October 18, 2013

Three PHRI faculty members: Veronique Dartois, PhD, Theresa Chang, PhD and Marila Gennaro, MD secure $11.5 million in NIH grants.

Veronique Dartois, PhD
Project Title: Lesion-centric imaging and PK-PD of pyrazinamide for TB/HIV co-infection
Project Period: 08/01/2013 – 07/31/2017
Total Award: $3,138,294

Abstract: Pyrazinamide is a central anti-tuberculosis drug which is part of most treatment regimens used against drug-sensitive and drug-resistant TB in HIV infected and non-HIV populations. It has a unique ability to shorten treatment of active disease and latent infection, but the pharmacological mechanisms underlying its sterilizing properties are poorly understood. TB lesion heterogeneity is one of the roots of bacterial persistence, owing to the presence of phenotypically drug resistant populations, which reside in remote infection sites where most drugs fail to penetrate. This proposal will test the idea that pyrazinamide reaches and kills these persistent subpopulations - suspected to be involved in reactivation of latent TB, cavitary disease progression, and disease transmission in TB/HIV patient populations - more effectively than other anti-TB agents. A rabbit model for active cavitary and latent TB will be used to measure lesion-specific distribution, bio-activation and activity of pyrazinamide. The results will provide answers to the puzzling sterilizing and synergistic properties of pyrazinamide, and inform the rational design of better and faster drug regimens containing pyrazinamide.

Theresa Chang, PhD
PIs: Theresa L Chang, Zhiheng Pei (NYU)
Co-investigator: Sally Hodder
Project Title: Modulation of innate immunity, microbiome and HIV transmission by Depo-Provera
Project Period: 08/09/2013 – 07/31/2018
Total Award: $2,785,332

Abstract: Depo-Provera may increase the risk of HIV acquisition and transmission, and can attenuate the vaccine efficacy to protect rhesus macaques against SIV infection. This application is designed to determine the impact of Depo-Provera on immune systems and microbiomes in a longitudinal study, and to elucidate the immune mechanisms of Depo-Provera-mediated modulation of HIV infection/transmission in vitro, ex vivo and in cervical explant models.

Maria Laura Gennaro, MD
Project Title: FISH-Flow platform for host-based tuberculosis diagnostics
Project Period: 08/15/2013 – 07/31/2018
Total Award: $5,523,914

Abstract: Diagnosis of infection is typically based on direct detection of the pathogen and/or its products in bodily fluids and tissues. This approach has limited sensitivity and speed of detection. In this grant, Dr. Gennaro's team, which include PHRI faculty members Drs. Yuri Bushkin, Richard Pine and Sanjay Tyagi, will develop a novel, host-based diagnostic assay platform for blood-based assays combining: (1) the precision of antigen-specific immune responses, (2) the versatility and robustness of nucleic acid detection by fluorescence in situ hybridization (FISH), and (3) the diagnostic and prognostic power of single-cell analysis by flow cytometry. Our preliminary work shows that stimulation of peripheral blood T lymphocytes *ex vivo* induces expression of mRNA for key cytokines (IL-2, IFNg and TNFa) that is detected by hybridization with gene-specific FISH probes and single- and multi-parameter flow cytometry. We now propose to move this platform through preclinical product development for diagnosis of tuberculosis (TB) in partnership with Oxford Immunotec, a world leader in FDA-approved, host-based TB diagnostics. TB is the selected pathology because: (i) it is a global health problem; (ii)

(continued)

M. tuberculosis infection is diagnosed by detecting antigen-specific T cell responses; and (iii) it has a complex clinical presentation ranging from asymptomatic, latent infection (LTBI) to active pulmonary tuberculosis (PTB). Application of our FISH-Flow platform to TB diagnosis is expected to yield a test with superior diagnostic accuracy and speed than the existing tests. Moreover, the platform has the unparalleled potential to distinguish between LTBI and PTB through its multi-parameter capacity, since stages/progression of infection are associated with different cytokine profiles. We will optimize key assay parameters and verify the resulting standardized assay for protocol robustness and reproducibility, assay duration, and initial assessment of diagnostic accuracy in comparison with an existing FDA-approved test. Future kit manufacturability will also be explored. The first outcome will be a simple and accurate single-cytokine assay for LTBI diagnosis that equals or exceeds the clinical accuracy of the existing LTBI diagnostics and surpasses them in speed and ease of execution. The second outcome will be the evaluation of the three-cytokine readout for distinguishing LTBI from PTB. Established collaborations at the PI site and at Oxford Immunotec with clinical sites in regions with low and high TB prevalence will provide blood samples from donors in all TB diagnostic groups. We have also shown that the FISH-Flow protocol is amenable to adaptation to microfluidics-based, automated platforms. A third outcome will be automation of all assay steps upstream of flow-cytometry detection (from sample preparation to nuclei acid staining) to obtain an semi-automated device operating with standard flow cytometry. This work, conducted by our bioengineer partner who is experienced in commercializing diagnostic devices, should eventually lead to a hand-held, fully automated device with sample-in-answer-out capability. The FISH-Flow platform in its manual and automated versions will be applicable to detection of receptor-mediated responses in many infectious and non-infectious pathologies.