# Approcci innovativi contro i batteri antibiotico-resistenti

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**Siena**

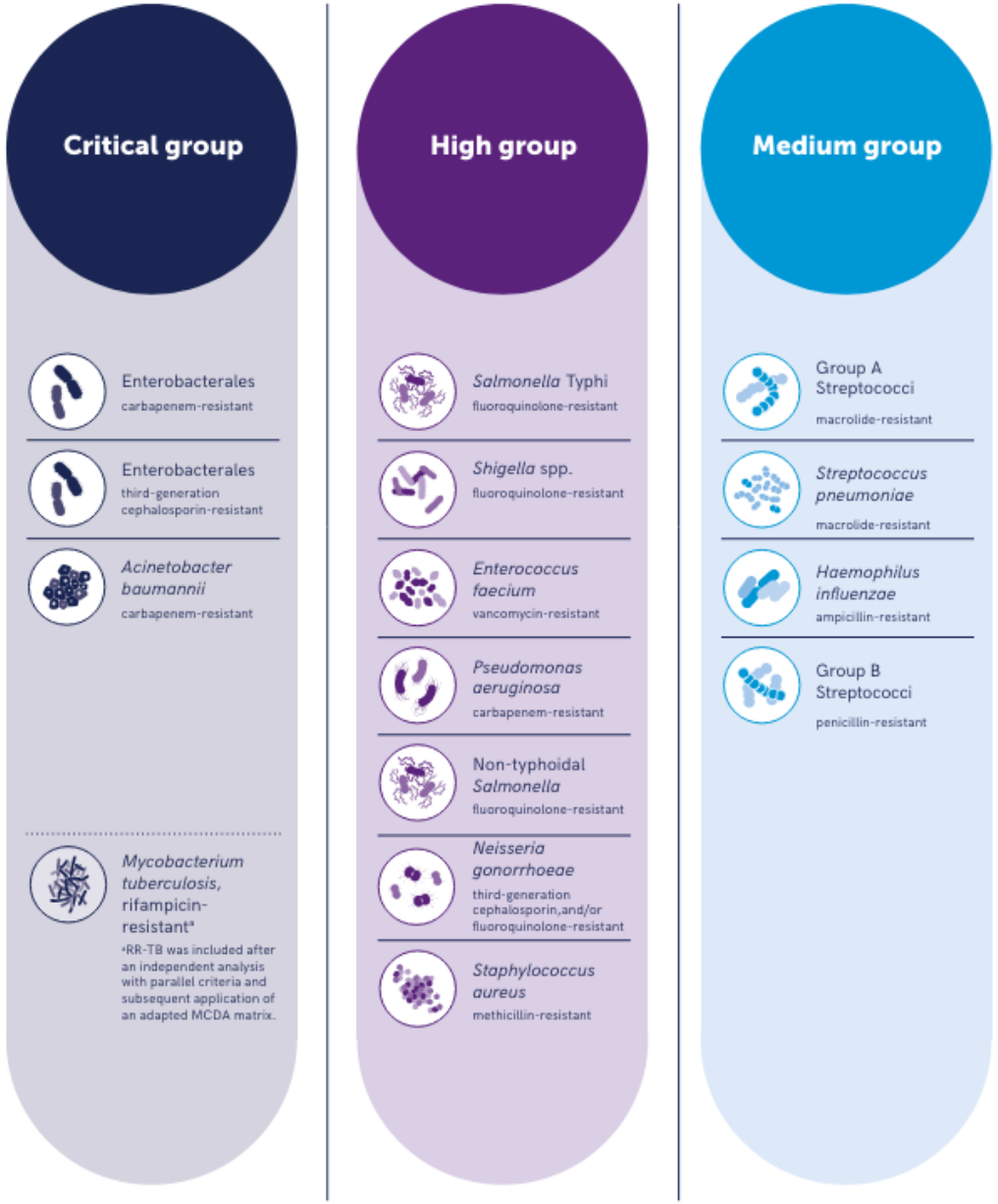
# The Siena cathedral: “memorial” to the plague

Plan for building larger cathedral in the XIV century

**Plague** in 1348 → socio-economic **crisis** → construction works interrupted and never resumed

**Warning against epidemics and pandemics**

# Today’s silent pandemic: antimicrobial resistance (AMR)




AMR kills **5 million people per year**

35,000 EU/EEA citizens (ECDC, 2022)

More than HIV and TB combined

*WHO Bacterial Priority Pathogen List 2024*

# What can we do when antibiotics are useless?

- Discover **new antibiotics**
- Develop **vaccines**

Explore new avenues:

- **monoclonal antibodies**
- **phages**
- **anti-virulence** compounds
- **antimicrobial peptides**
- **host-directed** therapies
- **CRISPR-Cas**-based approaches

# New antibiotics on the way?

Article

## A Gram-negative-selective antibiotic that spares the gut microbiome

https://doi.org/10.1038/s41586-024-07502-0

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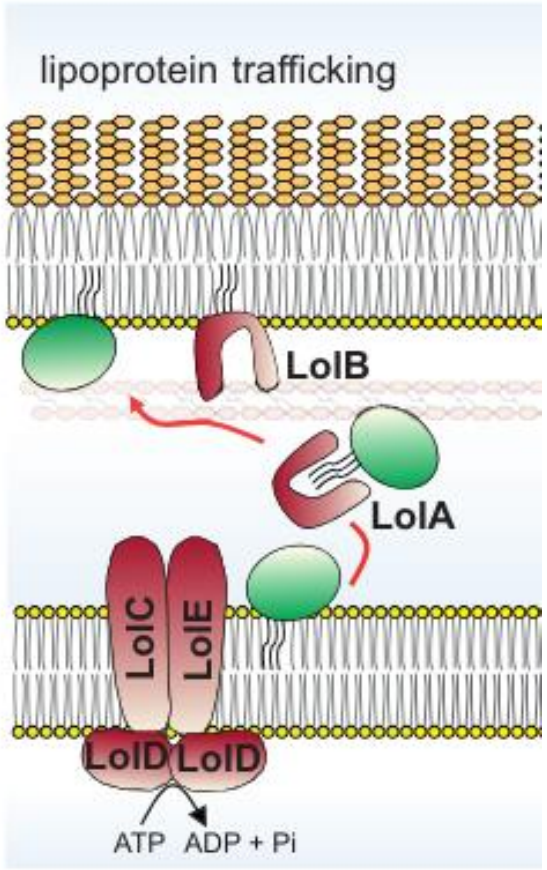
Kristen A. Muñoz[1,2], Rebecca J. Ulrich[1,2], Archit K. Vasan[3,4,5], Matt Sinclair[3,4], Po-Chao Wen[3,4,5], Jessica R. Holmes[6], Hyang Yeon Lee[1,2], Chien-Che Hung[7,8], Christopher J. Fields[6], Emad Tajkhorshid[1,3,4,5], Gee W. Lau[9] & Paul J. Hergenrother[1,2,4]




*Shrivastava et al, 2019*

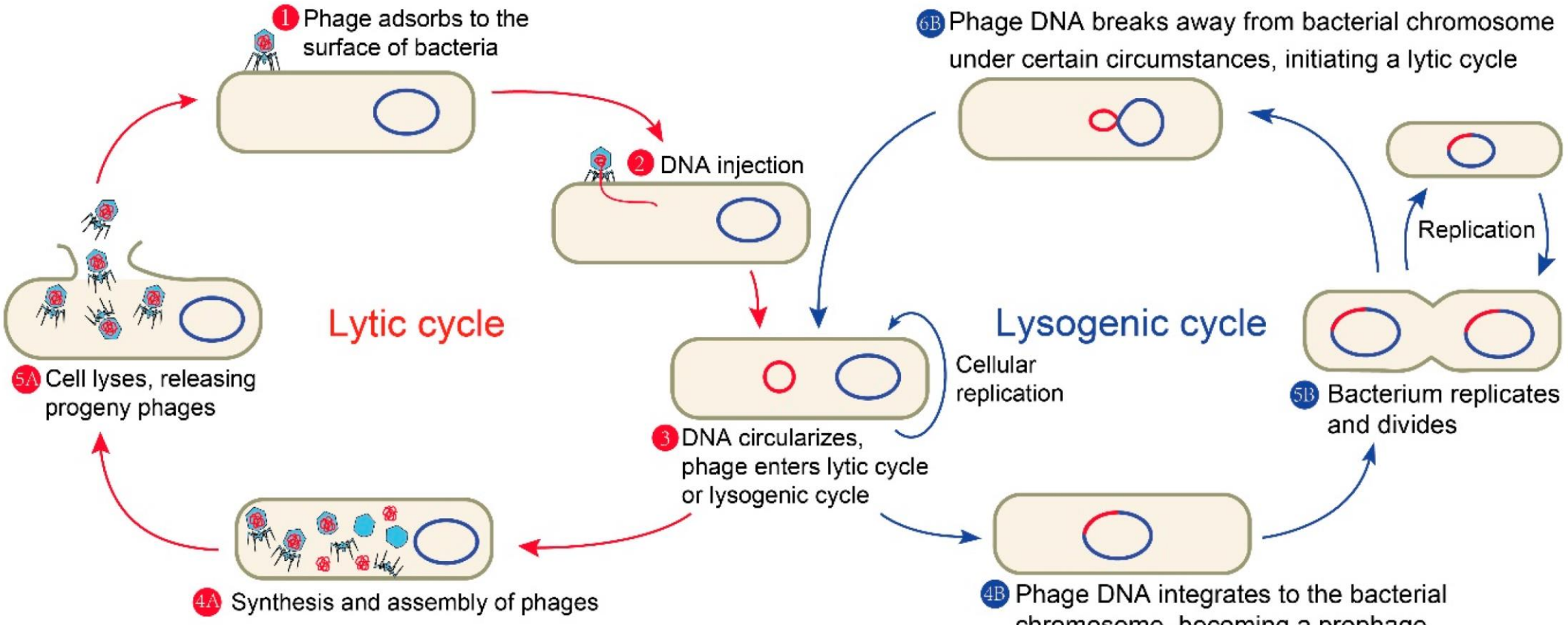
***Lolamicin**:*

- *Gram-negative-specific*
- *Targets **lipoprotein transport system** (Lol)*
- *Active vs. **MDR clinical isolates***
- *Effective in animals models*
- *Spares microbiota*


*Vacca, 2017*
*Ekiert et al., 2017*

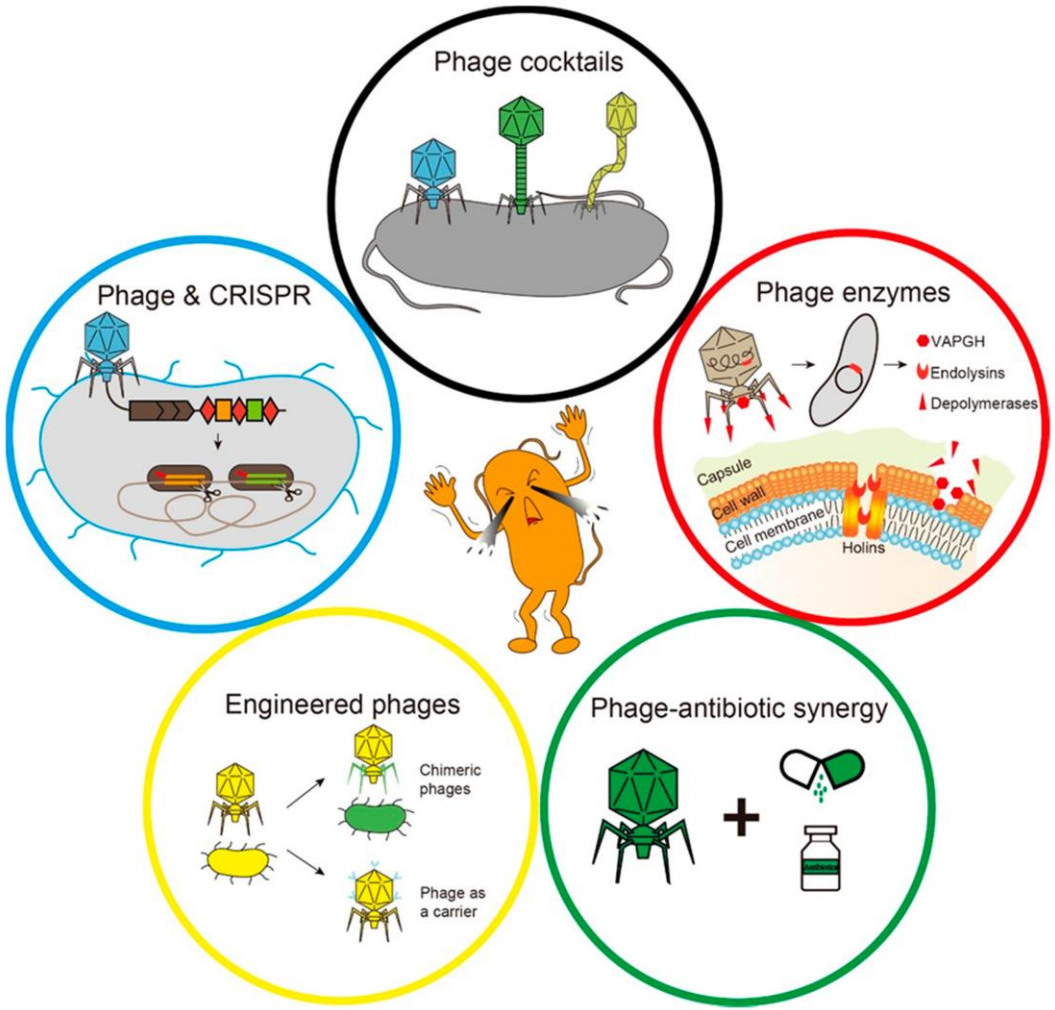
# Phages & phage therapy: what is a phage?

**Bacteriophage (phage) → virus that infects bacteria**




*Wei et al, 2020*

# Phages & phage therapy




**Specificity** of bacteriophages (advantages vs. drawbacks)

→ Need accurate identification of bacterial pathogen

→ Precise diagnosis → **precision/personalized medicine**

Bacterial **resistance** to phages

**Safety** and **effectiveness** of therapy

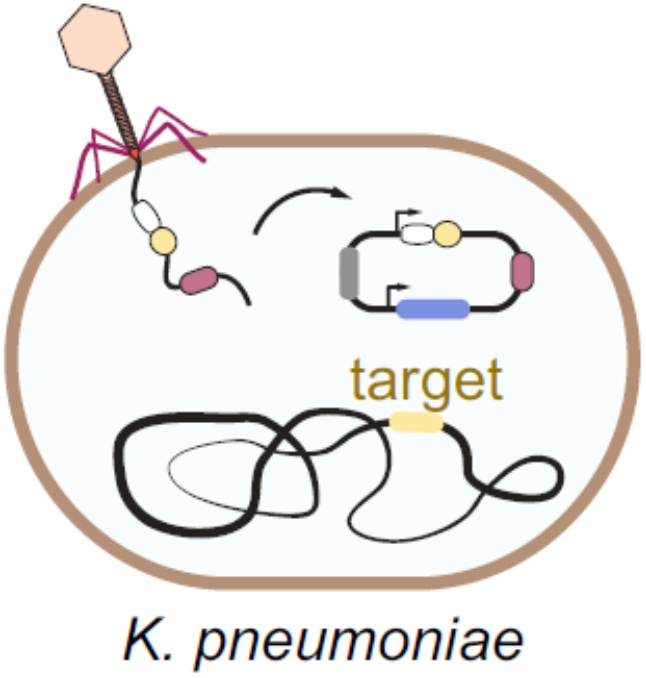
*A Phase 1b/2 Trial of the Safety and Microbiological Activity of **Bacteriophage Therapy in Cystic Fibrosis** Subjects Colonized With Pseudomonas Aeruginosa*

*clinicaltrial.gov NCT05453578 NIAID 2022*

*Wei et al, 2020*

# CRISPR-Cas-based approaches

**IDEA: cleave bacterial genomic DNA → kill bacteria**





**Phage** as a **vehicle of scissors and guide**:

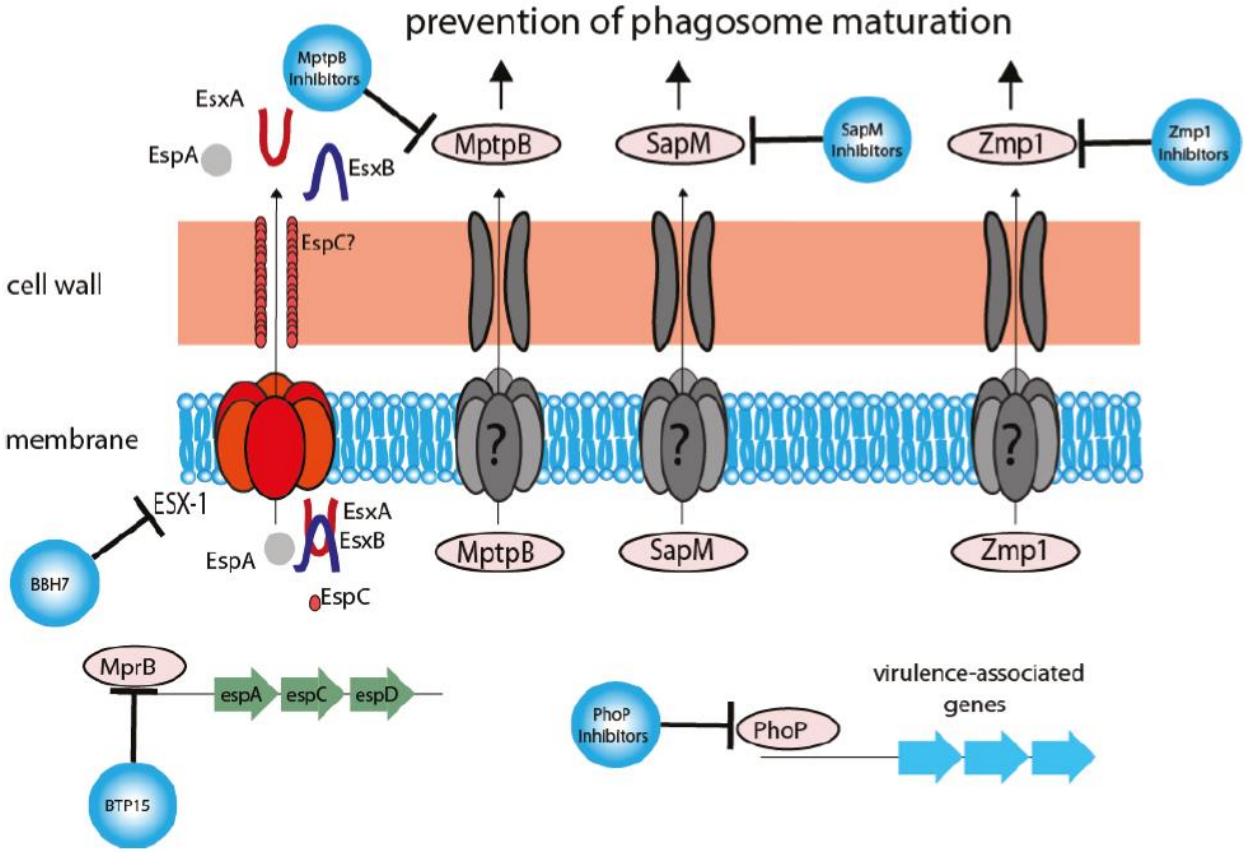
- **Cas12** protein: molecular **scissors**
- Guide RNA (**gRNA**) to direct Cas12 **cleavage** to specific target
- Target: genes on ***K. pneumoniae*** **chromosome**

*Vialetto et al, 2024*

# Anti-virulence targets and compounds

## Example: drugs that do not kill *M. tuberculosis* but reduce virulence

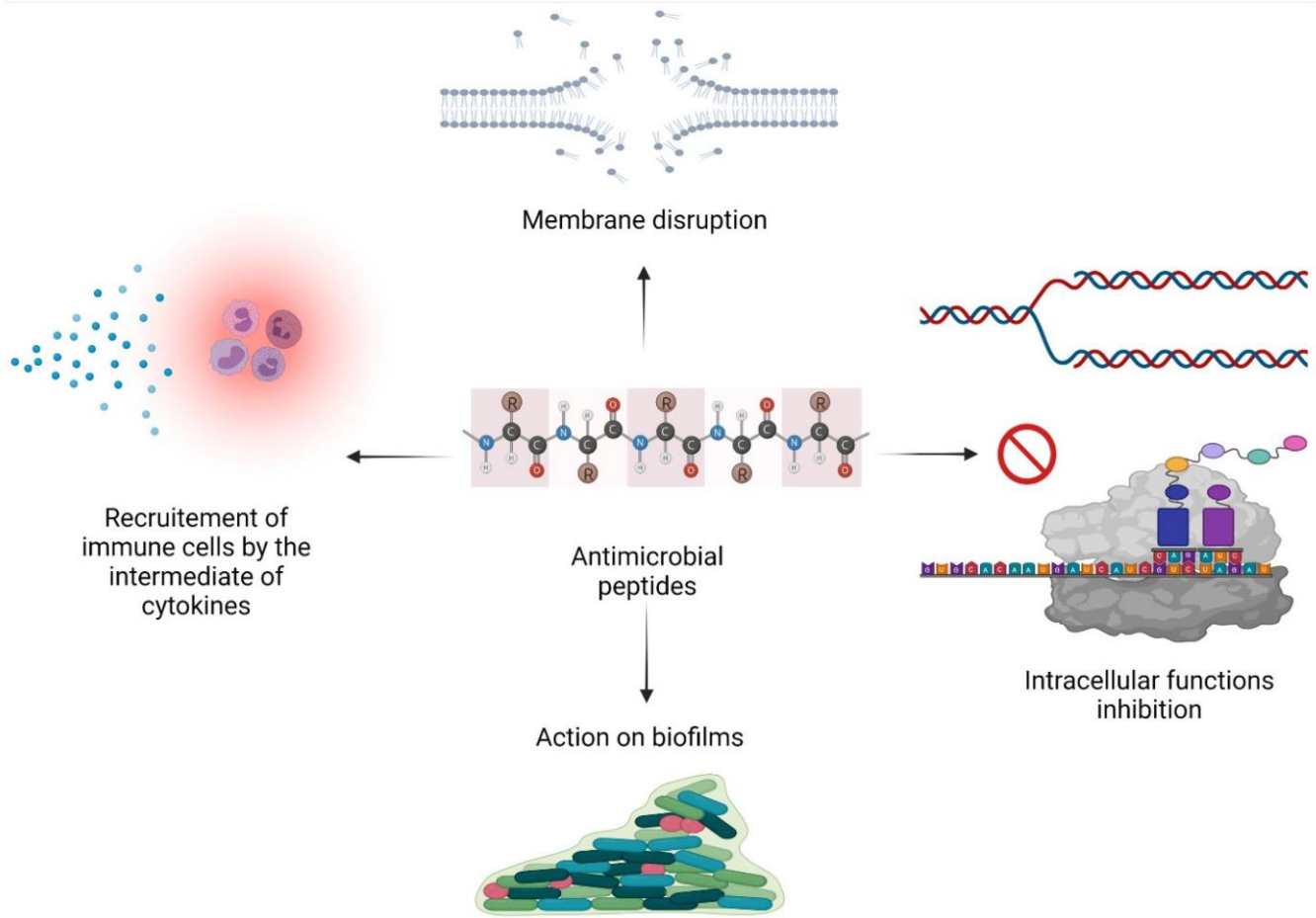
- Virulence factors
- **ESX-1** (type VII secretion system)
- **MptpB**: phosphatase required for survival *in vivo*
- **SapM**: phosphatase → targets phagosome maturation
- **Zmp1**: peptidase → targets phagosome maturation




*Gries et al., 2020*

# Antimicrobial peptides

- **Small molecules**, 15-50 aminoacid long
- Broadly found in nature
- Part of innate immune system
- **Natural** and **synthetic** AMPs
- Various AMPs in clinical trials

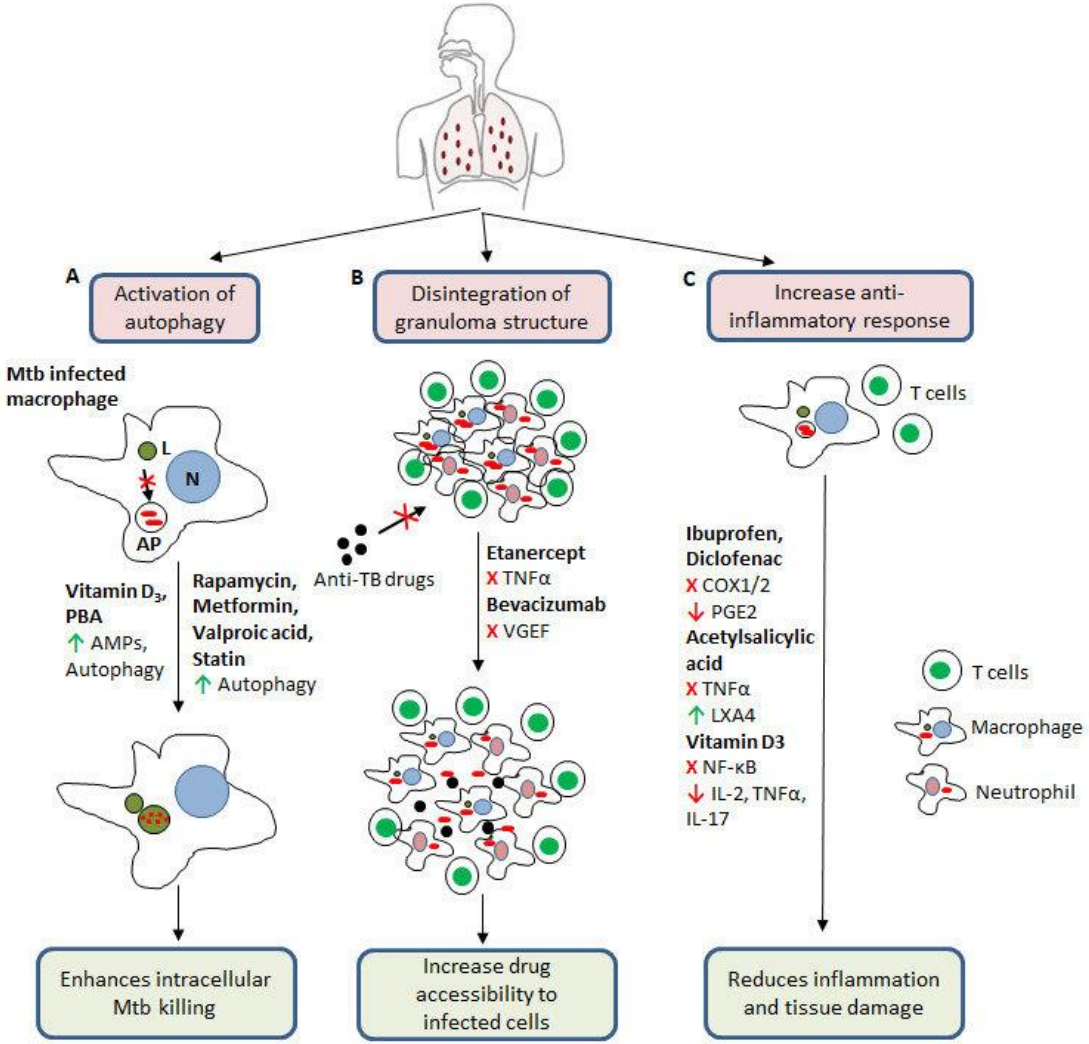



**Mode of action of AMPs**

*Rima et al, 2021; Dijksteel et al., 2021*

# Host-directed therapy

## Example: HDT vs. *M. tuberculosis*




- Several repurposed compounds (e.g. anticancer agents, diabetes therapy)
- **Clinical trials** ongoing (e.g. metformin on top of standard multidrug regimen)
- **Complementary** approach to standard drugs

*Ahmed et al., 2020*

# mAbs & passive immunization

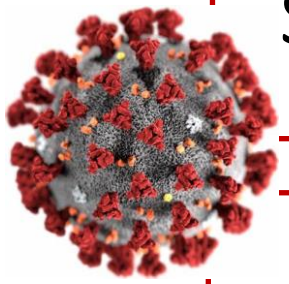
- Passive immunization with horse serum as an effective treatment against diphtheria and tetanus
- mAbs today: passive immunization and therapy vs. infectious diseases

Emil von Behring (1854 – 1917)
1901 Nobel prize in Physiology and Medicine

# mAbs against infectious diseases

| Advantages | Obstacles/open questions |
| --- | --- |
| Specificity (spare microbiota) | Accurate animal models for testing? |
| Only option for immunocompromised patients | Antigenic heterogeneity of pathogens |
| Enormous technological progress (cloning and expression) | Capsular layers may mask important antigens |
| Engineered mAbs → improved penetration, effector functions, conjugation to drugs | Precise timing for administration? Prophylaxis? Therapy? |

# MAD Lab ongoing projects overview



SARS-CoV-2

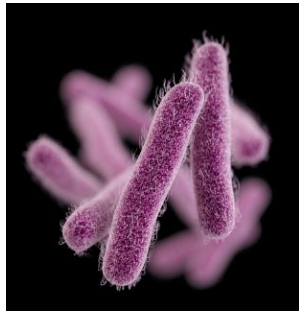
Monkeypox

*Neisseria gonorrhoeae*

*Klebsiella pneumoniae*

*Shigella spp.*

mRNA-mAbs



DaScH Lab

Advanced Data Analytics & Bioinformatics

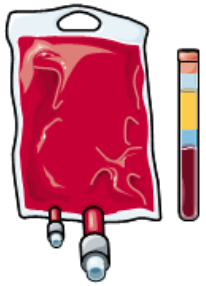
- mAbs for prophylaxis/therapy
- mAbs for antigen discovery → rational vaccine design

# mAb cloning pipeline

Blood collection & PBMC isolation

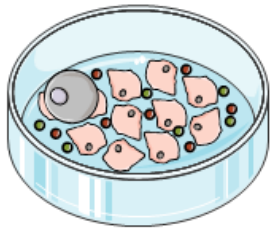
B cell proliferation *in vitro*

Production of mAbs



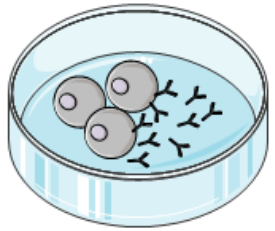
Memory B cells

sorting

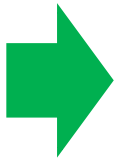


*In vitro*

mAb secretion

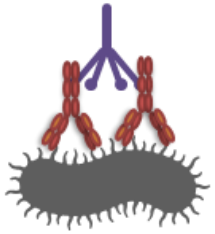


**mAbs are released into ≈ 50µL**



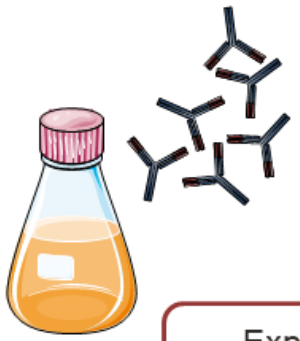
No need to take blood from patients anymore

Antigen binding ELISA assays



Assessment of activity

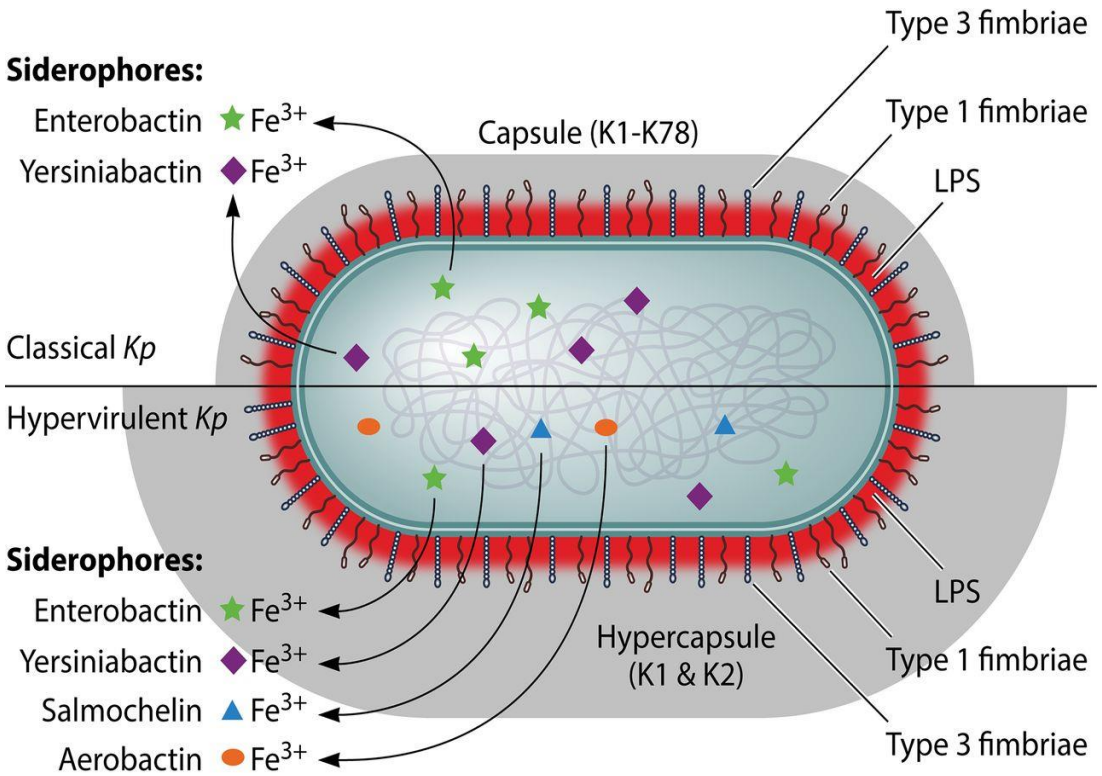
Sequence recovery



Expression as recombinant proteins

# mAbs vs. *Klebsiella pneumoniae*

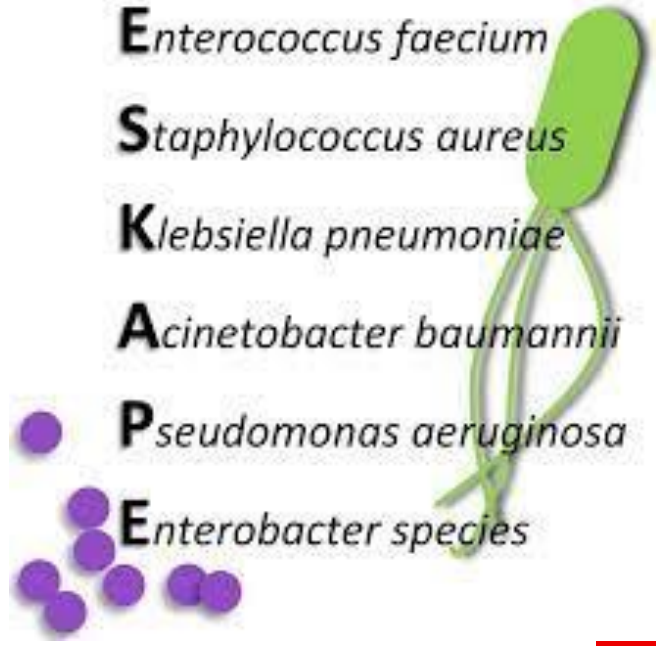
# *Klebsiella pneumoniae*: overview




→ Gram-negative, **encapsulated**, non-motile, **opportunistic** pathogen

→ Leading cause of **hospital-acquired infections** (i.e., pneumonia, UTI, bloodstream infections)

→ Kp acquired **resistance** to most classes of antibiotics, including carbapenems




**New Delhi metallo-beta-lactamase (NDM) - producing *K. pneumoniae***

Global concern

# Global spread of hypervirulent and pandrug-resistant ST147

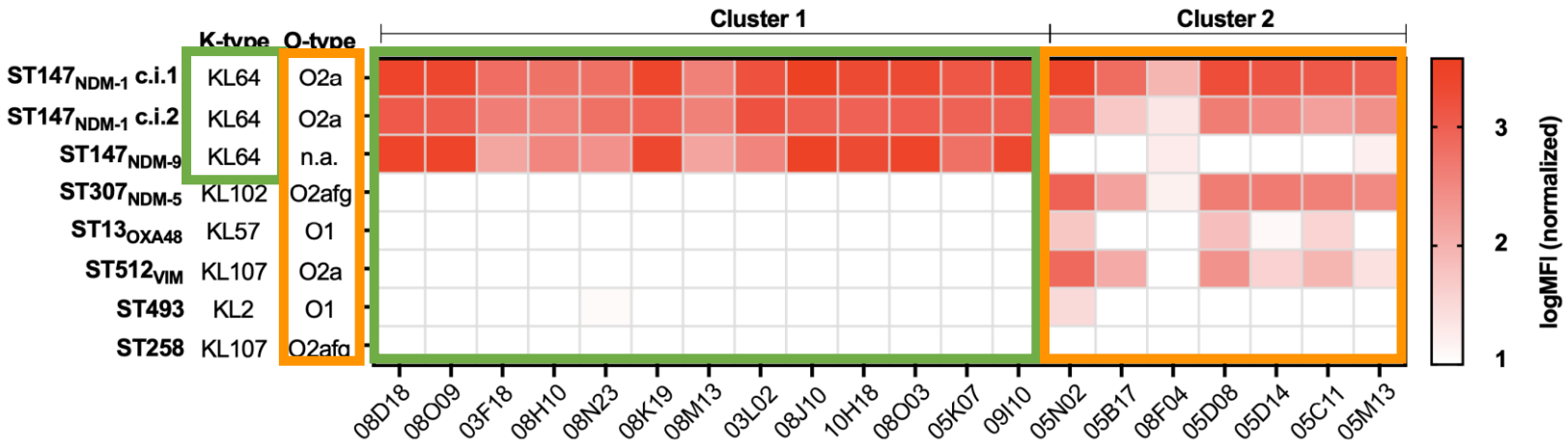

1,933 ST147 isolates
(6% of Kp genomes uploaded in PathogenWatch)

## Persistent nosocomial outbreak of ST147 Kp in Tuscany

- Colonization to bloodstream infection
- 499 blood infection cases (2018-2022) with 22.7% lethality
- Extensive AMR profile, genetically evolving

**mAb discovery in convalescent patients**

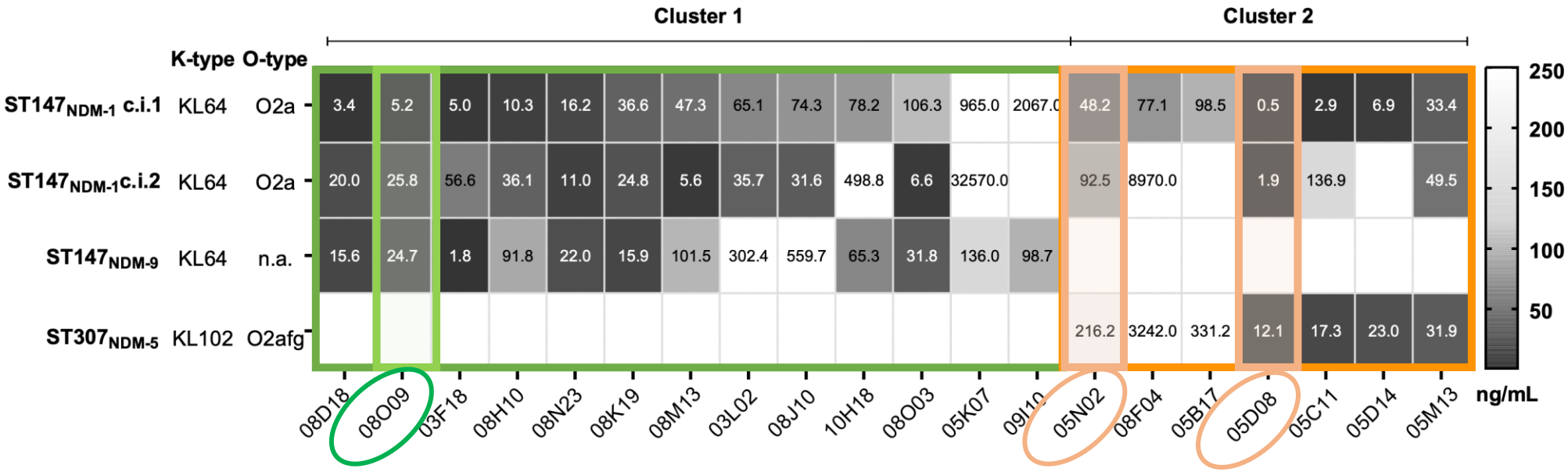
# Two mAb clusters targeting capsule and O-antigen with ng/mL bactericidal activity




FACS Binding

ELISA Binding

Capsule
O-antigen

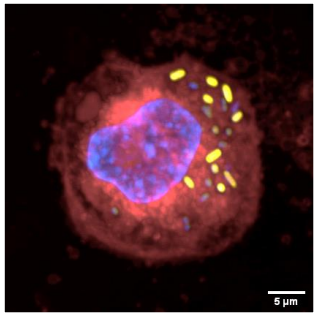


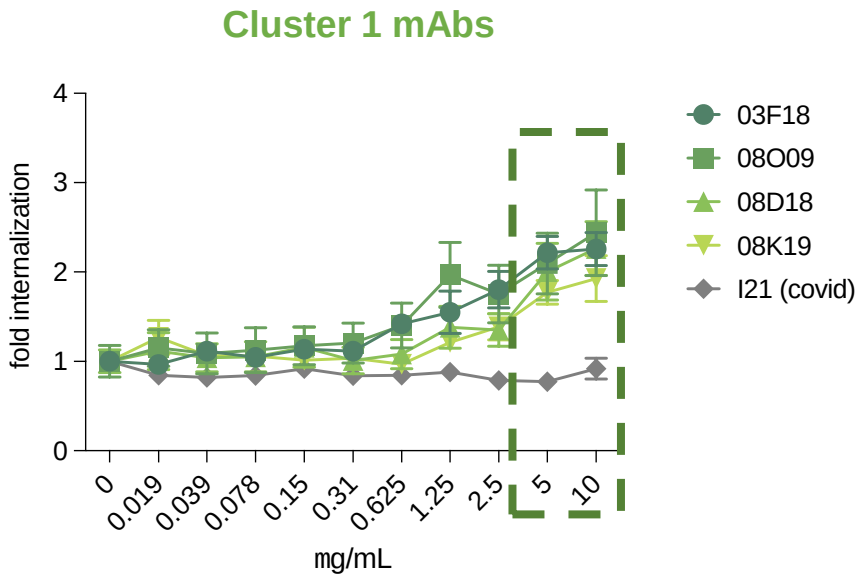

Complement-dependent killing

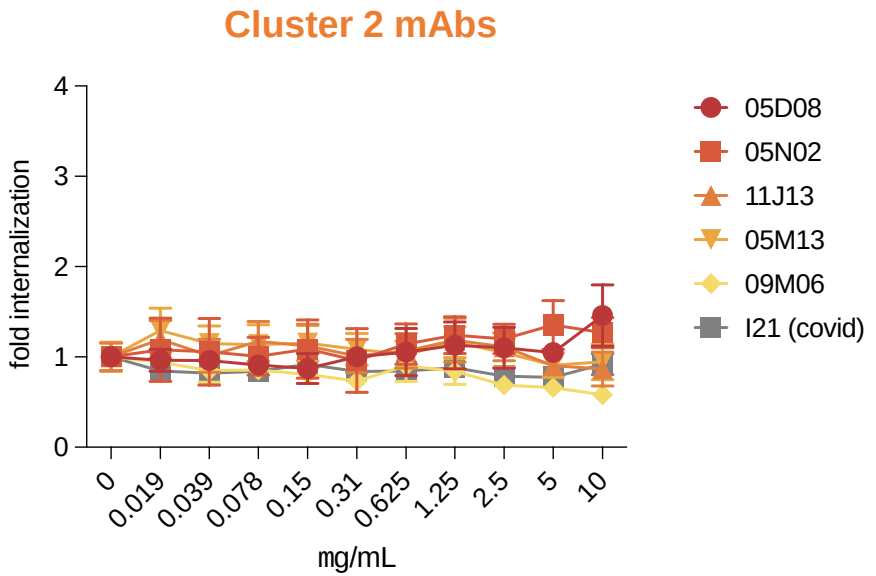
*Roscioli et al, submitted*

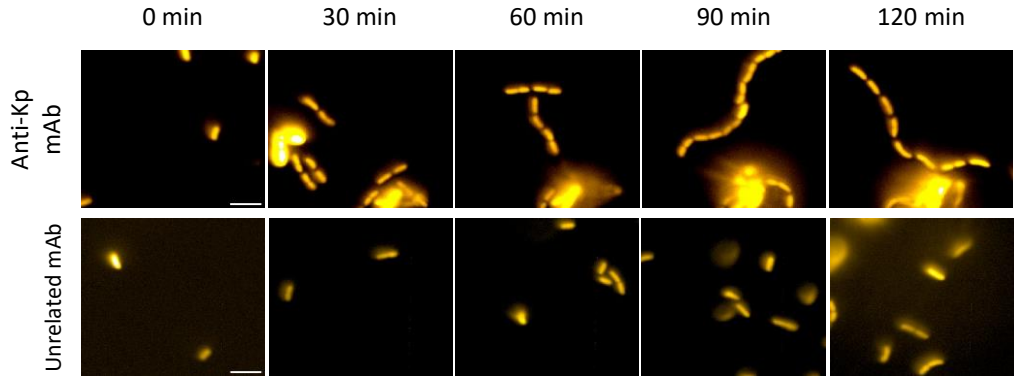
# Cluster 1 mAbs promote opsonophagocytosis and enchained growth

THP-1 cells

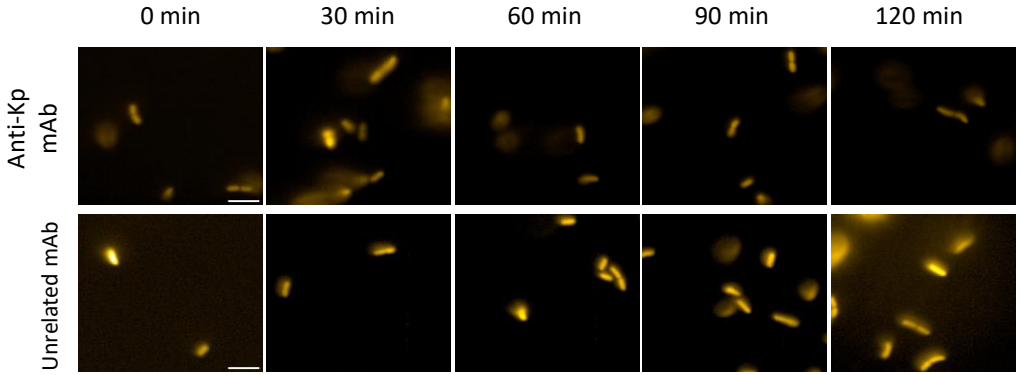


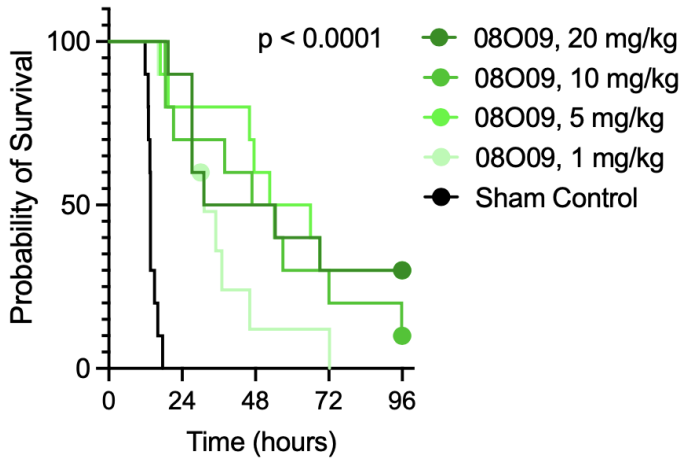

**Cluster 1 mAb**
**10-100 µg/mL**

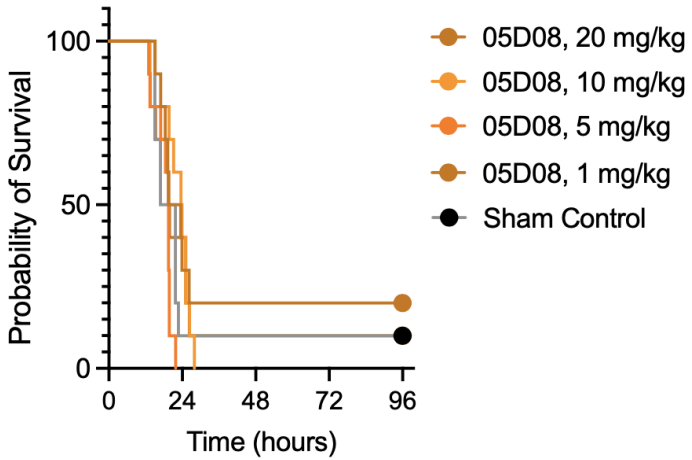


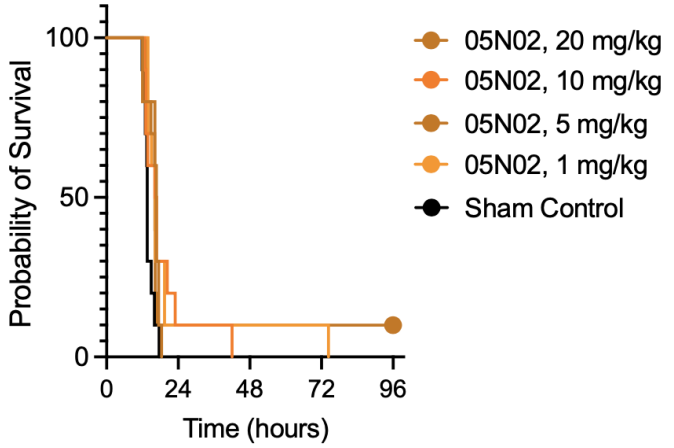

**Cluster 2 mAb**
**10-100 µg/mL**

*Roscioli et al, submitted*

# Cluster 1 mAbs protect from bacterial challenge *in vivo*

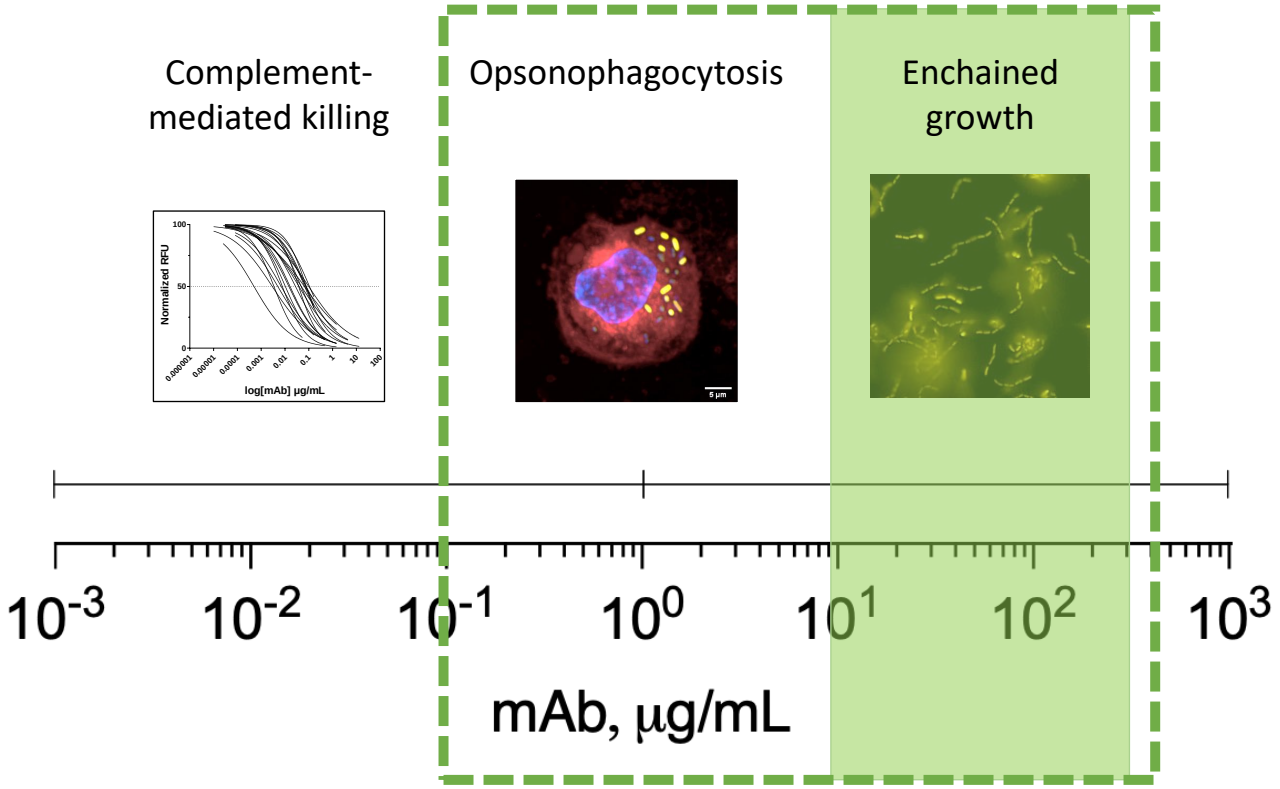




Serum mAb concentration 24h hours post ip injection*:* 50-100 μg/mL

*Roscioli et al, submitted*

# Protection against pandrug-resistant Kp correlates with mAb poly-functionality




Protective serum mAb concentration *in vivo*

1. Multi-functionality is important *in vivo,* right assays are important *in vitro*
2. Complement-based killing is not predictive of protection
3. KL64 shields O-antigen (and other antigens)

*Roscioli et al, submitted*

# Conclusions & next challenges

## Conclusions

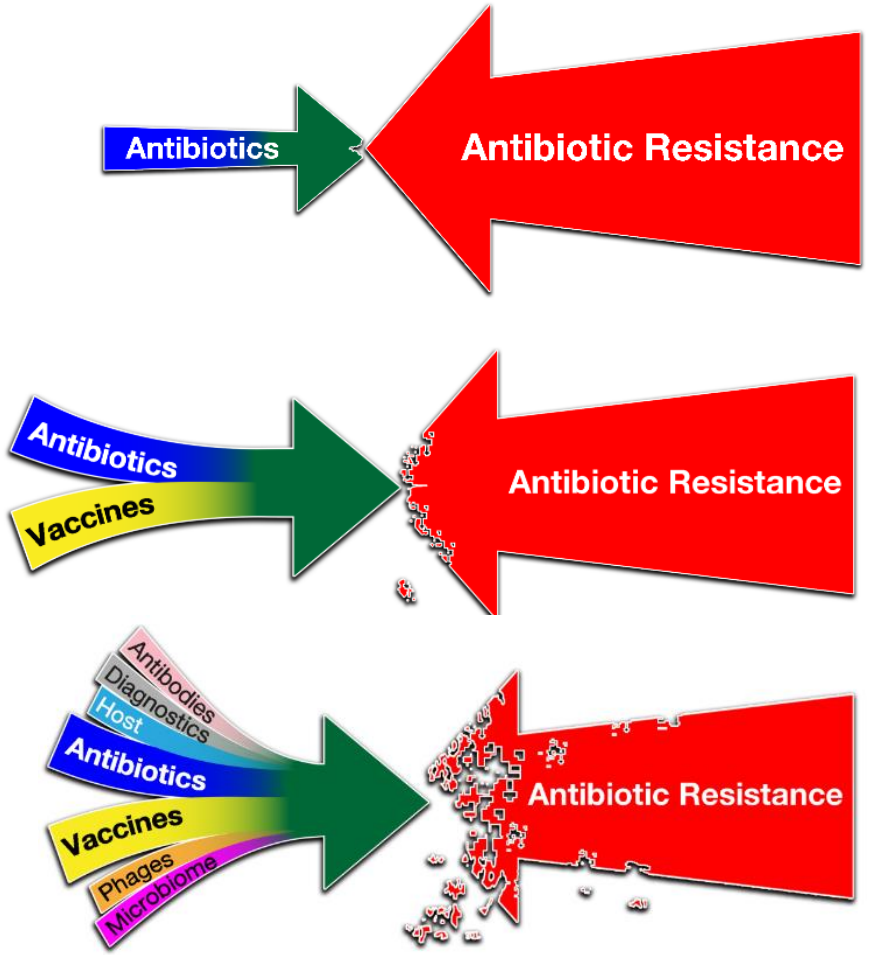
- mAbs for tackling **health challenges**
- mAbs for addressing **pandemic preparedness**
- mAbs for developing new **research tools**

## Next challenges

- Deliver **mRNA-encoded mAbs**
- Bring mAbs to **those in need**
- Promote **equitable access to mAbs**
- mAbs for **defining correlates of protection and assist vaccine design**

# Tackling AMR requires a joint effort

- AMR is a hard challenge for antibiotics alone
- Vaccines and Antibiotics together have a better chance to control AMR
- By joining forces we can control AMR




Source: Bloom et al, Antimicrobial resistance and the role of vaccines. PNAS 2018 115:51 12869. Adapted with permission of the authors.

# Acknowledgements



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