

Hypothesis or Research Question(s): Mycobacterium tuberculosis (Mtb), the cause of Tuberculosis diseases is an intracellular pathogen which infects alveolar macrophages. We have identified key macrophage proteins that control Mtb infection. We generated CRISPR knock outs in these genes and aim to analyse their infection properties using genomics, proteomics and imaging technologies. Our co applicant is an imaging expert and the candidate will evaluate the properties of each candidate protein.

PROJECT BACKGROUND & SUMMARY

Our program focuses on finding new TB drugs. One of the approaches we use is to identify key human genes and processes which enables the human host's own immune cells to fight Mycobacterium tuberculosis. This is called Host Directed Therapy and is currently on the forefront of infectious diseases research. We have screened millions of compounds and found multiple drug candidates termed lead compounds. Selected compounds were shown to target specific human proteins and activate them in the infected cells such that they are able to clear infection from infected macrophages. The student will select CRISPR knock-outs in selected targets identified by the drugs and genetically assess the identified drug target using microscopy imaging.

Our hypothesis is that the phenotype obtained by the lead compounds will be confirmed by testing the corresponding gene knock out strains thus genetically validating the host target. The project involves multidisciplinary training for the student between the Av-Gay lab (Medicine) and Elitza Tocheva laboratory at UBC Microbiology and immunology.

The student will be generating knock out strains in the Av-Gay lab and examining them in Dr. Tocheva lab using advanced Cryo-EM and fluorescence microscopy available in LSI.

Screening of a signalling inhibitors focused library resulted in identification of several inhibitors as compounds that kill Mtb intracellularly at low micromolar concentrations and in a dose dependent manner. We identified the targets of inhibitors using chemoinformatics. To validate each target as a drug target, we generated a CRISPR knock-out mutant of the candidate proteins in THP-1 cells .

The student will select for α knock outs from a CRISPR experiment conducted in our lab and in a multidisciplinary approach with Dr. Elitza Tocheva will use fluorescent and Cryo-Em microscopy to assess and compare Mtb infection in K/O macrophages vs their parental strain. The student will infect macrophages (WT and CRISPR K/O) with BCL-2 level mycobacteria and will assess infection progress in terms of uptake, bacterial replication, acidification and cell fate such as apoptosis, necrosis etc. This will be done using advance imaging technologies combined with specific fluorescence and Cryo-EM markers available at the Tocheva laboratory. The student will characterize Host pathogen interaction in correlation to molecular markers of phagocytosis, cell signaling and immune activation using fluorescence and Fluorescence/ Cryo-EM tomography applications.

BENEFIT TO THE STUDENTS

The student will receive advanced training in both genetic approaches (CRISPR knock outs and selection of clones) and Molecular imaging methods (Fluorescence and CRYO-EM microscopy). The student will be exposed to advanced chemical biology and drug discovery terminology, protocols and approaches and will be prepared for advanced studies in the field. Exposure to multidisciplinary collaborative research will expand the student understanding of cross disciplinary approaches to solving scientific and technological problems in the field of drug discovery. The student will get exposure to most advanced

Multidisciplinary Research Program in Medicine Project: *Host Directed Therapies in Tuberculosis*

tools and technologies available both at the Av-Gay Lab and The Tocheva Lab. The student will learn how to work individually and in a team. He / She will learn how to design and execute an experiment and how to assemble and analyze the experimental data. The student will learn how to compile a report and address technical, statistical and biological issues stemming from the experiments and how to use the information in designing new set of experiments. This training will provide the student with the required skills to either progress to advance degree or join the biotech/ pharmacology research community in Vancouver.