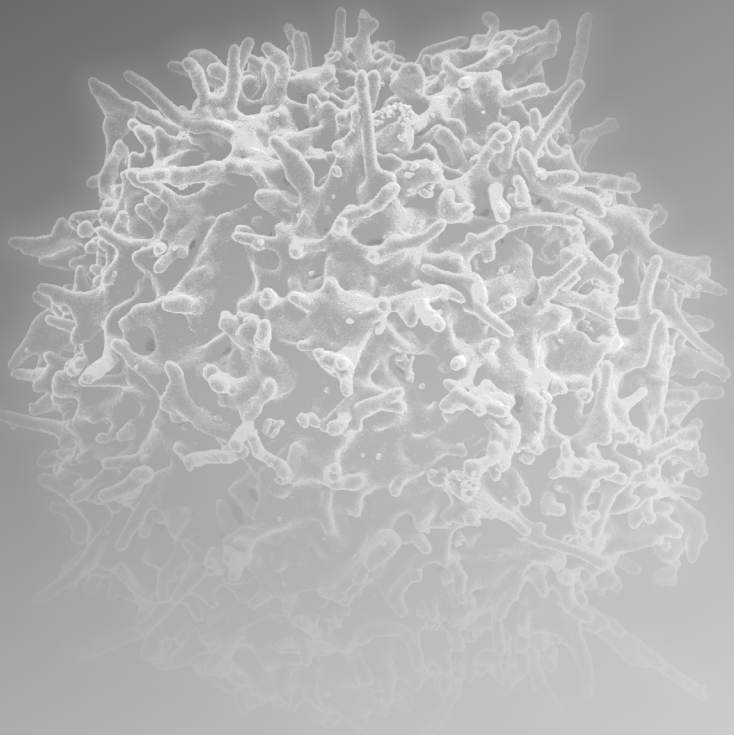
# CGT 4 HIV Cure

## Cell & Gene Therapy for HIV Cure 2017



August 17–18, 2017

Fred Hutchinson Cancer Research Center
Seattle, Washington

**cgt4hivcure2017.org**

defeatHIV

FRED HUTCH
CURES START HERE

CFAR
CENTER FOR AIDS RESEARCH
UNIVERSITY OF WASHINGTON

UW Medicine
LABORATORY MEDICINE
VIROLOGY

## The Conference on Cell & Gene Therapy would like to acknowledge Timothy Ray Brown and the 10th anniversary of his HIV cure in 2007.

"I think that you all agree with me that Timothy's case, as a proof of principle, has changed a lot of the field of HIV research. Timothy is the motivation for hundreds of researchers, fundraisers and activists to go forward to the big target that HIV/AIDS can be cured."

—Dr. Gero Hütter, the doctor who cured Timothy, commenting in a video he made for defeatHIV CAB to mark the 10th anniversary of the first HIV cure.

*Timothy Ray Brown celebrates his 10th "birthday" surrounded by friends and members of the defeatHIV CAB, marking the anniversary of the stem cell transplant that made him the first and so far only person in the world to be cured of the virus that causes AIDS.*

Photo Courtesy of Robert Hood / Fred Hutch News Service

# TABLE OF CONTENTS

On behalf of the Scientific Organizing Committee, we are pleased to welcome you to the 4th annual Conference on Cell & Gene Therapy for HIV Cure. As attendance grows each year, we are thrilled to see that this conference is becoming a mainstay in the community, and offering a platform for sharing groundbreaking discoveries that will advance our ultimate goal of ending HIV.

When the first-known case of AIDS was reported in the United States in 1981, the response was largely fear, ignorance, and discrimination. However, in that tumultuous time the groundwork was also laid for a global discussion leading to awareness, research, prevention, and advocacy. The past 35 years have seen great strides in our knowledge of HIV/AIDS, both scientifically and socially. In 1988, the World Health Organization declared December 1 to be World AIDS Day, helping raise awareness to increase prevention. Since 1996, we've seen inspiring treatments such as the development of highly active antiretroviral therapy (HAART). In 2007, the first known cure of HIV was achieved in Mr. Timothy Ray Brown and soon after, an entirely new field of cure research was born. In 2011, the first Martin Delaney Collaboratories were funded by the NIH to investigate potentially curative therapies for HIV. And in 2014, amfAR announced a research initiative aimed at finding a scientific basis for an HIV cure by the end of 2020. While there is still wide-spread stigma surrounding HIV/AIDS, we are working hard to create a dialogue and educate the public and ourselves about this disease.

Four years ago, this conference was created to bring attention to the evolving progress in HIV cell and gene therapies, and now we certainly have it. As we share and learn together, remember that our greatest resources are each other. Hopefully, everyone at this conference will all take the time to get to know one another, and build new partnerships to help further our goal of ending HIV. Finding a cure will require something from all of us, and we all have something to give. It is the researchers, clinicians, investigators, trainees, pre- and post-doctoral fellows, and community advocates, who hold the future of HIV.

Thank you all for your participation in this conference, and for taking the time out of your busy schedules to join us in reviewing the latest cell and gene therapy approaches targeting HIV. With these continued innovations, collaborations, and advocacies, we are making progress towards a cure.

Sincerely,

**Keith R. Jerome, MD, PhD**

*Conference Co-host*

**Hans-Peter Kiem, MD, PhD**

*Conference Co-host*

## SCHOLARSHIP RECIPIENTS

*The Conference on Cell & Gene Therapy for HIV Cure* is pleased to announce the 2017 scholarship recipients:

**Morgan Chateau, PhD**

**Adam Dingens, MS**

**Wannisa Khamaikawin, PhD**

**Anne-Sophie Kuhlmann, PhD**

**Nick Llewellyn, PhD**

**Paul Munson**

**Stefan Radtke, PhD**

**Daniel Reeves, PhD**

**Pavitra Roychoudhury, PhD**

**Eduardo Seclen, PhD**

**Gabriela Webb, PhD**

**Andrew Wong**

*Congratulations!*

**Catherine Bollard, MBChB, MD, FRACP, FRCPA**

*Chief, Division of Allergy and Immunology, Director, Program for Cell Enhancement and Technologies for Immunotherapy (CETI), Children's National Health System*

*Professor of Pediatrics and of Microbiology, Immunology and Tropical Medicine, George Washington University School of Medicine and Health Sciences*

**Smitty Buckler**

*defeatHIV Community Advisory Board*

**Paula Cannon, PhD**

*Professor of Molecular Microbiology & Immunology, Pediatrics, Biochemistry & Molecular Biology, University of Southern California*

**Deborah Fuller, PhD**

*Professor, Department of Microbiology, University of Washington*

*Core Scientist, AIDS Division, Washington National Primate Research Center*

**Keith R. Jerome, MD, PhD**

*Host, CGT4HIVCure 2017*

*Co-PI, defeatHIV Martin Delaney Collaboratory*

*Member, Vaccine & Infectious Disease Division, Program in Infectious Disease Sciences, Fred Hutchinson Cancer Research Center*

*Professor and Head, Virology Division, Department of Laboratory Medicine, University of Washington*

**Hans-Peter Kiem, MD, PhD**

*Host, CGT4HIVCure 2017*

*Co-PI, defeatHIV Martin Delaney Collaboratory*

*Endowed Chair for Cell and Gene Therapy, Director, Cell and Gene Therapy Program, Associate Head of Transplantation Biology, Fred Hutchinson Cancer Research Center*

*Professor of Medicine / Oncology and Pathology, University of Washington School of Medicine*

**James L. Riley, PhD**

*Associate Professor, Department of Microbiology, University of Pennsylvania*

**Geoff Symonds, PhD**

*Head of Scientific Affairs & Collaborations, Calimmune Inc.*

**Manuel Venegas**

*defeatHIV Community Advisory Board*

| | | |
|---|---|---|
| Pelton Auditorium | 7:15 – 8:00 | Registration and Continental Breakfast |
| | **SESSION 1** | **Opening remarks and HIV Cure Overview** |
| | 8:00 – 8:20 | Conference Welcome: Keith R. Jerome and Hans-Peter Kiem with special remarks by Timothy Ray Brown |
| | 8:20 – 8:40 | Michael Emerman |
| | 8:40 – 9:00 | DeMarc Hickson |
| | **SESSION 2** | **HIV Latency & Reservoirs** |
| | 9:00 – 9:20 | Mary Kearny |
| | 9:20 – 9:40 | James Mullins |
| | 9:40 – 10:00 | Ya-Chi Ho |
| | 10:00 – 10:15 | Benjamin Burwitz |
| | 10:15 – 10:30 | Chantelle Ahlenstiel |
| | 10:30 – 11:00 | BREAK |
| | **SESSION 3** | **Keynote** |
| | 11:00 – 11:05 | Keynote Introduction: Hans-Peter Kiem |
| | 11:05 – 12:00 | Keynote Speaker: Carl June |
| | 12:00 – 1:30 | LUNCH: WEINTRAUB B SUITES |
| | **SESSION 4** | **Immunotherapy for HIV Cure** |
| | 1:30 – 2:00 | PLENARY: Catherine Bollard |
| | 2:00 – 2:20 | James Riley |
| | 2:20 – 2:40 | Jerome Zack |
| | 2:40 – 2:55 | Edward Berger |
| | 2:55 – 3:30 | BREAK |
| | **SESSION 5** | **Therapeutic Development** |
| | 3:30 – 3:50 | Bruce Torbett |
| | 3:50 – 4:10 | David Rawlings |
| | 4:10 – 4:30 | Matthew Scholz |
| | 4:30 – 4:45 | Howard Gendelman |
| | 4:45 – 5:00 | Andrew Wong |

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| | **Evening Activities** | |
| | 5:00 – 6:20 | Poster Session: serving hors d'oeuvres, beer, and wine |
| | 6:20 – 7:00 | Walk to MOHAI dock and board the Argosy Cruise ship |
| | 7:00 – 10:00 | Lakes dinner cruise and reception |

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|---|---|---|
| Pelton Auditorium | 7:30 – 8:00 | Registration and Continental Breakfast |
| | **SESSION 6** | **Vaccines and Immunity** |
| | 8:00 – 8:30 | PLENARY: Michael Lederman |
| | 8:30 – 8:50 | Nancy Haigwood |
| | 8:50 – 9:10 | Karine Dubé |
| | 9:10 – 9:25 | Paul Munson |
| | 9:25 – 9:40 | Anne-Sophie Kuhlmann |
| | 9:40 – 10:10 | BREAK |
| | **SESSION 7** | **Transplantation and Gene Editing for HIV Cure** |
| | 10:10 – 10:30 | Leslie Kean |
| | 10:30 – 10:50 | Jonah Sacha |
| | 10:50 – 11:10 | Christopher Peterson |
| | 11:10 – 11:25 | Kamel Khalili |
| | 11:25 – 11:40 | Olivier Pernet |
| | 11:40 – 11:55 | Stefan Radtke |
| | 11:55 – 1:30 | LUNCH: WEINTRAUB B SUITES |
| | **SESSION 8** | **Animal Models** |
| | 1:30 – 2:00 | PLENARY: Guido Silvestri |
| | 2:00 – 2:20 | Genoveffa Franchini |
| | 2:20 – 2:40 | Francois Villinger |
| | 2:40 – 2:55 | Nick Llewellyn |
| | 2:55 – 3:00 | Closing Remarks: Keith R. Jerome and Hans-Peter Kiem |

Fred Hutch Communications and defeatHIV will be live-tweeting highlights during the conference. Follow us on Twitter @fredhutch, @defeatHIV, and the hashtag #cgt4hivcure.

The FHCRC offers free wireless access via the Fred Hutch Guest network.

## Carl H. June, MD

*Richard W. Vague Professor in Immunotherapy, Department of Pathology and Laboratory Medicine, Perelman School of Medicine, University of Pennsylvania*

*Director of the Center for Cellular Immunotherapies, Director of Translational Research, Abramson Cancer Center, University of Pennsylvania*

*Director of the Parker Institute for Cancer Immunotherapy, University of Pennsylvania*

Carl June is the Richard W. Vague Professor in Immunotherapy in the Department of Pathology and Laboratory Medicine. He is currently Director of the Center for Cellular Immunotherapies and Director of Translational Research in the Abramson Cancer Center of the University of Pennsylvania, and is an Investigator of the Abramson Family Cancer Research Institute. He is a graduate of the Naval Academy in Annapolis, and Baylor College of Medicine in Houston, 1979. He had graduate training in Immunology and malaria with Dr. Paul-Henri Lambert at the World Health Organization, Geneva, Switzerland in 1978 and 1979, and post-doctoral training in transplantation biology with E.

Donnell Thomas and John Hansen at the Fred Hutchinson Cancer Research Center in Seattle from 1983 to 1986. He is board certified in Internal Medicine and Medical Oncology. He maintains a research laboratory that studies various mechanisms of lymphocyte activation that relate to immune tolerance and adoptive immunotherapy for cancer and chronic infection. In 2011, his research team published findings which represented the first successful and sustained demonstration of the use of gene transfer therapy to treat cancer. Clinical trials utilizing this approach, in which patients are treated with genetically engineered versions of their own cells, are now underway for adults with chronic lymphocytic leukemia and adults and children with acute lymphoblastic leukemia. Early results in that group show that 90 percent of patients respond to the therapy, and more recently, trials of this approach have begun for patients with other blood cancers and solid tumors including pancreatic cancer, mesothelioma, and the brain cancer glioblastoma. In 2014, it became the first personalized cellular therapy for the treatment of cancer therapy to receive the U.S. Food and Drug Administration's prestigious Breakthrough Therapy designation.

He has published more than 350 manuscripts and is the recipient of numerous prizes and honors, including election to the Institute of Medicine in 2012, the William B Coley award, the Richard V Smalley Memorial Award from the Society for Immunotherapy of Cancer, the AACR-CRI Lloyd J. Old Award in Cancer Immunology, the Hamdan Award for Medical Research Excellence, and the Paul Ehrlich and Ludwig Darmstaedter Prize. In 2014, he was elected to the American Academy of Arts and Sciences.

## The Yin-Yang of Engineered T cells for Cancer and HIV

CH June

University of Pennsylvania

An overview of the status of immunotherapy and synthetic biology with engineered T cell therapy will be presented. The implications for HIV cure research based on advances in cancer therapy will be discussed.

## Catherine Bollard, MBChB, MD, FRACP, FRCPA

*Chief, Division of Allergy and Immunology; Director, Program for Cell Enhancement and Technologies for Immunotherapy (CETI), Children's National Health System*

*Professor of Pediatrics and of Microbiology, Immunology and Tropical Medicine, George Washington University School of Medicine and Health Sciences*

Catherine Bollard received her medical degree at the University of Otago in Dunedin, New Zealand. She is Board certified both in Pediatrics and Hematology. She worked both in New Zealand and London, England before moving to Baylor College of Medicine (BCM) in 2000 where she was Professor of Pediatrics, Medicine and Immunology and the Director of the Texas Children's Cancer and Hematology Center Pediatric Lymphoma Program. In August 2013, she moved to Children's National and The George Washington University School of Medicine and Health Sciences in Washington, DC. She is currently Chief, Division of Allergy and Immunology, Professor of Pediatrics and of Microbiology, Immunology and Tropical Medicine and Director of the Program for Cell Enhancement and Technologies for Immunotherapy (CETI). She is a member of the American Society for Clinical Investigation (ASCI) and is President of the International Society for Cellular Therapy (ISCT). She is on the Board of Directors of the Foundation for the Accreditation of Cellular Therapy (FACT) and Chairs the Non Hodgkins Lymphoma committee of the Children's Oncology Group. She is an Associate Editor for the journals Blood and Cytotherapy and is a member of the NCI Clinical Oncology Study Section and is a member of the Cellular, Tissues and Gene Therapies Advisory Committee for the Food and Drug Administration (FDA). Her bench and translational research focuses on improving outcomes for patients after hematopoietic stem cell transplantation as well as the development of novel cell therapies for viral diseases and hematologic malignancies.

## Development of HIV-specific T cell Therapy: Lessons from EBV

C Bollard

Children's National Medical Center, Washington, DC; George Washington University School of Medicine and Health Sciences, Washington, DC

Adoptive T cell therapy using virus specific T cells (VSTs) has been successful in boosting viral-specific immunity for the treatment of patients with EBV+ Lymphoma and for patients post-HSCT, effecting disease remissions and preventing viral reactivations of CMV and EBV. However, the therapeutic use of VSTs (non gene engineered) to boost HIV-specific T cell immunity in HIV+ patients has met with more modest success. Despite multiple attempts to eradicate HIV with allogeneic-HSCT, there is only one case of functional HIV cure. Hence, we hypothesized that broadly HIV-specific CD8 and CD4 T-cells (HXTCs) could be expanded from HIV+ individuals on ARVs, as well as HIV-negative adult and cord blood donors (dHXTC), employing a non-HLA restricted approach for the treatment of HIV+ individuals in the autologous or allogeneic settings. We adapted the manufacturing platform we had utilized successfully for the manufacture of EBV/LMP-specific T cells from patients with EBV+ lymphomas and expanded autologous HXTCs from HIV+ subjects under NCT02208167. To extend this approach to the allogeneic HSCT setting, we generated dHXTCs from HIV-naive adults and cord blood donors. IFNg-ELISPOT showed dHXTCs from adult donors were specific against Gag, Nef, and Pol versus irrelevant antigen actin. Similarly, we are able to produce cord dHXTCs that showed specificity to Gag, Nef and Pol compared to T cells-only in IFNg-ELISPOT. dHXTCs were polyfunctional producing proinflammatory TNFα, IL2, IL6, IL8, and perforin responses ($p<0.05$) to HIV stimulation. Importantly, dHXTCs derived from both adult ($p=0.0004$) and cord blood ($p=0.0003$) were able to suppress HIV replication compared with nonspecific CD8 T cells when cocultured with autologous CD4 T cells infected with HIV SF162 at an Effector-to-Target ratio of 20:1. Moreover, epitope mapping of both adult and cord dHXTC products revealed that products contained T cells recognizing unique epitopes not typically identified in HIV+ individuals, similar to our observations in the CMV setting, which may be critical in overcoming viral immune escape post-HSCT. In summary, building on our VST experiences for the treatment of patients with EBV+ lymphomas and viral infections post HSCT, we can now show that HIV-specific T cells can be expanded from HIV+ and HIVneg donors for clinical use and may offer a unique T cell therapeutic for HIV cure as well as a platform for gene modification.

## Michael Lederman, MD

*Scott R. Inkley Professor of Medicine, Professor of Biomedical Ethics, Pathology, Microbiology, and Molecular Biology, Case Western Reserve University School of Medicine and University Hospitals/Case Medical Center*

*Co-Director, Center for AIDS Research, Division of Infectious Diseases, Case Western Reserve University*

*Principle Investigator, AIDS Clinical Trials Unit, Case Western Reserve University*

Michael Lederman is the Scott R. Inkley Professor of Medicine at Case Western Reserve University School of Medicine and University Hospitals/Case Medical Center where he is also Professor of Biomedical Ethics, Pathology, Microbiology and Molecular Biology. Dr. Lederman received his bachelor's degree in Biology from Brandeis University and his M.D. from the Mt. Sinai School of Medicine. He trained in Internal Medicine at Case Western Reserve University, University Hospitals of Cleveland and the VA Medical Center where he served as chief resident in Medicine and completed fellowship training in Infectious Diseases. He received post-doctoral training in cellular immunology in the laboratory of Dr. Jerrold Ellner and he joined the faculty at CWRU in 1980. Dr. Lederman has been engaged in HIV/AIDS research since he and Dr. Oscar Ratnoff first described and characterized the occurrence of AIDS-related immune deficiency in otherwise healthy men with hemophilia in 1983. His work focuses on the mechanisms whereby HIV infection induces immune dysfunction and on strategies to correct and prevent it. Most recently, he launched a new journal – Pathogens and Immunity - that threatens to make life good for biomedical researchers.

## Immune Activation, Inflammation, Coagulation and HIV Pathogenesis

M Lederman

Case Western Reserve University School of Medicine, Cleveland, OH

Although immune activation in the presence of profound immune deficiency was recognized in the earliest reports describing AIDS, broad recognition of the importance of immune activation and inflammation in HIV pathogenesis was delayed.

Acute HIV infection is characterized by profound dynamic cellular changes in both circulating and tissue compartments and the frequent febrile acute infection syndrome is often characterized by a “cytokine storm”. This typically resolves but in untreated HIV infection, indices of immune activation, inflammation and coagulation remain elevated. Administration of suppressive antiretroviral therapy, attenuates these activation/inflammation and coagulation indices but many remain elevated and do not return to levels seen in otherwise healthy controls. Elevated plasma levels of these inflammatory markers are linked to morbidity in treated HIV infection and interestingly, even before administration of ART, elevated setpoints of these levels predict morbid outcomes after ART is initiated. Although predictive of outcomes, the cellular and tissue sources of plasma inflammatory mediators are not known nor is there clear understanding of the factors that drive these elevated levels. Persistent virus expression, systemic translocation of microbial products through a damaged gut mucosa, coinfections with other viruses, perturbations in lipid metabolism, altered expression of homeostatic cytokines and dysfunctional regulatory mechanisms have all been implicated in the persistent activation/inflammation and coagulation of treated HIV infection. And while elevated inflammatory and coagulation indices predict morbiditiy and mortality, the mediators of these outcomes are poorly understood. To gain mechanistic insight into the drivers of cardiovascular risk, we propose that that circulating monocytes and CD8 T cells engage in a pernicious collaboration that accelerates the development of atherosclerotic cardiovascular disease.

## Guido Silvestri, MD

*Georgia Research Alliance Eminent Scholar in Comparative Pathology*

*Professor and Vice-Chair for Research, Department of Pathology and Laboratory Medicine, Emory University School of Medicine*

*Chair, Division of Microbiology & Immunology, Yerkes National Primate Research Center*

Since 1993, Dr. Silvestri has been involved in studies of AIDS pathogenesis, prevention, and therapy, mostly using non-human primate models of SIV and SHIV infection, with particular focus on comparative studies of pathogenic and non-pathogenic primate lentiviral infections. Dr. Silvestri is currently the principal investigator of several NIH grants, including an R37 MERIT award, and he is involved in both the Consortium for AIDS vaccine studies in non-human primates, the CHAVI-ID, and the CARE Martin Delaney collaboratorium. He has authored or co-authored 216 peer-reviewed publications in this field, including some in the highest impact journals (Cell, Science, Nature, Nature Medicine, etc). His work has been quoted over 15,000 times, and has been presented in plenary sessions at all the major HIV/AIDS Conferences (CROI, IAS, Keystone, etc). Dr. Silvestri is an Editor of the Journal of Virology, an Associate Editor of PLoS Pathogens, and a past-Editor of the Journal of Immunology. He also serves in the Editorial Board of the Journal of Infectious Diseases, and Clinical Microbiology Reviews. He served as Chairman or Standing Member in several study sections at the NIH, CIHR, ANRS, AmFAR, and the European Commission. He is a member of the Scientific Committee of the Conference on Retrovirus and Opportunistic Infections (CROI), of the International AIDS Society Scientific Working Group on HIV Cure, and was Co-Chair of the 9th International AIDS Conference in Paris, July 2017.

## Immune-based interventions for HIV cure: lessons from NHP models

G Silvestri

Emory University and Yerkes Primate Center, Atlanta, GA

The availability of a large array of potent anti-retroviral drugs (collectively defined as anti-retroviral therapy, ART) has dramatically reduced the mortality and morbidity associated with HIV infection. However, no therapeutic strategy that can eradicate or functionally "cure" the infection has yet been developed. Our current inability to cure HIV infection is related to the presence of a persistent reservoir of latently infected cells that is resistant to both conventional anti-retroviral therapy (which targets specific phases of the "productive" virus life cycle) and immune-based interventions (which require expression of viral proteins as target antigens for either cellular or antibody responses).

Over the past few years, the non-human primate model of SIV or SHIV infection of rhesus macaques has been developed and successfully validated for studies of virus eradication in the setting of fully suppressive ART. In this presentation, I will briefly review: (i) the opportunities presented by the SIV and SHIV macaque models to conduct pre-clinical studies aimed at developing and testing novel interventions aimed at achieving a functional cure for HIV infection; (ii) the main immune-based strategies that are currently explored to reduce or eliminate the virus reservoir in the NHP model (i.e., shock & kill, block & lock; soothe & schmooze; push & vanish; as well as transplant and gene therapy); and (iii) the published and ongoing preclinical trials of immune-based interventions that are conducted by our team in ART-treated SIV-infected rhesus macaques with the goal of inducing a functional cure. Immune interventions that will be discussed include type I interferons, interleukin-21, FTY720, check-point blockade inhibition (i.e., inhibitors of PD-1, CTLA4, and LAG-3), CD8+ T cell depletion, CD4+ T cell depletion, agents that promote CD4+ T cell differentiation (i.e., beta-catenin and NOTCH inhibitors), and autologous stem cell transplantation.

**Michael Emerman, PhD** **INVITED SPEAKER**

*Division of Human Biology, Fred Hutchinson Cancer Research Center*

## HIV Ancient History and Lessons for a Cure

M Emerman

Division of Human Biology, Fred Hutchinson Cancer Research Center, Seattle, WA

While HIV is responsible for a modern pandemic, viruses related to HIV are ancient pathogens of primates. Even older retroviral infections of human ancestors have left remnants of their genetic material in the human genome. These ancient viral infections represent extinct viral lineages that have been defeated. HIV adapted to humans through a stepwise evolution that involved cross-species transmissions of an ancient virus from at least two kinds of monkeys into chimpanzees, followed by transmission of a precursor of HIV into gorillas and humans. Each of these steps was accompanied by changes in the viral genome to overcome the natural innate defense genes of the host. In this talk, I will discuss the consequences of these viral adaptations and the lessons to be learned for how primates might resist or be cured of retroviral infections.

**DeMarc A. Hickson, PhD** **INVITED SPEAKER**

*Executive Director, Us Helping Us, People Into Living, Inc.; Senior Research Scientist, My Brother's Keeper, Inc.*

## HIV Cure Research Perceptions Among HIV-Infected African American MSM, and Affected Communities, in the Deep American South: A Multi-level Mixed Methods Perspective

DA Hickson

Us Helping Us, People Into Living, Inc, Washington, DC; My Brother's Keeper, Inc., Jackson, MS

The successes and failures in HIV cure research in the past few years, such as the 'Berlin patient' and the Mississippi child, have challenged the paradigm that HIV is incurable and have prompted more organizations to actively engage in HIV cure research. The overall objective of this study is to quantify empirically the multi-level perceptions of HIV cure clinical research among HIV-infected African American MSM and affected communities (i.e., HIV-uninfected African American MSM and community residents) and to identify the salient correlates of knowledge and awareness of HIV cure research as well as the barriers and facilitators to participation in HIV cure clinical studies and to the uptake of HIV cure discoveries. The Specific Aims of the current study are to:

Specific Aim 1: Evaluate: a. HIV cure research knowledge and awareness; b. the willingness to participate in HIV cure research and to uptake cure discoveries; and c. the associations of demographics, intrapersonal beliefs and attitudes, and psychosocial factors with HIV cure research perceptions among 225 African American MSM in Atlanta, GA, Jackson, MS, and New Orleans, LA (75 per site: 25 HIV-infected and 50 HIV-uninfected).

Specific Aim 2: a. Quantify neighborhood-level HIV cure research perceptions and b. relate the associations of neighborhood-level HIV cure research perceptions with individual-level HIV cure research perceptions.

I will discuss our formative work and present the qualitative findings in the development of the surveys. The current study presents a remarkable opportunity to study the multi-level influences of HIV cure perceptions and willingness to participate in HIV cure research studies, and the uptake up HIV cure discoveries among an at significant risk population.

**Mary F. Kearney, PhD** **INVITED SPEAKER**

*Staff Scientist, Host-Virus Interaction Branch, HIV Dynamics and Replication Program; Head, Translational Research Unit, National Cancer Institute*

## No evidence for HIV replication in lymph nodes during ART

WR McManus[1], J Spindler[1], MJ Bale[1], A Wiegand[1], A Musick[1], X Wu[2], D Wells[2], SH Hughes[1], R Hoh[3], JM Coffin[4], JW Mellors[5], SG Deeks[3], MF Kearney[1]

[1]HIV Dynamics and Replication Program, CCR, National Cancer Institute, Frederick, MD; [2]Leidos Biomedical Research, Inc., Frederick National Laboratory for Cancer Research, Frederick, MD; [3]Department of Medicine, University of California, San Francisco, CA; [4]Department of Molecular Biology and Microbiology, Tufts University, Boston, MA; [5]Department of Medicine, University of Pittsburgh, Pittsburgh, PA

To better understand the mechanisms of HIV persistence and to further investigate the question on ongoing viral replication in lymph nodes, we characterized HIV proviral populations, their levels of expression, and their sites of host integration in paired lymph node mononuclear cells (LNMC) and peripheral blood mononuclear cells (PBMC) collected after long-term ART and compared to them to the HIV populations prior to ART initiation. Three donors initiated ART in chronic infection and had viremia suppressed for 4-12 years; one donor initiated ART in acute infection and had viremia suppressed for 18 years.

Proviral populations and expression were characterized by single-cell analyses and expanded clones were identified using the integration sites assay (ISA). Populations were compared phylogenetically, using a test for panmixia (well-mixed or divergent), by determining the fraction of expressing vs. latent proviruses, determining the levels of expression in single cells with actively-transcribing proviruses, and by comparing the expanded clonal populations.

Proviruses in LNMC and PBMC were well mixed and were not significantly divergence from the pre-ART populations. The fraction of proviruses that were latent vs. actively transcribing during ART were not different (5-8% in the PBMC and 2-20% in the LNMC) (p=0.4). The levels of expression in actively-infected cells were low in both compartments, typical of suppression of viral replication. The same clonal populations were detected in PBMC and LNMC (p=0.8).

These findings are not consistent with continued viral replication during ART in either the peripheral blood or the lymph nodes. Our results also suggest that infected cells migrate freely between the peripheral blood and the lymph nodes and demonstrate that the HIV reservoir is long-lived and proliferating populations of cells that were infected prior to initiating ART.

**James I. Mullins, MD** **INVITED SPEAKER**

*Professor, Departments of Microbiology, Medicine, and Laboratory Medicine, University of Washington*

## Selection for genomic sites, provirus orientation and cellular pathways of HIV integration sites in vivo

M Aid[1,§], PT Edlefsen[2], HA Aamer[3,4], W Deng[4], TR Sibley[4], EJ Silberman[4], S McLaughlin[3], ME Bull[3], H Huang[3], S Styrchak[3], R Rossenkhan[2], A Krumm[4], R Sekaly[1], LM Frenkel[3,4], JI Mullins[4]

[1]Case Western University, Cleveland, OH; [2]Fred Hutchinson Cancer Research Center, Seattle, WA; [3]Seattle Children's Research Institute, Seattle, WA; [4]University of Washington, Seattle, WA; §Present Address: Center of Virology and Vaccine Research, Harvard Medical School, Boston, MA

Proliferation of HIV-infected cells contributes to the persistence of infectious HIV reservoirs during antiretroviral therapy (ART). Factors leading to infected cell survival and proliferation are not clear, although these cells have been reported to be enriched for HIV integration sites (IS) within genes associated with cell proliferation. We conducted a meta-analysis of HIV IS in 55 individuals on prolonged suppressive ART and in cells infected with HIV in vitro to discern patterns associated with the integration of HIV into the human genome and with the survival of infected cells during ART. We found evidence for three levels of selection: (1) IS locations within genes were very similar in vitro and in vivo. Hence, IS location distributions within genes appear to be appropriately modeled from in vitro infection data. (2) The survival of infected cells was associated with distinct HIV provirus orientation biases found only in vivo. Proviruses with the same transcriptional orientation as the gene were underrepresented, suggesting a loss of infected cells in which the provirus provides a poly(A) signal within its 5' LTR that could truncate cellular transcripts. A notable exception to this pattern was in genes with the largest number of unique IS, in which most or all IS were found in the same transcriptional orientation as the gene. Such a pattern could alternatively lead to suppression of the provirus (latency), or production of native or aberrant cellular gene products controlled by the proviral LTR. (3) The biological pathways of genes with IS that distinguished in vitro and in vivo infections. IS were found at unexpectedly high levels within an integrated network of genes involved in the regulation of the generation of memory T cell differentiation and homeostasis. This suggests mechanisms by which the viral genome may impact T cell function and proliferation.

**Ya-Chi Ho, MD, PhD** **INVITED SPEAKER**

*Assistant Professor, Department of Medicine, Johns Hopkins University School of Medicine*

## The expression of defective HIV-1 proviruses complicates HIV-1 cure strategies

R Pollack[1], RB Jones[2], M Pertea[1], KM Bruner[1], RF Siliciano[1], Ya-Chi Ho[1]

[1]Johns Hopkins University School of Medicine, Baltimore, MD; [2]George Washington University, Washington D.C.

HIV-1 persists in the latent reservoir, such as resting memory CD4+ T cells, which is the major barrier to cure. The majority of HIV-1 proviruses are defective due to packaging signal deletions, APOBEC-mediated G-to-A hypermutations, large internal deletions, and point mutations. We recently showed that in HIV-1-infected individuals treated with suppressive antiretroviral therapy, the HIV-1 proviral landscape may change due to clonal expansion and differential immune selection pressure. Even in resting CD4+ T cells, HIV-1 transcription is not silent. Both intact and defective HIV-1 proviruses, particularly those containing packaging signal deletions and hypermutations, can be transcribed and translated, producing viral proteins which can induce cytotoxic T lymphocyte (CTL) recognition. Cells containing defective proviruses with large internal deletions do not produce sufficient amount of viral antigen and will increase in frequency over time due to the lack of immune selection pressure.

The expression of defective HIV-1 proviruses complicates the measurement and eradication of the latent reservoir. First, since defective HIV-1 proviruses can produce readily detectable cell-associated and supernatant HIV-1 RNA and proteins, detection of low levels of HIV-1 RNA and proteins may reflect the expression of defective, instead of infectious, HIV-1 proviruses. Second, the scope of CTL targets, including cells containing defective HIV-1 proviruses, is larger than the size of the latent reservoir. Whether cells containing defective HIV-1 proviruses may induce immune activation, distract CTL clearance of the latent reservoir, or boost CTL immune responses as a therapeutic vaccine strategy, requires further investigation. Whether gene therapy and cell therapy approaches may eliminate cells containing defective HIV-1 proviruses and hence reduces immune activation remains to be explored.

**Benjamin Burwitz, PhD** **ORAL ABSTRACT**
(Presenting on behalf of Gabriela M. Webb, PhD)

*Staff Scientist, Vaccine and Gene Therapy Institute, Oregon Health and Science University*

## The human IL-15 superagonist complex ALT-803 drives SIV-specific CD8+ T cells into B cell follicles

GM Webb[1], S Li[2], G Mwakalundwa[2], JS Reed[1], JJ Stanton[1], AW Legasse[1], BS Park[1], MK Axthelm[1], EK Jeng[3], HC Wong[3], JB Whitney[4], RB Jones[5], DF Nixon[5], E Connick[6], PJ Skinner[2], JB Sacha[1]

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**Background:** There is an urgent need for alternate approaches to activate and clear the HIV reservoir that do not negatively impact immune function. An added hurdle to clearing the reservoir is the exclusion of CD8+ T cells from B cell follicles, anatomical sites that harbor latently-infected CD4+ T cells. IL-15 is a key cytokine for homeostatic maintenance, proliferation, and expansion of memory CD4+ T cells, the primary HIV cellular reservoir. Here, we explored the human IL-15 superagonist complex, ALT-803, as an immunostimulatory molecule in chronically SIV-infected rhesus macaques.

**Methods:** SIV-infected rhesus macaques were treated with a single intravenous dose of 100 ug/kg ALT- 803 and were subsequently assessed for intrafollicular migration of SIV-specific CD8+ T cells via in situ immunofluorescence staining of lymph nodes with MHC-class-I tetramers. Additionally, RNA in situ hybridization was employed to determine the number of SIV-producing cells within the follicles and extrafollicular regions of the lymph node.

**Results:** ALT-803 activated NK cells and memory T cells, causing them to undergo proliferation and home to secondary lymphoid tissues, an anatomical location of the viral reservoir. In situ MHC-class-I tetramer staining confirmed increased numbers of SIV-specific CD8+ T cells in lymph node, and revealed that the effector cells trafficked into B cell follicles. Accordingly, lower numbers of SIV-producing cells were found within B cell follicles in elite controllers post ALT-803 treatment indicating immune mediated clearance.

**Conclusions:** IL-15 superagonist, ALT-803, triggers massive proliferation of NK cells and CD8+ T cells and also reactivates quiescent SIV. ALT-803 also drives activated NK and CD8+ T cells into lymph nodes, and allows for SIV-specific CD8+ T cells to enter B-cell follicles harboring latently-infected CD4+ TFH cells. The ability of ALT-803 to potentially mediate the “shock” and “kill” and to grant CD8+ T cells access to lymph node sanctuary sites makes it an appealing candidate for studies aimed at durable cART-free HIV remission.

**Chantelle Ahlenstiel, PhD** **ORAL ABSTRACT**

*Research Fellow/Lecturer in Virology, University of New South Wales*

## Direct visualization of nanoparticle delivery of epigenetic gene silencing RNA to the nucleus of HIV-1 infected activated and resting CD4+ T cells

CL Ahlenstiel[1], EI Czuba[2], V Klemm[1], C Cortez-Jugo[2], SG Turville[1], F Cavalieri[2], F Caruso[2], AD Kelleher[1]

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**Background:** The latent virus reservoir is a major barrier to HIV cure. We are investigating a targeted functional cure approach utilizing epigenetic silencing RNA, which potently inhibit virus transcription. Efficient delivery of anti-HIV agents, such as lentiviral vectors or siRNA, for gene therapy in CD4+ T cells and macrophages is a substantial challenge. This study visualises siRNA delivery in CD4+ T cells using nanoparticle technology.

**Methods:** Human primary CD4+ T cells were activated using anti-CD2/CD3/CD28 and infected with VSV-G pseudotyped HIV virus expressing an mOrange reporter or a live variant HIV-1NL4.3 expressing GFP and an envelope with high CD4 affinity. In parallel, resting human primary CD4+ T cells were infected using the same live virus. Epigenetic silencing siRNA, siPromA, or its scrambled control were delivered 24 h post-infection in activated cultures or 5 days post-infection in resting cultures using a newly designed nanostructured thin film composed of Poly-L-arginine and Poly(sodium 4-styrenesulfonate). siRNA is embedded and stabilized in the multilayer film. Poly-L-Arginine promotes endosomal escape, releasing the siRNA into the cytosol. CD4+ T cell cultures were imaged using a DeltaVision Elite microscope to assess both virus infection and subcellular localization of siRNA. Arbitrary line intensity profiles were utilized to determine signal overlap and subcellular location.

**Results:** 81% and 71% of activated CD4+ T cells were infected with Pseudotyped virus or live HIV-1NL4.3 virus, respectively. Nuclear localization of siPromA was observed only in infected CD4+ T cells, with 12% of mOrange positive cells and 40% of GFP positive cells showing nuclear siPromA signal. 23% of resting CD4+ T cells were infected with variant HIV-1NL4.3 virus as judged by GFP. 15% of these cells showed a nuclear siPromA signal, confirmed by arbitrary line analysis. In contrast, while siScrambled RNA was detected in all CD4+ cells, it was only detected in the cytoplasm.

**Conclusion:** This is the first study using nanoparticle technology to deliver epigenetic silencing siRNA into the nucleus of CD4+ T cells. These results provide a pathway for targeting latent reservoirs by achieving uptake and release of RNAi vectors into the nucleus of resting CD4+ T cells.

**James Riley, PhD** **INVITED SPEAKER**

*Associate Professor, Department of Microbiology, University of Pennsylvania*

## Engineering HIV Resistance: A Crucial Component of an HIV- Cure Strategy

James L. Riley is speaking for the UPENN and Sangamo collaborative team.

CD4 T cells can be rendered HIV-1 resistant by deletion of CCR5 using zinc finger nucleases (ZFN) into HIV-1 infected individuals. These modified T cells not only persisted during ART interruption, but also exerted some control of HIV-1 replication in vivo. Subsequent clinical studies have validated and improved this approach by altering the method of ZFN delivery and pretreating individuals with cyclophosphamide to improve T cell engraftment after adoptive transfer. Several individuals have had sustained low viral loads (<1000 copies per ml) for up to 8 months after analytical treatment interruption. More recently we developed novel approach to confer resistance to HIV-1 infection. We fused an inhibitory peptide (C34) from the HIV-1 gp41 heptad-repeat 2 domain to the N-terminus of CXCR4. This construct exerted the most potent and broadly-acting antiviral activity we have yet observed on both R5- and X4-tropic viruses. A clinical trial was recently opened to study the ability of C34-CXCR4 to protect T cell after ATI and these preliminary results will be discussed and contrasting with the ongoing CCR5 ZFN studies.

**Jerome A. Zack, PhD** **INVITED SPEAKER**

*Professor, Department of Medicine; Chair, Department of Microbiology, Immunology & Molecular Genetics, David Geffen School of Medicine at UCLA*

## Optimizing a "Kick and Kill" Approach to Target Latent HIV Reservoirs

MD Marsden[1], TW Chun[2], PA Wender[3], JA Zack[1,4]

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The ability of HIV to establish a long-lived latent infection within resting CD4+ T cells leads to persistence and episodic resupply of the virus in infected individuals treated with antiretroviral therapy, thereby preventing eradication of the disease. Protein kinase C (PKC) modulators such as prostratin or bryostatin 1 can activate these latently infected cells, potentially leading to their elimination by virus-mediated cytopathic effects, the host's immune response and/or therapeutic strategies targeting cells actively expressing virus. The use of naturally occurring PKC modulators as a means to flush out latent reservoirs has been hampered by the limited availability of these compounds, and equally significantly by sub-optimal activity and in vivo tolerability. We have found that a designed, synthetically-accessible analog of bryostatin 1 is better-tolerated in vivo than the naturally occurring product, and potently induces HIV expression from latently infected cells. In vivo assessment of this compound in humanized BLT mice demonstrated substantial increases in HIV expression from the latent HIV reservoir. Importantly, this induction of virus expression triggered the death of some of these newly HIV-expressing cells, without addition of an additional "kill" step. Thus, designed, synthetically-accessible bryostatin analogs can mediate both a "kick" and "kill" response in latently-infected cells and exhibit improved tolerability, suggesting unique promise as clinical adjuvants for HIV eradication.

**Edward A. Berger, PhD** **ORAL ABSTRACT**

*Chief, Molecular Structure Section, Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health*

## Toward an HIV Functional Cure with CAR-T Cells: Elucidating Mechanisms Underlying Anti-HIV CARs with Optimized Potency/Breadth and Minimized Immunogenicity

EA Berger, A Hajduczki, S Bolivar-Wagers, MH Ghanem, DA Vargas-Inchaustegui, DT Danielson, D Elias, V Bundoc, B Dey, JE Seedorff

Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD

Chimeric antigen receptor (CAR) technology offers the prospect to achieve profound and durable T cell- mediated suppression of HIV in the absence of antiretroviral therapy. We believe that durable (life-long?) CAR activity demands a CAR that approaching two ideal properties: escape-proof and non-immunogenic. We have designed bispecific CARs containing human CD4 linked to a second human moiety recognizing a distinct highly conserved binding site on Env. The second moiety enhances potency, and prevents the CD4 from acting as an entry/infection receptor on transduced CD8+ T.

We previously reported (Liu et al. JVirol. 2015) extremely potent and broad activity of a CD4-based bispecific CAR containing an scFv against a highly conserved CD4-induced epitope within the gp120 coreceptor-binding region. However, to avoid the concern that the scFv variable regions would elicit anti- idiotypic antibody responses, we designed CD4-based CARs in which the second moiety is the non- immunogenic carbohydrate-recognition domain of a human C-type lectin; this component recognizes the oligomannose patch obligatorily displayed on gp120 of all clinical HIV-1 variants. Of several lectins examined, we obtained particularly impressive results with a CAR in which the second domain is from human mannose binding lectin (MBL). The CD4-MBL CAR displayed extremely potent activity against all tested HIV-1 clinical isolates from genetically diverse clades. In HIV/PBMC spreading infection assays, p24 release was fully suppressed (>4 logs, below detection). CD4-MBL CAR potency significantly exceeded that of the corresponding monospecific CD4 CAR; moreover, the lectin component fully blocked the entry receptor activity of the CD4 moiety.

Mechanistic questions are underway regarding the extreme potency of the bispecific CARs. An inducible expression system indicated that CAR-mediated killing occurred with surface Env levels below flow cytometry detection. Moreover, the markedly superior suppression of spreading HIV infection by CAR-T cells compared to a targeted immunotoxin suggest kinetic advantages associated with “killing from the outside”. Preliminary experiments using replication-incompetent pseudoviruses have led us to investigate whether CAR-T cells can kill targets by recognizing Env molecules introduced by incoming virions, independent of, and prior to, synthesis of new virus-encoded Env. These findings support advancing the CD4-MBL CAR into preclinical studies in relevant animal models.

**Bruce E. Torbett, PhD, MSPH** **INVITED SPEAKER**

*Associate Professor, Department of Immunology and Microbiology, The Scripps Research Institute*

## β-Deliverin: A Small Molecule Approach for Relieving Lentiviral Vector Transduction Resistance in Human Hematopoietic Stem Cells

BE Torbett

Department of Immunology and Microbiology, The Scripps Research Institute, La Jolla, CA

Lentiviral vectors (LVs) have been used to deliver genes to patient CD34+ hematopoietic stem and progenitor cells (HSPCs) for treatment of a variety of hematologic disorders and for anti-HIV therapy. Therapeutic benefit is dependent upon efficient LV gene transfer to hematopoietic stem cells (HSCs). Achieving clinically relevant LV transduction levels in HSCs can require moderate to high amounts of LVs, as well as ex vivo culture to enhance LV transduction of HSCs. LV production is costly, thus a potential impediment for clinical protocols, while ex vivo exposure of HSCs to cytokines is cost limiting and prolonged culture periods may lower HSC engraftment. Methodology to enhance the efficacy of LV transduction of HSCs, while lowering the amounts of LV utilized and decreasing ex vivo culture time, is desirable for clinical translation.

The HSPC mechanisms that restrict efficient LV entry and gene delivery remain poorly understood. To overcome the problem of inefficient LV gene delivery we have identified small molecules, rapamycin and β-deliverin, that enhanced LV gene delivery to HSPCs. β-deliverin treatment during LV transduction of mobilized peripheral blood derived HSPCs resulted in ~2-fold increase in EGFP levels when compared to vehicle treatment. β-deliverin also enhanced LV transduction of cord blood and non-human primate bone marrow derived HSPCs. Consistent with our ex vivo findings with HSPCs, β-deliverin treatment enhanced LV transduction and EGFP gene delivery to human long-term repopulating cells ex vivo as evidenced by increased and prolonged EGFP expression in myeloid and lymphoid lineages in HSPC transplanted NSG mice. We are utilizing β-deliverin as a chemical probe to interrogate hematopoietic stem cells to identify the cellular mechanisms that restrict gene delivery and to ultimately improve therapeutic gene delivery to hematopoietic stem cells. I will discuss our recent progress.

**David Rawlings, MD** **INVITED SPEAKER**

*Children's Guild Association Endowed Chair in Pediatric Immunology; Director, Center for Immunity and Immunotherapies, Seattle Children's Research Institute; Chief, Division of Immunology and Director, SCRI Program for Cell and Gene Therapy, Seattle Children's Hospital; Professor of Pediatrics and Immunology, University of Washington*

## Engineering protein-secreting plasma cells by homology-directed repair in primary human B cells

D Rawlings

Seattle Children's Research Institute, Seattle, WA; Seattle Children's Hospital, Seattle, WA; University of Washington, Seattle, WA

We will describe our novel methods for high-efficiency, homology-directed genome editing in primary human B cells for the purposes of producing therapeutic proteins. Adoptive transfer of B cells expressing anti-HIV therapeutics (antibodies or other agents) might be useful in the future for preventive and cure strategies. Such autologous cell products might also be helpful in promoting immune tolerance to such agents- a problem that may limit long-term clinical efficacy.

## **SESSION 5** SPEAKERS

### **Matthew Scholz** INDUSTRY INVITED SPEAKER

*Chief Executive Officer, Immunosoft Corporation*

Matthew Scholz is the founder and Chief Executive Officer of Immusoft, a biotech start-up firm that is developing a breakthrough technology that will turn a patient's B cells into miniature drug factories. Longer term, this technology has the potential to transform the landscape of biomedicine.

He will discuss the technology being developed by Immusoft and the promising implications for HIV.

**Howard E. Gendelman, MD** **ORAL ABSTRACT**

*Margaret R. Larson Professor of Internal Medicine & Infectious Diseases; Chair, Dept. of Pharmacology & Experimental Neuroscience, University of Nebraska Medical Center*

## Long Acting Slow Effective Release Antiretroviral Therapy

HE Gendelman, B Kevadiya, A Bade, B Edagwa

Department of Pharmacology Experimental Neuroscience, University of Nebraska Medical Center, Omaha, NE

**Background:** A major unmet need in human immunodeficiency virus therapeutics rests in the development of long acting slow effective release antiretroviral therapy (LASER ART) formulations for improving drug regimen adherence, attenuating secondary toxicities and preventing new infections. Notwithstanding, LASER ART design can enhance tissue and cell penetrance by enhancing lipophilicity, hydrophobicity and particle viral reservoir targeting. Such formulation design serves to facilitate viral clearance. We now show improved cell penetrance and intracellular depots of antiretroviral drugs (ARV) by creating unique biodistribution screening and cell and tissue delivery schemes.

**Methods:** Multimodal nanoprobes were made with the intent of screening ARV platforms. This was made possible by employing fluorescence and magnetic resonance imaging with europium (Eu) doped cobalt ferrite (EuCF) nanoparticles. The combined florescence drug validation techniques were used to facilitate measures of drug content and biodistribution. EuCF particles enabled dolutegravir (DTG) drug carriage and as such serve as a theranostic screen. The theranostic particles were consistent in size. Macrophage drug particle uptake and retention in subcellular depots was determined. Following EuCF screening, pharmacokinetic (PK) and pharmacodynamic (PD) tests were performed in replicate particles encased only in decorated poloxamers. In these experiments, myristoylated prodrugs were made as nanoformuations (designated NMDTG) optimized for size, shape, polydispersity, stability and lipophilicity.

**Results:** Particles demonstrated paramagnetic properties and as such were used to assess drug arrival in tissue reservoirs of viral infection. Eu provided fluorescence imaging for particle biodistribution. The addition of DTG facilitated testing of “real time” antiretroviral responses. Confocal microscopy showed co- localization of particles in phagolysosomal compartments confirmed by measures of Eu, drug, ferrite and cobalt. NMDTG PK tests performed in Balb/cJ mice showed drug lymphoid tissue and plasma DTG levels, at or above, its inhibitory concentration90 of 64 ng/mL for up to 56 days. NMDTG particles protected humanized mice from parenteral challenge of a 2 x 104 tissue culture infective dose50 of HIV-1ADA beyond three weeks. No secondary metabolic effects and drug was readily delivered to viral reservoirs.

**Conclusion:** This innovative platform for drug formulation development provides effective screening of decorated antiretroviral drug and could be used to facilitate viral elimination treatment strategies.

**Andrew Wong** **ORAL ABSTRACT**

*PhD Candidate, University of New South Wales*

## Platform for Resting Immune Cell Gene Delivery for a HIV cure

AKH Wong[1], A Aggarwal[1], M Churchill[2], C Ahlenstiel[1], J Zaunders[1], G Symonds[3], A Kelleher[1], D Cooper[1], S Turville[1]

[1]The Kirby Institute, The University of New South Wales, AU; [2]RMIT University, Melbourne, AU; [3]Calimmune and St Vincent's Applied Medical Research Institute, Sydney, AU

**Background:** In lentiviral based gene therapy efforts towards a HIV cure, there are two limitations that presently limit lentiviral delivery: the fusogenic capacity of the viral vector with the target population (defined here as CD4 and CD8 resting T cells), and restriction of reverse transcription in resting immune cell types.

**Methods:** To increase the fusogenic capacity of lentiviral vectors for CD4 T cells, we short-listed 10 lead HIV envelopes based on their ability to use low levels of CD4; these were identified from a repository of over 1000 envelopes characterised using the HIV Affinofile assay. For CD8 T cells, we evolved lead HIV envelopes to be CD4 independent. To increase reverse transcription in resting T cells, we screened 164 Vpx variants for their ability to i) initially enhance HIV NL43 infection and ii) increase gene delivery using modified lentiviral vectors. From this pool, we selected 4 leads that would ensure this enhancement of infection was only mediated in the short term and would not render genetically modified cells more permissive to HIV upon re-infusion. Finally, we tested our lead HIV envelope/Vpx combinations using constructs that either transcriptionally silence (PromA) or render cells protected from HIV infection (Cal- 1).

**Results:** Using this combined bioprospecting/evolution approach, HIV envelope sequences were identified with fusogenic potential of greater than 90% for untouched resting memory and naïve T cells (n = 12) and that when combined with 4 lead Vpx variants resulted in greater than 30% gene delivery (n = 10). The majority of Vpx variants enhanced gene delivery and HIV infection over 14 days, and this directly led to antagonism of vectors that mediate HIV gene silencing. Importantly, we identified one Vpx variant that lacked this antagonism due to its ability to enhance gene delivery in the short term.

**Conclusion:** Herein we have identified a gene delivery pipeline for resting T cells that leverages the fusogenic potential of HIV-1 and the enhancing potential of Vpx. This has enabled us to produce gene delivery benchmarking at exceptionally high levels required for clinical trials, with combinations that ensure genetically modified cells are resistant to HIV infection.

**Nancy L. Haigwood, PhD** **INVITED SPEAKER**

*Senior Scientist; Adjunct Professor of Molecular Microbiology and Immunology; Director of the Oregon National Primate Research Center; Oregon Health and Science University*

## Antibodies as New Therapies For HIV

NL Haigwood

Oregon Health and Science University, Portland, OR

**Background:** Antibody therapy is being considered as a promising approach for the prevention of both mother-to-child HIV transmission and as PrEP, and may have applications in the clearance of established viremia. In infant rhesus macaques, we observed clearance of SHIV infectious centers and no establishment of tissue reservoirs following administration of broadly neutralizing human monoclonal antibodies (bNmAbs) at 24 hours after high-dose oral challenge with SHIV-SF162P3. We hypothesized that the timing of intervention is crucial to fully ablate infection.

**Methods:** To further examine how bNmAb treatment impacts viremia and influences the development of endogenous immunity, we tested the timing of intervention by delaying bNmAb treatment until 48 hours after infection. Infant macaques received 4 doses of 10 mg/kg bNmAb cocktail (PGT121 and VRC07-523) subcutaneously, beginning 48 hours after oral exposure to SHIV-SF162P3. We monitored blood and tissues to quantify viral replication and reservoirs.

**Results:** Postponing treatment yielded variable degrees of control with respect to set-point viremia, reservoir establishment, and endogenous antibody and T cell responses. Five of six infants exhibited attenuation in plasma- and cell-associated viral load, of which three developed no adaptive responses and showed near-complete control of SHIV with only a few tissues positive at necropsy. Moderate viremia was observed in conjunction with more potent NAb development and a stronger CD8+ T cell response. In contrast, an infant with low viremia had weak neutralization but strong antibody binding, indicating potential Fc-effector mechanisms that merit further study.

**Conclusion:** Together, these results suggest that moderate viremia favors the development of viral-specific CD8+ T cells and potent NAbs, while lower viremia promotes alternative protective responses. We conclude that varying levels of set-point viremia are associated with distinct mechanisms of immunological control in the context of delayed bNmAb treatment. Studies are currently underway to understand the mechanism(s) of action of bNmAbs in viral clearance.

**Karine Dubé, DrPH** **INVITED SPEAKER**

*Assistant Professor, UNC GIllings School of Global Public Health*

## 'We Need to Deploy Them Very Thoughtfully and Carefully': Perceptions of Analytical Treatment Interruptions in HIV Cure Research in the United States – A Qualitative Inquiry

K Dubé[1], D Evans[2,3], L Dee[2,4,5], L Sylla[6], J Taylor[5,7], A Skinner[1,8], BJ Weiner[1, 9], SB Greene[1], S Rennie[10], JD Tucker[11,12]

[1]University of North Carolina Gillings School of Global Public Health, Chapel Hill, NC; [2]Delaney AIDS Research Enterprise (DARE) Community Advisory Board (CAB), San Francisco, CA; [3]Project Inform, San Francisco, CA; [4]AIDS Action Baltimore, Baltimore, MD; [5]amfAR Institute for HIV Cure Research CAB, San Francisco, CA; [6]defeatHIV CAB, Seattle, WA; [7]Collaboratory of AIDS Researchers for Eradication (CARE), Palm Springs, CA; [8]Duke Clinical Research Institute (DCRI), Durham, NC; [9]Department of Global Health, University of Washington, Seattle, WA; [10]Department of Social Medicine and UNC Center for Bioethics, Chapel Hill, NC; [11]UNC Project China, Guangzhou, China; [12]UNC Institute of Global Health and Infectious Diseases (IGHID), Chapel Hill, NC

Strategies to control HIV in the absence of ART are needed to cure HIV. However, such strategies will require analytical treatment interruptions (ATIs) to determine their efficacy. We investigated how U.S. stakeholders involved in HIV cure research perceive ATIs. We conducted 36 in-depth interviews with three groups of stakeholders: 12 people living with HIV (PLWHIV), 11 clinician-researchers, and 13 policy-makers/bioethicists. Qualitative data revealed several themes. Our qualitative data revealed four main themes across all three stakeholder groups. First, there was little consensus on when ATIs would be ethically warranted. Second, the most frequent perceived hypothetical motivators for participating in research on ATIs were advancing science and contributing to society. Third, risks related to viral rebound were the most prevalent concerns related to ATIs. Stakeholders suggested ways to minimize the risks of ATIs in HIV cure research. Increased cooperation between scientists and local communities may be useful for minimizing risk. Further ethics research is necessary.

**Paul Munson** **ORAL ABSTRACT**

*Graduate Student, Department of Microbiology, University of Washington*

## T-cell Exhaustion Limits Therapeutic DNA Vaccine Immunogenicity in SIV Infected, cART Treated Macaques

P Munson[1], H Tunggal[2], N Hajari[2], M O'Connor[2], D Bratt[2], J Fuller[2], D May[2], S Wangari[2], B Agricola[2], J Smedley[2], X Hu[3], B Felber[3], G Pavlakis[3], J Mullins[1], D Fuller[2]

[1]Department of Microbiology, University of Washington, Seattle, WA; [2]Washington National Primate Research Center, Seattle, WA; [3]Human Retrovirus Pathogenesis Section, National Cancer Institute, Frederick, MD

**Background:** Therapeutic vaccines could augment HIV-specific immunity and prevent viremia in the absence of combination antiretroviral therapy (cART), otherwise known as "sustained remission" or a "functional cure." Specifically, improving HIV-specific CD8 T-cell responses may be necessary for increasing viral control. However, SIV/HIV infection causes T-cell exhaustion, defined as T-cells with poor effector function, that may limit therapeutic vaccine immunogenicity but may be restored, in part, by cART. To investigate, we analyzed the frequency of T cells expressing the exhaustion marker programmed death-1 (PD-1) prior to therapeutic vaccination to determine if the frequency of PD-1 expression limits magnitude and polyfunctionality of the SIV-specific T cell response subsequently induced by DNA vaccines expressing either a full length SIV Gag (FL) or conserved elements (CE) from SIV Gag.

**Materials & Methods:** SIV infected macaques initiated cART six weeks post infection and were vaccinated after 3 months on cART (week 18) received a series of 5 monthly doses of Mock (N=8), FL (N=8), CE (N=6) DNA vaccines by gene gun. FL and CE vaccines were co-formulated with the genetic mucosal adjuvant LT. PD-1 frequencies (%) were measured on CD4 and CD8 T-cells by flow cytometry prior to vaccination. Vaccine induced SIV-specific T cell polyfunctionality (T cells expressing 3 or more of the cytokines IFNg, TNFa, IL-2, and/or CD107a and Granzyme B) was measured by flow cytometry. Differences between time points were determined by a Wilcoxon signed rank test and correlations between the frequency of T cells expressing PD-1 and immune responses were analyzed by a Spearman test.

**Results:** Pre-vaccination (Week 18) PD-1 frequencies of CD4 ($P=0.008$, $r = -0.69$) and CD8 ($P=0.032$, $r=-0.58$) T-cells inversely correlated with the frequency of polyfunctional SIV specific T cells measured 2 weeks after the 5th dose DNA vaccine dose (Week 36), demonstrating that a T-cell exhaustion prior to vaccination may decrease vaccine responsiveness.

**Conclusion:** PD-1 frequencies on CD4 and CD8 T-cells prior to vaccination limit therapeutic DNA vaccine immunogenicity. Our results further suggest that approaches to limit T-cell exhaustion, may be an effective strategy for increasing the efficacy of therapeutic vaccines for HIV.

**Anne-Sophie Kuhlmann, PhD** **ORAL ABSTRACT**

*Post-Doctoral Research Fellow, Fred Hutchinson Cancer Research Center*

## Persistence of broadly neutralizing antibody-secreting hematopoietic cells in humanized mice

AS Kuhlmann, KG Haworth, C Ironside, MA Giese, HP Kiem

Fred Hutchinson Cancer Research Center, Seattle, WA

Broadly Neutralizing Antibodies (bNAbs) development by vaccination has been challenging but pre- or post-exposure prophylactic passive administration as an alternative has shown efficiency. bNAbs can neutralize circulating viral particles and actively target HIV-infected cells expressing HIV envelope. Importantly, bNAb therapy demonstrated an impact on the viral reservoirs which will be essential to achieve a cure. However, despite efforts to engineer antibodies to improve their potency and half-life in vivo, bNAbs do not persist leading to viral rebound. Gene-modified Hematopoietic Stem and Progenitor cells (HSPCs) could address this issue as their self-renewal capacity would facilitate sustained transgene delivery for the lifetime of an individual.

The potential of gene-modified CD34+ cells to support the secretion of bNAbs ex vivo and in vivo following transduction by a lentiviral vector was examined. Both human and non-human primates CD34+ cells can secrete bNAbs ex vivo. Antibody secretion was next evaluated in vivo using immunodeficient NOD-SCID-gamma (NSG) mice infused with unmodified (mock), gene-modified (GFP only) or bNAb- secreting human CD34+ cells producing PGT128 or VRC01 bNAbs. Similar engraftment efficiencies were observed and gene-marked cells from all groups efficiently gave rise to human lymphoid and myeloid lineages detected in the peripheral blood for up to eight months. Persistent engraftment was further confirmed by the presence of gene-modified cells in the bone marrow, spleen and thymus at the time of necropsy. Finally, PGT128 and VRC01 bNAb secretion was detected in the peripheral blood for over eight months, with bNAbs' mRNA observed in T and B lymphocytes.

The ability of the gene-modified antibody-producing hematopoietic cells to engraft, differentiate in vivo in humanized mice and to sustainably secrete antibodies highlight the promising potential of using HSPCs as a means to deliver anti-HIV therapeutics. Additionally, HSPCs mature and differentiate into all blood cell lineages with the potential to traffic to relevant viral reservoir sites. Future development will focus on optimizing the secretion and analyzing trafficking to the reservoir compartments. Importantly, this approach could be applied to other diseases in which current treatment include antibody expression, but require multiple injections for sustainable efficiency.

**Leslie S. Kean, MD, PhD** **INVITED SPEAKER**

*Associate Center Director, Ben Towne Center for Childhood Cancer Research, Seattle Children's; Associate Professor, Department of Pediatrics, University of Washington; Full Member, Fred Hutchinson Cancer Research Center*

## Non-Human Primate Model Reveals Increase of SHIV Reservoir Early after Allogenic Bone Marrow Transplantation

L Kean

Seattle Children's Research Institute, Seattle, WA; Seattle Children's Hospital, Seattle, WA; University of Washington, Seattle, WA

**Background:** Three components are thought to have contributed to the Berlin patient's cure following BMT: myeloablative conditioning, graft-versus-viral reservoir (GVVR), and the CCR5Δ32 donor cells, yet the contributions of each to reservoir clearance is unknown. To dissect the role of myeloablative conditioning, GVVR and viral resistance on the SHIV reservoir, we developed the first NHP model for allo-BMT in SHIV-infected, cART-treated rhesus macaques.

**Methods:** We intravenously infected 6 animals with SHIV-1157ipd3N4 and left them untreated for six months, followed by six months of cART (Raltegravir, PMPA and FTC). 3 animals remained untransplanted, and 3 animals received myeloablative TBI (1020 Gy) followed by haplo-identical BMT without ART discontinuation. Donor chimerism was monitored, and viral DNA and RNA were measured by qPCR in ~35 tissues following necropsy.

**Results:** All animals showed peak viral titers in the range of ~107 copies/ml ~2 weeks post-infection, which subsequently reached steady-state. 1 animal controlled viremia before ART initiation (day 184). In the other animals, plasma viral RNA became undetectable 2-3 weeks post ART initiation. The 3 transplanted animals were euthanized at day 47, 29, and 9 post-transplant, due to infection, acute graft-versus-host disease, and renal complications, respectively. All transplanted recipients engrafted with donor cells. Despite undetectable viremia post-transplant, viral DNA quantification in hematopoietic and other major organs including the CNS, revealed an increased reservoir in transplanted non-controllers versus untransplanted controls.

**Conclusions:** Our results indicate that the DNA reservoir may increase early after transplant, despite control of peripheral viremia. This suggests that transplant represents a significant initial 'shock' with loss of anti-HIV immunity contributing to reservoir enlargement, potentially explaining the rebound observed in the Boston patients. We believe that full recovery of anti-HIV immune control may be restored if additional HIV resistance factors and/or anti-HIV strategies are incorporated post-transplant to enhance the targeted killing of infected cells.

**Jonah B. Sacha, PhD** **INVITED SPEAKER**

*Associate Professor, Vaccine and Gene Therapy Institute, Oregon Health and Science University*

## A Fully MHC-matched Nonhuman Primate Allogeneic Stem Cell Transplantation Model: Understanding the Mechanism of HIV Cure in Timothy Brown

BJ Burwitz[1,2], HL Wu[1,2], S Abdulhaqq[1,2], C Shriver-Munsch[2], T Swanson[2], AW Legasse[2], KB Hammond[1,2], SL Junell[5], J Reed[1,2], BN Bimber[2], JM Greene[1,2], GM Webb[1,2], M Northrup[1,2], W Laub[5], P Kievit[2], R MacAllister[2], MK Axthelm[1,2], R Ducore[2], A Lewis[2], LMA Colgin[2], T Hobbs[2], LD Martin[2], CR Thomas Jr[5], A Panoskaltsis-Mortari[3], G Meyers[4], JJ Stanton[2], RT Maziarz[4], JB Sacha[1,2]

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Timothy Brown remains in full HIV remission following an allogeneic hematopoietic stem cell transplant (HSCT). Three potential reservoir-clearing mechanisms exist which may explain this remission: 1) myeloablative immune-conditioning chemotherapy 2) graft-versus-host immunity, or 3) the ccr5Δ32/Δ32 stem cell graft. Attempts to repeat Mr. Brown's HSCT-mediated cure in the clinic have failed, necessitating a clinically relevant animal model to understand the mechanisms of his cure. The complex immunogenetics of rhesus macaques (Macaca mulatta) precludes their use as a fully MHC-matched HSCT model to achieve stable full donor chimerism. Here, using reduced intensity conditioning and mobilized peripheral blood HSCT in unrelated, fully MHC-matched Mauritian-origin cynomolgus macaques (MCM), we demonstrate a spectrum of diverse clinical HSCT outcomes including primary and secondary graft failure, lethal GVHD, and stable, disease-free full donor engraftment. In durably donor-engrafted HSCT recipients, we observe complete replacement of recipient CD4+ T cells, including CD4+ T follicular helper cells in the lymph node, a known cellular reservoir of HIV. Utilizing this new preclinical allogeneic HSCT model HSCT, we are currently elucidating the mechanisms contributing to the full HIV remission seen in Timothy Brown.

**Christopher Peterson, PhD** **INVITED SPEAKER**

*Staff Scientist, Fred Hutchinson Cancer Research Center*

## Enhancing Active and Passive HIV Cure Approaches in the Nonhuman Primate Model

C Peterson[1,2], A Zhen[3], C Deleage[4], A Reik[5], MC Holmes[5], JD Estes[4], S Kitchen[3], HP Kiem[1,2]

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**Background:** A substantial body of evidence suggests that active targeting of persistent HIV reservoirs may be an important supplement to passive approaches, i.e. the generation of infection-resistant cells. We have applied and evaluated many such strategies in the nonhuman primate model, including active targeting using CD4-based Chimeric Antigen Receptors (CD4CAR), and passive protection of infection-susceptible cells using CCR5 editing and small peptide fusion inhibitors. We are interested in identifying the most promising and clinically relevant combinatorial therapies to eradicate persistently infected cells in our large animal model, and in patients.

**Methods:** In our studies, macaques are infected with simian/human immunodeficiency virus (SHIV) containing an HIV envelope, and suppressed by combination antiretroviral therapy. Following stable suppression, hematopoietic stem and progenitor cells (HSPCs) are CCR5-edited with Zinc Finger Nucleases, or transduced with lentiviral vectors expressing HIV/SHIV-specific CD4CAR and the peptide fusion inhibitor mC46. To quantify impacts on peripheral and tissue reservoirs, persistently infected cells are measured using cell-associated SHIV DNA and RNA, DNAscope and RNAscope, and quantitative viral outgrowth assays.

**Results:** Autologous transplantation with CCR5-edited HSPCs leads to significant decreases in tissue-associated SHIV DNA and RNA levels. However, in situ analyses clearly demonstrate that persistent viral reservoirs remain. In animals that receive CD4CAR modified HSPCs, CD4CAR expression persists for years, and is associated with decreased tissue-associated SHIV RNA levels and higher CD4:CD8 ratios in the gut. The number and function of these cells is proportional to the level of virus antigen. Finally, our data demonstrate that the toxicity of broadly cytotoxic conditioning regimens such as myeloablative irradiation outweighs their impact on reservoir size.

**Conclusions:** HSPCs can be gene-modified and rendered HIV/SHIV-resistant, but must persist in higher numbers in vivo in order to impact persistent infection. Combination approaches such as mC46 + CD4CAR engender a potent source of infection-resistant, virus-specific immune cells. Applying these strategies in the setting of reduced intensity conditioning will enable more effective reservoir targeting with substantially less toxicity. Most importantly, HSPC-based therapies provide a life-long source of “reservoir sentinels” that will augment the host response to recrudescent viral replication.

**Kamel Khalili, PhD** **ORAL ABSTRACT**

*Laura H. Carnell Professor and Chair, Department of Neuroscience; Director, Center for Neurovirology and Comprehensive NeuroAIDS Center, Lewis Katz School of Medicine at Temple University*

## Synergism between CRISPR/Cas9 and Long Acting ART leads to elimination of HIV-1 with no viral rebound in humanized mice

K Khalili[1], R Kaminski[1], R Bella[1], WB Young[1], P Dash[2], B Edagwa[2], S Gorantla[2], HE Gendelman[2]

[1]Department of Neuroscience, Lewis Katz School of Medicine at Temple University, Philadelphia, PA; [2]Department of Pharmacology and Experimental Neuroscience, University of Nebraska, Omaha, NE

**Background:** Advances in gene editing technology especially CRISPR/Cas9 and its in vivo delivery by viral vectors (Temple University, Philadelphia) and cell-based nanotechnology for long acting slow effective release antiretroviral therapy, LASER ART, and humanized mice (University of Nebraska, Omaha) were joined to facilitate eradication of HIV-1.

**Methods and Results:** First, optimal medicinal and polymer chemistry modified antiretroviral drug delivery to ensure maximal restriction of viral growth, biodistribution and pharmacokinetic through the generation of hydrophilic drug crystals. Second, the bioavailability/activity of the CRISPR/Cas9 designed for excision of intra-chromosomal HIV-1 DNA sequences were realized in tests of transgenic mice and rats. Intravenous administration of AAV9-CRISPR/Cas9 resulted in excision of a DNA fragment in various tissues and caused a substantial decrease in the level of viral gene expression in blood cells. Third, a humanized mouse model of HIV-1 disease was made whereas 17 week-old CD34 NOD/Scid-IL-2 RYcnull mice were infected with HIV-1NL4-3. After assessment of the immune profile reconstitution and stable viral load measures, animals were treated with LASER-ART and after seven weeks when virus was undetectable, fourteen animals (7 mice/group) were randomly chosen to receive no additional therapy or CRISPR/Cas9. Five weeks later, peripheral blood viral load, immune cell profile, measures of HIV-1 DNA/RNA, viral genotyping/sequencing were performed to evaluate the efficiency of viral excision and gene editing. Resurgence of virus in a range of 1.8x105-1.5x106 RNA copies/ml was observed in a group of animals receiving LASER ART or the additional AAV9-CRISPR/Cas9 control despite viral excision in the latter group. No excision was seen in the LASER-ART. In the group who received the combination therapy, two mice showed complete restoration of CD4+ T cells and no viral rebound. Results from RT-PCR, ddPCR, RNA/DNA scope failed to reveal any evidence for the presence of full length viral DNA. Examination of total genomic DNA for the presence of viral DNA showed robust excision of the viral DNA by CRISPR/Cas9 at the expected locations with respect to the signature PAM domain.

**Conclusion:** This is a proof-of-concept study indicates LASER ART and CRISPR/Cas9 performed in synergy is able to eliminate replication-competent HIV in an infected animal.

**Olivier Pernet, PhD** **ORAL ABSTRACT**

*Professional Researcher Assistant/Junior Faculty, UCLA AIDS Institute*

## Highly efficient Sendai Virus mediated CRISPR/CAS delivery for CCR5 editing in hematopoietic stem cells

O Pernet[1], A Park[2], R Watkinson[2], W Khamaikawin[1], P Thibault[2], P Hong[2], B Lee[2], DS An[1]

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**Background:** A HIV cure is possible as demonstrated by the Berlin patient who was cured of HIV after bone marrow transplantations with CCR5-Δ32/Δ32 homozygous deficient donor cells. The ease and versatility of the CRISPR/Cas9-mediated gene editing technology has spurred an immense interest in using it to edit the CCR5 gene for anti HIV human hematopoietic stem/progenitor cells (HSPCs) based gene therapy strategies. To replicate the success of the Berlin patient, a development of a highly efficient CRISPR/Cas9 delivery system is critical.

**Methods:** To maximize the levels of CCR5 gene modification in HSPCs, we recently developed a novel thermo sensitive/replication restricted Sendai virus vector for highly efficient CRISPR-Cas9 mediated editing of the CCR5 gene (ts SeV-Cas9-CCR5) in human cells with minimal off-target effects.

**Results:** Ts SeV-Cas9-CCR5 consistently transduces human fetal liver derived and G-CSF mobilized peripheral blood CD34+HSPCs with high efficiencies (~90%) at a multiplicity of infection of 5, including the CD34+/CD38-/CD90+(Thy1+)/CD49fhigh subpopulation capable of hematopoietic reconstitution by a single cell in humanized NSG mouse. Remarkably, our SeV-Cas9-CCR5 also edits at unprecedented frequencies (~80%) after 2days of vector transduction in the absence of any selection. Shifting to 37°C resulted in rapid loss of the SeV vector. Ts SeV-Cas9-CCR5 vector transduced CD34+ HSPCs differentiate into various lineage-specific CFUs by standard in vitro colony forming assays and CD14+/CD33+ macrophages in vitro, indicating no or minimum impact on hematopoietic differentiation potential.

**Conclusion:** Our current study demonstrated unprecedented efficiency (~80%) of CCR5 gene editing in human HSPCs by a newly developed SeV CRISPR/Cas9 delivery vector system. SeV is non-pathogenic in humans, has an established safety record, and has been extensively studied and modified for gene therapy applications. Introducing Ts mutations into replication-deficient SeV vectors (e.g. by removing the fusion protein essential for virus entry) will likely synergistically enhance the safety of our SeV-Cas9- CCR5 vector for clinical applications requiring highly efficient gene editing in HSPCs for HIV cure.

**Stefan Radtke, PhD** **ORAL ABSTRACT**

*Post-Doctoral Research Fellow, Fred Hutchinson Cancer Research Center*

## Identification and characterization of a blood stem cell-enriched cell population: Implications for gene therapy for HIV

S Radtke[1], JE Adair[1,2], MA Giese[1], Y Chan[1], ZK Norgaard[1], M Enstrom[1], KG Haworth[1], LE Schefter[1], HP Kiem[1,2,3]

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**Background:** The only functional cure for HIV so far has been reported for the Berlin patient who underwent a hematopoietic stem cell (HSC) transplant from a matched, allogeneic, HIV-resistant donor carrying the delta 32 mutation. Ever since, introducing the CCR5 mutation into the patient's own (autologous) HSCs has become a highly attractive approach to HIV Cure. Most if not all current HSC- mediated gene therapy strategies use CD34+ cells. However, most of these cells are not repopulating cells and do not contribute measurably to reconstitution. Thus, the goal of this study was to further refine the target cell population for gene therapy and gene editing strategies.

**Methods:** We first defined phenotypically and functionally distinct hematopoietic stem and progenitor cell (HSPC) subpopulations in our pre-clinical nonhuman primate (NHP) stem cell transplant and gene therapy model. Long-term multilineage engraftment potential of candidate cell fractions was tested in competitive repopulation experiments in the autologous, myeloablative setting. Finally, equivalent human HSC-enriched cell fractions were identified using RNAseq.

**Results:** Here, we demonstrate that the phenotype of CD34+CD45RA-CD90+ cells is highly enriched for HSCs, conserved between human and NHP, and exclusively required for rapid short-term as well as sustained long-term multilineage engraftment. This phenotype accounts for ~3-5% of the entire CD34+ cell population, reducing the number of cells targeted for gene therapy approaches by 20 to 30-fold. Most importantly, we established a flow-cytometry based strategy to identify, quantify and purify this HSC- enriched phenotype in human and NHP stem cell sources. This assay was able to quantitatively predict transplant success as well as the number of days to neutrophil and platelet recovery following myeloablative autologous transplantation in NHPs regardless of the stem cell source, gene modification or ex vivo expansion.

**Conclusion:** This novel strategy will allow us to overcome current limitations of HSC-mediated gene therapy approaches for HIV. Purification of CD34+CD45RA-CD90+ cells will improve targeting of primitive HSCs and thus likely increase efficacy and long-term outcome. Importantly, significant reduction in cell number will result in a dramatic decrease in reagents required for gene modification (i.e. lentiviral vectors or nucleases), lower overall costs, and ultimately improve feasibility.

**Genoveffa Franchini, MD** **INVITED SPEAKER**

*Senior Investigator, Vaccine Branch; Head, Animal Models and Retroviral Vaccines Section, National Cancer Institute, National Institutes of Health*

## Innate and adaptive responses associated with vaccine-mediated reduced risk of $SIV_{mac251}$ acquisition

G Franchini

Animal Models and Vaccine Section, National Cancer Institute, National Institutes of Health, Bethesda, MD

The RV144 HIV-vaccine trial, with a Canarypox-based vector (ALVAC-HIV) and AIDSVAX HIV B/E gp120 proteins in alum, resulted in limited but significant protection from HIV acquisition. Serum IgG against the envelope variable regions 1 and 2 (V1/V2) inversely correlated with the risk of HIV-1 infection, and sieve analysis demonstrated genetic markers of immunologic pressure within V2. We tested whether we could reproduce the protection observed in RV144 in macaques using ALVAC/gp120/alum SIV vaccine candidate, and whether we could improve the efficacy of this vaccine regimen by changing adjuvant (MF59), the prime (DNA or Ad26) or the vector (NYVAC rather than ALVAC). The combination of DNA /ALVAC/ gp120/Alum appeared to provide superior vaccine efficacy in this animal model. We exploited the macaque model to identify systemic and mucosal innate and adaptive correlates of risk of SIVmac251 acquisition. We used a system biology approach that combined cellular, humoral, and transcriptional outputs to identify such correlates of protection. Additionally, bioinformatics allowed us to generate an integrated model providing insights into the mechanisms leading to protection. We found that in the ALVAC/gp120/alum regimen vaccine efficacy was associated with alum-induced envelope-dependent mucosal Innate Lymphoid Cells (ILC) producing IL-17, mucosal V2 peptide-specific IgG and the expression of 12 genes in PBMCs, seven of which are part of the RAS pathway. Strikingly, the expression of 11 of the 12 genes was associated with the induction of ILC and of 3 of the 12 with mucosal V2 antibodies. In addition, monocytes' frequency and their associated genes were also associated with the risk of virius acquisition in the DNA /ALVAC/gp120/Alum regimen. The association of RAS activation and vaccine efficacy was independently observed for other efficacious vaccine modality, such as the DNA/ALVAC/gp120/alum. An independent functional validation of the association of RAS with a decreased risk of SIV acquisition was obtained by measuring activated RAS in extracellular vesicles (EV) from plasma of animals that had a delayed acquisition of SIVmac251. Thus, RAS activation, mucosal innate cells, and antibodies to V2 may be important hallmarks of SIV, and hopefully HIV vaccine efficacy.

**Francois Villinger, DVM/PhD** **INVITED SPEAKER**

*Director, New Iberia Research Center, University of Louisiana at Lafayette*

## Dynamics of generalized Simian Immunodeficiency Virus Replication in vivo mapped by PET/CT Imaging

P Santangelo[1], K Lindsay[1], K Rogers[2], C Zurla[1], S Byrareddy[3], J Arthos[4], C Cicala[4], A Ansari[5], F Villinger[2]

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The quantification of HIV replication relies on indirect methods such as plasma viral loads, which does not indicate the anatomic origin of the virus detected. In addition, potential residual virus replication seen during HAART or in Elite controllers cannot be confirmed or localized, nor are sites of functional reservoirs identified from which virus replication rekindles upon ART cessation. To address this issue, we developed a non invasive imaging technology to map SIV and HIV infection in real time, allowing for repeated evaluations, monitoring of treatment and treatment interruption and evaluation of the host cell depletion in vivo. Using an anti gp120 monoclonal F(ab')2 fragments labelled with Cu64, we have developed a novel strategy to image SIV, HIV in infected cells as well as CD4 T cells in vivo using positron emission tomography / computer tomography (PET/CT). PET/CT results were corroborated with SIV mRNA levels, and in situ detection and the method was extended to document the relative distribution of CD4+ T cells before and after infection. Using immunoPET/CT, maps of active viral replication are collected in real time, allowing for monitoring organ specific extinction of viral signals during ART inception, sites of residual virus signals during ART and prominently sites of (initial) viral rebound post ART interruption. The technique has also allowed us to monitor SIV and CD4 T cell dynamics during and post treatment of SIV infected monkeys with anti-α4β7 monoclonal antibodies, allowing for total body monitoring of the effects of the therapy and conditioning. We submit that immunoPET provides for a sensitive real time imaging method to evaluate and localize virus replication in vivo as well as the distribution and potential tissue specific reconstitution of target CD4+ T cells in vivo, as a novel tool to contribute to viral eradication in HIV infection.

**Nick Llewellyn, PhD** **ORAL ABSTRACT**

*Post-Doctoral Research Associate, University of Southern California*

## A Humanized Mouse Model of Latently HIV Infected Microglia

GN Llewellyn[1], D Alvarez-Carbonell[2], M Chateau[1], J Karn[2], P Cannon[1]

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The reservoir of latently infected cells that persists during ART is believed to consist mostly of infected memory T cells. However, autopsy samples have revealed that the brain also contains HIV reservoirs, in particular in microglia. These are the macrophage-like primary immune cells of the brain, and may also contribute to viral rebound after ART cessation. Studying microglia is challenging, since brain samples cannot easily be obtained from living patients, and cells of the brain die rapidly within hours of death. Thus, in order to better study the role of microglia in HIV persistence and latency, a small animal model would be immensely beneficial.

Using human HSC transplanted 'humanized' NSG mice, we readily identified cells in the brains of the mice that expressed both human and microglia specific markers. The number of such human microglia are low, ~0.5% of the total glial cells of the mouse brain, but can be increased 3-4-fold by conditioning the mice with busulfan instead of irradiation prior to injection of the HSC. By infecting humanized mice with HIV and then suppressing plasma viremia using ART, we were able to identify HIV-infected microglia in the brains of the mice, and demonstrate a significant reduction in the numbers of infected cells in the brain under ART.

In addition, it has recently been shown that the transcriptional repression pathways leading to HIV latency are distinct in T cells and microglia (Alvarez-Carbonell, 2017). An shRNA screen and follow-up analyses in microglial cell lines identified both monoamine oxidase and the CoREST complex, which is a transcriptional regulator, being specifically involved in latency in microglial but not T cell lines. Latently infected microglia isolated from the brains of the humanized mice on ART also responded to treatment with drugs that target these two pathways, leading to re-activation of HIV, and setting the scene for studies to evaluate the effect of such drugs on the latent reservoir in the brain in vivo.

**Morgan Chateau, PhD**

*Post-Doctoral Fellow, University of Southern California*

## Humanized mouse with Human B-cell follicles – a potential latency model to test HIV cure strategies

M Chateau, P Cannon

Department of Molecular Microbiology and Immunology, University of Southern California, Los Angeles, CA

**Background:** Progress in HIV cure strategies have highlighted how organization of the lymphoid tissues is a major obstacle for HIV eradication. Specifically, the "B-cell reservoir" is characterized as HIV infected T- cells which "hide" from immune surveillance and ART drugs inside the B-cell regions of lymphoid tissues (lymph nodes, spleen, MALT, etc). This level of organization does not exist in current humanized mouse models. To fill this need, we are developing a better humanized mouse that has human tonsil tissue implants which recreate the anatomical and immunological challenges for cure strategies to be tested on. Our pilot studies have given very encouraging results showing that 1) human tonsil tissue can be safely implanted into NSG animals, 2) implanted tonsil retain immune architecture for at least 5 weeks post implant, and 3) immune cells originating from the implanted tissue can rapidly repopulate the mouse systemically.

**Methods:** Immune deficient mice (NSG) are implanted with human tonsil tissues subcutaneously. As of now, two tonsils have been used to generate 8 mice. Various subcutaneous locations were tested along with various sizes of tissue implants.

**Results:** Of the nine mice operated on, we were able to recover two implanted tonsil pieces. One mouse developed necrosis immediately and was excluded from the study. Five of the remaining eight mice showed engraftment of human immune cells in the peripheral blood, likely expansion of human T-cells originating from the implanted tonsil. The recovered tonsil implants came from mice that had received the largest tonsil pieces. Histology showed initial retention of B-cell follicular structures but eventual loss of B-cells.

**Conclusion:** Surgical implant of human tonsil tissue is tolerable to NSG mice, although tissue persistence is dependent on size of implant. Implants from this pilot study showed excellent immune architecture persistence for 5 weeks post implantation (n=1) but dissolution by 8 weeks (n=1). The next step is to infect this mouse model with HIV to ensure 1) presence of HIV positive T-cells within the B-cell follicles of the implant and 2) evaluate whether co-transplanted HSC or T-cells will result in re-population of tonsil implant with donor cells.

**Harshana S. De Silva Feelixge**

*Research Technician II, Jerome Lab, Vaccine and Infectious Disease Division, Fred Hutchinson Cancer Research Center*

## Functional screen of CRISPR guide RNAs against HIV-1 LTR to account for global genetic diversity

HS De Silva Feelixge[1], P Roychoudhury[2], JT Schiffer[1,3], KR Jerome[1,2,3]

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A long-lived latent viral reservoir that harbors replication-competent provirus is the most significant obstacle towards developing an HIV cure. Various strategies to eliminate the reservoir, or at least to achieve permanent viral remission in the absence of antiretroviral therapy, are under investigation. One such approach, utilizing the CRISPRCas9 system intends to disable or excise HIV-1 proviruses by targeting the long terminal repeat (LTR) or essential viral genes, has shown promise in vivo. One of the major challenges of CRISPR/cas9 driven pro-viral genome eradication is identifying universal sets of highly specific and functional guide RNAs (sgRNAs) that could target the genetic diversity of HIV-1. Using computational techniques, we identified highly specific candidate S. pyogenes Cas9 sgRNAs from an LTR consensus sequence for HIV-1 group M, and from individual consensus sequences of the predominant subtypes A-C. Specifically, we determined how many individual group M LTR sequences could be targeted by each sgRNA identified within the consensus sequence. We then identified paired and triplet sgRNA combinations that would maximize the coverage of HIV sequences targeted at the group and subtype levels. These predicted individual and multiplexed sgRNAs were ranked based on their cumulative contribution to increasing sequence breadth. The top 59 single gRNAs were evaluated for their relative activity using a loss-of-function GFP reporter system in vitro. While in theory single gRNAs with high prevalence could be designed to target the majority of the available group M sequences, in vitro data suggests that a single gRNA alone is not sufficient to target a significant (>50% coverage) proportion of the group M sequences. However, a multiplexed gRNA regimen, composed of as few as three sgRNAs, is able cover a wider range of HIV-1 isolates, over 75 percent. Interestingly, even greater sequence coverage is achievable when gRNAs are multiplexed towards a specific subtype. In summary, CRISPR/Cas9 system has the potential to tackle the global genetic diversity of HIV-1 group M by utilizing a customized subtype specific gRNA regimen. Furthermore, a multiplexing approach would provide additional benefits, including increased potency of the antiretroviral therapy and circumvention of treatment resistance.

**Adam S. Dingens, MS**

*Graduate Student Fellow, Jesse Bloom and Julie Overbaugh Laboratories, Fred Hutchinson Cancer Research Center*

## Comprehensive mapping of HIV-1 escape from a broadly neutralizing antibody

AS Dingens, HK Haddox, J Overbaugh, JD Bloom

Basic Sciences Division, Fred Hutchinson Cancer Research Center, Seattle, WA

**Background:** Precisely defining how viral mutations affect HIV's sensitivity to antibodies is vital to the development and evaluation of vaccines and antibody-based immunotherapies. However, despite great effort, a full map of escape mutants has not yet been delineated for even a single anti-HIV antibody.

**Methods:** We developed a massively parallel experimental approach to quantify how all single amino-acid mutations to Envelope (Env) affect HIV's sensitivity to a neutralizing antibody. This approach, mutational antigenic profiling, involves creating libraries of all single amino-acid mutants of Env in the context of replication-competent HIV, selecting for mutations that promote antibody escape, and using deep sequencing to quantify the enrichment of each mutation.

**Results:** We applied this approach to PGT151, a broadly neutralizing antibody recognizing a combination of Env residues and glycans. We confirm sites previously defined by structural and functional studies and reveal additional sites of escape. We verified that the enrichment of each mutation in our high-throughput approach was well correlated with the effect of single mutations on neutralization sensitivity in TZM-bl neutralization assays. Further, evaluating the effect of each amino acid at each site lends insight into biochemical mechanisms of escape throughout the epitope, highlighting roles for charge-charge repulsions in the antibody-Env interface.

**Conclusion:** Comprehensive mapping of HIV antibody escape gives a quantitative, mutation-level view of the ways that Env can evade neutralization. We anticipate that this approach can be extended to define all possible HIV escape mutations from other broadly neutralizing antibodies (bnAbs). The resulting maps will be valuable in the development of immunogens and immunotherapeutic approaches, including passive and active transfer of bnAbs for treatment, cure, and prevention, as well as bnAb-based CAR T cells.

**Agnes Hajduczki, PhD**

*Postdoctoral Researcher, Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health*

## Targeting CAR T Cells to B cell follicles to cure HIV Infection

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**Background:** There is a need to develop improved methods to treat and potentially cure HIV infection. During chronic disease, HIV replication is concentrated within TFH cells in B cell follicles, where low levels of virus- specific CTL permit ongoing viral replication. We have previously shown that elevated levels of SIV- specific CTL in B cell follicles is linked to decreased levels of viral replication in follicles, and decreased plasma viral loads. We developed a strategy for targeting follicular viral-producing TFH cells using anti- viral chimeric antigen receptor (CAR) T cells co-expressing the follicular homing molecule CXCR5. We hypothesize that anti-viral CAR/CXCR5 expressing T cells when infused into an SIV-infected animal or an HIV-infected individual, will home to B cell follicles, suppress viral replication, and lead to long-term durable remission of SIV and HIV.

**Methods:** We engineered gammaretroviral transduction vectors for co-expression of an all-human bispecific anti- HIV CAR (designated CD4-MBL, displaying high potency and breadth) and human CXCR5 on human PMBC-derived T cells. We also generated vectors for expression of the corresponding rhesus macaque variants of these molecules on rhesus T cells. We measured viral suppression by CAR/CXCR5 T cells in vitro, and the ability of CAR/CXCR5 T cells to migrate to follicles using a novel ex vivo tissue migration assay.

**Results:** The CAR/CXCR5 T cells are functional as demonstrated by their potent suppression of SIVmac239 and SIVE660 replication in in vitro. The CAR/CXCR5 T cells also concentrated in B cell follicles ex vivo in tissues. We plan to test this cure strategy in SIV-infected macaques infused with CAR/CXCR5 T cells.

**Conclusion:** CAR/CXCR5 T cells exhibit virus suppression and follicular homing suggesting that they could provide long-term durable remission (functional cure) of HIV and SIV infections.

**Kevin G. Haworth, PhD**

*Research Associate, Kiem Lab, Fred Hutchinson Cancer Research Center*

## In Vivo Selection of Human Hematopoietic Stem/ Progenitor Cells Engineered Through Targeted Gene Integration

KG Haworth, S Radtke, ZK Norgaard, MA Atkins, SE Weitz, C Ironside, CE McAllister, HP Kiem

Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, WA

Achieving exogenous gene expression in a target cell population typically utilizes retroviral transduction to deliver transgene cassettes. An alternative, and potentially safer, approach is using targeted nucleases to direct DNA double-stranded breaks at predetermined chromosomal locations for gene integration though the homologous repair pathway. It is thought that this technique, also known as Targeted Integration (TI), will enable permanent gene expression without the long-term threat of oncogenesis due to insertional mutagenesis.

The challenge with TI in human CD34+ hematopoietic stem and progenitor cells (HSPCs) has been achieving persistent levels of modified cells high enough to observe a therapeutic benefit. This is especially important in protective gene therapy strategies for HIV treatment where a significant frequency of modified cells will be required for efficacy. We aim to address this low level of gene modification in two ways. First, to ensure we are engineering true, long-term HSPCs, we have utilized a new panel of surface markers to identify these cells and monitor for TI events in the most primitive fraction. Second, we have incorporated a selectable marker into the integration cassette enabling in vivo selection of modified cells after transplant.

In order to test engraftment potential of engineered HSPCs, we used the humanized NSG mouse model. Combining engineered nucleases (ZFNs) with AAV-delivered donor template DNA, we have achieved upwards of 30% integration in cells with a 2kb fragment expressing both GFP and our selectable marker. After one round of low-dose selection, mice exhibited a 2-3x fold increase of modified cells in blood and over 12% of engrafted HSPCs in bone marrow contained the integration cassette. Additionally, using next-generation sequencing, we can track unique clones over time in blood and tissues.

In these studies, we have for the first time specifically engineered HSPCs with a large integration cassette, and enabled direct in vivo selection of modified cells after transplant. Such in vivo selection strategies may prove beneficial for HIV cure strategies designed to achieve a protective threshold of CCR5-modified cells to resist infection, or incorporate an exogenous gene such as anti-HIV chimeric antigen receptors (CAR) to actively eliminate virally infected cells.

**Wannisa Khamaikawin, PhD**

*Postdoctoral Scholar, School of Nursing, University of California at Los Angeles*

## Modeling Anti-HIV-1 HSPC Based Gene Therapy in Humanized Mice Pre-Infected with HIV-1

W Khamaikawin[2,4], S Shimizu[1,2,4], M Kamata[3,4], R Cortado[2,4], Y Jung[2,4], J Lam[2,4], J Wen[3,4], P Kim[1,2,3,4], Y Xie[3,4], S Kim[3,4], H Arokium[3,4], AP Presson[5,6], ISY Chen[1,3,4], D An[1,2,4]

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**Background:** Humanized mice have been utilized as a small animal model to investigate anti-HIV-1 hematopoietic stem/progenitor cell (HSPC) based gene therapy. Previous investigations of anti-HIV-1 HSPC based gene therapy have been performed by first repopulating anti HIV-1 gene modified human hematopoietic cells in humanized mice and followed by an HIV-1 challenge. Although, previous experiments have provided important results, the practical clinical application of gene therapy is to treat HIV-1 already infected patients. Therefore, we investigate anti-HIV-1 HSPC based gene therapy in already HIV-1 infected humanized mice in a more clinically relevant experimental setting.

**Methods:** First, to create HIV-1 infected humanized mice, 1-3 day old neonatal NOD. Cg-Prkdcscid Il2rgtm1Wjl/SzJ (NSG) mice were injected with human fetal liver derived CD34+ cells. After human hematopoietic cell reconstitution at 10 weeks, humanized mice were injected with R5 tropic HIV-1 NFNSX (200 ng of p24). HIV-1 infection was confirmed by viral load (1.25x10e7±4.10x10e7(copies /ml±SD) at 3 weeks post HIV infection. HIV-1 infected mice were myeloablated by busufan and transplanted with anti HIV-1 gene modified human CD34+ HSPC transduced with a lentiviral vector expressing two anti HIV-1 short hairpin RNAs (shRNA) against CCR5 and HIV-1 LTR along with human thymus tissue under the kidney capsule to reconstitute HIV-1 already infected humanized mice. Vector transduction efficiency was 72.92±10.84 (%±SD).

**Results:** Anti-HIV gene modified HSPCs successfully engrafted and reconstituted human hematopoietic cells in HIV-1 infected humanized mice from 10-12 weeks post-transplant with 31.68±16.26 (%±SD) and 43.00±5.36 (%±SD) in human CD45+ hematopoietic cells, 38.61±19.93 (%±SD) and 40.43±26.03 (% ±SD) in human CD3+ T, and 17.70±5.14 (%±SD) and 36.22±8.75 (%±SD) % in human CD19+ B cells, in peripheral blood and lymphoid tissues, respectively. Anti HIV-1 gene modified CD4+T cell showed selective advantages by 4.7 and 4 fold relative to non-anti-HIV-1 gene control cells in peripheral blood and tissues, respectively.

**Conclusion:** Our results demonstrated anti-HIV-1 gene modified HSPC engrafted and differentiate into mature lymphocytes in HIV-1 infected mice. The anti-HIV-1 gene-modified CD4+ T cells were selected over unprotected cells. This new humanized mouse model will be useful for investigations of anti-HIV-1 gene therapy to test in more clinically relevant experimental settings.

**Mark D. Pankau**

*Graduate Research Assistant, Fred Hutchinson Cancer Research Center*

## The dynamics of the HIV latent reservoir in a cohort of superinfected Kenyan women

MD Pankau[1], K Ronen[1], C Warth[1], F Matsen[1], W Jaoko[2], K Mandaliya[3], RS McClelland[4], J Overbaugh[1], DA Lehman[1]

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**Background:** A major barrier to HIV cure is establishment of a reservoir of latently infected cells early in HIV infection which persists despite suppressive cART. Understanding the dynamics and composition of the reservoir will help aid HIV cure strategies. Here we characterize the seeding dynamics of the latent reservoir using longitudinal samples from before and after cART initiation in individuals infected sequentially with genetically distinct HIV variants.

**Methods:** Cases of HIV superinfection in a cohort of high-risk women in Mombasa, Kenya were previously identified and the dates of both initial infection and superinfection were determined. Six of these cases were subsequently treated with cART (5.4-18 years after initial infection and 5.1-13 years after superinfection). We performed next-generation sequencing of gag, pol, and env from HIV plasma RNA every 1.5-5.02 years prior to cART initiation and from HIV proviral DNA from PBMCs collected 0.9-4.8 years after viral suppression on cART. Phylogenetic analysis was used to compare HIV DNA and RNA sequences over time and to determine the proportion of initial and superinfecting variants in circulation and in the reservoir at each time point.

**Results:** The proportion of HIV DNA sequences for gag, pol, and env from the initial and superinfecting viruses was correlated with the proportions observed in the RNA at the time point just prior to cART initiation ($r = 0.83$, $p = 0.0008$). HIV DNA proviral sequences were phylogenetically closest to the RNA sequences just prior to cART initiation.

**Conclusion:** Our data suggest that the HIV latent reservoir is predominately comprised of viruses present in plasma just prior to cART initiation.

**Daniel B. Reeves, PhD**

*Post-Doctoral Research Fellow, Clinical Research Program, Fred Hutchinson Cancer Research Center*

## Reservoir ecology shows HIV persists on long-term ART due to cellular proliferation

DB Reeves[1], ER Duke[1], T Wagner[2], S Palmer[3], A Spivak[4], JT Schiffer[1]

[1]Vaccine and Infectious Disease Division, Fred Hutchinson Cancer Research Center, Seattle, WA; [2]Pediatric Infectious Diseases, Seattle Children's Hospital, Seattle, WA; [3]Westmead Institute for Medical Research, University of Sydney, Australia; [4]Department of Medicine, University of Utah, Salt Lake City, UT

**Background:** A latently infected cellular population survives even in HIV patients treated with suppressive antiretroviral therapy (ART). Some data from patients early on ART attributes signatures of viral evolution in the first six months of treatment to an insufficiently dosed ART sanctuary. These data contrast with other data from time points after several years on ART that show no ongoing viral evolution.

**Methods:** Using published integration site data from patients on multiple years of ART (Wagner et al. 2014), we performed an ecological analysis to extrapolate the richness and abundance distribution of distinct HIV sequences. We used several mathematical models to recapitulate these distributions in the context of a typical HIV reservoir size.

**Results:** Ecological estimates find that there may be up to 10 thousand different HIV sequences archived in the HIV reservoir. However, because this richness is still significantly lower than the population size of the reservoir (~1 million cells), most of these sequences are likely from clonal populations. In observed data, while the fraction of unique sequences may be 60-80%, this is an artifact of the sampling such that observed unique sequences are more likely from clones that, by chance, were sampled only once. Unique sequences are indicative of viral replication. However, our abundance distribution fitting shows that in all cases, patients beyond 1 year of ART have a percentage of unique sequences below 0.1%, which indicates that most reservoir sequences are clonal, and therefore most are generated by faithful cellular proliferation.

**Conclusions:** In making the decision to pursue a candidate therapy for HIV cure, we must understand the biological mechanisms underlying the persistence of the HIV reservoir. Our mathematical modeling demonstrates that latent proliferation should be a target of curative regimens to achieve functional HIV cure.

**Pavitra Roychoudhury, PhD**

*Senior Fellow, Department of Laboratory Medicine, University of Washington*

## Rational design and evaluation of CRISPR/Cas9 strategies for HIV cure

P Roychoudhury[1], HS De Silva Feelixge[2], D Reeves[2], JT Schiffer[2], KR Jerome[1]

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A number of gene therapy approaches have recently been proposed in the search for a cure for HIV. One approach, which has now been demonstrated in vivo, involves using RNA-guided CRISPR/Cas9 enzymes to target the HIV-1 long terminal repeat (LTR) in order to excise the integrated genome from latently infected cells. In order to take this strategy to the clinic, a number of practical challenges must be surmounted. One important consideration is HIV genetic diversity, which presents challenges at the population and intra-host levels: selection of multiplexed set of single guide RNAs (sgRNAs) must account for diversity of circulating strains across a wide range of infected persons, while dosing strategies must consider within-host diversity of HIV to maximize the probability of a functional cure. To address these issues, we used computational techniques to predict and rank a set of single and multiplexed sgRNAs most likely to cleave HIV LTR in subtypes A-C and recombinant strains in group M based on an alignment of 1126 sequences from the LANL HIV database. We then evaluated the activity of the top 59 single sgRNAs using a loss-of-function GFP reporter system. We found that predicted activity scores mostly correlated with measured activity. We then identified and ranked pairs, triplets, and quadruplet sgRNA combinations to optimize activity and percentage of HIV sequences targeted at the group and subtype level. We also identified potential off-target cleavage sites in the human genome for these sgRNAs. Lastly, we developed a mathematical model to predict, based on hypothetical distributions of within-host HIV sequence diversity, the number of doses required with each guide or guide combination to deplete the latent reservoir to achieve functional cure thresholds. Our preliminary results show that both reservoir composition and target cleavage efficiency will determine the number of doses required for a functional cure and that inadequate targeting of rare strains could lead to rebound upon cessation of ART.

**Eduardo Seclen, PhD**

*Postdoctoral Research Associate, Keck School of Medicine, University of Southern California*

## Systemic expression of HIV entry inhibitors by site specific gene editing of hematopoietic cells

E Seclen[1], GL Rogers[1], CX Wang[1], N Llewellyn[2], A Gadon[2], PM Cannon[1,2]

[1]Keck School of Medicine, University of South California, Los Angeles, CA; [2]Department of Medical Microbiology and Immunology, Keck School of Medicine, University of South California, Los Angeles, CA

Site-specific gene editing of T cells or hematopoietic stem cells (HSC) can provide protection to individual CD4+ T cells from HIV infection, most notably by CCR5 knockout. Gene editing can also be used to provide more systemic protection, by engineering cells to secrete soluble anti-HIV factors such as broadly neutralizing antibodies or the receptor mimic eCD4-IgG (Gardner et al., Nat 2015). To date, approaches for systemic secretion tend to have focused on AAV vector delivery to muscle cells. We are instead evaluating the ability of hematopoietic cells to act as factories to produce secreted anti-HIV factors, and evaluating the anti-HIV efficacy of combining these with editing strategies that protect the individual producer cells.

Using adeno-associated viruses (AAV) together with ZFN mRNA electroporation, we can site-specifically edit CD34+ HSC, CD4+ T cells and B cells. Plasma cells are of particular interest as producers of secreted factors, and we have been able to effectively edit B cells at CCR5 with rates of 25%, which successfully differentiated into plasma cells in vitro. We are currently evaluating eCD4-IgG insertion and expression in these cells. Of note, eCD4IgG requires sulfonation for activity, making it necessary to simultaneously co- deliver a sulfotransferase enzyme for successful production in certain cell types. We are currently evaluating these approaches using in vivo models of HIV infection based on injection of edited cells into NSG mice followed by HIV challenge.

**Sai Vikram Vemula, PhD**

*Associate Research Scientist, Merck*

## Changes in expression of small non-coding RNA during HIV infection and latency in a CD4+ T cell model of HIV latency

SV Vemula, B Howell

Infectious Diseases & Vaccines, Merck & Co., Inc., West Point, PA

**Background:** Non-coding RNAs (ncRNAs) comprise a large group of functional RNA molecules that are not transcribed into proteins, but have been shown to be play crucial functions in post-transcriptional regulation of various biological processes. Over the years, several ncRNAs have been shown to have important roles in pathogenesis of various human diseases including cancer and HIV. A better understanding of ncRNA expression and functional implications during active HIV infection and latency could aid in development of novel strategies for HIV treatment/cure.

**Methods:** We used an RNA seq-based approach to identify the various classes of small ncRNA (miRNA, tRNA, yRNA) modulated during active and latent stages of HIV infection in a primary CD4+ T cell model of HIV latency. We further assessed expression of some of these ncRNA by qPCR in HIV infected primary CD4+ T cells, cell lines (Jurkat 2C4, MOLTIIIB), and ex vivo isolated CD4+ T cells from viremic and ART suppressed donors.

**Results:** A total of 304 small RNAs were modulated > 2-fold (up or down) during active and latent HIV infection compared to uninfected CD4+ T cells in a primary latency model. yRNAs were the most abundant class by total number of reads and miRNAs were the most abundant class based on diversity of sequences, with over 140 modulated (93 upregulated and 45 downregulated) during active and latent stages of HIV infection. miRNAs enriched in latently infected cells included miR-29b (previously shown to interfere with nef protein) and miR-125b (imparts an HIV-1 transcriptional block), with additional miRNAs identified and unknown roles in HIV biology (e.g., miR-4657, miR-6500, and miR-6513). miR-29b and miR-125b are both aberrantly expressed in the majority of human cancers and display oncogenic potential. Work is progressing to further validate and explore miRNA differentation in physiologically relevant model systems of HIV infection as well as CD4+ T cells isolated from HIV infected patients.

**Conclusions:** Overall, our findings could provide new insights into the role of novel ncRNA in HIV and cancer biology and treatment.


Cell & Gene Therapy for HIV Cure 2017


## defeatHIV, the Delaney Cell and Genome Engineering Initiative

Founded in 2011 and led by Drs. Keith Jerome and Hans-Peter Kiem at the Fred Hutchinson Cancer Research Center, the defeatHIV Martin Delaney Collaboratory is a consortium of scientific investigators and clinicians from both public and private research organizations who are committed to finding a cure for HIV. Our collaboratory believes that cell and gene therapies represent perhaps the most promising approach to HIV cure. We are focused on evaluating these approaches to meet the dual goals of eliminating latently-infected cells from the body, while improving an individual's ability to control HIV reactivation from viral reservoirs.

We are supported by the NIH program, Martin Delaney Collaboratory: Towards an HIV-1 Cure. The program is named after the late HIV/AIDS activist Martin Delaney, who worked tirelessly as an educator and advocate for HIV/AIDS patients. The Martin Delaney Collaboratory program provides support for translational and clinical HIV cure research, fostering partnerships between public and private research institutions.

defeatHIV is one of six collaboratories funded in 2016 by NIH as part of the second iteration of the Martin Delaney Collaboratory program. The others include:

- **George Washington University**
  BELIEVE: Bench to Bed Enhanced Lymphoctye Infusions to Engineer Viral Eradication
- **University of California, San Francisco**
  Delaney AIDS Research Enterprise to Cure HIV (DARE)
- **Wistar Institute**
  BEAT-HIV: Delaney Collaboratory to Cure HIV-1 Infection by Combination Immunotherapy
- **Beth Israel Deaconess Medical Center**
  Combined Immunologic Approaches to Cure HIV-1
- **University of North Carolina, Chapel Hill**
  Collaboratory of AIDS Researchers for Eradication (CARE)

**defeathiv.org**

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At Fred Hutchinson Cancer Research Center, home to three Nobel laureates, interdisciplinary teams of world-renowned scientists seek new and innovative ways to prevent, diagnose and treat cancer, HIV/AIDS and other life-threatening diseases. Fred Hutch's pioneering work in bone marrow transplantation led to the development of immunotherapy, which harnesses the power of the immune system to treat cancer with minimal side effects. An independent, nonprofit research institute based in Seattle, Fred Hutch houses the nation's first and largest cancer prevention research program, as well as the clinical coordinating center of the Women's Health Initiative and the international headquarters of the HIV Vaccine Trials Network. Private contributions are essential for enabling Fred Hutch scientists to explore novel research opportunities that lead to important medical breakthroughs.

**fredhutch.org**


## CFAR – Center for AIDS Research

The focus of the Curative Therapies for HIV (Cure) Scientific Working Group is to accelerate work toward a cure of HIV, by linking local investigators of curative therapies for HIV to the comprehensive UW/FHCRC CFAR. Additionally, we strive to connect Seattle investigators with international leaders in the field, in order to develop critical local expertise and enhance areas of local strength. These collective activities have helped establish an international center of excellence in the study of curative therapies for HIV at the UW/FHCRC CFAR.

The Cure Scientific Working Group leverages a large NIH investment in the Seattle-led consortium defeatHIV, one of six Martin Delaney Collaboratories focused on the cure of HIV. The Cure Scientific Working Group synergizes with CFAR to utilize expertise in the clinical, basic science, and developmental cores, and to develop novel research questions for the study of curative therapies for HIV.

**depts.washington.edu/cfar/**

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Department of Laboratory Medicine

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The University of Washington Virology Division is one of 11 divisions that comprise the Department of Laboratory Medicine in the University's School of Medicine. The Virology Division's twelve faculty members and over 100 staff are actively engaged in the Department's three-fold mission of clinical service, education, and research.

The Division performs clinical diagnostic testing for a full range of human pathogens including Herpes group, HIV, respiratory, and enteric viruses. Techniques used are molecular PCR diagnostics and both Sanger and Next Generation Sequencing for standard pathogens and esoteric or non-culturable viruses, tissue culture with direct antigen detection, and serological assays such as Western blot for HSV types 1 and 2. The patient care services provided exemplify the highest achievable quality and serve as a model of excellence for other clinical virology laboratories across the nation.

As part of the School of Medicine, educational opportunities are available for undergraduate and graduate students and post-doctoral trainees within the Virology Division. UW Medicine teaching programs were ranked among the best in the country in the 2016 U.S. News & World Report annual rankings of medical schools.

An Environment conducive to the performance of high quality research and development is fostered within the Division. The faculty, staff, and trainees are involved in research and development activities that include developing the latest laboratory tests, creating new vaccines, inventing and patenting new technology, and elucidating basic cellular processes in health and disease. The Division's faculty is internally recognized for their clinical and basic science research.

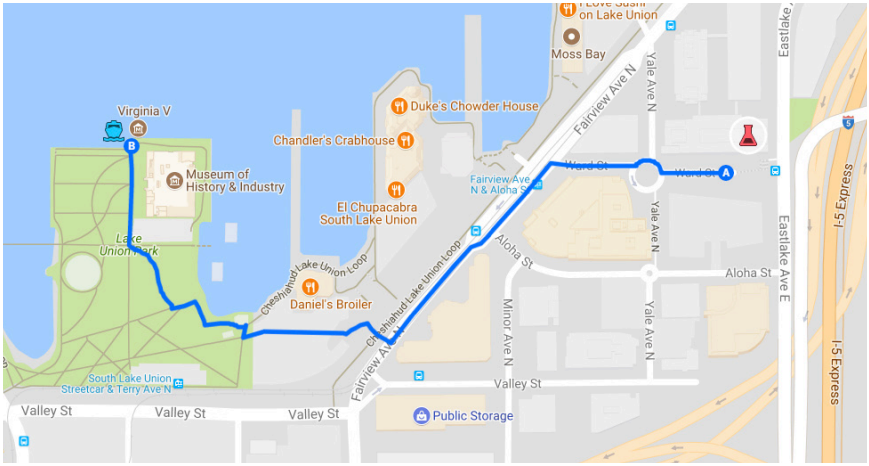
**uwvirology.org**

## Thursday, August 17th from 7-10pm

Please join us as we set sail on an Argosy Cruises adventure around scenic Lake Union and Lake Washington on the Lady Mary. Dinner and drinks will be provided and the pick-up location is conveniently located at the dock of the nearby Museum of History and Industry.

**Location and Transportation:**

The boarding location for our Argosy Cruise is at South Lake Union on the dock at the Museum of History and Industry (MOHAI). After the poster session on Day 1, attendees will be led on a walk from the Fred Hutch campus to the MOHAI dock.




**Dinner and Reception:**


As we cruise past Seattle's historic waterfront through Lake Union, Portage Bay, the Montlake Cut, and past the University of Washington, please enjoy hors d'oeuvres, a buffet dinner, and a fully-hosted bar. The Lady Mary cruise ship will depart from the MOHAI dock at precisely 7:00pm and not return until 10:00pm.

**Schedule:**

5:00pm: Poster Session begins

6:20pm: Poster Session ends and attendees may start walking to the MOHAI dock

7:00pm: The Lady Mary boat launches and cruise begins

10:00pm: Cruise concluded and attendees de-board at the MOHAI dock

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amfAR, The Foundation for AIDS Research, is one of the world's leading nonprofit organizations dedicated to the support of AIDS research, HIV prevention, treatment education, and advocacy. Since 1985, amfAR has invested more than $480 million in its programs and has awarded more than 3,300 grants to research teams worldwide.

Today amfAR's research focus is on the search for a cure for HIV/AIDS. Following the establishment of the amfAR Research Consortium on HIV Eradication (ARCHE) in 2010, amfAR launched the Countdown to a Cure for AIDS initiative in 2014 and established the amfAR Institute for HIV Cure Research in 2015. Backed by a $100 million research investment strategy, the Countdown is aimed at developing the scientific basis for a cure by 2020.

**amfar.org**

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For more information, please visit **www.rocketpharma.com**.

The conference is pleased to be supporting the attendance and participation of Community Advisory Board (CAB) members from the Martin Delaney Collaboratories of CARE, DARE, and defeatHIV.

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*defeatHIV CAB*

**Steve S. Muchnick, PhD**
*DARE CAB*

**David Palm, MSc**
*CARE CAB*

**Dawn Renee Weinman**
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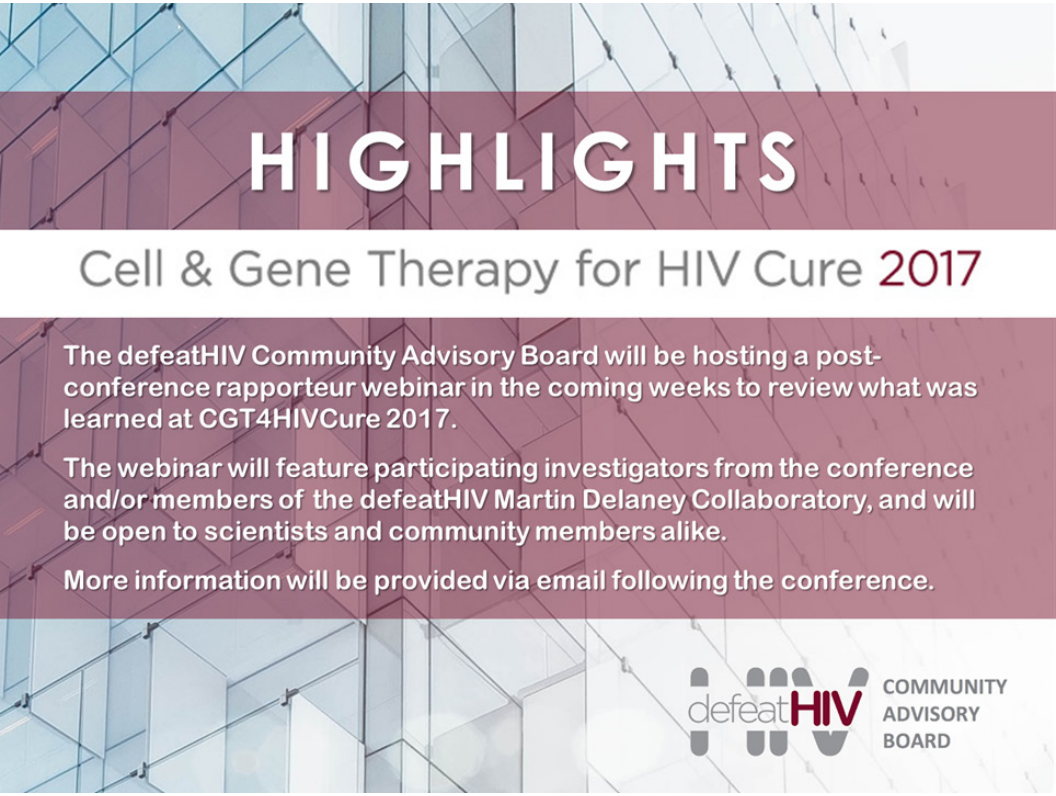

## NOTES

## NOTES

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