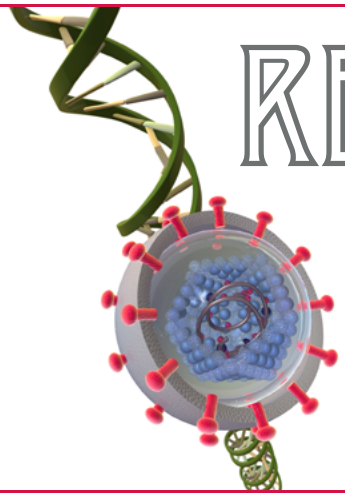

RETRO-ACTIVE NEWS

Newsletter of the Center for Retrovirus Research
at The Ohio State University

2018 Highlights

Dr. Sanggu Kim awarded NIH grants focused on ground-breaking sequencing methodology and HIV detection and surveillance

Dr. Sanggu Kim, Assistant Professor in the Department of Veterinary Biosciences and Center member, received a three-year **R21 grant ($624,000)** entitled "High-accuracy, long-range sequencing for HIV-1 genotyping" and a four-year **R01 grant ($2.9M)** entitled "On-site, high-fidelity target sequencing and absolute quantitation for HIV-1 surveillance" (both from NIH-National Genome Research Institute).

Microbial detection/classification and drug-resistance surveillance have long been plagued by erroneous and limited read-length genotyping assays. These awards will enable Dr. Kim and his collaborators to develop improvements for the new, so-called "third-generation" nanopore sequencing technology.

Unlike other forms of sequencing, nanopore sequencing can read long strands of DNA or RNA all at once and in real time. However, it comes with a high error rate. By continuing to refine his "twin barcoding" method, Dr. Kim's research will improve sequencing accuracy, detection and quantitation of target genetic molecules. In addition to making nanopore sequencing more accurate, he is also working to make it more accessible—eventually hoping to create a portable sequencing laboratory.

Dr. Kim's collaborators include co-investigator **Dr. Li Wu (Veterinary Biosciences and Center member)**, and Drs. Matthew Sullivan (Microbiology and Civil, Environmental and Geodetic Engineering), and Marcel Yotebieng (Public Health). Successful completion of these projects will produce a low-cost, portable sequencing and quantitation technology that may prove ground-breaking for biomedical sequence analysis, providing a novel means of resolving key issues in broad areas of science and medicine.

Dr. Shan-Lu Liu elected fellow of the AAM

Center member, Dr Shan-Lu Liu, Professor in the Departments of Veterinary Biosciences and Microbial Infection and Immunity and Center member has been elected Fellow of the American Academy of Microbiology.

The Academy, the honorific leadership group within the American Society for Microbiology, recognizes excellence, originality, and leadership in the microbiological sciences, and Dr. Liu's election to this group is a mark of distinction for his many important contributions to the field of virology, especially in HIV pathogenesis, retroviral oncogenesis, virus fusion, and IFN restriction of viral infection.

Other Center Members that are AAM Fellows include Patrick Green.

Dr. Amit Sharma, PhD joins the Ohio State faculty and Center

Dr. Amit Sharma was recruited to join the Departments of Veterinary Biosciences and Microbial Infection and Immunity and the Center for Retrovirus Research as part of the University's Discovery Themes initiative.

Dr. Sharma received his postdoctoral training with Dr. Julie Overbaugh at the Fred Hutchinson Cancer Research Center, working on the non-human primate models of HIV-1 infection. Dr. Sharma is the recipient of an NIH career transition grant (K99/R00). During the K99 phase of the grant, Dr. Sharma employed clinically relevant, circulating HIV-1 strains and studied their interactions in the non-human primate models of HIV-1 infection. His research identified that CD4 alleles of Spix's owl monkeys encode functional receptors for entry by globally circulating HIV-1 variants. His research also characterized the role of host interferon response in restricting SHIV replication in macaque lymphocytes.

He identified that in contrast to the SHIVs encoding lab-adapted HIV-1 variants, the SHIVs encoding circulating HIV-1 variants have lower replication kinetics in macaque lymphocytes and are sensitive to type-1 interferon (IFN). Moreover, he demonstrated that serial macaque-passage selects for IFN-resistant SHIV variants that have higher replication kinetics. He also identified macaque Interferon-induced Transmembrane Proteins (IFITMs) as host-factors that limit replication of SHIVs in macaque lymphocytes.

As Dr. Sharma begins his independent program here at Ohio State, his laboratory will continue to characterize the host-viral interactions in the SHIV/macaque model. His work will focus on: 1) characterizing of viral and host factors that drive selection, adaptation, and pathogenicity of SHIVs in macaque lymphocytes; 2) identifying the molecular and mechanistic basis of adaptation of SHIVs to the macaque immune responses; and 3) exploring the genetic repertoire of non-human primate species to identify novel innate immune factors that limit the cross-species transmission of lentiviruses. This research will be critical for developing more relevant SHIV/macaque models of HIV-1 infection.

Welcome Amit!

'Molecular scissors' could be key to cutting off diseases including HIV infection

A new study from Dr. Li Wu (Professor, Center Member) revealed how the cellular enzyme SAMHD1 influences proteins that stimulate the immune response. Previous research established SAMHD1 as a key player in inhibiting HIV replication in immune cells.

The new study illuminated the way in which SAMHD1 interacts with cellular proteins that play a critical role in regulating innate immune responses to viral infections. Interestingly, SAMHD1 acts as an inhibitor of potentially harmful responses during viral infection.

The study was published in **Proceedings of the National Academy of Sciences** in April 2018 and co-lead authors are postdoctoral researchers Shuliang Chen and Serena Bonifati in the Wu lab. Other researchers in the Wu lab contributed to the research, including Zhihua Qin, Corine St. Gelais, Karthik Kodigepalli, Sun Hee Kim, and Jenna Antonucci. The interdisciplinary study was performed in collaboration with Drs. Jacob Yount and Denis Guttridge at Ohio State, Yong Xiong at Yale University, and Mario Santiago at the University of Colorado.

Chen S, Bonifati S, Qin Z, St. Gelais C, Kodigepalli KM, Barrett BS, Kim SH, Antonucci JM, Ladner KJ, Buzovetsky O, Knecht KM, Xiong Y, Yount JS, Guttridge D, Santiago ML, and Wu L., **SAMHD1 suppresses the innate immune responses to viral infections and inflammatory stimuli by inhibiting the NF-kB and interferon pathways. Proc Natl Acad Sci U S A. 2018; 115(16) E3798-E3807.**

Drs. Ross Larue and James Fuchs funded to develop novel scaffolds as tools for HIV-1 integrase inhibition

Dr. Ross Larue, Research Assistant Professor, College of Pharmacy and member of the Center for Retrovirus Research and colleague, Dr. James Fuchs, Associate Professor Medicinal Chemistry and Pharmacognosy, have been awarded a NIH R21 exploratory grant.

HIV-1 integrase (IN) is a key target in the viral life cycle for the development of new therapeutics. Despite the successful development of FDA approved active site inhibitors like raltegravir, elvitegravir, and dolutegravir, resistance to these agents threatens their long-term utility. Allosteric IN inhibitors that bind at the LEDGF/p75 site of IN represent an alternative strategy for the development of compounds that will not share the same resistance profile.

This R21 proposal is focused on the development of compounds designed to probe the IN CCD dimer/CTD interface in an effort to more efficiently inhibit IN. A key component of this proposal is also the development of a completely new scaffold capable of binding to the LEDGF/p75 pocket and predicted to show a unique resistance profile compared to previously synthesized allosteric inhibitors.

Dr. Jian Zhu, PhD joins the Ohio State faculty and Center for Retrovirus Research

Dr. Jian Zhu (Associate Professor) was recruited from the University of Rochester to join the Department of Pathology and the Center for Retrovirus Research.

Dr. Zhu's lab studies the host-virus interactions, particularly for HIV and herpesviruses, by using multidisciplinary systematic approaches. His group works at the interface of virology, immunology, cellular and molecular biology, proteomics, and functional genomics. One of the major directions of his research program is to identify key host factors that epigenetically regulate viral latency and reactivation for HIV and herpesviruses, by combining genomic RNAi and CRISPR/Cas9 screening technologies together with various modern proteomic approaches including PLATO (ParalleL Analysis of Translated ORFs) synthetic display method. Small-molecule compounds targeting these epigenetic regulators are also tested for their potentials to eliminate viral latent reservoirs as well as to treat virus-associated pathogenesis.

Dr. Zhu is currently PI of three NIH grants investigating 1) latency promoting genes in HIV oral reservoirs cell; 2) the role of FACT proteins in regulating HIV transcription and latency, and; 3) the cooperation of BRD4 and Tat associated proteins in HIV transcription and latency Welcome Jian!

Dr. Shan-Lu Liu awarded NIH R01 to investigate TIM-mediated inhibition of HIV-1 release

Dr. Shan-Lu Liu, MD, PhD (Professor, Veterinary Biosciences and Center member, received a four-year, $1.33M R01 grant from NIH-National Institute of General Medicine Sciences to study inhibition of HIV particle release.

TIM (T-cell immunoglobulin and mucin domain) and SERINC (serine incorporator) family proteins directly interact with or possibly regulate the synthesis of phosphatidylserine (PS), thus inhibiting HIV release or infectivity. Recent published work from the group showed that lentiviral Nef proteins effectively antagonize the restriction by TIMs and SERINCs. Moreover, Dr. Liu recently observed that SERINC proteins potentiate the ability of TIM-1 to block HIV-1 release and that SERINCs do this by stabilizing the TIM expression in the viral producer cells.

This grant will test several novel hypotheses that address the possible link between TIM, SERINC, PS and Nef. Aim 1 will determine how HIV-1 Nef antagonizes TIM-mediated inhibition of viral release. Aim 2 will focus on understanding of the role of endogenous SERINC proteins in CD4+ T cells that regulates the TIM expression and stability. Aim 3 will define the molecular interplay between SERINC and TIM proteins in viral producer cells, and dissect how HIV-1 Nef protein down-modulates this process to promote HIV-1 production and infection.

Results from the proposed experiments will provide novel and unified mechanistic insights into the interplay between TIM, SERINC and HIV Nef, and will enhance the understanding of virus-host interaction and AIDS pathogenesis.

Dr. Patrick Green awarded an NIH R13 meeting grant to help support 30th Workshop on Retroviral Pathogenesis

The International Workshop on Retroviral Pathogenesis has long served as a forum for the exchange of new research findings and concepts on all aspects of retroviral pathogenesis, particularly oncogenesis and immunodeficiencies, on topics ranging from molecular mechanisms to the immunological parameters of host-virus interaction. The size and format of the conference, generally between 90 – 120 attendees, supports concentrated interaction and deep engagement over four days.

Pathogens of humans and animals in all retroviral genera are the subject of scientific presentation and vibrant discussion. The conference has long fostered the professional development of junior investigators by affording them the opportunity to present their current work to a panel of engaged colleagues, many of whom will be assessing their work through peer review, and to serve as s Session Chairs. The 30th Workshop was held October 8-12, 2018 Awaji City, Japan. The 31st Workshop is scheduled for October 13-16, 2019 in Padova, Italy.

Dr. Nicholas Funderburg receives NIH R21 grant to study HIV and inflammation in transgender women

Dr. Nicholas Funderburg (Associate Professor), School of Health and Rehabilitation Sciences and Center member was awarded an NIH R21 grant entitled "Comprehensive HIV+/- Analysis of inflammation in transGender women on estrogen supplementation: the changes study."

The physiologic intersections of HIV, antiretroviral therapy (ART) and feminizing hormonal therapy (FHT) are critical but incompletely understood elements of optimizing care for transgender women (TW). Worldwide, HIV prevalence rates among TW have reached epidemic levels. HIV infection is characterized by persistent inflammation and immune activation that contributes to multiple metabolic disturbances. ART also contributes to these disturbances, and FHT modulates inflammatory, metabolic, and coagulation pathways and causes gain of fat and loss of lean mass.

Using data and stored samples from Féminas participants (220 adult TW in Lima, Peru) and complementary *in vitro* experiments, this proposal will determine how the intersections of HIV infection, ART and FHT affect inflammatory pathways and cardiometabolic risk in TW. This project will elucidate mechanisms of cardiometabolic disease risk and immuno- metabolic perturbations in TW on FHT, including whether inflammatory and metabolic changes induced by FHT and ART are antagonistic, additive or synergistic with the ultimate goal to inform care and improve quality of life for TW.

CRR collaborative study of leukemia using a mouse model

In collaboration with the labs of Drs. Patrick Green and Shan-Lu Liu, Dr. Li Wu's lab reported in **Cell Cycle** that the host protein SAMHD1 inhibits epithelial cell transformation *in vitro* and affects leukemia development in xenograft mice. SAMHD1 functions as a negative regulator in the efficacy of cytarabine treatment of acute myeloid leukemia (AML), but the function of SAMHD1 in modulating AML leukemogenesis remains unclear.

This study showed that SAMHD1 expression significantly inhibited cell transformation caused by a retrovirus envelope protein. Bioluminescence imaging and quantification analysis of xenografted immunodeficient mice revealed that SAMHD1 knockout AML cell-derived tumors had similar growth and metastatic potential compared with control cells. However, mice xenografted with SAMHD1 knockout cells showed greater survival compared to mice injected with control cells, suggesting that SAMHD1 affects AML tumorigenicity and disease progression *in vivo*.

Kodigepalli KM, Li M, Bonifati S, Panfil A, Green P, Liu S-L, and Wu L. SAMHD1 inhibits epithelial cell transformation in vitro and affects leukemia development in xenograft mice. Cell Cycle, 2018; 17(23):2564-2576.

The Center for Retrovirus Research

2018 Distinguished Research Career Award

Dr. Daria J. Hazuda, Vice President and Therapeutic Area Head, Infectious Diseases, and Chief Scientific Officer of the Merck Exploratory Science Center was the 18th recipient of the annual award for her seminal contributions to the field of retroviruses and antiviral therapeutics.

Dr. Hazuda received her PhD in biochemistry from the State University of New York, Stony Brook, where she trained with Dr. Cheng-Wen Wu. She performed her postdoctoral studies at Smith, Kline, and French in their Department of Molecular Genetics. In 1989, Dr. Hazuda joined Merck Research Laboratories as a Senior Research Biochemist and is now Vice President and Therapeutic Area Head, Infectious Diseases, and Chief Scientific Officer of the Merck Exploratory Science Center.

While at Merck Research Laboratories, Dr. Hazuda focused on the development of antiviral therapeutics. Her leadership led to the development of the first HIV-1 integrase inhibitor Isentress (Raltegravir) as well as HCV inhibitors Elbasvir and Grazoprevir. In addition to her work in drug development, Dr. Hazuda made seminal contributions to our understanding of the HIV-1 integrase mechanism, including the roles of divalent cations in integration complex assembly and enzymatic activity. In 2008, her group performed one of three landmark genome scale siRNA screens to identify host factors that influence HIV-1 infection.

Dr. Hazuda is an author of over 190 research articles, reviews and book chapters. She holds 28 U.S. patents. She serves on the amfAR, American Foundation for AIDS Research, Advisory Council and the editorial boards of *ACS Infectious Diseases* and the *Journal of Virus Eradication*. She has previously served on NIH study sections and the HCV Drug Resistance Advisory Group.

In 2010 Dr. Hazuda was elected as a Fellow to the American Academy of Microbiology. She has been awarded several international honors, including the David Barry DART Development of Antiretroviral Therapies Achievement Award and the Italian Premio Galeno Award for her work with Isentress.

Dr. Hazuda's visit was sponsored by the Center for Retrovirus Research, Department of Veterinary Biosciences, Infectious Diseases Discovery Theme, Infectious Disease Institute, and the Comprehensive Cancer Center.

Dr. Hazuda receives Career Award crystal from members of the Center for Retrovirus Research. Shown from left are (kneeling) Jesse Kwiek, Sanggu Kim, (standing) Michael Oglesbee, Karin Musier-Forsyth, Lawrence Mathis, Patrick Green, Daria Hazuda, Ross Larue, Kristine Yoder, Li Wu, Namal Liyanage. Dr. Hazuda's distinguished award lecture was entitled "HIV Drug Discovery: Past, Present, and Future".

Selected Grants and Recognitions

Sanggu Kim
NIH/OD R21HG010108 "High-accuracy, long-range sequencing for HIV-1 genotyping" (2018-2021)

Sanggu Kim
NIH/NHGRI R01HG010318 "On-site, high-fidelity target sequencing and absolute quantitation for HIV-1 surveillance" (2018-2022)

Shan-Lu Liu
R01GM132069, "TIM-mediated inhibition of HIV Release: Cooperation with SERINC and antagonism by Nef" (2018-2022)

Patrick Green
R13CA232205, "30th International Workshop on Retroviral Pathogenesis" (2018)

Nicholas Funderburg
R21AI143452 "Comprehensive HIV+/- Analysis of inflammation in transGender women on estrogen supplementation: the changes study" (2018-2020)

Li Wu
R01GM128212-S1 Equipment supplemental grant, "Mechanisms of HIV-1 RNA methylation in regulating viral replication" (2018-2019)

Li Wu
R21AI136737 (subaward, Y. Xiong [PI], Yale University) "Comparative structure and function analyses of human and mouse SAMHD1 proteins" 2018-2020

Li Wu
SBIR HHSN272201800033C (subaward, T. Ferguson [PI], Luna Innovations Inc) "Vectored delivery of mRNA for HIV-1 vaccines" (2018-2019)

Li Wu
(Co-I), R01HG010318 (Sanggu Kim [PI]) "On-site, high-fidelity target sequencing and absolute quantitation for HIV-1 surveillance" (2018-2022)

Shan-Lu Liu was elected as Fellow of the American Academy of Microbiology

Shan-Lu Liu was elected as the Founding President of Association of Chinese Virologists in America (ACVA).

Student, Post-doc and Research Scientist Awards

Jenna Antonucci (PhD student; Wu Lab) Poster Award, 2018 The American Society of Microbiology Ohio Branch Meeting.

Jingyou Yu (PhD student; Liu Lab) - American Society for Virology (ASV) travel award and selected for an oral presentation at the 37th ASV meeting.

Jingyou Yu (PhD student; Liu Lab) Winner Oral Platform Presentation, College of Veterinary Medicine Research Day (2018).

Serena Bonifati, PhD (Postdoctoral Researcher; Wu Lab). Recipient of the Ohio State Center for RNA Biology Travel Award. Presented an oral platform at the Frontiers of Retrovirology Conference, Belgium, September 2018.

Zhihua Qin (PhD student; Wu Lab). Recipient of the C. Glenn Barber Fellowship (2018-2020)

2018 Graduates and Passage of Candidacy Exam

Jenna Antonucci, PhD (Wu Lab) "Mechanisms of HIV-1 Restriction by SAMHD1". Currently Postdoctoral Researcher, Dr. Lee Gehrke's lab at the Massachusetts Institute of Technology.

Erik Olson, PhD (Musier-Forsyth Lab) "Retroviral 5´-untranslated region RNA structure and interactions with viral and host proteins". Currently attending Law School at Ohio State.

Jingyou Yu, PhD (Liu Lab) "The Multifaceted Roles of IFITMs and LY6E in HIV Infection". Jingyou has recently accepted a postdoctoral position with Dr. Daniel Barouch at Harvard Medical School.

Randi Mackler, PhD (Yoder Lab) "Understanding prototype foamy virus integrase site selection, activity, and stability".

Yingke Tang (Musier-Forsyth Lab) successfully passed her PhD candidacy exam.

Yu-Ci Syu (Musier-Forsyth Lab) successfully passed her PhD candidacy exam

Selected Upcoming Meetings

Symposium on HIV/AIDS
March 7-9, 2019, Palm Springs, CA

Cold Spring Harbor Laboratory "Retroviruses"
May 20-25, 2019, Cold Spring Harbor, NY

American Society for Virology
July 20-24, 2019, Minneapolis, MN

11th International Retroviral Nucleocapsid Protein and Assembly Symposium
August 15-17, 2019, Boston, MA

31st Workshop on Retroviral Pathogenesis
October 13-16, 2019, Padova, Italy

Selected Publications

Antonucci JM, Kim SH, St. Gelais C, Bonifati S, Li T-W, Buzovetsky O, Knecht KM, Duchon AA, Xiong Y, **Musier-Forsyth K**, **Wu L**. SAMHD1 suppresses HIV-1 gene expression and negatively modulates reactivation of viral latency in CD4+ T-cells. J Virol. 2018; 92(15): e00292-18.

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