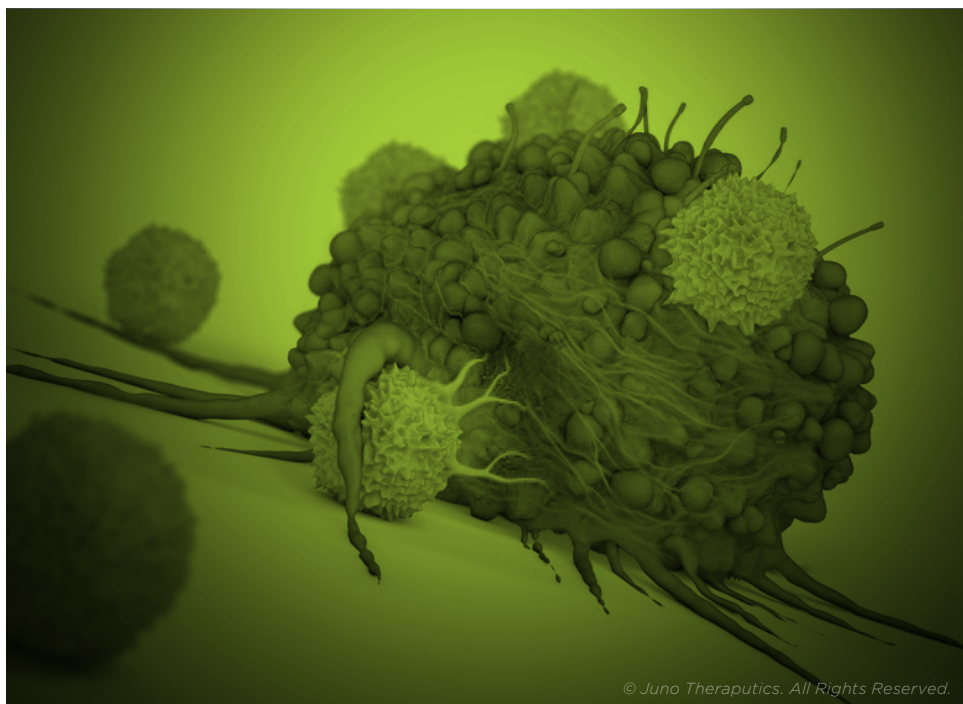


# CGT 4 **HIV** Cure

Cell & Gene Therapy for HIV Cure **2016**



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August 4-5, 2016  
Fred Hutchinson Cancer  
Research Center  
Seattle, Washington

[cgt4hivcure2016.org](http://cgt4hivcure2016.org)



## UM1 MARTIN DELANEY COLLABORATORIES

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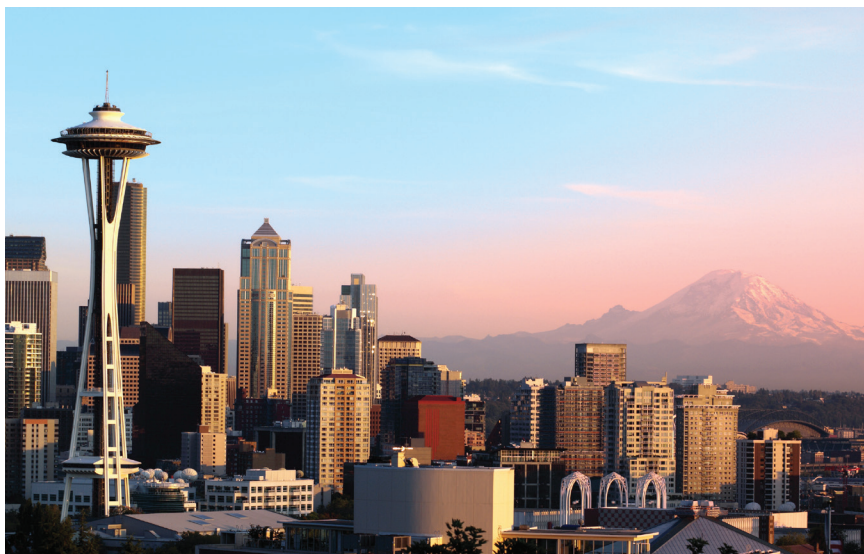
We at defeatHIV are proud to announce our participation in the second iteration of the Martin Delaney Collaboratory: Towards an HIV-1 Cure.

Over the next five years, defeatHIV will partner with the five other collaboratories of this program to launch novel investigations and bring the world closer to finding a cure for HIV.

Congratulations to our fellow collaboratories:

- **Fred Hutchinson Cancer Research Center**  
defeatHIV, Cell and Gene therapies for HIV Cure
- **George Washington University**  
BELIEVE: Bench to Bed Enhanced Lymphocyte 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)

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On behalf of the Scientific Organizing Committee, we are pleased to welcome you to the third Conference on Cell & Gene Therapy for HIV Cure (CGT4HIVCure.) We are proud to host this growing conference and look forward to facilitating the innovative collaborations that will advance our shared goal of eliminating HIV.

With each passing year, great strides are made towards achieving a cure for HIV. Cell and gene therapies are at the forefront of this effort, and investigators from all over the world are using these approaches to guide their research in a variety of novel ways. Advancements in immunotherapy, gene editing, and vaccines highlight only a handful of the ways that HIV cure research has progressed in 2016 alone. Meetings like this are crucial platforms for investigators to share their findings with a forum of experts in order to refine their techniques and make tangible progress in their research.

In addition to hosting many of the world's leading cell and gene therapy experts, CGT4HIVCure aims to make this research accessible to the community as a whole. Community engagement was one of the pillars upon which the HIV cure-focused Martin Delaney Collaboratories were founded, and each year this conference has emphasized the importance of soliciting community feedback and spreading awareness of the current state of HIV cell and gene therapy research. Community involvement in this endeavor will be critical in generating the momentum and resources necessary for our work to achieve its goal.

We hope CGT4HIVCure 2016 will be another stepping stone on the way to finding an HIV cure. Leveraging the infrastructure built through the original Martin Delaney Collaboratory U19, we at defeatHIV are excited and honored by the opportunity to move forward with a new UM1 Collaboratory and accelerate our efforts over the next several years.

Thank you all for your participation at this conference, and we hope you find the program to be thoughtful and engaging!

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

*Conference Co-host*



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

*Conference Co-host*



## SCHOLARSHIP RECIPIENTS

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*The Conference on Cell & Gene Therapy for HIV Cure* is pleased to announce the 2016 scholarship recipients:

Hadega Aamer, MSc

Nick Llewellyn, PhD

Hadia Abdelaal, MSc

Nixon Niyonzima, MD

Malika Aid, PhD

Biswajit Paul, PhD

Mayra Carrillo, PhD

Pavrita Roychoudhury, PhD

Morgan Chateau, PhD

Chelsea Spragg, PhD

Venkata Viswanadh Edara, MSc

Jim Sun, PhD

Pargol Hashemi, MSc

James Voss, PhD

Wannisa Khamaikawin, PhD

Cathy Wang, PhD

Shengbin Li, MSc

Gabriela Webb, PhD

*Congratulations!*

## SCIENTIFIC ORGANIZING COMMITTEE

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### **Paula Cannon, PhD**

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

### **Michael Farzan, PhD**

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

### **Timothy Henrich, MD**

*Assistant Professor, Division of Experimental Medicine, University of California San Francisco*

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

*Host, CGT4HIVCure 2016*  
*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*

### **Rowena Johnston, PhD**

*Vice President, Director of Research, The Foundation for AIDS Research (amfAR)*

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

*Host, CGT4HIVCure 2016*  
*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*  
*Associate Head, Heme Malignancy Program, UW / Fred Hutch Cancer Consortium*

### **Scott Kitchen, PhD**

*Director, UCLA CFAR/JCCC Humanized Mouse Core Laboratory; Associate Professor of Medicine; Member, CTSI, Tumor Immunology Program at the Jonsson Comprehensive Cancer Center, University of California Los Angeles*

### **Semih Tareen, PhD**

*Associate Director and Head of Gene Engineering & Delivery, Juno Therapeutics*

### **Manuel Venegas**

*Youth Scholar, National Minority AIDS Council;*  
*defeatHIV Community Advisory Board*

## AGENDA | DAY 1:

Pelton Auditorium	7:15 - 8:00	Registration and Continental Breakfast
	<b>SESSION 1</b>	<b>Opening remarks and HIV Cure Overview</b>
	8:00 - 8:20	Conference Welcome: Keith R. Jerome and Hans-Peter Kiem
	8:20 - 8:40	HIV Cure Overview: Rowena Johnston
	8:40 - 9:00	NIAID Cure Priorities: Lillian Kuo
	<b>SESSION 2</b>	<b>HIV Latency &amp; Reservoirs</b>
	9:00 - 9:20	Jacob Estes
	9:20 - 9:40	Pamela Skinner
	9:40 - 10:00	Rafick-Pierre Sekaly
	10:00 - 10:20	Florian Hladik
	10:20 - 10:35	Jim Sun
	10:35 - 11:00	Break
	<b>SESSION 3</b>	<b>Keynote</b>
	11:00 - 12:00	Keynote Speaker: Lawrence Corey
	12:00 - 1:00	Lunch: Weintraub B Suites
	<b>SESSION 4</b>	<b>CAR T Cell Development for HIV Cure</b>
	1:00 - 1:20	James Riley
	1:20 - 1:40	Thor Wagner
	1:40 - 2:00	Edward Berger
	2:00 - 2:15	Mayra Carrillo
	<b>SESSION 5</b>	<b>Transplantation for HIV Cure</b>
	2:15 - 2:45	Plenary Speaker: Timothy Henrich
	2:45 - 3:05	Laurie Sylla
	3:05 - 3:25	Annemarie Wensing
	3:25 - 3:55	Break
	<b>SESSION 6</b>	<b>Animal Models</b>
	3:55 - 4:15	Priti Kumar
	4:15 - 4:30	Nick Llewellyn
	4:30 - 4:45	Gabriela Webb
	4:45 - 5:00	Kevin Haworth

<b>Evening Activities</b>	
5:00 - 6:30	Poster Session, Cocktails, and Hors d'Oeuvres (SZE Conference Room)
6:30 - 7:00	Shuttle from Fred Hutch to Space Needle
7:00 - 10:00	Dinner Reception - Space Needle



Pelton Auditorium	7:30 - 8:00	Registration and Continental Breakfast
	8:00 - 8:30	Plenary Speaker: Warner Greene
	<b>SESSION 7</b>	<b>Vaccines and Immunity</b>
	8:30 - 8:50	Alan Perelson
	8:50 - 9:10	Mario Roederer
	9:10 - 9:30	Michael Farzan
	9:30 - 9:50	Deborah Fuller
	9:50 - 10:05	James Voss
	10:05 - 10:30	Break
	<b>SESSION 8</b>	<b>Gene Editing</b>
	10:30 - 10:50	Kamel Khalili
	10:50 - 11:10	Christopher Peterson
	11:10 - 11:25	Biswajit Paul
	11:25 - 11:40	Cathy Wang
	11:40 - 11:55	Nixon Niyonzima
	11:55 - 1:00	Lunch: Weintraub B Suites
	1:00 - 1:30	Plenary Speaker: Mark Kay
	<b>SESSION 9</b>	<b>Therapeutic Development &amp; Gene Therapy</b>
	1:30 - 1:50	Semih Tareen
	1:50 - 2:10	Matt Sharp
	2:10 - 2:25	Pavitra Roychoudhury
	2:25 - 2:40	Joumana Zeidan
	2:40 - 3:00	James Whitney
	3:00	Closing Remarks: Keith R. Jerome and Hans-Peter Kiem



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



### **Lawrence Corey, MD**

*President and Director Emeritus,  
Fred Hutchinson Cancer Research Center;  
Professor of Medicine and Laboratory Medicine,  
University of Washington*

Dr. Lawrence Corey is president and director emeritus of Fred Hutchinson Cancer Research Center and professor of medicine and laboratory medicine at the University of Washington. He is also principal investigator of the HIV Vaccine Trials Network, an international collaboration of scientists and institutions dedicated to accelerating the development of HIV vaccines.

An internationally renowned expert in virology, immunology and vaccine development, his research focuses on herpes viruses, HIV and other viral infections, particularly those associated with cancer.

His honors and awards include election to the American Academy of Arts and Sciences and to the Institute of Medicine. He is also the recipient of the Pan American Society Clinical Virology Award, the American Society for STD Research Parran Award, the University of Michigan Medical School Distinguished Alumnus Award, the Infectious Diseases Society of America Ender's Award and the Cubist Award from the American Society of Microbiology.

Corey received his medical degree from the University of Michigan and his infectious diseases training at the University of Washington. He has authored more than 700 scientific publications and has served on numerous editorial boards and national committees, along with serving as head of both the NIH-sponsored AIDS therapy and HIV vaccine programs.

### Immunologic Approaches to HIV Cure

L Corey

Vaccine and Infectious Disease Division, Fred Hutchinson Cancer Research Center, Seattle, WA

While retroviral latency is a sine qua non of cell lentiviral infections, there is evidence that HIV RNA is detected in many latently infected cells and that some HIV proteins/peptides may be expressed. The high rate of indirect killing of T cells during all phases of HIV suggests that HIV antigens may be displayed on such cells; even among persons with optimal clinical and virological control. Immunological approaches that would enhance recognition of these intermittent bursts of viral reactivation might lead to a functional eradication of HIV. A functional cure might be achieved through the development of HIV specific T cells that perform immune surveillance for infected cells expressing HIV proteins or peptides. Such effector T cells might provide both direct and indirect mechanisms of elimination of HIV reservoirs. Chimeric Antigen Receptor (CAR) T cells offer some potential advantages for such an approach. CAR T cells can be designed as "off the shelf" products that allow one to "improve" host T-cell immunity; genetically engineered T cells that contain a combination of antigens that can be made or mixtures of such CARs to overcome antigenic diversity. The targets can be directed to conserved regions of the virus, thus obviating selection of immune escape variants. In addition, CAR T cells can be engineered to enter immunologically privileged sites as well as to resist HIV replication when encountering an HIV infected cell. Several candidate CARs have been developed and exhibit in vitro killing, but optimizing them and developing pre-clinical models is still a nascent field. Key scientific questions such as long term persistence, eventual escape, potency of killing, and what HIV-1 or host antigen could be used to be clinically relevant require defining.

This talk will discuss the potential of developing CAR T-cell therapy for long term benefit for HIV control and help define what scientific questions are needed to define if genetically engineered CAR T cells can provide a path toward achieving an HIV-1 "cure."



### **Timothy Henrich, MD**

*Assistant Professor, Department of Medicine*

*Division of Experimental Medicine*

*University of California, San Francisco*

Timothy Henrich, MD is an Assistant Professor of Medicine in the Division of Experimental Medicine at the University of California, San Francisco. Dr. Henrich is engaged in basic and translational HIV eradication research with an emphasis on stem cell transplantation and cytotoxic chemotherapy for malignancies on HIV persistence, viral evolution, and immune responses. Dr. Henrich is also engaged in the design and implementation of novel platforms for the detection and characterization of HIV reservoirs in collaborative studies involving bioengineering and nano/microtechnology.

Lastly, he is determining the role of immune modulatory agents and antibody-drug conjugates in targeting and eliminating latent HIV reservoirs. After graduating from the Yale University School of Medicine, Dr. Henrich completed internal medicine residency at the BWH and infectious disease fellowship at the BWH and Massachusetts General Hospital. He then joined the infectious disease faculty at the BWH as an Assistant Professor of Medicine at Harvard Medical School prior to relocating to San Francisco.

## Almost Cured: Hematopoietic Stem Cell Transplantation Provides Important Insights into HIV Persistence and Eradication Strategies

T Henrich

Division of Experimental Medicine, University of California, San Francisco, San Francisco, CA

Hematopoietic stem cell transplantation (HSCT) provides unique opportunities to investigate the impact of various immune components on HIV-infected cells, and inform the design of novel studies involving immunotherapeutic and cell modification approaches to clear or control HIV and other viral infections. Allogeneic HSCT remains one of the few HIV curative strategies that leads to marked, sustained decline in HIV reservoir size. Thus far, HSCT studies have provided important insights into HIV persistence and various curative strategies. For example, beneficial graft-versus-host (GvH) effects that target and clear cells capable of harboring HIV are mediated largely by the innate immune system following allogeneic HSCT. NK cells reconstitute rapidly in the months following HSCT, a time of decreased T lymphocyte numbers and function. GvH responses can selectively activate latent HIV infection and target residual HIV-infected cells; NK and NKT cells appear to shoulder the burden of responding to these reactivated cells following HSCT. In addition, the study of the humoral immunity following allogeneic HSCT and HIV reservoir reduction using chip-based broad antibody characterization and next-generation B cell receptor sequencing provides unique opportunities to understand HIV tissue persistence and reservoir size. Lastly, studies of HIV acquisition and progression in individuals with CCR5 mutations provides unique insights into challenges surrounding CCR5 modification strategies.

## PLENARY SPEAKERS

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### **Warner C. Greene, MD, PhD**

*Director, Senior Investigator*

*Gladstone Institute of Virology and Immunology*

*Nick and Sue Hellmann Distinguished Professor of  
Translational Medicine*

*Professor of Medicine, Microbiology and  
Immunology*

*University of California, San Francisco*

Dr. Warner C. Greene is the founding Director and the Nick and Sue Hellmann Distinguished Professor of the Gladstone Institute of Virology and Immunology. He is also a Professor of Medicine, Microbiology and Immunology at the University of California, San Francisco (UCSF) and is co-director of the federally funded UCSF-Gladstone Center for AIDS Research.

Dr. Greene received his BA degree with great distinction from Stanford University and his MD and PhD degrees with honors from Washington University School of Medicine. He completed his internship and residency training in Internal Medicine at the Massachusetts General Hospital at Harvard. Next, he served as a senior investigator at the National Cancer Institute from 1979-1986 where he started his own laboratory. In 1987, he became Professor of Medicine at Duke University Medical Center and an Investigator in the Howard Hughes Medical Institute.

Dr. Greene's studies focus on HIV pathogenesis including new insights into how CD4 T cells die during HIV infection and new approaches to curing HIV infection. He has authored more than 370 scientific papers and is a member of the American Academy of Arts & Sciences, the National Academy of Medicine and a fellow of the American Association for the Advancement of Science, and a past president of the Association of American Physicians. Dr. Greene is most proud of having mentored more than 120 students and fellows during his 30+ year career in science.

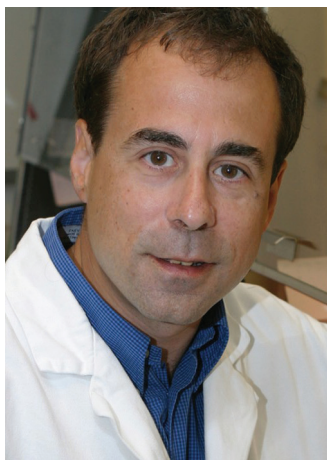
## Harnessing RIG-I Innate Immunity to Promote Selective Killing of Reactivated HIV Reservoir Cells

Li P<sup>1</sup>, Kaiser P<sup>1</sup>, Lampiris HW<sup>2</sup>, Kim P<sup>1</sup>, Yukl, SA<sup>1,2</sup>, Havlir DV<sup>2,3</sup>, Greene WC<sup>2,4,5</sup>, Wong JK<sup>1,2</sup>

<sup>1</sup>Infectious Diseases Section, Medical Service, San Francisco Veterans Affairs Medical Center, San Francisco, CA, <sup>2</sup>Department of Medicine, University of California, San Francisco, San Francisco, CA <sup>3</sup>HIV/AIDS Division, San Francisco General Hospital, San Francisco, CA, <sup>4</sup>Department of Microbiology and Biology, University of California, San Francisco, San Francisco, CA <sup>5</sup>Gladstone Institute of Virology and Immunology, San Francisco, CA

Shock without kill is a failed, potentially dangerous, strategy for attacking the latent HIV reservoir. Early findings indicate that HIV reservoir cells exposed to latency reversing agents (LRAs) like HDAC inhibitors do not spontaneously die. While stronger LRAs may be key for eliciting a viral cytopathic effect, it seems prudent to prepare for the possibility that a selective killing strategy will be needed. Potential approaches include the induction of CTL activity with vaccines, the activation of ADCC through the binding of broadly neutralizing antibodies to Fc receptors on NK cells, and “higher tech” genetic approaches including engineered bispecific antibodies and T cells bearing chimeric antigen receptors. We have explored an alternative approach that seeks to harness the RIG-I innate immune defensive system to selectively kill reservoir cells expressing HIV RNA. We find that acitretin, a retinoic acid derivative approved by the US Food and Drug Administration (FDA) for psoriasis, enhances RIG-I expression *ex vivo*, acts as a mild shocking agent, and induces preferential apoptotic cell death in reactivated reservoir cells including cells from HIV infected patients on suppressive therapy. These effects are observed at doses of acitretin readily achieved *in vivo*. RNAi-mediated knockdown of RIG-I sharply curbs acitretin induced apoptotic death of these reservoir cells and impairs production of downstream soluble factors produced when the RIG-I pathway is activated (interferon- $\alpha$  and CXCL10). Event greater cell killing is observed when acitretin is combined with SAHA. Thus, one possible approach to inducing the death of reservoir cells could be to use combinations of LRAs and acitretin to preferentially ‘shock and kill’ CD4 T cells harboring HIV. Such an approach could eliminate cells that produce both infectious and noninfectious forms of HIV.

This work was supported by the NIH (grants 1R21AI104445-01A1 (P.L.), R56 AI116342 and R21 AI116218 (J.K.W.)), the Department of Veterans Affairs Merit Review Award 5101 BX001048 (J.K.W.), the UCSF-Gladstone Center for AIDS Research Virology Core P30AI027763 (W.C.G. and J.K.W.), U19 AI096113 (W.C.G.) and grant 109301 from amfAR establishing the amfAR Institute for HIV Cure Research (W.C. G., J.K.W., and P.L.)



### **Mark Kay, MD, PhD**

*Dennis Farrey Family Professor*

*Department of Pediatrics and Genetics*

*Vice Chair for Basic Research (Pediatrics)*

*Stanford University*

Dr. Kay is the Head of the Division of Human Gene Therapy and the Dennis Farrey Family Professor in the Departments of Pediatrics and Genetics at Stanford University. He received a PhD in Developmental Genetics, and MD from Case Western Reserve in Cleveland, Ohio. Before coming to Stanford in 1998, Dr. Kay was at the University of Washington as Associate Professor in the Departments of Medicine, Biochemistry and Pathology. Dr. Kay has received many awards. Dr. Kay was on the founding board of directors of the American

Society for Gene Therapy and served as the Society's Vice-President, President-Elect, and President in 2003-2006. He has served on the Oligonucleotide Therapeutics Board of Directors during the last 5 years. He was elected to the Association of American Physicians in 2010. Dr. Kay received the American Society for Gene and Cell Therapy's Outstanding Investigator Award in 2013.

Dr. Kay has worked on the development of many DNA gene transfer vectors and the mechanism by which they transduce tissues in mammals. His group has performed two Phase I/II gene therapy trials for hemophilia B. His laboratory was the first to establish therapeutic RNAi in whole non-embryonic mammals, and RNAi-mediated inhibition of a human viral pathogen (HBV) in animals. His work continues towards defining the molecular limits of delivered and expressed RNAi in vivo as well as the mechanisms involved in si/shRNA-mediated gene silencing and the biological mechanisms involved in miRNA-mediated gene repression. In addition, his laboratory is studying the role that the newly defined small tRNA-derived RNAs play in mammalian gene regulation.



## Using Novel AAV approaches for therapeutic passive immunity against HIV and other pathogens

MA Kay

Department of Pediatrics and Genetics, Stanford University, Stanford, CA

Recombinant AAV vectors (rAAV) have provided some successes in early clinical trials for the treatment of serious diseases. Importantly, rAAV-mediated gene transfer has the potential for achieving long-term and perhaps life-long gene expression after single dose administration. As a result, there is interest in using these vectors to express recombinant proteins (e.g. pan neutralizing antibodies) to provide passive immunization against viral pathogens such as HIV. One of the difficulties in using various rAAV vectors is that when moving from animals to humans a number of unanticipated responses and/or discordance in the dose-response ultimately resulting in a sub-therapeutic outcome. Much of the discordance between animal and humans is based on the sequence variations in the AAV capsid protein used for delivering the therapeutic payload. Based on our published (Lisowski et al., Nature 2014) and more recent unpublished studies, we propose that murine-human xenotransplant models for muscle and liver represent a robust animal model that may more closely predict clinical trial outcomes. Moreover, such models in combination with multi-species DNase-shuffled AAV capsid libraries have allowed us to select for AAV vectors that show 10-20 times enhanced transduction of human muscle and liver. These vectors are currently being tested in more advanced preclinical studies before progressing into the clinic.

The episomal nature of the vector genomes in transduced cells restrict classical rAAV-mediated gene transfer to quiescent tissues. Moreover, even with the low rate of AAV integration, high rates of hepatocellular carcinoma resulting from promoter activation of oncogenic loci in young mice, and loss of episomal AAV genomes (and hence transgene expression) with normal growth and development has raised concerns about their long term safety and efficacy profiles when treating infants. To circumvent these concerns and provide a viable approach to treating infants, we have developed an AAV promoterless gene targeting approach without the use of nucleases (Barzel et al Nature 2015) and use this tactic to successfully treat mice with hemophilia B and more recently express therapeutic levels of pan-neutralizing anti-HIV antibodies. The discovery and characterization of novel AAV vectors as well as their use in both classical gene transfer and genome-editing approaches broadens their application in both biological discovery and therapeutic applications, and may play an important role in designing passive immune strategies against pathogenic viruses.

## SESSION 1 SPEAKERS

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### **Rowena Johnston, PhD** INVITED SPEAKER

*Vice President and Director of Research, amfAR, the Foundation for AIDS Research*

### amfAR's HIV cure research funding

R Johnston

amfAR, the Foundation for AIDS Research, New York, NY

amfAR is dedicated to developing a cure for HIV as part of a strategy to end the HIV/AIDS pandemic. The Countdown to a Cure (C2C) initiative, with a commitment to strategically invest \$100 million in focused, goal-directed cure research, is an intensification of amfAR's cure research funding program that began in 2002. The first C2C grants were awarded in 2015. The initiative includes a range of support for: preliminary investigations; mature projects; encouraging researchers from outside HIV to apply their expertise to the challenge of curing HIV; investigator-initiated and amfAR-directed research programs. amfAR aims to support those projects with the most promise to help build the scientific basis of an HIV cure by 2020. Given that HIV is an integrated virus, and a gene therapy-like approach has resulted in the only apparent sterilizing cure to date, gene therapy may represent a particularly promising approach to cure HIV.

### **Jacob Estes, PhD INVITED SPEAKER**

*Senior Principal Investigator, AIDS and Cancer Virus Program, Frederick National Laboratory for Cancer Research, Leidos Biomedical Research, Inc.*

## **Imaging Lymphoid Tissues to Understand Viral Persistence and Impaired Function**

J Estes

Frederick National Laboratory for Cancer Research. Leidos Biomedical Research Inc. Frederick, MD

A primary obstacle to curing HIV infection is the early establishment of long-lived viral reservoirs from which infection rebounds if antiretroviral therapy (ART) is interrupted. There is currently considerable effort directed to devising strategies to eliminate or greatly reduce these reservoirs so it would be possible to discontinue ART for extended or indefinite periods of time, referred to as a functional cure. To date, these strategies have largely been assessed by monitoring changes in T cell HIV reservoirs from peripheral blood (PB), but the lymphoid tissues (LT) are the major tissue compartments where latently and persistently infected cells reside and infectious virus persists on the follicular dendritic cell network (FDCn) in lymphoid follicles. Because HIV is primarily a disease of lymphoid tissues, a detailed understanding of HIV reservoir establishment and persistence is particularly difficult to study in humans but can be readily studied in SIV nonhuman primate (NHP) models. This talk will focus on the invaluable utility of NHP models in understanding the timing of viral seeding, viral dynamics and compartmentalization, and cell populations involved in viral persistence within LTs following infection, and will highlight B cell follicles in viral persistence both before and during ART, with infected TFH cells and viral particles bound to the FDCn as potentially important cells harboring infectious virus. Novel strategies to purge virus from these tissues may be hindered by the pathologic damage to these immune organs induced by persistent chronic inflammation and immune activation, which leads to a profound impairment of normal lymph node function. Adjunctive strategies to reverse LT damage will likely need to be considered to realize the full potential of HIV cure therapeutic approaches, which will need full access to these important immune organs where most HIV reservoirs reside.

## SESSION 2 SPEAKERS

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### **Pamela Skinner, PhD** INVITED SPEAKER

*Associate Professor, Department of Veterinary and Biomedical Sciences,  
University of Minnesota*

### Targeting virus-specific CTL to B cell follicles to treat HIV

P Haran<sup>1</sup>, S Li<sup>1</sup>, E Rakasz<sup>2</sup>, TA Reinhart<sup>3</sup>, E Connick<sup>4</sup>, and PJ Skinner<sup>1</sup>

<sup>1</sup>University of Minnesota, Minneapolis, MN; <sup>2</sup>University of Wisconsin-Madison, Madison, WI; <sup>3</sup>University of Pittsburgh, Pittsburgh, PA <sup>4</sup>University of Arizona, Tucson, AZ

During chronic HIV and SIV infections, virus-producing cells are most highly concentrated inside of B cell follicles within lymphoid tissues. Whereas virus-specific CTL are often largely excluded from B cell follicles and unable to clear all HIV- and SIV-producing cells located within B cell follicles. These findings indicate that B cell follicles are somewhat of an immune privileged site. Although typically relatively low in numbers, our studies suggest that subsets of follicular SIV-specific CTL are functional, in that 1) they express functional markers including Ki67, perforin, and granzyme B, and 2) upon CD8 depletion, levels of follicular SIV-producing cells increase. Based on these data, we are now working to develop a cellular therapy to treat HIV. We hypothesize that targeting HIV-specific CTL to B cell follicles will lead to long-term durable remission from HIV. In order to begin to test this hypothesis, we created human and rhesus macaque CXCR5-transducing retroviral vectors pseudotyped with either the vesicular stomatitis virus G protein (VSV-G) or the feline endogenous retrovirus RD114 envelope protein to enable transduction of CD8 T cells. These CXCR5 encoding virions are being used to transduce primary human and rhesus macaque CD8 T cells. We are characterizing the phenotype of these cells and evaluating the ability of these cells to migrate to the ligand CXCL13 in vitro. We will also evaluate the ability of autologous CXCR5-transduced rhesus macaque CD8 T cells to migrate to B cell follicles and suppress virus replication in vivo. We are hopeful that these studies will lead to a novel cellular therapy to effectively treat HIV and lead to long-term durable remission.

### **Rafick-Pierre Sékaly, PhD INVITED SPEAKER**

*Professor, Department of Pathology; Co-Director, Proteomics and Systems Biology Core, Center for AIDS Research, Case Western Reserve University*

## **The Duality of interferons as drivers and antagonists of immune senescence and HIV persistence**

RP Sekaly<sup>1</sup>, K Ghneim<sup>1</sup>, SP Ribeiro<sup>1</sup>, A Talla<sup>1</sup>, M Aid<sup>2</sup>, SA Younes<sup>1</sup>, J Zeidan<sup>1</sup>, C Benne<sup>1</sup>, M Lederman<sup>1</sup> and S Deeks<sup>3</sup>

<sup>1</sup>Departments of Pathology and Medicine, Case Western Reserve University, Cleveland, OH; <sup>2</sup>Beth Israel Deaconess Medical Center, Boston, MA; <sup>3</sup>Department of Medicine, University of California, San Francisco, San Francisco, CA.

Interferons are the most potent mediators of antiviral immune responses. They are also immune modulators as they can trigger pro and anti-inflammatory effector molecules such as cytokines and cell surface markers including immune check point blockers. I will focus my presentation on providing evidence for the duality of interferons and show the cells and pathways that contribute to the interferon response that contributes to driving innate and adaptive immune responses that contribute control of viral dissemination and persistence. This presentation will highlight also interferon responses that trigger to immune senescence and viral persistence. The upstream regulators and drivers of these different responses will be discussed. Emphasis will be placed on immune-metabolism.

This work has been funded by DARE U19 AI096109 and CAVR U19 AI095985, U01 AI105937 , R01 AI110334 and U19 HL129902

## SESSION 2 SPEAKERS

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### **Florian Hladik, MD, PhD INVITED SPEAKER**

*Research Associate Professor, Departments of Obstetrics & Gynecology and Medicine, University of Washington; Affiliate Investigator, Vaccine and Infectious Disease Division, Fred Hutchinson Cancer Research Center*

## Anti-proliferation and NRTI-sparing ART to treat HIV latency

F Hladik<sup>1,2</sup>

<sup>1</sup>University of Washington, Seattle, WA; <sup>2</sup>Fred Hutchinson Cancer Research Center, Seattle, WA

The latent HIV reservoir is largely sustained by proliferation of CD4+ T cells containing integrated, non-replicating HIV DNA. Using a mathematical model describing the decay dynamics of the HIV reservoir during suppressive ART, developed by collaborator Dr. Joshua Schiffer and his team, we predict that partially curbing latent cell proliferation continuously for three years will shrink the reservoir 100 to 10000-fold. This would allow ART-free remission in some patients. When testing anti-proliferative therapy for this purpose, which we are proposing in a current grant application, the choice of suppressive ART may also matter. In my group, we are investigating the NRTI tenofovir, an ART mainstay drug, in vivo in humans. We discovered that tenofovir has some activities that may slow down the decay rate of the latent cell pool. If confirmed, we conclude that combining anti-proliferative therapy with an NRTI-free ART regimen, fully suppressive versions of which are becoming available, offers a new approach to HIV cure.

**Jim Sun, PhD ORAL ABSTRACT**

*Post-Doctoral Fellow, University of Alabama at Birmingham*

## Targeted therapeutic elimination of HIV-infected reservoir macrophages

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**Background:** Macrophages are readily infected by HIV-1, but are resistant to the cytopathic effect of the virus, a phenomenon that allows the virus to hijack macrophages to establish persistent infection in a variety of tissues, thereby forming viral reservoirs. We recently described that the host phosphatase PPM1A reduces the innate cellular antiviral and antibacterial immune response, rendering macrophages highly susceptible to HIV-1 infection (1). Interestingly, HIV-1 infected macrophages showed upregulated production of PPM1A. However, the functional consequences of HIV-infection induced PPM1A overproduction remain unclear. This study aims to address these questions and evaluate the potential for therapeutic targeting of the PPM1A signaling network as a means to eradicate persistently HIV-infected macrophages.

**Methods:** Kinome analysis was used to build protein-protein interaction networks (MetaCore) to (i) understand how HIV-1 infection induced PPM1A upregulation renders macrophages resistant to apoptosis and (ii) guide the identification of drug targets to restore the ability of HIV-1 infected macrophages to undergo apoptosis. Functional characterization of PPM1A was addressed using genetic manipulation of THP-1 monocytes/macrophages, and all effects were confirmed in primary human macrophages.

**Results:** Here we demonstrate that upregulation of PPM1A in response to HIV-1 infection renders macrophages resistant to apoptotic cell death. Accordingly, targeted depletion of PPM1A by shRNA induced apoptosis of HIV-1 infected macrophages. Kinome analysis revealed a role for the c-Jun N-terminal kinase (JNK) in the PPM1A signaling pathway, which was confirmed by multiplex assays showing abrogation of JNK phosphorylation in HIV-1 infected macrophages. Importantly, pharmacologic activation of JNK by anisomycin, similar to genetic depletion of PPM1A, specifically promoted cell death of HIV-1 infected macrophages.

**Conclusions:** Our results demonstrate that the resistance of macrophages to the cytopathic effect of HIV-1 is a result of PPM1A controlled suppression of pathogen-induced apoptosis. Restoring the intrinsic ability of HIV-1 infected macrophages to undergo apoptosis by modulating the PPM1A signaling axis would thus provide a host-cell directed therapy approach to specifically eliminate persistently HIV-1 infected macrophages.

**References:** Sun J, et al. Protein Phosphatase, Mg<sup>2+</sup>/Mn<sup>2+</sup>-dependent 1A controls the innate antiviral and antibacterial response of macrophages during HIV-1 and Mycobacterium tuberculosis infection. *Oncotarget*, (2016).

## SESSION 4 SPEAKERS

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

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

## **Supraphysiologic Control over HIV Replication Mediated by CD8 T cells Expressing a Re-engineered CD4 based Chimeric Antigen Receptor**

RS Leibman<sup>1</sup>, MW Richardson<sup>1</sup>, CT Ellebrecht<sup>2</sup>, JA Glover<sup>1</sup>, A J Secreto<sup>1</sup>, SR Akkina<sup>1</sup>, Y Yi<sup>1</sup>, F Shaheen<sup>1</sup>, J Wang<sup>3</sup>, KA Dufendach<sup>1</sup>, MC Holmes<sup>3</sup>, RG Collman<sup>1</sup>, AS Payne<sup>2</sup>, JL Riley<sup>1</sup>

<sup>1</sup>Department of Microbiology and Center for Cellular Immunotherapies, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA <sup>2</sup>Department of Dermatology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA; <sup>3</sup>Sangamo BioSciences Inc., Richmond, CA

HIV is adept at avoiding naturally generated T cell responses; therefore, there is a need to develop HIV-specific T cells with greater potency for use in HIV cure strategies. Starting with a CD4-based chimeric antigen receptor (CAR) that was used safely in several clinical trials, we optimized the vector backbone, promoter, HIV targeting moiety, and transmembrane and signaling domains to determine which components augmented the ability of T cells to control HIV replication. T cells expressing this optimized CAR were at least 50-fold more potent in vitro at controlling HIV replication than the original CD4 CAR or TCR-based approaches and substantially better than broadly neutralizing antibody-based CARs. In a humanized mouse model of HIV infection, optimized CD4 CAR T cells that contained the CD28 costimulatory domain were able to protect CD4 T cells from HIV-mediated destruction better than CD4 CARs containing 4-1BB or just the CD3 zeta signaling domain. Additionally, we observed significant enrichment of CAR-expressing CD8 T cells that were protected from HIV infection using CCR5 zinc finger nucleases. Importantly, T cells expressing the original CD4 CAR demonstrated improved control over HIV replication when pre-treated with CCR5 ZFNs. Together, these data indicate that potent HIV-specific T cells can be generated using improved CAR design and suggest that these highly active effector T cells will need to be protected from HIV infection to enable durable control of HIV.



**Thor Wagner, MD INVITED SPEAKER**

*Assistant Professor, Pediatric Infectious Diseases, University of Washington & Seattle Children's Hospital*

**Engineering HIV-resistant anti-HIV CAR T cells**

M Hale<sup>1</sup>, T Mesojednik<sup>2</sup>, G Romano Ibarra<sup>1</sup>, J Sahni<sup>1</sup>, A Bernard<sup>2</sup>, K Sommer<sup>1</sup>, AM Scharenberg<sup>1,3,4</sup>, DJ Rawlings<sup>1,3,4</sup>, TA Wagner<sup>2,3</sup>

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Recent advances in gene editing, chimeric antigen receptor (CAR) technology, and broadly neutralizing antibodies (bNAbs) provide opportunities to improve cellular therapeutics for the treatment of HIV. We engineered primary human T cells to express anti-HIV CARs based on bNAbs (HIVCAR) that target several different epitopes on Env. We show that HIVCAR T cells are specifically activated and kill HIV-infected versus uninfected cells, even in the absence of HIV replication. We also show that disabling CCR5 expression in these T cells, by nuclease-mediated NHEJ or that homology-directed recombination of the HIVCAR gene expression cassette directly into the CCR5 locus, enhances suppression of replicating virus compared to HIVCAR expression alone. This work demonstrates that HIV immunotherapy combining potent bNAb-based single chain variable fragments (scFv) fused to second generation CAR signaling domains, delivered directly into the CCR5 locus of T cells by homology-directed gene editing is feasible and effective. This strategy might be able to eradicate the HIV-infected cell reservoir in HIV-infected individuals, and could help cure HIV.

### **Edward Berger, PhD INVITED SPEAKER**

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

## **Bi-Specific Anti-HIV Chimeric Antigen Receptors Designed for Optimal Breadth and Potency, and Minimal Immunogenicity**

M Ghanem, S Bolivar-Wagers, B Dey, V Bundoc, B Patel, D Vargas-Inchaustegui, EA Berger

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

Durable control of HIV after cessation of antiretroviral therapy is a much sought-after goal toward a 'functional cure'. We are developing strategies based on T cells engineered to express a chimeric antigen receptor (CAR) targeting the HIV Env glycoprotein on the surface of infected cells. When adoptively transferred back to the infected person, these cells will potentially provide the long-term control of infection needed for a functional cure.

For a CAR against HIV, the ideal targeting motif should be inescapable, non-immunogenic, and devoid of undesired activities. We designed CARs with novel bi-specific targeting motifs composed of sequences from invariant human proteins, and directed against distinct highly conserved determinants on the HIV Env glycoprotein. These targeting motifs consist of a segment of human CD4 (which binds to the requisite primary receptor binding site on HIV-1 gp120) linked to the carbohydrate recognition domain (CRD) of a human C-type lectin (which recognizes the high-mannose glycans universally displayed on gp120).

Compared to a monospecific CD4 CAR, several bi-specific CD4-CRD CARs exhibited extraordinary potency against genetically diverse HIV-1 isolates in HIV/PBMC spreading infection assays. Analyses of mutant CAR constructs revealed the absolute requirement for the functional CD4 moiety, and the augmenting activity of the CRD. Importantly, the CRD prevented the CD4 moiety from acting as an HIV entry receptor and rendering transduced CD8+ T cells susceptible to infection.

The minimal immunogenicity predicted for all-human invariant sequences, coupled with limits on virus escape imposed by targeting two highly conserved Env determinants, highlight the potential value of these bi-specific CARs toward a functional cure of HIV. Collaborative efforts are underway to test these CARs relevant animal models. Such studies will inform strategies to optimize the complex requisites for in vivo efficacy of CAR-T cells against HIV, including their engraftment, expansion, persistence, trafficking, and durable functionality.

**Mayra Carrillo, PhD ORAL ABSTRACT**

*Post-Doctoral Scholar, Department of Medicine, Hematology, and Oncology, University of California, Los Angeles*

## Hematopoietic stem cell based chimeric antigen receptor therapy for HIV infection

A Zhen<sup>1</sup>, C Peterson<sup>2</sup>, MA Carrillo<sup>1</sup>, N Chang<sup>1</sup>, B Lam<sup>1</sup>, C Youn<sup>1</sup>, V Rezek<sup>1</sup>, M Kamata<sup>1</sup>, J Zack<sup>1</sup>, HP Kiem<sup>2</sup>, S Kitchen<sup>1</sup>

<sup>1</sup>Department of Medicine, Hematology, and Oncology, University of California, Los Angeles, Los Angeles, CA; <sup>2</sup>Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, WA

HIV-1 specific cytotoxic T lymphocytes are a key host immune response to HIV infection and are crucial for the elimination of HIV infected cells. We are currently investigating therapeutic approaches directed at enhancing HIV specific CTL response in hope of eradicating the virus. Previous studies have shown that a chimeric antigen receptor (CAR) containing the CD4 molecule linked to the signal domain of the TCR  $\zeta$  chain can be used to redirect T cells to target HIV infected cells. CD4 CAR modified T cells can inhibit viral replication and kill HIV infected cells in vitro, but showed limited efficacy in clinical trials primarily due to their susceptibility to HIV infection through the expression of the CD4 CAR.

Stem cell based gene therapy has several advantages over adoptive T cell transfer therapy as it provides long-term sustained engraftment of modified stem cells that undergo normal T cell differentiation and selection. Our previous studies using humanized mice demonstrated that stem cell based gene therapy with a protective CD4 CAR resulted in successful hematopoietic differentiation of CD4 CAR expressing T cells and resulted in significant suppression of HIV replication. Here we report the testing of a lentiviral vector that expresses the CD4 CAR and the highly effective anti-HIV protective peptide C46 (C46CD4CAR) in the non-human primate model of SHIV infection. We determined that engraftment of pigtailed macaques with C46CD4CAR-modified hematopoietic stem cells is safe and the animals have normal transplant recovery. We observed long-term engraftment and stable production of C46CD4CAR expressing cells without any significant toxicities. We found that C46CD4CAR modified HSCs could differentiate into multiple hematopoietic lineages, including T cells, NK cells, and B cells. Following SHIV challenge, we observed significant expansion of C46CD4CAR expressing cells and were capable of killing infected cells. We believe this demonstrates the safety and feasibility of a stem cell based therapy utilizing an HIV-specific CAR for chronic HIV infection in non-human primates. These results set the stage for future investigational new drug (IND) development and future use in human clinical trials in an attempt to eradicate viral infection and provide more effective immune surveillance of HIV.

## SESSION 5 SPEAKERS

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### Laurie Sylla **INVITED SPEAKER**

*Member, defeatHIV and Martin Delaney Collaboratory Community Advisory Boards*

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

L. Sylla<sup>1</sup>, J. Taylor<sup>2</sup>, D. Evans<sup>3</sup>, A. Skinner<sup>4</sup>, K. Dubé<sup>4</sup>

<sup>1</sup>defeatHIV Community Advisory Board (CAB), Seattle, WA; <sup>2</sup>Collaboratory of AIDS Researchers for Eradication (CARE) CAB, Palm Springs, CA; <sup>3</sup>Delaney AIDS Research Enterprise (DARE) CAB, San Francisco, CA <sup>4</sup>UNC Gillings School of Global Public Health, Chapel Hill, NC

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

**Methods:** A cross-sectional survey of 400 HIV+ adults was conducted, followed up by 10 focus groups with participants in four cities. Focus group discussions included questions specific to what individuals expected of HIV cure researchers and what they wanted researchers to know.

**Results:** Participants wanted research teams to understand the psychological trauma experienced by many people living with HIV, particularly past and ongoing stigma, to be respectful of them as whole human beings, to "get them," to be honest and transparent about all study risks and procedures, to compensate them fairly, and to have study team members and visits available to them after hours. Participants also endorsed having someone with a mental health background as part of the study team. Non-white participants expressed desire for study teams to include members who were reflective of participants. Participants expressed significant concerns about risk to their health, study procedures, side effects, and lack of durability of a functional cure. Women had specific concerns related to reproductive health and the impact a side effect might have on their responsibility to care for others. Some wanted researchers to be mindful that they were as essential to the research as the researchers.

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

**Annemarie Wensing, MD, PhD INVITED SPEAKER***Clinical Virologist, Department of Medical Microbiology, University Medical Center Utrecht***Allogeneic Stem Cell Transplantation in HIV-1 Infected Individuals; the EpiStem Consortium**

AM Wensing<sup>1</sup>, JL Diez-Martin<sup>2</sup>, G Huetter<sup>3</sup>, J Kuball<sup>4</sup>, M Kwon<sup>2</sup>, M Nijhuis<sup>1</sup>, A Saez-Cirion<sup>5</sup>, V Rocha<sup>6</sup>, M Salgado<sup>7</sup>, J Schulze zur Wiesch<sup>8</sup>, A Stam<sup>1</sup>, J Martinez-Picado<sup>7,9</sup>, EpiStem Consortium

<sup>1</sup>University Medical Center Utrecht, Microbiology/Virology, Utrecht, Netherlands, <sup>2</sup>Hospital General Universitario Gregorio Marañon, Madrid, Spain, <sup>3</sup>Cellex, Dresden, Germany, <sup>4</sup>University Medical Center Utrecht, Utrecht, Netherlands, <sup>5</sup>Institute Pasteur, Paris, France, <sup>6</sup>Oxford University Hospital, Oxford, United Kingdom, <sup>7</sup>AIDS Research Institute IrsiCaixa, Badalona, Spain, <sup>8</sup>University Medical Center Hamburg-Eppendorf, Hamburg, Germany, <sup>9</sup>ICREA, Barcelona, Spain

**Background:** To date, the only and most compelling evidence of a medical intervention that has been able to cure HIV-1 infection (the “Berlin patient”), involved an allogeneic stem cell transplant (SCT) from a donor who was homozygous for CCR5 $\Delta$ 32. Although this high-risk procedure is only indicated for certain hematological malignancies, the strategy raised tremendous scientific potential to gain insight in the mechanisms of HIV eradication.

**Methods:** The EpiStem consortium aims to guide clinicians of HIV infected patients who require an SCT in donor search and CCR5 screening, ethical regulations, the SCT procedure, sampling procedures and in depth investigations to study HIV persistence. The patients are included in the EPISTEM observational cohort. Detailed analysis of the cohort should provide insight as to whether additional factors such as conditioning regimen, total body irradiation and graft versus host disease may contribute to the eradication of the potentially infectious viral reservoir in addition to the lack of a functional CCR5 receptor.

**Results:** Nearly 30,000 cord blood units in multiple European blood banks and more than 1,000,000 adult donors have been genotyped for CCR5 to generate a registry of CCR5 $\Delta$ 32 available donors. Twenty HIV positive patients with diverse hematological malignancies have been registered to the EPISTEM cohort. Since 2012, 22 individuals have been included in the cohort. 15 patients have been transplanted; 5 with a CCR5 $\Delta$ 32, 1 with a heterozygous, and 9 with a CCR5 WT donor. In 3 cases the donor cells came from cord blood and in 12 cases from an adult donor. So far, 5 patients have successfully passed the 12 months follow-up after transplantation, and 8 patients have died after transplantation, despite achieving full donor chimerism in most cases. Preliminary analysis of virological and immunological data from blood and tissue samples shows a systematic reduction of HIV-1 reservoirs to very low levels.

**Conclusions:** EPISTEM is actively recruiting new cases and continues to systematically investigate HIV persistence over time to gain insight in potential HIV-1 eradication.

Countries of research: Belgium, Brazil, France, Germany, Italy, Netherlands, Spain, United Kingdom.

## SESSION 6 SPEAKERS

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### **Priti Kumar, PhD** INVITED SPEAKER

*Associate Professor, Department of Internal Medicine/Section of Infectious Diseases, Yale School of Medicine*

## Direct in-vivo T cell engineering for gene therapy of HIV-1

P Kumar

Department of Internal Medicine/Section of Infectious Diseases, Yale School of Medicine, Yale University, New Haven CT

Human T cells play an important role in controlling HIV replication and spread, however, they are themselves targets of the virus. Genetic manipulation of T cells for adoptive cellular therapies is being pursued with the aim of generating HIV-1-resistant cells or re-inforcing HIV-1-specific immune responses. It is hoped that one or a combination of these approaches may enable a functional cure for HIV-AIDS in the absence of antiretrovirals. However, at present, engineering of T cells for sustained control of HIV-1 replication can be achieved only ex-vivo. This talk will focus on an approach for direct gene transfer into human T cells in vivo and their use in humanized mouse models of HIV infection.

**Nick Llewellyn, PhD ORAL ABSTRACT**

*Post-Doctoral Research Associate, Department of Molecular Microbiology and Immunology, University of Southern California.*

**An Improved Humanized Model of HIV Latency**

GN Llewellyn<sup>1</sup>, E Seclen<sup>1</sup>, S Wietgrefe<sup>2</sup>, H Ping<sup>1</sup>, M Chateau<sup>1</sup>, Siyu Liu<sup>1</sup>, K Perkey<sup>1</sup>, J Zack<sup>3</sup>, S Louie<sup>1</sup>, A Haase<sup>2</sup>, P Cannon<sup>1</sup>

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Current anti-retroviral therapy (ART) is very effective at suppressing HIV in infected patients. However, ART is not curative, and viral rebound to pre-treatment levels is observed quickly after therapy withdrawal. One of the primary causes of this phenomena is likely HIV latency. Therefore, ridding the body of these latently infected cells in combination with current antiretroviral therapy is one avenue for a cure.

To study HIV latency and anti-latency therapies, a small animal model is crucial. We have developed a model using antiretroviral therapy drugs administered through mouse food pellets and have thoroughly characterized virus suppression and drug levels in the blood and tissues. Latency was measured via an ex vivo latency assay of spleen cells. An important focus of latency research is on identifying and studying surrogate markers of latently infected cells. In this study, we show that PD1+ and TIGIT+ T cells, markers of T cell exhaustion and negative regulators of cell activation and proliferation, contribute significantly more to the latent reservoir than PD1- and TIGIT- T cells in a humanized mouse model of latency. In addition to spleen cells, we also measured latency specifically in lymph nodes. Finally, we were also able to use the ex vivo latency assay to demonstrate the effect of anti-HIV TALENs at reducing the latent reservoir, an important milestone for nuclease-mediated anti-latency strategies.

### Gabriela Webb, PhD **ORAL ABSTRACT**

*Post-Doctoral Researcher, Vaccine and Gene Therapy Institute, Oregon Health and Science University*

## The human IL-15 superagonist, ALT-803, activated NK cells, memory T cells, and latency SIV-infected CD4+ T cells in the macaque model of HIV

GM Webb<sup>1</sup>, JM Greene<sup>1</sup>, JJ Stanton<sup>1</sup>, AW Legasse<sup>1</sup>, BS Park<sup>1</sup>, MK Axhelm<sup>1</sup>, EK JEng<sup>2</sup>, HC Wong<sup>2</sup>, JB Whitney<sup>3</sup>, RB Jones<sup>4</sup>, JB Sacha<sup>1</sup>

<sup>1</sup>Oregon Health & Science University, Portland OR; <sup>2</sup>Altor BioScience Corporation; Miramar, FL; <sup>3</sup>Harvard Medical School, Boston, MA; <sup>4</sup>The George Washington University, Washington, DC.

**Background:** A major focus of HIV cure research is the “shock and kill” approach, whereby small molecule latency reversing agents (LRAs) activate latent virus for recognition and clearance by immune mediators such as CD8+ T cells. However, certain LRAs such as HDAC inhibitors retard antiviral T cell immunity, and many archived viruses carry escape mutations rendering them invisible to CD8+ T cells. Therefore, there is an urgent need to develop alternate approaches to activate and clear the latent HIV reservoir that do not negatively impact immune function and are independent of viral sequence. 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, ALT-803, as a potential LRA in cART-suppressed SIV-infected macaques.

**Methods:** SIV-naïve and SIV-infected macaques were administered ALT-803 IV and subsequently monitored for NK and T cell proliferation. ALT-803 was tested as an LRA in vitro with primary CD4+ T cells from cART-suppressed macaques, and in vivo in SIV-infected, cART-suppressed macaques.

**Results:** ALT-803 activated and induced robust proliferation in NK cells, and both effector and central memory T cells. ALT-803 redirected activated cells to secondary lymphoid tissues, a known anatomical location of the viral reservoir. ALT-803 did not affect viral loads in macaques with uncontrolled SIV infection; instead, ALT-803 potentiated low-level viral replication in elite controllers. In experiments using PBMC from cART-suppressed macaques, ALT-803 induced robust viral replication in vitro. When administered to macaques with cART-suppression of plasma viremia, ALT-803 treatment resulted in plasma viral “blips” and unlike previous reports of IL-7, ALT-803 did not cause an increase in the size of the latent viral reservoir.

**Conclusions:** ALT-803, an IL-15 superagonist, is well tolerated in SIV-infected, cART-suppressed macaques and induces virus reactivation both in vitro and in vivo. In addition to reactivating quiescent virus, ALT-803 potentially activates NK and memory CD8+ T cells, which traffic to lymph nodes where latently-infected CD4+ T cells reside. The ability of ALT-803 to potentially mediate both the “shock” and “kill” make it an appealing candidate for inducing durable cART-free HIV remission.



**Kevin Haworth, PhD ORAL ABSTRACT**

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

## Comprehensive Integration Site Analysis of Human Immunodeficiency Virus During *In Vivo* Infections of Humanized NSG Mice

KG Haworth<sup>1</sup>, LE Scheffter<sup>1</sup>, ZK Norgaard<sup>1</sup>, JE Adair<sup>1,2</sup>, HP Kiem<sup>1,2</sup>

<sup>1</sup>Fred Hutchinson Cancer Research Center, Seattle, WA; <sup>2</sup>University of Washington, Seattle, WA

**Background:** A key event in the lifecycle of Human Immunodeficiency Virus (HIV) is the permanent integration into the infected cell's genome. In addition to allowing for long-term persistence of the virus, this integration event results in a trackable mark carried in infected cells. By analyzing the patterns of integration sites detected in both cell cultures and tissue samples from animal models of infection, it is possible to better understand the basic biology of integration and determine what may drive infected cells to persist despite effective treatment regimens.

**Methods:** NSG mice were infused with human CD34+ cells as neonates, reconstituting a human immune system including high levels of CD4+ T cells capable of sustaining HIV infection. After 16 weeks post-challenge, tissues were collected and subjected to integration site analysis for HIV proviral DNA. Identified integration sites were mapped and compared across multiple parameters to identify areas enriched for integration events, as well as clonally expanded cells *in vivo*.

**Results:** Genomic wide analysis of HIV integration sites reveals a remarkably similar landscape both in tissue culture infection of Jurkat cells to *in vivo* infection data, and across HIV strains including both CXCR4 and CCR5 tropic viruses. As previously observed, the majority of integrations occur near or within gene transcripts demonstrating HIV's preference to integrate near actively transcribed genes. However, certain areas of the genome, and even unique genes are specifically enriched for IS in the samples and across multiple animals and infection conditions. In addition to specific genomic regions of enrichment, we also observe specific clonal outgrowth of unique integration events in genes previously unidentified in the literature.

**Conclusions:** We have cataloged the most extensive HIV integration library to date in both tissue culture and *in vivo* infection studies, including over 80,000 unique integration events. Genome wide correlation studies reveal regions significantly enriched for HIV integration and genes which repeatedly exhibit clonal outgrowth in multiple animals. These types of studies are now being applied to human patient samples undergoing anti-retroviral therapy studies to determine if latency and persistence of infection can be mapped to unique integration events or genes of interest.

### **Alan Perelson, PhD** INVITED SPEAKER

*Senior Fellow, Los Alamos National Laboratory*

## Using anti-PD-L1 to switch from a high to a low viral load steady state: A possible means to functional cure

Y Cao<sup>1,2</sup>, J Conway<sup>1,3</sup>, S Mason<sup>4</sup>, JB Whitney<sup>5</sup>, AS Perelson<sup>1</sup>

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During chronic viral infections, CD8 T cells are driven into an exhausted state in which they express PD-1 and have impaired effector functions. Exhaustion can be reversed by monoclonal antibodies that block the PD-1/PD-L1 interaction. In a recent study, 4 of 8 SIV-infected rhesus macaques on ART and treated with 5 infusions of anti-PD-L1 (BMS-936559) controlled their viral load after ceasing ART.

To understand if the reversal of T cell exhaustion contributed to viral control, we generalized our previous post-treatment control (PTC) viral dynamics model (Conway and Perelson, PNAS 2015) so that it explicitly incorporates an exhausted cell population. We used this model to analyze the data generated from the anti-PD-L1 study.

Fitting our model to the data in order to estimate parameters showed our model exhibits bistability for the parameters estimated for the majority of responder animals, with one high VL and one low VL steady state. Surprisingly, from model simulations we found that PTC would not be obtained in these animals without anti-PD-L1 and that between 2 and 5 doses of anti-PD-L1 were needed to switch the responders from the high VL steady state to the low VL one. Detailed analysis of the effector and exhausted cell population dynamics showed that the effector cell population generated from the anti-PD-L1 reversal of exhaustion contributed to the VL control in responder animals. Overall, our modeling and data fitting suggests that the reversal of T cell exhaustion can help to achieve VL control in some groups of patients after cessation of ART. The model also suggests that it may be possible to switch “bistable patients” from high to low VL steady states and thereby achieve sustained control with only a limited number of antibody infusions.

**Mario Roederer, PhD INVITED SPEAKER**

*Acting Director, Translational Research Program Chief, ImmunoTechnology Section,  
Vaccine Research Center, National Institute of Allergy and Infectious Diseases*

## **The SIV Model to Evaluate Chimeric Antigen Receptor-Based Intervention**

N Iwamoto, B Patel, R Mason, H Welles, E Berger, and M Roederer

Vaccine Research Center, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD

To develop the NHP model to evaluate antibody-based interventions, and take advantage of the well-characterized genetically diverse swarms (and clones) with varying neutralization profiles, we undertook to isolate elicited NHP mAbs against the SIV envelope. Using a combination of targeted approaches, we isolated over 100 distinct mAbs that target multiple epitopes each in: CD4 binding site, V1/V2, V3, and the high mannose patch region. Using a panel of 20 highly diverse SIV and HIV-2 strains, we defined both strain-specific and broadly neutralizing highly potent antibodies.

We down-selected a panel of these mAbs for application to immunological interventions, including adoptive transfer of autologous chimeric antigen receptor (CAR) transduced CD8 T cells. A range of mAbs targeting different epitopes and having different potencies were tested in vitro. To our surprise, there was no correlation between neutralization potency of the mAb and its ability to suppress SIV as a CAR; the latter was dependent solely on structural constraints.

Three CARs were selected for in vivo testing. We compared a variety of parameters such as retroviral vs lentiviral transduction, introduction of multiple CARs per cell, and ex vivo stimulation and expansion protocols to determine which resulted in optimal phenotype and numbers of transferred cells following infusion. In SIV-infected animals, we could detect cells for months following infusion; they appeared to be activated and dividing. However, to date, the cells did not expand over time nor did they have a measurable impact on viral load, either when infused in the setting of chronic or acute infection.

## SESSION 7 SPEAKERS

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### **Michael Farzan, PhD** INVITED SPEAKER

*Professor and Vice Chairman, Department of Immunology and Microbial Science, The Scripps Research Institute*

## AAV-expressed eCD4-Ig as an HIV-1 vaccine alternative

M Farzan

The Scripps Research Institute, Jupiter, FL

Long-term in vivo expression of a broad and potent entry inhibitor could circumvent the need for a conventional vaccine for HIV-1. Adeno-associated virus (AAV) vectors can stably express HIV-1 broadly neutralizing antibodies (bNAbs). However even the best bNAbs neutralize 10-50% of HIV-1 isolates inefficiently ( $IC_{80} > 5 \mu\text{g/ml}$ ), suggesting that high concentrations of these antibodies would be necessary to achieve general protection. bNAbs are also heavily hypermutated, and in primate studies, they tend to elicit high levels of anti-idiotypic antibodies which interfere with their long-term expression.

eCD4-Ig, a fusion of CD4-Ig with a small CCR5-mimetic sulfopeptide, binds avidly and cooperatively to the HIV-1 envelope glycoprotein (Env) and is more potent than the best bNAbs (geometric mean  $IC_{50} < 0.1 \mu\text{g/ml}$ ). Because eCD4-Ig binds only conserved regions of Env, it is also much broader than any bNAb. For example, eCD4-Ig efficiently neutralized 100% of a diverse panel of neutralization-resistant HIV-1, HIV-2, and SIV isolates, including a comprehensive set of isolates resistant to the CD4-binding site bNAbs VRC01, NIH45-46, and 3BNC117, and a panel of 200 clade C isolates. Moreover, in in vitro studies, eCD4-Ig-resistant viruses did not emerge under conditions where escape from CD4-Ig and NIH45-46 was readily observed.

Rhesus eCD4-Ig was also markedly less immunogenic than rhesus forms of four well characterized bNAbs in rhesus macaques. All rhesus macaques inoculated with an AAV vector expressing rhesus eCD4-Ig were protected after more than a year post-inoculations from escalating intravenous challenges with SHIV-AD8 and SIVmac239 that infected all 12 control macaques. Our data suggest that AAV-delivered eCD4-Ig can function like an effective HIV-1 vaccine.

**Deborah Fuller, PhD INVITED SPEAKER**

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

## The Potential for Achieving a Functional Cure of HIV Through Therapeutic Vaccination

D Fuller

Department of Microbiology, University of Washington, Seattle, WA

Therapeutic vaccines that increase T cell responses may improve treatment of HIV. We have shown that therapeutic immunization of SIV-infected macaques with DNA vaccines administered by particle mediated epidermal delivery (gene gun) during antiretroviral drug therapy (ART) increases mucosal and systemic T cell responses and can afford durable protection from viral rebound with no evidence of disease in ~50% of the animals for years after stopping ART. In addition, we found that when SIV infected macaques are immunized both before infection and then again post-infection during ART with a single drug, up to 88% of the animals are protected from viral rebound after stopping ART suggesting that vaccines that fail to prevent infection, may nevertheless improve the effectiveness of post-infection therapy or cure strategies. To understand the host and viral mechanisms underlying the apparent viral remission that occurs in some vaccinated animals but not others, our laboratory is investigating biomarkers and host responses in animals that exhibit a viral remission/functional cure and comparing those results to responses in animals that are not protected and exhibit viral rebound shortly after stopping ART. Our results, to date, indicate broadly specific mucosal CD8+ T cell response that is localized in mucosal tissues likely contributes to a functional cure but that the benefits of these responses are diminished in animals exhibiting strong inflammatory responses in the gut. Our results show the challenges of developing an effective therapeutic HIV vaccine and suggest an effective approach will need to achieve a fine balance between increasing host immunity to improve control HIV infection without inducing detrimental responses that can benefit the virus.

### James Voss, PhD ORAL ABSTRACT

*Post-Doctoral Fellow, Department of Immunology and Microbial Science, The Scripps Research Institute.*

## Engineering broad HIV binding specificity into the B-cell Receptor: Proof of concept for a cell therapy approach to HIV Immunity

JE Voss<sup>1</sup>, R Andrabi<sup>1</sup>, LE McCoy<sup>1</sup>, RP Fuller<sup>1</sup>, M Chateau<sup>2</sup>, A Feeney<sup>1</sup>, D Nemazee<sup>1</sup>, PM Cannon<sup>2</sup>, DR Burton<sup>1</sup>

<sup>1</sup>The Scripps Research Institute, La Jolla, CA; <sup>2</sup>University of Southern California, Los Angeles, CA

**Background:** Efforts to develop an HIV vaccine focus on; 1) eliciting broadly neutralizing antibodies (bNAbs) through engagement of the B-cell receptor (BCR); 2) developing methods to express bNAbs from vectors like AAV.

Elicitation of bNAbs traditionally requires the engagement of rare precursor B-cell BCRs with the potential to evolve through the introduction of mutations into their immunoglobulin (Ig) variable genes. While mutations here occur randomly through the activity of AID, specific mutations must be introduced and selected to convert the precursor into a bNAb. In this study we bypass these by engineering bNAb specificity directly into the BCR.

**Methods:** We identified bNAbs whose heavy chains (HC) are predominantly responsible for HIV binding breadth and which pair with a diversity of light chains allowing us to engineer only the heavy chain variable locus. We optimized an engineering strategy in the human Ramos B-cell line using homologous recombination (HR) repair induced by Crispr/cas9 in the presence of PG9 HC variable donor DNA. Engineering strategies were designed to accommodate the diversity of VDJ recombination events in primary B-cells.

**Results:** An HR strategy which places the PG9 gene directly between the most 5' V-gene promoter (V7-81) and the J6 splice site to drop-out 0.5MB of the HC variable locus was almost as efficient as removing the native VDJ gene alone (400bp). All primary B-cells would have the homology regions required for the V781-J6 drop-out and polyclonal EBV immortalized cells have also been engineered using this strategy. Engineered cells were selected by FACS using HIV envelope probes from diverse clades confirming surface expression and broad HIV binding specificity. gDNA analysis confirmed correct editing and mRNA sequencing shows correct splicing of the new variable gene to the native cell constant region. Because AID is active in Ramos cells, low affinity HIV probes purified mutations in Ig genes that improve the affinity of the chimeric BCR for antigen and optimize pairing between the LC and novel HC.

**Conclusions:** A strategy for engineering chimeric bNAb HIV BCRs has been established and is ready for testing in primary B-cells as a potential cell therapy for HIV cure and prevention.

### **Kamel Khalili, PhD INVITED SPEAKER**

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

## **Excision of HIV-1 DNA by Gene Editing: In Vitro, Ex Vivo, and In Vivo Studies**

R Kaminski<sup>1</sup>, Wi Hu<sup>1</sup>, J Karn<sup>2</sup>, K Khalili<sup>1</sup>

<sup>1</sup>Department of Neuroscience/Center for Neurovirology, Lewis Katz School of Medicine at Temple University, Philadelphia, PA <sup>2</sup>Department of Molecular Biology and Microbiology/Case Center for AIDS Research, Case Western Reserve University, Cleveland, OH

**Background:** It is now well accepted that the cure strategy for HIV-1 infection and AIDS should include methods that directly eliminate the proviral genome from HIV-1 positive cells and/or eliminate the infected cells harboring latent virus.

**Methods:** We have modified the CRISPR/Cas9 system to enable recognition of specific DNA sequences positioned within the HIV-1 promoter spanning the 5' long-terminal repeats (LTR) and the various viral genes including Gag. We have applied CRISPR/Cas9 by a variety of methods including plasmid, lentiviral and Adeno-associated virus to cell models for latency, in vitro HIV-1 infection of CD4+ T-cells from HIV-1 positive patients and transgenic animals encompassing multiple integrated copies of HIV-1 to assess the efficacy of our gene editing molecule in excising a segment of HIV-1 for cells in in vitro, ex vivo and in vivo systems.

**Results:** We demonstrated complete elimination of HIV-1 DNA from the latently infected cells, a drastic decrease in HIV-1 replication in in vitro replication of PBMCs and CD4+ T-cells, suppression of HIV-1 expression in PBMCs and CD4+ T-cells for HIV-1+/AIDS patients due to the InDel mutations in the viral genome and the excision of viral DNA positioned between the LTR and Gag gene in various tissues of HIV-1 transgenic mice upon tail vein injection of AAV-CRISPR/Cas9.

**Conclusion:** CRISPR/Cas9 can offer an effective, precise, efficient and safe strategy for eradication of HIV-1 in several laboratory model systems and can be considered for its advancements toward clinical trials.

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

*Staff Scientist, Clinical Research Division, Fred Hutchinson Cancer Research Center*

## Engraftment and Positive Selection of CCR5 Gene Edited Hematopoietic Stem Cells in a Nonhuman Primate Model of HIV/AIDS

CW Peterson<sup>1</sup>, J Wang<sup>2</sup>, P Polacino<sup>3</sup>, SL Hu<sup>3</sup>, MC Holmes<sup>2</sup>, HP Kiem<sup>1,3</sup>

<sup>1</sup>Fred Hutchinson Cancer Research Center, Seattle, WA, <sup>2</sup>Sangamo Biosciences, Richmond, CA <sup>3</sup>University of Washington, Seattle, WA

**Background:** Genetic targeting of the CCR5 co-receptor locus in hematopoietic stem/progenitor cells (HSPCs) is a promising therapy for HIV cure. We have previously demonstrated that Zinc Finger Nuclease (ZFN) edited autologous HSPCs engraft long-term in the pigtailed macaque, *M. nemestrina*. Here, we present our findings regarding the clonal persistence and trafficking of gene-edited cells in animals infected with Simian/Human Immunodeficiency Virus (SHIV). Our objectives were to understand the spatiotemporal mechanism of gene-edited HSPC engraftment following autologous transplantation and virus infection, the impact of transplantation and gene editing on viral reservoirs, and develop strategies to increase the number of gene edited cells *in vivo*.

**Methods:** CCR5 ZFN mRNA is delivered into macaque HSPCs by electroporation, and gene edited HSPCs are transplanted into autologous animals either prior to infection with SHIV, or in SHIV-infected animals that are treated with a combination antiretroviral therapy (cART) regimen designed to approximate a well-suppressed HIV+ patient. Edited cells are measured in peripheral blood, bone marrow, and a broad array of tissue sites, using methods including deep sequencing and HSPC subset sorting.

**Results:** Gene edited cells are found in a broad array of anatomical sites. Memory CD4+ T-cell subsets are enriched up to 14-fold for CCR5-gene edited cells following SHIV-dependent selection. Kinetic tracking of CCR5 mutations suggests that gene edited cells persist long-term, and are polyclonal. Sequencing of CCR5 alleles from HSPC subsets suggests that the efficiency of gene editing in long-term engrafting macaque HSPCs is lower than in equivalent human HSPC subsets.

**Conclusions:** Our gene editing strategy results in stable engraftment of CCR5-mutated and SHIV-resistant HSPCs *in vivo*, supporting the validity of this approach in the clinic. HSPC subset data suggest that improving the efficiency of CCR5 editing in long term HSPCs may increase the percentage of gene-edited cells that persist long-term in animals. As CCR5-edited memory CD4+ T-cells undergo SHIV-dependent positive selection even at current levels of HSPC editing, we believe that increasing editing efficiency in long term HSPC subsets will dramatically increase the systemic anti-SHIV response in gene edited animals.



**Biswajit Paul, PhD ORAL ABSTRACT**

*Graduate Student, Molecular Cell Biology Graduate Program, Fred Hutchinson Cancer Research Center*

## Targeted Gene Insertion & Chemoselection for the enrichment of HIV-resistant CCR5-null human CD4 T cells

B Paul<sup>1</sup>, HP Kiem<sup>2</sup>

<sup>1</sup>Molecular Cell Biology Graduate Program, Fred Hutchinson Cancer Research Center, Seattle, WA;

<sup>2</sup>Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, WA

Human Immunodeficiency Virus (HIV) infection remains a substantial health problem worldwide. The CCR5 receptor serves as a co-receptor for HIV entry into CD4+ T cells and represents an alternative therapeutic target. Early clinical trials using CCR5-targeting zinc finger nucleases demonstrated transient control of HIV infection in the course of antiretroviral treatment interruption (Tebas, NEJM, 2014). Our current work improves on these advances by combining high level of CCR5 gene disruption with preferential selection of gene modified cells.

The CCR5-targeting megaTAL combines a LAGLIDADG homing endonuclease scaffold with an eleven repeat transcription activator-like (TAL) effector array to achieve site specific DNA cleavage. This nuclease produces highly efficient CCR5 targeting in primary human CD4+ T cells in vitro (70-90% disruption). We tested the protective effects of megaTAL treatment of primary human CD4+ T cells in NOD/SCID/γc-null (NSG) mice challenged with HIV-1. We observed a 100- fold increase of megaTAL-treated cells compared to untreated controls during an active in vivo infection demonstrating the functionality of this approach. Next we hypothesized that coupling CCR5 knockout with drug selection will help us achieve therapeutically relevant levels of HIV protected cells by enabling efficient selection only of CCR5-modified T-cells. We used the mutant human dihydrofolate reductase (mDHFR) chemoselection system, which renders cells resistant to lymphotoxic concentrations of the drug methotrexate (MTX). We combined megaTAL treatment with adeno-associated virus (AAV) transduction to integrate mDHFR into the CCR5 locus, producing a population of MTX-resistant cells that also lack CCR5. We found that CD4+ T cells transfected with CCR5-megaTAL mRNA and transduced with AAV6 containing a mutant DHFR donor template flanked by 0.6kb CCR5 homology arms, demonstrated between five to six-fold enrichment after chemoselection in 0.01μM MTX compared to untreated controls ex vivo.

In conclusion, the CCR5-megaTAL nuclease platform produces very high levels of gene-modified CD4+ T-cells and protects these cells from subsequent HIV infection in vivo. Furthermore, combining targeted integration and chemical selection results in the specific selection of gene modified primary human T cells. To our knowledge we are the first group to report MTX-mediated chemoselection and expansion of CD4+ T cells following targeted integration at the CCR5 locus.

## SESSION 8 SPEAKERS

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### **Cathy Wang, PhD ORAL ABSTRACT**

*Post-Doctoral Fellow, Department of Molecular Microbiology and Immunology, University of Southern California*

## **Genome editing to enhance human HIV-1 restriction factors in hematopoietic stem and progenitor cells**

CX Wang, KJ Tatioussian, PM Cannon

University of Southern California, Los Angeles, CA

Genome editing approaches to counteract HIV-1 has shown significant promise in recent years, especially in the form of CCR5 disruption. The advancement in targeted nuclease technology is now allowing more sophisticated forms of precision genome engineering. We are exploring genome editing-based HIV therapies that can be used in combination with, or as an alternative to, CCR5 disruption. In particular, we focus on editing human restriction factors tetherin and TRIM5a to enhance their HIV-1 restrictive activity. Despite being powerful barriers against cross-species lentivirus transmission, human tetherin and TRIM5a are only weakly active against HIV-1 due to evolution of viral countermeasures. We propose to edit endogenous human tetherin and TRIM5a to mimic their highly HIV-restrictive simian homologs. We target human hematopoietic stem and progenitor cells (HSPCs), for which we have previously developed efficient protocols for genome editing mediated by homologous recombination. Transplantation of restriction factor-edited autologous HSPCs could represent a potential anti-HIV therapeutic strategy.

To optimize genome editing reagents at the human tetherin and TRIM5a loci, we screened a panel of CRISPR/Cas9, zinc finger nucleases (ZFNs), and homology template constructs targeted to each locus in K562 cells. Identification of highly efficient ZFN and homology template constructs allowed successful genome editing in primary human CD34+ HSPCs, by delivering ZFN using mRNA electroporation and homology template using AAV6 vectors. At each restriction factor locus, we were able to achieve both point mutation editing to introduce specific amino acid changes, as well as insertion of larger cassettes to create fusion genes such as TRIMCyp. Importantly, we demonstrate the feasibility of "editing-at-a-distance", where the DNA break site is located within an intron at a distance from the intended mutation site, in order to minimize inadvertent gene disruption due to mutagenic repair.

Genome editing of HIV-1 restriction factors represents a novel addition to the ever expanding toolbox of anti-HIV genetic therapies. Demonstration of homologous recombination-mediated genome editing in primary human CD34+ HSPCs at the tetherin and TRIM5a loci allows us to investigate the biological role of these factors in an in vivo context, and to further explore their utility as anti-HIV genetic therapy targets.

**Nixon Niyonzima, MD ORAL ABSTRACT**

*Graduate Student Research Assistant, Vaccine and Infectious Disease Division, Fred Hutchinson Cancer Research Center*

## Structural and functional basis of meganuclease-mediated disruption of the integrated HIV provirus

N Niyonzima<sup>1,2</sup>, AR Lambert<sup>3</sup>, HSDS Feelixge<sup>1</sup>, P Roychoudhury<sup>1</sup>, D Stone<sup>1</sup>, BL Stoddard<sup>3</sup>, KR Jerome<sup>1,4</sup>

<sup>1</sup>Vaccine and Infectious Disease Division, Fred Hutchinson Cancer Research Center, Seattle, WA; USA;

<sup>2</sup>Uganda Cancer Institute, Kampala, Uganda; <sup>3</sup>Basic Sciences Division, Fred Hutch, Seattle, WA USA;

<sup>4</sup>Department of Laboratory Medicine, University of Washington, Seattle, WA, USA

**Background:** The long-lived latent viral reservoir is a major barrier to the eradication of HIV. Modeling studies suggest that a four log reduction in HIV viral reservoir size might be sufficient to lead to long-term virologic remission without cART. Engineered endonucleases; TALENs, ZFNs, CRISPR/Cas9 and meganucleases can be used to disrupt the integrated provirus and reduce the size of the functional HIV reservoir. When engineered endonucleases bind to target DNA sequences, they introduce double strand breaks, which can be repaired via error prone NHEJ and introduce mutations at the site of the double strand break. Mutations in essential genes will lead to nonviable viral progeny. We demonstrate here the on-target activity of HIV-integrase specific engineered meganucleases, their off-target cleavage activity and sequential protein engineered engineering to improve target specificity of these HIV-specific engineered meganucleases.

**Methods and Results:** A wild type meganuclease, I-OnuI, was re-targeted to a 22-nucleotide sequence in the HIV pol gene. The re-engineered meganuclease, eOnu\_v1, was fused to a TALE domain to create a fusion megaTAL that we have called eOnu\_v1\_7.5 MT. To demonstrate activity of the engineered fusion megaTAL, we co-transfected 293T cells with pDHIV3, and plasmids containing eOnu\_v1\_7.5 MT. We demonstrate that the engineered fusion megaTAL eOnu\_v1\_7.5 MT cleaves target HIV sequences both by the mismatch cleavage assay and Illumina sequencing. We have demonstrated similar activity on integrated HIV provirus in SupT1 CD4+ cells. To determine the off-target cleavage activity of eOnu\_v1\_7.5 MT, we have performed Illumina sequencing on the closest matched homology sites identified using PROGNOS and have detected mutations at predicted offtarget sites. Using iterative protein engineering we have developed eOnu\_v2 which has also been fused with a TALE domain to create eOnu\_v2\_7.5 MT. We have further characterized the activity, both on and off-target, of these two new enzymes using yeast surface display and Illumina sequencing. We have shown that eOnu\_v2\_7.5 MT has better specificity than eOnu\_v1\_7.5.

**Conclusions:** We have demonstrated that engineered meganucleases cleave target HIV sequences but have significant off-target activity. Through iterative protein engineering we have developed a new version of the HIV-specific engineered meganuclease that has a better off-target specificity profile.

## SESSION 9 SPEAKERS

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### **Matt Sharp** INVITED SPEAKER

*Long Term Survivor of HIV/AIDS; Independent HIV Education & Advocacy Consultant*

## The (mis)Understanding of Cell & Gene Therapy

M Sharp

Independent HIV Education & Advocacy Consultant, San Francisco, CA

Over the last several years cell and gene therapy medical research has increased. In HIV/AIDS, cell and gene therapy is one of the main theoretical pathways towards a cure. Still, there is less understanding and more misunderstanding among the many stakeholders in HIV research as to the importance and the possibilities in applicable approaches that can be used globally.

This presentation will discuss pros and cons of HIV gene therapy, some common misconceptions, and will discuss the author's own success in a gene therapy trial, and advocacy to push for further studies in HIV immunologic non-responders.

### **Pavitra Roychoudhury, PhD ORAL ABSTRACT**

*Post-Doctoral Research Fellow, Vaccine and Infectious Disease Division, Fred Hutchinson Cancer Research Center*

## **Pharmacodynamics of anti-HIV gene therapy using viral vectors and targeted endonucleases**

P Roychoudhury, HSDS Feelixge, H Pietz, D Stone, KR Jerome, JT Schiffer  
Vaccine and Infectious Disease Division, Fred Hutchinson Cancer Research Center, Seattle, WA

**Background:** A promising curative approach for HIV is to use designer endonucleases that bind and cleave specific target sequences within latent genomes, resulting in mutations that render the virus replication-incompetent. We developed a mathematical model to describe the expression and activity of endonucleases delivered to HIV-infected cells using engineered viral vectors in order to guide dose-selection and predict therapeutic outcomes.

**Methods:** We developed a mechanistic model that predicts the number of transgene copies expressed at a given dose in individual target cells from fluorescence of a reporter gene. We fit the model to flow cytometry datasets to determine the optimal vector serotype, promoter and dose required to achieve maximum expression.

**Results:** We showed that our model provides a more accurate measure of transduction efficiency compared to gating-based methods, which underestimate the percentage of cells expressing reporter genes. We identified that gene expression follows a sigmoidal dose-response relationship and that the level of gene expression saturation depends on vector serotype and promoter. We also demonstrated that significant bottlenecks exist at the level of viral uptake and gene expression: only ~1 in 220 added vectors enter a cell and of these, depending on the dose and promoter used, between 1 in 15 and 1 in 1500 express transgene.

**Conclusions:** Our model provides a quantitative method of dose selection and optimization that can be readily applied to a wide range of other gene therapy applications. Reducing bottlenecks in delivery will be key to reducing the number of doses required for a functional cure.

**Joumana Zeidan, PhD ORAL ABSTRACT** (*Rafick-Pierre Sekaly presenting*)

*Postdoctoral Fellow, Department of Pathology, Case Western Reserve University*

### CD45RA<sup>int</sup>RO<sup>int</sup> T Memory Stem Cells Correlate with Decay of the HIV Reservoir and Control of Viral Replication During Analytical Treatment Interruption Post Autologous Transfer of ZFN CCR5-Modified CD4<sup>+</sup> T-cells (SB-728-T)

J Zeidan<sup>1</sup>, G Lee<sup>2</sup>, S Fourati<sup>1</sup>, M Aid<sup>1</sup>, R Fromentin<sup>3</sup>, C Benne<sup>1</sup>, J Lalezari<sup>4</sup>, R Mitsuyasu<sup>5</sup>, S Wang<sup>2</sup>, W Tang<sup>2</sup>, N Chomont<sup>3</sup>, S Deeks<sup>6</sup>, D Ando<sup>2</sup>, RP Sekaly<sup>1</sup>

<sup>1</sup>Case Western Reserve University, Cleveland, OH, USA; <sup>2</sup>Sangamo Biosciences Inc. Richmond, CA, USA,

<sup>3</sup>Centre hospitalier de l'Université de Montréal, Montreal, Canada; <sup>4</sup>Quest Clinical Research, San Francisco, CA, USA; <sup>5</sup>University of California Los Angeles, Los Angeles, CA, USA; <sup>6</sup>University of California San Francisco, San Francisco, CA, USA.

**Background:** Nine aviremic HIV+ participants on ART received 10-30 billion SB-728-T cells (902 trial). CCR5-modified cells expanded and persisted in peripheral blood up to 3.5 yrs post infusion (median = 2.1%). We have previously identified a novel TSCM-like CD4<sup>+</sup> subset (CD45RA<sup>int</sup>CD45RO<sup>int</sup>CCR7<sup>+</sup>CD27<sup>+</sup>CD95<sup>+</sup>CD58<sup>+</sup>) that expanded post-infusion and correlated with long-term CD4 reconstitution and CCR5-modified cell persistence.

**Methods:** TSCM phenotyping was performed on PBMCs pre- and post-infusion. Total HIV-DNA was measured using ddPCR. Levels of CCR5 modification and integrated HIV-DNA were determined in sorted CD4<sup>+</sup> T-cell subsets by qPCR. Gene array analysis was performed to determine pathways modulated by SB-728-T infusion in immune subsets.

**Results:** Persistence of TSCM CD4<sup>+</sup> T-cells was associated with global restoration of T-cell homeostasis, as shown by reduction of genes and pathways associated with activation and inflammation, including Interferon Stimulated Genes, in multiple CD4<sup>+</sup> and CD8<sup>+</sup> T-cell subsets. Six out of 9 subjects displayed a significant decrease in levels of total HIV-DNA over time (range of decay 0.5-3.6 log at months 33-44), which correlated with expansion of TSCM-like CD4<sup>+</sup> T-cells at month 6 and levels of CCR5-modified cells in total PBMCs at m33-44. In a separate cohort (1101 trial), participants underwent analytical treatment interruption (ATI) at 6 weeks post-infusion of SB-728-T. The levels of CCR5-modified cells and the absolute numbers of this novel TSCM subset at week 6 correlated with viral load (VL) control post-SB-728-T treatment. Twelve weeks post-ATI, the frequency of CCR5-modified TEM, but not of other subsets, correlated with VL control suggesting that protection of TEM from infection is crucial for VL control. Tracking of CCR5-modification unique to TSCM at week 6 showed persistence of these cells at week 22 and a potential to differentiate into other memory subsets during viremia.

**Conclusions:** An HIV-resistant TSCM population can reduce the latent reservoir by differentiating into TEM cells, reducing immune activation and limiting reservoir replenishment. Our data suggest that generation and protection of early CD4<sup>+</sup> memory cells will improve T-cell homeostasis, accelerate the decline of the host HIV reservoir, and lead to control of viral replication during ATI.

**Acknowledgement:** amFAR#108833-55-RGRL, DAREU19#AI096109, and an unrestricted grant from Sangamo Biosciences.

**James Whitney, PhD INVITED SPEAKER**

*Assistant Professor of Medicine, Harvard Medical School; Associate Member, Ragon Institute of MGH, MIT, and Harvard*

## **Toll-Like Receptor 7 Agonists Induce Transient Plasma Viremia and Reduce the Viral Reservoir in SIV-Infected Monkeys on Antiretroviral Therapy**

SY Lim<sup>1</sup>, J Hesselgesser<sup>2</sup> CE Osuna<sup>1</sup>, PT Hrabec<sup>3</sup>, JM Gerold<sup>4</sup>, AL Hill<sup>4</sup>, TL Barnes<sup>2</sup>, S Sanisetty<sup>1</sup>, MS Seaman<sup>1</sup>, MG Lewis<sup>5</sup>, MD Miller<sup>2</sup>, T Cihlar<sup>2</sup>, WA Lee<sup>2</sup>, R Geleziunas<sup>2</sup> JB Whitney<sup>1,6</sup>

<sup>1</sup>Center for Virology and Vaccine Research, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA; <sup>2</sup>Gilead Sciences Inc., Foster City, CA; <sup>3</sup>Los Alamos National Laboratory, Los Alamos, NM; <sup>4</sup>Program for Evolutionary Dynamics, Harvard University, Cambridge, MA; <sup>5</sup>Bioqual, Rockville, MD; <sup>6</sup>Ragon Institute of MGH, MIT, and Harvard, Cambridge, MA

**Background:** The persistence of an HIV-1 reservoir in the anatomic tissues of infected patients on antiretroviral therapy (ART) represents the major obstacle to HIV remission. The identification of pharmaceutical agents capable of safely reversing HIV-1 latency in ART-treated patients is urgently needed. We have previously reported that an oral TLR7 “tool” agonist (GS-436986) can induce transient viremia in SIV-infected, ART-treated rhesus monkeys (RMs).

**Methods:** We have conducted a follow up study using 11 SIV-infected RMs on ART to directly compare multiple low-dose oral administrations of GS-436986 to the clinical TLR7 agonist (GS-9620), for their ability to induce transient plasma viremia and perturb the viral reservoir. Eleven RMs were mucosally infected with SIVmac251 and received ART at 65 days post-infection (PI). Virological suppression (< 50 SIV RNA copies/mL) was maintained through ~67 weeks post infection. At 67 weeks PI, our RM cohort was divided into 4 groups and then received up to 19 bi-weekly doses of: vehicle alone, GS-436986 (at 0.1mg/kg) or GS-9620 (at 0.05 or 0.15mg/kg), while on ART. We longitudinally assessed multiple endpoints including: viral outgrowth (VOA) using lymph node (LN) mononuclear cells and PBMC as well as total viral DNA in PBMC, LN and colon. Two weeks after the final TLR7 dose, ART was discontinued to assess the kinetics of any rebound viremia.

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## SESSION 9 SPEAKERS

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**Results:** Collectively, the first 2 doses of TLR7 agonist (GS-436986 or GS-9620) administered to ART-suppressed RMs did not impact plasma viremia. However, doses 3 through 10 led to transient, albeit inconsistent activation of plasma virus in all TLR7-treated animals. Additional TLR7 doses (11 through 19) did not induce further virologic “blipping”. Post-TLR7 dosing, SIV DNA levels were reduced in PBMC, colon and LN biopsies of all treated animals. SIV DNA levels remained unchanged in control RMs. To assess the impact of TLR7 agonist treatment on the “live” reservoir, ART was discontinued. The timing of plasma virus rebound in most TLR7-treated RMs was similar to that of control RMs. However, 2 TLR7-treated RMs, that received 0.1mg/kg GS-436986 and 0.15mg/kg GS9620 respectively, remained SIV negative after ART cessation. Our VOA findings correlate with both reductions in proviral DNA and SIV rebound kinetics. To date, both TLR7-treated RMs have remained SIV plasma negative for more than 200 days after ART cessation.

**Conclusions:** Repeated low-dose oral administration of GS-436986 or GS-9620 agonists in SIV-infected ART-suppressed RM is safe, induces transient plasma viremia and impacts viral DNA levels in blood and tissues. Importantly, GS-436986 or GS-9620 agonist treatment can significantly delay viral rebound after ART cessation in some RMs. These novel findings underscore the need for continued clinical investigation of GS-9620 in HIV-1 infected patients on ART.



**Hadega Aamer, MSc***Graduate Student, Pathobiology Program, University of Washington***HIV Proviral Orientation Biases Confer Selective Advantage**HA Aamer<sup>1</sup>, M Aid<sup>2</sup>, S McLaughlin<sup>1</sup>, S Styrchak<sup>1</sup>, H Huang<sup>1</sup>, T Sibley<sup>3</sup>, E Silberman<sup>3</sup>, W Deng<sup>3</sup>, RP Sekaly<sup>4</sup>, LM Frenkel<sup>1</sup>, JI Mullins<sup>3</sup><sup>1</sup>Center for Global and Infectious Disease Research/Seattle Children's Research Institute; Seattle WA; <sup>2</sup>Harvard Medical School and Beth Israel Deaconess Medical Center, Boston, MA; <sup>3</sup>Department of Microbiology, University of Washington, Seattle, WA; <sup>4</sup>Case Western Reserve University, Cleveland, OH.

**Background:** Although antiretroviral therapy can be effective at suppressing virus to undetectable levels, the virus is not eradicated and persists in CD4+ T cells. Studies show HIV integration sites may play an important role in driving infected cell persistence and proviral latency. One mechanism contributing to HIV persistence is cellular proliferation. We are interested in understanding how cells with latent HIV persist despite therapy and whether HIV-infected cells have a survival advantage based on the integration site (IS) and proviral orientation relative to human genes. We hypothesized that HIV integration in the positive orientation into genes involved with cell growth and CTL escape allows for proliferation and persistence by immune evasion.

**Methods:** We compiled the in vitro and in vivo-generated IS data from published and unpublished work into a database and evaluated integration orientation biases and enriched biological pathways. We identified pathways that were enriched in genes that integrate HIV for in vivo and in vitro datasets from MsigDB using a Fisher's exact test.

**Results:** A total of 62,210 IS were identified from the in vitro and in vivo datasets. The degree of proliferation, detected by identical IS in separate cells, was higher in vivo (11.5%) compared to in vitro (<1%). While there was no orientation bias in vitro, there was a significant bias toward integration in the reverse orientation with respect to the gene in vivo (59%,  $p=0.005$ ). However, genes associated with cell proliferation following HIV integration showed a bias for proviruses found in the forward orientation (68%). Pathway analysis of the IS revealed significant enrichment in pathways responsible for cancer, Treg-modulation, IL10 targets, MHC-I/II, and G2M checkpoint. Further analysis of provirus orientation in these pathways revealed a bias for integration in the reverse orientation except for the IL10 targets pathway, where proviral integration was significantly higher in the forward orientation in vivo ( $p=0.03$ ).

**Conclusion:** Our data suggest that HIV integrated into specific genes provides an advantage for enhanced proliferation and persistence during ART. Specifically, integration in the forward direction in IL10-target genes suggests expression of these proteins may be associated with protection of infected cells from immune clearance.

### **Hadia Abdelaal, MSc**

*Graduate Student, Veterinary and Biomedical Science Department, University of Minnesota*

### **CTL-based vaccine-induced control is associated with induction of high follicular to extra-follicular ratios of virus-specific CD8 T cells.**

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There is an urgent need to develop an effective HIV vaccine. We previously showed that during chronic HIV-1 and SIV infections, HIV and SIV replication is concentrated within B cell follicles, whereas HIV and SIV-specific CTL are largely excluded from these sites suggesting that the inability of HIV and SIV-specific CTL to fully suppress virus replication may be due to their deficiency in B-cell follicles. We hypothesize that a successful HIV vaccine will either prevent the seeding of B cell follicles or induce high levels of virus-specific CTL in B cell follicles. Here we investigated whether control associated with three CTL inducing SIV vaccines was associated with levels of SIV-specific CTL in follicular (F) and extrafollicular (EF) compartments in lymph nodes of vaccinated animals after challenge with pathogenic SIV relative to a cohort of non-vaccinated chronically infected animals using in situ tetramer staining with MHC tetramers combined with immunohistochemistry. Control was defined a set point plasma viral load of  $\leq 104$ . We found lower levels of tetramer+ cells in F compared to EF areas of unvaccinated animals ( $P=0.0002$ ), but not in vaccinated animals ( $P=0.63$ ). Although similar levels of tetramer+ cells were detected in F areas between vaccinated and unvaccinated animals ( $P=0.8$ ), the vaccinated animals had significantly higher F: EF ratios of tetramer+ cells ( $P=0.0099$ ). Also, there was a significant inverse correlation between F: EF ratio of tetramer+ cells and plasma VL in vaccinated ( $P=0.04$ ) but not in unvaccinated animals ( $P=0.85$ ). These results support developing CTL-based HIV vaccines that augment relative levels of virus-specific CTL within B-cell follicles.

**Malika Aid, PhD**

*Post-doctoral Fellow, Center for Virology and Vaccine Research, Beth Israel Deaconess Medical Center, Harvard Medical School*

**Baseline (Pre-Vaccination) Gene Expression Signature that Predicts Protection from Acquisition of SIV Infection in Challenged Rhesus Macaques Model**

M Aid

Center for Virology and Vaccine Research, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA.

Human immunodeficiency virus type 1 (HIV-1) vaccine development has been hampered by the lack of correlates of immune /vaccine mediated protection. Using the non-human primate (NHP) model, we have identified a baseline gene expression signature that correlates with vaccine-mediated protection against acquisition of simian immunodeficiency virus (SIV) infection. The baseline expression of these markers segregates significantly NHPs that required high (4:7) or low (1:3) challenges to establish infection.

We found that this gene signature correlated with surrogate markers of protection including SIV-specific neutralizing antibody (nAb) titers as well as viral load upon immunization. We validated the predictive power of this signature using an independent cohort immunized with different vectors. In addition, this signature correlated with the nAb response to yellow fever vaccination using an independent human cohort.

This signature revealed the enrichment of diverse immune related genes including B cell and interferon signaling involved in the innate immune control of viruses. Analysis of the baseline transcriptome of NHPs that required high challenge number was characterized by antiviral and inflammation related gene expression patterns with transcriptional nodes of genes regulated by IRF7 and Foxo3. We found a broad-spectrum of antiviral activity do to a potent stimulation of the inflammatory and antiviral response. In parallel, we observed an inhibition of genes involved in Foxo3 signaling. These results highlighted the balance between the transcriptional repressors Foxo3 and the interferon type I inducer, IRF-7 and their target genes as a critical factor in predisposing to vaccine responses that were associated with protection or lack thereof.

Overall, this study provides evidence that vaccine response can be predicted using a baseline transcriptomic profile and showed that a specific baseline immune environment can lead to a better vaccine response, by enhancing its efficacy or by creating a milieu, which favors the immune response.

### Benjamin Burwitz, PhD

*Research Assistant Professor, Oregon Health & Science University Vaccine & Gene therapy Institute*

## A nonhuman primate model of fully MHC-matched allogeneic stem cell transplantation to study HIV reservoir clearance

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**Background:** 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-versushost immunity, or 3) the ccr5 $\Delta$ 32/ $\Delta$ 32 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, we present a nonhuman primate model of fully MHC-matched allogeneic HSCT to define the mechanisms of Timothy Brown's functional HIV cure.

**Methods:** Fully MHC-matched Mauritian cynomolgus macaques (MCM – *Macaca fascicularis*) donor-recipient pairs were used to perform HSCT. Mobilized peripheral stem cells were collected from donors by leukopheresis and transplanted into recipients following chemotherapy and total body irradiation. Donor engraftment was monitored by Illumina deep sequencing across 12 single nucleotide polymorphisms to distinguish donor cells. Immune subset reconstitution was assessed longitudinally using multiple flow cytometric phenotyping panels.

**Results:** We performed seven HSCT between MCM with four distinct outcomes (listed in chronological order): 1) engraftment failure (N=1), 2) graft rejection (N=2), 3) lethal acute GVHD (N=2), and 4) long-term stable chimerism (N=2). We observed strong engraftment in all immune subsets, including T cells, in our long-term stable chimeras. Importantly, with our improved GVHD prophylaxis we have observed no evidence of pathologic acute GVHD despite full donor engraftment for over 100 days.

**Discussion:** We have built a physiologically relevant model of fully MHC-matched allogeneic HSCT to elucidate the mechanisms contributing to the full HIV remission seen in Timothy Brown. We have achieved high frequencies of CD3+ T cell donor chimerism using this model, which will be a crucial component of any studies looking at SIV reservoir clearance. We are generating CCR5-null transgenic MCM for use as donors, and allogeneic HSCT are now underway in fully combination antiretroviral therapy-suppressed MCM.

**Morgan Chateau, PhD**

*Post-Doctoral Research Fellow, Department of Molecular Microbiology and Immunology, University of Southern California.*

**Development of a humanized mouse model for investigating follicular T-helper cell HIV infection**

M Chateau, GN Llewellyn, A Gadon, P Cannon

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**Background:** Follicular T-helper cells (Tfh) have been proposed to be the major contributor to persistent HIV infection. Actively infected Tfh cells have been found in patients despite excellent ART adherence and even in patients with an effective CD8 T cell response such as in elite controllers. Tfh cells are resistant to ART and CD8 T-cell response due to their physical “sanctuary” locations within B-cell follicles in lymph nodes. Novel therapies designed to target Tfh cells must be tested in animal models with the correct immunological architecture to simulate what is found in humans (lymph nodes with separate T/B zones as well as organized B-cell follicles). Despite containing cells with the Tfh flow cytometry markers, humanized mice lack the proper immunological architecture to simulate the “sanctuaries” seen in humans and non-human primates. Our first hypothesis is that transplant of human lymph node or spleen tissue will provide the needed organization to recreate B-cell follicles in humanized mice and therefore provide a more accessible small animal model for novel cure research therapies. Our secondary hypothesis is that lymph node or spleen implants will improve B-cell development and antibody production in humanized mice.

**Methods:** Traditional huNSG mice were made by irradiating (250cGy, whole body) mice then transplanting with human hematopoietic stem cells (HSC). LN-huNSG mice were irradiated, implanted with human lymph node tissue, and then transplanted with donor matching human HSC.

**Results:** Presence of Tfh cells were confirmed in traditional huNSG mice by flow cytometry. Preliminary experiments have shown NSG mice can support human lymph node tissue engraftment. LN-huNSG mice had similar humanization kinetics in peripheral blood with traditional huNSG mice. Both mouse models had similar development of T cell and B cell populations in peripheral blood. One LN-huNSG mouse developed a very large growth at the human lymph node implant site. The growth in addition to spleen and endogenous lymph nodes are currently being evaluated histologically.

**Conclusions:** Surgical implant of human lymph node tissue is well tolerated (no GVHD) and effectively recruits human immune cells after HSC transplant. Alternative lymph node implantation sites as well as human splenic tissue are being evaluated.

### Harshana De Silva Feelixge

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## Accounting for genetic diversity when designing CRISPR gRNA against HIV-1

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RNA-guided CRISPR/Cas9 DNA editing platform has shown incredible success in excising HIV-1 genome from latently infected CD4+ T cells ex-vivo and recently in-vivo, a potential gene therapy strategy towards a HIV cure. One of the major challenges of CRISPR/cas9 driven HIV-1 pro-viral genome eradication is the clinical feasibility of designing guide RNAs (gRNAs) to successfully cover HIV-1 genetic diversity across the world. Excision of the pro-viral genome is most efficient, with gRNAs designed against the highly conserved long terminal repeats (LTR) of HIV-1. We identified highly specific candidate *S. pyogenes* Cas9 gRNAs from an LTR consensus sequence for HIV-1 group M, the major contributor to the worldwide pandemic, and from individual consensus sequences of the predominant subtypes A-C. Particularly, we determined how many individual group M LTR sequence could be targeted by each guide identified within the consensus sequence and identified individual guides that target over 70% of all sequences with minimal predicted off-target activity. While single gRNAs with high prevalence may be designed to encompass the majority of group M, gRNAs designed towards a specific subtype are able to target a wider range of HIV-1 isolates. To assess whether greater sequence coverage could be achieved through multiplexing either two or more gRNAs we further analyzed various permutations of single gRNAs for either the entire group M or for each subtype. Higher sequence coverage up to 90% could be achieved through multiplexing with additional gRNA regardless of group M or subtype specificity. Our observations suggest that CRISPR/Cas9 system has potential to tackle the genetic diversity of HIV-1 group M by utilizing a customized subtype specific gRNA regimen. Furthermore, multiplexing approach would not only allow greater sequence coverage including targeting of recombinant LTRs but other added benefits such as increased potency of the antiretroviral therapy and circumventing potential treatment resistance.

**Elizabeth Duke, MD**

*Senior Fellow, Vaccine and Infectious Disease Division, Fred Hutchinson Cancer Research Center.*

**A compound interest approach to HIV cure**

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**Background:** Antiretroviral therapy (ART) suppresses HIV viral replication but does not cure HIV due to the presence of a viral reservoir of latently infected cells. On ART, the viral reservoir sustains itself predominantly through homeostatic and antigen-driven proliferation of latently infected cells. Thus, we propose the use of the anti-proliferative drug mycophenolate mofetil (MMF) in combination with suppressive ART to deplete the latent reservoir and cure HIV infection.

**Methods:** We created a mathematical model composed of a standard system of ordinary differential equations to describe the dynamics of productive and latent HIV infection. With analytical methods, we simplify our model to a single equation that predicts time to cure and corresponds to the continuous compound interest formula. Using our model, we predict times to cure from various latency eradication strategies including anti-proliferative therapy.

**Results:** Our model predicts that continuous-time latency eradication strategies result in faster cure than abrupt, one-time reductions in the reservoir. We frame the concept in terms of compounding interest: if a small change in interest rate is applied over a long period of time, the ultimate return on investment is profound. Additionally, natural rates of proliferation and death of reservoir cells are orders of magnitude larger than the natural reactivation rate of HIV in these cells. Thus, applying the compound interest approach by reducing proliferation rates (or by increasing death rates) eliminates the reservoir much more rapidly than increasing latency reactivation rates. In fact, our model predicts functional cure in patients receiving combination therapy with MMF and ART in 2-10 years as opposed to a predicted 70 years on ART alone. We also found that heterogeneity in the composition of reservoir CD4+ T cells (e.g. naïve versus central memory versus effector memory) affects the rate at which cure could be achieved.

**Conclusions:** Contingent on the subtypes of cells that compose the reservoir and their respective proliferation rates, our model predicts that coupling MMF with ART would result in functional cure within 2-10 years, rather than several decades on ART alone.

### **Khader Ghneim, MSc, MBA**

*Senior Research Associate, Department of Pathology, Case Western Reserve University*

## **The sooner the better: innate immunity as a path towards the HIV cure**

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Despite the development and implementation of new therapies, HIV infection leads to high incidence of morbidity and mortality worldwide (WHO 2014). Currently available drugs used continuously are able to control the virus to undetectable levels. Interruption of ART culminates in virus rebound, confirming the presence of long-lived viral reservoir. Rapid immune subversion and manipulation by HIV can inhibit the generation of antiviral immunity promoting the establishment and maintenance of the HIV/SIV reservoir. Utilizing detailed necropsy studies of intravaginally SIVmac251 infected RMs, we have shown that SIV rapidly disseminates following infection and triggers components of the inflammasome, both at the site of inoculation and distal sites of viral spread. A pro-inflammatory signature lacking antiviral restriction factors was observed in viral RNA-positive tissues 24-hours post-infection. The early innate response included expression of NLRX1 and activation of the FOXO3a/Transforming Growth Factor $\beta$  (TGF $\beta$ ) pathway. NLRX1 expression correlated with lack of induction of antiviral ISGs (at days 1 and 3). We have also probed the expression of NLRX1 in an independent study in which a Gly and Tyr deletion within a highly conserved trafficking motif (GYRPV) in the SIVmac239 envelope (Env) cytoplasmic tail, produced a virus ("ΔGY") that in pigtail macaques (PTM) replicates acutely to wild type levels, but becomes highly controlled. SIVmac239 infected-animals up-regulated NLRX1 expression as early as 48 hours post infection. Expressions of NLRX1 as well as several pro-inflammatory pathways (interferon  $\gamma$ , TNFA signaling via NFK $\beta$ ) were all down-regulated in the ΔGY model compared to SIVmac239. Furthermore, we were able to identify a number of proliferation pathways (E2F targets, G2M checkpoints) as positive correlates between pre-infection gene expression signatures and KI67 levels. These data suggest a model in which rapid triggering of NLRX1 by PRRs sensing HIV/SIV will suppress the generation of protective innate and adaptive immune responses and promote infection. NLRX1 and TGF $\beta$  may serve as central regulators of immune activation signatures in both SIV and HIV infection. Identifying the upstream and downstream signals that perturb these pathways during SIV/HIV is crucial for understanding viral seeding/dissemination and will guide future therapy development.



**Pargol Hashemi, MSc**

*Graduate Student, Department of Biochemistry and Molecular Biology, University of British Columbia*

## **Novel Potent Latency Reversing Agents Targeting HIV-1 Latent Reservoirs to Disrupt Viral Latency both in vitro and ex vivo**

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**Introduction:** Despite nearly 35 years of world awareness of Acquired Immunodeficiency Syndrome (AIDS), mankind still faces major challenges in developing a cure. One significant challenge to an HIV/AIDS cure lies with the latency ability of the virus. During replication in CD4+ T lymphocytes, the main HIV-1 target cells, a small fraction of activated cells revert to a resting state, in which transcription of the chromosomally integrated provirus is repressed, and the virus becomes latent. The long life span of these cells presents a major challenge, since they are unrecognizable by the immune system and highly active antiretroviral therapy (HAART). Cessation of HAART will cause the virus to reappear, which makes patients dependent on lifetime therapy. Without strategies for eliminating this latent population, current treatments are ineffective, because resistant strains emerge and prolonged use of HAART can cause significant side effects, making it impractical for long-term use.

**Methods:** In pursuing efforts directed at devising ways to wipe out latent populations, we focused on the strategy referred to as “shock and kill.” The strategy uses latency reversing agents (LRAs) in combination with HAART to reawaken latent reservoirs (the “shock”), which are purged by either cytotoxic T lymphocytes or viral-induced cytopathic effects (the “kill”). For the initial attempt, a high throughput screening of small molecules was conducted to identify potent LRAs. The capability of these compounds has been evaluated on both reporter cell lines carrying dual-labeled mini-virus (in vitro) and resting CD4+ T cells purified from aviremic HIV-1 infected patients on HAART (ex vivo).

**Results:** Through intensive work assessing latency reversing activity of compounds, we have discovered five novel LRAs showing promising results for disrupting latency both in vitro and ex vivo while causing minimal toxicity. These compounds do not cause massive production of pro-inflammatory cytokines and exhibit synergism with other previously known LRAs — particularly Ingenol-3-Angelate/PEP005 — when used on latent HIV-1 infected cellular models.

**Conclusion:** We have identified new potent compounds to reverse HIV-1 latency, making these LRAs ideal candidates to abrogate persistent viral infection when used in advanced antiretroviral therapies.

### **Wannisa Khamaikawin, PhD**

*Post-Doctoral Scholar, School of Nursing, University of California Los Angeles*

## **A novel Chemoselection Strategy for Combinatorial Genetically Modified HIV Protected Hematopoietic Stem/Progenitor Cells**

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

Our novel selection strategy exclusively employs 6-thioguanine (6TG) for in vivo chemoselection of hypoxanthine-guanine phosphoribosyltransferase (HPRT) down-regulated HSPC using a short hairpin RNA (shRNA) to enable 6TG-mediated positive selection. In addition, a novel feature of our HPRT knockdown is a negative selection strategy to eliminate unexpected adverse effects of the gene-modified HPRT knockdown cells as a safety mechanism using a clinically available drug, methotrexate (MTX), which inhibits the enzyme dihydrofolate reductase (DHFR) in the purine de novo synthetic pathway. shRNAs were delivered through a lentiviral vector to stably knockdown CCR5 and HPRT in human T-cell lines, primary PBMC, as well as in primary CD34+ HSPC in the presence of 6-TG to select vector-transduced cells. Furthermore, we then added a second anti-HIV gene, the fusion inhibitor C46, and tested whether the newly engineered cells were able to block HIV-1 of both R5 and X4 tropism.

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

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

**Shengbin Li, MSc**

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**Subsets of follicular SIV-specific CTL display an effector memory phenotype and suppress viral replication in vivo**

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The inability of HIV- and SIV-specific CTL to fully suppress virus replication may be due to a deficiency of CTL in B-cell follicles. The majority of HIV and SIV replication is concentrated within B-cell follicles in secondary lymphoid tissues during chronic disease; whereas HIV- and SIV-specific CTL typically fail to accumulate in large numbers within B-cell follicles. Further, large regions of follicles are often completely devoid of virus-specific CTL. It is not known whether virus specific-CTLs can migrate throughout the entire B cell follicle area including germinal centers (GC). It is also not known that whether virus specific-CTLs within B cell follicles are functional. We evaluated these questions using in situ tetramer and immunohistochemical staining in lymphoid tissues from chronically SIV infected rhesus macaques. We found that SIV-specific CTLs were able to migrate into GC at similar levels detected in other follicular areas. We further found that many follicular SIV-specific CTL expressed programmed death-1 (PD-1), indicating that they may have been exhausted or recently stimulated by antigen. In addition, we found that some follicular SIV-specific CTL were in direct contact with Foxp3+ T regulatory cells (Tregs) that can inhibit T cell function, and a small subset were themselves Foxp3+. However, many follicular SIV-specific CTL expressed low to medium levels of perforin, consistent with being effector memory CD8+ T cells (TEM), and some were activated and proliferating. More importantly, we found an increase in follicular SIV-producing cells after CD8 depletion suggesting that follicular CD8+ T cells have a suppressive effect on follicular SIV replication. Taken together, these results strongly suggest that during chronic SIV infection, despite a milieu of inhibitory factors, subsets of follicular SIV-specific CTL are functional and suppress viral replication. These findings support HIV cure strategies that augment follicular virus-specific CTL to enhance viral control.

### Mark Pankau

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## Short treatment interruption in a cohort of Kenyan infants does not increase the size of the HIV latent reservoir

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During infection, HIV integrates into resting CD4+ T cells and establishes a latent reservoir that persists despite antiretroviral treatment (ART). Upon treatment interruption, reactivation of latently infected cells can lead to viral recrudescence. Previous data suggest that limiting the size of the latent reservoir through early ART may allow post-treatment control. However, assessing post-treatment control requires treatment interruption, which could lead to an increased size of the latent reservoir, decreasing the benefits of early treatment. Here, we measured reservoir size in a cohort of early treated Kenyan infants, prior to, and after randomization to treatment interruption. All infants were treated with ART for 24 months prior to randomization, and infants randomized to treatment interruption had ART re-started when their CD4 count dropped below 25% (average 112 days off treatment). Infant reservoir size was quantified prior to randomization for all 14 infants, and again 18 months later in infants randomized to treatment interruption (n=7) or continuous treatment (n=7). Reservoir size was measured by ddPCR using cross-subtype pol primers that accurately detect 5 copies of HIV DNA/1e6 PBMCs. Prior to randomization, infants randomized to the continuous ART arm had a median HIV DNA of 168 HIV DNA copies/1e6 PBMCs (IQR: 100-235) and infants randomized to treatment interruption had a median HIV DNA of 90copies/1e6 PBMCs (IQR: 54-485). 18 months after randomization, infants randomized to the continuous ART arm had a median of 50 HIV DNA copies/1e6 PBMCs (IQR: 24-173) and those randomized to the interruption arm had a median of 184 copies/1e6 PBMCs (IQR: 92-280). Median HIV DNA fold change in infants who continued cART following randomization was 0.23 (IQR: 0.15- 0.51) while the median HIV DNA fold change in infants undergoing treatment interruption was 1.04 (IQR: 0.71-1.6) (p=0.073 for comparison). Our data suggest that while the latent reservoir decayed during continuous cART, short treatment interruption did not cause a large increase in the size of the latent reservoir.

**Susan Pereira Ribeiro, PhD***Research Associate, Department of Pathology, Case Western Reserve University***Memory CD4<sup>+</sup> T cell subsets show differential responses to HIV latency reversing agents**SP Ribeiro<sup>1</sup>, Sally Yuan<sup>2</sup>, Aarthi Talla<sup>1</sup>, Jessica Brehm<sup>3</sup>, AG Bebin-Blackwell<sup>4</sup>, Richard Bar-nard<sup>5</sup>, M Miller<sup>5</sup>, D Hazuda<sup>5</sup>, N Chomont<sup>6</sup>, RP Sékaly<sup>1</sup>, DA. Kulpa<sup>7</sup><sup>1</sup>Department of Pathology, Case Western Reserve University, Cleveland, OH, USA; <sup>2</sup>Janssen Pharmaceuticals, Springhouse, PA, USA; <sup>3</sup>Glaxo Smith Kline, NC, USA; <sup>4</sup>University of Georgia, Athens, GA, USA; <sup>5</sup>Infectious Disease, Merck Research Laboratories, West Point, PA, USA; <sup>6</sup>Centre de Recherche du CHUM and Université de Montréal, Department of microbiology, infectiology and immunology, Montréal, QC, Canada; <sup>7</sup>Infectious Disease Research Department, Southern Research Institute, Frederick, MD, USA.

HIV-1 persists in individuals on ART in infected central memory (CM), transitional memory (TM) and effector memory (EM) CD4<sup>+</sup> T cells. Latency reversing agents (LRAs) used in HIV eradication strategies will need to activate viral replication in this heterogeneous pool of cells. To facilitate the evaluation of LRA latency reversal efficiency in all memory CD4<sup>+</sup> T cell compartments, we developed LARA (latency and reversion assay), a primary cell based in vitro model of HIV latency that comprises the CM, TM and EM subsets. Validation experiments confirmed that cells generated in this assay show significant homology with ex vivo sorted memory CD4<sup>+</sup> T cell subsets, and transcriptional profiles that differentiate CM and EM were highly preserved in our culture conditions. Using LARA, we demonstrate robust latency induction and reversion in all memory CD4<sup>+</sup> T subsets with a hierarchy similar to that generated in ex vivo samples. Significantly, latently infected cells generated in LARA demonstrate distinct responses to different LRAs between memory CD4<sup>+</sup> T cell subsets. Specifically, interleukin-15 and the protein kinase C agonist bryostatin reversed latency in all three subsets, whereas the histone deacetylase inhibitors panobinostat and romidepsin were only active in CM cells. Understanding of pathways that can allow virus seeding and spreading can be of fundamental importance to counteract HIV infection. We found that the expression of the anti-inflammatory molecule, NLRX1, on CD4<sup>+</sup> memory cells 2 days after HIV infection in vitro, was simultaneously upregulated with the p24 HIV protein. NLRX1 inhibits antiviral innate immune responses and its expression one-day post in vivo infection of NHPs correlates with systemic viral dissemination. Our results support the identification of compounds that can trigger HIV replication in all memory CD4<sup>+</sup> T cell subsets, a critical parameter for the success of HIV eradication strategies; as well we have identified pathways associated to HIV dissemination thereby providing novel targets for eradication strategies.

### Chelsea Spragg, PhD

*Post-Doctoral Research Fellow, Vaccine and Infectious Disease Division, Fred Hutchinson Cancer Research Center.*

## Multiplexed droplet digital PCR for estimation of the functional latent viral reservoir

CJ Spragg<sup>1</sup>, N Niyonzima<sup>1</sup>, SM Hughes<sup>2</sup>, RH Sedlack<sup>1</sup>, KR Jerome<sup>2</sup>

<sup>1</sup>University of Washington, Seattle, WA; <sup>2</sup>Fred Hutchinson Cancer Research Center, Seattle, WA

**Background:** A cure for HIV is hindered by a reservoir of viral genomes integrated into host DNA of long-lived memory T cells. HIV cure research demands accurate quantitation of the latent viral reservoir, but the current gold standard assay, the quantitative viral outgrowth assay, is extremely resource and labor intensive. Additionally, assay underestimates the true reservoir size. PCR based methods are far easier to perform. However, in patients with suppressed viremia, most HIV genomes contain large deletions that render them replication incompetent which may or may not include the PCR target, leading to inaccurate quantitation of the true viral reservoir.

**Methods:** Droplet digital PCR (ddPCR) is a powerful technology in which nucleic acid, PCR mix, and fluorescent probes are encapsulated into tens of thousands of nanoliter sized oil droplets, each acting as an individual “well” on which qPCR can be performed. Fluorescent droplets are then read and counted, similar to flow cytometry. We designed fluorescent probes to bind short sequences of supreme conservation in HIV-1 subtype B or SHIV and optimized multiplexed reactions that interrogate individual genomes for the simultaneous presence of three viral genes.

**Results:** We used lentiviral plasmid constructs to develop and optimize multiplexed ddPCR assays to simultaneously probe SHIV genomes for presence of gag, env, and 3’LTR, and in a separate assay 5’LTR, pol, and vpu. By assaying genomic DNA derived from lymph node biopsies of SHIV infected pigtail macaques, we were able to provide estimations of the size of the viral reservoir in several macaques while on combination antiretroviral therapy and after autologous stem cell transplant. Additionally, using alignments of HIV subtype B sequence, we have designed primer probe sets that target highly conserved regions of the HIV genome, allowing reliable interrogation of diverse patient samples.

**Conclusions:** The presence of three genomic regions in a lentiviral genome is a stronger indicator that the genome may be intact than a single PCR assay, allowing for a more accurate PCR-based estimation of the latent viral reservoir of patients on cART. Multiplexed ddPCR is a promising alternative assay for measuring the latent viral reservoir in HIV cure research.

**Venkata Viswanadh Edara, MSc***Graduate Research Assistant, University of North Texas Health Science Center***Resolving Astrocyte Latent Reservoirs in The Brain for HIV Cure**

VVC Edara, A Ghorpade

Department of Cell Biology and Immunology, University of North Texas Health Science Center, Fort Worth, Texas

HIV-1 infection of the central nervous system (CNS) impairs brain function and leads to HIV associated neurocognitive disorders (HAND). While the anti-retroviral therapy (ART) has increased the life expectancy of the HIV-1 infected individuals, eradication of latent reservoirs is impossible as the current therapy targets only active infection. Astrocytes are the most abundant cell type in CNS and provide structural and metabolic support under homeostasis and in diseases including HAND. Several studies have shown the validity of astrocytes as a major reservoir of HIV-1 in CNS. Methamphetamine (Meth) abuse and HIV-1 infection increases neuroinflammation through several molecular and cellular mechanisms. Given our prior work on astrocyte inflammatory responses in HAND, we sought to investigate the role of astrocyte HIV-1 reservoirs in HAND pathogenesis. We hypothesize that astrocyte HIV-1 reservoirs contribute to HAND pathogenesis; also Meth abuse during HIV-1 infection exacerbates astrocyte reservoirs and gene-editing techniques such as CRISPR/Cas9 can eradicate HIV-1 genome from astrocyte reservoirs and reverse HAND pathogenesis. A doubly labeled fluorescent reporter Red/Green-HIV-1 (R/G-HIV-1) was used as a model of latency in primary human astrocytes. VSV- pseudotyped R/G-HIV-1 virions were produced by transfecting HEK 293 cells with both the R/G-HIV-1-WT vector and a vector to express VSV coat proteins and used to infect astrocytes by spinoculation. Time kinetic studies of pseudotyped R/GHIV- 1 infected HPAs showed establishment of latency over a period of time. Active (mCherry+/GFP+) and latently infected (mCherry+/GFP-) astrocytes were enriched using fluorescence activated cell sorting (FACS). mCherry+/GFP- astrocytes whose HIV-1 LTR is silent, they are also devoid of late viral proteins such as p24. Vorinostat a histone deacetylase inhibitor (HDACi) reactivated the silenced HIV-1 LTR in a mixed population of pseudotyped R/G-HIV-1- infected astrocytes. This data suggests that R/G-HIV-1 can be used as a relevant model of latency in astrocytes as it mimics virus reactivation in inflammation leading to expression of viral proteins. However we propose that healthy versus latently infected astrocyte reservoirs respond differentially to inflammation. Elucidation of these mechanisms will prove instrumental in developing gene-editing strategies to eradicate HIV-1 from latently infected astrocytes.



### 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 recently announced 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 Lymphocyte 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](http://defeathiv.org)





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CURES START HERE™

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.

**[www.fredhutch.org](http://www.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.

<http://depts.washington.edu/cfar/>

# UW Medicine

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## LABORATORY MEDICINE

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### VIROLOGY

#### **Virology Division**

Department of Laboratory Medicine

University of Washington School of Medicine.

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 sequencing for both 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](http://uwvirology.org)**

## DINNER AND RECEPTION

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### Conference for Cell & Gene Therapy for HIV Cure Dinner and Reception

Please join us on Thursday, August 4, 2016 for the Conference on Cell & Gene Therapy for HIV Cure Dinner and Reception at Seattle's iconic Space Needle in downtown Seattle.

Space Needle  
400 Broad Street  
Seattle, WA 98109

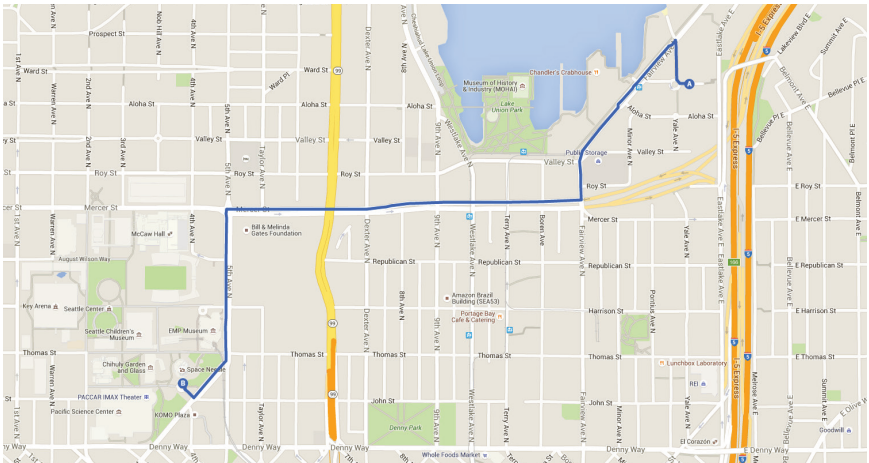


### Transportation:

Complimentary coaches will be provided for conference attendees for transportation between Fred Hutch and the Space Needle.

Coaches will arrive at Fred Hutch in the roundabout outside the Thomas Building at 6:30pm and begin shuttling attendees to the Space Needle for the 7:00pm reception. Starting at 9:00pm, coaches will be available to transport conference attendees back to Fred Hutch until the reception concludes at 10:00pm.

You are free to use any other mode of transportation that you please; however, Space Needle taxi/parking will not be reimbursed.



### Reception:

The reception on the Space Needle Skyline level will include hors d'oeuvres, carefully selected entrees and a hosted bar. Gluten-free and vegetarian options will be available.

Attendees will have complimentary access to the observation deck beginning at 8:00pm and throughout the reception until it concludes at 10:00pm.

*7:00 Reception Begins – drinks and hors d'oeuvres served*

*7:45 Entrees served*

*8:00 Observation deck opens for viewing*

*8:45 Dessert served*

*10:00 Reception concludes, observation deck closes*

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## MAKING AIDS HISTORY

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 the advocacy of sound AIDS-related public policy. Since 1985, amfAR has invested more than \$450 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, last year amfAR launched the Countdown to a Cure for AIDS initiative. 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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Abbott Molecular is an emerging leader in molecular diagnostics and the analysis of DNA, RNA, and proteins at the molecular level. We are committed to exploring new clinical frontiers through the development and delivery of system and assay solutions that are designed to provide earlier disease diagnosis, selection of appropriate therapies and monitoring of disease recurrence. Our expanding portfolio brings multiples technologies that enable fast, accurate results. Our state of the art research and manufacturing facilities have hundreds of scientists who are dedicated to producing quality and reliable products.

**[abbott.com](http://abbott.com)**



Gilead Sciences, Inc. is a research-based biopharmaceutical company that discovers, develops and commercializes innovative medicines in areas of unmet medical need. We strive to transform and simplify care for people with life-threatening illnesses around the world. Gilead's portfolio of products and pipeline of investigational drugs includes treatments for HIV/AIDS, liver diseases, cancer, inflammatory and respiratory diseases, and cardiovascular conditions.

Our portfolio of marketed products includes a number of category firsts, including complete treatment regimens for HIV infection available in a once-daily single pill and the first oral antiretroviral pill available to reduce the risk of acquiring HIV infection in certain high-risk adults.

**[Gilead.com](http://Gilead.com)**





Juno Therapeutics is building a fully integrated biopharmaceutical company focused on re-engaging the body's immune system to revolutionize the treatment of cancer. Founded on the vision that the use of human cells as therapeutic entities will drive one of the next important phases in medicine, Juno is developing cell-based cancer immunotherapies based on chimeric antigen receptor and high-affinity T cell receptor technologies to genetically engineer T cells to recognize and kill cancer. Juno is developing multiple cell-based product candidates to treat a variety of B-cell malignancies as well as solid tumors.

**[Junotherapeutics.com](http://Junotherapeutics.com)**



Sangamo is a clinical stage biopharmaceutical company focused on the research, development and commercialization of engineered zinc finger DNA-binding proteins (ZFPs) as novel ZFP Therapeutics® targeting various monogenic and infectious diseases with unmet medical needs.

We are worldwide leaders in the development of our proprietary ZFP technology platform that enables highly specific regulation of gene expression and genome modification. Our technology platform is based on a naturally occurring class of transcription factors, ZFPs, which we can engineer to bind to any DNA sequence with singular specificity and drive desired therapeutic outcomes.

Our ZFPs can be linked to functional domains that normally activate or repress gene expression to create ZFP transcription factors (ZFP TFs) capable of turning genes on or off. We can also link ZFPs to endonuclease domains to create zinc finger nucleases (ZFNs) which enable precise genome editing in cells. Sangamo's engineered ZFNs can modify a cell's DNA at a precise location, thereby facilitating the correction or disruption of a specific gene or the targeted addition of a new DNA sequence, without the unwanted consequences of off-target DNA binding activity.

## GOLD SPONSORS

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Calimmune Inc. is a clinical-stage, international biotechnology company focused on developing novel gene therapies that have the potential to improve and protect the lives of patients by enhancing their immune system. The company was founded in 2006 from technology developed in the labs of Nobel Laureate David Baltimore, PhD (California Institute of Technology), Irvin Chen, PhD (University of California, Los Angeles) and Inder M. Verma, PhD (Salk Institute for Biological Studies). Calimmune's lead product candidate for HIV, now in Phase I/II studies, is being evaluated as a one-time treatment to prevent HIV progression to AIDS. The company is headquartered in Tucson, Arizona with labs and branch offices in Pasadena, California and Sydney, Australia.

**Calimmune.com**



Editas Medicine is a transformative genome editing company founded by world leaders in the fields of genome editing, protein engineering, and molecular and structural biology, with specific expertise in CRISPR/Cas9 and TALENs technologies.

The company's mission is to translate its genome editing technology into a novel class of human therapeutics that enable precise and corrective molecular modification to treat the underlying cause of a broad range of diseases at the genetic level. The company has generated substantial patent filings and has access to intellectual property covering foundational genome editing technologies, as well as essential advancements and enablements that will uniquely allow the company to translate early findings into viable human therapeutic products.

**Editasmedicine.com**

## COMMUNITY REPRESENTATION & FINANCIAL SUPPORT

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The conference is pleased to be supporting the attendance and participation of Community Advisory Board members from the Martin Delaney Collaboratories.

**Steve Muchnick, PhD**

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# CURED **NOT**CURED

A TALE OF TWO DIAGNOSES



FOR SEVEN MONTHS **HE BELIEVED**  
**HE WAS CURED OF HIV**

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## NOTES

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## NOTES

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[cgt4hivcure2016.org](http://cgt4hivcure2016.org)

