Date of Submission: 2018-10-24

Principal Investigators: Elsje Pienaar
Department: Biomedical Engineering

Project Area: Disease progression and treatment

Project Title: Patient derived mechanistic simulations of mycobacterial infections

Goal/Specific Aim:
The goal of this proposal is to simulate mycobacterial infections within virtual lung lesions based on CT scans. These simulations will provide preliminary data for an R01 proposal that, in collaboration with Mayo clinic physician Dr. Patricio Escalante, will simulate and optimize antibiotic treatment in these virtual lesions. Within the scope of this Showalter award, we will 1) build simulation boundaries based on lung lesion CT scans, and 2) find parameter combinations that produce stable infections within these lesion boundaries.

Brief Background:
Prevalence of pulmonary disease due to non-tuberculous mycobacteria (NTM-PD) in the United States has doubled in the last 10 years and the disease outnumbers tuberculosis (TB) in many developed countries 1-4. In 2010, 86,000 cases of NTM-PD in the United States resulted in $815 million in direct medical costs 5. Yet, these important pathogens remain vastly understudied compared to TB 6.

NTM-PD typically manifests as lung lesions where the bacteria reside and antibiotics must act. The structure of these lesions affect drug susceptibility as well as antibiotic efficacy7. Treatment requires a cocktail of at least 3 antibiotics for >12 months and efficacy remains as low as 40%-8-11. The complex nature of the disease and necessary combination therapy makes it challenging and expensive to systematically optimize treatment regimens in animal models or clinical trials 8,12-14. Therefore, regimen recommendations remain largely based on expert opinion, informed by TB studies. These practical challenges in NTM-PD regimen development are likely to persist and therefore require innovative approaches to treatment optimization.

Mechanistic computational simulations are well-suited to leverage available in vitro, animal and human data to make actionable predictions in complex biological systems. Such simulations can extrapolate predictions over time and for new treatment approaches, and incorporate diverse data from other fields like microbiology, pharmacology and immunology. We have previously used such simulations of antibiotic treatment in TB to inform regimen design and successfully predict relative efficacy of different fluoroquinolone antibiotics 15-17. However, it is vital that virtual lesions accurately represent the size, structure and dynamics of lung lesions in NTM patients. Our long-term goal is therefore to use mechanistic simulations of NTM infection and treatment to optimize treatment regimens and predict patient responses based on their CT scans, bacterial load measurements and therapeutic drug monitoring.

Approach and Methodology:
Within this Showalter Award we propose to develop the computational capacity to simulate chronic NTM infection within virtual lesions generated from patient CT scans.

1) Define simulation boundaries using CT scans
My lab has developed an agent-based computational model (ABM) of NTM infection in the lung. ABMs are stochastic models that track individual agents (cells), define interaction rules and produce emergent behavior at the population level. Our ABM currently tracks individual mycobacteria, macrophages and T cells as well as their interactions including bacterial growth,
phagocytosis, host cell recruitment, and bacterial killing. Current simulations define virtual infections in regular 3D boundaries e.g. cubes (representing subsections of lung parenchyma) or cylinders (representing bronchioles) (Figure 1). Here, we will expand this capacity to define simulation boundaries using CT scans of lung lesions.

We will take advantage of a published database of >10,000 CT scans with annotated lesions, including >2300 lung lesions (Figure 2). We will use available scripts to convert CT images to 3D volumes in open-source software 3D Slicer (slicer.org). We will use these volumes to define lesion outlines in the ABM environment. For bronchiectatic lesions, the outlines will be defined by virtual epithelial cells and virtual immune cells and bacteria will populate, move and interact in the lesion interiors. For nodules, the entire lesion volume will be populated by a mixture of bacteria and immune cells (macrophages and T cells).

2) Simulate chronic NTM infection within CT-based lesions

In order to emulate chronic infection our simulations must maintain stable infections in cell numbers, bacterial load and lesion geometry in the absence of treatment. We will identify model parameters that produce stable infections by sampling the multi-dimensional parameter space and simulating infection over 6 months (typical window between clinic visits). To account for uncertainty in lesion composition we will sample model parameters (bacterial growth rate, rate of infection, etc.) as well as initial conditions (T cell:macrophage ratio, number and location of bacteria etc.). Feasible parameter ranges will be based on in vitro and animal NTM studies where available, and further informed by related studies in TB.

Significance:

With this preliminary data we will be well positioned to submit an R01 proposal in collaboration with Dr. Escalante at the Mayo clinic. In the R01 we will propose to incorporate NTM-PD patient CT-scans from the Mayo clinic and to simulate treatment within these virtual lesions. This research is in line with NIH NTM and mycobacterial research priorities (NOT-AI-17-016, PAR-16-254). The methodology developed within the Showalter award will be the first to integrate diagnostic imaging with mechanistic simulations, and will provide the foundation for future applications to other mycobacterial pathogens, drug screening and virtual clinical trials.

Past Showalter Awardee: No

Will the project involve use of: human subjects or vertebrate animals? No
References:


NAME: Elsje Pienaar

eRA COMMONS USER NAME (credential, e.g., agency login): epienaar

POSITION TITLE: Assistant Professor

EDUCATION/TRAINING (Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)

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<td>BSc</td>
<td>12/2004</td>
<td>Financial and Actuarial Mathematics</td>
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<tr>
<td>University of Nebraska, Lincoln, NE</td>
<td>MS</td>
<td>08/2007</td>
<td>Chemical and Biomolecular Engineering</td>
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<td>University of Nebraska, Lincoln, NE</td>
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<td>Linkoping University, Linkoping, Sweden</td>
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<td>Postdoc</td>
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A. Personal Statement

My expertise is in designing and building multi-scale computational models of biological systems. My models have spanned molecular to population scales, including spatio-temporal representations of biological mechanisms like receptor-ligand interactions, intracellular signaling, molecular transport in tissues, host-pathogen interactions, host immune responses, drug dynamics and community infrastructure. I have successfully applied these methods to predict improved treatment regimens for tuberculosis, including multiple antibiotics. My computational approaches include deterministic and stochastic models, as well as hybrid models combining ordinary and partial differential equations with individual-based models. While my focus has thus far been on infectious diseases, my computational experience translates to a variety of fields studying complex biological systems. Finally, my research experience has been in close collaboration with experimental scientists, including microbiologists, chemical engineers, immunologists and physicians. Through such collaborations, I have addressed challenges of data integration across experimental systems, especially in biological systems that often produce noisy data. My interdisciplinary training and publication record in systems biology and systems pharmacology speak to my expertise and dedication to successfully complete the proposed project to simulate non-tuberculous mycobacterial pulmonary disease in CT scan-based lesion boundaries.


B. Positions and Honors

Positions and Employment
2015-2017 Research Investigator, Microbiology and Immunology, Chemical Engineering, Univ. of Michigan
2017- Assistant Professor, Weldon School of Biomedical Engineering, Purdue Univ.

Other Experience and Professional Memberships
2009- Member, American Institute of Chemical Engineers
2013-2017 Member, University of Michigan Postdoctoral Association
2014- Member, Biomedical Engineering Society
2015-2017 Member, University of Michigan College of Engineering Postdoc Advisory Group

Honors
2009 NIH-NIAID Scholarship, Keystone Symposia
2010 Outstanding Doctoral Dissertation Award, UNL College of Engineering
2014 Innovation and Career Development Award, Biomedical Engineering Society

C. Contributions to Science

1. TB treatment
   There are many antibiotics available to treat tuberculosis (TB), and these antibiotics need to be given in combination to reduce the risk of drug resistance development. Each antibiotic can be given at different dose sizes and frequency, and it is impossible to intuit which antibiotic combinations, doses and frequencies are optimal. The design of antibiotic regimens is further complicated by the TB site of infection, granulomas, which are dense collections of bacteria and host immune cells that could limit the distribution of antibiotics. I designed, implemented, calibrated and applied a computational model of antibiotic treatment in TB granulomas. This model highlighted the power of computational approaches by providing the first systematic and side-by-side comparison of TB treatment regimen efficacy for two main anti-TB antibiotics, isoniazid and rifampin. This work, in collaboration with non-human primate studies of TB, showed that small increases in dosing frequency could improve treatment outcomes while limiting the amount of total antibiotics given to patients. We also quantify treatment outcome metrics that are currently impossible to determine in patients or animal models of TB, including time to sterilization and risk of drug-resistance development for individual lesions. These predictions provide quantitative guidance for which antibiotic regimens to advance to animal or clinical trials. The potential impact of this work extends to the field of medicinal chemistry since we predict which physicochemical properties of the drug, such as its ability to penetrate host cells, should be targeted to improve treatment outcomes. The work was further applied to explore the potential of inhaled nano-particle delivery of antibiotics, which revealed that such delivery is not feasible for Rifampin, one of the major anti-TB drugs. This work lays the foundation for more expansive future studies including more antibiotics, and combination of antibiotic treatment with host-directed therapy.
2. Host-pathogen interaction

Much is known about how *Mycobacterium tuberculosis* responds *in vitro* to stresses like intracellular conditions, hypoxia etc. However, how these adaptations arise *in vivo* and what their implications are for the long-term survival of *M. tuberculosis* in host granulomas remains a mystery, and extremely difficult to study experimentally. Using *in vitro* methods, I have shown *M. tuberculosis* could take advantage of low multiplicity of infection to elude host immune mechanisms in macrophages. These findings were implemented in a simple computational model predicting how such dynamics could result in oscillatory dynamics between host and bacterium *in vivo*. These early findings are consistent with my more recent work in a sophisticated model capturing mycobacterial metabolism and growth adaptations in hypoxic conditions emerging in TB granulomas. I designed, implemented and analyzed the model in close collaboration of metabolic model experts. This model predicts long-term fluctuations in bacterial numbers, bacterial growth phenotypes and host responses in the granuloma. We predict that the bacterium’s ability to slow its growth rate, but not its ability to accumulate lipid reserves, is essential for long-term survival in the host. This work could shift the perception of latent TB infection from being perceived as a static balance between host and pathogen, to being a highly dynamic balancing act between host and pathogen. The work also has strong implications for antibiotic treatment strategies as the shifting metabolic activity of bacterial population would lead to fluctuations in antibiotic susceptibility over time.


3. TB diagnosis

It is clear that early diagnosis of TB specifically at the first clinic visit is key to dampening an epidemic. I helped build and analyze an epidemiological model of TB, specific to a community resembling informal settlements common in countries such as South Africa where TB incidence is among the highest in the world. The model predicted that disease transmission during daily commutes to work is a significant contributor to the spread of disease and confirmed the need for early diagnosis. Such predictions motivated a collaborative effort to design, build and test in South Africa a rapid nucleic acid amplification diagnostic test for TB. My contributions to the project included design and analysis of computational models of PCR amplification and bacterial lysis, experimental design and optimization for PCR amplification and bacterial lysis. My work laid the foundation for others to continue the diagnostic method development, and testing on clinical samples is currently ongoing in South Africa.


Complete List of Published Work in MyBibliography:

D. Additional Information: Research Support and/or Scholastic Performance

**Ongoing Research Support**
NIH U01HL121072-01 (Kirschner, Linderman, Flynn and Dartois PIs) 2/2016 – 8/2021

Title: A multi-scale systems pharmacology approach to TB therapy
Goals: To integrate state-of-the-art computational modeling and experimental data from humans, primates and rabbits to identify optimal antibiotics and regimens to improve TB treatment.
Role: Co-investigator

Indiana Center for AIDS Research (CFAR) Pilot Grant 02/2018-06/2019
Title: Computational approaches to advance HIV research toward a cure
Goals: Develop a multi-compartment computational model that accurately represents infection progression in the absence of cART. Specific focus of this model will be the establishment and maintenance of latent viral reservoirs.
Role: PI

Indiana Clinical and Translational Sciences Institute – Project Development Team 08/2018-07/2019
Title: Toward an integrated experimental and computational approach to target Ebola virus assembly
Goals: Develop an interdisciplinary systems pharmacology approach combining experimental and computational methods to explore drug repurposing to treat Ebola virus disease by targeting virus assembly.
Role: PI
Proposed budget

Salary and Wages

*Senior personnel*

Pienaar: 1 mo summer + fringe $13,486

*Other personnel*

Graduate student: salary, fringe and fees $38,543

Supplies and Expenses

High-performance computing access (Brown cluster) $5,600
Publication $1,000

Travel $1,000

Total direct $59,629
Total indirect $15,000

Total $74,629

Budget justification:

Full-time support for one graduate student is requested for 1 year. Research resources includes access to one node on Purdue’s high-performance computing cluster (Brown). Travel costs are included to help defray travel cost of PI and student to national meetings to present this research. Publication costs include papers and printing costs for research posters.