State Pilot Awards – January 2015

Sapna Deo, Ph.D.

TITLE: Highly Sensitive and Rapid Detection of HIV Persistence Using Bioluminescent Nanosensing Platform

ABSTRACT: The HIV-1 virus is known to hide in reservoir cells resulting in persistent infection even when patients are on antiretroviral therapy. These patients have undetectable levels of plasma HIV RNA and the cells that are HIV reservoirs are low in number. Current available methods of detection of HIV latency/persistence are cumbersome, have low sensitivity, and the time from test-to-answer is long. Thus, highly sensitive and specific diagnostic tools are needed to identify low level of RNA in HIV reservoir cells in a timely accurate manner.

Herein, we propose to design a nanosensing platform to overcome this challenge. The nanosensing platform is composed of a novel bioluminescent stem-loop probe (BSLP) that provides for unprecedented sensitivity and selectivity for direct detection of HIV-1 RNA splice junctions. Bioluminescent sensing has been demonstrated highly sensitive detection at attomole (10-18 moles) levels and without any significant background interference.

The latter affords highly sensitive bioluminescence-based detection in complex sample matrices, such as physiological fluids, without any background signal interference. The specificity of detection in the proposed nanosensing platform will be achieved through the use of stem-loop probes that will open only in the presence of the target HIV RNA resulting in the generation of bioluminescence emission. To achieve the overall objective of this work we propose to evaluate two specific aims: Aim 1. Design, synthesize, and characterize bioluminescent stem-loop probe corresponding to HIV RNA and Aim 2. Test the efficacy of the nanosensing platform for the detection of HIV RNA in PBMCs and cells infected with HIV; evaluation of primary patient samples.

Jose Martinez-Navio, Ph.D.

TITLE: Does Infectious HIV-1 Trapped on Follicular Dendritic Cells Represent a Long-Term Reservoir?

ABSTRACT: There is intense interest at the current time in defining the sources of HIV-1, the so-called ‘reservoirs”, that are responsible for the rebound in HIV-1 replication following removal from HAART. Strategies to prevent this rebound, i.e. affecting a “functional cure”, are heavily dependent upon knowing what these sources are.

Usually absent from these discussions is the possibility that infectious HIV-1 can remain trapped on follicular dendritic cells for prolonged periods. In a publication dating back to 2001, Smith et al found that infectious HIV-1 can remain trapped on follicular dendritic cells in lymph nodes of mice for as long as they looked, as late as 9 months following the HIV-1 administration. We will confirm and extend this observation with sophisticated pilot studies in monkeys. We will employ a replication-defective strain of HIV-1 that can only be grown and recovered in a complementing cell line in order to ensure the absence of any replication in the monkeys. We will analyze lymph node biopsies at regular intervals for a period of nine months for the presence of recoverable HIV-1 using the complementing cell line. Populations of cells harboring recoverable HIV-1 will be defined by use of sorted cell populations. The absence of any reversional replication-competent virus will be confirmed by testing the inability of recovered virus to

replicate in the parental cell line from which the complementing cell line was derived. Extensive sequencing will be performed on the recovered virus. If follicular dendritic cells are found to be a long-term reservoir of recoverable HIV-1, schemes will be devised to try to purge this reservoir using the monkey model.

Savita Pahwa, M.D.

TITLE: Characterization of HIV Reservoirs in Lymph Nodes

OBJECTIVE: Emerging data in the literature suggests that T follicular helper (Tfh) CD4 T cells in lymph nodes represent a major site for HIV latency. We hypothesize that the B cell follicle provides an environment for active replication of the virus as well as formation of latently infected CD4 T cells. We propose to perform a comprehensive analysis of the topology of lymph node CD4 T cells harboring integrated or actively transcribed HIV and to analyse HIV RNA and DNA content in sort-purified specific CD4 T cell populations.

STUDY DESIGN: This study will be performed in lymph node tissue and cells from HIV+ individuals using paraffin blocks and cryopreserved cell suspensions. Samples from 25 Viremic and 10 aviremic participants are available for the proposed study to be performed in collaboration with Dr Petrovas in the laboratory of Dr Richard Koup at the NIH.

Analysis: Cutting edge confocal-based assays for a detailed analysis of the localization of infected cells within particular areas of the B cell follicle as well as the surrounding cells, especially CD8 T cells will be followed by HIV RNA and DNA analysis in defined CD4 T cell populations purified by cell sorting based on phenotypic antibody markers corresponding to T cell zone and the intra-follicular zones for characterization of the HIV reservoirs (active transcription sites as well as “latent” pools of virus).

Significance: An accurate estimation of the numbers of HIV infected (actively and latent) cells as well as their anatomical localization is an absolute requirement in order to design new tools for the virus mobilization and develop interventions for the elimination of the infected cells to eradicate HIV (eradication strategies) or for equipping the immune system to control the virus after discontinuation of antiretroviral treatment (functional cure).

Geoffrey Stone, Ph.D.

TITLE: Correlates of Reduced Viral Reservoir in a HIV-1 Therapeutic Vaccine Clinical Trial

ABSTRACT: HIV-1 combination anti-retroviral therapy (cART) cannot eliminate the viral reservoir. HIV-persistence, including viral latency and low-level viral replication, presents an important barrier to the elimination of this reservoir. Therapies that reduce persistence will likely be a critical component of an HIV Cure strategy, in particular the possibility of using vaccination to reduce low-level viral replication and latency.

Our long-term goal is to understand the immune mechanisms that impact the viral reservoir in HIV-infected patients. The objective of this proposal is to evaluate patients receiving a novel HIV therapeutic vaccine. Preliminary data show that chronic HIV-1 infected virally suppressed patients on cART given single cycle HIV-1 reduced their viral reservoir (both 2-LTR and total genomic HIV-1 DNA). Patients that received the vaccine, but not placebo controls, also showed reduced systemic immune activation and microbial translocation and improvement in HIV-1 specific CD8 T cell function. Our hypothesis is that

therapeutic vaccination reduced low-level viral replication, reducing systemic immune activation and reduced re-seeding of the latent reservoir. Samples are stored and ready for immediate analysis by established collaborators. This proposal will test remaining patient PBMC samples for 2-LTR and total genomic DNA. We will analyze key CD4 memory T cell markers of the viral reservoir and HIV -1 specific CD8 T cell function. We will also examine plasmacytoid DC and alpha-4-beta-7 levels in PBMC as a correlate for gut inflammation. Finally, we will analyze samples for cell associated HIV-1 multispliced RNA using a sensitive PCR-based assay. This project is innovative because it provides human clinical data on the role of anti-HIV immune responses in reducing the viral reservoir. The proposed research is significant because understanding mechanisms of reduced viral reservoir will provide important information on the link between HIV-1 latency, systemic immune activation and anti-HIV immune responses.

Lydie Trautman, Ph.D.

TITLE: Combined Effect of Reactivating Agents of HIV Latency on CD8-Mediated Killing of Reactivated Latently-Infected CD4 T Cells

ABSTRACT: Increasing evidences suggest that purging the latent HIV reservoir will require both the induction of viral replication from its latent state and the elimination of these reactivated latently infected cells (shock and kill strategy). The “shock” of the latent reservoir requires the development of new latency reversing agents (LRAs) that consists in reactivating the latent HIV reservoir to produce HIV antigens while preserving or enhancing HIV-specific CD8 T cells to “kill” the reactivated latently infected cells. But current LRAs have shown little efficacy in reducing the latent reservoir and some have been also shown to dampen the cytolytic responses. There is a need for identifying LRAs that can reactivate latently infected CD4 T cells and preserve or enhance the cytolytic function of HIV-specific CD8 T cells to achieve the elimination of HIV reservoirs. Assays that measure the CTL-mediated killing of latently HIV-infected primary CD4+ T cells from individuals under ART are not widely available. We have developed in our laboratory a novel assay to reliably quantify the depletion of reactivated latently-infected CD4 T cells by autologous HIV-specific CD8 T cells. Using this assay, we will test 10 LRAs alone or in combination and measure: 1- The impact of LRAs on the reactivation of HIV multi-spliced RNA expression and viral particles HIV RNA in primary CD4+T cells from aviremic HIV-infected donors; 2- The impact of LRAs on the transcriptional profile of HIV-specific CD8 T cells; and 3- The impact of LRAs on the killing capacity of reactivated HIV infected CD4 T cells by HIV-specific CD8 T cells. These results will provide the rationale to select the best LRA candidates for their potency not only to increase HIV antigen expression but for their capacity to render the latently infected cells susceptible to CTL-mediated killing.

David Watkins, Ph.D.

TITLE: Isolation of Neutralizing Antibodies from Non-Human Primates After Immunization

ABSTRACT: Currently, there are no potent neutralizing antibodies (nAbs) against SIV and, consequently, nAb efficacy studies remain limited to humanized mice and Simian-Human Immunodeficiency Viruses (SHIV) AIDS models. These models might not fully recapitulate the intricacies of the sequential appearance of nAb and envelope (Env) escape, due to the absence of natural immune responses in the murine model and the structurally constrained nature of SHIV Env. Our only goal of this proposal is, therefore, to isolate novel potent SIVmac239-specific nAbs. Having a nAb against SIVmac239 would facilitate the study of nAb efficacy in SIVmac239- infected rhesus macaques. The development of potent

nAb with increased neutralization breadth is thought to occur in response to successive waves of Env escape and diversification. Our strategy is to select Env gp160 immunogens that may elicit nAbs (Aim 1), engineer recombinant vesicular stomatitis virus (VSV) vectors to express these variant Env gp160 proteins (Aim 2), and immunize long-term SIV-infected macaques to isolate antibodies (Aim 3). We have assembled an exceptional group of researchers for this pilot proposal and, if successful, we will open up an entirely new field of nAb prevention and cure studies using SIVmac239-infected rhesus macaques, the best animal model to study therapeutic interventions against the AIDS virus.