## Simulations

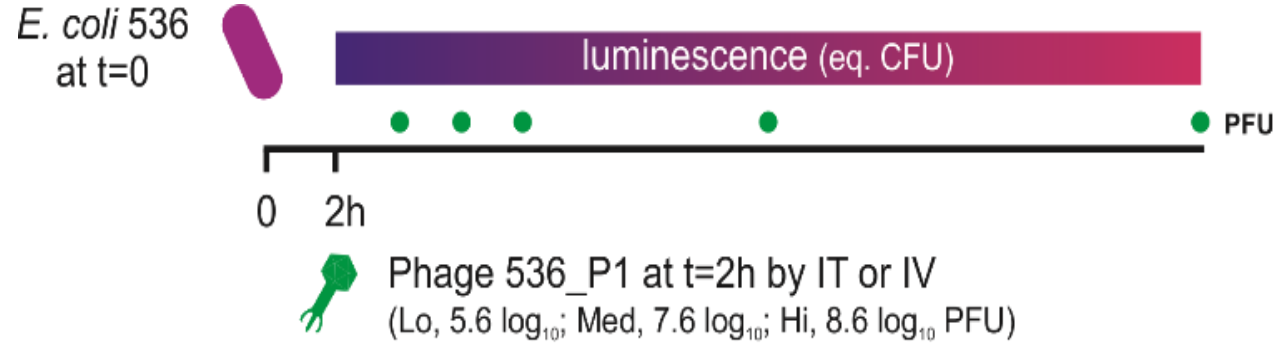


“Immunophage synergy”: the immune response (via neutrophils)

is essential to control **phage-resistant bacteria**

# Towards a pharmacometric model

Infection by IT



Frequent luminescence recording

Pulmonary PFU and CFU counts

uninfected and treated

IT (8 log<sub>10</sub> PFU; n=16)

IV (8 log<sub>10</sub> PFU; n=37)

infected and untreated

(7.6 log<sub>10</sub> CFU; n=45)



infected and treated

IT Hi (n=10)

IT Med (n=25)

IT Lo (n=24)

IV Med (n=29)

IV Lo (n=50)

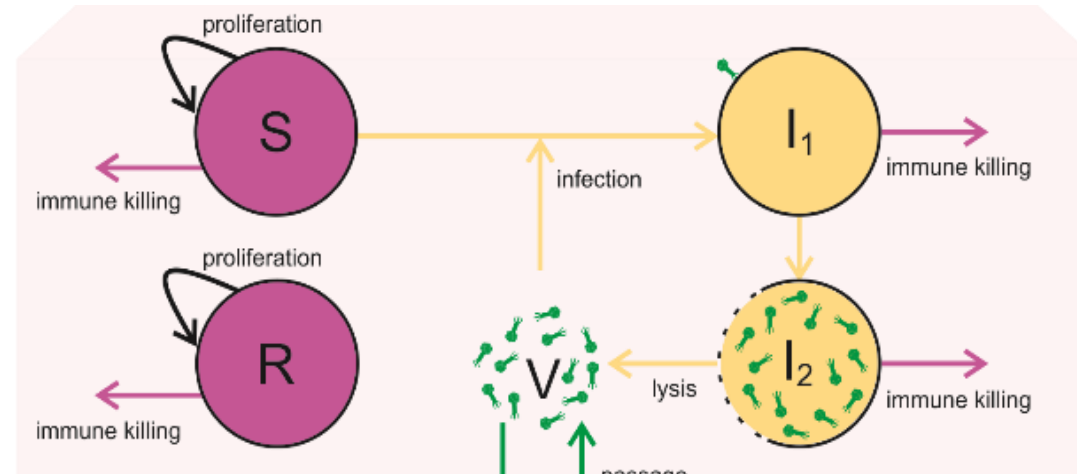


J r mie Guedj  
J r my Seurat

# Defining several compartments to build a model

S: susceptible bacteria

R: refractory bacteria  
(resistant + inaccessible)

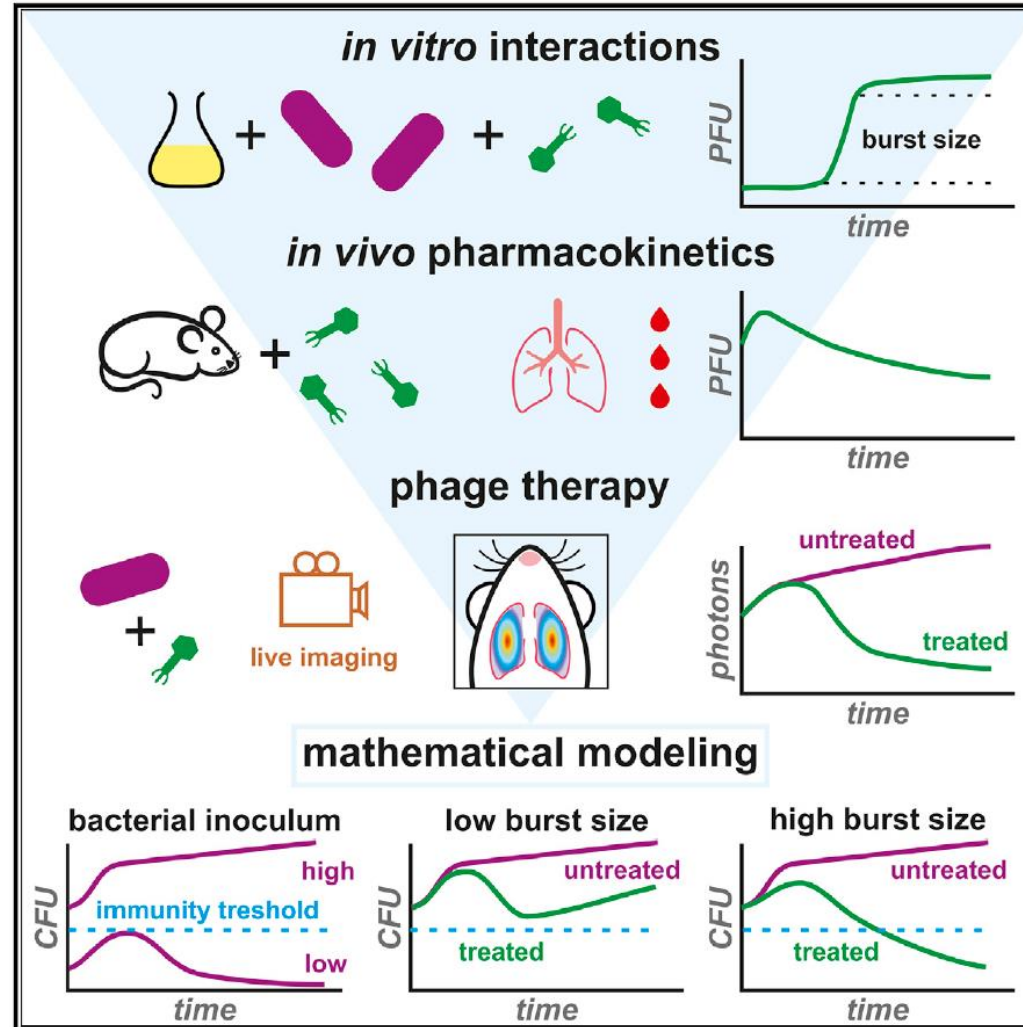


I<sub>1</sub>: infected  
and latent

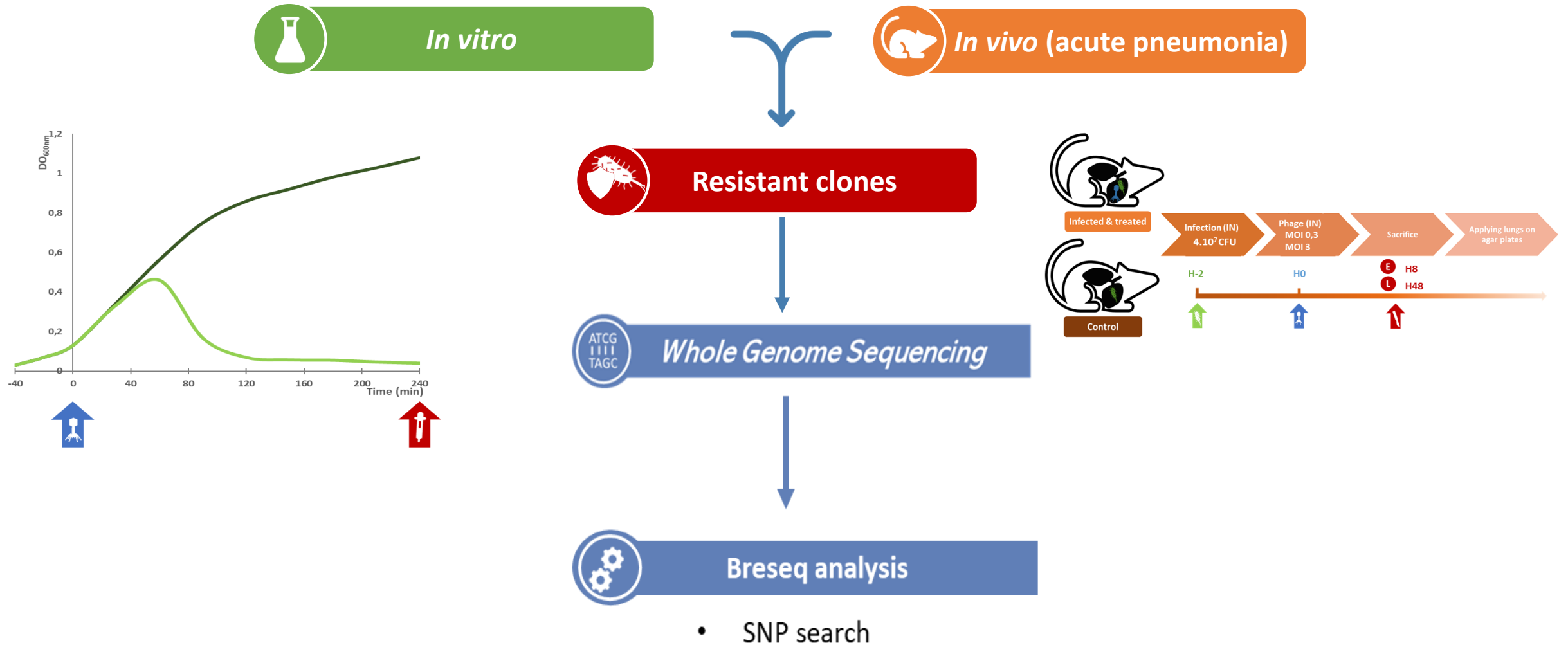
I<sub>2</sub>: infected  
and producer

V: phage

# The model predicts treatment outcome for any bacteriophage

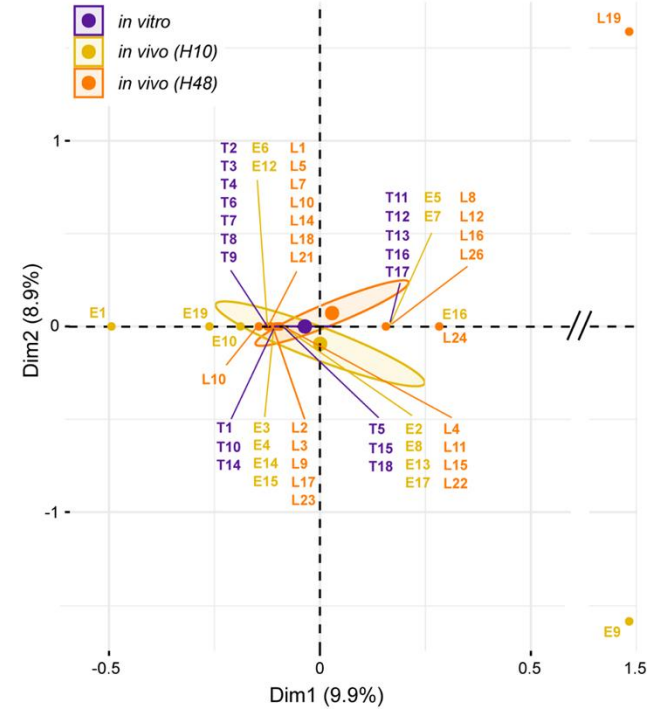
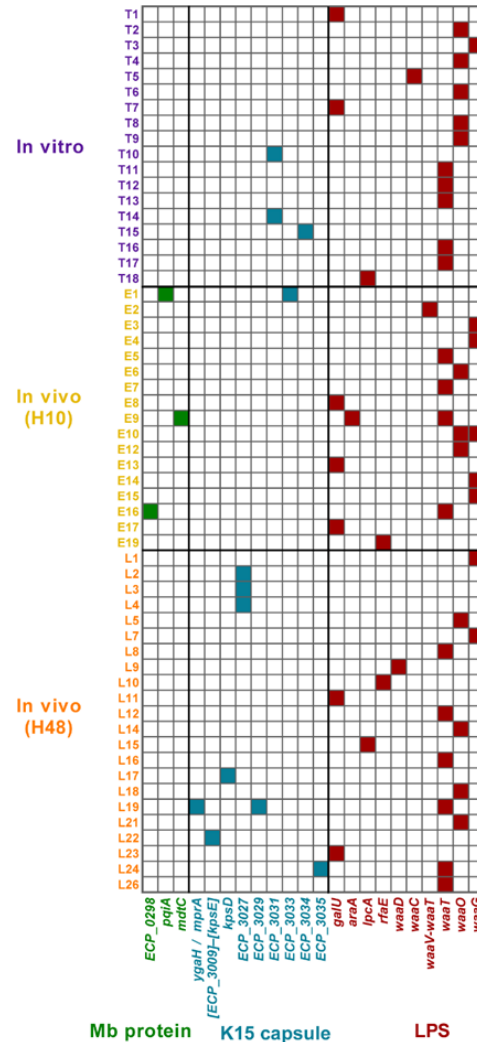
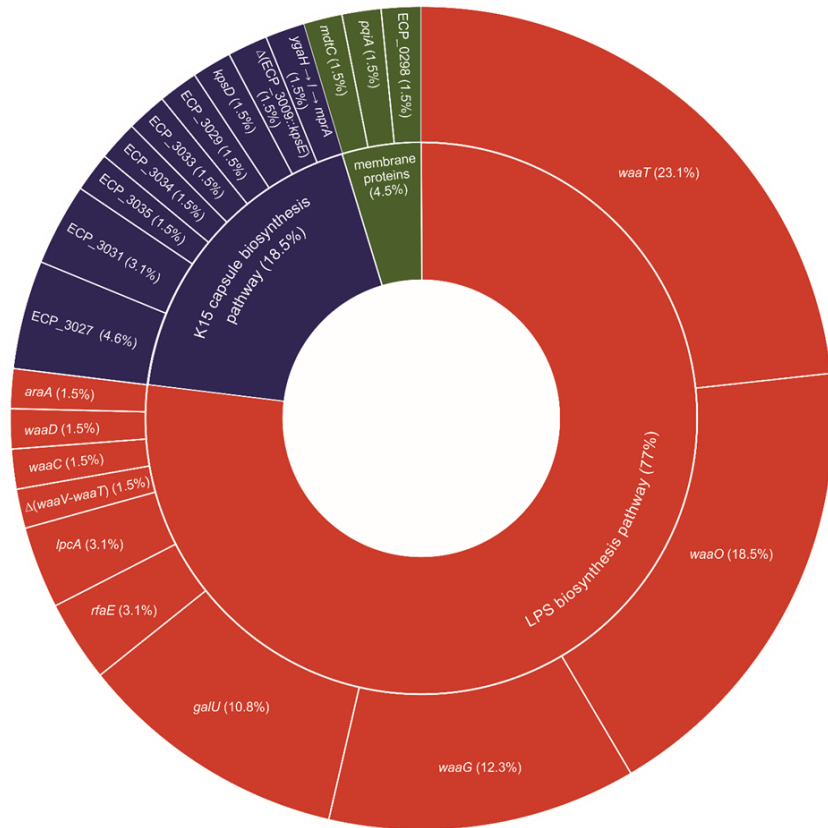


# What about bacteria becoming resistant to bacteriophages ?



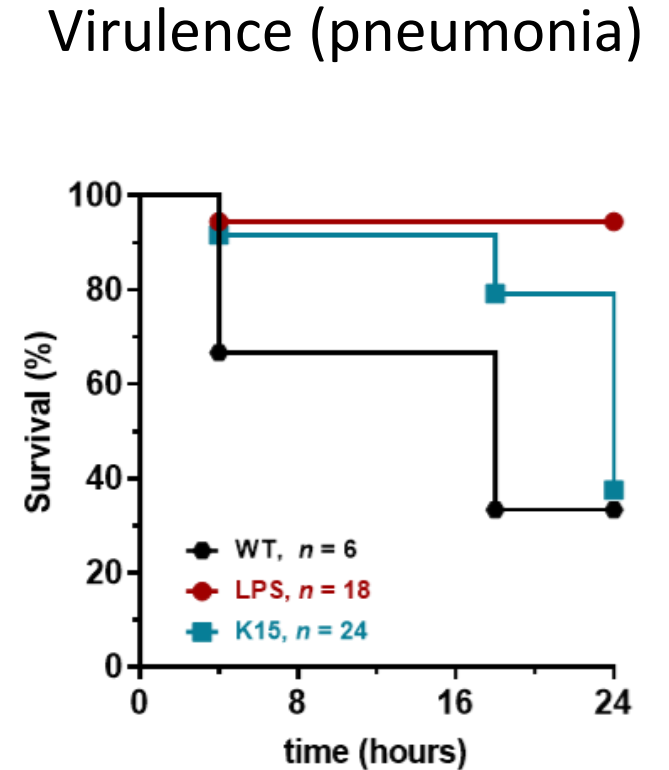
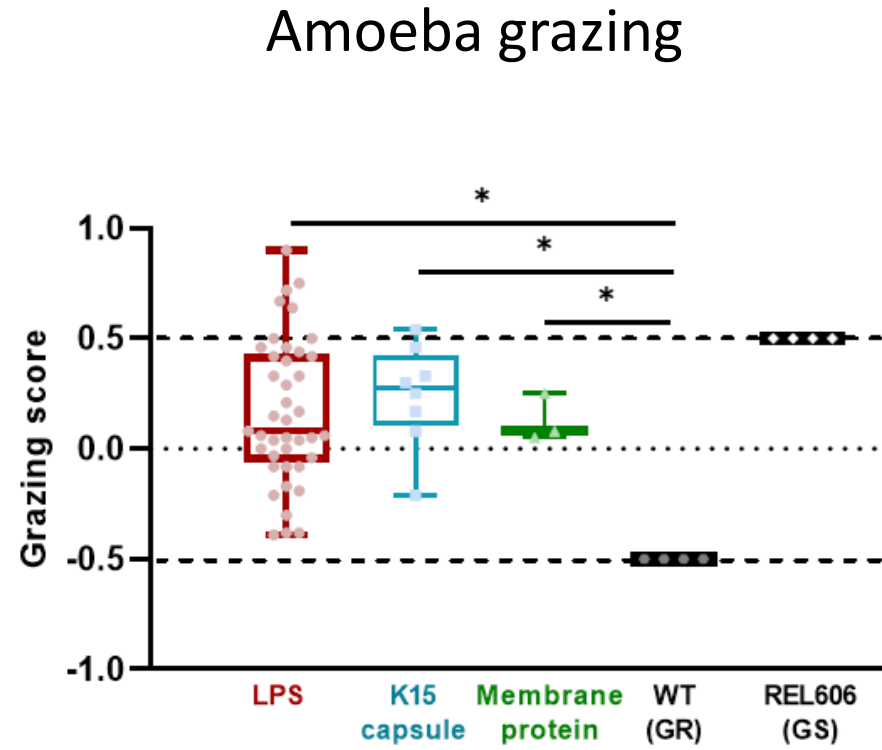
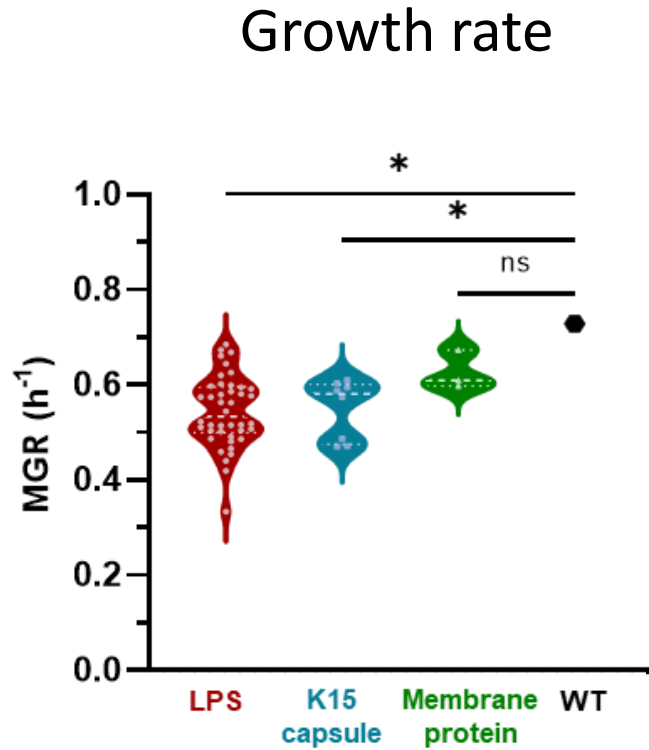
# Only two main pathways: LPS and K15 capsule

77% in LPS  
20% in K15 capsule



No difference between *in vitro* and *in vivo* clones

# Major differences between LPS and K15 capsule mutants



**LPS but not K15 capsule mutants are less virulent**

# Few conclusions and perspectives

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Phage therapy **is not a “killer” therapy**, but instead a **“back to control” approach**

**The immune response** can control the growth of phage resistant clones

But, phage resistant bacteria **are not systematically less virulent**

## Next steps

- Quantification of **immune cells** (neutrophils/macrophages)
- Assessment of **phage diffusion** in the lungs
- Control of **phage resistance**



# Phage Therapy: current status in EU and USA

## Increasing number of clinical cases published from France, Belgium, USA...

Jennes S et al. *Crit Care*. **2017 Jun** 4;21(1):129. doi: 10.1186/s13054-017-1709-y.

Schooley RT et al. *Antimicrob Agents Chemother*. **2017 Sep** 22;61(9):e01511-17. doi: 10.1128/AAC.00954-17.

Ferry T et al. *J Antimicrob Chemother*. **2018 Oct** 1;73(10):2903-2910. doi: 10.1093/jac/dky263

Dedrick RM et al. *Nat Med*. **2019 May**;25(5):730-732. doi: 10.1038/s41591-019-0437-z

Corbellino M et al. *Clin Infect Dis*. **2019 Aug** 15;68(4):e12-13. doi: 10.1093/cid/ciz782.

Khatami A et al. *EMBO Mol Med*. **2021** 13(11):e125252/emmm.202113936

Ferry T et al. *Nat Commun*. **2021** 12(1):3838/s41467-022-31837-9.

Uyttebroek S et al. *Lancet Infect Dis*. **2021** 21(11):e208-e220. doi: 10.1016/S1473-3099(21)00612-5 (Review)

Pirnay et al., **2023** *Journal of Clinical Microbiology*, observational analysis of the first one hundred consecutive cases of personalized bacteriophage therapy of difficult-to-treat infections facilitated by a Belgian consortium

**Next Talk !!!**

But still no convincing Phase II clinical trial published !

Regulatory agencies (EMA, FDA):

not opposed to phage therapy but... not ready to approve without clinical trials

**One path:** magistral preparation officially authorized in Belgium

**European Pharmacopeia:** will release recommendations (early 2024)

# What about in France ?

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- ~ 2010: ANSM “rediscover” bacteriophages
- ~ 2017: ANSM sets a CSST to respond to Clinicians/Patients demands
- ~ 2018: ANSM agrees on using phages from Belgium/Pherecydes
- ~ 2021: PhagOne (Lyon) Grant from AMR priority axis to support phage therapy (setting a public service to produce phages for compassionate treatments) (Tristan Ferry and Frédéric Laurent, Hôpitaux Civils de Lyon)
- ~ 2024: increasing phage treatment in France ?

**Annonce:** plusieurs PHRC sur cette thématique ont été financés !!!

# Bacteriophage, Bacterium, Host Laboratory

## Lab members (October 2022)



## Current Team members

Devon Conti  
Solène Ecomar  
Emma Evrard  
Caroline Henrot  
Céline Mulet  
Chau Nguyen  
Camille Sivelle  
Sophia Zborowsky

## Collaborators

Bärbel Stecher  
Martial Marbouty  
Rob Lavigne  
Joshua Weitz  
Alain Bousquet-Melou  
James Di Santo  
Philippe Schmitt-Kopplin  
Martin Witzernath  
Luisa De Sordi  
Marie-Agnès Petit  
Jérémy Guedj  
Eric Oswald  
Jean-Damien Ricard



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