Using Viruses to Select for Reduced Virulence of Bacterial Pathogens in Human Patients

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Microbiology Faculty, Yale School of Medicine.
Virus genetics, genomics and evolution (current study systems)

- vesicular stomatitis virus
- rhinovirus
- dengue virus
- Sindbis virus
- chikungunya virus
- phage phi-6
- phage M13
- phage OMKO1
Evolutionary Trade-offs

• Evolution involves *compromises*.

• Natural selection can improve one trait at the expense of reduced performance in another trait.
Evolutionary Trade-offs

- Evolution involves *compromises*.

- Natural selection can improve one trait at the expense of reduced performance in another trait.
Virus example: phage phi-6 infection of plant pathogenic bacteria

- Infects *Pseudomonas syringae* bacteria
- Initiates lytic (lethal) infection cycle by binding to pilus

*P. syringae* bacteria attached to leaf surface

Phage particles attached to host type-IV pilus

*P. syringae* host cell
Resistance to phage phi-6 reduces *P. syringae* virulence

- *P. syringae* can evolve resistance to phi-6 by mutational loss of pilus

Pilus loss reduces conditional virulence (cannot traverse leaves, but still grows in plant) *Evolutionary Trade-off*
Resistance to phage phi-6 reduces *P. syringae* virulence

- *P. syringae* can evolve resistance to phi-6 by mutational loss of pilus

Pilus loss reduces conditional virulence (cannot traverse leaves, but still grows in plant)

*Evolutionary Trade-off*

**Shows phages can select for REDUCED virulence in plant-pathogenic bacteria**
Can phage therapy similarly exploit evolutionary trade-offs?
Antibiotic Resistance

- Increasing proportion of bacteria show resistance to common antibiotics
- Global problem
Phage Therapy

- Phages are viruses that kill only bacteria
- Alternative to chemical antibiotic drugs
Phage Therapy

• **Problem**: bacteria can evolve phage resistance, similar to antibiotic failure

• *Can we develop a strategy that works, although phage resistance is inevitable?*

Antibiotic resistance mechanisms
Sherrard et al. (2014)

Phage resistance mechanisms
Seed (2015)
Our Approach to Phage Therapy

• **Innovation**: discover phages that attack bacteria by binding to virulence factors

• **Selects for bacteria to evolve phage resistance by compromising virulence (evolutionary trade-off)**

Can evolved change cause reduced virulence?

Selects for increased phage resistance
Our Approach to Phage Therapy

• Many bacterial structures serve as virulence factors
Our Approach to Phage Therapy

- We are discovering phages that bind to these structures
Example: *Pseudomonas aeruginosa*

- Antibiotics are failing and multi-drug resistant (MDR) bacteria pathogens are on the rise
- MDR *Pseudomonas aeruginosa* is a priority pathogen (World Health Organization, 2017)
- Hospital-acquired infections with high mortality rate, especially immune compromised people
- Cystic fibrosis, severe burns, infected prosthetics
Example: *Pseudomonas aeruginosa*

- Efflux pumps are transport proteins that remove wide variety of drugs from the cell
- Also function in host colonization, immune escape and biofilm formation

![Diagram of P. aeruginosa biofilm](image)
Example: *Pseudomonas aeruginosa*

- In 2016, we discovered phage OMKO1 where outer protein (OprM) of MexAB and MexXY efflux pumps important in binding.

- Found in Dodge Pond lake, Connecticut, USA.

Bioinformatics analysis shows oprM gene under strong **stabilizing selection**.

Chan et al. 2016, *Scientific Reports*
Example: *Pseudomonas aeruginosa*

- Phage OMKO1 (*Myoviridae*) forces bacteria to trade phage resistance for antibiotic sensitivity

**Antibiotic Resistance/Virulence**

**Phage Resistance**

Chan et al. 2016, *Scientific Reports*
Measuring Change in Antibiotic Sensitivity

- Minimum inhibitory concentration (MIC)

Growth of MDR strain in presence of drug (no zone of inhibited growth)

Growth of phage-resistant MDR strain; kill zone measures MIC

Chan et al. 2016, *Scientific Reports*
### Efficacy of Discovery in Clinical, Environmental and Model-Strain Isolates

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* Chan et al. 2016, *Scientific Reports*
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<tr>
<td>Ampicillin</td>
<td>Penicillin</td>
<td>&gt;256</td>
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ONLY drugs exported by **Multi-drug efflux (Mex) pump system** were affected.

* > 1 isolate showed reversal from clinical resistance to susceptibility (EUCAST 2015 breakpoints)

Chan et al. 2016, *Scientific Reports*
Efficacy of Discovery in Attacking Biofilms

Phage + antibiotic synergy against biofilms
Efficacy of Discovery in Attacking Biofilms

CAZ = ceftazidime
CIP = ciprofloxacin

Chan et al. 2018 Evolution, Medicine & Public Health
Efficacy of Discovery in Mouse Lung-Infection Model

P. aeruginosa lung pneumonia model
(see Lawrenz et al. 2015 Pathogens & Disease)

Results:

Goal: Test safety/efficacy of phage OMKO1 in leukopenic mouse model of MDR P. aeruginosa respiratory disease.
Efficacy of Discovery in Mouse Lung-Infection Model

*P. aeruginosa* lung pneumonia model
(see Lawrenz et al. 2015 *Pathogens & Disease*)

Results:

Phage generally improves rescue, regardless of meropenem dosage:

Kortright, Warawa, Lawrenz, Chan et al. (unpublished)
Efficacy of Discovery in Human Volunteer

CASE 1

• Phage + ceftazidime used to treat patient infected by MDR *P. aeruginosa*

• U.S. FDA approved experimental therapy

Chan et al. 2018 *Evolution, Medicine & Public Health*
See also: Zimmer, STAT Online News 2016;
NPR Science Friday, 2016, 2018

*aortic arch replacement*

*intraoperative photo*
Efficacy of Discovery in Human Volunteer

CASE 1

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- U.S. FDA approved experimental therapy

CT image showing infected collection and site of targeted aspiration during therapy

Chan et al. 2018 *Evolution, Medicine & Public Health*
See also: Zimmer, STAT Online News 2016; NPR Science Friday, 2016, 2018
Efficacy of Discovery in Patient Lung-Infection Volunteer

CASE 2
- Dec 2017: U.S. FDA approved experimental phage therapy
- 22 year-old female with cystic fibrosis and failing pulmonary function

Pre Therapy (day 0)
_________________________
64% lung function
70% exercise performance
High bacterial density (4+)
in lung
*TDR (total drug resistant)*
*bacteria in sputum*

| Resistance of *P. aeruginosa* lung community pre-treatment: 4+ | Aminoglycoside | Amikacin | R | R |
| | Gentamycin | R | R |
| Fluoroquinolone | Ciprofloxacin | R | R |
| | Levofloxacin | R | R |
| Cephalosporin | Ceftazadime | R | R |
| | Cefepime | R | R |
| Beta lactam | Piperacillin | R | R |
| | Imipenem | R | R |
| | Meropenem | R | R |
| | Aztreonam | R | R |
| Polymixin | Colistin | S | R |

Kanu, Chan et al. (submitted)
Efficacy of Discovery in Patient Lung-Infection Volunteer

Treatment and post-treatment observations:

• 3mL doses every 10 days; first dose in hospital and remainder at home

• On day 2, reported significant increase in produced mucus and coughed up numerous plugs; this had not been reported in years.

• On Day 3, thinner mucus no longer green in color and notable absence of cough.

• One week after final dose, notable increased energy (parents observed changed attitude).

• Two weeks after last dose, joined a gym (!).

• Three weeks after last dose, lungs sounded clearer on exam than ever previously reported.

Kanu, Chan et al. (submitted)
Efficacy of Discovery in Patient Lung-Infection Volunteer

Treatment and post-treatment observations:

FEV1: expiratory volume exhaled during first forced breath

Kanu, Chan et al. (submitted)
## Efficacy of Discovery in Patient Lung-Infection Volunteer

### Sensitivity of *P. aeruginosa* lung community post-treatment: 1+

<table>
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<th>Sensitivity (R for Resistant, S for Sensitive)</th>
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<td>Amikacin</td>
<td>R, R</td>
</tr>
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<td></td>
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### Post-treatment observations:

- **Post Therapy (day 8)**
  - 80% lung function
  - 90% exercise performance
  - Low bacterial density (1+)
    - in lung

**ANTIBIOTIC SENSITIVE**

*bacteria in sputum***

Kanu, Chan et al. (submitted)
Efficacy of Discovery in Patient Lung-Infection Volunteer

CASE 3 (Sept 2018)
• 70 year-old male with chronic obstructive pulmonary disease (COPD)
• Small cell lung cancer diagnosis (2016) and history of MDR *P. aeruginosa* infections
• One lung (massive abscess) needed removal but fear of infection spread to other lung

Efficacy of Discovery in Patient Lung-Infection Volunteer

CASE 3 (Sept 2018)

- 70 year-old male with chronic obstructive pulmonary disease (COPD)
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- One lung (massive abscess) needed removal but fear of infection spread to other lung
- Treated with phage H6: binds type-IV pilus and selects against pyocyanin production

![Chronic Obstructive Pulmonary Disease (COPD)](chart.png)

**Relative Pyocyanin/Cell after 48 hrs**

- **Cip** = ciprofloxacin
- **Caz** = ceftazidime
- **Ery** = erythromycin

Chan et al. (unpublished)
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• 10-day nebulizer treatment: reduced infection $10^4$-fold; reduced $O_2$ dependency; undetectable pyocyanin

---

**COPD**

- **Chronic Bronchitis**
  - Healthy
  - Inflammation & excess mucus

- **Emphysema**
  - Healthy
  - Alveolar membranes break down

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• 71 year-old male retired firefighter with history of bronchiectasis
• Lung damaged by significant smoke inhalation 42 years ago during building fire
• History of MRSA and MDR *P. aeruginosa* infections, causing pulmonary exacerbations
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• Nebulizer treatment: 7 days using phage OMK01, followed by phage H6
• 2 log decrease in PA after first 48 hrs; discharged Oct 12 and follow up in Dec

Chan et al. (unpublished)
Clinical Trials / Future Applications

- **Seeking Investigational New Drug status and broad approval for clinical trials targeting MDR P. aeruginosa:**
  - Cystic fibrosis associated pulmonary infections
  - Hospital acquired pneumonia
  - Catheter-associated urinary tract infections
  - Burns

Phage OMK01, TEM image by K. Kortright
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• **Breadth of applications:**
  - Emergency compassionate-care therapy
  - Prophylaxis
Future Work

Basic research on synergy between MDR *P. aeruginosa* and antibiotics:

- *Genetic mechanisms of bacteria/phage resistance/sensitivity*
- *Experimental (co)evolution*
- *Pharmacodynamics, pharmacokinetics and minimal dosing*
- *Interactions with human immune system*
- *Longitudinal studies in treated patients*
Other Bacterial Targets for Discovering Phage-based Trade-offs

- *Pseudomonas aeruginosa*
- *Vibrio cholerae*
- *Streptococcus pneumoniae*
- Pathogenic *Escherichia coli*
- *Klebsiella pneumoniae*
- *Shigella strains*
- *Salmonella strains*
- *Enterobacteriaceae*, ESBL-producing*

*World Health Organization (2017) priority pathogens*
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Sampling Locations

- Sewage treatment plants, USA
- Caribbean
- East Africa
- Cuatro Cienegas, Mexico
- Refugee Trails / Camps
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Turner Lab

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Matthew Lawrenz  
Jon Warawa  
Adaobi Kanu, MD

Project High Hopes

NSF

ANR

BEACON

Yale Innovation