

HIV Projects

1. What HIV-1 Vpr Interactive proteins mediate G2 arrest?

Background: Vpr induced G2 arrest is a key feature of HIV pathogenicity through binding DCAF1 ubiquitin ligase.

Methods:

1. Use Vpr-BioID (biotin ligase) fusion protein as well as mutants that fail to induce G2 arrest (Q65R, R80A) to transfect 293T cells to confirm biotin labeling of DCAF-1 (ubiquitin ligase substrate) and UNG2 (uracil glycosylase 2)
2. Submit biotin labeled proteins for mass spec analysis to identify proteins identified with wt but not mutant Vpr
3. Confirm mass spec identified proteins by colP
4. Assess effect of siRNA against mass spec identified protein on ability of Vpr to induce G2 arrest

Ref: PMID: 28075409, 10864665

HTLV Projects

2. What co-activators mediate IRF4 transcriptional activity in ATL

Background: IRF4 is amplified or mutated (K59R) in 33% of HTLV-1 associated adult T cell leukemias (ATL) and over-expressed in all cases.

Methods:

1. Use IRF4-Apex (ascorbate peroxidase) fusion protein as well as mutant (K59R) to transfect Jurkat T cells and confirm interaction with BATF
2. Transfect IRF4-Apex and IRF4 (K59R)-Apex in ATL cell lines for mass spec analysis to identify interactive proteins
3. Confirm mass spec identified proteins by colP
4. Assess effect of siRNA against mass spec identified proteins on ability of IRF4 to activate gene targets in ATL cells

Ref: PMID: 26437031, 27826752

3. HTLV-1 Infection of Human Embryonic Stem Cells

Background: HTLV-1 infects T cell precursor to produce clonal expansion of mature CD4+ T cells

Questions: At what stage of hematopoietic stem cell differentiation can HTLV-1 infect and what is the consequence?

1. Infect human induced pluripotent stem cells with HTLV and assess hematopoietic progenitor numbers and clonality

Ref: PMID: 28408465

4. How does HTLV-1 Tax activate the alternative NFkB pathway

Background: HTLV-1 replication of alt NFkB is important to confer resistance to apoptosis

Methods:

1. Make BioID (biotin ligase) fusions with wt and Tax mutant (deficient in alt NFkB activation)
2. Transfect Jurkat T cells to examine protein expression and alt NFkB activation (by p100 cleavage)
3. Biotin label transfected Jurkat cells to determine if known interactors bind Tax-BioID e.g. p100, NEMO
4. Perform mass spec analysis to identify interactive proteins

Ref: PMID: 24060211, 16751281

5. What cellular proteins regulate HTLV-1 entry into human cells

Background: Although Glut1 and Nrp1 have been proposed as HTLV-1 receptors, coreceptor and entry mechanisms remain obscure. Methods:

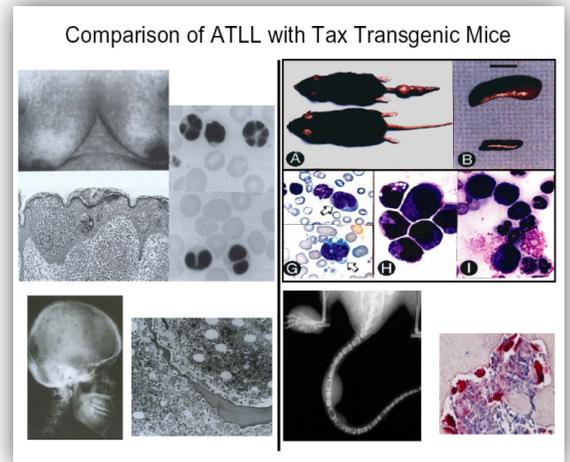
1. Screen Crispr/Cas9 knockout library for HTLV-1 entry using a herpes simplex virus thymidine kinase
2. Compare results with prior genetic screen of genes that promote HTLV-1 entry into mouse cells

Ref: PMID: 21114861

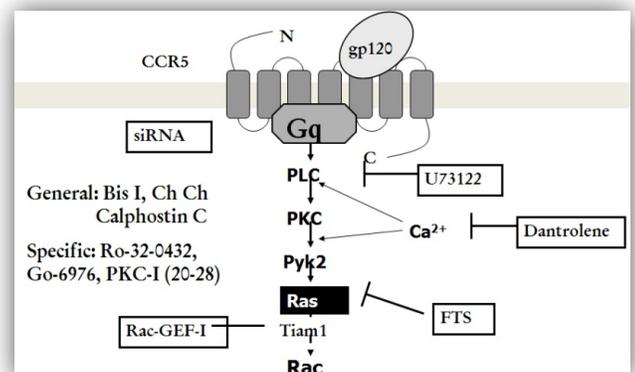
Research Program for Lee Ratner, M.D., Ph.D.

I joined Washington University in July of 1985. I graduated from Yale University in 1979 and trained in Internal Medicine at Barnes Hospital. After completing Medical Oncology training at the National Cancer Institute, I worked as a research associate with Drs. Flossie Wong-Staal and Robert Gallo. My laboratory is studying human retrovirus infections.

My **basic research is focused on the genetic basis of HTLV-1 lymphomagenic activity**. This includes basic molecular biology studies, animal models, and multicenter clinical trials. My interests in HTLV-1 began during postdoctoral studies in 1983, and my independent laboratory has been critical in establishing a strong molecular understanding of the pathogenic effects of this virus over the last 32 yrs. Our studies of **HTLV-1** are concerned with analysis of the mechanisms of leukemogenesis through studies of transforming determinants in an infectious immortalizing HTLV-1 proviral clone in culture and humanized mice; and transgenic models utilizing the HTLV-1 trans-activator protein, which results in a lymphoproliferative malignancy; and identification and characterization of the receptor for the virus. We established the first infectious molecular clone of HTLV-1 and the first animal model of HTLV-1-associated leukemia-lymphoma. These tools have been provided to almost every group in the field throughout the world since their discovery, and a number of current innovative variations of these reagents include studies of the pathogenesis of the infectious molecular clone in humanized mice and an inducible transgenic mouse model of HTLV-1 Tax induced lymphoma. The major focus over the last 10 yrs has been deciphering the role of Tax activation of NFkB in lymphoma



Our studies of **HIV-1** focus on two distinct questions: 1) How does viral protein X (Vpx) enhance virus replication in quiescent cells? 2) How does the viral envelope interact with the receptor (CD4) and coreceptor (CXCR4 or CCR5) to allow virus entry? For these studies, we utilize infectious molecular clones of HIV-1 and HIV-2, cell culture and animal studies, small molecule and siRNA inhibitors, as well as viral and cellular cofactor mutants. Information from these studies has been applied to developing novel therapeutic approaches for HTLV-1 and HIV-1 infections. Information from these studies has been applied to developing novel therapeutic approaches for HTLV-1 and HIV-1 infections.



My **clinical research is focused on human virus-associated cancers**. I participated and/or led clinical trials HIV-associated lymphomas, anal carcinomas, and Kaposi's sarcoma. I have also led multicenter trials of HTLV-1 associated leukemia/lymphoma. I am **Director of Molecular Oncology** with a faculty of 16 individuals. I am PI of the NCI T32 funded Molecular Oncology Training Program, and Co-Leader of the Solid Tumor Therapeutics Research Program of the Siteman Cancer Center. I am a former American Cancer Society Research Professor. I mentored 16 graduate students (4 were MD PhD students) who completed their thesis and more than 50 postdoctoral researchers have trained in my laboratory. I sponsored postdoctoral trainees who successfully competed for F32 National Research and K08 Clinical Investigator Awards, and fellowship/scholar grants from the Leukemia Society, Keck Foundation, Lymphoma Research Foundation, and American Foundation for AIDS Research.

Recent Ratner Lab Pubs: PMID:27015285, 27128349, 26324707, 26265053, 25532805