# Nebulized Bacteriophage D29 Provides Prophylactic Protection Against *Mycobacterium tuberculosis* Aerosol Challenge in a Preclinical Mouse Model

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## Introduction

**Tuberculosis** has led to the **most deaths worldwide** of any infectious disease over the last four years [1]

***Mycobacterium tuberculosis*** (*Mtb*) is becoming increasingly **drug-resistant** and **BCG vaccine has limited effectiveness**

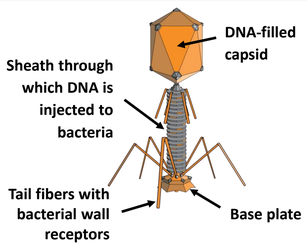
**Bacteriophage** (phage) D29 is a parasitic virus of mycobacteria, including *Mtb,* and infects and **lyses regardless of drug-resistance**

**Aerosolization** with a **vibrating mesh nebulizer** resulted in less phage D29 inactivation than a jet nebulizer [2]

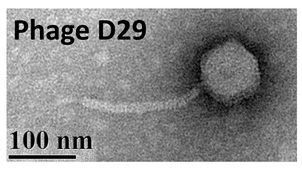
**Nose-only inhalation** exposure provides most **uniform aerosol deposition** in the **lungs of mice** [3]

**Prophylactic** phage D29 delivery to the lungs may allow for lysis of *Mtb* **before granuloma formation**

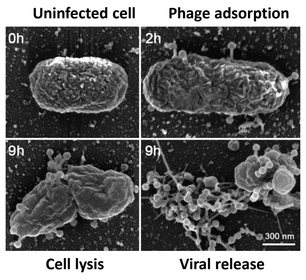
## Materials & Methods



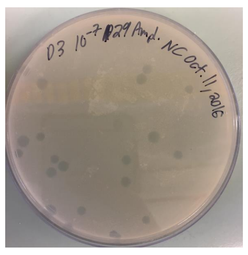

Morphological components of tailed phage [4]



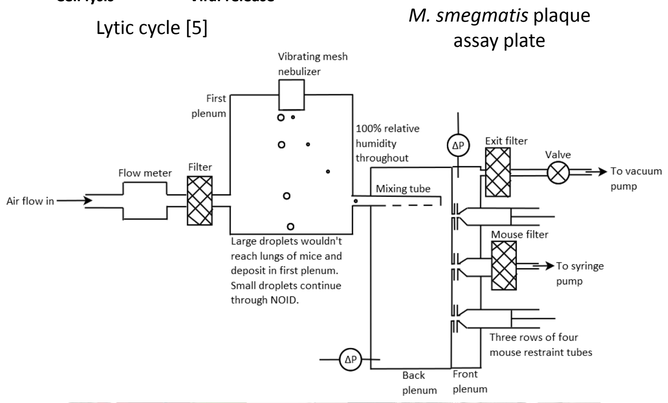

TEM of *siphoviridae* phage D29 [2], which was amplified on *Mycobacterium smegmatis* to a titer of 1.6x10$^{12}$ PFU/mL

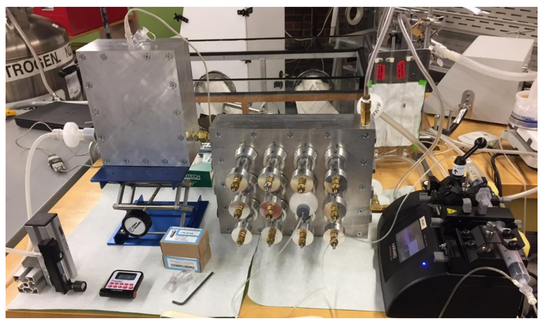



Lytic cycle [5]



*M. smegmatis* plaque assay plate



Schematic and picture of nose-only aerosol exposure system, set-up for dose simulation experiments with aerosolized tryptophan tracer; the air flow in was 500 mL/min to minimize convection of aerosol by the noses of the mice

Female C57BL/6 mice age 4-6 weeks, weighing 14-16 grams, have a minute ventilation of ~22 mL/min [6]; the mice were trained to remain calm in the restraint tubes.

*Mtb* H37Rv delivered to mice within 30 minutes of phage D29 pre-treatment using a Wisconsin-Madison aerosol chamber; low dose challenge delivered ~50-100 CFU and ultra-low dose challenge delivered ~5-10 CFU (image from [7])


Lung samples removed, placed in 5 mL buffer, homogenized, centrifuged, and plated for assay of D29 and H37Rv levels (image from [8])

## Results & Discussion

### Lung Homogenization Does Not Inactivate D29

- Phage D29 titer after lung homogenization was 12.08 ± 0.03 log(PFU/mL)
- Control titer before homogenization was 12.12 ± 0.04 log(PFU/mL)
  - No significant difference ($p > 0.5$; n=3 each)

➢ **Lung homogenization did not cause phage D29 inactivation**

➢ No innate tissue factor influencing phage activity or the properties of the plaque assay for phage quantification

### *Mtb* H37Rv Susceptible to Phage D29

- Control plate with *Mtb* (no phage added) incubated for 3 weeks resulted in 38 CFU
- Plate with *Mtb* and phage D29 addition (2 replicates) resulted in 1 CFU or 2 CFU

➢ **Phage D29 efficiently lyses *Mtb* H37Rv**

### Dose Simulation Matches *In Vivo* Experiment

**Dose Simulation**

- Model developed to predict number of phage D29 per alveolus, $T_{m/A}$, delivered with the nose-only exposure system
  - $T_0$ is the initial titer of the lysate input into the vibrating mesh nebulizer in PFU
  - $f_n$ is the fraction of the phage not inactivated by the nebulizer taken as 0.319 [2]
  - $f_i$ is the fraction of the breathing cycle spent inhaling approximated as 0.5
  - $f_m$ is the fraction of the aerosol emitted from the nebulizer that is inhaled by a single mouse from tryptophan tracer dose simulation experiments (from mouse filter results in Table 1)
  - $f_l$ is the fraction of aerosol that is inhaled by a mouse that reaches its lungs taken as 0.08 [9]
  - $A_m$ is the number of alveoli per mouse taken as 4×10$^7$ [10]

$$T_{m/A} = \frac{T_0 \cdot f_n \cdot f_i \cdot f_m \cdot f_l}{A_m}$$

Relatively high dose predicted to reach lungs of a mouse by nose-only inhalation based on tryptophan tracer deposition experiments and a mathematical model to simulate phage D29 delivery

| First plenum width (mm) | Nebulizer reservoir (%) | First plenum (%) | Mixing tube (%) | Back plenum (%) | Nosepiece & adapter (%) | Mouse filter (%) | Exit filter (%) | Unaccounted (%) | Predicted dose to a mouse $T_{m/A}$ (PFU/alveolus) |
|---|---|---|---|---|---|---|---|---|---|
| 95 | 1.0 ± 0.8 | 61.2 ± 7.5 | 1.9 | 5.1 ± 1.2 | 0.062 ± 0.008 | 0.013 ± 0.005 | 1.07 ± 0.05 | 30 ± 6 | 0.4 ± 0.2 |
| 32 | 1.2 ± 0.6 | 60.3 ± 0.4 | 3.2 | 6.3 ± 0.6 | 0.073 ± 0.004 | 0.033 ± 0.004 | 1.50 ± 0.023 | 27 ± 3 | 1.0 ± 0.1 |

➢ Use of smaller first plenum width resulted in **1 PFU/alveolus** predicted to be delivered

➢ Total dose reaching all 12 noseports was **~2.5%** of dose input to nebulizer

➢ Small standard deviation indicated **dosing was repeatable**

**_In Vivo_ Experiment**

Phage D29 dose in lungs of mice after delivery with nose-only inhalation device

| Time between exposure and euthanization (min) | Phage D29 dose in mouse lungs in log(PFU/mouse)* |
|---|---|
| 0 | 6.6 ± 0.3 |
| 30 | 7.3 ± 0.1 |
| 90 | 7.0 ± 0.4 |

*avg ± SD of n=3 mice per time point

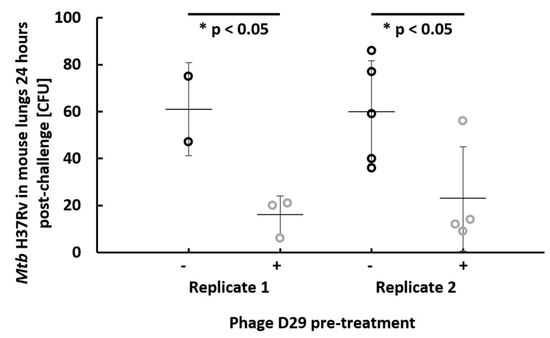
➢ Measured **dose *in vivo* matched dose simulation** for amount of phage nebulized

➢ **Phage active in lungs 90 minutes post-exposure**, indicating challenging mice with *Mtb* 30 minutes after phage exposure would be acceptable

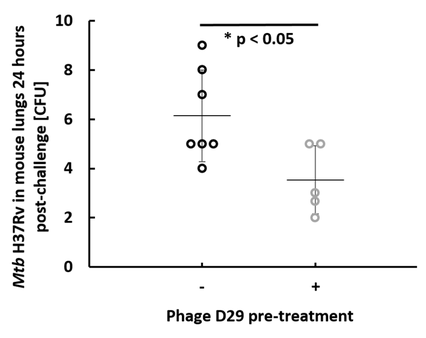
➢ **Higher dose** of phage in the lungs of mice **than in any other study** that used nose-only inhalation

### Prophylactic Respiratory Delivery of D29 Provides *In Vivo* Protection against *M. tuberculosis*

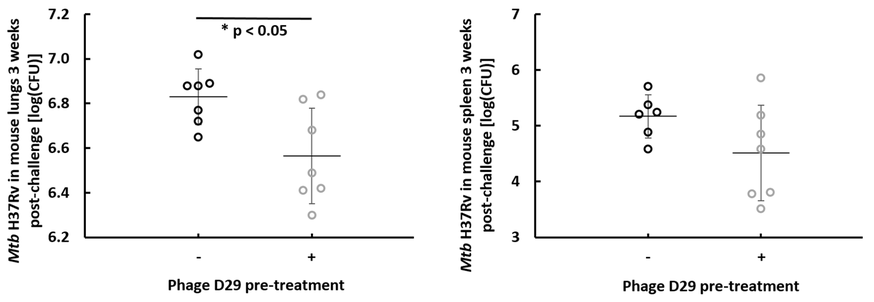
Approximately **1 PFU/alveolus**, or 7.6 log(PFU/mouse), **of phage D29** measured to be **delivered prior to all bacterial challenge experiments**.
***In vivo* results show a prophylactic effect.**




Prophylactic protection demonstrated by reduced bacterial burden 24 hours post-challenge with a low dose of H37Rv




Evidence of prophylactic protection against challenge with an ultra-low dose of H37Rv




Decrease in bacterial burden in the lungs still observed 3 weeks post-challenge

➢ ~67% reduction in bacterial burden was observed *in vivo* for pre-treatment with ~1 PFU/alveolus

➢ Predicted alveolar coverage according to Poisson statistics is 63%

➢ In agreement if **all bacteria that deposit in an alveolus in the presence of phage are inactivated**

➢ **Complete prophylaxis in humans appears possible** based on doses achievable and average PFU/alveolus predicted to be required by Poisson statistics

- Poisson statistics [11] useful for estimating the probability, $P$, that an alveolus will contain a certain number of phages, $x$, knowing the average number of PFU/alveolus, $\lambda$,

$$P = \frac{e^{-\lambda} \cdot \lambda^x}{x!}$$

Poisson statistics predictions of alveolar coverage of phage in the lungs of a mouse for different average PFU/alveolus.

| Average PFU/alveolus, λ | Alveolar Coverage |
|---|---|
| 0.001 | 0.1% |
| 0.01 | 1% |
| 0.1 | 10% |
| 0.5 | 39% |
| 0.7 | 50% |
| **1** | **63%** |
| 2 | 86% |
| 5 | 99% |
| 7 | 99.9% |
| ≥18 | Complete |

## Conclusions

➢ **Phage D29 appears promising for prophylactic protection against *Mtb***, exhibiting capacity to significantly reduce bacterial levels in the lungs of mice

➢ **Vibrating mesh nebulizer coupled with a nose-only exposure system** is a good choice for delivering phage D29 in animal exposure experiments; delivered on average **7.6 log(PFU) to lungs of each of 12 mice in ~20 minutes**

➢ **Lysis of bacteria in the alveoli by phage is effective**

➢ Development of **cocktails active against tuberculosis** is of interest; developed exposure system may be used to test efficacy of each anti-tuberculosis phage

➢ Large doses of nebulized mycobacteriophage cocktail aerosol to the lungs may be a valuable intervention to provide **extra protection to health care professionals** frequently exposed to infectious active cases of tuberculosis **and to individuals in regions with high tuberculosis transmission rates**

➢ **Complete prophylaxis in humans may be achievable** as higher doses could be more easily achieved; human clinical trials are of interest

## References

Further details regarding this poster is available in:
**Carrigy NB, Larsen S, Reese V, Pecor T, Harrison M, Kuehl PJ, Hatfull GF, Sauvageau D, Finlay WH, Coler RN, Vehring R. Prophylaxis of *Mycobacterium tuberculosis* H37Rv Infection in a Preclinical Mouse Model via Inhalation of Nebulized Bacteriophage D29. *Antimicrobial Agents and Chemotherapy.* Submitted April 26, 2019.**
[1] World Health Organization. Global tuberculosis report 2018. Available from: https://www.who.int/tb/publications/global_report/en/
[2] Carrigy *et al.* 2017. Anti-tuberculosis bacteriophage D29 delivery with a vibrating mesh nebulizer, jet nebulizer, and soft mist inhaler. *Pharm Res* 34:2084-2096.
[3] Leong *et al.* 1998. Quantitative morphometric analysis of pulmonary deposition of aerosol particles inhaled via intratracheal nebulization, intratracheal instillation or nose-only inhalation in rats. *J Appl Toxicol* 18:149-160.
[4] Image from: https://en.wikipedia.org/wiki/Bacteriophage
[5] Sabehi *et al.* 2012. A novel lineage of myoviruses infecting cyanobacteria is widespread in the oceans. *PNAS* 109:2037-42.
[6] Fairchild. 1972. Measurement of respiratory volume for virus retention studies in mice. *Appl Microbiol* 24:812-818.
[7] Image from: http://www.sacmm.org/pdf/SOP/3%20Guinea%20Pig%20Inhalational%20Pulmonary%20Aspergillosis%20Version%201_1.pdf
[8] Image from: https://www.omni-inc.com/omni-prep.html
[9] Nadithe *et al.* 2003. Evaluation of nose-only aerosol inhalation chamber and comparison of experimental results with mathematical simulation of aerosol deposition in mouse lungs. *J Pharm Sci* 92:1066-1076.
[10] Soutiere *et al.* 2004. Differences in alveolar size in inbred mouse strains. *Respir Physiol Neurobiol* 140:283-291.
[11] Wheeler, Ganji. 2010. *Introduction to engineering experimentation, 3rd edition.* Pearson Higher Education, Upper Saddle River, NJ, USA.

## Acknowledgements

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