POSTER ABSTRACTS

Table of Contents

Abstract #	Presenting Author	Title
1	Sardo	Investigating the Mechanisms of HIV-1 Latency by High Resolution Microscopy
2	Misra	Inhibition of HIV-1 by Glycosylphosphitidylinositol (GPI)-Anchored Anti-HIV Env scFv
3	He	Efficient Transduction of Human and Rhesus Macaque Primary T Cells by a Modified Human Immunodeficiency Virus Type 1 (HIV-1)-Based Lentiviral Vector
4	Shu	The Role of HIV-1 Nef Dimerization in AP2 Recruitment, CD4 Downregulation and Infectivity in Cell Culture and Humanized Mice
5	Lan	Protease Inhibitors and Neutralizing Antibodies Effectively Block Cell-Free and Cell-to-Cell Transmission of HIV-1 From Provirus-Activated Latently Infected Cells to Uninfected Cells
6	Kyei	Inhibition of SF3B1 Modify the Epigenome and Prevent HIV Provirus Reactivation
7	Ho	Cells Containing Hypermutated HIV-1 Proviruses Can Be Preferentially Targeted by Cytotoxic T Lymphocytes
8	Musick	A Small Fraction of Proviruses in Expanded Clones Express Unspliced HIV RNA in Vivo
9	Romerio	The HIV-1 Antisense Transcript Ast Recruits the Polycomb Repressor Complex 2 to the HIV-1 5'LTR and Acts as a Viral Latency Factor
10	Lee	Quantification of the Latent HIV-1 Reservoir Using Primer-ID Based Ultra Deep Sequencing Assay
11	Zhang	An Affordable and Sensitive Assay to Detect Replication-Competent HIV Reservoir in CD4+ T Cells From HIV+ Subjects on ART
12	Sahu	A Simple Viral Outgrowth Assay (simVOA) to Monitor the Levels of Inducible Latent HIV Reservoir in Patients
13	Takata	A Novel HIV Latency Model Using Primary CD4+ T Cells From HIV-Infected ART-Suppressed Treated Individuals
14	Pache	Identification of Smac Mimetics as a Novel Class of HIV-1 Latency Reversing Agents
15	Feng	Crosstalk Between Histone Modifications Indicates That the Arginine Methyltransferase CARM1 Is a Pharmacological Target for Reactivation of HIV Latency
16	Nikolai	Host Cell Deubiquitinases Are Modulators of HIV Infection and Latency
17	Huang, H.	A Novel Bromodomain and Extra-Terminal Domain Inhibitor That Reverses HIV-1 Latency
18	Archin	Interval Dosing Allows Effective Latency Reversal by Vorinostat
19	Boucau	Latency Reversing Agents and Cellular Activation Affect Antigen Processing in Primary CD4 T Cells
20	Galardi	Evaluation of Ingenol Based PKC Agonists for HIV Latency Disruption
21	Larragoite	Reducing Pro-Inflammatory Cytokine Release During PKC-induced HIV-1 Latency Reactivation
22	Young	In Vivo Visualization and Quantification of HIV Infection and Viremia Rebound in Humanized Mice
23	Clutton	CD8 T Cells from HIV+ Individuals on ART Have a Skewed Differentiation Phenotype and Impaired Proliferative Responses

24	Simon	HIV-1 Susceptibility of Primary CD4+ T Lymphocytes Is Enhanced by IL15 Through Phosphorylation of SAMHD1
25	Shen	Lymphatic Endothelial Cells Induce HIV Infection in Resting CD4+ T Cells Without Signs of Activation
26	Skinner	SIV-Producing Cells in Follicles Are Partially Suppressed by CD8+ Cells in Vivo
27	Spivak	Anti-HIV-1 Effector Potential of Mucosal-Associated Invariant T (MAIT) Cells
28	Liao	Novel in Vivo Model to Evaluate Human Gut Microbiota's Role in HIV Persistence and the Efficacy of HIV Cure Strategies
29	Routy	Immune Tolerance of Testicular Tissue and HIV Persistence
30	Ancuta	HIV Persists in CCR6+CD4+ T Cells from Colon and Blood During Antiretroviral Therapy
31	Goswami	CD4+ T Cell Subsets and Tissue-Associated Viral Loads in SHIV/HIV-1 Infected Tonsillar Tissue
32	Ling	Multiple Lymphoid Tissue Viral DNA and RNA Reservoirs in SIV-Infected Rhesus Macaques of Chinese Origin on Suppressive Antiretroviral Therapy
33	Soriano-Sarabia	Contribution of Gamma/Delta T Cell Subpopulations to the Latent HIV Reservoir
34	Wahl	Steps Towards the In Vivo Eradication of HIV Tissue Reservoirs: Differential Restoration of CD4+ Tissue-Resident Memory T Cells After Antiretroviral Treatment
35	Clayton	HIV-Infected Macrophages Resist Perforin-Mediated Killing via Necrosis and Promote Pro-inflammatory Cytokine Release by Cytotoxic T Lymphocytes
36	Clavel	Genetic Analysis of HIV-1 Env Sequences Expressed from the T-Cell Reservoir
37	Maldarelli	No Evidence of Ongoing HIV Replication in Tissue Compartments During Combination Antiretroviral Therapy
38	Liu	Potential Application of VRC01-CAR for Eradication of HIV-1 Reservoirs
39	Leibman	Optimized, Second Generation Chimeric Antigen Receptors Improve CAR T Cell Expansion and Control Over HIV-1 Replication
40	Vargas-Inchaustegui	CD4-Based Bispecific Chimeric Antigen Receptors: Towards a Functional Cure of HIV
41	Garrido	Natural Killer Cells for Clearing the HIV Reservoir
42	Huang, S.	CTLs Pare Defective HIV Proviruses Without Impacting Infectious Latent Reservoirs
43	Patel	HIV-Specific T Cells Expanded from HIV+ and HIV-Naive Donors Target a Range of Viral Epitopes: Implications for a Cure Strategy After Allogeneic HSCT
44	Kuruc	Safety and Tolerability of Reinfused Ex-Vivo Expanded, HIV-1-Specific T-Cell Therapy (HXTC)
45	Tcherepanova	Development of an Autologous HIV Outgrowth Process for the Manufacture of Targeted Immunotherapy for Latent Reservoir Clearance
46	Fuller	Factors Influencing the Immunogenicity of Therapeutic DNA Vaccination and Induction of Viral Remission After Stopping Drug Therapy
47	Sivanandham	Treg Depletion as an Effective Strategy to Induce SIV Reactivation and Clearance
48	Pavlakis	Heterodimeric IL-15 Induces Effector Cell Activation and Trafficking to the Germinal Centers of SIV infected Macaques
49	Alvarado	Small-Molecule Antagonists of the HIV-1 Nef Accessory Protein: Targeting the Nef Dimer

50	Emert-Sedlak	Orally Active HIV-1 Nef Inhibitors Rescue Cell-Surface MHC-I Expression and CTL Responses to HIV Infected Cells
51	Mediouni	Interaction of Didehydro-Cortistatin A with HIV-1 Tat and Inhibition of Tat Neurotoxic Activity
52	Valente	Activity of the Tat Inhibitor dCA on Primary Lymphocytes Isolated from Infected Individuals
53	Kessing	The Tat Inhibitor Didehydro-Cortistatin A Is a Potent Suppressor of SIV Replication
54	Nixon	In Vivo Analysis of the Efficacy of the Tat Inhibitor Didehydro-Cortistatin A
55	Giavedoni	Efficient Proviral Inactivation with SIV-Specific RNA-Guided Cas9 Nucleases and Nickases
56	Hu	In Vivo Excision of HIV Provirus by SaCas9 and Multiplex sgRNAs in Pre-Clinical Animal Models
57	Akala	Antiretroviral Drugs-Loaded Nanoparticles Fabricated by Dispersion Polymerization for Galt Targetable Drug Delivery System
58	Pernet	A Novel Chemoselection Strategy for Combinatorial Genetically Modified HIV Protected Hematopoietic Stem/Progenitor Cells
59	Stoddart	Inhibition of Heat Shock Protein 90 Prevents HIV Rebound
60	Dunham	Pharmacologic Inhibition of IDO Blunts Features of SIV-Related Chronic Inflammation
61	Hataye	HIV Exponential Growth Dependent on Burst Size Breakthrough of the Allee Threshold
62	Cardozo-Ojeda	Modeling HIV-1 Kinetics After the Infusion of VRC01 in Chronically Infected Individuals
63	Cao	Control of SIV with Short-Term Anti-PD-L1 Treatment
64	Zurakowski	Optimal Design of HIV Experiments with Design Constraints
65	Rosenbloom	Assessing Intra-Lab Precision and Inter-Lab Repeatability of Outgrowth Assays of HIV-1 Latent Reservoir Size
66	Flint	Human Tissue and Organ Research Resource (HTORR): Enabling Biomedical Research and Development
67	Lewin	Alemtuzumab Treatment for Sezary Syndrome: Effect on Latently Infected CD4+ T Cells
68	Sommerfelt	Approaching HIV Functional Cure Applying Vacc-4x, a Peptide-based Therapeutic HIV Vaccine: Clinical Development Methodological Considerations
69	Steens	Antiviral Activity and Safety of ABX464
70	Manak	Absence of Anti-HIV Antibody Following Treatment at Early HIV Acute Infection Presents Challenges to Monitoring HIV Infection
71	Saadatmand	The Impact of Exposure to Community and Interpersonal Violence on Alcohol and Drug Use, and HIV Risk Behaviors Among Young Adult African Americans
72	Mathews	An Innovation Contest as Community Engagement for HIV Cure Research in North Carolina: A Mixed Methods Evaluation
73	Dubé	Perceived Motivators, Deterrents, Concerns and Barriers Related to HIV Cure Research Participation in the United States: A Qualitative Study
74	Sylla	What Do Participants Want? Participant Hopes, Concerns, and Expectations of HIV Cure Researchers
75	Bromwich	HIV Cure Research and the Dual Aims of the Informed Consent Process

#1 Sardo, Luca

Investigating the Mechanisms of HIV-1 Latency by High Resolution Microscopy

Luca Sardo[1], Angel Lin[1], Svetlana Khakhina[1], Lucas Beckman[1], Luis Ricon[1], Weam Elbezanti[1], Tara Jaison[1], Harshad D Vishwasrao[2], Hari Shroff[3], Christopher J Janetopoulos[1], Zachary A Klase[1]

1. Department of Biological Sciences, McNeil Science and Technology Center, University of the Sciences, Philadelphia, Pennsylvania; 2. Advanced Imaging and Microscopy Resource, National Institutes of Health, Bethesda, Maryland; 3. Section on High Resolution Optical Imaging, National Institute of Biomedical Imaging and Bioengineering, National Institutes of Health, Bethesda, Maryland

The microscopic mechanisms that control HIV-1 latency in-vivo are still undefined. Biochemical evidence showed that HIV-1 expression is controlled by the state of chromatin condensation and by the recruitment on the LTR of negative and positive regulators of transcription. Microscopy allows to observe a single cell, but traditional techniques require fixation and the cell body hampers visualization of the nucleus. High resolution microscopy tools to study live transcriptional events at a nuclear level are in need.

To overcome these limitations we isolated live, transcriptionally competent nuclei from cell line models of HIV-1 latency and used antibody staining to track nuclear events in real-time. Nuclei were stained for factors and histone modifications involved in the control of HIV-1 transcription. Lamin and nucleic acids dyes were used to define shape, integrity and content of the nuclei.

High resolution images for a panel of chromatin markers were obtained and a distinct architecture was observed that has not been visualized by standard methods. Software assisted image analysis was used to identify diffraction limited spots and to obtain sub-nuclear position, co-localization and intensity. The effects of drugs that relieve HIV-1 latency were visualized in live nuclei and confirmed by flow cytometry and biochemical assays. In similar experiments we transfected a tagged version of HIV-1 Tat to visualize the location of the HIV-1 LTR.

We have developed a high resolution, multi-color imaging approach to investigate the in-vivo mechanisms involved in HIV-1 transcriptional latency.

#**2 Misra, Anisha**

Inhibition of HIV-1 by Glycosylphosphitidylinositol (GPI)-Anchored Anti-HIV Env scFv

Anisha Misra[1], Emile Gleeson[1], Weiming Wang[2], Chaobaihui Ye[2], Paul Zhou[2], Jason T Kimata[1]

1. Department of Molecular Virology and Microbiology, Baylor College of Medicine, Houston, Texas; 2. Unit of Antiviral Immunity and Genetic Therapy, Institut Pasteur of Shanghai-Chinese Academy of Sciences, Shanghai, China

Protecting HIV-specific CD4+ T cells from infection is necessary to maintain immunity without antiretroviral therapy. In previous studies, we demonstrated that single-chain variable fragments (scFv) from anti-HIV Env monoclonal antibodies could function as potent entry inhibitors when engineered to be tethered to the surface of target cells by a glycosyl-phosphitidylinositol (GPI) anchor. Interestingly, even if a virus escapes inhibition at entry, its replication is ultimately controlled. We hypothesized that in addition to functioning as fusion inhibitors, anti-HIV GPI-scFvs can interact with Env in an infected cell, thereby interfering with infectivity of newly produced virions. To test this hypothesis, we cotransfected 293T cells with HIV and SIV proviruses and plasmids expressing anti-HIV GPI-scFvs or a negative control GPI-scFv, and analyzed the expression level of the GPI-scFv constructs by flow cytometry, the level of virus production by HIV p24 ELISA, infectivity of the released virions using TZM-bl indicator cells, and viral protein expression by immunoblot analysis. We found that the anti-HIV Env GPI-scFvs reduced the release of HIV from cells 5-22-fold, and infectivity of the virions that were released was inhibited by 74% to 99%. By contrast, SIV production and infectivity were not affected by the anti-HIV Env GPI-scFvs. Furthermore, loss of infectivity of HIV was associated with a reduction in the amount of virion-associated Env gp120. Interestingly, an analysis of Env expression in cell lysates demonstrated that the anti-Env GPI-scFvs interfered with processing of Env gp160 precursors in cells. These data indicate that GPI-scFvs have the ability to inhibit Env processing and function, thereby restricting production and infectivity of newly synthesized HIV. Anti-Env GPI-scFvs therefore appear to be unique anti-HIV molecules, as they derive their potent inhibitory activity by interfering with both early (receptor binding/entry) and late (Env processing and incorporation into virions) stages of the HIV life cycle. GPI-anchored scFv-based gene therapy may also be useful for protecting CD4+ T cells from HIV in vivo.

#**3 He, Huan**

Efficient Transduction of Human and Rhesus Macaque Primary T Cells by a Modified Human Immunodeficiency Virus Type 1 (HIV-1)-Based Lentiviral Vector

Huan He[1], Jing Xue[2], Weiming Wang[1], Lihong Liu[1], Chaobaihui Ye[1], Zhe Cong[2], Jason T Kimata[3], Chuan Qin[2] , Paul Zhou[1]

1. The Unit of Anti-Viral Immunity and Genetic Therapy, the Key Laboratory of Molecular Virology and Immunology, the Institut Pasteur of Shanghai, Chinese Academy of Sciences, Shanghai, China; 2. Key Laboratory of Human Disease Comparative Medicine, Institute of Laboratory Animal Science, Chinese Academy of Medical Sciences and Comparative Medicine Center, Peking Union Medical College, Beijing, China; 3. Department of Molecular Virology and Microbiology, Baylor College of Medicine, Houston, Texas

Human immunodeficiency virus type-1 (HIV-1)-based lentiviral vectors efficiently transduce genes to human, but not rhesus macaque, primary T cells and hematopoietic stem cells (HSCs). The poor transduction of HIV-1 vectors to rhesus macaque cells is mainly due to species-specific restriction factors such as rhesus TRIM5α. Previously, several strategies to modify HIV-1 vectors were developed in order to overcome rhesus TRIM5α restriction. While the modified HIV-1 vectors efficiently transduce genes to rhesus HSCs, to rhesus primary T cells they remain suboptimal. Recently, HIV-1 variants that encode combinations of LNEIE mutations in capsid (CA) protein and SIVmac239 Vif were found to replicate efficiently in rhesus primary T cells (Soll et al. PLoS Pathog 2013;9:e1003667). Therefore, in the present study, we tested whether HIV-1 vectors packaged by a packaging construct containing these CA substitutions could efficiently transduce both human and rhesus primary CD4 T cells. To accomplish this, we made LNEIE mutations in the packaging construct CEMRΔ8.9, and generated recombinant HIV-1 vectors packaged by∆8.9 WT or ∆8.9 LNEIE. We then compared transduction rates, stability of CA, and vector integration in CEMss-CCR5 and CEMss-CCR5-rhTRIM5α/GFP cells as well as transduction rates using human and rhesus primary CD4 T cells by ∆8.9 WT or ∆8.9 LNEIE-packaged HIV-1 vectors. Finally, we studied the influence of rhesus TRIM5α variations and other host factors in transduction rates to primary CD4 T cells from a cohort of 37 Chinese rhesus macaques. Here, we report that while it maintains efficient transduction to human T cell line and primary CD4 T cells, Δ8.9 LNEIE-packaged HIV-1 vector overcomes rhesus TRIM5α-mediated CA degradation, resulting in significantly higher transduction efficiency of rhesus primary CD4 T cells than Δ8.9 WT-packaged HIV-1 vector. We also show that rhesus TRIM5α variations strongly influence transduction efficiency of rhesus primary CD4 T cells by both∆8.9 WT or ∆8.9 LNEIE-packaged HIV-1 vectors. Thus, we conclude that ∆8.9 LNEIE-packaged HIV-1 vectors indeed overcome rhesus TRIM5α-mediated species-specific restriction, resulting in high transduction efficiency into both human and rhesus macaque primary T cells. (Supported by CNSF - NIH joint grant 81361120406 – R01 AI106574 to PZ and JTK.)

#**4 Shu, Sherry**

The Role of HIV-1 Nef Dimerization in AP2 Recruitment, CD4 Downregulation and Infectivity in Cell Culture and Humanized Mice

Sherry T Shu[1], Lori A Emert-Sedlak[1], Thomas E Smithgall[1]

1. Department of Microbiology and Molecular Genetics, School of Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania

Background: The HIV-1 Nef accessory factor enhances viral infectivity, immune evasion, and AIDS progression. Nef hijacks host cell trafficking pathways to downregulate membrane-bound receptors, including CD4 via the adaptor protein 2 (AP2) complex. CD4 downregulation is linked to enhanced viral infectivity and immune escape from ADCC, identifying this important Nef function as a rational target for new antiretroviral drug discovery.

Methods: We developed a cell-based bimolecular fluorescence complementation (BiFC) assay to visualize the interaction of Nef with the AP2 complex and CD4. Interacting protein pairs were fused to complementary, non-fluorescent fragments of YFP and co-expressed in 293T cells. Nef interactions with CD4 or AP2 result in complementation of YFP and a bright fluorescent signal that can be detected by confocal microcopy. To test infectivity and CD4 T cell depletion in vivo, we generated and infected BLT (bone marrow-liver-thymus) and hu-PBMC humanized mice with HIV-1 harboring wild type or mutant Nef alleles.

Results: Nef interaction with both CD4 and the AP2 alpha and sigma2 subunits was readily visualized in live cells by BiFC assay. Co-expression of the AP2 alpha subunit enhanced the Nef:AP2 sigma2 BiFC signal and vice versa, suggesting that the AP2 alpha/sigma2 hemi-complex interacts cooperatively with Nef. Mutagenesis of Nef residues R134, E174, and D175, which stabilize the Nef docking surface for AP2 in a co-crystal structure (PDB: 4NEE), substantially reduced AP2 interaction without affecting CD4 binding. Remarkably, a dimerization-defective mutant of Nef failed to interact with either CD4 or AP2 in the BiFC assay, indicating that the quaternary structure of Nef is required for CD4 and AP2 recruitment as well as CD4 downregulation. Small molecule inhibitors of Nef dimerization also reduced Nef interactions with AP2 and CD4 by BiFC, providing further support for this idea and identifying the Nef dimer interface as a target for inhibitor action. In addition, preliminary data show that humanized mice infected with HIV-1 containing wild type Nef developed greater viral load and CD4+ T cell depletion compared to mice infected with viruses harboring dimerization-deficient Nef, suggesting an important role for the Nef dimer on infectivity and AIDS progression in vivo.

Conclusions: Here we describe a fluorescence complementation assay to visualize interactions between Nef, CD4 and the AP2 complex in live cells. Using this assay, we observed that the AP2 alpha and sigma2 subunits interact cooperatively with Nef, and that the interaction is dependent upon an internal Nef salt bridge present in a recent crystal structure of the Nef:AP2 complex. Complex assembly also requires an intact Nef dimerization interface, as a dimerization-defective Nef mutant failed to downregulate CD4 in cells. Infection of humanized mice with HIV-1 harboring this same dimerization-defective Nef mutant failed to generate a high viral load and deplete CD4+ T cells. Small molecules that inhibit Nef dimerization also suppress AP2 and CD4 recruitment in the BiFC assay, which may provide a mechanistic explanation for their effects on HIV infectivity.

#**5 Lan, Jie**

Protease Inhibitors and Neutralizing Antibodies Effectively Block Cell-Free and Cell-to-Cell Transmission of HIV-1 From Provirus-Activated Latently Infected Cells to Uninfected Cells

Jie Lan[1], Nicole Shepherd[1], Yanyan Xing[1], Wei Li[1], Sushmita Rane[1], Qigui Yu[1]

1. Indiana Center for AIDS Research and Department of Microbiology and Immunology, Indiana University School of Medicine, Indianapolis, Indiana

Latently HIV-1-infected cells harboring replication-competent proviruses represent a major obstacle for HIV-1 eradication. Efforts to purge these latently infected cells have focused on development of “Shock and Kill” strategies to reactivate the proviruses. These latently infected cells would be killed after reactivation of virus gene expression by viral cytopathic effects (CPEs), host immune responses, or both. Several latency-reversing agents (LRAs) including histone deacetylase inhibitors (HDACi) such as romidepsin (RMD), valproic acid (VPA), suberoylanilide hydroxamic acid (SAHA) and protein kinase C activators such as prostratin have been explored to force activation of proviruses in latently-infected resting CD4+ T cells that constitute the major reservoir of HIV-1 in vivo. These LRAs must be used in combination with combined antiretroviral therapy (cART) to prevent new infections in HIV-1 patients. In the present study, we evaluated the effects of different classes of cART drugs and broadly neutralizing antibodies (nAbs) on blockage of cell-free and cell-to-cell transmission of HIV-1 from provirus-activated ACH-2 cells (a human T cell line latently infected with HIV-1) to A3.01 cells (the parental uninfected cell line). Western blot revealed the bands of Nef and Env proteins in RMD-treated ACH-2 cells at 3 h post-exposure, indicating that RMD triggers expression of Nef and Env early. The intensity of Nef and Env increased over time and reached the highest levels 24 h post-activation. Cell-free and cell-associated virions became detectable at 6 h post-exposure of RMD and gradually increased over time. Within 24 h cell-cell or trans well co-culture, RMD-treated ACH-2 cells effectively transferred HIV-1 virions to uninfected A3.01 cells via cell-to-cell transmission, while cell-free virus infection was minimal. The anti-fusion drug T20 and certain protease inhibitors (PIs), but not all PIs, effectively blocked cell-free and cell-to-cell transmission of HIV-1. In contrast, reverse transcriptase inhibitors did not show any blocking activity. Broadly nAbs including 2G12, 4E10, and VRC-01, but not non-nAbs such as A32, effectively blocked cell-free and cell-to-cell transmission HIV-1 from provirus-activated ACH-2 cells to uninfected A3.01 cells. Our results suggest that PIs, T20, nAbs, or their combinations should be used in the “Shock and Kill” strategies to prevent new infection of cells with HIV-1 virions from provirus-activated cells.

#**6 Kyei, George**

Inhibition of SF3B1 Modify the Epigenome and Prevent HIV Provirus Reactivation

Rashmi Ramani[1], Shanshan Meng[1], Thomas Webb[2], Lee Ratner[1], George Kyei[1]

1. Department of Medicine, Washington University in St. Louis, St. Louis, Missouri; 2. Division of Biosciences, SRI International, Menlo Park, California

The main obstacle to HIV cure is the presence of latent proviruses in resting CD4+ T cells. Modifying the T cells epigenome to silence HIV proviruses may help achieve a functional cure. Although this approach has received less attention in the HIV cure efforts, the abundance of apparently inactive endogenous retroviruses in the vertebrate genome provides an evolutionary validation for this hypothesis. To advance this approach, there is the need to discover new epigenetic targets that control HIV transcription and reactivation from latency. Using a screen of splicing inhibitors, we found that splicing factor 3b subunit 1 (SF3B1) could be a viable target for this approach. SF3B1 is involved in chromatin modifications and guides nascent transcripts to appropriate splice sites. When SF3B1 is inhibited, trimethylation of histone H3 lysine 36 (H3K36me3) is excluded from some genes. Since H3K36me3 is associated with HIV integration sites, we hypothesized that SF3B1 may play a role in HIV latency. Our results show that knockdown of SF3B1 severely attenuate HIV replication in multiple cell types and abrogate Tat-mediated HIV transcription. Using sudemycin D6, a specific inhibitor of SF3B1, we found a dose-dependent inhibition of HIV reactivation in cell lines and in a primary cell model of latency, irrespective of the latency reversing agent used. In addition, SF3B1 inhibition blocks RNA polymerase II recruitment to the HIV promoter and reduces the trimethylation of histone H3K36. Thus inhibitors of SF3B1 could play a role in silencing HIV proviruses for a functional cure.

#**7 Ho, Ya-Chi**

Cells Containing Hypermutated HIV-1 Proviruses Can Be Preferentially Targeted by Cytotoxic T Lymphocytes

R Brad Jones[1], Mihaela Pertea[2], Katherine M Bruner[2], Ross A Pollack[2], Adam A Capoferri[2], Robert F Siliciano[2], Ya-Chi Ho[2]

1. The George Washington School of Medicine and Health Sciences, Washington, DC; 2. Johns Hopkins University School of Medicine, Baltimore, Maryland

Background: The majority of HIV-1 proviruses in individuals under suppressive antiretroviral therapy (ART) are defective due to APOBEC-mediated G-to-A mutations, large internal deletions, packaging signal deletions or point mutations. We have previously shown that defective HIV-1 proviruses, having intact LTR promoter function and the lack of promoter CpG methylation, can be transcribed and translated. It has been shown that HIV-1 infected cells may undergo clonal expansion through integration into cell proliferation genes, and the frequency of clonally expanded cells increases over time. However, it has also been shown that the frequency of HIV-1 DNA remains stable over time in individuals under ART, raising the question of how the frequency of clonally expanded HIV-1-infected cells may increase over time but the overall frequency of HIV-1-infected cells remains unchanged. Furthermore, in some clinical trials using the shock-and-kill strategy, a significant decrease in the frequency of HIV-1 proviral DNA is observed (up to 30%), given that the frequency of replication competent HIV-1 provirus is 10% or less. We propose that cytotoxic T lymphocytes (CTLs) can preferentially target certain subpopulations of defective HIV-1 proviruses and shape the landscape of HIV-1 proviruses and thus contribute to the dynamics of HIV-1 DNA levels in HIV-1-infected individuals.

Methods/Results: To understand whether HIV-1 proviral landscape in virally suppressed HIV-1-infected individuals may change over time, we first examined the frequency of each defective proviral subsets in HIV-1-infected individuals under suppressive ART. We found that the frequency of hypermutated proviruses correlates negatively with the duration of infection, while the frequency of proviruses containing large internal deletions correlates positively with the duration of infection, implying that the HIV-1 proviral landscape may change during the course of infection despite suppressive ART.

To examine whether defective HIV-1 proviruses can be recognized by CTLs in vitro, we co-cultured CD4+ T cells transfected with defective HIV-1 proviruses with autologous CTL clones known to recognize specific epitopes. CTL recognition of cells containing defective HIV-1 proviruses were measured by CD107a expression. Surprisingly, we found that cells containing hypermutated proviruses which has a premature stop codon or mutated start codon 5’ to an intact CTL epitope can be recognized by CTLs, while cells containing large internal deletions cannot be recognized by CTLs.

To understand whether hypermutated HIV-1 proviruses may be recognized by CD8+ T lymphocytes ex vivo, we used targeted deep-sequencing to examine the frequency of APOBEC-mediated nonsense mutations in gag. We compared resting and activated CD4+ T cells treated with and without autologous pre-stimulated CD8+ T cells. We found that the frequency of HIV-1 RNA containing premature stop codons 5’ to the CTL recognition site decreased upon CD8+ T cell co-culture, indicating that cells containing hypermutated proviruses can be eliminated by CD8+ T cells ex vivo.

Conclusions: We found that hypermutated HIV-1 proviruses may be targeted by CTLs. HIV-1 proviral landscape may be shaped, at least in part, by CTL negative selection pressure on cells containing hypermutated HIV-1 proviruses. HIV-1 DNA measurement may in part reflect the effect of HIV-1 elimination strategies.

#**8 Musick, Andrew**

A Small Fraction of Proviruses in Expanded Clones Express Unspliced HIV RNA in Vivo

Andrew Musick[1], Jon Spindler[1], Michele Sobolewski[2], Brandon Keele[1], Michael J Bale[1], Wei Shao[3], Ann Wiegand[1], Stephen Hughes[1], John Mellors[2], John M Coffin[4], Frank Maldarelli[1], Mary F Kearney[1]

1. HIV Dynamics and Replication Program, CCR, NCI-Frederick, Frederick, Maryland; 2. Department of Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania; 3. Advanced Biomedical Computing Center, Leidos Biomedical, Inc., Frederick, Maryland; 4. Department of Molecular Biology and Microbiology, Tufts University, Boston, Massachusetts

Introduction: The vast majority of proviruses that persist on ART are defective. Of the minority that are intact (~2%), the fractions that are latent or transcriptionally active are not known. To address this question, we determined the fraction of proviruses that express HIV RNA in vivo in cell populations carrying either intact or defective proviruses.

Methods: PBMC were obtained from Patient #1 in Maldarelli, et al. (Science, 2014). This donor had multiple clones of cells that contain intact or defective proviruses. Proviral expression was determined by single-genome pro-pol sequencing (SGS) of HIV DNA and RNA from multiple aliquots of PBMC diluted to an endpoint such that each aliquot contained one to a few HIV RNA expressing cells. Intact proviruses were identified using viral outgrowth assays (VOA). The levels and fractions of cells expressing HIV RNA were determined for probable clones (identified by identical sequence matches) carrying intact and defective proviruses.

Results: A total of 77 million PBMC were analyzed, of which 10,450 contained HIV pro-pol sequences. Fourteen percent of the infected cells expressed HIV RNA. The median levels of expression in single cells was 1 RNA/cell (ranging from 1-16). We identified 412 different WT or hypermutant RNA species in infected cells. Of these, 3 were from expression of intact proviruses, 81 from obviously defective proviruses, and 328 from proviruses that were likely defective (did not grow out in VOA but did not contain stop codons in the region analyzed). The median fraction of cells in the probable clones (those with matching DNA and RNA) that carried intact proviruses (N=3) and expressed HIV RNA was 2.3% (1.2%-8.8%). For clones carrying defective proviruses (N=5), the median expressing was 3.5% (0.9%-7.0%), and for presumptive clones carrying likely defective proviruses (N=26), the median was 6.6% (1.3%-66.7%) (p =0.51 for a difference across groups).

Conclusion: The large majority (>80%) of infected cells that persist on ART are either latent or incapable of HIV RNA expression. A small fraction of proviruses within infected clones expressed unspliced HIV RNA, but this fraction was not significantly different between clones carrying intact proviruses from clones containing obviously defective proviruses, indicating that HIV RNA expression appears similarly detrimental (or non-detrimental) for infected cells regardless of whether the provirus they carry is intact.

#**9 Romerio, Fabio**

The HIV-1 Antisense Transcript Ast Recruits the Polycomb Repressor Complex 2 to the HIV-1 5'LTR and Acts as a Viral Latency Factor

Juan Zapata[1], Federica Campilongo[1], Robert Barclay[2], Catherine DeMarino[2], Maria Iglesias-Ussel[1], Fatah Kashanchi[2], Fabio Romerio[1]

1. Institute of Human Virology, University of Maryland School of Medicine, Baltimore, Maryland; 2. Laboratory of Molecular Virology, George Mason University, Manassas, Virginia

The HIV-1 Tat protein is necessary for exit from latency, and for efficient viral replication. On the other hand, the prevailing view has been that HIV-1 does not encode a factor that promotes entry into latency, and that environmental stimuli indirectly control this process. During viral latency the expression of the integrated HIV-1 provirus is restricted via epigenetic events that position the nucleosome Nuc-1 at the 5' long terminal repeat (5'LTR). These events include DNA methylation, and histone acetylation and methylation. Enhancer of zest homolog 2 (EZH2) – a core component of the polycomb repressor complex 2 (PRC2) – is the dominant histone methyltransferase that regulates nucleosome assembly at the HIV-1 5'LTR via trimethylation of lysine 27 on histone H3 (H3K27me3). In higher eukaryotes, long noncoding RNA molecules (lncRNA) regulate the expression of coding transcript encoded on the opposite strand by recruiting PRC2 to their promoter. Two very important and possibly related questions are still open. First, how is Nuc-1 precisely and invariably positioned at the 5'LTR irrespective of the site and orientation of HIV-1 integration into the host genome? Second, what is the lncRNA that recruits PCR2 to the HIV-1 5'LTR? An attractive hypothesis that would answer both questions is that HIV-1 has evolved the ability to encode for its own lncRNA as an autonomous mechanism to recruit PRC2 to the 5'LTR, and to establish latency regardless of the chromatin context, integration site and orientation into the host genome. The HIV-1 proviral genome directs the expression of an antisense transcript (Ast) from a promoter located within the 3'LTR. Here we show that Ast suppresses HIV-1 expression, and promotes viral latency by acting as an lncRNA. We found that Ast is expressed in many infected cell types, including chronically infected cell lines, primary CD4+ T cells infected in vitro, as well as resting memory CD4+ T cells from HIV-1 infected individuals under suppressing antiretroviral therapy. HIV-1 infection of Jurkat cells stably transduced with a lentivirus expressing Ast results in reduced viral replication. Moreover, reactivation of HIV-1 in latently infected Jurkat E4 cells stably transduced with the same lentiviral construct (JE4AST) is significantly reduced, whereas return to latency is accelerated. ChIP assays showed that RNA Pol II is nearly absent at the HIV-1 promoter in JE4AST cells, and that its presence does not increase following stimulation with latency reversing agents. On the other hand, EZH2 and H3K27me3 persist at high levels at Nuc-1 in JE4AST cells even after exposure to viral reactivation agents. Finally, we demonstrate that the HIV-1 antisense RNA Ast interacts with members of PRC2 (EZH2 and SUZ12). Altogether, these results show that Ast is an HIV-encoded inducer of viral latency that acts by recruiting PRC2, thus leading to nucleosome assembly and chromatin silencing at the 5'LTR. In addition, they could guide in the design of new therapeutic strategies aimed at permanent stabilization of latency by exploiting Ast.

#**10 Lee, Sook-Kyung**

Quantification of the Latent HIV-1 Reservoir Using Primer-ID Based Ultra Deep Sequencing Assay

Sook-Kyung Lee[1], Shuntai Zhou[1], Pedro L Baldoni[3], Ean Spielvogel[1], Nancie M Archin[2], Michael G Hudgens[3], David M Margolis[2], Ronald Swanstrom[1]

1. Department of Biochemistry and Biophysics, and the UNC Center for AIDS Research, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina; 2. Departments of Medicine, Microbiology & Immunology, and UNC HIV Cure Center, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina; 3. Department of Biostatistics, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina

HIV-1 persistence in viral reservoirs such as resting memory CD4+ T cells is the major hurdle to virus eradication. In this study, we measured the latent HIV-1 reservoir harboring replication-competent HIV-1 in resting CD4+ T cells in participants on highly active antiretroviral therapy (HAART), quantitating the frequency of latent infection through the use of a Primer ID-based Ultra Deep Sequencing Assay (UDSA), in comparison to the readout of the quantitative viral outgrowth assay (QVOA). Viral RNA derived from culture wells of QVOA that scored as HIV-1 p24-positive were tagged with a specific barcode during cDNA synthesis, and the sequences within theV1-V3 region of HIV-1 env gene were analyzed using the Primer ID-based paired-end MiSeq platform. We analyzed samples from a total of 19 participants, 2 initially treated with HAART in acute infection and 17 treated during chronic infection. Phylogenetic trees were generated with all viral lineages detected from culture wells derived from each participant to determine the number of distinct viral lineages (DVLs) growing out in each well, thus capturing another level of information beyond the well being positive for viral antigen. The infectious units per million cells (IUPM) values estimated using a maximum likelihood approach, based on the number of DVLs detected (VOA-UDSA), were compared with those obtained from QVOA measured using limiting dilution. The frequency of method-introduced recombination was also examined by performing a cDNA reaction with a mixture of two genetically distinct parental viral lineages. IUPM estimates determined by VOA-UDSA ranged from 0.14 to 3.66 and strongly correlated with the IUPM estimates determined by QVOA ($r=0.94$; $P<0.0001$). Only 0.08% of all template consensus sequences were the result of method-introduced recombinant sequences. VOA-UDSA can be an alternative readout for that currently used for QVOA. Future work will determine if this approach can improve the accuracy of measurements of the latent reservoir and/or reduce the number of cells needed to attain a desired level of accuracy.

#**11 Zhang, Yuwei**

An Affordable and Sensitive Assay to Detect Replication-Competent HIV Reservoir in CD4+ T Cells From HIV+ Subjects on ART

Yuwei Zhang[1,2], Vanessa Sue Wacleche[1,2], Huicheng Chen[1,2], Delphine Planas[1,2], Annie Gosselin[1,2], Jean-Pierre Routy[3], Petronela Ancuta[1,2]

1. Department of Microbiology, Infectiology, and Immunology, Faculty of Medicine, Université de Montréal, Montréal, QC, Canada; 2. CHUM-Research Centre, Montréal, QC, Canada; 3. McGill University Health Centre-Glen Site, Montréal, QC, Canada

Background: Despite the fact that current antiviral therapies (ART) successfully control viral replication in HIV infected subjects, the persistence of viral reservoir represent a major barrier for the cure. The high risk of viral rebound upon treatment interruptions imposes a life-long treatment. Thus, the development of HIV eradication strategies represents a major research priority. To assess the efficiency of such strategies, quantitative and qualitative assays are needed to detect replication-competent HIV reservoirs responsible for viral rebound. Here we describe a modified viral outgrowth assay (VOA) that measure replication-competent viral reservoirs in low numbers of cells from HIV-infected individuals receiving ART.

Methods: CD4+ T-cells were isolated by MACS from PBMCs of HIV+ subjects on ART with no detectable viral load in the plasma. A fraction of cells (0.5x10e6) was used for quantification of integrated HIV-DNA ex vivo using real-time nested PCR. Another fraction of cells (10e6) was cultured in complete RPMI1640 medium in the presence of plate-bound CD3 and soluble CD28 Abs (1 µg/ml) for 3 days. Cells were washed and split then cultured in complete medium plus IL-2 (5 ng/ml) for 9 more days. To ensure the viability of cells and efficiency of viral expansion, cells were split every three days. Each split was kept as a replicate. Supernatants were collected to measure levels of HIV-p24 by ELISA. At day 12, cells were harvested and intracellular HIV-p24 staining and flow cytometry analysis were performed. All-trans retinoic acid (ATRA; 100nM) was added to facilitate reactivation and viral transmission in culture.

Results: HIV reactivation, confirmed by both HIV-p24 ELISA and FACS, was detected in CD4+ T cells from 8 out of 10 HIV+ subjects on ART using this protocol at day 9-12 post-stimulation. Levels of viral replication varied in different donors and did not correlate with integrated HIV-DNA levels measured ex vivo. This assay was highly reproducible and results from independent experiments were well correlated ($r=0.96$, $p<0.0001$). Finally, ATRA greatly accelerated HIV reactivation that was detected at day 6 post-stimulation. The cost of this assay is estimated at 200$ per donor.

Conclusions: Here we described an easy, affordable and highly reproducible assay that measures replication-competent HIV reservoirs in CD4+ T cells of ART-treated individuals. This assay may be used to assess the efficiency of HIV eradication strategies and evaluate the risk of viral rebound upon treatment interruption. Future studies should compare the efficacy of this assay to that of the existent and well-established “gold standard” VOA.

#**12 Sahu, Gautam**

A Simple Viral Outgrowth Assay (simVOA) to Monitor the Levels of Inducible Latent HIV Reservoir in Patients

Gautam K Sahu[1], Roberto Ramirez[1], Gail Skowron[1]

1. HIV Biology and Persistence Laboratory, Roger Williams Medical Center, Providence, Rhode Island

Background: The latent HIV reservoir remains a major impediment to curing the infection. Currently, the frequencies of inducible latent proviruses residing in CD4 T cells are measured through a quantitative viral outgrowth assay that remains a fairly complex and lengthy procedure. This assay generally requires a high volume (>150ml) of patient's blood to isolate and stimulate resting memory CD4 T cells, and also some amounts of a normal donor's PBMCs (γ-irradiated) and stimulated CD4 T cells to use them as feeders for cell stimulation and virus expansion, respectively. It would be desirable to have a simple method for viral outgrowth to measure the levels of inducible latent reservoir present in vivo.

Methods: The robust growth of patients' CD4 T cells is achieved by stimulating these cells with a cocktail of stimulators (COS) ex vivo. These cells are found to proliferate in culture for an extended period of time, allowing the reactivated latent virus to spread solely in the autologous CD4 T cells. About 5x106 of CD4 T cells purified from ~30 ml of blood are equally distributed among 25 wells in a 48-well plate and stimulated and cultured for 18 days. Media from each well are then tested for HIV-p24 by ELISA. From the number of HIV-p24+ wells, the frequencies of inducible latently infected cells are calculated.

Results: By using this simVOA, we have quantified the frequencies of cells carrying inducible latent proviruses in four patients on suppressive antiretroviral therapy, and found that the mean frequencies ranged from 0.6 to 2 per 106 CD4 T cells, which are very much similar to the frequencies generally reported for many treated patients. Interestingly, some of the replication-competent viruses outgrown from each of two patients' CD4 T cells in independent wells were genetically identical.

Conclusion: Our straightforward method for viral outgrowth from CD4 T cells could be useful for monitoring the levels of inducible latent proviruses in patients in the efforts of HIV eradication. In addition, the detection of identical replication-competent proviruses in independent wells in the assay may suggest that the clonally expanded CD4 T cells that are usually observed in treated patients can often harbor replication-competent proviruses.

#**13 Takata, Hiroshi**

A Novel HIV Latency Model Using Primary CD4+ T Cells From HIV-Infected ART-Suppressed Treated Individuals

Hiroshi Takata[1,2], Cari Kessing[3], Aaron Sy[1,2], Julia Garnett[1,2], Nicolas Chomont[4], Susana Valente[3], Lydie Trautmann[1,2]

1. Henry M. Jackson Foundation for the Advancement of Military Medicine, Bethesda, Maryland; 2. U.S. Military HIV Research Program, Walter Reed Army Institute of Research, Silver Spring, Maryland; 3. Scripps Research Institute Florida, Jupiter, Florida; 4. Université de Montréal et Centre de Recherche du CHUM, Montréal, QC, Canada

Background: The small number of latently HIV-infected cells in vivo has setback studies of HIV latency and robust measurements of viral reactivation with latency-reversing agents (LRAs). Cell line models of latency have been very useful due to their tractability; however, their inability to cycle between quiescent and active phases and their clonal nature has prompted the development of primary cellular models of latency. The current primary cell models of latency utilizing endogenous virus and CD4+ T cells from HIV infected individuals require leukapheresis to obtain the large amount of cells needed to test LRAs. To overcome these limitations, we developed a primary CD4+ T cell model of HIV latency with in vitro expanded memory CD4+ T cells isolated from virally suppressed HIV-infected individuals in the presence of Antiretroviral drugs (ARVs).

Methods: PBMCs from 5 HIV-infected individuals on stable suppressive ART for more than 3 years were used to isolate memory CD4+ T cells. None of the subjects had detectable plasma viremia at the time of blood sampling. The CD4+ T cells were expanded with phytohemagglutinin (PHA) and rhIL-2 for 14 days to establish primary CD4+ T cell lines in the presence of ARV treatment (Azidothymidine, Efavirenz, and Raltegravir). The CD4+ T cell lines were further expanded as in the initial expansion, and T cell activation was monitored by expression of Ki-67 and CD25 by flow cytometry. Integrated and total HIV DNA were measured by real time nested PCR. HIV production in culture supernatant was assessed by ultrasensitive RT-PCR.

Results: Primary memory CD4+ T cells from virally suppressed individuals were polyclonally expanded 159 (47-255) times from their initial input after primary and secondary expansions. The CD4+ T cells were sustained in culture for more than 36 days after the secondary stimulation, and retained the ex vivo frequencies of cells harboring integrated and total HIV DNA after the expansion (Integrated: 634.5, 159-1197; Total: 409.0, 39-926). T cell activation peaked at 7 days after the second stimulation, with 63.2% (49.4-70.5) Ki-67+ cells and 77.7% (68.3-77.7) CD25+ cells at day 7. Both activation markers decreased over time during the culture period. T cell activation status was concomitant with virus production in the culture supernatant (472 copies/ ml, 24-880), and decreased below 10 copies/ ml in 4 out of 5 individuals 36 days after the expansion. Importantly, these resting CD4+ T cells retained the capacity to produce HIV after anti-CD3/28 stimulation, or treatment with Prostratin. This model allowed us to test 22 LRAs and measure HIV production on a large population of latently infected CD4+ T cells initially generated from only 0.4-2.4 x106 primary memory CD4+ T cells.

Conclusions: Using this primary CD4+ T cell culture model, we generated large numbers of latently HIV infected CD4+ T cells that are able to maintain original HIV provirus content. The initial and secondary expansion with primary memory CD4+ T cells enables us to generate enough CD4+ T cells to screen a relatively large number of LRAs without requiring leukapheresis, immortalized cell lines, in vitro HIV infection, or genetic modification of CD4+ T cells. Importantly, HIV latently infected CD4+ T cells generated in this model harbor endogenous HIV in genuine memory CD4+ T cells from virally suppressed HIV-

infected individuals. This model should be useful not only to evaluate potential HIV latency-reversing agents, but also to understand the mechanisms responsible for the persistence of latency in primary CD4+ T cells.

#**14 Pache, Lars**

Identification of Smac Mimetics as a Novel Class of HIV-1 Latency Reversing Agents

Lars Pache[1], Miriam S Dutra[1], Adam M Spivak[2], John M Marlett[3], Jeffrey P Murry[3,7], Young Hwang[4], Ana M Maestre[5], Lara Manganaro[5], Mitchell Vamos[1], Peter Teriete[1], Laura J Martins[2], Renate König[1,6], Viviana Simon[5], Alberto Bosque[2], Ana Fernandez-Sesma[5], Nicholas D P Cosford[1], Frederic D Bushman[4], John A T Young[3,8], Vicente Planelles[2], Sumit K Chanda[1]

1. Sanford Burnham Prebys Medical Discovery Institute, La Jolla, California; 2. University of Utah School of Medicine, Salt Lake City, Utah; 3. The Salk Institute for Biological Studies, La Jolla, California; 4. Perelman School of Medicine at the University of Pennsylvania, Philadelphia, Pennsylvania; 5. Icahn School of Medicine at Mount Sinai, New York, New York; 6. Paul-Ehrlich-Institut, Langen, Germany; 7. Gilead Sciences, Foster City, California; 8. F. Hoffmann-La Roche Ltd., Basel, Switzerland

Replication of HIV-1 is highly dependent on components of the host cell machinery. To identify constitutively expressed factors that limit HIV-1 replication we selected a set of candidate genes that have the potential to impede viral replication based on a meta-analysis of multiple genome-wide screening datasets. Employing a targeted RNAi screen we identified 139 factors that interfere with early stages of HIV-1 replication. A subset of these factors were subjected to additional validation and mapped to specific stages of the viral life cycle.

One of these factors, BIRC2, limits HIV-1 transcription through an NF-κB-dependent mechanism. BIRC2 is a negative regulator of non-canonical NF-κB signaling, suggesting that this pathway activates HIV-1 transcription. Consistently, activation of non-canonical NF-κB signaling through BIRC2 depletion enhances HIV-1 replication. Interestingly, treatment of latently infected Jurkat (JLat) cells with small-molecule BIRC2 antagonists known as Smac mimetics led to a reactivation of the virus in this latency model. Moreover, Smac mimetics acted synergistically with HDAC inhibitors to reverse latency in JLat cell lines. We further confirmed the latency-reversing activity of these compounds in the more physiological context of resting CD4+ T cells isolated from aviremic HIV-infected patients. We have found that Smac mimetic treatment, in combination with the HDAC inhibitor panobinostat, resulted in synergistic activation of the latent reservoir in this ex vivo system.

While previous studies have demonstrated that canonical NF-κB signaling is utilized by HIV-1 to increase viral replication, the role of the non-canonical NF-κB pathway has been more elusive. Taken together, our findings suggest that non-canonical NF-κB signaling increases HIV transcription. Moreover, activating the non-canonical NF-κB pathway by targeting BIRC2 with Smac mimetics appears as a promising new therapeutic strategy to reactivate latent HIV-1 while limiting toxicity risks associated with systemic activation of canonical NF-κB signaling.

#**15 Feng, Qin**

Crosstalk Between Histone Modifications Indicates That the Arginine Methyltransferase CARM1 Is a Pharmacological Target for Reactivation of HIV Latency

Zheng Zhang[1], Bryan Nikolai[1], Leah Gates[1], Sung Yun Jung[2], Edward Siwak[3], Bin He[4], Andrew Rice[3], Bert O'Malley[1], Qin Feng[1]

1. Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, Texas; 2. Department of Biochemistry and Molecular Biology, Baylor College of Medicine, Houston, Texas; 3. Department of Molecular Virology and Microbiology, Baylor College of Medicine, Houston, Texas; 4. Department of Medicine-Hematology & Oncology, Baylor College of Medicine, Houston, Texas

During HIV infection, proviral DNA is integrated into the host genome and host transcriptional machinery is utilized to transcribe HIV mRNAs for production of viral proteins and mature virions. In a small population of cells, HIV provirus remains transcriptionally silent and does not produce viral antigen or proteins to make new virions. This reservoir of latent HIV provirus is resistant to the currently widely used Combination Antiretroviral Therapy (cART), making it difficult to eradicate and thus a major obstacle toward achieving a functional cure within patients. HIV latency is largely determined by the repressive status of chromatin at the proviral locus. In eukaryotic cells, gene expression is strictly controlled by epigenetic modifications on chromatin, and modification of histone H3K27 acts as a key molecular switch for activation or suppression of many cellular genes. In this study, we found that K27-acetylated histone H3 specifically recruits the Super Elongation Complex (SEC), the transcriptional elongation complex essential for HIV-1 long terminal repeat (LTR)-mediated and general cellular transcription. Interestingly, H3K27 acetylation further stimulates H3R26 methylation, which subsequently abrogates the recruitment of SEC, forming a negative feedback regulatory loop. Importantly, by inhibiting methyltransferase activity of CARM1, the enzyme responsible for H3R26 methylation, HIV-1 transcription is induced in several HIV latency cell models, including a primary resting CD4+ T cell model. When combined with other latency-disrupting compounds such as JQ1 or vorinostat/SAHA, the CARM1 inhibitor achieved synergistic effects on HIV-1 activation. This study suggests that coordinated and dynamic histone modifications orchestrate HIV-1 LTR mediated transcription at the chromatin level, and potentially opens a new avenue to disrupt latent HIV-1 infection by targeting specific epigenetic enzymes.

#**16 Nikolai, Bryan**

Host Cell Deubiquitinases Are Modulators of HIV Infection and Latency

Bryan C Nikolai[1,2], Qin Feng[1], Brian York[1], Subhamoy Dasgupta[1], Ed Siwak[2], David M Lonard[1], Andrew P Rice[2], Bert W O'Malley[1]

1. Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, Texas; 2. Department of Molecular Virology and Microbiology, Baylor College of Medicine, Houston, Texas

Human Immunodeficiency Virus (HIV) is an obligate parasite of host cell molecular biology. Proviral latency and infection are thus subject to the signaling and epigenetic state of the host cell, which is pharmacologically malleable. Post-translational modification (PTM) of proteins with phosphorylation, methyl, acetyl, and ribosylations, and ubiquitin-type protein moieties remains a major focus for reversal or induction of HIV latency and infection. In addition to the role of ubiquitin in proteasomal degradation, modification of proteins by ubiquitin and ubiquitin-like peptides can alter protein structure and function. Ubiquitination is involved in many essential cell processes, including histone ubiquitination during transcription and the 'ubiquitination code' for sorting of proteins in membrane-bound vesicles during cellular trafficking. Importantly, the ubiquitination mark does not always flag the protein for destruction and is subject to reversal by a class of nearly 100 enzymes, the deubiquitinases (DUbs). These proteolytic enzymes cleave or trim monoubiquitin or entire ubiquitin chains from diverse substrates and have been suggested to be involved in the life cycle of viruses, including HIV. We recently showed that the transcriptional coactivator Steroid Receptor Coactivator 3 (SRC-3) participates in HIV proviral transcription and we identified small molecule compounds capable of increasing SRC-3 intrinsic activity. The lead compound MCB-613 activates HIV reporter activity synergistically with other known latency reversing agents (LRAs) JQ1 and SAHA. Mechanistic work on this drug has revealed striking structural and activity similarities to a selective class of DUb inhibitors and we find that the structurally unrelated, broad-spectrum DUb inhibitor PR-619 also can function as an LRA. When treated with MCB-613 or PR-619, viral transcripts are induced in HIV-infected primary T cells but viral outgrowth does not occur. Moreover, infectious lentiviral vectors are not produced from transfected 293T/17 packaging cells and p24 levels are reduced in DUb-treated cells and supernatants. We report that MCB-613 and PR-619 (DUb inhibitors) increase total cellular ubiquitination as well as ubiquitinated SRC-3, which is a highly active form of this coactivator. A knockdown screen of DUbs revealed several candidates that inhibit the intrinsic activity of SRC-3 coactivator, presumably by cleaving ubiquitin from its transcriptionally active form. Knockdown and selective inhibition of DUbs from the screen identifies a potential role for USP14 in HIV transcriptional latency, while other DUbs are thought to regulate Gag trafficking. Importantly, we observe low toxicity of MCB-613 and its derivatives in vitro and treated mice are normophagic and healthy. Our results suggest that enzymatic cleavage of ubiquitin by host cell deubiquitinases (DUbs) is an essential, pharmacologically targetable, and bifunctional step during HIV-1 infection and proviral transcriptional latency. Collectively, the results imply that DUb inhibitors can be bifunctional and selective against HIV latency or against production of new virus.

#**17 Huang, Huachao**

A Novel Bromodomain and Extra-Terminal Domain Inhibitor That Reverses HIV-1 Latency

Huachao Huang[1], Shuai Liu[2], James Bradner[3,4], Wei Zhang[2], Jian Zhu[1]

1. Department of Microbiology and Immunology, University of Rochester Medical Center, Rochester, New York; 2. Department of Chemistry, University of Massachusetts Boston, Boston, Massachusetts; 3. Dana-Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts; 4. Novartis Institutes for BioMedical Research, Cambridge, Massachusetts

Although combinatory antiretroviral therapy (cART) is effective to reduce HIV-1 viremia, it does not eliminate HIV-1 infection.HIV-1 remains latent with the presence of cART, impeding the cure of HIV-1/AIDS. Recently, latency-reversing agents (LRAs) have been developed to purge latent HIV-1, providing an intriguing strategy for eradication of residual, latent viral reservoirs. Our earlier studies show that antagonism of HIV-1 competitive factor bromodomain containing 4 (BRD4) using bromodomain and extra-terminal domain inhibitor (BETi) JQ1 may facilitate the reversal of HIV-1 latency. BETis have recently emerged as a new class of compounds that are promising for both the treatment of anticancer and HIV-1 eradication. However, the current BETis, including JQ1, only modestly reverse HIV-1 latency as a single drug, which complicates the study of the underlining mechanisms. Thus, BETis, which are more potent and easier for synthesis, are currently under active development. One such compound is UMB-32, a novel BETi based on an imidazo[1,2-a]pyrazine scaffold. We screened 37 UMB-32 derivatives and identified that UMB-136 reactivates HIV-1 in multiple cell models of HIV-1 latency with better efficiency than JQ1 and UMB-32. UMB-136 enhances HIV-1 Tat-LTR mediated transcription and increases HIV-1 viral production. Furthermore, UMB-136 enhances the latency-reversing effect of PKC agonists (prostratin, bryostatin-1) in CD8-depleted PBMCs containing HIV-1 latent reservoirs. Thus, our results illustrate that structurally improved BETis, such as UMB-136, would be used as promising LRAs for HIV-1 eradication.

#**18 Archin, Nancie**

Interval Dosing Allows Effective Latency Reversal by Vorinostat

N M Archin[1], J Kirchherr[1], G Clutton[1], K Sholtis[1], Y Xu[1], J Murphy[1], B Allard[1], E Stuelke[1], A Crooks[1], JD Kuruc[1], C Gay[1], J Eron[1], J Sung[1], N Goonetilleke[1], DM Margolis[1]

1. UNC HIV Cure Center, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina

Epigenetic restrictions at the HIV promoter (LTR), together with the sequestration of host transcription factors necessary for LTR activity, contribute to the maintenance of HIV latency. Epigenetic modulators such as histone deacetylase (HDAC) inhibitors can relieve repression at the LTR and promote HIV transcription, and have advanced into clinical trials seeking to reverse latency in humans. We demonstrated that in vivo administration of the class I HDAC inhibitor vorinostat (VOR) results in an increase in HIV RNA within the resting CD4+ T cells of aviremic HIV+ participants, and similar studies using VOR and other HDAC inhibitors have reported reversal of latency in total circulating CD4+ T cells. However, in a follow-up study where VOR was given in cycles of 3 days on/4 days off for 8 weeks, a reduction in the ability of VOR to induce HIV RNA expression was observed. Several studies using HDAC inhibitors, including one that even incorporated a vaccine designed to increase clearance of infected cells, have failed to show clearance of latent infection following the administration of HDAC inhibitors.

It is unclear whether this failure is related to insufficient reversal of HIV latency, ineffective clearance of infected cells, or a combination of these or other deficiencies. VOR concentration-response measurements made during a clinical study suggested an exposure-effect relationship consistent with clock-wise hysteresis, signifying tolerance to VOR over time. Therefore, we conducted a three-step study to measure the response to 1) a single dose of VOR; 2) the second of a pair of doses separated by 48 or 72 hours; and 3) the tenth of a series of doses separated by the effective interval (either 48 or 72 hours). We found that serial VOR exposures separated by 72 hours results in sustained increases in cell-associated HIV RNA within circulating resting CD4+ T cells. Importantly, serial dosing with VOR had very little effect on the effector capacity of CD8 T cells. However, this serial reversal of latency over a month did not result in a measurable depletion of the frequency of latent infection within resting CD4+ T cells.

These results suggest that reversal of latency may need to act on a broader population of cells, or be employed for a longer period of time. However, as suggested by other studies the clearance of latent infection may require additional measures, including at a minimum the sustained induction of potent antiviral immune responses capable of recognizing and clearing the rare cells in which HIV latency has been reversed.

#**19 Boucau, Julie**

Latency Reversing Agents and Cellular Activation Affect Antigen Processing in Primary CD4 T Cells

Julie Boucau[1], Julien Madouasse[1], Daniel Wambua[1], Matthew Berberich[1], Sylvie Le Gall[1]

1. The Ragon Institute of MGH, MIT and Harvard, Cambridge, Massachusetts

Strategies to purge HIV reservoirs commonly rely on reactivation of HIV provirus by latency-reversing agents (LRA) followed by death or immune clearance of reactivated CD4T cells. LRAs such as Histone deacetylase (HDAC) inhibitors or Protein Kinase C (PKC) agonists showed variable efficacy in vitro and limited efficacy in clinical trials. While productive infection mostly occurs in activated CD4T cells, reactivation occurs in memory resting cells receiving LRA and HAART. How these parameters shape HIV antigen processing and presentation by CD4T cells is unknown despite its critical role in immune recognition after reactivation.

We measured hydrolytic activities of cellular proteases involved in antigen processing in live primary CD4T cells that were either resting, CD3/28-activated, LRA-treated, or in conditions used in the Lewin or Planelles latency models. To assess how changes in activities affect antigen processing we compared the degradation of HIV peptides in extracts of primary CD4T cells treated as stated above. The degradation products were quantified by mass spectrometry and their antigenicity measured by Chromium release killing assay with HIV-specific CTL.

The peptidase activities of HDACi-treated primary CD4T cells decreased by 20-60% compared to resting cells while PKCa-treated cells displayed enhanced activities comparable to activated cells. Combination treatment of panobinostat (HDACi) and bryostatin (PKCa) resulted in similar peptidase activities to the mock-treated cells. Memory-like CD4T cells of the Lewin latency model showed activities level similar to resting CD4T cells. CD4T cells of the Planelles model displayed activities similar to activated cells. In vitro degradation of HIV epitope-containing peptides showed that resting primary CD4T cells produced longer, more antigenic peptides and unique cleavage sites compared to activated cells, and that some LRAs caused discrete changes in degradation patterns.

These variations in peptidase activities and degradation patterns suggest that LRA-reactivated and productively infected CD4T cells might process antigen differently, thus requiring different CTL responses for effective clearance.

#**20 Galardi, Cristin**

Evaluation of Ingenol Based PKC Agonists for HIV Latency Disruption

Jessica Brehm[1,2], Vincent Tai[1], David Irlbeck[1,2], Robert Ferris[1,2], Nancie Archin[2], Jennifer Kirchherr[2], Katherine Sholtis[2], Erin Stuelke[2], Cristin Galardi[1,2], James Schawalder[1,2], Shari Gordon[1,2], Richard Dunham[1,2], Milloni Chhabra[1,2], Jean-Pierre Routy[3], David M Margolis[2], Brian Johns[1,2], Jun Tang[1,2], David Favre[1,2]

1. GlaxoSmithKline; 2. University of North Carolina HIV Cure Center, Chapel Hill, North Carolina; 3. McGill University Health Center, Montréal, QC, Canada

Background: Protein kinase C agonists (PKCa) are potent in vitro latency reversing agents (LRAs) that act synergistically with histone deacetylase inhibitors (HDACi) and bromodomain inhibitors (iBET), suggesting their potential use in HIV cure strategies. Here we confirm and extend prior testing of the PKCa Ingenol-B (IngB) in vitro. Because IngB is unstable at physiological pH conditions, a stabilized form of Ingenol (GSK445A) was developed and tested in vitro and in the non-human primate model.

Methods: The ability of IngB to reverse latency in vitro was tested in reporter cell lines and in total or resting CD4+ T cells from stably-treated HIV-infected donors. Reactivation was measured by cell-associated RNA (caRNA), tat/rev induced limiting dilution assay (TILDA) and quantitative viral outgrowth assays (QVOA). IngB, GSK445A and iBET151 as single agents, fixed-dose combination and dose-response curves were measured. Cellular responses were profiled in vitro for cytotoxicity and cell signaling. The pharmacology and biomarkers of GSK445A were analyzed in blood and tissues of macaques.

Results: The effective concentrations 50 (EC50) of GSK445A and IngB were 1 and 13 nM, respectively, in HIV-Jurkat cell lines compared with ~200 nM for IngB in primary CD4+ T cells of HIV donors (caRNA), with latency reversal confirmed by TILDA and QVOA. Cell signaling demonstrated rapid and potent activation of NFkB, Akt and MAPK pathways in CD4+ T cells. Combination activity of IngB with iBET151 showed a 5 to 10-fold increase in potency and maximal response by caRNA and was confirmed by TILDA, suggesting synergy in the reactivation of a large pool of latently infected cells. In vivo, a brief infusion of GSK445A at 5 to 20 µg/kg did not result in observable toxicity in rhesus macaques (n=20). Pharmacokinetics of GSK445A displayed a biphasic decline with exposure above the EC50 for 3-6 hours. Rapid increase in plasma IL-6, T cell trafficking and up-regulation of CD69 (10-60% in T cells) revealed dose-dependent responses in blood and lymphoid tissues, which returned to baseline after 6 to 48 hours.

Conclusions: In vitro, GSK445A and IngB reversed HIV latency; activity was augmented by iBET151. GSK445A was developed as a stable Ingenol derivative that was well tolerated at an effective dose in rhesus macaques making GSK445A a candidate for single and combination studies to test latency reversal and clearance in vivo.

#**21 Larragoite, Erin**

Reducing Pro-Inflammatory Cytokine Release During PKC-induced HIV-1 Latency Reactivation

Erin T Larragoite[1], McKenna L Coletti[1], Amanda B Macedo[1], Laura J Martins[1], Alberto Bosque[1], Vicente Planelles[1], Adam M Spivak[2]

1. Department of Pathology, University of Utah School of Medicine, Salt Lake City, Utah; 2. Department of Medicine, University of Utah School of Medicine, Salt Lake City, Utah

Background: Antiretroviral therapy (ART) has significantly improved the lives of HIV patients over the last two decades by reducing plasma viremia levels to below the limit of detection (~20-50 copies/ml) and allowing for immune reconstitution. However, ART is not curative due to the persistence of a latent HIV reservoir, forcing patients to remain on ART indefinitely in order to prevent viral rebound. Cure strategies, such as the “shock and kill” strategy, attempt to eliminate HIV through transcriptional reactivation of the latent HIV reservoir using latency reversing agents (LRAs) and targeting of cells harboring transcriptionally reactivated HIV via immune-mediated mechanisms while patients remain on ART. In a recent study comparing LRA classes using in vitro models of latency, Protein Kinase C (PKC) agonists demonstrated consistent and potent proviral reactivation. Despite their in vitro efficacy, PKC agonists can induce T cell activation and pro-inflammatory cytokine release which has limited their use in pilot HIV eradication trials. In this work we sought to identify compounds that could attenuate these off-target effects, which would in turn allow for PKC agonists to be safely evaluated for latency reversal in vivo.

Methods: We first evaluated PKC agonists bryostatin-1, prostratin and ingenol-3,20-dibenzoate with regard to induction of pro-inflammatory cytokine release in peripheral blood mononuclear cells (PBMCs) from aviremic patients on ART. We subsequently conducted an in vitro screening using chemical libraries of known kinase inhibitors and epigenetic modifiers to identify pathways that could be targeted or individual compounds that decrease pro-inflammatory cytokine production during PKC activation. We further examined the effect of one screening hit on cytokine release and latency reversal using HIV-positive donor cells ex vivo.

Results: At concentrations shown in vitro to reverse HIV latency, ingenol-3,20-dibenzoate, bryostatin-1 and prostratin each induced pro-inflammatory cytokine release in patient PBMCs, with ingenol-3-20-dibenzoate inducing significantly higher pro-inflammatory cytokine release than bryostatin-1 or prostratin. Twelve kinase inhibitors and 8 epigenetic modifiers were identified from the screening that potently reduced cytokine release induced by ingenol-3,20-dibenzoate. One compound, ruxolitinib, was identified that significantly dampened cytokine production and did not affect cellular viability. Ruxolitinib is FDA-approved for the treatment of myelofibrosis. Treatment of cells from HIV-1 infected patients with ruxolitinib and ingenol-3,20-dibenozate showed that ruxolitinib dramatically reduced pro-inflammatory cytokine secretion while still allowing ingenol to potently reactivate latent viruses in cells from aviremic patients.

Conclusions: PKC agonists are promising candidates for HIV latency reversal, however PKC-induced T cell activation and pro-inflammatory cytokine release must be addressed before in vivo clinical trials can be considered. Ingenol induced higher pro-inflammatory cytokines than bryostatin-1 or prostratin. In vitro epigenetic modifier and kinase inhibitor screens identified several pathways and specific compounds to attenuate ingenol-induced cytokine release including the FDA-approved JAK inhibitor ruxolitinib. Co-treatment of resting CD4+ T cells from HIV-1 positive patients with ingenol-3,20-dibenzoate and ruxolitinib largely abrogated pro-inflammatory cytokine release without impairing a strong latency reversal effect.

#**22 Young, Won-Bin**

In Vivo Visualization and Quantification of HIV Infection and Viremia Rebound in Humanized Mice

Xiying Qu[1], Won-Bin Young[1]

1. Department of Radiology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania

Introduction: Currently, the only functional cure for HIV is by suppressing the virus to an undetectable level via antiretroviral therapy (ART); however, the viremia could still rebound after ART withdrawal despite a life time use of ART. In vivo imaging can provide real time information and non-invasive images of deep tissues and organs for visualizing and quantifying HIV transmission and the tissue-resident infected cells as potential reservoirs. Knowing where HIV-infected cells are located allows for monitoring how the infection spreads from the infection site to others in the same individual. The whole-body examination or monitoring dynamics of HIV infection in real time over the course of infection provides an opportunity for investigating where the viral infection starts, where the HIV sanctuaries are during the suppressive ART and, when and where viremia rebound occurs after ART withdrawal. This in vivo imaging can be used as a readout for the efficacy of ART, vaccines and HIV eradication strategies, e.g. genome editing.

Materials and Methods: We previously demonstrated that in vivo bioluminescence imaging (BLI) based on an enhanced firefly luciferase is sensitive for visualizing as few as 5 single cells in a niche(Song, Cai et al. 2015). In this study, we engineered replication-competent HIV-BaL (CCR5/macrophage tropism) carrying this luciferase reporter for infecting humanized Bone marrow-Liver-Thymus (BLT) mice via vaginal mucosa or intraperitoneal injection. A combination of ART consisting of Truvada and Raltegravir was used to treat the infected BLT mice to reveal the HIV resistance in different tissues and organs.

Results: Compared to the intraperitoneal route, penetration of mucosal barrier by HIV vaginal transmission requires at least 17 fold more virus. Infection in the female reproductive tract (FRT) could be visualized in the vaginal vulva/canal 48 hrs post inoculation (p.i.). Robust infection from FRT to initiate a systemic infection was visualized as early as 21 days, p.i., presumably an eclipse phase during local HIV expansion. Compared to intraperitoneal inoculation, the spatial-temporal dynamics of HIV mucosal transmission is significantly different, which is unlikely to be observed via conventional measurement of viral load in the blood. Treating the inoculated hu-BLT mice with combination ART, consisting of Truvada plus Raltegravir, resulted in declined HIV dissemination in only two weeks. HIV rebound was observed in the neck lymph node two weeks after ART withdrawal. Using in vivo imaging, we also demonstrated that HIV can escape from the clinically approved Pre-Exposure Prophylaxis (PrEP) Truvada, if not adhering to daily PrEP schedule, resulting in a systemic HIV infection three weeks after the PrEP.

Conclusions: Mucosal barrier in the female reproductive tract is significantly resistant to HIV infection comparing to non-mucosal routes. Almost 60% of local mucosal transmission in this BLT mouse model does not result in successful systemic HIV dissemination, which implies a potential confinement in the early stage of HIV infection as an opportunity for PrEP or antiretroviral therapy. The viremia rebound occurs mainly in the lymph nodes in two weeks after ART withdrawal. The mutagenesis of HIV proviral DNA harvested from the tissue-resident cells indicated active HIV dissemination among them.

References: Song, J., Z. Cai, A. G. White, T. Jin, X. Wang, D. Kadayakkara, C. J. Anderson, Z. Ambrose and W. B. Young (2015). Visualization and quantification of simian immunodeficiency virus-infected cells using non-invasive molecular imaging. J Gen Virol 96(10): 3131-3142.

#**23 Clutton, Genevieve**

CD8 T Cells from HIV+ Individuals on ART Have a Skewed Differentiation Phenotype and Impaired Proliferative Responses

G Clutton[1], D Lee[1], Y Xu[1], M Abad Fernandez[1], A Crooks[1], B Turner[1], N Maponga[1], A Cope[1], S Fiscus[1], K McGee[2], M McKellar[2], J Kuruc[1], G Ferrari[2], D Margolis[1], C Hicks[2], J Eron[1], C Gay[1], N Goonetilleke[1]

1. The University of North Carolina at Chapel Hill, Chapel Hill, North Carolina; 2. Duke University, Durham, North Carolina

In HIV-infected individuals, anti-retroviral therapy (ART) is effective in maintaining low viral loads, restoring CD4 T cell counts, and prolonging life. However, chronic inflammation and immune dysfunction persist even after years of successful ART, and there are conflicting data concerning whether CD8 T cell function is fully restored. In a cross-sectional study, we compared the differentiation state (CD45RA, CCR7, T-bet, Eomes), exhaustion (PD-1, Tim-3, CD160), and proliferation of CD8 T cells from HIV+ participants who initiated ART during acute infection, HIV+ participants who initiated ART during chronic infection, and HIV-seronegative controls. CD8 T cell effector function (cytokine and lytic molecule production) and exhaustion marker expression was similar in HIV+ and HIV- participants. However, T cells from HIV+ participants exhibited a more differentiated phenotype, with fewer naive and more terminally differentiated effector cells compared with HIV- participants. CD8 T cells from HIV+ participants were skewed toward a T-bet high Eomes low phenotype that has previously been associated with high cytotoxic activity but reduced proliferative capacity. Accordingly, antigen-specific proliferation of CD8 T cells from HIV+ participants was reduced compared with that of CD8 T cells from HIV- participants. Collectively, these data suggest that effector CD8 T cell function is retained in ART-treated HIV+ individuals, but that memory responses are not fully restored. These observations have important implications for the efficacy of therapeutic vaccines designed to elicit long-lived protective T cell responses in HIV-infected individuals. Funding acknowledgement: P30 CNIHR AI0227763.

#**24 Simon, Viviana**

HIV-1 Susceptibility of Primary CD4+ T Lymphocytes Is Enhanced by IL15 Through Phosphorylation of SAMHD1

Lara Manganaro[1,3], Patrick Hong[1], Dionne Argyle[1,3], Dabeiba Bernal[1], Uma Potla[1], Lubbertus C F Mulder[1,3], Felipe Diaz-Griffero[2], Benhur Lee[1], Ana Fernandez-Sesma[1], Viviana Simon[1,3,4]

1. Department of Microbiology, Icahn School of Medicine at Mount Sinai, New York, New York; 2. Department of Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, New York; 3. Global Health and Emerging Pathogens Institute, Icahn School of Medicine at Mount Sinai, New York, New York; 4. Department of Medicine, Icahn School of Medicine at Mount Sinai, New York, New York

HIV persists in CD4+ T memory cells despite effective highly active therapy. Resting CD4+ T memory cells are refractory to HIV infection due to several blocks that occur throughout the viral life cycle while fully activated, cycling CD4+ T cells are permissive to productive HIV infection. Little is currently known about how more physiological stimulation with gamma cytokine modulates the susceptibility of CD4+ T memory cells to HIV infection.

We found that IL15 stimulation increased CD4+ T cells susceptibility to HIV infection by releasing an early block to infection in primary human CD4+ T cells. IL15 induced phosphorylation of the restriction factor SAMHD1 was identified as the underlying mechanism of action. Systematic analysis of the upstream signaling pathways using small molecule inhibitors identified the Janus kinases JAK1/JAK2 as being responsible for SAMHD1 phosphorylation in the presence of IL15. Conversely, ruxolitinib, an inhibitor of JAK1/JAK2, reduced significantly late reverse transcript products in CD4+ T cells in the presence of IL15.

We next studied the effect of IL15 on the composition of CD4+ T cell subpopulations using Mass Cytometry by time of Flight (CYTOF). IL15 not only increases the general proliferation capacity of CD4+ T stem memory cells (CD4+ TSCM), but also increased their percentage in the HIV infected CD4+ T cell population. Lastly, by combining surface and intracellular staining, we determined that CD4+ TSCM express high levels of phosphorylated SAMHD1.

Taken together, IL15 modulates the susceptibility of primary human CD4+ T memory cells in a SAMHD1 dependent manner. Given that high levels of IL15 are observed during acute HIV infection, it is conceivable that this gamma cytokine plays a pivotal role in rendering CD4+ TSCM subset susceptible to HIV infection early upon transmission when the persistent HIV reservoir is seeded. Future studies are needed to test whether short-term chemotherapeutic interventions with FDA approved JAK1/JAK2 inhibitors can limit the creation of a self-renewing persistent HIV reservoir in CD4+ TSCM.

#**25 Shen, Anding**

Lymphatic Endothelial Cells Induce HIV Infection in Resting CD4+ T Cells Without Signs of Activation

Seth Verkaik[1], Bo Ram Kim[1], Mackenzie Geels[1], Derrick Kamp[1], Anding Shen[1]

1. Biology Department, Calvin College, Grand Rapids, Michigan

HIV could only infect proliferating CD4+ T cells in vitro because viral reverse transcription and integration are blocked in resting T cells. However, in vivo resting T cells are productively infected, due to interactions with other cell types and cytokines. Previous studies showed that resting CD4+ T cells stimulated by endothelial cells (HUVEC) could be infected by HIV. In this study we examined the roles of lymphatic endothelial cells (LEC) in HIV infection of resting CD4+ T cells. LEC are isolated from lymphoid tissue and have more relevance in in vivo interactions with T cells. We found that LEC were able to induce both productive and latent infection, though to a lesser extent compared with HUVEC. Similar to HUVEC stimulation, LEC stimulation of resting T cells involves IL6. Though induction of infection in naïve CD4+ T cells was low with LEC stimulation, induction of infection was much more substantial in memory T cells, which is consistent with the effect in HUVEC stimulation and in vivo findings. Also similar to the effect of HUVEC, minimal expressions of CD69, CD25 or HLA-DR were found on LEC stimulated resting T cells. Such results confirmed the importance of endothelial cells in HIV infection of resting T cells, and in latency formation.

#**26 Skinner, Pam**

SIV-Producing Cells in Follicles Are Partially Suppressed by CD8+ Cells in Vivo

Shengbin Li[1], Joy M Folkvord[2], Eva G Rakasz[3], Hadia M Abdelaal[1], Reece K Wagstaff[1], Katalin J Kovacs[1], Hyeon O Kim[1], Ryoko Hatsugai[1], Samantha MaWhinney[4], David Masopust[1], Elizabeth Connick[2], Pamela J Skinner[1]

1. University of Minnesota, Minneapolis, Minnesota; 2. University of Arizona, Tucson, Arizona; 3. University of Wisconsin-Madison, Madison, Wisconsin; 4. University of Colorado, Denver, Colorado

CD8+ T cells are critical to controlling HIV and simian immunodeficiency virus (SIV) infections, but are not able to eradicate all virus-producing cells. HIV- and SIV-specific CD8+ T cells are typically largely excluded from lymphoid B cell follicles where HIV- and SIV-producing cells are most highly concentrated, indicating that B cell follicles are somewhat of an immune privileged site. To gain insights into virus-specific follicular CD8+ T cells, we determined the location and phenotype of follicular SIV-specific CD8+ T cells in situ, the local relationship of these cells to Foxp3+ cells, and effects of CD8 depletion on levels of follicular SIV-producing cells in chronically SIV infected rhesus macaques. We found that follicular SIV-specific CD8+ T cells were able to migrate throughout follicular areas including germinal centers. Many expressed PD-1, indicating they may have been exhausted. A small subset was in direct contact with and likely inhibited by Foxp3+ cells and a few were themselves Foxp3+. In addition, subsets of follicular SIV-specific CD8+ T cells expressed low to medium levels of perforin and subsets were activated and proliferating. Importantly, after CD8 depletion, SIV-producing cells increased in B cell follicles and extrafollicular areas, suggesting that follicular and extrafollicular CD8+Tcellshave a suppressive effect on SIV replication. Taken together, these results suggest that during chronic SIV infection, despite high levels of exhaustion and likely inhibition by Foxp3+ cells, a subset of follicular SIV-specific CD8+ T cells are functional and suppress viral replication in vivo. These findings support HIV cure strategies that augment functional follicular virus-specific CD8+ T cells to enhance viral control. Supported by NIH-AI-096966-01A1 and AI-080418.

#27 Spivak, Adam

Anti-HIV-1 Effector Potential of Mucosal-Associated Invariant T (MAIT) Cells

Adam M Spivak[1], Michael S Bennett[1], McKenna L Coletti[1], Jordan A Berg[1], Matthew T Rondina[1], Daniel T Leung[1]

1. University of Utah School of Medicine, Salt Lake City, Utah

Background: Antiretroviral therapy (ART) is not curative due to the persistence of replication-competent proviruses primarily in long-lived memory CD4+ T cells. One strategy to eradicate this latent reservoir, termed 'shock and kill,' involves administration of compounds able to reverse proviral latency in order for the immune system to target these cells. In a recent clinical trial of panobinostat, the frequency and activity of NK cells and plasmacytoid dendritic cells, but not HIV-1-specific CD8+ T cells, were associated with decreased HIV-1 proviral DNA. This in vivo data identifies the cytotoxic antiviral response of innate lymphocytes as a highly promising means to target the latent reservoir in the setting of LRAs. MAIT cells are recently described innate-like T cells that account for approximately 5% of circulating T cells and are enriched in the mucosa. MAIT cell activation is associated with pro-inflammatory cytokine secretion and direct cytotoxic activity via granzyme B and perforin. Recent reports have identified that MAIT cells are able to target virally infected cells. We hypothesize that MAIT cells are uniquely-armed to aid in the clearance of the HIV-1 reservoir in the setting of latency reversal.

Methods: We isolated peripheral blood mononuclear cells (PBMCs) from HIV-1-infected individuals and healthy controls and stimulated with microbial antigen. We isolated MAIT cells (defined as CD3+CD4-TRAV1-2+CD161++) using flow cytometric sorting, and used a high-throughput sequencing method with bar coding to link the expression of TCR-alpha, TCR-beta, and functional genes of interest at the single cell level. We used limiting dilution cloning to isolate and expand distinct clones of MAIT cells, and confirmed identity by Sanger sequencing. We activated MAIT clones with IL-12, IL-18, microbial antigen, and/or anti-CD3/CD28 beads, and subsequently co-cultured clones for eight hours with a latently infected cell line (J-Lat 10.6 cells). Cell surface expression of CD107a was measured by flow cytometry to quantify cytotoxic potential of each MAIT clone when exposed to latently infected cells undergoing HIV-1 viral reactivation.

Results: In exploratory RNA-Seq studies of stimulated compared to unstimulated MAITs, we identified a large number of innate viral sensing and antiviral response genes among the top upregulated genes. Using single-cell paired clonality-functionality sequencing, we show that MAIT cells have a unique clonal distribution in HIV-1-infected individuals who control viremia and maintain normal T cell counts without ART (known as elite controllers or EC), distinct from both aviremic patients on ART and uninfected individuals. We also observed that MAIT clones derived from HIV-1-infected persons, when activated in both TCR-dependent and TCR-independent manners, exhibit potent cytotoxic activity against HIV-1-infected cells in vitro.

Discussion: We show that MAIT cells are capable of antiviral activity, and observed unique clonal populations of MAIT cells among EC compared to HIV-1-infected patients on ART, raising the possibility that these cells may contribute to endogenous viral control observed in EC. We have also demonstrated antiviral cytotoxicity of activated MAIT clones against latently infected cells induced to undergo viral reactivation in vitro. MAIT cells represent a previously unexplored effector cell population that may contribute to recognition and clearance of HIV-1-infected cells in the context of latency reversal.

#**28 Liao, Baolin**

Novel in Vivo Model to Evaluate Human Gut Microbiota's Role in HIV Persistence and the Efficacy of HIV Cure Strategies

Baolin Liao[1,2], Morgan Chateau[1], J Victor Garcia[1], Angela Wahl[1]

1. Division of Infectious Diseases, Center for AIDS Research, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina; 2. Guangzhou No. 8 People's Hospital, Guangzhou Medical University, Guangzhou, China

Commensal microbiota protect the gut from pathogenic organisms. During HIV infection, the gut is a significant site of HIV replication, CD4+ T cell depletion, inflammation, and microbial translocation. While it is well-documented that the composition of the gut microbiome is altered in HIV-infected patients, the role of gut microbiota in HIV persistence and the efficacy of HIV cure strategies is unknown. In vitro studies indicate that HIV expression can be affected by bacterial metabolites and stimulation of toll-like receptors.

It is not possible to perform the direct experimentation that is needed to establish gut microbiota's role in HIV persistence in humans. Bone marrow/liver/thymus (BLT) humanized mice are systemically reconstituted with human immune cells and have been utilized to study HIV persistence and evaluate novel curative strategies in vivo. However, the gut microbiome of BLT mice is murine. Therefore, we developed germ-free BLT mice and BLT mice colonized with human gut microbiota (HuM-BLT mice) to study human gut microbiota's role in HIV infection in vivo. We 1) derived and utilized a germ-free immune deficient mouse strain to generate germ-free BLT mice, 2) characterized human immune cell reconstitution of germ-free BLT mice, 3) colonized germ-free mice with human gut microbiota to create HuM-BLT mice, 4) determined the susceptibility of HuM-BLT mice to HIV acquisition, and 5) evaluated the effect of HIV infection and antiretroviral therapy (ART) on the composition of gut microbiota.

A germ-free NOD/SCID/IL2Rγc-/- (NSG) mouse strain was surgically derived by sterile embryo transfer. Germ-free BLT mice were generated in a germ-free surgical isolator by transplanting human hematopoietic stem cells into irradiated germ-free NSG mice implanted with autologous human thymus and liver tissue under their kidney capsule. Systemic reconstitution of germ-free BLT mice with human cell populations was analyzed by flow cytometry with an emphasis on HIV target cells (CD4+ T cells, dendritic cells, macrophages). Germ-free mice were colonized with human gut microbiota via oral fecal transplantation to generate HuM-BLT mice. Colonization of HuM-BLT mice following fecal transplantation of human gut microbiota was analyzed longitudinally in fecal pellets with 16S rRNA gene amplicon sequencing. HuM-BLT mice were exposed vaginally to the HIV-1 transmitted/founder virus CH040. HIV infection was monitored longitudinally in peripheral blood by measuring plasma HIV-RNA levels with real-time PCR and CD4+ T cell levels with flow cytometry. Fecal pellets were collected from mice longitudinally from HIV infected mice and mice administered daily systemic ART(tenofovir/emtricitabine/raltegravir) to evaluate the composition of the gut microbiome

Our data demonstrate: 1) successful generation of germ-free BLT mice, 2) robust systemic reconstitution of germ-free BLT mice with human HIV target cells; 3) the gut microbiome of HuM-BLT mice is stable and recapitulates the human donor inoculum; and 4) HuM-BLT mice are susceptible to HIV acquisition which results in a decline in peripheral blood CD4+ T cell levels. Analyses evaluating the effect of HIV infection and systemic ART on the composition of the gut microbiome are ongoing. In conclusion, our data validate germ-free BLT and HuM-BLT mice as in vivo platforms for the study of the human gut microbiome and for the evaluation of HIV persistence in the context of no microbiota and human gut microbiota.

#**29 Routy, Jean-Pierre**

Immune Tolerance of Testicular Tissue and HIV Persistence

Jean-Pierre Routy[1-3], Rosalie Ponte[1,2], Mohammad-Ali Jenabian[4], Franck Dupuy[1,2], Vikram Mehraj[1,2], Pierre Brassard[5], Maud Bélanger[5], Nicolas Chomont[6,7], Petronela Ancuta[6,7]

1. Research Institute of the McGill University Health Centre, Montréal, QC, Canada; 2. Chronic Viral Illness Service, McGill University Health Centre, Montréal, QC, Canada; 3. Division of Hematology, McGill University Health Centre, Montréal, QC, Canada; 4. Department of Biological Sciences and BioMed Research Centre, Université du Québec à Montréal (UQAM), Montréal, QC, Canada; 5. Metropolitan Centre for Plastic Surgery, Montréal, QC, Canada; 6. Department of Microbiology, Infectiology and Immunology, University of Montréal, Montréal, QC, Canada; 7. University of Montréal Hospital Research Centre (CR-CHUM), Montréal, QC, Canada

Background: Antiretroviral therapy (ART) has significantly improved the lives of millions of individuals living with HIV by controlling viral replication, preventing the development of AIDS and partially reducing the risk for non-AIDS events. However, immune dysfunction and HIV persistence in the tissues remain major obstacles to an HIV cure in patients on long-term ART. In addition, certain organs such as CNS, eyes and testes display a structural barrier and a distinct immunometabolic milieu restraining immune cell infiltration, therefore presenting an immune privileged environment that can facilitate HIV persistence. Testicular tissue represents a viral sanctuary site owing to its blood-testis-barrier, and constitutes an immune privilege organ. We assessed lymphoid and myeloid immune tolerance properties of the testicular tissue in ART-treated individuals.

Methods: Testicular tissue and blood samples were collected from 6 virally suppressed ART-treated adults and 16 controls following their elective bilateral orchiectomy for gender reassignment. T-cells were purified from freshly isolated testicular interstitial cell suspensions by mechanical disruption followed by CD3 enrichment by microbeads, while myeloid cells were collected after enzymatic digestion. T-cell subsets including CD4, CD8 and regulatory T cells (Tregs), macrophages, myeloid dendritic cells (mDCs), plasmacytoid dendritic cells (pDCs) and myeloid-derived suppressor cells (MDSCs) with expression of immune activation, and of adenosine/ATP immunosuppressive markers implicated in energy metabolism were assessed by multi-color flow cytometry. The mRNA expression levels of indoleamine 2 3-dioxygenase (IDO) enzyme, which catabolizes essential amino acid tryptophan into immunosuppressive kynurenine was measured in PBMCs and testicular tissues by qPCR. The frequency of cells harboring total and integrated HIV DNA as a measure of viral persistence was quantified in matched testicular cells and PBMCs by using a well-established ultra-sensitive PCR-based assay.

Results: Testes in comparison to PBMCs were characterized by a significantly lower CD4 T-cell proportion among total T-cells ($p<0.001$), a decrease in the frequency of naïve cells ($p<0.001$), an increase in the frequency of effector-memory T-cells ($p<0.001$) and a dramatic increase of up to 77 fold ($p<0.001$) in CCR5 expression in both the HIV-infected and control groups. Only in HIV-infected individuals on ART, higher expression levels of immune activation markers (CD38/HLA-DR) on both CD4 and CD8 T-cells was observed in testes than PBMCs. In both groups, testes vs. blood were characterized by higher frequencies of immunosuppressive CD39+Tregs (64±22% vs. 37±27%, $p=0.002$ and 62.9±11.5%, vs. 42.6±10%, $p<0.001$) and a massive increase in CD73 expression on CD8 T-cells (24.6±13.2 vs. 77.4±4% and 13.8±4 vs. 67.6±14.6%, $p<0.001$). Immunosuppressive macrophages (lin- HLA-DR+ CD14+ CD163+) represented 20% of the leucocytes in testes. Frequency of mDCs (lin- CD14- HLA-DR+) was elevated contrasting with low frequency of pDCs (CD123+) and absence of MDSCs in testes. Compared to the blood a remarkable increase in IDO mRNA expression was observed in testes. Rare cells harbouring HIV DNA were detected in testes from 5 out 6 patients.

Conclusions: We demonstrate for the first time that human testicular tissue provides an immune tolerant environment with a distinct immune profile. We show that the adenosine/ATP and tryptophan/kynurenine immune-metabolic pathways contribute to the testicular immune tolerance and that the testes represent a distinctive HIV sanctuary site during ART. Such findings increasing our understanding of the mechanisms of HIV persistence will pave the way towards specific strategies aimed at HIV eradication.

#30 Ancuta, Petronela

HIV Persists in CCR6+CD4+ T Cells from Colon and Blood During Antiretroviral Therapy

Annie Gosselin[1], Tomas Raul Wiche Salinas[1,2], Delphine Planas[1,2], Vanessa Sue Wacleche[1,2], Yuwei Zhang[1,2], Rémi Fromentin[1], Nicolas Chomont[1,2], Éric A Cohen[2,3], Barbara Shacklett[4], Vikram Mehraj[5], Maged Peter Ghali[6], Jean-Pierre Routy[5,7], Petronela Ancuta[1,2]

1. CHUM-Research Centre, Montréal, QC, Canada; 2. Department of Microbiology, Infectiology and Immunology, Faculty of Medicine, Université de Montréal, Montréal, QC, Canada; 3. Institut de Recherche Clinique de Montréal, Montréal, QC, Canada; 4. University of California Davis, Davis, California; 5. Chronic Viral Illness Service and Research Institute, McGill University Health Centre, Montréal, QC, Canada; 6. Division of Gastroenterology and Hepatology, McGill University Health Centre, Montréal, QC, Canada; 7. Division of Hematology, McGill University Health Centre, Montréal, QC, Canada

Objectives: To investigate the contribution of colon and blood CD4+ T-cell subsets expressing the chemokine receptor CCR6 to HIV persistence during ART.

Design: Matched sigmoid biopsies and blood samples (n=13) as well as leukapheresis (n=20) were collected from chronically HIV-infected individuals receiving ART. Subsets of CD4+ T-cells with distinct differentiation/polarization profiles were identified using surface markers as follows: memory (TM, CD45RA), central memory (TCM; CD45RA-CCR7+), effector memory (TEM; CD45RA-CCR7-), Th17 (CCR6+CCR4+), Th1Th17 (CCR6+CXCR3+), Th1 (CCR6-CXCR3+), and Th2 (CCR6-CCR4+).

Methods: We used polychromatic flow cytometry for cell sorting, nested real-time PCR for HIV-DNA quantification, ELISA and flow cytometry for HIV-p24 quantification. HIV reactivation was induced by TCR-triggering in the presence/absence of all-trans retinoic acid.

Results: Compared to blood, the frequency of CCR6+TM was higher in the colon. In both colon and blood compartments, CCR6+TM were significantly enriched in HIV-DNA when compared to their CCR6- counterparts (n=13). In blood, integrated HIV-DNA levels were significantly enriched in CCR6+ versus CCR6- TCM of 4/5 individuals and CCR6+ versus CCR6- TEM of 3/5 individuals. Among blood TCM, Th17 and Th1Th17 contributed the most to the pool of cells harboring integrated HIV-DNA despite their reduced frequency compared to Th2 which were infected the least. HIV reactivation was induced by TCR triggering and/or retinoic acid exposure at higher levels in CCR6+ versus CCR6- TM, TCM, and TEM.

Conclusions: CCR6 is a marker for colon and blood CD4+ T-cells enriched for replication-competent HIV-DNA. Novel eradication strategies should target HIV persistence in CCR6+CD4+ T-cells from various anatomic sites.

#**31 Goswami, Ria**

CD4+ T Cell Subsets and Tissue-Associated Viral Loads in SHIV/HIV-1 Infected Tonsillar Tissue

Ria Goswami[1], Holly Jakubowski[1], Joshua Eudailey[1], Maria Blasi[1], Jonathon E Himes[1], Riley J Mangan[1], Pyone Aye[2], Mary Klotman[1], Sallie R Permar[1]

1. Human Vaccine Institute, Duke University Medical Center, Durham, North Carolina; 2. Tulane National Primate Research Center, Tulane University, Covington, Louisiana

Introduction: Transmission of HIV-1 via breastfeeding accounts for more than half of the 150,000 pediatric infections occurring annually. Unlike transmission during delivery, HIV-1 transmission via breastfeeding is not readily diagnosed after infection. Thus, early treatment with ARV to limit the size of viral reservoir is not possible. As oral lymphoid tissues, including tonsils, are the initial sites of viral exposure in breastfed infants, strategies to limit establishment of viral reservoir in these tissues might be a key to prevent systemic viral spread and achieve long term remission or “cure”. Thus, this study aims to delineate mechanisms of viral spread in tonsillar tissue to identify strategies for prevention of productive HIV-1 replication.

Materials and Methods: Infant rhesus macaques (RMs) were orally challenged, and adult RMs were rectally challenged with SHIV1157ipd3N4, which expresses an HIV envelope sequence originally isolated from a postnatally infected infant. Oral lymphoid and GI tissues were collected 8-week post-infection. Tissue-associated infectious viral load was measured through coculture of tissue mononuclear cells and TZM-bl reporter cells. Mononuclear cells isolated from oral lymphoid and GI tissues were subjected to flow cytometric analysis for characterization of CD4+ T cell subsets. Tissue-associated viral loads were quantified using digital droplet DNA PCR and in situ hybridization. Mononuclear cells from discarded bilateral tonsil tissues from children <6 years of age were infected with CXCR4-tropic or CCR5-tropic-GFP-labeled strain of HIV-1. Tonsillar CD4+ T cells were analyzed for productive infection (GFP+). CD4+ Naïve (TN:CD28+ CD95-), central memory (TCM:CD28+ CD95+), transitional memory (TTM: CCR7- CD28+), effector memory (TEM: CD28-CD95+), activated (HLA-DR+) T cells and Tregs(CD25+) were quantified by flow cytometry.

Results: Our results indicate that palatine tonsil tissues from both orally challenged infant and rectally challenged adult RMs had higher infectious tissue-associated viral load compared to other oral lymphoid and GI tissues. Palatine tonsil also had a higher proviral DNA (Mean=3.2x106copies/million CD4+ T cells) and RNA (Mean=7.13 SHIV gag RNA transcribing cells per 1000 CD3+ T cells) load compared to other tissues. RM Tonsillar CD4+ T cells consisted of a higher proportion of TCM and TTM cells and lower proportion of TN and TEM cells compared to other oral lymphoid tissues. Uninfected human tonsillar CD4+ T cells were predominantly of TCM (94.7%) and TTM (53.5%) phenotype, yet had a lower proportion of TN (3.11%), and TEM (1.32%) cells. Approximately 3.32% and 0.23% of the CD4+T cells were infected by HIVNLGI and HIVJRFL, respectively and infection was predominant in the TCM (HIVNLGI: 4.1% cells infected; HIVJRFL: 0.22% cells infected) and TTM (HIVNLGI: 1.17% cells infected; HIVJRFL: 0.15% cells infected) subsets. Viral infection resulted in depletion of TN (HIVNLGI: 39.54% reduction; HIVJRFL: 47.59% reduction), TTM (HIVNLGI: 67.1% reduction; HIVJRFL: 17% reduction) and Tregs (HIVNLGI: 20.96% reduction; HIVJRFL: 16.59% reduction), and an increase in the proportion of activated HLA-DR+ (HIVNLGI: 136% increase; HIVJRFL: 86.44% increase) CD4+ T cells. However, the TCM population remained unchanged.

Conclusions: Our findings suggest that tonsil is an important tissue reservoir of HIV-1 upon both oral and rectal virus exposure. Infection of tonsil tissues leads to loss of TN and resting memory CD4+ T cells leading to disruption of Treg balance and accumulation of highly activated T cells. Whether blocking

CD4+T cell immune activation pathways inhibits establishment of viral reservoir in tonsils warrants further investigation.

#**32 Ling, Binhua**

Multiple Lymphoid Tissue Viral DNA and RNA Reservoirs in SIV-Infected Rhesus Macaques of Chinese Origin on Suppressive Antiretroviral Therapy

Summer Siddiqui[1], Stefanie Perez[1], Ann-Marie Johnson[1], Lara Doyle-Meyers[1,2], Qingsheng Li[3], Ronald Veazey[1,2], Binhua Ling[1]

1. Tulane National Primate Research Center, Covington, Louisiana; 2. Tulane University School of Medicine, New Orleans, Louisiana; 3. Nebraka Center for Virology, School of Biological Sciences, University of Nebraska-Lincoln, Lincoln, Nebraska

Background: Virus persistence in cellular and anatomical compartments is a major obstacle to HIV cure. Understanding tissue viral RNA and DNA reservoirs is essential for development of new strategies targeting these sites. Here we used the SIV-infected rhesus macaques of Chinese origin (ChRM) to assess dynamic changes and the size of these reservoirs in multiple tissues. We also studied viral mutation and diversity in lymphoid tissues and different sites of the gut to determine whether aviremic animals on ART have residual viral replication.

Methods: SIV-infected ChRM including long-term nonprogressors were treated with Tenofovir and Emtricitabine daily for up to 24 weeks. Blood, spleen, peripheral and mesenteric lymph nodes (LNs) were collected. Samples from 6 sites of the gut were also obtained from 10 animals by the end of ART. PCR-based assays, RNAscope and quantified viral outgrowth assays were used to evaluate size of viral RNA and DNA reservoirs. Single genome amplification (SGA) was used to amplify SIV env gp120. Variants isolated from multiple tissues were sequenced and analyzed.

Results: Plasma viral load was maintained undetectable during ART in majority of animals, however, in the gut, viral DNA was higher in large intestine than small intestine. SGA derived viral DNA sequences analysis revealed that viral diversity across different sites of the gut even in animals with sustained undetectable pVL after received 24 weeks of ART. When compared with blood, spleen and peripheral LNs, mesenteric LNs had the largest size of reservoir with the range of 1 – 4.2 IUPM (infectious units per million cells) in resting CD4+ T cells by the end of ART.

Conclusions: Multiple lymphoid organs and tissues especially mesenteric LNs are major reservoirs. Different sections of the guts may serve as reservoirs at different levels. The SIV/ChRM nonhuman primate model is excellent for testing novel therapeutic interventions for HIV cure and eradication.

#**33 Soriano-Sarabia, Natalia**

Contribution of Gamma/Delta T Cell Subpopulations to the Latent HIV Reservoir

K Sholtis[1], C Garrido[1], J Kirchherr[1], E Stuelke[1], N M Archin[1], D M Margolis[1], Natalia Soriano-Sarabia[1]

1. UNC HIV-Cure Center. University of North Carolina at Chapel Hill, Chapel Hill, North Carolina

Background: We previously reported that gamma/delta T cells, specifically the Vdelta2 T cell subpopulation, harbor replication-competent HIV (PLoS Pathog, 2015). This was demonstrated by performing viral outgrowth assays of ex vivo isolated Vdelta2 T cells in 18 suppressed HIV-donors on successful ART. As these cells are mainly memory cells, and virus was recovered from patients that had been suppressed for more than one year, they may constitute a long-term cellular reservoir of HIV infection. However, longitudinal analyses were not performed. In addition, whether the other main human gamma/delta subset Vdelta1 cells can also be infected and contribute to the total HIV reservoir was not addressed and it is currently not known.

Objective: To assess the specific contribution of gamma/delta T cell subpopulations to the HIV reservoir.

Methods: Vdelta2 T cells or Vdelta1 T cells were FACS-sorted and viral outgrowth assays performed, as previously reported. Patients treated in the acute or in the chronic phase of the infection and suppressed for at least one year were included in the study. Briefly, isolated cells were plated in 5-10 replicates, activated with HMBPP in the case of Vdelta2 T cells, or PHA and IL-2 for the Vdelta1 cell subset. After 24 hours, healthy PBMC depleted of CD8 T cells were added to the culture to increase virus production. After 19 days of culture, supernatants were recovered and viral replication was assessed by HIVp24 ELISA. In parallel, viral outgrowth assays of total isolated resting (r) CD4 cells were performed. In addition, total HIV levels in Vdelta2 and Vdelta1 cell subpopulations and rCD4 cells were quantified by digital droplet PCR using primers for HIV pol.

Results: We measured the frequency of infection in Vdelta2 T cells in six patients at two different time points at least one year apart. Our results show no statistical differences between the frequency of infection in the first (37.7 IUPM) and second visits (18.4 IUPM). Mean contribution of Vdelta2 T cells to the total HIV reservoir was 0.1 IUPM CD3+ T cells while rCD4 cells showed a mean contribution of 0.78 IUPM CD3+ T cells. Total pol HIV DNA levels were assayed in five acutely treated patients and six chronically treated patients showing high levels of HIV DNA within gamma/delta T cell populations. In addition, in three patients assayed so far, we also recovered replication-competent HIV from the Vdelta1 subset.

Conclusions: A slight reduction in the frequency of infection was detected in the six patients assayed. Inclusion of more patients as well as repeated measures within the same patients are critical to obtain a definitive conclusion of the contribution of gamma/delta T cell subsets to the latent HIV reservoir. Interestingly, Vdelta1 cells also harbor replication-competent HIV. However, the mechanism of Vdelta1 infection is currently unknown and under study. We are also starting to analyze whether these cells will require specific strategies to disrupt latency, different than those used in resting CD4 cells.

#**34 Wahl, Angela**

Steps Towards the In Vivo Eradication of HIV Tissue Reservoirs: Differential Restoration of CD4+ Tissue-Resident Memory T Cells After Antiretroviral Treatment

Angela Wahl[1], J Victor Garcia[1]

1. Division of Infectious Diseases, Center for AIDS Research, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina

The decline of peripheral CD4+ T cells is a hallmark of HIV infection. Sustained suppression of plasma viremia by antiretroviral therapy (ART) promotes CD4+ T cell reconstitution in peripheral blood. However, in most cases, peripheral blood CD4+ T cell levels are not informative of the extensive destruction of tissue resident CD4+T cells or their reconstitution during ART. Similarly, analysis of the HIV reservoir or the impact of CURE strategies on peripheral blood cells is not informative about the magnitude, the dynamics, or the effect of CURE strategies on the HIV reservoir in tissues.

Immunological interventions aimed at eradication of cellular HIV reservoirs in tissues require the enhanced clearance of HIV infected cells by tissue-resident memory T cells (TRM). TRM serve to provide optimal pathogen specific immunity in tissues. However, due to the intrinsic difficulties in accessing internal organs for analysis, CD4+ TRM restoration in tissues during suppressive ART is difficult to assess. Specifically, TRM do not recirculate and are absent from peripheral blood. To address this critical need, we utilized BLT humanized mice to investigate the effect of ART on CD4+ TRM reconstitution in tissue. HIV infection significantly decreased CD4+ T cell levels in the tissue BLT mice ($p=0.006$), and in particular, memory CD4+ T cell levels (75% reduction, $p=0.0002$). HIV-infection resulted in decreased CD4+ TRM ($p=0.0003$) levels in the tissues of BLT mice. CD4+ TRM were specifically depleted in non-lymphoid tissues (87%-93%) compared to lymphoid (29- 55%) tissues. Given that TRM provide optimal immunity, their depletion during HIV-infection may increase the risk of infection with other pathogens, especially those that target non-lymphoid tissues. Daily suppressive ART significantly increased memory CD4+ T cell levels in tissue ($p=0.03$). ART significantly increased CD4+ TRM ($p=0.02$) in the tissues of BLT mice. The increase in CD4+ TRM was primarily due to restoration of CD4+ TRM levels in non-lymphoid tissues (liver: $p=0.0017$ and lung: $p=0.0002$) as CD4+ TRM levels in lymphoid tissues (spleen: $p=0.1568$, lymph nodes: $p=0.7277$, and bone marrow: $p=0.2777$) of ART-suppressed HIV-infected BLT mice were not significantly different compared to those of ART-naïve HIV infected BLT mice.

Collectively, these data demonstrate that CD4+ TRM are depleted during HIV infection. Although suppressive ART increased CD4+ TRM levels, CD4+ TRM were not restored in all tissues. These results have significant implications for the design of effective HIV eradication approaches. Specifically, current ART approaches are not sufficient to restore CD4+ TRM to normal levels. Adjunctive therapies will be needed to augment restoration of TRM to achieve optimal clearance of HIV-infected cells in tissue.

#**35 Clayton, Kiera**

HIV-Infected Macrophages Resist Perforin-Mediated Killing via Necrosis and Promote Pro-inflammatory Cytokine Release by Cytotoxic T Lymphocytes

Kiera L Clayton[1], David R Collins[1], Bruce D Walker[1,2]

1. Ragon Institute of MGH, MIT and Harvard, Cambridge, Massachusetts; 2. Howard Hughes Medical Institute, Chevy Chase, Maryland

Although CD4+ T cells represent the main target cell for HIV infection, macrophages also contribute to both SIV and HIV reservoirs. Given CD8+ cytotoxic T lymphocytes (CTL) are essential for controlling viral replication, characterizing how they interact with HIV-infected targets will provide insights into mechanisms of reservoir persistence and cure strategies. In SIV infection, CTL killing of SIV-infected macrophages is less efficient than CD4+ T cells, but the mechanism of this differential killing and whether this applies to HIV is not known. Here we employed flow cytometric and ELISA-based techniques to compare target cell killing and pro-inflammatory cytokine production by CTL following co-culture of HIV-infected or peptide-loaded target cells and ex vivo or expanded HIV-specific CTL lines. Further we used imaging flow cytometry to visualize the target-effector interaction and longitudinally assess immunological synapse formation. We show that HIV-infected macrophages are more resistant to CTL-mediated killing, specifically via necrosis, compared to infected CD4+ T cells ($p = 0.0005$ for ex vivo CTLs and $p = 0.0241$ for expanded CTLs), a characteristic independent of HIV infection and intrinsic to macrophages. Furthermore, this differential killing of target cells is a consequence of the relative resistance of macrophages to perforin-induced necrosis compared to CD4+ T cells. Indeed, differential killing is not a consequence of poor recognition. On the contrary, CTL interaction of antigen-loaded macrophages is associated with longer effector-target contact time and increased production of pro-inflammatory cytokines ($p = 0.0047$), which further drives production of macrophage pro-inflammatory chemokines CXCL9, and CXCL10 ($p = 0.0274$ and $p = 0.0036$, respectively). These results suggest that infection of perforin-resistant macrophages may contribute to reservoir persistence and chronic inflammation even in the presence of HIV-specific CTL responses.

#**36 Clavel, François**

Genetic Analysis of HIV-1 Env Sequences Expressed from the T-Cell Reservoir

Anne Huard de Verneuil[1], Julie Migraine[1], Constance Delaugerre[1], Jean-Michel Molina[1], Sébastien Gallien[1], Fabrizio Mammano[1], Allan J Hance[1], François Clavel[1]

1. Inserm U941, Hôpital Saint Louis, Paris, France

Background: The HIV-1 T-cell reservoir is mainly constituted of defective viral genomes with large deletions or hypermutation. Little is known, however, of structure and genetic profile of HIV sequences expressed from the reservoir following activation of the resting host T-cells that harbor silent HIV-1 proviruses.

Methods: Resting CD4+ T-cells were collected from 4 HIV-infected subjects in whom viral replication had been fully suppressed by ART for more than 4 years. Env sequences were obtained from two sources: clonal replicative virus isolated by VOA, and Env mRNAs amplified by limiting-dilution PCR from activated CD4 T-cells. Env sequences were subjected to phylogenetic analyses and tropism was determined by the GtoP algorithm. Recombination profiles were analyzed using Simplot.

Results: No obvious genetic defects were seen in VOA-derived Env sequences. All mRNA-derived Env sequences appeared to be full-length, with no obvious deletions. A number of mRNA-derived Env sequences were heavily hypermutated, showing that such sequences can be readily expressed. Hypermutation profiles ranged from extensive (introducing numerous stop codons across the Env sequence) to shorter local stretches of G to A mutations. Env diversity was widest in patients with longest infection time before suppressive ART. In patients with highest diversity, extensive recombination was seen within separate clusters of sequences, revealing serial accumulation of « layers » of contemporary viruses in the reservoir over time. Several instances of clonally expanded sequences were seen both in VOA viruses and mRNAs. Only one patient expressed Env sequences with dual/X4 tropism, all of which, interestingly, were mutated and non-functional.

Conclusion: Our results offer further insight into the complexity and potential functionality of archived HIV sequences in the resting T-cell reservoir.

#**37 Maldarelli, Frank**

No Evidence of Ongoing HIV Replication in Tissue Compartments During Combination Antiretroviral Therapy

G Bozzi[1], S Watters[1,2], F Simonetti[1], E Anderson[1], R Gorelick[4], W Shao[3], J Bell[1], C Rehm[6], R Yarchoan[5], T Uldrick[5], F Maldarelli[1]

1. HIV Drug Resistance Program, NCI, NIH, Frederick, Maryland; 2. Dept. Infection and Immunity, University College London, UK; 3. Advanced Biomedical Computing Center, Leidos Biomedical Research Inc., Frederick National Laboratory, Frederick, Maryland; 4. AIDS and Cancer Virus Program, Leidos Biomedical Research, Inc., Frederick National Laboratory, Frederick, Maryland; 5. HIV and AIDS Malignancy Program, NCI, NIH, Bethesda, Maryland; 6. Laboratory of Immunoregulation, NIAID, NIH, Bethesda, Maryland

Introduction: The sources of HIV persistence during combination antiretroviral therapy (cART) remains uncertain, and the contribution of active HIV replication in tissue compartments is unknown. Genetic analyses represent sensitive measures to detect ongoing cycles of virus replication, particularly in individuals who initiate cART early after infection, when HIV populations are relatively monomorphic and increases in genetic diversity are easily detectable. We investigated whether ongoing replication occurs in tissues, we investigated HIV populations in blood and anatomic compartments from 3 individuals who initiated cART shortly after HIV infection occurred.

Methods: Patients (N=3) had HIV RNA levels <50 c/ml for 2-16 years and underwent autopsy for primary effusion lymphoma (N=1), or routine colonoscopy (N=2). HIV from autopsy was quantified using real time PCR, and HIV sequences (pro-pol, 1200 nt) were obtained from tissue and peripheral blood lymphocytes (PBL) using single genome sequencing (SGS) techniques. Sequences were aligned, subjected to phylogenetic (MEGA), and compartmentalization analyses (Slatkin-Maddison, FST, geographic subdivision). A total of 266 sequences from the autopsy and 100 from colonoscopy were analyzed.

Results: HIV DNA was readily recovered in most tissues at autopsy with median 1.8 copies/1e6 cells, range 1-75/million cells) All patients had relatively monomorphic virus populations with limited genetic diversity in both tissues and PBL (average pairwise difference range 0.1 – 0.5%). From the autopsy, a total of 263 SGS were obtained, PBL (N=124) gut ileum, jejunem, colon (40), spleen (38), lymph node (30), effusion cells (10), kidney (5), lung (3), testes (1). HIV populations were well-mixed and were not divergent from PBL-derived HIV, with no evidence of compartmentalized replication in any tissue examined. Identical hypermutated sequences were present in PBL and several tissues, demonstrating distribution of clonally expanded cells had occurred. In 2 individuals undergoing colonoscopy, comparative analysis of HIV from ileum, colon, and PBL revealed no evidence of ongoing replication and no divergence from pretherapy plasma RNA obtained 10-12 years prior to colonoscopy.

Conclusions: No evidence of ongoing replication was detected in tissues compared to peripheral blood, in individuals undergoing cART, suggesting cART blocks active HIV replication in tissues.

#**38 Liu, Dongfang**

Potential Application of VRC01-CAR for Eradication of HIV-1 Reservoirs

Shuo Tian[1], William K Decker[3], Jason Kimata[4], Andrew Rice[4], Jacob Couturier[5], Roberto Arduino[5], Dorothy E Lewis[5], Dongfang Liu[1,2]

1. Center for Inflammation and Epigenetics, Houston Methodist Research Institute, Houston, Texas; 2. Department of Microbiology and Immunology, Weill Cornell Medical College, New York, New York; 3. Department of Pathology & Immunology, Baylor College of Medicine, Houston, Texas; 4. Department of Molecular Virology and Microbiology, Baylor College of Medicine, Houston, Texas; 5. Department of Internal Medicine, University of Texas Health Center at Houston, Houston, Texas

Eradication of HIV-1 from infected individuals remains a major medical challenge. Although cART has dramatically reduced HIV-related morbidity and mortality, cART fails to eliminate HIV in vivo due to persistence of replication-competent proviruses in long-lived latently infected cells, also known as HIV reservoirs. Recently, the use of chimeric antigen receptors (CARs) to redirect CTLs toward cancers has become a promising approach of cancer immunotherapy and represents a broad-based approach by which T cells can be engineered to overcome antigen restriction or mutation. We developed CAR cytotoxic lymphocytes that express the single-chain variable fragment (scFv) of VRC01 (a broadly HIV-1-neutralizing antibody).We hypothesize that when used with pharmacological “Shock” approaches, such as the use of chromatin remodeling enzymes like histone deacetylases (HDACs) to reactivate viruses, the resulting CAR cytotoxic lymphocytes will effectively kill reservoirs via VRC01 neutralization of free wild-type and mutant virions. Preliminary data will demonstrate efficacy of CAR cytotoxic lymphocytes. Successful development of the proposed “kill two birds with one stone” CAR cytotoxic lymphocytes will not only address latency in HIV-1, but will provide proof-of-concept for similar approaches in other disease states associated with viral latency.

#**39 Leibman, Rachel**

Optimized, Second Generation Chimeric Antigen Receptors Improve CAR T Cell Expansion and Control Over HIV-1 Replication

Rachel S Leibman[1], Max W Richardson[1], Christoph T Ellebrecht[1], Keith A Dufendach[1], Joshua A Glover[1], Anthony J Secreto[1], Yanjie Yi[1], Jianbin Wang[2], Michael C Holmes[2], Ronald G Collman[1], Aimee S Payne[1], James L Riley[1]

1. University of Pennsylvania, Perelman School of Medicine, Philadelphia, Pennsylvania; 2. Sangamo Biosciences Inc., Richmond, California

Chimeric Antigen Receptors (CARs) consist of an extracellular antigen binding domain fused to intracellular T-cell activation domains. These synthetic receptors have the ability to redirect T cell activity to recognize intact membrane proteins independent of antigen processing in a MHC independent manner. The first CAR tested in humans fused CD4 to the CD3 zeta intracellular domain as a means to re-direct CD8 T cells to target HIV Env. While modest antiviral activity and excellent patient safety were observed, further clinical development was abandoned. We have optimized the original construct's expression vector, promoter, transmembrane, and costimulatory domains, and have demonstrated that a second generation CAR has over 50 fold more potency. Complete suppression of HIV-1 replication and spread was observed when CAR-expressing CD8 T cells were cocultured at a 1:100 effector to target ratio with infected CD4 T cells. We then compared the ability of ScFv extracellular domains derived from the VRC01, PG9, PGT128, 3BNC60, and PDGM1400 antibodies to the ability of CD4 to re-direct the CD8 T cell response. All of the ScFv based CARs were functional and the PGT128 ScFv had the most anti-viral activity; However, all ScFv based CARs were inferior to the CD4 based CARs. Finally, we compared the ability of CD4 based CARs containing either the CD28 or the 4-1BB costimulatory domain to control HIV-1 replication in vivo using a humanized mouse model of HIV-1 infection. We observed greater in vivo control of HIV-1 infection and antigen specific expansion in T cells expressing the second generation, optimized CD4 CARs. These data form the basis and rationale for pursuing second generation CD4 based CAR T cell approaches in human clinical trials.

#**40 Vargas-Inchaustegui, Diego**

CD4-Based Bispecific Chimeric Antigen Receptors: Towards a Functional Cure of HIV

Diego A Vargas-Inchaustegui[1], Agnes Hajduczki[1], David S Elias[1], David T Danielson[1], Virgilio G Bundoc[1], Edward A Berger[1]

1. Molecular Structure Section, Laboratory of Viral Diseases, NIAID, NIH, Bethesda, Maryland

As one approach to achieve a “functional cure” against chronic HIV infection, cells of the immune system can be engineered for durable identification and elimination of HIV-infected cells, even after discontinuation of antiretroviral therapy. To achieve this goal, our laboratory focuses on the design and validation of novel bispecific chimeric antigen receptors (CARs) that reprogram T cells to detect the HIV-1 envelope glycoprotein on the surface of infected cells. By using a bispecific targeting moiety composed of CD4 linked to the carbohydrate binding domain of mannose-binding lectin (CD4-MBL CAR), we aim to approach durable, escape-proof and non-immunogenic anti-HIV-1 suppressive activity. In vitro, our CD4-MBL CAR displays high potency and extensive breadth for suppression of genetically diverse HIV-1 isolates in spreading infection assays. Moving forward, we have designed and analyzed an all-rhesus version of the CD4-MBL CAR for in vivo pre-clinical NHP studies and also developing novel assays to extensively test the specificity and sensitivity of human CD4-MBL CARs. Here, we summarize our preliminary in vitro studies with the rhesus CD4-MBL CAR and show, as in the human system, that a rhesus CD4-MBL CAR is capable of re-programming rhesus T cells into strong and specific anti-SIV effectors. Moreover, we are developing a live imaging in vitro system that allows us to visualize and quantify CAR-mediated specific killing of Env-expressing cells within a mixture of target and non-target cell populations. Finally, using a tetracycline-controlled system for Env expression on target cells, we seek to elucidate the minimum surface expression levels required for effective CAR-mediated target cell identification and elimination. Overall our laboratory efforts are aimed at validating CD4-based bispecific CARs as tools for targeted killing of infected cells toward a functional cure of HIV.

#**41 Garrido, Carolina**

Natural Killer Cells for Clearing the HIV Reservoir

Carolina Garrido[1], Natalia Soriano-Sarabia[1], Nancie Archin[1], David Margolis[1]

1. UNC HIV Cure Center and Department of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina

Natural killer (NK) cells are the main effectors of the innate immune system and play an important role in host defense against HIV infection. The innate immune response may be a useful tool to target HIV infection that persists despite durable, successful antiretroviral therapy (ART). We are investigating the potential of NK cells to clear persistent HIV infection following latency reversal.

To assess effector function of NK cells we performed cytotoxicity analysis, viral inhibition assays and latency clearance experiments. The antiviral activity of NK cells was assessed directly, or following 24 hours of exposure to 25 ng/mL of IL-15. Statistical analysis was performed using the Wilcoxon matched-pairs signed rank test.

Cytotoxicity was assessed measuring the degranulation marker CD107a. NK cells were cultured with the MHC-I deficient cell line K562 for 4 hours in the presence of CD107a antibody, adding the protein transport inhibitor GolgiStop after the first hour of incubation. Expression of CD107a analyzed by flow cytometry showed that IL-15 stimulation improved NK cells cytotoxic potential from a mean of 48.3% to 65.7% ($p<0.0001$). Cytotoxicity was also analyzed using HIV-infected autologous CD4+T cells as targets. CD4+T cells were stimulated with PHA and IL-2 and then superinfected by spinoculation with the viral strain JR-CSF. Using these autologous infected targets, IL-15 also improved NK cell degranulation, from 26.9% to 40.9% ($p=0.002$).

Antiviral activity was analyzed using viral inhibition assays. Autologous CD4+T cells were infected as described for cytotoxicity, and left in culture alone or with NK cells for 7 days, after which supernatant was collected to measure viral replication by HIV gag p24 ELISA. Untreated NK cells reduced viral replication 70.9%, while IL-15 stimulated NK cells reduced viral production 94% ($p=0.008$).

Clearance of persistent infection was evaluated using the autologous latency clearance assay system [Sung et al. JID 2015]. Resting CD4+T cells and NK cells were isolated from HIV-infected individuals on antiretroviral therapy and suppressed viral load. Resting CD4+T cells were reactivated with PHA or latency was reversed with Vorinostat (VOR), and plated in 12 replicates in the presence or absence of NK cells for 15 days. In cells reactivated with PHA, NK cells reduced the number of HIV-positive wells 75% ($p=0.06$), while IL-15 stimulated cells mediated a 95% ($p=0.03$) reduction of viral recovery. In cells reactivated with VOR, NK cells reduced number of HIV-positive wells by 50% ($p=0.008$) and IL-15 stimulated NK cells by 75% ($p=0.004$).

Effector function in the presence of latency reversal agents. Given that immune clearance strategies must be implemented in concert with latency reversal, it is essential to investigate whether compounds used to disrupt latency have an impact of effector cell function. We explored the impact of VOR exposure into NK cell function, both ex vivo and in vivo. We conducted cytotoxicity and viral inhibition assays as previously described, and in addition IFN-gamma production was evaluated. For the ex vivo assessment, we exposed isolated NK cells to VOR for 24 hours, while in vivo determination was performed analyzing blood samples from HIV-infected patients before and after clinical administration of VOR. There was no significant effect of ex vivo exposure of VOR on NK cell function, either as degranulation and IFN-gamma production after culture with K562 cells, or antiviral activity using autologous infected HIV CD4+T cells. In vivo exposure to

VOR showed no significant change in degranulation capacity of NK cells against K562 cells but interestingly there was a significant improvement in IFN-gamma production (fold change of 1.9 after 11 doses of VOR and FC=2 after 22 doses, p=0.008).

Encouragingly, our results show that NK cells can recognize and target autologous HIV-infected cells, and that their function is improved significantly upon stimulation with IL-15. Of interest, exposure to the latency reversing agent Vorinostat did not impair NK function.

#**42 Huang, Szuhan**

CTLs Pare Defective HIV Proviruses Without Impacting Infectious Latent Reservoirs

Szuhan Huang[1], Allison Thomas[2], Ya-Chi Ho[3], Sara Karandish[1], Dora Chan[1], Adam Ward[1], Colin Kovacs[4], Erika Benko[4], R Brad Jones[1]

1. George Washington University, Washington, DC; 2. Boston University, Boston, Massachusetts; 3. Johns Hopkins University, Baltimore, Maryland; 4. Maple Leaf Medical Clinic, Toronto, ON, Canada

The majority of “shock-and-kill” studies have been performed in primary-cell models of HIV latency, which are imperfect representations of natural viral reservoirs. A need remains for a rigorous investigation of whether immune effectors, in combination with latency-reversing agents (LRAs), can drive reductions in replication-competent proviruses from natural reservoirs. Here, we treat ex vivo CD4+ T-cells from HIV+ individuals on long-term ARV therapy (>5 years), with LRAs and autologous HIV-specific CTL clones (targeting non-escaped epitopes), and assessed the impact on total and intact-inducible proviruses. Method: HIV-specific CTL clones targeting known HIV epitopes were isolated from ARV-treated subjects by limiting dilution, and killing activities were confirmed by flow cytometric assays. We developed an HIV eradication (HIVE) assay to test the abilities of these CTLs to reduce viral reservoirs in combination with romidepsin, vorinostat, bryostatin, ALT-803, or Pam3CSK4. In short, resting CD4+ T-cells from HIV+ leukapheresis samples are co-cultured with LRAs + CTLs for 5 days with ARVs, and activation/memory phenotypes are monitored. CD4+ T-cells are isolated after treatment, and total/intact-inducible reservoirs are measured by cell-associated HIV DNA (ddPCR) and by quantitative viral outgrowth assay (QVOA). Results: Combinations of bryostatin and ALT-803+Pam3CSK4 with HIV-specific CTL generally led to significant decreases in cell-associated HIV DNA, with the greatest effects observed for bryostatin (up to 50% reductions, $p < 0.01$). Critically, these decreases in HIV DNA were not associated with measurable reductions in intact-inducible virus, regardless of the CTL clone or LRA combination used (powered to detect ~50% reductions with 95% confidence). Even when combined with PMA/ionomycin, CTLs were unable to drive reductions in intact-inducible virus. CTLs degranulated (CD107a) in response to autologous activated CD4+ T-cells that had been infected with virus from positive QVOA wells, ruling out a role for immune escape in our observation. Conclusions: Recently, it has been demonstrated that some defective proviruses can be expressed as antigens, enabling CTL recognition. Data from our ex vivo experiments are consistent with the preferential depletion of defective proviruses by CTLs, leading to reductions in HIV DNA without impacting intact proviruses. Understanding and overcoming the mechanisms limiting CTL against the intact-inducible reservoir may be key to successful CTL-based shock and kill interventions.

#**43 Patel, Shabnum**

HIV-Specific T Cells Expanded from HIV+ and HIV-Naive Donors Target a Range of Viral Epitopes: Implications for a Cure Strategy After Allogeneic HSCT

Shabnum Patel[1,2], Sharon Lam[1], Julia A M Sung[3], Conrad Russell Y Cruz[1,2], Nilu Goonetilleke[3], Yinyan Xu[3], JoAnn D Kuruc[3], Cynthia L Gay[3], David M Margolis[3], Richard F Ambinder[4], Elizabeth J Shpall[5], Catherine M Bollard[1,2]

1. Center for Cancer and Immunology Research, Children's National Health System, Washington, DC; 2. The George Washington University, Washington DC; 3. University of North Carolina HIV Cure Center, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina; 4. Sidney Kimmel Comprehensive Cancer Center, The Johns Hopkins University School of Medicine, Baltimore, Maryland; 5. Department of Stem Cell Transplantation and Cellular Therapy, The University of Texas MD Anderson Cancer Center, Houston, Texas

Adoptive T cell therapy has been successful in boosting viral-specific immunity post-hematopoietic stem cell transplant (HSCT), preventing viral rebound of CMV and EBV. However, the therapeutic use of T cells to boost HIV-specific T cell immunity in HIV+ patients has been met with limited success. Despite multiple attempts to eradicate HIV infection with allogeneic HSCT, the Berlin patient remains the only case of functional HIV cure. Previous infusions of HIV-specific T cells have resulted in immune escape from single epitope specificity and limited persistence of the T cell product. Hence, we hypothesized that ex vivo expanded, broadly HIV-specific CD8+ and CD4+ T cells (HXTCs) could be expanded from patients on antiretrovirals (ARVs), as well as HIV negative adult and cord blood (dHXTCs) donors, employing a non-HLA restricted approach for the treatment of HIV+ individuals after autologous or allogeneic HSCT.

We have expanded autologous HXTCs from HIV+ subjects underNCT02208167.To extend this approach to the allogeneic HSCT setting, we expanded dHXTCs from HIV naive adult (n=8) and cord blood donors (n=8). IFNg ELISPOT showed dHXTCs from adult donors were specific against Gag (mean=331.25 SFC/105cells) and Nef (mean=242.63 SFC/105cells) versus irrelevant antigen (mean=13 SFC/105cells) (n=8). Similarly, we are able to produce dHXTCs from cord blood units (n=8) that reveal significant levels of IFNg when stimulated with Gag (mean=103.252 SFC/105cells), Nef (mean=109.43 SFC/105cells), or Pol (mean=130.56 SFC/105cells), ($p<0.0001$ compared to control). dHXTCs produced pro- inflammatory responses ($p<0.05$) to gag/nef stimulation, as determined by levels of TNF-a, IL-2, IL-6, IL-8, and perforin. Importantly, dHXTCs derived from both adult (n=4, p=0.0004) and cord blood (n=2, p=0.0003) were able to suppress HIV replication more than non-specific CD8+ T-cells when co-cultured with autologous CD4+ T-cells infected with HIV SF162 at an effector-to-target ratio of 20:1. Exhaustion analysis of cord-derived dHXTC lines revealed minimal expression of exhaustion markers PD-1 (0.25-0.32%), TIM-3 (0.84-1.65%), LAG-3 (1.05-1.89%, and KLRG1 (0.91-4.35%), emphasizing the potential of cord blood as a source for therapeutic HIV-specific T cells post-HSCT (n=3). Epitope mapping of HXTCs (HIVpos) versus dHXTCs (HIVneg) revealed that dHXTC products contained T cells recognizing unique epitopes not typically identified in HIV+ subjects. Comparing these unique epitopes to known HIV epitope maps (based on the Los Alamos National HIV Database) revealed novel HIV epitopes, which may be critical in overcoming viral immune escape post HSCT.

In summary, HIV--specific T cells can be expanded from HIVpos and HIVneg donors for clinical use. Focusing on donors with HLA types that are associated with well characterized HIV responses (HLA A02) or associated with delayed progression to AIDS (HLA B27, B51, B57) may allow us to identify HLA-restricted epitopes critical for the successful development of a potent HIV-specific T cell therapeutic. Hence, the administration of dHXTCs derived from adult and cord blood donors could offer a unique curative strategy post allogeneic HSCT.

#44 Kuruc, JoAnn

Safety and Tolerability of Reinfused Ex-Vivo Expanded, HIV-1-Specific T-Cell Therapy (HXTC)

JoAnn D Kuruc[1], Cynthia L Gay[1], Julia M Sung[1], Amanda Crooks[1], Shabnum Patel[2], Lauren Roesch[2], Patrick Hanley[2], C Russell Cruz[2], Joseph J Eron[1], Nilu Goonetilleke[1], Cliona M Rooney[3], Catherine M Bollard[2], David M Margolis[1]

1. UNC HIV Cure Center, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina; 2. Center for Cancer and Immunology Research, Children's National Health System, Washington, DC; 3. Department of Molecular Virology and Microbiology, Department of Immunology, Section of Hematology-Oncology, Baylor College of Medicine, Houston, Texas

Background: The administration of autologous T cells specific for multiple HIV antigens and expanded ex vivo (HXTCs) derived from HIV-infected participants on suppressive combination antiretroviral therapy (cART) is a novel strategy employed in research aimed to cure HIV.

Methods: This is a Phase 1, ongoing, proof of concept study (NCT02208167) of the administration of HXTCs to HIV-infected participants with undetectable plasma viremia (<50 copies/mL) on stable cART. Autologous T cells are stimulated ex vivo with autologous dendritic cells pulsed with a consensus Gag, Pol and Nef peptide mix to reflect the high sequence diversity of the HIV-1 virus. T cell expansion is performed in the presence of indinavir and raltegravir to inhibit HIV replication in cultures. After several rounds of stimulation (24–26 days), ex vivo expanded HXTCs showed a mean expansion of 146-fold (range: 37–287). Participants meeting eligibility criteria receive two HXTC infusions 2 weeks apart. Each HXTC dose is 2x107 cells/m². In addition, all participants undergo two leukapheresis procedures to measure the frequency of resting cell infection by the Quantitative Viral Outgrowth Assay (QVOA), at baseline and 12 weeks after the second HXTC infusion. All participants remain on cART throughout the study, classified by the stage of HIV infection during which treatment was initiated. All participants have sustained HIV suppression. Treatment during acute HIV infection was defined as starting cART within 30 days of the first positive HIV test identifying acute HIV infection. Leukapheresisis a safe and well-tolerated procedure in which leukocytes, or specific cell fractions, are selectively harvested and the red cells and other blood components are returned to the participant.

Results: Four male participants have received two HXTC infusions to date; 2 participants completed follow-up through week 48 and 2 participants completed follow-up through week 13 following the 2nd infusion. Among the 4 participants, 2 initiated cART during acute HIV infection and 2 initiated during chronic HIV infection. All four participants are white males with a median age of 52 years (range 35– 65); two participants self-identified as heterosexual, and two self-identified as men who have sex with men (MSM). At the time of cART initiation, the median nadir CD4 was 226 cells/mm3 (range 168 – 427) and the median viral load was 479,000 copies/mL (range 24,905 -14,538,865). All participants were aviremic for greater than one year at the time of enrollment. Overall, there were few (n=14) adverse events (AEs) observed among these 4 participants. Ten of 14 (71%) AEs were related to the leukapheresis procedure and the other 4 AEs (29%) were associated with the HXTC infusions. Thirteen (93%) of 14 AEs resolved without treatment or intervention; one participant took Naproxen for pain associated with infusion site ecchymosis experienced after the second HXTC infusion. Seven of the ten (70%) AEs attributed to the leukapheresis were grade 1 in severity. Four of the 10 (40%) reported events were attributed to the needle insertion sites and 6 were associated with the blood pressure readings obtained during the leukapheresis procedure. The needle insertion site AEs included ecchymosis (1/10, 10%), swelling (10%) and tenderness (2/10, 20%). The six (60%) reported blood pressure related AEs were transient elevations; two were grade 3 (20%), one was grade 2 (10%) and 3 were grade 1 (3/10, 30%). These were comparable to AEs seen in other populations undergoing leukapheresis. The eight HXTC infusions accounted for four AEs and all were

Grade 1 in severity. These included ecchymosis in the area of the infusion site (2/4, 50%) and one report each of grade 1 fatigue (1/4, 25%) and subjective report of fever (25%). AEs were comparable to those seen in other populations receiving cryopreserved antigen-specific T-cell products.

Conclusion: Harvest, expansion, and reinfusion of HXTCs from ART-treated, aviremic HIV-infected participants appears safe and well-tolerated. AEs were minimal and typically associated with the leukapheresis and the infusion of cryopreserved T-cells. HXTC infusion did not induce clinically perceptible signs of immune activation perhaps due to the low HIV antigen burden expressed during ART. Evaluations of the virological and immunological impact of HXTC infusion in this study is ongoing. Preliminary data suggests that HXTCs confer a measurable augmentation of HIV-specific immune response. Following confirmation of safety of HXTCs in HIV-infected participants, we plan to co-administer HXTCs with a latency-reversing agent.

#**45 Tcherepanova, Irina**

Development of an Autologous HIV Outgrowth Process for the Manufacture of Targeted Immunotherapy for Latent Reservoir Clearance

John Krisko[1], Nancie M Archin[2], Joe Horvatinovich[1], Mark DeBenedette[1], Joann Kuruc[2], Cynthia Gay[2], David M Margolis[2], Charles Nicolette[1], Irina Tcherepanova[1]

1. Argos Therapeutics, Durham, North Carolina; 2. University of North Carolina HIV Cure Center, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina

One strategy for eradicating HIV from latently infected reservoirs is the combination of suppressive antiretroviral therapy (ART) with latency-reversing agents (LRAs) to induce the expression of viral antigen in latently infected cells, making them susceptible to immune clearance. A known barrier to this strategy is that existing CD8+ T cell responses may not be sufficient to clear the latent reservoir following virus reactivation. Approaches that strengthen HIV-specific CD8+ T cell responses may be instrumental in overcoming this barrier.

AGS-004 immunotherapy comprises autologous DCs electroporated with autologous amplified HIV RNAs (Gag, Vpr, Rev and Nef). In a previous clinical trial, administration of AGS-004 was shown to induce HIV-specific effector/memory CD8+ T cell responses in the majority of participants. The manufacture of AGS-004 involves the amplification of autologous HIV RNA from virus present in patient plasma that is obtained prior to initiation of ART. As the initiation of ART is currently recommended for all HIV-infected individuals, regardless of viral load or CD4+ T cell count, pre-ART plasma may not be available for use in manufacturing for some participants.

As an alternate source of HIV RNA for the manufacture of AGS-004, virus can be obtained from the reservoir of latently infected cells. Mitogen activation of these cells ex-vivo results in production of virus, which can be harvested and used for RNA amplification. This approach would not only eliminate the need for archived pre-ART plasma, a scarce resource, but may allow specific targeting of the HIV antigens present in latent reservoir. Here, we describe the development of an autologous system for HIV outgrowth for the purpose of immunotherapy for investigation of latent reservoir clearance.

Methods: Peripheral blood mononuclear cells (PBMCs) were obtained by Ficoll separation of leukapheresis samples from aviremic HIV-positive participants on suppressive ART. CD8+ T cells were depleted from PBMCs by positive selection using magnetic bead separation. CD8-depleted PBMCs were cultured in Iscove's Modified Dulbecco's Medium (IMDM) supplemented with phytohemagglutinin (PHA) with or without IL-2. For one participant, CD8-depleted PBMCs were stimulated using 2 micrograms/mL PHA and 20 U/mL Interleukin-2 (IL-2). CD4+ cells were enriched from PBMCs by positive selection using magnetic bead separation. Purified CD4+ cells were plated in replicate cultures (24 cultures) and stimulated for 2 days in IMDM supplemented with PHA and IL-2. Following PHA activation, cultures were maintained for up to 18 additional days in IMDM supplemented with IL-2. CD4+ cell cultures were supplemented with PHA-stimulated autologous CD8-depleted PBMCs on both day 2 and day 8. CD4+ cell cultures were assayed for HIV outgrowth at day 14 and at day 20 using an HIV-1 p24 ELISA. Cultures positive for virus outgrowth at day 14 were harvested and the supernatant stored for future pooling of culture supernatants. CD4+ cultures that were negative for virus outgrowth at day 14 were reevaluated by p24 ELISA at day 20. All cultures positive for HIV outgrowth at day 20 were harvested and the supernatant stored for future pooling of culture supernatants. HIV supernatant to be used for RNA amplification to generate AGS-004 was prepared by pooling individual supernatants of CD4+ cultures positive for virus outgrowth.

Results: This novel procedure was developed using leukapheresis product from three different non-treatment participants. The first participant material used in this process gave two CD4+ cultures positive for virus outgrowth at day 14, with an additional 8 cultures becoming positive for HIV at day 20. The overall result in the first development procedure was 10 out of 24 (42%) CD4+ cell cultures produced HIV at levels suitable for RNA amplification and AGS-004 production. A leukapheresis product from a participant known

to have a low number of infected resting T cells was also tested in this procedure. All 24 cultures remained negative for p24 for the duration of the procedure, demonstrating that this HIV outgrowth process can be limited by a low frequency of infected resting cells. We sought to increase the number of positive CD4+ cell cultures in the next procedure, as having a greater number of CD4+ cell cultures grow out HIV potentially improves the sampling of antigens present in the viral reservoir. We had observed cell loss in CD8-depleted PBMC cultures during activation with the first two participants and attempted to mitigate this loss with the addition of IL-2 to the culture media during mitogen stimulation with our 3rd participant. HIV outgrowth was substantially improved, as 21 CD4+ cell cultures were positive for p24 by day 14. Evaluation of the remaining 3 cultures at day 20 found that 2 additional cultures had become positive for virus outgrowth to give an overall result of 23 out of 24 (96%) cultures yielding HIV.
Conclusions: We report the development of a novel, autologous system for recovering replication competent HIV from the latent reservoir for use in the manufacture of AGS-004 immunotherapy. In this system, replicate cultures of ex-vivo stimulated CD4+ cells allow the recovery of HIV from multiple distinct sources, potentially improving the representation of the viral antigens used to manufacture AGS-004. In the two non-treatment procedures where HIV replication was induced, virus was recovered from 42% and 96% of cultures respectively. AGS-004 immunotherapy, manufactured using diverse HIV antigens recovered with this novel system, will be combined with the LRA Vorinostat in an ongoing trial (NCT02707900) to determine if this combination treatment can induce immune responses that facilitate clearance of the latent reservoir in the setting of latency reversal.

#**46 Fuller, Deborah**

Factors Influencing the Immunogenicity of Therapeutic DNA Vaccination and Induction of Viral Remission After Stopping Drug Therapy

Deborah H Fuller[1,2], Paul Munson[1,2], Amithi Narendran[3], Premeela Rajakumar[4], Michael Murphey-Corb[4], Viraj Kulkarni[5], Barbara K Felber[6], George N Pavlakis[6], James I Mullins[1]

1. Department of Microbiology, University of Washington, Seattle, Washington; 2. University of Washington National Primate Research Center, Seattle, Washington; 3. Albany Medical College, Albany, New York; 4. University of Pittsburgh, Pittsburgh, Pennsylvania; 5. Human Retrovirus Pathogenesis Section and 6Human Retrovirus Section, Vaccine Branch, Center for Cancer Research, Frederick National Laboratory for Cancer Research, Frederick, Maryland

There is now evidence in both nonhuman primates and humans that a cure or durable viral remission of HIV may be possible. Our studies show that DNA vaccines expressing highly conserved sequences of SIV (Conserved Elements or CE) and/or full length SIV antigens increase mucosal and systemic T cell responses against conserved and/or variable sequences and, to date, have suppressed viral rebound or induced durable viral remission after stopping ART in 10/19 (53%) of vaccinated animals. Among these animals, we have observed up to a 5 log reduction in mean viral RNA in the gut and lymphoid tissues and four animals maintained undetectable viral load in the blood (<10 viral RNA copies/ml) for over 30 months after treatment was stopped (end of study). When combined with adjuvants and with improvements in DNA vaccine immunogen design, we may be able to increase the ability of DNA vaccination to induce durable viral remission. Furthermore, when combined with gene therapy, latency reversing agents or immune modulators, we may be able to eliminate residual SIV/HIV reservoirs. However, a consistent observation in our therapeutic vaccine studies, to date, is that viral remission is achieved in only a subset (40-50%) of animals, an outcome that raises questions about what factors are driving success versus failure of therapeutic vaccination? We have investigated this question at two phases in our studies: 1) After initiation of ART but prior to vaccination and 2) after completing vaccination but before stopping ART. In the first phase, responsiveness to ART with respect to either viral load or the duration in which plasma viremia was suppressed to very low or undetectable levels prior to initiating vaccination correlated with the immunogenicity of the therapeutic vaccine and its ability to induce de novo specificities in the T cell repertoire. In the second phase, breadth of the T cell response and the level of inflammation in the gut correlated with duration of viral remission after stopping ART. Understanding the factors influencing success vs. failure in achieving viral remission in the nonhuman primate model may lead to new strategies to improve success rate and the effectiveness of therapeutic vaccines in combination with other therapies.

#**47 Sivanandham, Ranjit**

Treg Depletion as an Effective Strategy to Induce SIV Reactivation and Clearance

Ranjit Sivanandham[1], Benjamin B Policicchio[1], Egidio Brocca-Cofano[1], Kathryn J Martin[1], Tianyu He[1], Kevin D Raehtz[1], George H Richter[1], Tammy Dunsmore[1], Zhirui Wang[2], Ivona Pandrea[1], Cristian Apetrei[1]

1. Center for Vaccine Research, University of Pittsburgh, Pittsburgh, Pennsylvania; 2. Massachusetts General Hospital, Harvard University, Boston, Massachusetts

T regulatory cells (Tregs) may be involved in formation of the latent reservoir, being susceptible to HIV/SIV infection. Resting Tregs harbor high levels of HIV/SIV. During acute infection, Tregs reverse CD4+ T cell immune activation status, increasing the pool of resting CD4+ T cells and contribute to the establishment of HIV reservoir. During chronic infection, Tregs limit CTL responses (Treg expansion correlates with loss of CTL function and theirex vivo depletion enhances T cell responses to HIV/SIV antigens). We hypothesized that Treg depletion is a valid approach for HIV cure, in which a single intervention reduces the size of the reservoir, reactivates the virus and boosts cell-mediated immune responses.

Methods: Seven SIVsab-infected RMs, in which spontaneous supercontrol of virus replication (< 3 copies/ml plasma) associates complete control of immune activation, were depleted of Tregs with an engineered protein combining IL-2 (the ligand of CD25, a marker for Tregs) and diphtheria toxin (IL-2 DT). Treg depletion was monitored by flow cytometry and immunohistochemistry; plasma viral load was measured by single copy assay; specific cellular immune responses to SIV antigens were monitored flow-cytometrically by intracellular cytokine staining after stimulation with SIVsab peptides.

Results: IL-2 DT administration to SIVsab-infected RMs resulted in significant depletion (>75%) of the circulating Fox-P3+CD25highCD4+ T cells. Up to 60% and >50% of Tregs were depleted from gut and the lymph nodes, respectively. IL-2 DT impact on overall CD8+ T cell counts was minimal. Treg depletion resulted in a major increase of the levels of CD4+ T cell activation (Ki-67). In the absence of antiretroviral therapy, virus rebound to 103 vRNA copies/ml of plasma occurred by day 10 post-IL-2 DT administration. Importantly, Treg depletion resulted in a significant boost of the SIV-specific CD8+ T cells and rapid clearance of the reactivated virus.

Conclusions: Treg depletion in chronically SIV-infected super elite controller RMs resulted in both reactivation of latent virus and a boost of CTL responses. The overall Treg ability to control immune responses was significantly impaired despite the fact that Treg depletion was incomplete. Furthermore, incomplete Treg depletion probably prevented a significant increase in autoimmunity. As no latency reversing agent in development has such a dual activity, our strategy holds great promises for cure research and other strategies for Treg depletion are currently being explored.

#48 Pavlakis, George

Heterodimeric IL-15 Induces Effector Cell Activation and Trafficking to the Germinal Centers of SIV infected Macaques

George N Pavlakis[1], Antonio Valentin[1], Dionysios C Watson[1], Eirini Moysi[1], Cristina Bergamaschi[2], Constantinos Petrovas[3], Shakti Singh[2], Xintao Hu[2], Jenifer Bear[2], Barbara K Felber[2]

1. Human Retrovirus Section, Vaccine Branch, CCR, NCI at Frederick, Frederick, Maryland; 2. Human Retrovirus Pathogenesis Section, Vaccine Branch, CCR, NCI at Frederick, Frederick, Maryland; 3. Vaccine Research Center, NIAID, NIH, Bethesda, Maryland

IL-15 stimulates the growth and activation of NK cells and cytotoxic lymphocytes. We have produced heterodimeric IL-15 (hetIL-15), the form of IL-15 found in the circulation, and demonstrated that administration of hetIL-15 in macaques results in improved pharmacokinetics and pharmacodynamics compared to previous preparations of IL-15 without significant side effects. Several groups have proposed the use of IL-15 in the effort to control and eradicate HIV-1.

Heterodimeric IL-15 (hetIL-15) was purified and tested in macaques upon subcutaneous (SC) administration. Phenotype and functional changes in lymphocyte subsets were monitored by flow cytometry, and their location within the lymph nodes by multiplexed confocal imaging (MCI). HetIL-15 was also combined with DNA vaccination to test the hypothesis that it will increase antigen-specific cytotoxic cells in the tissues, including virus reservoir sites.

Treatment with hetIL-15 resulted in a significant increase of CD8+ effector T cells and NK cells with activated cytotoxic phenotype (Granzyme+Ki67+). This expanded T lymphocyte population was distributed in the tissues and was also present in secondary lymphoid organs where an increased frequency of Ag-specific effector and total effector CD8 T cells could be observed by flow cytometry. A subset of CD8 T cells present in lymph nodes expresses CXCR5, indicating ability to migrate into germinal centers where chronically infected CD4+Tfh cells reside. Effective levels of hetIL-15 can be delivered without side effects. Multiplexed confocal imaging confirmed the presence of effector CD8 in germinal centers and showed that these cells are cytotoxic (GrzmB+) and actively proliferating (Ki67+) in response to hetIL-15.

The results suggest that hetIL-15 has potential as a viral reservoir reducing agent in ART-treated SIV infected macaques due to its ability to increase cellular immunity and deliver cytotoxic cells in areas of virus persistence.

#**49 Alvarado, John**

Small-Molecule Antagonists of the HIV-1 Nef Accessory Protein: Targeting the Nef Dimer

John J Alvarado[1], Haibin Shi[1], Lori A Emert-Sedlak[1], H Marie Loughran[2], Jay E Wrobel[2], Allen B Reitz[2], Thomas E Smithgall[1]

1. Department of Microbiology & Molecular Genetics, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania; 2. Fox Chase Chemical Diversity Center, Inc., Doylestown, Pennsylvania

The HIV-1 accessory protein Nef supports high-titer viral replication, immune evasion of HIV-infected cells, and is essential for AIDS progression. Efficient replication of M-tropic HIV-1 in primary human macrophages requires expression of the myeloid Src-family kinase, Hck. Nef provides a crucial link between HIV-1 and this host cell kinase, interacting with Hck through its SH3 domain and driving constitutive kinase activation. In addition to Hck, Nef also interacts with the cellular endocytic adaptor proteins, AP-1 and AP-2, which are responsible for downregulation of MHC-I and CD4, respectively. Mutagenesis and fluorescence complementation studies, based on previous crystal structures of Nef dimers, have suggested that Nef dimerization is required for Hck activation, MHC-I and CD4 downregulation as well as enhancement of virus replication. The requirement for dimerization in multiple Nef functions identifies the dimerization interface as an attractive target for small molecule inhibitors. We recently described a class of Nef inhibitors that bind directly to Nef in vitro and inhibit Nef-dependent enhancement of HIV-1 infectivity and replication as well as Hck activation and MHC-I downregulation in cell culture. These compounds, based on a diphenylpyrazole scaffold, bind to recombinant Nef with sub-micromolar affinity by surface plasmon resonance in vitro. Computational docking studies predict that the inhibitors occupy a pocket formed by the HIV-1 Nef dimer interface, suggesting that compound binding may alter the structure of the functional dimer. Recently we reported an X-ray crystal structure of Nef in complex with the Hck SH3-SH2 regulatory region, which formed a dimer of complexes. In this structure, the Hck SH2 domains impinge on the N-terminal region of Nef to stabilize a dimer conformation that exposes Nef Asp123, a residue critical for Nef functions related to both MHC-I and CD4 downregulation. This structure suggests that in addition to serving as a kinase effector for Nef, Hck may reorganize the Nef dimer for functional interaction with AP-1 and other signaling partners. This hypothesis is consistent with the previous observation that RNAi-knockdown of Hck interferes with Nef-mediated downregulation of MHC-I via AP-1 in vitro. Reorganization of the Nef dimer may be inhibited by our small-molecule antagonists and prevent interaction with cellular signaling partners such as Hck, AP-1 and AP-2. In an effort to understand the molecular interactions between our small-molecule inhibitors, Nef, and Hck, we are actively exploring the X-ray crystal structures of Nef in complex with the Hck regulatory domains and in the presence of inhibitors of the diphenylpyrazole class. To date, we have identified conditions that produce single crystals of Nef:SH3 complexes in the presence of diphenylpyrazole ligands suitable for diffraction studies. Successful determination of these structures will provide crucial insight regarding inhibitor mechanism of action as well as a basis for structure-guided design of more potent compounds with favorable pharmacological profiles for testing in vivo.

#**50 Emert-Sedlak, Lori**

Orally Active HIV-1 Nef Inhibitors Rescue Cell-Surface MHC-I Expression and CTL Responses to HIV Infected Cells

Lori A Emert-Sedlak[1], Shariq Mujib[2], H Marie Loughran[3], Haibin Shi[2], John L Kulp III[4], Sherry T Shu[1], Jay E Wrobel[3], Allen B Reitz[3], Mario Ostrowski[2], Thomas E Smithgall[1]

1. Department of Microbiology and Molecular Genetics, University of Pittsburgh, Pittsburgh, Pennsylvania; 2. Institute of Medical Science (IMS), Department of Medicine, University of Toronto, Toronto, ON, Canada; 3. Fox Chase Chemical Diversity Center, Inc., Doylestown, Pennsylvania; 4. Conifer Point Pharmaceuticals, Doylestown, Pennsylvania

HIV-1 Nef promotes internalization of class I MHC receptors from infected cell membranes, contributing to immune system evasion and viral reservoir formation. Small molecule antagonists of this Nef function may restore cell-surface MHC-I and promote viral clearance by the host immune system. Recently we described the discovery of a novel class of diphenylpyrazolodiazene (DPP) compounds that bind to Nef in vitro and inhibit Nef-dependent enhancement of HIV-1 infectivity and replication in cell culture. These first-generation DPP-based Nef antagonists have several structural liabilities, including an azo linkage that led to poor oral bioavailability. The azo linker was therefore replaced with either a one- or two-carbon bond. The resulting non-azo DPP analogs retained sub-micromolar binding affinity for Nef by surface plasmon resonance, while inhibiting HIV-1 infectivity in TZM-bl cells and viral replication in primary human macrophages with similar potency. Rescue of Nef-dependent MHC-I downregulation by these Nef antagonists was demonstrated in HIV-infected CEM-GFP reporter cells and in exogenously latently infected CD4+ T cells from HIV patients. In addition, inhibitor treatment followed by autologous CD8 T cell coculture stimulated enhanced INF-gamma release. Latently HIV infected CD4 T cells' elimination by autologous HIV-specific CD8 T cells was also significantly enhanced following Nef blockade in multiple patient samples ex vivo, measured as HIV-1 p24 in the supernatant or as the frequency of HIV-1 infected cells by flow cytometry in a viral outgrowth assay. Pharmacokinetic evaluation of these Nef inhibitors in mice showed that replacement of the azo linkage dramatically enhanced oral bioavailability without substantially affecting plasma half-life or clearance. The improved oral bioavailability of the non-azo DPP Nef antagonists provides a starting point for further drug lead optimization to enable efficacy testing in humanized mouse models of AIDS.

#**51 Mediouni, Sonia**

Interaction of Didehydro-Cortistatin A with HIV-1 Tat and Inhibition of Tat Neurotoxic Activity

Sonia Mediouni[1], Joseph Jablonski[1], Jason Paris[2], Mark Clementz[1], Guillaume Mousseau[1], Souvik Maiti[3], Jay McLaughlin[4], Susana Valente[1]

1. Department of Immunology and Microbiological Sciences, The Scripps Research Institute, Jupiter, Florida; 2. Department of Pharmacology and Toxicology, Virginia Commonwealth University, Richmond, Virginia; 3. Institute of Genomics and Integrative Biology, New Delhi, India; 4. Department of Pharmacodynamics, University of Florida, Gainesville, Florida

The viral Tat protein, detected in HIV infected individuals even under suppressive anti-retroviral therapy (ART), plays essential roles in HIV-1 pathogenesis. Tat binds the viral RNA structure, the Trans-Activation Responsive element (TAR), and recruits a series of cellular cofactors resulting in more than 100-fold increase of viral mRNA expression. In addition to this main activity, Tat also triggers much of the HIV related neuropathogenesis observed in more than 50 % of individuals on ART. The basic domain of Tat has been shown to be responsible for the interaction with TAR and to trigger neurotoxicity. So far in the clinic, there is no therapeutic agent to counteract Tat's activities. Recently, our laboratory reported that didehydro-Cortistatin A (dCA), an analog of a natural steroidal alkaloid, inhibits viral production from integrated viral genomes at low nanomolar concentrations and promotes a state of persistent latency, refractory to viral reactivationby the usual panel of latency reverting agents. Importantly, discontinuation of dCA treatment does not result in viral rebound, suggesting the chromatin environment of the HIV promoter is epigenetically repressed.

Here, using biophysical assays, we first characterized the interaction of Tat with dCA. We show by several methods that dCA binds to Tat's basic domain (immobilized Tat in ELISA and Dot Blot assays, and in solution by isothermal titration calorimetry and pull-down assays). As expected from dCA's in vivo activity, we confirmed that it blocks Tat binding to TAR RNA by electrophoretic mobility shift assays (EMSA).The crystallization of full-length Tat has historically been a very difficult endeavor, given the unstable nature of the Tat protein. Interestingly, dCA stabilizes the flexible structure of Tat observed in Tryptophan fluorescence and proteolysis protection assays, giving us a unique opportunity to attempt the crystallization of full-length Tat complexed with dCA.

We have also assessed the activity of dCA on Tat-mediated neuropathogenesis. We demonstrated that dCA crosses the blood brain barrier in mice and inhibits Tat-induced neuroinflammation in glial cell lines. Importantly, using a Tat transgenic mouse model, we demonstrated that dCA reverses Tat potentiation of cocaine and morphine reward.

In sum, dCA is as a potent inhibitor of a multitude of HIV-1 Tat activities and may represent a novel class of compounds that could add significant benefits to a clinical functional cure strategy.

#**52 Valente, Susana**

Activity of the Tat Inhibitor dCA on Primary Lymphocytes Isolated from Infected Individuals

Cari Kessing[1], Hiroshi Takata[2,3], Guillaume Mousseau[1], Remi Fromentin[4], Nicolas Chomont[4], Lydie Trautmann[2,3], Susana Valente[1]

1. Department of Immunology and Microbial Sciences, The Scripps Research Institute, Jupiter, Florida; 2. Henry M. Jackson Foundation for the Advancement of Military Medicine, Bethesda, Maryland; 3. U.S. Military HIV Research Program, Walter Reed Army Institute of Research, Silver Spring, Maryland; 4. CR-CHUM, Montréal, QC, Canada

Antiretroviral therapy (ART) effectively blocks HIV-1 replication, although it fails to eradicate the virus. Memory CD4+T cells are considered a critical reservoir for maintenance of latent HIV-1 proviral DNA due to the long life span and ability to reactivate upon encounter with antigen. We have identified didehydro-Cortistatin A (dCA) as a specific HIV-1 Tat inhibitor, that inhibits transcription at low nanomolar concentrations (Mousseau et al, cell Host and Microbe, 2012). We have shown that over time dCA drives HIV-1 gene expression into an induced state of persistent latency, refractory to viral reactivation by the usual panel of latency reverting agents (LRAs) (cytokines, HDAC inhibitors, PKC activators), in several cell line models of latency and in primary CD4+T cells isolated from infected individuals. Moreover, discontinuation of dCA treatment does not result in viral rebound in a period of 6 days, suggesting the chromatin environment of the HIV promoter is epigenetically repressed (Mousseau et al, mBio, 2015).

The small number of latently infected cells in vivo has setback studies of HIV latency and reactivation. Unfortunately, so far no single primary cellular model alone captures accurately the response characteristics of latently infected T cells from patients. Current primary models use clonal HIV laboratory strains, grow cells in various cytokine cocktails perturbing T cell subsets representation or transform the CD4+T cells to prolong lifespan. To overcome these limitations, we took advantage of a novel latency model departing from primary cells from infected individuals developed in our collaborator's laboratory, Dr. Trautmann at the Military HIV Research Program (see Hiroshi Takata's poster for model and details). Using these culture conditions, primary CD4+T cells carrying autologous HIV reservoir proliferate for 2 weeks, return to a resting state after 3 weeks of culture, and can be maintained for up to 10 weeks. We showed that these cells stop producing HIV particles after 3 weeks and preserve their in vivo representation of the HIV reservoir, and viral production can be reinduced in these cells after T-cell receptor (TCR) stimulation or with LRAs.

Using this cell model we studied whether a dCA "blocked" HIV promoter would remain "locked" even in the absence of treatment for a longer period of time (over the previously published 6 days). As such, CD4+T cells were explanted from successfully treated subjects with complete viral suppression (n=5) and expanded in cell culture with "feeder" cells, IL-2, and PHA. CD4+T cells were treated with a cocktail of ARVs or ARVs plus dCA for 28 days and HIV RNA levels from the supernatant were quantified every 7 days by real-time qPCR. Once viral suppression was achieved, drug treatments were either maintained or removed for another 21 days. Practically no viral rebound was observed in CD4+T cells previously cultured in the presence of ARVs+dCA from all five individuals, as opposed to cell treated with ARVs alone. The cell phenotype was monitored weekly by FACS to ensure central memory T cell phenotype was maintained using markers (CD38+, CD27+, HLA-DR+, CD127+). Gene expression was monitored using a Biomark Assay of 60 genes related to various signaling pathways including T cell activation, cell cycle, apoptosis, transcription factors, and TCR signaling. No significant differences in phenotype or gene expression was seen in cells treated with ARVs or ARVs+dCA.

In a parallel experiment, cells were treated for 35 days with either ARVs dCA or ARVs alone prior to treatment removal and subsequent overnight stimulation with a potent PKC activator, prostratin. Reactivation was 97% inhibited on average (n=5) in cells previously treated with ARVs + dCA compared to cells treated with ARVs alone. Collectively, our results suggest that long-term treatment of primary human CD4+T cells in vitro with dCA establishes a state of sustained latency that nearly or completely hinders the provirus from reactivation without significantly affecting phenotype or gene expression.

Based on dCA's mode of action in latently infected cells, our proposed work highlights a strategy of eradication without reactivation dubbed "block and lock". In this model, the use of specific HIV-1 Tat inhibitors, would promote a more durable state of latency, less susceptible to spontaneous reactivation during ART and when ART is discontinued ("deep" latency). By directly silencing viral transcription the size of the latent reservoir might decrease in vivo through the efficient suppression of transient viral reactivations episodes ("blips") suspected to participate in reservoir replenishment. It is now clearly emerging from studies of individuals on very early ART treatment that a smaller reservoir size translates directly into better viral control supporting the concept that supplement of the current therapy with an HIV Tat inhibitor could add significant benefits to a clinical functional cure strategy.

#**53 Kessing, Cari**

The Tat Inhibitor Didehydro-Cortistatin A Is a Potent Suppressor of SIV Replication

Sonia Mediouni[1], Suzie Houssier-Thenin[1], Mark Clementz[1], Melia Dora Kovak[1], Ian de Vera Michel[2], Guillaume Mousseau[1], Joseph Jablonski[1], Doug Kojetin[2], Susana Valente[1]

1. Department of Immunology and Microbial Sciences, The Scripps Research Institute, Jupiter, Florida; 2. Department of Molecular Therapeutics, The Scripps Research Institute, Jupiter, Florida

Persistent viral reservoirs remain a major obstacle in eradicating HIV-1 despite the many antiretrovirals (ARVs) available. We have previously demonstrated that the HIV Tat inhibitor, didehydro-Cortistatin A (dCA), in combination with ARVs, induces a state of persistent latency in vitro at low nanomolar concentrations in cell line models and in primary human HIV+ CD4+T cells. Latent virus becomes refractory to viral reactivation by a standard panel of activators, and importantly, discontinuation of dCA treatment does not result in viral rebound, suggesting the chromatin environment of the HIV promoter is epigenetically repressed (Mousseau et al, Cell Host and Microbe 2012; and mBio, 2015). We propose a strategy for HIV eradication without reactivation, dubbed “Block and Lock”, where the combination ofART with a Tat-inhibitor reduces the size of the latent reservoir by blocking ongoing viral replication, reactivation and reservoir replenishment.

Simian immunodeficiency virus (SIV) infection of rhesus macaques (RHM) is so far the best animal model that closely resembles HIV-1 infection in humans. Here, we show that dCA efficiency inhibits acute infection of several SIV strains in cell lines and in primary RHM CD4+T cells. Addition of dCA to ART has an additive inhibitory activity on SIV replication and dCA potently inhibits viral reactivation from CD4+T cells explanted from ART-naïve RHM chronically infected with SIVmac239. Similarly to what was found for HIV-1 Tat, we demonstrate that dCA binds SIVmac239 Tat through its basic domain blocking its transactivation activity. Our results suggest that dCA is similarly potent in HIV and SIV infections, encouraging the use of SIV infected RHM for the pre-clinical evaluation of dCA to reduce residual virus replication and reservoir size under ART.

#**54 Nixon, Christopher**

In Vivo Analysis of the Efficacy of the Tat Inhibitor Didehydro-Cortistatin A

Christopher C Nixon[1], Cari F Kessing[2], Susana T Valente[2], J Victor Garcia[1]

1. Division of Infectious Diseases, Center for AIDS Research, University of North Carolina at Chapel Hill School of Medicine, Chapel Hill, North Carolina; 2. Department of Immunology and Microbial Sciences, The Scripps Research Institute, Jupiter, Florida

Despite successful suppression of HIV-1 infection by current antiretroviral therapy (ART) regimens, there persists a small reservoir of latently infected cells that represents a major barrier to curing HIV-1 infection. It follows that most HIV-1 infected individuals must remain on ART for the duration of their lives. Even though ART has greatly improved the well-being and longevity of infected individuals, there is significant toxicity and financial burden associated with lifelong treatment. Therefore, new treatments must be explored.

Long have Tat inhibitors been considered attractive therapeutics for the treatment of HIV disease. Tat is a powerful transactivator of viral gene expression, which is severely diminished in its absence. As ART is unable to impact viral transcription directly, residual viral RNA production is detectable even under therapy. In trying to achieve silencing of the HIV promoter, Tat is a particularly attractive target because it has no cellular homolog and is expressed early in the viral life cycle. Didehydro-Cortistatin A (dCA) is an analogue of a natural steroidal alkaloid isolated from the marine sponge Corticium simplex that targets the HIV Tat protein. dCA inhibits Tat-mediated transactivation of the HIV integrated provirus by binding specifically to the basic region of Tat which is responsible for nucleolar localization and binding to the transactivation response element (TAR). In this way, dCA prevents Tat recruitment to the TAR stem-loop structure, hindering the recruitment of positive transcription elongation factor-b (P-TEFb) and thus transcription elongation. Additionally, dCA has a drug-like structure, is highly soluble in water, and displays good bioavailability in mice.

A number of in vitro experiments have indicated the tremendous promise of dCA as an HIV therapeutic. It has been established that dCA can inhibit HIV replication in tissue-culture adapted cells and in primary cells at nanomolar concentrations with no observed toxicity. Moreover, the abilities of dCA to almost entirely inhibit viral replication in chronically infected cells and to reduce transcriptional initiation and elongation from the viral promoter have both been demonstrated. When ART is interrupted both in vitro and in vivo, rebound viremia results. It has been documented that when dCA administration is terminated, there is no immediate viral rebound as the viral promoter is transcriptionally silenced in the absence of Tat activity. Additionally, using multiple latently infected cell lines as well HIV infected patient derived cells, dCA was shown to induce a state of latency with an extremely impaired ability to rebound. In latently infected primary CD4+ T cells isolated from HIV infected patients on suppressive ART, dCA reduced viral reactivation an average of 92.3% upon anti-CD3/CD28 stimulation.

While the in vitro data are certainly promising, the in vivo efficacy of the compound has remained untested. Here, we present data that suggest that dCA is also effective in vivo in HIV-1 infected bone marrow-liver-thymus (BLT) humanized mice. The BLT model is well characterized and established as a useful model to study HIV transmission, prevention, and persistence. In our study, mice were infected with HIVJRCSF (n=14) and suppressed two weeks later with ART (tenofovir, emtricitabine, and raltegravir) delivered daily by intraperitoneal (IP) injection and monitored weekly for viral load and peripheral T cell levels. For two weeks following suppression, mice were either treated with both ART and dCA (n=7) or ART and vehicle (n=7). Both were delivered IP and dCA was administered at a dose of 0.5mg/kg daily. Importantly, there was no noticeable toxicity associated with dCA administration, nor did dCA treatment alter T cell levels.

Upon sacrifice, blood and tissues were examined for levels of drug and viral RNA. dCA was found to be well distributed in all tissues at concentrations well above the EC50. In blood and each tissue analyzed, there is a clear pattern of reduction of viral RNA in dCA treated compared to control mice. Moreover, when blood and tissues are analyzed in aggregate, there is a significantly lower level of viral RNA detected in treated compared to control mice ($p=0.0297$, Mann-Whitney u test).

Importantly, these results confirm for the first time the activity of dCA in an in vivo model of HIV-1 infection. Future studies will include examining the impact of dCA treatment on the kinetics of viral rebound following cessation of ART. These data are certainly encouraging, seeing that previous studies highlight the fact that dCA is more effective the longer it is administered. While much attention has been paid to sterilizing cure strategies, a functional cure strategy whereby viremia is controlled without ART is equally compelling. As dCA potentially represents a functional cure for HIV infection, further studies are clearly warranted.

#**55 Giavedoni, Luis**

Efficient Proviral Inactivation with SIV-Specific RNA-Guided Cas9 Nucleases and Nickases

Lisa M Smith[1,2], Vida L Hodara[2], Laura M Parodi[2], Zhao Lai[1], Eric Carnes[3,4], Christopher Lino[4], Keith Reimann[5], Luis D Giavedoni[2]

1. University of Texas Health Science Center, San Antonio, Texas; 2. Texas Biomedical Research Institute, San Antonio, Texas; 3. University of New Mexico, Albuquerque, New Mexico; 4. Sandia National Laboratories, Albuquerque, New Mexico; 5. University of Massachusetts Medical School, Boston, Massachusetts

Background: The rhesus macaque/SIV AIDS model offers the best option for testing potentially provocative methods for virus eradication. CRISPR/Cas9 reagents offer a versatile set of tools for the manipulation of cellular genomes, including the possibility of inactivating integrated proviral DNA. However, delivery of therapeutic materials to infected CD4+ T cells found deep within the body has proved difficult. We have developed sets of pairs of RNA-guided Cas9 nucleases (Cas9Nu) specifically targeting the integrated SIV provirus. After identifying efficient pairs of guiding RNAs (gRNAs) for targeting Cas9Nu to SIV, we used the same gRNAs for the delivery of Cas9 nickases (Cas9ni) mutants, which can only introduce a break in a single DNA strand. We have also developed mesoporous silica nanoparticles encased within supported lipid bilayers (protocells) conjugated with anti-CD4 antibodies for the targeted delivery of these vectors expressing RNA-guided nucleases.

Materials and Methods: We designed RNA-guided Cas9Nu targeting highly conserved regions of the SIVmac genome, with the goal of disrupting infectious virus production via insertions and deletions (indels). In vitro assays in HEK293T cells co-transfected with a plasmid containing the SIVmac239 proviral genome and a plasmid encoding GFP and RNA-guided Cas9Nu were used to select the most effective constructs. Cells were sorted for GFP expression and their DNA was analyzed by next generation sequencing to characterize indel formation. Protocell internalization was tested with rhesus white blood cells (WBC) and PBMCs by incorporating the fluorophore Dylight 633 inside particles coated with anti-CD4 antibodies. Guiding-RNAs were also tested in CD4 T cell lines chronically infected with SIV.

Results: RNA-guided Cas9Nu targeting ribosome slip site (RSS) and LTRs showed almost total inhibition of SIV p27 production. Cas9Nu targeting LTRs showed a remarkable incidence of insertions and deletions at the Cas9 cleave site up to 134-fold higher than control. Cas9Nu were effective in chronically infected cell lines, reducing SIV p27 production and increasing CD4 expression. Protocells conjugated with an anti-CD4 IgG4 antibody entered more than 97% of rhesus CD4+ T-cells, whether in whole blood or in purified PBMCs. Cas9ni guided by the LTR gRNAs inhibited SIV is a similar fashion.

Conclusions: Results show that RNA-guided Cas9 nucleases and nickases can reduce viral replication, correlating with a high incidence of indels in the proviral DNA. This inhibition was seen on both plasmids carrying a proviral DNA as well as cells with integrated proviral DNA. Protocells conjugated with anti-CD4 IgG4 show great promise for facilitating preferential delivery of therapeutic materials into CD4+ T cells.These protocells will be loaded with vector encoded Cas9 to test whether the protocells can deliver effectively biomaterials to latently CD4+ T cells and reduce the viral reservoir in chronically SIV-infected rhesus macaques.

#**56 Hu, Wenhui**

In Vivo Excision of HIV Provirus by SaCas9 and Multiplex sgRNAs in Pre-Clinical Animal Models

Chaoran Yin[1], Ting Zhang[1], Xiying Qu[2], Yonggang Zhang[1], Raj Putatunda[1], Fang Li[1], Huaqing Zhao[4], Shen Dai[1], Xuebin Qin[1], Xianming Mo[3], Jennifer Gordon[1], Won-Bin Young[2], Kamel Khalili[1], Wenhui Hu[1]

1. Department of Neuroscience, Center for Neurovirology and The Comprehensive NeuroAIDS Center, Temple University Lewis Katz School of Medicine, Philadelphia, Pennsylvania; 2. Department of Radiology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania; 3. Laboratory of Stem Cell Biology, State Key Laboratory of Biotherapy, West China Hospital, West China Medical School, Sichuan University, Chengdu, China; 4. Department of Clinical Science, Temple University Lewis Katz School of Medicine, Philadelphia, Pennsylvania

Cas9/gRNA-mediated genome editing provides a promising cure for HIV-1/AIDS by excising the proviral DNA from host genome. Here, we demonstrate the feasibility of excising the HIV-1 proviral genome in experimental animal models using saCas9 along with 2-4 multiplex sgRNAs in a single AAV vector. Both duplex and quadruplex sgRNAs/saCas9 can be efficiently packaged into AAV-DJ or AAV-DJ/8 with high titer at 1013-14 genome copy (GC)/ml. The quadruplex sgRNA/saCas9 vector outperformed the duplex one in cleaving the integrated HIV-1 genome in cultured neural stem/progenitor cells and various organs of HIV Tg26 transgenic mice. Excision of HIV-1 proviral DNA by quadruplex AAV-DJ/8 (i.v.) was demonstrated by PCR genotyping in liver, lung, brain and spleen of the mice inoculated (i.v.) with chimeric EcoHIV carrying a firefly luciferase reporter (eLuc). Live bioluminescence imaging showed a significant reduction of EcoHIV infection in the neck lymph nodes and whole body. To demonstrate if Cas9/sgRNAs excise HIV provirus in latently infected cells in vivo, we administrated quadruplex sgRNA/saCas9 AAV-DJ/8 (2x1012 GC/mouse, i.v.) in humanized Bone marrow-Liver-Thymus (BLT) mice with intravaginal inoculation of HIV-1NL-BaL-eLuc. These HIV-infected BLT mice showed significant vaginal infection during the first 3 weeks of inoculation but no observed systemic HIV dissemination for at least 60 days prior to AAV/saCas9 treatment. Successful excision of HIV-1 provirus was detected by PCR genotyping in spleen, lung, heart, colon and brain as well as human thymic organoid on the left kidney as early as 2 weeks after AAV injection. In conclusion, in vivo excision of HIV proviral DNA in solid tissues/organs can be achieved via a single intravenous injection of AAV-DJ/8 carrying quadruplex sgRNA/saCas9. This is an important step moving forward to the clinical application of the CRISPR/Cas9 gene editing strategy.

#**57 Akala, Emmanuel**

Antiretroviral Drugs-Loaded Nanoparticles Fabricated by Dispersion Polymerization for Galt Targetable Drug Delivery System

Emmanuel Akala[1], Sergei Nekhai[1], Winston Anderson[1]

1. Howard University, Washington, DC

Highly active antiretroviral (ARV) therapy (HAART) for chronic suppression of HIV replication has revolutionized the treatment of HIV/AIDS. HAART is no panacea; treatments must be maintained for life. Although great progress has been made in ARV therapy, HIV continues to replicate in anatomical and intracellular reservoirs where ARV drugs have restricted access. Nanotechnology has been considered a platform to circumvent some of the challenges in HIV/AIDS treatment. Dispersion polymerization was used to fabricate two types (PMM and ECA) of polymeric nanoparticles. Each type was successfully loaded with four ARV drugs (zidovudine, lamivudine, nevirapine, and raltegravir) simultaneously, followed by physicochemical characterization: scanning electron microscope, particle size distribution, zeta potential, drug loading, and in vitro availability. These nanoparticles efficiently inhibited HIV-1 infection in CEM T cells and peripheral blood mononuclear cells. They hold promise for the treatment of HIV/AIDS. The ARV-loaded nanoparticles with polyethylene glycol on the corona may facilitate tethering ligands for targeting specific receptors expressed on the cells of HIV reservoirs for HIV eradication.

#**58 Pernet, Olivier**

A Novel Chemoselection Strategy for Combinatorial Genetically Modified HIV Protected Hematopoietic Stem/Progenitor Cells

O Pernet[1,2], S S Yadav[1,2], W Khamaikawin[1], S Shimizu[1,2], M Ehsaninia[1,2], J Lam[1,2], M Narukawa[1,2], K Hacke[3], N Kasahara[3], DS An[1,2,4]

1. School of Nursing, University of California, Los Angeles, Los Angeles, California; 2. UCLA AIDS Institute, Los Angeles, California; 3. Department of Cell Biology, Miller School of Medicine, University of Miami, Miami, Florida; 4. Hematology-Oncology, David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, California

After the successful cure of the Berlin Patient, hematopoietic stem/progenitor cells (HSPC) based gene therapy holds great hopes for HIV cure. However, the low efficiency of hematopoietic reconstitution with modified HSPC limits the success of related-clinical trials. We present here a novel 6-TG-chemoselection approach for anti-HIV HSPC gene therapy to overcome this limitation.

Our novel selection strategy exclusively employs 6-thioguanine (6-TG) for in vivo chemoselection of hypoxanthine-guanine phosphoribosyltransferase (HPRT) down-regulated HSPC, using a short hairpin RNA (shRNA) to enable 6-TG-mediated positive selection. In addition, a novel feature of our HPRT knockdown is a negative selection strategy to eliminate unexpected adverse effects of the gene-modified cells. This safety mechanism uses a clinically available drug, methotrexate (MTX), which inhibits the enzyme dihydrofolate reductase (DHFR) in the purine de novo synthetic pathway.

To stably knockdown HPRT and CCR5, shRNAs were delivered through a lentiviral vector in human T-cell lines, primary PBMC, as well as in primary CD34+ HSPC in the presence of 6-TG to select vector-transduced cells. Furthermore, we then added a second anti-HIV gene, the fusion inhibitor C46, and tested whether the newly engineered cells were able to block HIV-1 of both R5 and X4 tropism.

Lentivirus vector delivery of HPRT-shRNA and CCR5-shRNA results in efficient CCR5 knockdown and confers the ability to select vector transduced cells by 6-TG in human T-cell lines and primary cells. We also confirmed that selected cells inhibited R5 tropic HIV-1 infection. Importantly, cells transduced with the two shRNAs together with C46 inhibited infection by HIV in both tropisms. Transduced cells are also negatively selectable by MTX. In vivo 6-TG selection resulted in higher reconstitution of CCR5-shRNA and HPRT-shRNA co-expressing vector-modified human hematopoietic cells than the control no shRNA vector modified cells in humanized BLT mice.

Our results demonstrate that our newly developed HPRT-shRNA can be combined with anti-HIV genes in a lentiviral vector for positive selection in vivo. Our current results provide a small RNA based novel chemoselection strategy that can be used for improving the engraftment of genetically protected cells for HSPC based anti-HIV gene therapy strategies.

#**59 Stoddart, Cheryl**

Inhibition of Heat Shock Protein 90 Prevents HIV Rebound

Pheroze Joshi[1], Ekaterina Maidji[1], Cheryl A Stoddart[1]

1. Division of Experimental Medicine, Department of Medicine, Zuckerberg San Francisco General, University of California, San Francisco, San Francisco, California

Background: HIV evades eradication in the body because transcriptionally dormant integrated proviral genomes persist in long-lived reservoirs. Nevertheless, transcriptionally silent HIV genomes can readily produce infectious virus upon cellular activation because full-length HIV transcription is mediated by host transcription factors that are specifically induced by cell-activating agents and heat shock. The molecular chaperone heat shock protein 90 (Hsp90) activates inducible cellular transcription factors that are essential for HIV transcription. We identified Hsp90 in a functional genetic screen for host factors that could completely rescue the highly impaired replication of protease-mutant and capsid-defective HIV in unstimulated T cells(1,2). We have since reported that heat shock conditions enhance HIV replication up to 30-fold in primary human cells(3), and concurrent work by others in a transformed cell line model of HIV latency has shown that heat shock controls virus replication and that Hsp90 inhibition suppresses reporter gene expression in these cells(4,5). Here, we provide evidence that Hsp90 activates a specific subset of host transcription factors that specifically accelerate HIV transcription and more importantly, show that vivo inhibition of Hsp90 prevents HIV rebound from a tissue reservoir of replication-competent HIV(6).

Results: We systematically investigated the effect of heat shock and Hsp90 activity on HIV transcription using a chronically infected cell line and found that heat shock accelerated HIV transcription from the viral promoter, and that tanespimycin (17-AAG), a Hsp90 inhibitor in clinical trials, greatly reduced HIV gene expression. Importantly, we show that during heat shock, only Hsp90-activated transcription factors (NFkB, NFAT, and STAT5) selectively accelerated HIV transcription and that treatment with 17-AAG prevented gene expression by these transcription factors. Subsequently, we used HIV-viremic humanized mice to demonstrate that pharmacologic inhibition of Hsp90 prevents HIV rebound in vivo. We show that Hsp90 controls virus transcription in vivo by administering Hsp90 inhibitors to HIV-infected humanized NSG-BLT mice with fully suppressed plasma viremia. To achieve complete viral suppression in peripheral blood, we treated groups of HIVJR-CSF-infected NSG-BLT mice with an ultrapotent (50 pM IC50) nucleoside analog reverse transcriptase inhibitor 4'-ethynyl-2-fluoro-2'-deoxyadenosine (EFdA). In our first study in 28 humanized mice, we employed two different regimens: 1) suppression of viremia with EFdA for 2 weeks before 17-AAG administration for 2 weeks or 2) suppression with EFdA for 4 weeks with concurrent administration of 17-AAG for the last 2 weeks. We observed that: a. Seven weeks after treatment cessation, 6 of 18 17-AAG-treated mice had no detectable plasma HIV RNA(<75 copies per 100 µl). b. The lack of rebound in plasma HIV RNA was confirmed by a highly sensitive infectious unit per million cells (IUPM) virus outgrowth assay that quantifies the number of cells with replication-competent HIV in the peripheral blood. c. The long-term pharmacodynamic effects of Hsp90 inhibitors have been extensively studied in preclinical oncology trials, and we show that 17-AAG pretreatment inhibited HIV replication in vitro for at least 6 days (the longest interval studied) after the drug was removed from the cells.

In a second study in 29 NSG-BLT mice, we compared 17-AAG to AUY922, a second-generation Hsp90 inhibitor with more potent (>3.5-fold) anti-HIV activity than 17-AAG in vitro. We observed that: a. Both Hsp90 inhibitors prevented sustained rebounds in plasma HIV RNA for up to 11 weeks after treatment cessation. More importantly, 17-AAG and AUY922 exerted this effect while preserving peripheral blood human CD4+ T-cell counts in the mice, so the lack of viral rebound was not merely the result of the loss of these cells from the peripheral blood. b. The lack of plasma HIV RNA rebound was confirmed by the IUPM assay and interestingly, we were able to recover infectious HIV from PBMC with heat shock. c. Low IUPM

levels (200–1,000) were detected in spleen cells from the nonviremic, Hsp90 inhibitor-treated mice, indicating the presence of a tissue reservoir of persistent infection that was durably suppressed by Hsp90 inhibition. d. RNAscope in situ hybridization confirmed the presence of a replication-competent reservoir in the spleen, and AUY922 treatment of the mice appeared to reduce this persistent reservoir to a greater extent than 17-AAG in the side-by-side comparison. We did not observe any adverse effects in the mice; mean human CD4+ T cell counts at the time of treatment cessation were similar between the untreated, 17-AAG-treated and AUY922-treated groups, and no signs of toxicity or weight loss were seen in the mice after 2 weeks of treatment with either 17-AAG or AUY922 at the dosage level (10 mg/kg/day) we used. Finally, we used the 8E5/LAV and ACH-2 chronically infected cell lines to show that heat shock increased Hsp90 activity which in turn, accelerated HIV transcription. The human 8E5/LAV cell line is reminiscent of a latently HIV-infected cell that requires cellular activation to resume virus production. We show that heat shock increased 8E5/LAV virus production greater than cellular activation and higher virus load was a direct result of Hsp90-mediated accelerated HIV transcription and not the pleiotropic effects of heat shock. We confirmed the potent effect of heat shock on HIV transcription in the ACH-2 cell line that similarly requires cell activation to produce infectious virus. Heat shock specifically accelerated HIV transcription resulting in increased the viral load, and we confirmed the ACH-2 virus was infectious and replication competent. These findings provide evidence that Hsp90 activity is required for HIV transcription and confirm our in vivo observations that Hsp90 inhibition prevents HIV rebound from persistently infected tissue reservoirs.

Conclusions: Our novel findings provide in vivo evidence for the key role of Hsp90 in HIV persistence in human cells, in vitro and in vivo, and show that inhibition of Hsp90 prevents HIV gene expression in replication-competent cellular reservoirs that would typically cause rebound in plasma viremia after ARV cessation. Alternating or supplementing Hsp90 inhibitors with current ARV regimens could potentially suppress the rebound of viremia from persistent HIV reservoirs.

1. Stoddart, C. A., Liegler, T. J., Mammano, F., Linquist-Stepps, V. D., Hayden, M. S., Deeks, S. G., Grant, R. M., Clavel, F., and McCune, J. M. (2001) Impaired replication of protease inhibitor-resistant HIV-1 in human thymus.Nat Med7, 712-718.
2. Joshi, P., and Stoddart, C. A. (2011) Impaired infectivity of ritonavir-resistant HIV is rescued by heat shock protein 90AB1. J Biol Chem 286, 24581-24592.
3. Joshi, P., Sloan, B., Torbett, B. E., and Stoddart, C. A. (2013) Heat shock protein 90AB1 and hyperthermia rescue infectivity of HIV with defective cores. Virology 436, 162-172.
4. Roesch, F., Meziane, O., Kula, A., Nisole, S., Porrot, F., Anderson, I., Mammano, F., Fassati, A., Marcello, A., Benkirane, M., and Schwartz, O. (2012) Hyperthermia stimulates HIV-1 replication. PLoS Pathog 8, e1002792.
5. Anderson, I., Low, J. S., Weston, S., Weinberger, M., Zhyvoloup, A., Labokha, A. A., Corazza, G., Kitson, R. A., Moody, C. J., Marcello, A., and Fassati, A. (2014) Heat shock protein 90 controls HIV-1 reactivation from latency. Proc Natl Acad Sci U S A 111, E1528-1537.
6. Joshi, P., Maidji, E., and Stoddart, C. A. (2016) Inhibition of Heat Shock Protein 90 Prevents HIV Rebound. J Biol Chem 291, 10332-10346.

#60 Dunham, Richard

Pharmacologic Inhibition of IDO Blunts Features of SIV-Related Chronic Inflammation

Richard M Dunham[1], Kevin W Brown[1], Shari Gordon[1], James M Billingsley[2], Zhong-Min Ma[3], Gregory K Tharp[2], Chris Miller[3], Steven Bosinger[2], Brian Johns[1], David Favre[1]

1. GlaxoSmithKline, Research and Development, HIV Discovery Performance Unit, Research Triangle Park, North Carolina; 2. Yerkes National Primate Research Center, Nonhuman Primate Genomics Core Laboratory, Atlanta, Georgia; 3. University of California, Davis, Center for Comparative Medicine, Davis, California

Background: The immunomodulatory and neurotoxic activities of kynurenines derived from tryptophan catabolism by indoleamine-2,3-dioxygenase (IDO1) play a role in sustaining a cycle of inflammation and immune dysfunction during HIV infection. This inflammatory environment is likely a major barrier to HIV cure, as inflammation underlies several mechanisms of persistence. A major barrier to HIV cure is the dysfunctional antiviral immune response associated with chronic inflammation that is incapable of clearing cells reactivated with latency reversal strategies. We hypothesized that inhibition of IDO1 may break this cycle, reversing immune dysfunction and decreasing systemic inflammation. We tested this hypothesis in a pilot study of virologically suppressed, SIV-infected rhesus macaques treated with an optimized regimen of an IDO1 inhibitor (IDOi) currently under clinical investigation (INCB024360).

Methods: Twelve rhesus macaques were infected with SIVmac251, treated with cART, and divided into two groups after virologic suppression to receive either placebo or IDOi for 8 weeks while continuing ART. Blood, lymphatic tissue, and mucosal tissues were evaluated to determine the pharmacologic, immunologic, metabolic, microbiological, and transcriptomic features of IDOi during SIV infection.

Results: SIV infection increased plasma levels of kynurenine even after ART and kynurenine was transiently suppressed after each IDOi dose. Pharmacologic inhibition of IDO1 led to increased CD4/CD8 ratio in blood and significantly decreased markers of T cell and monocyte activation in blood and lymphoid tissues. In gut mucosa, we observed a trend to increased frequency of CD4+ T cells producing IL-17, IL-22, and/or IL-21. We studied the magnitude and quality of the SIV-specific T cell response using multiparametric flow cytometry following SIV peptide stimulation. We found that while there was not an overt increase in the CD8+ SIV-specific T cell response after IDOi treatment.

Conclusions: These results support the hypothesis that IDOi disrupts the vicious cycle of IDO1-mediated inflammation and immune dysfunction during SIV infection. Resolution of this inflammatory environment will impact impacting multiple mechanisms maintaining HIV persistence and is an important consideration for HIV cure. These data suggest further investigation of IDOi as a strategy to blunt or prevent inflammation in suppressed HIV infection to decrease residual inflammatory disease and enable HIV cure.

#**61 Hataye, Jason**

HIV Exponential Growth Dependent on Burst Size Breakthrough of the Allee Threshold

Jason M Hataye[1], Joseph P Casazza[1], David R Ambrozak[1], Takuya Yamamoto[2], Daniel C Douek[1], Alan S Perelson[3], Richard A Koup[1]

1. Vaccine Research Center, Bethesda, Maryland; 2. Laboratory of Adjuvant Innovation, National Institutes of Biomedical Innovation, Health, and Nutrition, Osaka, Japan; 3. Theoretical Biology, Los Alamos National Laboratory, Los Alamos, New Mexico

Exponential growth is the mode by which a population establishes itself following arrival in a new environment. For HIV, establishment is inevitable by the time exponential growth is detected, during both acute infection following transmission, and viral rebound from the latent reservoir following interruption of suppressive antiretroviral therapy (ART). Although fundamental, the processes leading up to overt exponential growth versus extinction have eluded definition because they are simultaneously low amplitude, rare, and fleeting.

To investigate viral release and growth, resting CD4+ T cells from 8 donors on ART were stimulated with antibodies against CD3, CD2, and CD28. The cells were cultured in limiting dilution with ART to quantify initial HIV release in the absence of new infections, or with IL-2 plus exogenous cells for outgrowth. To distinguish replication-competency from establishment, supernatant positive for HIV RNA by RT-PCR on day 8 of the primary outgrowth culture was transferred to new stimulated CD4+ cells from HIV-uninfected donors. Fitting the experimental results to deterministic and stochastic models, we tested key assumptions of HIV release and early growth, and present an adapted model that not only fit existing data but was predictive of more complex experiments.

Despite extensive cell division, most CD4+ T cells initially present in culture died before virus could be released, which on average began 4 days following activation. The duration of HIV release due to one latent cell ranged from less than one day up to 7 consecutive days. Such sustained detections resulted from sequentially occurring progeny releasing on average 1000 HIV RNA copies per cell for a total 3900 HIV RNA copies. Many primary culture releases consisted of virus which was confirmed intact by secondary culture, but did not result in exponential growth. Released HIV in a well was most likely to undergo exponential growth if it exceeded a critical threshold of 6000 HIV RNA copies. Establishment dependence on a low threshold is called the Allee effect and has been previously reported across diverse biological taxa including animals, plants, and non-pathogenic microorganisms.

This work demonstrates why integrated and intact provirus may not result in ex-vivo outgrowth, advances the theoretical foundation for rebound prediction following ART interruption, and identifies the Allee growth threshold as a population dynamically defined target for an HIV functional cure.

#**62 Cardozo-Ojeda, Erwing**

Modeling HIV-1 Kinetics After the Infusion of VRC01 in Chronically Infected Individuals

Erwing Fabian Cardozo-Ojeda[1], Robert Bailer[2], Emily Coates[2], Rebecca Lynch[2], John Mascola[2], Julie Ledgerwood[2], Richard Koup[2], Alan S Perelson[1]

1. Theoretical Biology and Biophysics, Los Alamos National Laboratory, Los Alamos, New Mexico; 2. Vaccine Research Center, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland

VRC01 is a broadly neutralizing antibody that targets the CD4 binding site of HIV-1 gp120. Passive administration of VRC01 in humans has assessed the safety and viremic effect of this monoclonal antibody in a phase 1 clinical trial. In this study, the plasma HIV RNA presented either a delay of a few days or fast decline after a single infusion of VRC01. After that, the viral load then decays while the plasma antibody concentration is sufficiently high. As the antibody level decreases, the virus returns slowly to baseline. To explain these kinetics, we fit the VRC01 PK data in each treated individual to a two-compartmental PK model, and then incorporated the predicted Ab profile into a viral kinetic model. The viral kinetic model included reversible VRC01 binding to HIV-1 to form neutralized immune complexes that were then phagocytosed in a concentration-dependent manner. Our model predicts that VRC01 may enhance the clearance of HIV-complexes up to three-fold by the formation of Ab-virus complexes, explaining the initial viral decay observed immediately after antibody infusion in some patients. By assuming a homogeneous viral population sensitive to VRC01, the model provided good fits to all of the patient data. In some cases, however, the fits are improved by assuming that there were two populations of virus, one more susceptible to antibody-mediated neutralization than the other. In these cases, the model estimates that the susceptible virus comprises about 90% of the initial virus population.

#**63 Cao, Youfang**

Control of SIV with Short-Term Anti-PD-L1 Treatment

Youfang Cao[1], Jessica M Conway[2], Stephen Mason[3], James B Whitney[4], Alan S Perelson[1]

1. Theoretical Biology and Biophysics (T-6), Los Alamos National Laboratory, Los Alamos, New Mexico; 2. Department of Mathematics, Pennsylvania State University, University Park, Pennsylvania; 3. Discovery-Virology, Bristol-Myers Squibb, Wallingford, Connecticut; 4. Center for Virology and Vaccine Research, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston Massachusetts

In chronic HIV infection, effector cells, such as CD8+ T cells, are driven into an exhausted state, in which their effector functions are impaired and viral load (VL) control is lost. Monoclonal antibodies against the PD-1/PD-L1 inhibitory checkpoint pathway can reverse the exhausted state. Based on our previous post-treatment control (PTC) model, we developed a viral dynamic model explicitly incorporating immune cell exhaustion and antibody-induced reactivation to study the effects and mechanisms of anti-PD-L1 immunotherapy on VL control. We fit our model to longitudinal VL data obtained from 12 ART-suppressed simian immunodeficiency virus (SIV)-infected rhesus macaques (RM) treated with short term anti-PD-L1 therapy during ART. We estimated 9 biologically important parameters for each RM by maximizing the log-likelihood between simulated viral dynamics trajectories and the VL data. Stability analyses using the estimated parameters suggest that 3 of 8 RMs in the anti-PD-L1 treatment group have two stable VL set points, i.e. two stable steady states with one at high VL and one at low VL. Our modeling shows that after short term anti-PD-L1 treatment a subset of animals is able to control the infection and establish a low VL steady state. Our model predicts that without anti-PD-L1 treatment, the VL will rebound back to baseline, i.e. the high VL set point. Our findings are consistent with the hypothesis that some HIV infected patients, namely post-treatment controllers (PTC), might have two different VL set points as suggested by the VISCONTI study. We also studied the optimal treatment schedule for anti-PD-L1 treatment during ART. We found that PTC can be achieved more effectively if anti-PD-L1 is infused immediately after cessation of ART. Overall, our modeling suggests that PTC might be induced by boosting the immune responses using short-term immunotherapy in some patients.

#**64 Zurakowski, Ryan**

Optimal Design of HIV Experiments with Design Constraints

LaMont Cannon[1], Aditya Jagarapu[1], Ryan Zurakowski[1]

1. Department of Biomedical Engineering, University of Delaware, Newark, Delaware

We present a novel approach to experiment design for testing dynamic hypotheses, subject to experimental constraints. Classical experiment design methods are poorly suited for testing hypotheses on systems where measurement time and frequency strongly effect the information contained in the measurements. The few methods that do exist, such as sensitivity analysis, are local methods whose accuracy is dependent on a particular guess for the values of the unknowns that the experiment is designed to interrogate. We present a novel approach to designing experiments with design constraints that maximize the probability of achieving a particular experimental goal, subject to constraints on possible experiment designs. This method returns a design that is robust to the level of uncertainty in the model prior to performing the experiment.

The method described can be applied to hypotheses described by differential equations with uncertain parameters, with well-characterized measurement error. First, a mathematical model of the hypothesis is developed, including measurement error. Next, the prior distributions of the model parameters whose posterior distributions will be used to test the hypothesis are described. A virtual patient library is developed with statistics representative of the prior distribution's variation. A constrained set of possible experimental protocols is described. A cost function is developed to measure how well a given experimental design meets the objectives for a given parameter set drawn from the prior distribution. Monte-Carlo simulation is used to generate an estimate of the cost of candidate protocols, averaged across the virtual patient library. Genetic Algorithms are applied to find protocols that maximize the experiment's probability of achieving the objective encoded in the cost function.

We have applied this method to two HIV-related experiments with significant design constraints. The first is a prospective design of a clinical trial to test the hypothesis that ongoing HIV replication in sanctuary sites can be revealed through the dynamics of Peripheral Blood Mononuclear Cells containing circularized 2-LTR HIV DNA. For this experiment, the objective is to obtain the most information possible about the unknown model parameters, so the cost function used is the expected Kullback-Leibler divergence, which measures the information content of an experiment based on the difference between a prior and posterior distribution. The prior distributions are based on fits to patient data from prior experiments described in (Buzon et al. 2010) and (Hatano et al. 2013). Experiment design is constrained to 10 or fewer ddPCR measurements over a 20-week period.

The second experiment considered is an in vitro experiment design for measuring the rates of molecular events in early HIV infection. In this case, the main objective is to discriminate between competing models, so the cost function used is the expected difference in Akaike's Information Criterion. The prior distributions are based on a fit to data from an experiment described in (Pace et al. 2013). This experiment is constrained by a total sample size in terms of cultured T-Cells.

The method consistently returns experiment designs that yield significantly more information compared to ad hoc designs using the same number of measurements.

This dramatically increases the likelihood that the experiment will yield enough information to test the hypothesis without increasing the cost of the experiment. This method may be adapted to many similar experimental design challenges.

#**65 Rosenbloom, Daniel**

Assessing Intra-Lab Precision and Inter-Lab Repeatability of Outgrowth Assays of HIV-1 Latent Reservoir Size

Daniel Rosenbloom[1], Peter Bacchetti[2], Mars Stone[3], Melanie Dimapasoc[3], Xutao Deng[3], Ron Bosch[4], Doug Richman[5], Janet Siliciano[6], John Mellors[7], Steve Deeks[8], Rebecca Ho[8], Marion Lanteri[3], Sheila Keating[3], Marta Massanella[9], Jun Lai[6], Michele Sobolewski[7], Michael Busch[3], for the Reservoir Assay Validation and Evaluation Network (RAVEN) Study Group

1. Department of Biomedical Informatics, Columbia University College of Physicians and Surgeons, New York, New York; 2. Department of Epidemiology and Biostatistics, University of California San Francisco, San Francisco, California; 3. Blood Systems Research Institute, San Francisco, California; 4. Department of Biostatistics, Harvard T.H. Chan School of Public Health, Boston, Massachusetts; 5. Department of Pathology, University of California San Diego, La Jolla, California; 6. Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland; 7. Department of Medicine, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania; 8. Department of Medicine, University of California San Francisco School of Medicine, San Francisco, California; 9. Department of Microbiology, Infectious Diseases, and Immunology, University of Montréal, Faculty of Medicine, Montréal, QC, Canada

Introduction: Quantitative viral outgrowth assays (QVOA) use limiting dilutions starting from millions of resting CD4+ T cells to measure the size of the latent reservoir (LR) of replication-competent HIV-1. Although QVOA is regarded as a "gold standard" for LR measurement, little is known about the accuracy and precision of this measurement or about how cell storage conditions or laboratory-specific practices affect results. Owing to this lack of knowledge, confidence intervals around LR size estimates – as well as judgments of the ability of therapeutic interventions to alter the size of the LR – rely on theoretical statistical assumptions about dilution assays. In particular, it is unknown whether the predominant assumption that QVOA follows Poisson-binomial variation holds in practice. To address this gap, we have carried out a Bayesian statistical analysis of QVOA reliability across three separate labs, estimating assay precision and the effect of frozen cell storage on estimated LR size.

Methods: The RAVEN study performed QVOA on 20 samples derived from a single leukapheresis collection from 5 longstanding ART-suppressed HIV^+ participants, with one aliquot of fresh cells and 3 aliquots of cryopreserved cells tested at each lab under code. The frozen panels, which also included 3 replicates of an HIV-negative control, were blinded as to participant and aliquot identity, and samples were assayed in balanced batches of 2. We used Markov chain Monte Carlo methods in the Stan programming language to estimate the size of random effects at the aliquot, batch, and lab levels; fixed effects of different labs and of frozen cell storage prior to the assay; and LR size in infectious units per million (IUPM) for each of the 5 HIV^+ participants. The random effects in this model represent experimental variation in IUPM measurement beyond the unavoidable Poisson-binomial variation in an idealized dilution assay. To validate this approach, we performed estimates on 10,000 simulated data sets with different levels of aliquot and batch variation. We report the magnitude of extra-Poisson variation as the standard deviation of the random effects.

To quantify the evidence for aliquot-, batch-, and lab-level extra-Poisson variation, we fitted eight models, one for each possible combination of these 3 effects. We computed Bayes factors from wAIC (Watanabe-Akaike information criterion) to assign a probability to each model. The probability that an effect is present is deemed to be the sum of probabilities for all models that include the effect. Effect sizes were estimated from a probability-weighted ensemble of the eight models.

Results: Model-based estimates converged rapidly, and overstated actual levels of variation in simulated datasets by only 3 – 8% (aliquot variation) and 10 – 14% (batch variation). Based on analysis of the three-lab dataset, the probability that QVOA follows idealized Poisson-binomial variation is < 10^{-18}. The probability that extra-Poisson variation exists at the aliquot, batch, and lab levels is 95%, 79%, and 83%, respectively.

Extra-Poisson variation between aliquots of the same participant sample, assayed together in the same batch had standard deviation 1.5-fold up or down (95% credible interval, CI, 1.0 – 2.2). Assaying the sample in two separate batches in the same lab added a further 1.7-fold variation (95% CI 1.0 – 2.4). Performance of the assay by two separate labs added an additional 1.4-fold variation (95% CI 1.0 – 2.2). Accounting for all these sources of variation, we found that credible intervals of log(IUPM) for each participant were 2.4- to 2.8-fold wider than would have been estimated had idealized Poisson-binomial variation been assumed. We estimated that frozen storage did not substantially alter IUPM (median effect size = 1.1-fold change, 95% CI 0.6 – 2.1).

Conclusions: Considerable experimental effort, involving replicate assays of samples taken from the same study participants, performed in multiple batches and labs, is required to study precision and repeatability of QVOA. Although QVOA has more variation than typically assumed, we found that running samples in the same batch can be expected to limit the extra variability to about 1.5-fold up or down, which would make the assay remarkably consistent in the context of the very large reductions in LR sought by cure strategies. Additional variation of similar magnitude should be expected from assaying samples in different batches or labs. We found strong evidence that frozen storage of cells alters IUPM at most twofold (up or down), which again is a minor effect in context. This finding supports the use of frozen shipping and storage in studies of the LR, which, among other benefits, allows batched QVOA of longitudinal samples to minimize variation. Nevertheless, incorrectly assuming only Poisson-binomial variation could exaggerate the evidence for effects of interventions designed to alter the size of the LR, particularly if samples must be assayed in different batches and/or at different labs. Our findings point the way toward more precise assessments of HIV cure strategies.

#**66 Flint, Ashley**

Human Tissue and Organ Research Resource (HTORR): Enabling Biomedical Research and Development

Ashley Flint[1], Honesto Nunez III[1], Saboor Shad[1], Jessica Lyons[1], Cristina Kelly[1], Randall Sassaman[1], Melissa VonDran[1], Gene Kopen[1]

1. National Disease Research Interchange, Philadelphia, Pennsylvania

The National Disease Research Interchange (NDRI) is a non-profit organization that serves as the link between individuals wishing to donate organs and tissues for research and the nation's leading investigators who are working to find new treatments or cures for a wide range of diseases, including HIV/AIDS. NDRI receives funding and oversight from the National Institutes of Health, including NIAID, as a Human Tissue and Organ Research Resource (HTORR) to make biospecimens available to researchers in academia and non-profit organizations. NDRI's Donor Programs give individuals and their families an opportunity to leave a meaningful legacy for their loved ones, by providing a simple mechanism through which tissues and organs can be donated for research after death. NDRI works with patients and next-of-kin to obtain consent for donation and takes care of recovering tissues and providing them to approved researchers. To identify donors and coordinate these recoveries, NDRI partners with a nationwide network of Organ Procurement Organizations (OPOs), Tissue Banks, Hospitals, Medical Examiners and funeral homes; these industry professionals help champion the last wishes of donors and ultimately serve NDRI's registered research projects with biospecimens for their studies. NDRI's Private Donor Program is an excellent resource to support HIV/AIDS research. Our private donor registry allows our recovery sites to collect various tissues from each donor with recoveries completed < 24 hours post mortem. Our high-quality biospecimens are commonly utilized to establish primary cell cultures and for rigorous molecular methodologies, such as RNAseq. NDRI works with researchers to determine donor eligibility parameters for both HIV positive and control donors. Tissues collected for our HIV researchers include brain, CSF, liver, blood, lymph nodes, kidney, adipose, lung, spleen, and bone marrow. NDRI specializes in establishing researcher specific protocols for donor eligibility and organ and tissue collection procedures for optimal biospecimens sampling and preservation. These well annotated samples are needed to continue the vital work to discover new therapies for HIV/AIDS.

#**67 Lewin, Sharon R**

Alemtuzumab Treatment for Sezary Syndrome: Effect on Latently Infected CD4+ T Cells

Thomas A Rasmussen[1,2], James McMahon[1,3], Judy Chang[1], Fiona Wightman[1], Jori Symons[1], Ajantha Solomon[1], Bonnie Hierner[4], Sarah Palmer[4], Carrie Van Der Weyden[5], Paul Cameron[1,3], Miles Prince[5], Sharon Lewin[1,3]

1. The Peter Doherty Institute for Infection and Immunity, University of Melbourne and Royal Melbourne Hospital, Melbourne, Australia; 2. Department of Infectious Diseases, Aarhus University Hospital, Aarhus, Denmark; 3. Department of Infectious Diseases, Alfred Hospital and Monash University, Melbourne, Australia; 4. The Westmead Institute, University of Sydney, Westmead, NSW, Australia; 5. Peter MacCallan Cancer Centre, Melbourne, Australia

Background: Alemtuzumab, an anti-CD52 monoclonal antibody used for the treatment of cutaneous T cell lymphoma, causes extensive depletion of T cells. Here, we describe the effects of alemtuzumab on the frequency of latently infected CD4+ T cells in an HIV-infected individual on antiretroviral therapy (ART) with Sezary syndrome, a malignancy of CD4+ T-cells.

Methods: We analyzed PBMCs collected 30 and 18 months prior to presentation with Sezary syndrome, at the time of presentation and during alemtuzumab. T cell subsets were analyzed using flow cytometry. We quantified HIV DNA by PCR in total CD4+ T cells, in sorted malignant and non-malignant CD4+ T cells, and performed full-length sequencing of HIV DNA. We infected mitogen stimulated malignant and non-malignant cells in vitro with NL4,3 eGFP virus and quantified eGFP expression by flow cytometry.

Results: HIV-HBV co-infection was diagnosed and ART initiated 4 years prior to presentation with Sezary syndrome with a disseminated erythematous rash. The patient subsequently developed multifocal nodules and biopsy of both was consistent with Sezary syndrome and concomitant anaplastic large cell lymphoma. The patient received alemtuzumab 10 mg s.c. three times per week for 4 weeks but died 6 weeks post-alemtuzumab of multi-organ failure in the setting of progressive lymphadenopathy. The malignant CD4+ T cells were characterized by lack of CD7 and CD26 expression and by clonal expression of TCR-VBeta2. Malignant cells (CD7-CD26-TCR-VBeta2+) expressed CXCR4 and CCR5 but contained no HIV DNA, consistent with expansion of a non-infected CD4+ T cell clone. In vitro, the malignant cells were not permissive to HIV infection. Alemtuzumab led to a drastic depletion of T-cells including a >1 log reduction in total CD3+ T-cells and >3 log reduction in CD4+ T cells. Among memory CD4+ T cells, central memory cells were most consistently depleted. Four weeks of alemtuzumab led to a decrease in HIV DNA from 289 to 25 copies per 106 CD4+ T cells after 18 days on alemtuzumab and 127 copies per 10^6 CD4+ T cells after 25 days on alemtuzumab (a final 57% reduction). Levels of HIV DNA were 2.7 to 5.0-fold higher in CD4+ T-cells collected prior to developing malignancy compared to non-malignant cells obtained pre-alemtuzumab. Full-length HIV DNA sequencing demonstrated no clonal expansion and most HIV sequences contained lethal mutations.

Conclusions: Expansion of malignant CD4+ T cells was not associated with clonal expansion of the HIV reservoir and these cells were resistant to HIV infection. Despite massive depletion of CD4+ T-cells following alemtuzumab, only a modest decrease in HIV DNA was observed, although the majority of this virus was defective.

#**68 Sommerfelt, Maja**

Approaching HIV Functional Cure Applying Vacc-4x, a Peptide-based Therapeutic HIV Vaccine: Clinical Development Methodological Considerations

Maja Sommerfelt[1], Alejandra Mork[1], Mats Okvist[1], Unni Hjelmaas[1], Shahin Gharakhanian[2]

1. Bionor Pharma ASA, Oslo, Norway; 2. Pharmaceutical Medicine and Infectious Diseases, CIC, Cambridge, Massachusetts

Background: The clinical development of novel therapeutic agents for HIV functional cure poses unique methodological and operational challenges. Functional cure has been defined as the "host-mediated control of HIV replication in the absence of ART" during which viral replication should be suppressed for a predefined period of time, and effective immune function should be stabilized. It is a prerequisite that HIV-induced chronic inflammation will be at very low levels and that levels of HIV RNA not allow for transmission of infection. Clinical proof-of-principle includes data from unique subsets of individuals who effectively control HIV infection. Bionor Pharma ASA is developing a therapeutic vaccine comprising four modified HIV peptides (Vacc-4x). Our group's working hypothesis follows: 1) Functional cure therapy will comprise several therapeutic components that can be safely combined in the absence of clinically meaningful drug-drug or drug-biological interactions. 2) Therapeutic vaccination represents an important component of a cure combination therapy as an immune-mediated component for the removal of infected cells.3) Functional HIV cure will probably be maintained through repeated administration of the combination selected over time.
Methods: Our group is approaching functional cure by stimulating cellular immunity. We are developing Vacc-4x which is designed to induce cell-mediated immune responses to conserved regions on the HIV capsid/core protein p24. Vacc-4x is administered intradermally (ID) using recombinant human granulocyte colony stimulating factor (rhuGM-CSF) as a local adjuvant. Intradermal injection allows for uptake by resident dendritic cells of the skin in situ. Following cross presentation within dendritic cells, Vacc-4x epitopes can be presented on human leukocyte antigens (HLA)-I and II for presentation to CD8+ and CD4+ T-cells respectively, leading to antigen-specific T-cell responses that can remove HIV-1 infected cells presenting these viral antigens. The clinical working hypothesis is that Vacc-4x may be combined with other interventions, such as latency reversing agents, romidepsin and/or other immunomodulatory agents (e.g., lenalidomide, a thalidomide analogue enhancing T-cell function) to achieve long-term control or Sustained Virologic Remission of HIV, in the absence of ART.
Results: Vacc-4x has been investigated in seven Bionor-sponsored clinical studies to date and shown to be safe and well tolerated in approximately 200 participants. Safety (phase I/II - University of Bergen) and immunogenicity studies (Phase II- Oslo University Hospital) were carried out on HIV positive participants in Norway. Delayed Type Hypersensitivity (DTH) responses involving intradermal injection of Vacc-4x peptides in the absence of rhuGM-CSF was associated with preserved CD4 count and reduction in plasma viral load on treatment interruption. There was limited immune escape. Participants could be safely re-boosted where memory responses of at least 7 years were evident. Vacc-4x was safe in one of the largest therapeutic HIV vaccine studies to date and shown to induce a statistically significant reduction in viral load compared to placebo on treatment interruption lasting up to 6 months (NCT00659789). The Statistical Center for HIV/AIDS Research and Prevention (SCHARP) has independently demonstrated the observations on viral load, and showed that CD4 counts were better preserved in the Vacc-4x group compared to placebo over time. Re-boosting of Vacc-4x followed by a second treatment interruption showed that viral load was reduced compared to the first study, but the reduction was not statistically significant (NCT01712256). The viral load set point for individuals participating in both studies remained significantly below pre-ART levels. Furthermore, a statistically significant reduction in total DNA was observed following booster immunizations during ART. Vacc-4x has been combined with the immune modulator lenalidomide in patients that fail to regain CD4 counts despite being well controlled on ART.

Increases in CD4 counts in the Vacc-4x/lenalidomide group were numerically higher than that of the Vacc-4x alone group, but the difference between the groups was not statistically significant. The CD4 count from baseline to end of study in the Vacc-4x/lenalidomide group showed an increase that was statistically significant. The increase from baseline in the Vacc-4x alone group was not statistically significant. There was an increase in the naive CD4 T-cell subset in the Vacc-4x/lenalidomide group compared to Vacc-4x alone (NCT01704781). Vacc-4x was combined with latency reversal to assess the HIV "shock and kill" concept towards functional HIV cure. This study was carried out in two parts. Part A showed that the HDAC inhibitor romidepsin was able to reverse latency in the presence of ART, leading to viral load increases that could be measured in plasma using standard viral load assays. Part B of the study showed a statistically significant reduction in total HIV DNA compared to baseline of 40 per cent with Vacc-4x pretreatment. Similarly, in participants where it was possible to measure the outgrowth of replication competent virus, there was a 38 percent reduction compared to baseline with Vacc-4x pretreatment. There was a 19 percent reduction in integrated HIV DNA, but this was not statistically significant (NCT02092116).
Conclusions: Safety of Vacc-4x as a stand-alone agent has been established during the course of seven studies included in a robust database from approximately 200 patients having received over 1600 injections of the peptide-based therapeutic vaccine Vacc-4x. Initial demonstration of the safety of Vacc-4x combined with the immunomodulatory agent lenalidomide as well as romidepsin during the REDUC study has been shown. Vacc-4x in combination with romidepsin led to reductions in total HIV DNA, however, addition of a third component that can complement the effects of Vacc-4x may lead to even greater reservoir reductions. Agents that may be considered include broadly neutralizing antibodies, immune modulators, cytokines, Toll-like receptors, PD1/CTLAs and other potential approaches in development. Our group's approach of immunologically controlling HIV is consistent with the current, non-mutually exclusive hypotheses that have been proposed in order to achieve a functional cure: (a) "Shock and Kill" which involves latency activation and control of peripheral viral replication, or (b) "Soothe and Snooze" which includes quiescence of latent virus and durable viral suppression. Central to clinical development towards functional cure is the latent reservoir of HIV-1 in resting CD4+ cells, and at various anatomical sites, that constitute the main barrier to eradication or the absence of rebound after ART interruption. HIV latency is maintained by several mechanisms, essentially through suppression of transcription. Lowering the quantifiable viral reservoir below a critical threshold, coupled with the activation of specific immune responses in selected patients treated with potent ART regimens, could enable the peripheral control of HIV RNA synthesis such that ultimately ART could be safely discontinued.

#**69 Steens, Jean-Marc**

Antiviral Activity and Safety of ABX464

J-M Steens[1], S Khuanchai[2], R Winai[3], K Ruxungtham[4], R Rouzier[5], D Scherrer[6], P Gineste[7], P Pouletty[7], H Ehrlich[7], R Murphy[8]

1. ABIVAX SA, Chief Medical Officer, Paris, France; 2. Chiang Mai University, Research Institute for Health Sciences, Chiang Mai, Thailand; 3. Faculty of Medicine Siriraj Hospital, Preventive and Social Medicine, Bangkok, Thailand; 4. Chulalongkorn University, Faculty of Medicine, Bangkok, Thailand; 5. Cap Research, Mauritius, Mauritius; 6. ABIVAX, Montpellier, France; 7. ABIVAX, Paris, France; 8. Northwestern Feinberg Medical School, Chicago, Illinois

Background: ABX464 is a first-in-class antiviral drug candidate for the treatment of patients with HIV-infection. It is an orally available small molecule that blocks HIV replication through an entirely novel mechanism, inhibition of Rev activity. Preclinical data in humanized mice showed that ABX 464 monotherapy had an antiviral effect which was sustained after treatment interruption (Campos et al, Retrovirology 2015 12:30) A prior food-effect study demonstrated a 3-fold increase in parent drug exposure when administered with food without a significant impact on the active glucuronide metabolite.

ABX464 also protects DSS-treated mice from inflammation by triggering overproduction of IL22 in LPS activated macrophages. Given that reduced IL-22 production in the gut mucosa is an important factor in HIV mucosal immunopathogenesis, the anti-inflammatory properties of ABX464 may have a significant impact on the chronic inflammation observed in HIV patients, on top of its antiviral effects.

Methods: The objective of this study was to evaluate the safety of ABX-464 at ascending doses versus placebo in HIV-infected treatment-naive patients. Patients were randomized into successive cohorts of 8 patients where 6 received 14-or 21 days of ABX 464 and 2 placebo. Patients from Mauritius and Thailand were included in the study after confirmation of HIV infection and no history of prior antiretroviral therapy. At day 0, patients received the first dose of ABX-464/ placebo in a once daily schedule. Safety assessments and laboratory parameters were recorded throughout the study. After completion of each cohort, a DSMB reviewed safety data and recommended whether the next cohort be initiated at a higher dose. Successive cohorts received 25, 50, 75, 100 and 150 mg QD. The 25, 50 and 100 mg. cohorts took drug fasting for 21 days, the 75 and 150 mg cohorts took drug with food for 14 days.

Results: Safety and Tolerability: No grade 3 or 4 events were noted. No patient stopped the study due to adverse events and all patients completed at least 14 days of treatment. Viral load reduction was observed in 3/12 patients in the 75 and 100 mg cohorts and 4/6 in 150 mg cohort; there were no significant viral load changes in the 6 placebo patients from these cohorts.

Conclusions: ABX 464 was well tolerated in this first study in HIV-infected patients.
ABX 464 monotherapy showed early antiviral activity in HIV-infected treatment naïve patients. These results warrant the further planned development of this novel acting antiretroviral drug. Abivax has launched a new Phase II a study in Europe (Spain, France and Belgium) where 27 patients whose HIV viral load is well controlled under boosted Darunavir will receive either ABX464 or Placebo for 28 days. All antiviral treatments are then stopped. Patients' viral load is being measured twice a week and receive antiretroviral treatment as soon as viral load is above 1000 copies/ml. Safety and tolerability as well as time to viral load rebound is being evaluated. Preliminary study results are expected by end 2016.

#70 Manak, Mark

Absence of Anti-HIV Antibody Following Treatment at Early HIV Acute Infection Presents Challenges to Monitoring HIV Infection

Mark Manak[1], Ashley Shutt[1], Rapee Trichavaroj[3], Jintanat Ananworanich[1], Sheila A Peel[2]

1. Henry M Jackson Foundation, Silver Spring, Maryland; 2. Walter Reed Army Institute of Research, Silver Spring, Maryland; 3. Armed Forces Research Institute of Medical Sciences (AFRIMS), Bangkok, Thailand

Initiation of highly active antiretroviral therapy (HAART) at very early times in acute HIV infection (AHI) rapidly reduces viral load to undetectable levels and may delay or prevent emergence of HIV diagnostic markers in blood. HIV infected infants who initiated treatment before 12 weeks of age frequently become HIV seronegative by 2 years of age, but have not cleared virus from latent reservoirs.

In a study conducted among high risk individuals presenting for HIV testing in Bangkok, Thailand, we examined the evolution of serological markers of infection in HIV-1 viremic volunteers who initiated HAART prior to establishment of confirmed HIV-1 antibody responses. Individuals treated at early stages of infection were tested for reactivity to the Fourth Generation (4th Gen) EIA (Ab/Ag Combo), Western blot, and MultiSpot (all fromBio-Rad Laboratories, Inc., Redmond, WA), and retested at 2, 12 and 24 weeks after initiation of antiretroviral therapy.

% Individuals Serologically Negative

	Fiebig I N=20			Fiebig II N=41			Fiebig III/IV N=20		
	4th Gen	MS	WB	4th Gen	MS	WB	4th Gen	MS	WB
Week 12	60%	85%	75%	10%	46%	41%	0%	65%	35%
Week 24	50%	90%	70%	2%	51%	34%	5%	80%	40%

Table 1. Percent individuals who remain seronegative at weeks 12 and 24 when treated at early times in Acute HIV infection.

Sixty percent (60%) of individuals who entered treatment at the earliest stage of AHI [RNA Reactive (R), but 4th Gen EIA Non-Reactive (NR): Fiebig I], remained 4th Gen EIA NR at 12 weeks, and 50% by 24 weeks post initiation of treatment. Of these 85% and 90% remained NR by MS, and 75% and 70% negative by Western Blot at week 12 and 24. Treatment at Fiebig Stage II (p24 R, 3rd Gen antibody NR) or Fiebig State III/IV [4th Gen R; WB NR or Indeterminate (IND)], all of which were initially R by 4th Gen, demonstrated some loss of reactivity yielding 10% NR results by 4th Gen at 12 weeks, and 2% NR at 24 weeks, while treatment at Fiebig III/IV yielded 5% NR results at week 24. MultiSpot remained NR in 51% of individuals treated at Fiebig II and 80% of those treated at Fiebig III/IV by 24 weeks post treatment. The percent MultiSpot reactivity increased between Wk 12 and 24 for all AHI stages. Western Blot reactivity followed a similar pattern, with 35% and 40% being IND or NR by weeks 12 and 24, respectively.

Absence of serological markers in individuals treated early in infection poses difficulties in monitoring status of infection, as some individuals may test serologically non-reactive, and RNA negative, but are infected. Even in cases where the 4th Gen EIA is reactive, it may be difficult to confirm reactivity by Western Blot, MultiSpot, or RNA for individuals under therapy. MultiSpot has now been withdrawn from the market, and replaced with the Geenius assay, which will need to be evaluated in the context of serological response after therapy. Caution is urged in interpretation of negative serological results in individuals on ART as the absence of infection. These findings may also have implications in monitoring individuals on Pre-exposure Prophylactics (PrEP) by serological tests alone.

#**71 Saadatmand, Forough**

The Impact of Exposure to Community and Interpersonal Violence on Alcohol and Drug Use, and HIV Risk Behaviors Among Young Adult African Americans

Forough Saadatmand[1], Roderick Harrison[2], Jennifer Bronson[3], Deanna Crouse[1], Joseph Wright[1]

1. Department of Pediatrics and Child Health, Howard University College of Medicine, Washington, DC; 2. 2M Research Services, Arlington, Texas; 3. U.S. Department of Justice, Bureau of Justice Statistics, Washington, DC

Background: This presentation discusses baseline preliminary analysis of data from 440 African Americans young adults ages 18-25 living in Washington DC collected in a longitudinal study.

Objective: The objective is to understand how exposure to community and interpersonal violence impacts alcohol, tobacco and drug use and HIV risk behaviors and if there are differences in the strength of associations based on violence type.

Methods: We first conducted a factor analysis of the interpersonal and community violence scales to identify domains or types of violence. We then conducted a series of regression analyses to assess the various interpersonal and community violence types (e.g., witnessing a murder) on the dependent variables representing Alcohol Tobacco and Other Drugs (ATOD) use, and knowledge and perception of HIV risk factors.

Results: Survey data showed that as children, 32%of the participants reported seeing a parent get hit by another parent or a boyfriend/girlfriend and 40% said someone close to them was murdered. As adults, 18% have seen someone shot, 27% reported seeing a dead person in the community, 35% witnessed someone getting beaten up or mugged, and 50% heard the sound of gunfire outside or near their home. Factors representing exposure to community violence were strikingly more likely to influence ATOD use and HIV risk behaviors than those representing interpersonal violence. Direct experiences of interpersonal violence were associated with alcohol use, younger age of first alcohol use, tobacco or marijuana use, and limited knowledge of HIV risk behaviors. However, community violence proved to have a stronger effect on the dependent variables than interpersonal violence.

Conclusion: The results suggest that a large number of young African American adults are exposed to community and interpersonal violence and that community violence in particular impacts ATOD use and HIV risks. Implications for improving public health through targeted HIV prevention, trauma-informed care, and violence prevention are discussed.

#**72 Mathews, Allison**

An Innovation Contest as Community Engagement for HIV Cure Research in North Carolina: A Mixed Methods Evaluation

Allison Mathews[1,2,6], Donaldson Conserve[1], Lisa Hightow-Weidman[3], Kate Muessig[4], Alexandria Anderson[5], Samantha Farley[4], Stuart Rennie[2,6], Joe Tucker[1,7]

1. Institute for Global Health and Infectious Diseases, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina; 2. Department of Social Medicine, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina; 3. School of Medicine, Division of Infectious Diseases, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina; 4. UNC Gillings School of Global Public Health, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina; 5. Lincoln Community Health Center, Early Intervention Clinic, Durham, North Carolina; 6. Center for Bioethics, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina; 7. UNC Project-China, 2 Lujing Road, Guangzhou, China

Background: Optimal ways to engage communities about HIV cure research remain unclear. The early stages of HIV cure research require an examination of alternative community engagement strategies that adopt a bottom-up approach. Crowdsourcing contests solicit contributions from the community, then evaluate and celebrate them. This pilot study evaluates community engagement in a crowdsourcing contest on HIV cure using social media analytics and qualitative methods.

Methods: The crowdsourcing contest solicited images and videos of what HIV cure meant to people. Participants submitted entries to an Ideascale site, an encrypted online platform specifically designed for innovation contests. We primarily engaged Black young adults between 18 to 35 years old in North Carolina because they are at the highest risk for acquiring HIV in the state. Recruitment included radio interviews and in-person and online engagement. Google, Twitter, and Facebook analytics assessed social media contest engagement. Online engagement included: page follows (unique users who subscribe to page update alerts), page visits, video views, reach (unique users who saw any contest-related material), and contest submissions. Qualitative research included focus groups and community forums that were transcribed, coded, and analyzed using MaxQDA.

Results: The crowdsourcing contest resulted in substantial in-person and online engagement, including in-person events (n=258 participants), focus groups and community forums (n=172 participants), and a reach of 168,364 unique users online. We had 31 contest submissions. Facebook analytics showed 277 page followers, 1297 page visits, 1409 video views, and a combined 112,041 unique users who viewed any contest-related media. Similar trends were observed on Twitter, Youtube, and Google analytics. Qualitative findings reveal the crowdsourcing contest was perceived as feasible and inclusive. In-person and online engagement about HIV cure research facilitated contest participation. Low HIV cure literacy and HIV-related stigma were barriers to contest participation.

Conclusion: Crowdsourcing contests may be useful for HIV cure community engagement. Findings suggest that combining recruitment through in-person events and multimedia platforms reach a broad range of individuals for potential participation in crowdsourcing contests. Community contributions to crowdsourcing contests may provide useful content for culturally relevant and locally responsive social marketing campaigns.

#**73 Dubé, Karine**

Perceived Motivators, Deterrents, Concerns and Barriers Related to HIV Cure Research Participation in the United States: A Qualitative Study

Karine Dubé[1], Jeff Taylor[2], David Evans[3], Laurie Sylla[4], Lynda Dee[5]

1. UNC Gillings School of Global Public Health, Chapel Hill, North Carolina; 2. Collaboratory of AIDS Researchers for Eradication (CARE) CAB, Palm Springs, California; 3. Delaney AIDS Research Enterprise (DARE) CAB, Los Angeles, California; 4. defeatHIV Community Advisory Board (CAB), Seattle, Washington; 5. AIDS Action Baltimore, Baltimore, Maryland

Background: Using qualitative research methods, we investigated perceptions of potential motivators, deterrents, concerns and barriers related to participation in HIV cure research in the United States. Given the complexity of HIV cure research, we approached a broad group of stakeholders, including people living with HIV, biomedical researchers and policy-makers/bioethicists. The findings inform the effective and ethical implementation of HIV cure studies and complement a quantitative survey on willingness to participate and take risks in HIV cure research among Americans living with HIV.

Methods: We implemented 36 in-depth interviews with a purposive sample of 12 people living with HIV, 11 biomedical researchers and 13 policy-makers/bioethicists in 2015 – 2016. We transcribed interviews verbatim and performed concurrent thematic analysis to compare perceptions of factors affecting participation in research among the three groups of informants.

Results: In the in-depth interviews, general factors affecting participation in HIV cure research included: 1) good quality HIV treatment available to potential participants; 2) the fact that translational studies of HIV cure interventions will not be curative; 3) quality of the science being undertaken; 4) current health status; 5) impact of research on one's health and 6) involvement of significant others. The possibility of risks combined with the lack of prospect of direct benefit was an important factor affecting decision-making. There was a tension between a people's anticipated willingness and interest to participate in studies versus actual opportunities (or lack thereof) to join studies. Motivators related to HIV cure research participation were centered on tangible factors, such as money or compensation. Intangible motivators included: 1) a desire to contribute to science and give back, 2) altruism (including social altruism, mixed altruism and self-altruism), 3) clinical contact factors (including communication with research team) and 4) hope and optimism. Deterrents focused on 1) the modality under investigation, 2) the scientific merit of the approach, 3) the early phase of experimentation, and 4) the stringent exclusion criteria for most research protocols. Main practical and logistical deterrents included 1) financial incentives, 2) intensity of visits, 3) time commitments, and 4) location of the research site. A subset of potential deterrents included factors related to study procedures, including perceived side effects and risks. Possible clinical risks, psychological risks, financial risks and social risks were also highlighted as deterrents. The three most prevalent negative clinical contact factors that would deter participation were: 1) poor treatment of study participants, 2) inadequate communication about risks, burdens and benefits (or lack thereof) and 3) breaches in confidentiality. Concerns related to HIV cure research participation concentrated on 1) unforeseen and unintended consequences of the research, 2) complex and unclear terminology around HIV cure/remission, 3) safety issues, 4) impacts on current health status, 5) consequences of treatment interruption, and 6) circumstances after study participation or post-cure discovery. Concerns around curative misconception and "failed cures" were prevalent. Main perceived burdens focused on 1) time commitments, 2) intensity of study visits and 3) travel-related constraints. Perceived barriers to HIV cure research participation included 1) geographical availability, 2) ineligible status due to health condition or co-morbidities; 3) stigma, 4) lack of family support, 5) concerns about the greed of pharmaceutical companies,

6) negative prior experience with the clinical research site, 7) incremental scientific progress in the field, 8) fatigue, and 9) difficulty maintaining long-term interest in research.

Conclusions: Findings can inform study design, informed consent, implementation, recruitment and retention efforts related to HIV cure research in the United States. Several motivators/deterrents, concerns and barriers associated with HIV cure research participation were non-medical in nature and existed at multiple level: personal, familial, social, structural, economic and cultural. They played a major role in whether people living with HIV would participate in cure studies. More formative research is needed to improve access to HIV cure studies in women and diverse ethnic groups across the United States. More interventions should be implemented to remove non-medical barriers to participation.

#**74 Sylla, Laurie**

What Do Participants Want? Participant Hopes, Concerns, and Expectations of HIV Cure Researchers

Laurie Sylla[1], David Evans[2], Jeff Taylor[3], Adam Lloyd Gilbertson[4], Karine Dubé[4]

1. defeatHIV Community Advisory Board (CAB), Seattle, Washington; 2. Delaney AIDS Research Enterprise (DARE) CAB, Los Angeles, California; 3. Collaboratory of AIDS Researchers for Eradication (CARE) CAB, Palm Springs, California; 4. UNC Gillings School of Global Public Health, Chapel Hill, North Carolina

Background: Clinical trials exploring methodologies leading to sustained viral suppression or an eradicating cure are a new field in HIV research, with unique physiological and psychological risks and potential benefits to participants. This study endeavored to learn about potential participants' motivations to participate in these trials, barriers to participation, perceptions of what an HIV cure would mean, and expectations related to participating in HIV cure research.

Methods: A cross sectional survey of 400 US adults living with HIV was conducted, followed up by a sample of structured in-depth interviews. These were followed up by nine focus groups conducted in California, North Carolina, and Washington State. Focus group discussions included questions specific to what individuals expected of HIV cure researchers and what they wanted researchers to know.

Results: These results are from a subset of questions asked of four Seattle focus groups that included 31 participants. Participants wanted research teams to understand the psychological trauma experienced by many people living with HIV, particularly past and on-going stigma; to be respectful of them as whole human beings; to "get them;" to be honest and transparent about all study risks and procedures; to compensate them fairly; and to have study team members and visits available to them after hours. Participants also endorsed having someone with a mental health background as part of the study team. Hispanic participants expressed desire for study teams to include members who were reflective of participants. Participants expressed significant concerns about potential risks to their health, study procedures, reproductive health, and side effects, including impact on mental health. Some wanted researchers to be mindful that they (participants) are as essential to the research as the researchers.

Conclusions: Honest, respectful relationships between participants and researchers, transparency, appropriate compensation, and safety planning will be necessary for recruiting and retaining cure trial participants. Taking participant concerns and expectations into account will contribute to the success of HIV cure research.

#**75 Bromwich, Danielle**

HIV Cure Research and the Dual Aims of the Informed Consent Process

Danielle Bromwich[1], Joseph Millum[2]

1. Department of Philosophy, University of Massachusetts, Boston, Massachusetts; 2. Clinical Center Department of Bioethics & Fogarty International Center, National Institutes of Health, Bethesda, Maryland

A cure for HIV would be tremendously valuable. Approximately 37 million people worldwide are HIV-positive and 15 million are currently on antiretroviral therapy. Until recently it was assumed that this therapy would be the extent of HIV treatment and that those with access to it would need to take their drugs for life. But what once seemed impossible is now in early phase clinical trials: interventions designed to completely eradicate HIV from the immune system. Excitement surrounding these “HIV cure” studies is tempered by ethical concern. They require participants to come off their antiretroviral therapy and undergo highly risky interventions using gene transfers or stem cell therapy. These are currently proof of concept studies—no one expects the participants to be cured. Their purpose is to provide essential information about safety and pharmacokinetics, but in doing so they expose participants to high risks with little prospect of direct benefit.

If we could be confident that participants understood their trials’ true risk-benefit ratio, these high risks might be less troubling. But such confidence would be misplaced. Decades of data show poor comprehension of risk among participants in clinical trials. The fact that HIV is still a stigmatized condition amplifies this concern. Potential participants may be desperate to be rid of their disease and so downplay the risks and exaggerate the potential benefits. Understandably, HIV cure researchers and research ethics committees are worried. What should they do with a patient-participant who wants to come off his medication and receive a high-risk experimental intervention because he thinks that he’ll be “the one” who is cured?

Informed consent is generally thought to be one key protection for participants enrolled in risky studies. The standard view of informed consent says that valid consent requires the person giving consent to understand the risks and benefits of study participation. According to this view, someone who seriously misunderstands the study’s true risk-benefit ratio can be excluded on the grounds that he has not given valid consent to study participation.

In this presentation, we analyze a range of concerns about informed consent for HIV cure trials. Against the standard view on understanding, we argue that it is not necessary to understand risks in order to give valid consent. In fact, if you take this widely accepted view seriously, you end up committed to the conclusion that it is never possible to give valid consent to medical research participation. Such a view would be absurd.

If understanding all the risks is not necessary for valid informed consent, does that mean that researchers and research ethics committees should be unconcerned about whether the people enrolling in HIV cure studies understand? Not at all. This case illuminates a further function of the informed consent process that explains, without absurdity, why understanding matters. The primary goal of the process is to obtain valid consent. This is an ethical requirement of most studies, and proceeding without it often involves serious rights violations. But there’s an important secondary goal. In disclosing information clearly and carefully and in discussing the study with participants, we aim to facilitate good decision-making—helping participants make decisions that are consistent with and promote their values and preferences. Good decision-making is an ethical aspiration. While we cannot force a competent adult to do what she should, we know that she is more likely to make a good decision about study enrolment if she understands the

information that is relevant to that decision. We respect autonomy by getting valid consent; we enhance autonomy by helping people make good decisions.

Given the unfavorable risk-benefit ratio they face, testing potential participants' comprehension of facts like risks and benefits would be a worthwhile check on whether they are making a decision that is genuinely consistent with their values and preferences. Such checks might seem paternalistic if people do not need to understand risks in order to exercise their autonomy rights. But patients whose HIV is controlled on medication do not have a right to enroll in "cure" research, and so delaying or refusing to enroll on these grounds does not violate their rights. If a participant is seriously confused, researchers can supplement the informed consent process with an extended one-on-one discussion, retesting understanding after this intervention. This would no doubt add to the researcher's workload. But given the low numbers of participants enrolled in early phase trials, it is unlikely to be too burdensome. The benefits to participants are worth it.