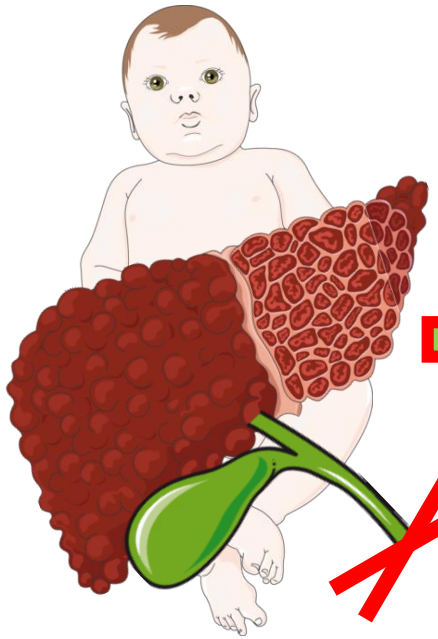
Phage therapy to allow liver transplantation in a toddler infected by an extensively drug-resistant *Pseudomonas aeruginosa*

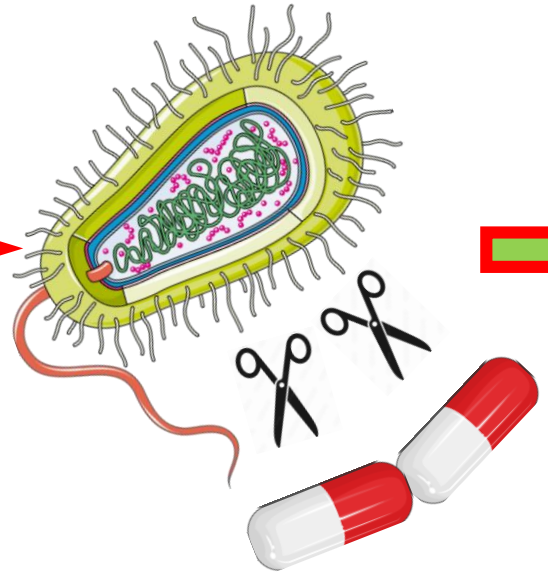
« The enemy of my enemy is my friend »

1yo child w/ Biliary Atresia



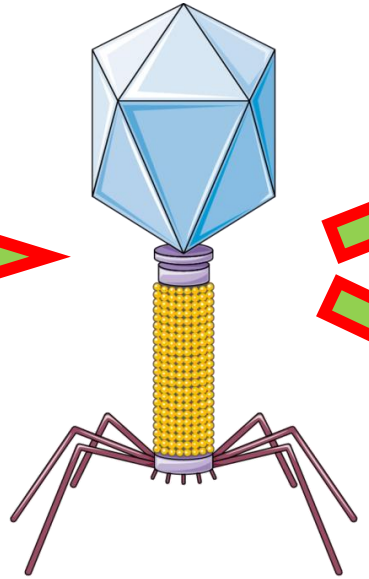
ABOi LDLT

XDR *Pseudomonas aeruginosa* systemic infection (liver, blood)



Failure of targeted antibiotic therapy (including colistin)

Phage Therapy (PT) : the use of bacteriophage viruses as bactericidal therapeutic agents



Durable eradication of *P. aeruginosa* from the bloodstream

Successful retransplantation !

Translational research on PT's contribution :

- Resistance to phages ?
- Immune reaction ?
- Synergies with AB's ?
- and more …

Brieuc Van Nieuwenhuyse et al., 2022

UCLouvain

1. Clinical Story

Key messages

- **Bacterial infections** are a major cause of mortality/morbidity after LT, and antimicrobial resistance causes therapeutic challenges.
- **Phage Therapy (PT)** uses lytic bacteriophage viruses as bactericidal agents, which is safe and can be efficient in the treatment of resistant bacterial infections.

Background

A 1 year old boy undergoes a 1st Liver Transplantation (LT) after biliary atresia diagnosis.

Several early complications occur, mainly on the infectious plan, leading to **repeated stays in the Pediatric Intensive Care Unit.**

On D+20 post-LT, we detect fecal carriage of an extensively drug-resistant **(XDR)** ***Pseudomonas aeruginosa*** *(Pa)* producing VIM-type Carbapenemase.

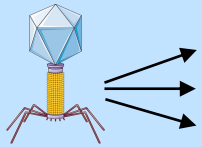
Acute problematic situation

On D+53, the child presents a severe septic state.

Bacteremia with the aforementioned VIM *Pa* strain.

➔ Minimum Inhibitory Concentration (MIC) analysis suggest **resistance to colistin**
➔ MIC's suggest susceptibility to gentamycin but its addition to treatment leads to **no improvement after 4 days.**.

Phage Therapy : administration and results

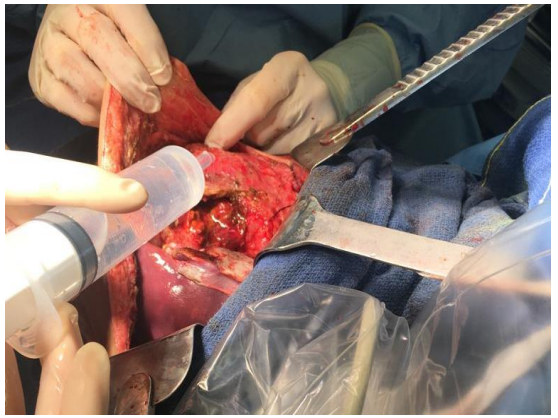


BFC-1

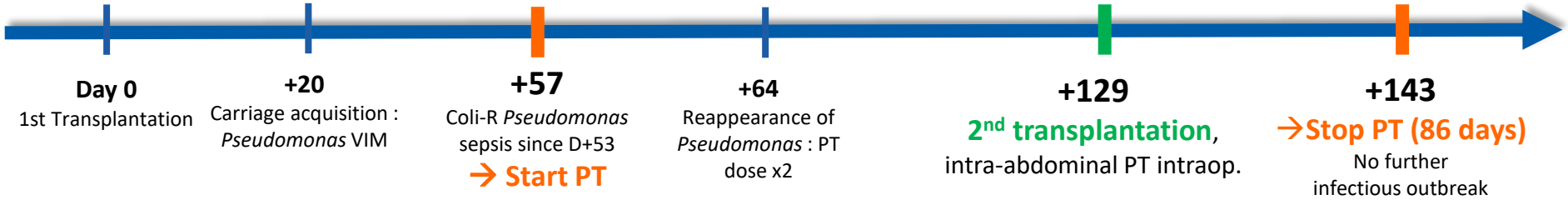
- **Intravenous** : **86 days** in total ; 72 before 2nd LT / 14 after
- **Intrahepatic** : via biliary pig-tail drain, **7 days**, poor tolerance
- **Intraperitoneal** : phage bath during anhepatic phase of 2nd LT

We used phage cocktail "BFC-1", which contained one **anti-*Pa* phage (phage PNM)** that had lytic activity on the child's *Pa* strain.

➔ Blood cultures (BC) **sterile in ~24h** after start of PT.
➔ **Transient re-occurrence of the *Pa* strain in BC** was overcome by doubling of the PT dose. Previous antibiotic regimen was pursued all along.
➔ **2nd LT successfully performed at D+129 after 42 days of sterile BC.**



Intraperitoneal phage bath (250 mL) during the anhepatic phase of 2nd Liver Transplantation (DDLT)



2. Bacterial Phage Resistance and Phage-Induced Virulence Tradeoffs

➔ **7 *Pa* isolates** were retrieved before and during PT, both from blood and liver abscess pus culture. **4 of them show complete resistance to phage PNM**, the only efficient anti-*Pa* phage in phage cocktail BFC-1.

➔ Sequencing revealed all of the phage-resistant *Pa* isolates had **mutations in the Type IV pilus complex.**

Name	Isolation site	Isolation date	Time relative to PT start	Phage lytic activity		
				PNM (10^9 pfu/mL)	PNM (10^7 pfu/mL)	PNM EOP
$Pa1_{BS}$	Blood	23-nov-18	- 4 hours	++++	++	0.3
$Pa2_{BR}$	Blood	24-nov-18	+ 8 hours	-	-	-
$Pa3_{LS}$	Liver	24-nov-18	+ 11 hours	++++	+++	0.2
$Pa4_{BR}$	Blood	27-nov-18	+ 4 days	-	-	-
$Pa5_{LR}$	Liver	27-nov-18	+ 4 days	-	-	-
$Pa6_{LR}$	Liver	27-nov-18	+ 4 days	-	-	-
$Pa7_{BS}$	Blood	1 Dec 18	+ 8 days	++++	+++	0.27

EOP, efficiency of plating; pfu, plaque forming unit; ++++, confluent lysis; +++, semi-confluent lysis; ++, opaque lysis; -, no lysis

The nomenclature of these isolates consists of : [Pa] for *P. aeruginosa*; [number] for chronological order of isolation; [first letter: B/L] for origin of isolate, either from Blood culture or Liver abscess pus culture; [second letter: S/R] for phenotypic Susceptibility or Resistance of the isolate to phage PNM.

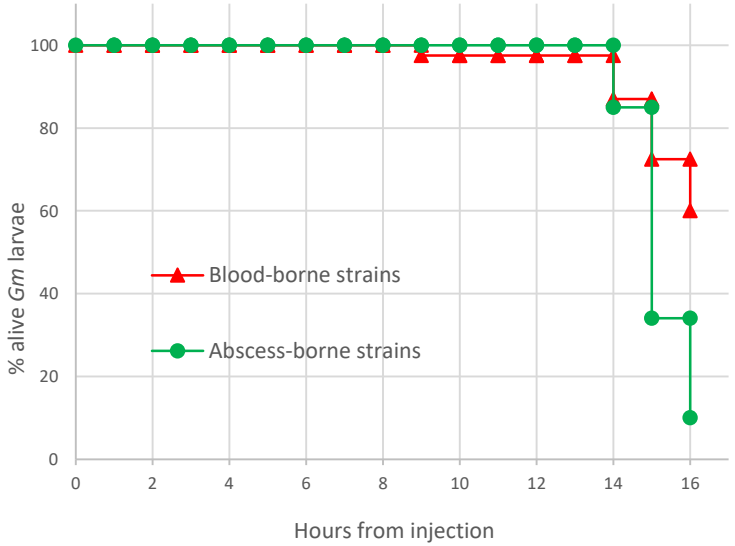
Key messages

- Development of **Bacterial Phage Resistance** is frequent but does not necessarily lead to the failure of PT.
- **Phage-Induced Virulence Tradeoffs** in the target bacteria could explain this, and were investigated in an *in vivo* model.

The "Phage-Induced Virulence Tradeoffs" paradigm

Is the idea that obtaining phage-resistance through certain mutations has a **"fitness cost"** that lowers bacterial virulence. This could explain the successful outcome of PT despite some isolates being phage-resistant.

We tested the virulence of each of the 7 isolates by injecting them in larvae (*Galleria mellonella)* and found that **liver abscess-borne isolates were more virulent** than bloodborne isolates (Log-rank p-value : 0,0011).
It is safe to assume that bloodborne isolates were the most exposted to PT.

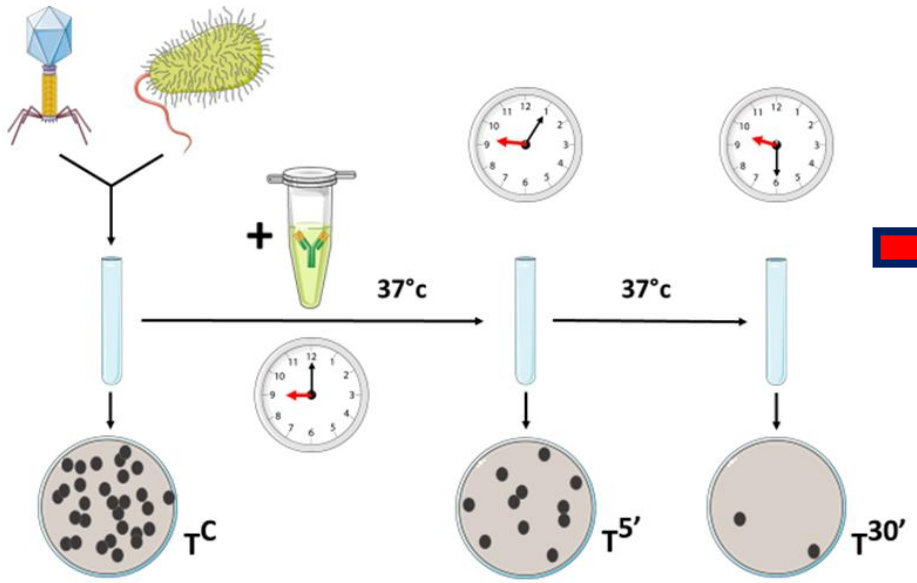


Live (left) and dead (right) larvae. Larvae injected with *Pa*'s isolated from liver pus have poorer survival (p = 0,0011)

3. Phage Immune Neutralization (PIN)

➔ **10 serum samples** retrieved from the patient before, during and after PT, were tested for their ability to neutralize any of the phages composing phage cocktail BFC-1.

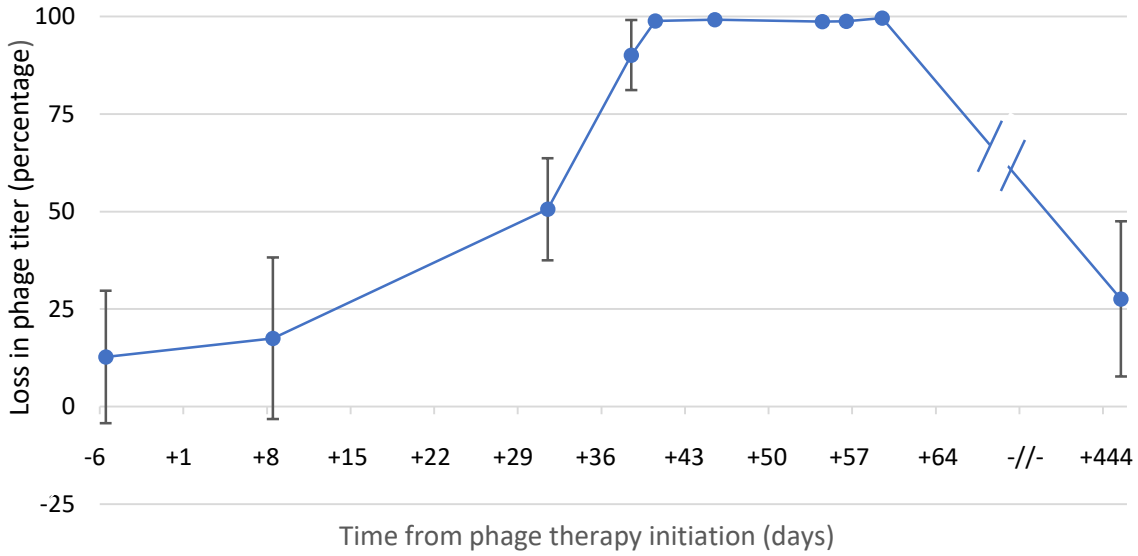
➔ Anti-*Staphylococcus aureus* phage ISP triggered such a PIN reaction, but anti-*Pa* phage PNM did not.



➔ Schematic methodology of the double-agar overlay assay that was used to quantify the phage-neutralizing power of the serum samples.

Key messages

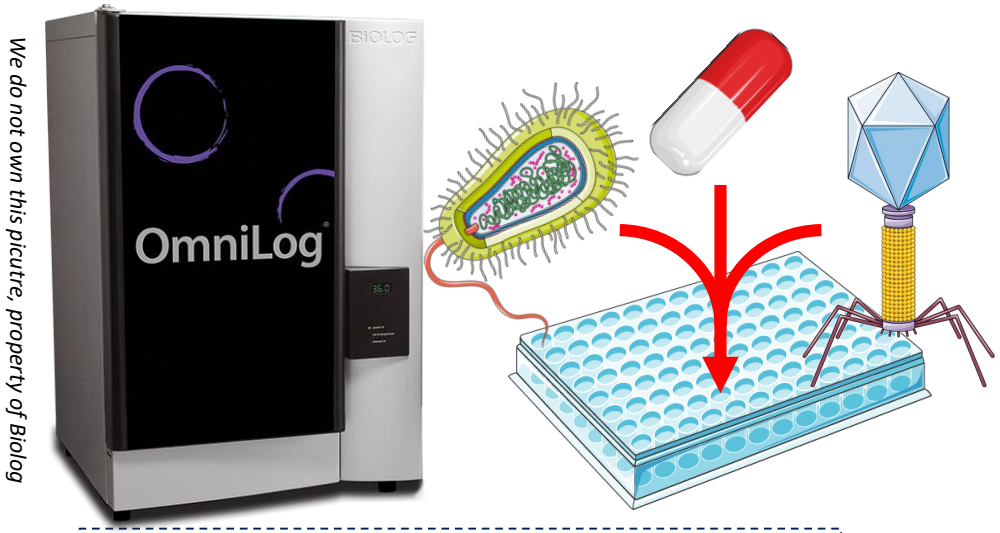
- **Development of phage-targeting antibodies** in the patient is frequent in case of intravenous PT, but does not always have a negative impact on the patient's outcome.
- **The incomplete pattern of phage immunization** in this patient is fortunante, and is probably due to his persistent and multifactorial immune suppression (lymphopenia and hypogammaglobulinemia during PT, *data not shown*).



➔ Development of a PIN reaction against anti-*Staphylococcus aureus* **phage ISP** (present in phage cocktail BFC-1) over time. Neutralization reached a maximum after 5 weeks of PT and had almost disappeared on a >1 year control serum sample. Phage PNM, which was the « useful » anti-*Pa* phage, fortunately triggered no PIN reaction (*not shown*).

4. Phage –Antibiotic Synergy (PAS)

- We investigated whether the addition of phage PNM to the patient's previous antibiotic therapy could have resulted in **synergistic bactericidal effect.**
- This was tested with phage PNM and any of the three antibiotics that were pursued along with PT that could have had an anti-*Pa* effect : **colistin, gentamycin and aztreonam.**
- **All three antibiotics showed potent synergistic effect** on isolate $Pa1_{BS}$ in combination with phage PNM when tested through Biologs' OmniLog® *in vitro* automated culture system. Results for PNM+colistin are represented.

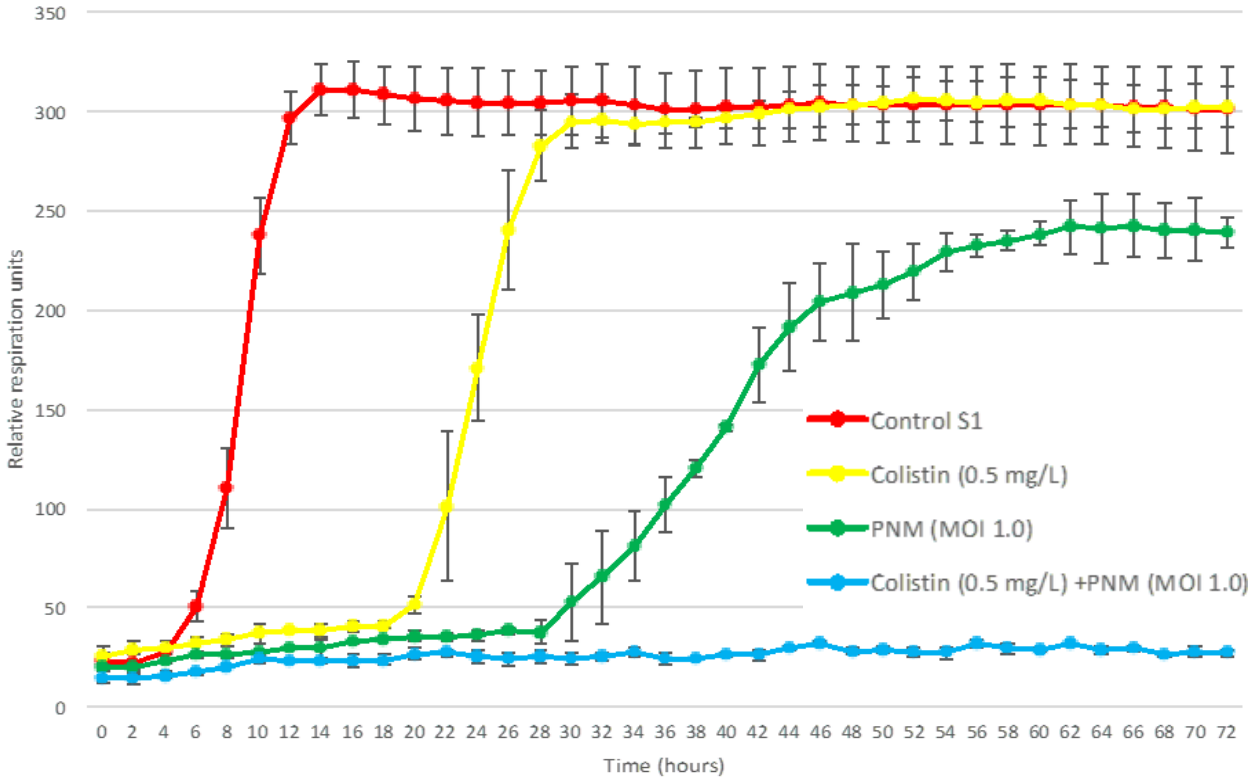


We do not own this picutre, property of Biolog

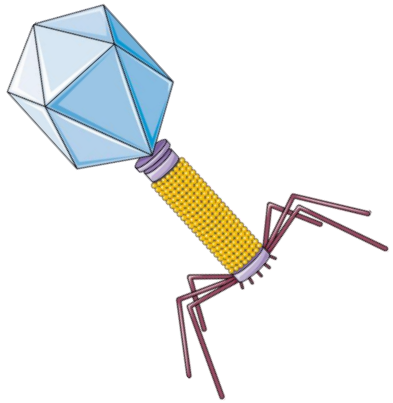
Biolog's OmniLog® measures bacterial growth in 96-well microplates through cellular respiration

Key messages

- **Phages and antibiotics can achieve synergistic bactericidal effect**, but these synergies probably depend on the type and titers of both the phage and the antibiotic used.
- Fundamental mechanisms underlying these synergies are still incompletely understood, as well as their relevancy to « clinically realistic » titers of phages and antibiotics.



Combination of certain titers of colistin and phage PNM leads to potent synergistic effect, with only the phage-antibiotic combination being able to achieve sustained bacterial suppression over 72 hours.



Thank you for your attention !

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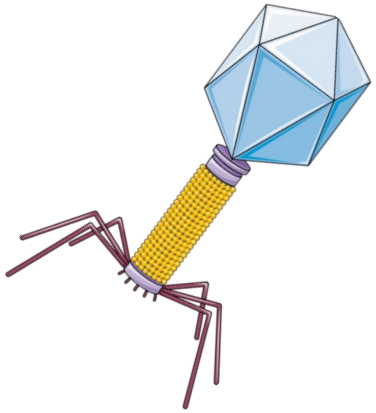

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