Combining wet lab and computational simulations to predict optimal antibiotic drug regimens for *Mycobacterium tuberculosis*

Denise Kirschner and Jennifer Linderman  
University of Michigan Medical School  

JoAnne Flynn  
University of Pittsburgh School of Medicine  

Veronique Dartois  
Rutgers University  

Systems biology mechanisms funding this work from NIH/NHLBI:  
MSM : 1U01HL131072
Tuberculosis (TB):
Infectious disease caused by *Mycobacterium tuberculosis* (Mt) and new infections occur at a rate of one per second.

*One–third of the world’s population is infected with Mt, and 3 people die every minute, i.e. 2 million deaths/year.*
Granulomas are a result of multi-scale dynamics in both space & time.
GranSim (2D/3D)

Cellular/tissue scale Model--
A stochastic **model** that captures discrete cellular dynamics via a set of well-described interactions between immune cells and Mtb leading to tissue scale outcomes

**Leads to “emergent behavior”**

*Segovia-Juarez et al. J. Theor Biol. 2004*
*Ray et al, J. Immunol. 2009*
Experimental & computer generated granuloma

A.

- red - CD68
- green - CD3
- blue - calprotectin

caseum
Granuloma formation

*Calibrated to NHP data
*Simulation begins with a single infected macrophage with one intracellular bacteria

*Video simulates 200 days of infection

*2mmx2mm lung tissue
Visualizing simulated molecule chemical gradients at day 50 post-infection for one simulation (using DataTank).

Viewing chemical gradients in 3D space 50 days post infection

BLACK: a 2D, yz-slice at x=50,

GREEN: we use 3D level sets to depict the 3D spatial distributions of two biologically-relevant TNF concentrations for macrophage recruitment.

*TNF concentration is 0.05 or greater (i.e. level set = 0.05); this is the minimum concentration that macrophages can sense, a concentration below this value is not detectable by macrophages.

PURPLE: TNF concentration is 50 or greater (i.e. level set = 50); this is the maximum concentration that macrophages can sense.
Why is TB so hard to treat?

1. Mycobacterium
   - Slow growing bacterium
   - Acquires antibiotic resistance

2. Granulomas
   - Granulomas present physiological barrier to antibiotic diffusion

3. Patient-unfriendly treatment
   - Long -9 months
   - multiple drugs given
   - many side-effects
   - 4-drug standard regimen: INH, RIF, PZA, EMB

Antibiotic plasma concentration

\[ \text{Oral Dose} \xrightarrow{k_a} \text{Transit 1} \xrightarrow{k_a} \text{Transit 2} \xrightarrow{CL} \text{Plasma} \xrightarrow{Q} \text{Peripheral Tissue} \]

Antibiotic tissue concentration in granuloma simulation

Bacterial death from local concentration

\[ \text{Drug concentration} \xrightarrow{E_{\text{max}}} \text{Antibiotic killing rate constant} \]

\[ 0.5E_{\text{max}} \xrightarrow{\text{Hill constant}} \]

2. Predicting granuloma antibiotic exposure

Orange trace – INH concentration over time
Purple trace – Total bacteria over time

Concentration oscillates between above and below effective concentrations

Antibiotic exposure inside granuloma much lower than outside

Antibiotic spatial dynamics during daily dosing

RIF
Color scale: 0 – 10 mg/L

INH
Color scale: (0 – 0.5 mg/L)
Can we predict what can improve drugs? i.e. which PK and PD properties are good targets for modification?

OUTCOMES

Use sensitivity and uncertainty analysis to determine this
3. What’s the best antibiotic regimen?

- Current regimen is 4 drugs 6-9 months
- Too many options to test
  - Clinically or computationally
- Optimization problem

Regimen design space (RDS)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment segments (M)</td>
<td>2</td>
</tr>
<tr>
<td>Number of drugs (c)</td>
<td>10</td>
</tr>
<tr>
<td>Drugs per segment (n)</td>
<td>4</td>
</tr>
<tr>
<td>Dose (D, mg/kg)</td>
<td>5</td>
</tr>
<tr>
<td>Frequency (F, week(^{-1}))</td>
<td>7</td>
</tr>
</tbody>
</table>

Number of possible regimens:

\[
RDS = \left( \binom{c}{n} (D \times F)^n \right)^M = 9.9 \times 10^{16}
\]

Cicchese et al. CMBE (2017)
Defining the optimization problem

Regimen of \( n \) antibiotics (2\( n \)-dimensional)

\[ x = \left\{ D_1, F_1, D_2, F_2, \ldots, D_n, F_n \right\} \]

Antibiotic 1 Dose
Antibiotic 1 Dose Frequency

INH and RIF regimen (4-dimensional)

\[ x = \left\{ 10 \text{ mg/kg, 7 wk}^{-1}, 15 \text{ mg/kg, 2 wk}^{-1} \right\} \]

INH
- 10 mg/kg given
- 7 times per week

RIF
- 15 mg/kg given
- 2 times per week

Objective function is a function of model outputs

- Time to sterilize granuloma of bacteria
- Antibiotic dose
- Measures of resistance
- Potentially more

Cicchese et al. CMBE (2017)
Optimization algorithm options

Genetic Algorithm

1. Initialize population

2. Population evolution
   i. Evaluation fitness
   ii. Select parents
   iii. Generate new population
   iv. Check stopping criteria

3. Solution found

Surrogate-Assisted Optimization

- Sample Points
- Unknown Function
- Prediction (from sample points)

Forrester et al. (2008)
Test problems for comparing algorithms

- Single-antibiotic test problems, optimize dose and frequency
- Based on published simulations (Pienaar et al. *BMC Syst. Biol.* (2015))
- Generate objective function surface based on simulation output
- Use to test performance of optimization algorithms
  - Accuracy: distance from the known solution
  - Efficiency: how much of the design space sampled

\[ f(x) = \frac{t_c(x)}{t_{c,\text{max}}} + \frac{D(x)}{D_{\text{max}}} \]

\[ f = \text{(time to clear)} + \text{(dose)(freq.)} \]

INH test problem surface

Cicchese *et al.* Cellular and Molecular Bioengineering (2017)
GA most accurate in predicting solutions

Surrogate-Assisted Optimization

- Each algorithm solved the test problem with 30 independent optimizations
- Each “x” corresponds to the estimated optimal solution

Genetic Algorithms

Cicchese et al. CMBE (2017)
Surrogate-assisted optimization requires fewer function evaluations

<table>
<thead>
<tr>
<th>Optimization method</th>
<th>Average number of function evaluations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surrogate-assisted optimization</td>
<td>21</td>
</tr>
<tr>
<td>Default genetic algorithm</td>
<td>3,855</td>
</tr>
<tr>
<td>Relaxed genetic algorithm</td>
<td>347</td>
</tr>
</tbody>
</table>

With modifications, surrogate assisted models can efficiently predict optima with sufficient accuracy

Cicchese et al. CMBE (2017)
Predicted optimal regimens for 2 drugs

Identifies two major regions:
1. 20-25 mg/kg INH daily, low dose RIF twice a week
2. 20-25 mg/kg INH weekly, ~15 mg/kg RIF daily

*Major regions similar to human equivalent doses for CDC regimens

Surrogate model predictions are fast, accurate and relevant

Cicchese et al. CMBE (2017)
1. GranSim

2. /PD of Antibiotics

3. Surrogate-assisted optimization
   - Test different regimens
   - Objective function
   - Resample regimen space
   - Build surrogate model

4. Predicted optimal regimen(s)

5. Test in virtual treatment trial

6. Animal model testing
Capturing Multiple granulomas-

A multi-scale systems pharmacology approach to tuberculosis therapy

Joseph M. Cicchese¹, Véronique Dartois², JoAnne L. Flynn³, Denise E. Kirchner⁴, Jennifer J. Linderman¹

¹Department of Chemical Engineering, University of Michigan, Ann Arbor, Michigan, USA
²Public Health Research Institute and New Jersey Medical School, Rutgers, The State University of New Jersey, Newark, New Jersey, USA
³Department of Microbiology and Molecular Genetics, University of Pittsburgh, Pittsburgh, Pennsylvania, USA
⁴Department of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor, Michigan, USA

Introduction

Tuberculosis (TB) is a bacterial infection, primarily affecting lung tissue, that is considered one of the world’s deadliest infections. Caused by infection with the pathogen Mycobacterium tuberculosis (Mtb), standard treatment of TB often requires six months or longer of multiple antibiotics. Length of treatment and the emergence of drug-resistant TB indicate a need for better antibiotic regimens.

Granuloma formation in lung tissue during infection leaves that forms due to immune response - hallmark of TB, location of Mtb - physical barrier to antibiotic diffusion

Design of better regimens requires modeling at multiple scales:
1. Molecular scale - pharmacokinetic (PK) and pharmacodynamic (PD) models to predict antibiotic distribution in granulomas, secretion and diffusion of cytokines (TNF, IL-10, etc.)
2. Cellular and tissue scale - agent-based models to simulate cellular interactions and rules, with emergent granuloma formation representing tissue-level structure
3. Whole-body - combination of granuloma simulations through linked antibiotic PK to simulate treatment of infected host

Using computational modeling and surrogate-assisted optimization algorithms, we can assess the regimen design space for optimal therapies and test efficacy in a virtual clinical trial.

Modeling granuloma formation and PK/PD

Plasma PK compartmental model

Granuloma agent-based model

PD model - killing rate constant based on RC curve

Optimizing antibiotic regimens

Surrogate-assisted optimization of antibiotic regimens

Optimization algorithms predict better treatments to simulate

Antibiotic Treatment Vector

x = [t₁, t₂,...] 

Oral dose and dosing frequency of antibiotics in regimen

Treatment simulation determines optimal effective and efficacy

Multi-objective optimization

A single-objective function that simultaneously attempts to minimize sterilization time and weak antibiotic dose shows how changing dose-size and dosing frequency affects regimen optimality. Black dots represent regimens used to build surrogate, the green circle is the surrogate-predicted optimum compared to the true global optimum (red circle).

Predicting in vivo antibiotic distributions

Parameters of PK models are fit to capture the range of antibiotic concentrations observed in vivo.

(A-D) Black dots represent experimentally measured antibiotic concentrations in granulomas after oral dosing in human volunteers (INH, RIF and PZA) or rabbits (EMS) (Fig. D). Blue-shaded regions are range of simulation, with the solid blue line representing the simulation’s mean concentration based on varying plasma PK parameters.

(E-H) Spatial distribution of the antibiotic in the agent-based simulation around the time of peak average granuloma concentrations (3 to 4 hours depending on the antibiotic). Lighter shades indicate higher concentrations. Many antibiotics have lower concentrations in granulomas, or fail to diffuse completely into the granuloma.

First-line TB Antibiotics:

INH = isoniazid

RIF = rifampin

EMS = ethambutol

PZA = pyrazinamide

Conclusions

- Multi-scale modeling of granuloma formation and antibiotic treatment combines agent-based models of cellular interactions, pharmacokinetic models involving ODE’s and PDG’s, and optimization algorithms to search for better regimens.

- The model quantitatively captures range of antibiotic concentration experimentally observed, as well as qualitative spatial distributions.

- Surrogate-assisted optimization provides an efficient way to search for optimal antibiotic regimens.

- Treatment simulations can be used to compare antibiotics in sterilizing granulomas. Grouping granulomas together into hosts provides a computational framework to compare regimens through virtual clinical trials.

References

Acknowledgments

Fantastic Collaborators:
• Simeone Marino, (U of M)
• Elsje Pienaar, (Purdue)
• Veronique Dartois (Rutgers)
• Mark Miller (Washington University)
• JoAnne Flynn, Ling Lin, Josh Matilla,
• Hannah Gideon (Univ of Pitt)

LAB MEMBERS:
• Joseph Cicchese
• Louis Joslyn
• Paul Wolberg
• Tim Wessler
• Caitlin Hult
• Stephanie Evans
• Marissa Renardy
• Joe Waliga

*Generous funding from the NIH

U01 (NHLBI)
Publish your QSP in JTB and BMB!