



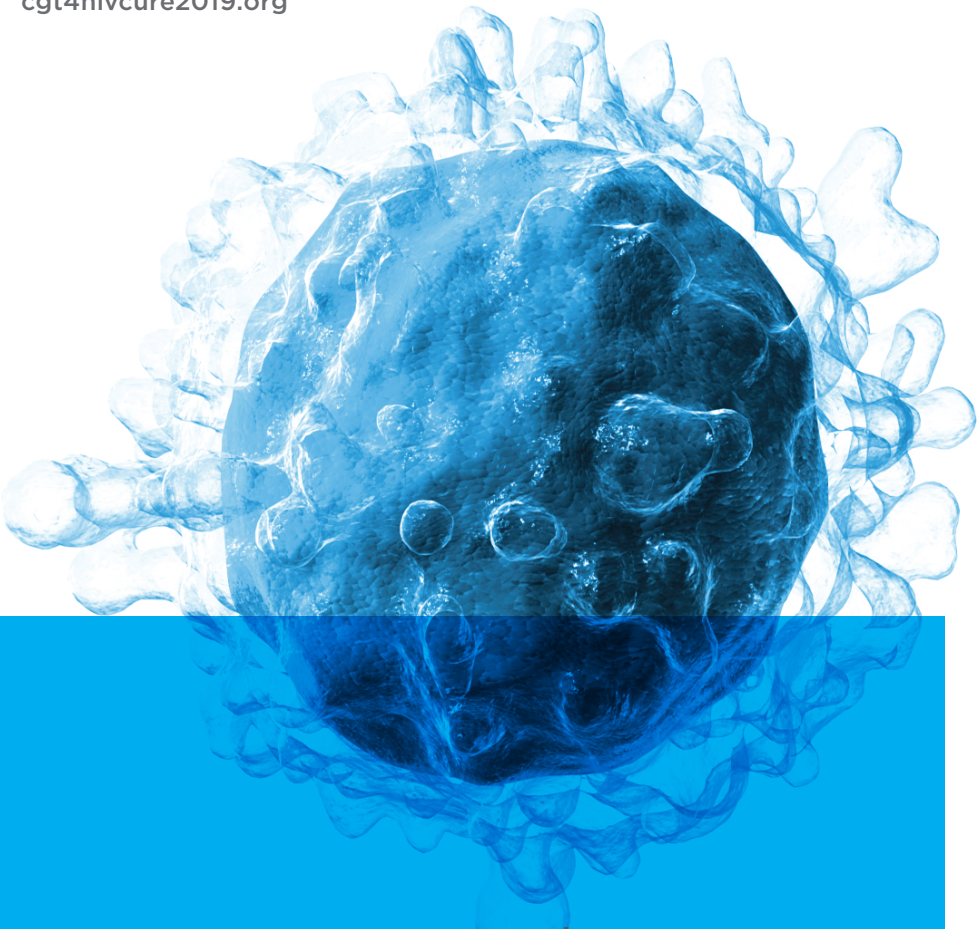
5<sup>th</sup> Conference on  
Cell & Gene Therapy  
for HIV Cure 2019

**August 22-23, 2019**

Seattle, Washington

Marriott Residence Inn Seattle  
Downtown/Convention Center

[cgt4hivcure2019.org](http://cgt4hivcure2019.org)





In 2020, the plaza over Seattle's Capitol Hill light rail station and the north edge of Cal Anderson Park will become home to THE AMP: AIDS MEMORIAL PATHWAY.

The AMP, community driven and collaboratively funded, will use public art to create a physical place for remembrance and reflection; utilize technology to share stories about the epidemic and the diverse community responses to the crisis; and provide a call to action to end HIV/AIDS, stigma, and discrimination.

Artists have been commissioned to create work for four art zones within The AMP, which will be installed in 2020. Meanwhile, The AMP has started to gather stories about those in our region who lived and died with HIV/AIDS, who fought and continue to fight the virus, and who are living with HIV today. Videos and more info can be found at [www.theamp.org](http://www.theamp.org).

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## WELCOME

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On behalf of the Scientific Organizing Committee, we are pleased to welcome you to the 5th Conference on Cell and Gene Therapy for HIV Cure. We are proud to host this growing conference, as we showcase cutting-edge research in cell and gene therapy that will advance us closer to our goal of ending HIV.

This year marks the 12th anniversary of the first HIV cure in Timothy Ray Brown, formerly known as the Berlin patient; as well, it brought us reports of potential cure in a second and third person, the London and Düsseldorf patients. This is truly exciting, but the fact that it took over a decade to repeat this success indicates the vast amount of work still to do. We have learned that bone marrow transplantation from a matched unrelated donor with the CCR5 delta 32 deletion can result in durable remission and cure. Translating these findings to the broader population of people living with HIV remains the critical, urgent next step to developing a cure that is safe, durable, affordable, and accessible to everyone who needs it. We need to understand where HIV hides in the body, as much as we need to devise optimal strategies to make a patient's cells resistant to HIV and harness the immune system to fight and cure HIV. To date our investigations in bone marrow transplantation, cell therapy, and gene therapy / editing have been done with *ex vivo* approaches, taking cells from patients and bringing them to appropriately equipped facilities for modification. This year we will dedicate a full day of our conference to the discussion of *in vivo* gene therapy / editing approaches, which will include considerations of resource-limited settings. We are grateful for the Bill and Melinda Gates Foundation for sponsoring this effort on our second day of this year's conference.

We hope that CGT4HIVCure 2019 will bring us one step closer to designing, developing, and testing an HIV cure. Just as the Martin Delaney Collaboratory program encourages collaborative efforts to address the multifaceted puzzle of curing HIV, we hope that our conference inspires you to bring your expertise in HIV molecular virology, gene therapy, or the immune system into play over the next two days, so that together we can accelerate progress in this research endeavor on which so many dreams depend.

Thank you all for your participation in our conference. We hope you find the program thoughtful and engaging.



**Keith R. Jerome**

*MD, PhD  
Conference Co-host*



**Hans-Peter Kiem**

*MD, PhD  
Conference Co-host*



**Michael Louella**

*Community Engagement  
Project Manager, defeatHIV*

Three handwritten signatures in black ink, corresponding to the individuals above: Keith R. Jerome, Hans-Peter Kiem, and Michael Louella.

## SCHOLARSHIP RECIPIENTS

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*The Conference on Cell & Gene Therapy for HIV Cure* is pleased to announce the 2019 scholarship recipients:

**Hadia M. Abdelaal, PhD**

**Hsu-Yu Chen, MS**

**Pooja Bhardwaj, PhD**

**Sandra Dross, PhD**

**Cheriko Boone, MSW, MPH**

**Qi Guo, PhD**

**Mohamad Bouzidi, PhD**

**Eytan Herzig, PhD**

**Mayra Carrillo, PhD**

**Rebecca Olson, MS**

*Congratulations!*

## SCIENTIFIC ORGANIZING COMMITTEE

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### **Catherine Bollard, MD, MBChB**

*Director, Center for Cancer and Immunology, Director, Program for Cell Enhancement and Technologies for Immunotherapy, Children's National Medical Center*

*Professor of Pediatrics & Microbiology, Immunology and Topical Medicine, George Washington University*

### **Paula Cannon, PhD**

*Professor, Molecular Microbiology & Immunology, Keck School of Medicine, University of Southern California*

### **Mike McCune, MD, PhD**

*Senior Advisor, Global Health, Bill & Melinda Gates Foundation*

### **Rowena Johnston, PhD**

*Vice President and Director of Research, amfAR*

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

*HOST, Conference on CGT4HIVCure 2019*

*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, Dept. of Laboratory Medicine, University of Washington*

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

*Stephanus Family Endowed Chair for Cell and Gene Therapy*

*Director, Stem Cell and Gene Therapy Program*

*Associate Head of Transplantation Biology*

*Associate Head, Heme Malignancy Program Cancer Consortium*

*Professor of Medicine and Pathology, University of Washington*

### **Romas Gelezianas, PhD**

*Executive Director, Biology, Gilead Sciences*

### **Ya-Chi Ho, MD, PhD**

*Assistant Professor, Microbial Pathogenesis & Medicine, Yale School of Medicine*

### **James Riley, PhD**

*Associate Professor, Microbiology, Perelman School of Medicine, University of Pennsylvania*

### **Manuel Venagas**

*defeatHIV Community Advisory Board*

Marriott Residence Inn Seattle, Downtown/Convention Center	7:15 – 8:00	Registration and Breakfast
	<b>SESSION 1</b>	<b>Opening Remarks &amp; HIV Cure Overview</b>
	8:00 – 8:20	Conference Welcome: Keith R. Jerome and Hans-Peter Kiem
	8:20 – 8:40	Rowena Johnston
	<b>SESSION 2</b>	<b>HSC Transplantation &amp; Gene Editing</b>
	8:40 – 9:10	PLENARY: Monique Nijhuis
	9:10 – 9:30	Paula Cannon
	9:30 – 9:50	John A. Zaia
	9:50 – 10:05	Tricia Burdo
	10:05 – 10:15	Sandra Dross (oral abstract)
	<b>10:15 – 10:40</b>	<b>BREAK</b>
	<b>SESSION 3</b>	<b>Keynote</b>
	10:40 – 10:45	Keynote Introduction: Keith R. Jerome
	10:45 – 11:45	Keynote Speaker: Robert F. Siliciano
	<b>11:45 – 1:00</b>	<b>LUNCH</b>
	<b>SESSION 4</b>	<b>HIV Latency &amp; Reservoirs</b>
	1:00 – 1:20	Pamela Skinner
	1:20 – 1:30	Hadia M. Abdelaal (oral abstract)
	1:30 – 1:40	Michael Farzan (oral abstract)
	1:40 – 2:00	Laurie Sylla
	<b>SESSION 5</b>	<b>CAR T Cells &amp; Other T Cell Therapies for HIV</b>
	2:00 – 2:20	Catherine Bollard
	2:20 – 2:40	James Riley
	2:40 – 2:55	Christopher Peterson
	2:55 – 3:05	Kim Anthony-Gonda (oral abstract)
	3:05 – 3:15	Mayra Carrillo (oral abstract)
	3:15 – 3:25	Eytan Herzig (oral abstract)
	<b>3:25 – 3:50</b>	<b>BREAK</b>
<b>SESSION 6</b>	<b>Lessons from Cell &amp; Gene Therapy for Other Conditions</b>	
3:50 – 4:10	Aude Chapuis	
4:10 – 4:30	Keith R. Jerome	
4:30 – 4:45	Holly Peay	
4:45 – 5:00	Olivier Humbert	
<b>Evening Activities</b>		
5:00 – 6:20	Poster Session: serving hors d'oeuvres, beer and wine	
6:30 – 7:00	Shuttle to MOHAI dock and board the Argosy Cruise ship	
7:00 – 10:00	Lakes dinner cruise and reception	



## Workshop on In Vivo Gene Therapy

### Sponsored by Bill & Melinda Gates Foundation

Marriott Residence Inn Seattle, Downtown/Convention Center	7:30 – 8:00	Registration and Breakfast
	<b>SESSION 7</b>	<b>In Vivo Gene Therapy Overview</b>
	8:00 – 8:15	Workshop Overview: Mike McCune and Hans-Peter Kiem
	8:15 – 8:45	PLENARY: David Baker
	<b>SESSION 8</b>	<b>Viral Delivery Vectors</b>
	8:45 – 9:05	Hildegard Büning
	9:05 – 9:25	Guangping Gao
	9:25 – 9:40	Koki Morizono
	9:40 – 10:00	Priti Kumar
	10:00 – 10:20	André Lieber
	<b>10:20 – 10:45</b>	<b>BREAK</b>
	10:45 – 11:05	Leah Sabin
	11:05 – 11:25	Els Verhoeven
	11:25 – 11:35	Hsu-Yu Chen (oral abstract)
	<b>SESSION 9</b>	<b>Non-Viral Delivery Vectors</b>
	11:35 – 11:55	Daniel Anderson
	11:55 – 12:15	Drew Weissman
	<b>12:15 – 1:15</b>	<b>LUNCH</b>
	1:15 – 1:35	Chris Jewell
	1:35 – 1:50	Jing Wen
1:50 – 2:10	Ross Wilson	
2:10 – 2:20	Sarah Slack (oral abstract)	
<b>SESSION 10</b>	<b>In Vivo Gene Therapy Q&amp;A Discussion</b>	
2:20 – 2:45	Moderators: Romas Gelezianas, Manuel Venegas, Hans-Peter Kiem	
2:45 – 2:50	Closing Remarks: Keith R. Jerome and Hans-Peter Kiem	



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



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**Robert F. Siliciano MD, PhD**

*Professor of Medicine, Molecular Biology and Genetics, Johns Hopkins University School of Medicine*

*Investigator, Howard Hughes Medical Institute*

Dr. Robert F. Siliciano is a Professor of Medicine and Molecular Biology and Genetics at the Johns Hopkins University School of Medicine and a member of the Howard Hughes Medical Institute. In 1995, his laboratory provided the first demonstration that latently infected memory CD4+ T cells were present in patients with HIV-1 infection. He showed that latently infected cells persist even in patients on prolonged antiretroviral therapy (ART). These studies indicated that eradication of HIV-1 infection with ART alone would never be possible, a finding which led to a fundamental change in the treatment strategy for HIV-1 infection. This latent reservoir is now recognized as the major barrier to curing HIV-1 infection and is the subject of an intense international research effort. Dr. Siliciano's laboratory has gone on to characterize the reservoir and to explore strategies for eradicating it. In addition, Dr. Siliciano has developed a theoretical foundation for understanding the success of ART in controlling HIV-1 replication.

Dr. Siliciano graduated from Princeton and received his MD and PhD degrees from Johns Hopkins. After a postdoctoral fellowship at Harvard, he joined the Hopkins faculty. He has received the Distinguished Clinical Scientist Award from the Doris Duke Charitable Foundation and two NIH Merit Awards. He is a past Chairman of the NIH AIDS and Related Research Study Section. For 16 years, he directed the Hopkins MD-PhD Program, and he now serves as an advisor for MDPH students. In 2008, he received a major award in AIDS research, the Bernard N. Fields Memorial Lecture at the Conference for Retroviruses and Opportunistic Infections.

### Understanding Barriers to HIV Cure

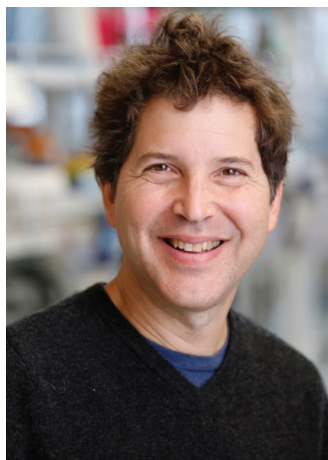
Robert F. Siliciano MD, PhD

Johns Hopkins University School of Medicine and Howard Hughes Medical Institute, Baltimore MD

The latent reservoir for HIV in resting CD4+ T cells is a major barrier to curing the infection. One approach to cure involves the shock and kill strategy in which expression of latent proviruses is induced by latency reversing agents so that infected cells can be recognized and eliminated by the immune response. Alternative strategies envision the using of genetic engineering approaches to directly target latent proviruses, generate HIV-resistant cells, or produce effector cell populations that will more effectively eliminate infected cells. Implementing these approaches requires an understanding of the nature and distribution of latent proviruses and mechanisms by which latently infected cells persist. This talk will review aspects of the latent reservoir that are relevant to the search for a cure.

## PLENARY SPEAKERS

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### **David Baker, PhD**

*Director, Institute for Protein Design*

*Professor of Biochemistry*

*Adjunct Professor of Bioengineering, Genome Sciences, Physics, Chemical Engineering, Computer Science*

*University of Washington*

*Investigator, Howard Hughes Medical Institute*

David Baker is the Henrietta and Aubrey Davis Endowed Professor in Biochemistry, Director of the Institute for Protein Design, Investigator of the Howard Hughes Medical Institute, and adjunct professor of Genome Sciences, Bioengineering, Chemical Engineering, Computer Science, and Physics at the University of Washington. He received his Ph.D. in biochemistry with Randy Schekman

at the University of California, Berkeley and did postdoctoral work in biophysics with David Agard at UCSF. His research group is focused on the prediction and design of macromolecular structures, interactions and functions. Dr. Baker received young investigator awards from the National Science Foundation and the Beckman Foundation, and the Packard Foundation fellowship in Science and Engineering. He has also received the Irving Sigal Young Investigator award from the Protein Society and the Overton Prize from the International Society of Computational Biology. He is a recipient of the Feynman Prize from the Foresight Institute, the AAAS Newcomb Cleveland prize, the Sackler prize in biophysics, and the Centenary award from the Biochemical society. He is a TED speaker and member of the National Academy of Sciences and the American Academy of Sciences. His research group is a world leader in computational protein design and protein structure prediction.

## The coming of age of de novo protein design

In this talk Dr. Baker will address how de novo protein design is being applied to the challenge of targeted biological delivery of small molecules, proteins and nucleic acids. He will describe the computational design of new, self-assembling icosahedral protein cages that encapsulate and protect their own RNA genomes, and how these extremely stable structures are being transformed into vehicles for targeted nucleic acid delivery. He will also detail the development of dynamic protein assemblies that respond to local changes in pH, including when take up into the endosome, which can trigger their membrane-lytic endosomal escape. Finally, he will discuss an approach for high-specificity cell targeting using combinatorial logic on the cell surface. With this system, cells displaying two different markers but not a third can be distinguished from all other cells, for example.



### **Monique Nijhuis, PhD**

*Associate Professor, Department of Virology, Medical Microbiology, University Medical Centre Utrecht, The Netherlands*

Monique Nijhuis studied Biology and obtained her PhD in 1999 at the University of Utrecht on the investigation of the impact of antiretroviral therapy on HIV fitness. Following postdoctoral research training she initiated a research line at the interface between fundamental and clinical research and became an honourable Professor of the University of the Witwatersrand in Johannesburg and an associate Professor of Virology at the University Medical Center Utrecht in The Netherlands.

Her research interest are centred on  
(1) Viral reservoirs and eradication,

including stem cell transplantation and gene-therapy and (2) Mechanisms of viral resistance and evolution.

Monique Nijhuis was granted by the Netherlands Organization for Scientific Research (NWO) with the VIDI and ASPASIA award. She is part of the scientific advisory board of the Dutch Aids Fonds. She is a member of the IAS HIV Cure International Scientific Working Group. She is Associated Editor of *Retrovirology* and published over 100 original papers in peer reviewed journals and has guided numerous students, PhD students and Postdoctoral Fellows.

## Potential for HIV Cure by allogeneic stem cell transplantation, the IciStem perspective

M. Nijhuis, M. Salgado, M. Kwon, J.M. Eberhard, A. Saez-Cirion, J. Schulze zur Wiesch, G. Huetter, J. Badiola-González, A. Bandera, L. Barrett, R. Gupta, B.E. Jensen, J.H.E. Kuball, L. Martín-Carbonero, M. Nabergoj, K. Raj, R. Saldaña-Moreno, G. Scarlatti, L. Vandekerckhove, J.L. Diez-Martin, J. Martinez-Picado and A.M.J. Wensing on behalf of IciStem Consortium

**Background:** Allogeneic Stem Cell Transplantation (SCT) in HIV infected individuals has the potential to cure HIV as has been observed in the Berlin patient. Although this high-risk procedure is only indicated for certain hematological malignancies, the strategy raised scientific potential to gain profound insight in the mechanisms of HIV eradication.

**Methods:** The IciStem 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 registered to the IciStem observational cohort. Viral tropism, the remaining HIV reservoir and cellular and humoral immunity are investigated thoroughly.

**Results:** As part of IciStem efforts, 32,000 cord blood units and 2,200,000 bone marrow adult donors have been genotyped for CCR5 in multiple blood banks around Europe to increase CCR5 $\Delta$ 32 donor availability. 44 HIV-positive patients with diverse hematological malignancies have been registered to the IciStem cohort. 38 patients have been transplanted with CCR5 $\Delta$ 32 (n=9), heterozygous (n=3) or CCR5 WT donors. So far, 19 patients have successfully passed the 12 months follow-up after transplantation, and 13 patients have died after transplantation, despite achieving full donor chimerism in most cases. Analysis of virological and immunological data from blood and tissue samples shows a systematic reduction of HIV-1 reservoirs and a diminishing humoral immunity with (partial) seroreversion. Out of the 4 patients with a CCR5 $\Delta$ 32 homozygous donor that are still on follow up, two patients have interrupted antiviral therapy and did not rebound during the observation time. Five CCR5WT participants with proven undetectable viral reservoirs are in the process of initiating a combination of an immune therapy (bNAbs: 10-1074+3BNC117) with ATI.

**Conclusions:** IciStem is the largest registry studying in depth the HIV reservoir and the respective immune system after allo-HSCT. A potential eradication of the reservoir is currently evaluated by treatment interruption alone or in combination with bNAbs.

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

*Vice President and Director of Research, amfAR,  
The Foundation for AIDS Research, New York, NY*

## Where is cell and gene therapy in HIV cure?

Clinical precedent set by the Berlin and London patient cases suggest that cell and gene therapy may constitute the most promising avenue towards a cure for HIV. Economic precedent suggests that these approaches may encounter significant implementation challenges in low and middle income settings, where most of the individuals who stand to benefit from a cure live. How have these two opposing forces affected the pursuit of an HIV cure via cell and gene therapy? Through the lens of network analyses, we use bibliometric and altmetric techniques to explore the productivity, citation patterns, influence and newsworthiness of efforts by specific individuals and networks active in research on cell and gene therapy for HIV cure. While Robert Siliciano is the overall most productive HIV cure researcher, Keith Jerome, Hans-Peter Kiem, and John Rossi have emerged as the most productive HIV cure researchers working in gene therapy since the publication of the Berlin patient case. Network analyses indicate that gene therapy is currently peripheral to the HIV cure research enterprise, and its network structure shows signs of “immaturity” and “fragility”. Our analyses allow us to identify individuals who are most influential, are power-brokers, or are newsmakers. The analyses enable recommendations to strengthen and mature cell and gene therapy for HIV cure, and suggest some likely future directions of cell and gene therapy research for HIV cure.



**Paula M. Cannon, PhD INVITED SPEAKER**

*Distinguished Professor of Molecular Microbiology & Immunology; Associate Director of Cross School Programs for the MESH Academy, University of Southern California, Los Angeles, CA*

### HSC genome editing to control HIV

Genome editing of hematopoietic stem cells (HSC) combines the rapid advances that are being made in genome editing technologies with the long-standing clinical experience of autologous HSC transplantation. Because HSC give rise to both HIV target cells and the effector cells of the immune system, genome editing of HSC can both protect cells from infection and improve anti-HIV immune response. My lab is exploring multiple approaches aimed at controlling HIV infection by engineering these cells.

## SESSION 2 SPEAKERS

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### **John A. Zaia, MD INVITED SPEAKER**

*Professor, Department of Pediatrics; Director, Center for Gene Therapy, City of Hope, Duarte, CA*

### **Use of zinc finger nuclease CCR5-modified CD34+ hematopoietic stem/progenitor cells in HIV-1 (R5) Infected subjects: preliminary results**

Among the strategies for HIV CURE is the engineering and engraftment of HIV-resistant immune cells via gene editing of autologous hematopoietic stem/progenitor cells (HSPC). A Zinc Finger Nuclease (ZFN) mRNA construct was designed by Sangamo Therapeutics INC to selectively disrupt the chemokine receptor 5 (CCR5). ZFN-CCR5-HSPC were manufactured and infused at City of Hope as an autologous HSPC transplant (aHCT) after busulfan conditioning into 8 healthy persons living with HIV (PLWH) (NCT02500849). These PLWH were aviremic R5-tropic HIV-1 infected and had CD4 counts  $\geq 200$  and  $< 750$  cells/ $\mu\text{L}$  while on antiretroviral therapy. They received busulfan to a targeted area-under-the-curve (AUC) of either 8,000 (+/- 1000)  $\mu\text{mol}\cdot\text{min}$  (cohort 1, n=3) or 12,000 (+/- 1000)  $\mu\text{mol}\cdot\text{min}$  (cohort 2, n=5) and followed for safety, engraftment, and presence of CCR5-disrupted alleles, among other endpoints. The ZFN-CCR5-HSPC dose was between  $2.15\text{E}+06$  and  $11.5\text{E}+06$  CD34+ cells/kg body weight, and the median CCR5 disruption evaluated by MiSeq was -25% indels for the investigational products. All subjects received the anticipated number of busulfan doses and appropriate busulfan exposure. All subjects engrafted by Day +15 (ANC  $\geq 500/\mu\text{l}$  for 3 consecutive days), and there were no serious adverse events in this outpatient study. However, 3 persons had brief hospitalization for fever or mucositis. All showed levels of disruption of CCR5 in peripheral blood and in CD4 cells. This is the first-in-human use of ZFN genome editing of HSPC, and preliminary data shows that conditioning with busulfan and infusion of autologous CCR5-ZFN-modified HSPC are safe and well tolerated in HIV-infected subjects.

**Tricia Burdo, PhD INVITED SPEAKER**

Associate Professor of Neuroscience; Associate Chair for Education; Associate Professor, Center for Neurovirology, Temple University

**Ex vivo and in vivo editing of the SIV genome in non-human primates by CRISPR-Cas9**

T Burdo<sup>1</sup>, Pietro Mancuso<sup>1</sup>, R Kaminski<sup>1</sup>, C Chen<sup>1</sup>, S Iqbal<sup>2</sup>, J Williams<sup>2</sup>, T Peterson<sup>2</sup>, J Gordon<sup>1</sup>, B Ling<sup>2</sup>, A MacLean<sup>2</sup> and K Khalili<sup>1</sup>

<sup>1</sup>Temple University Lewis Katz School of Medicine, <sup>2</sup>Tulane National Primate Research Center

**Background:** Antiretroviral therapy (ART) has increased survival, but is a non-curative approach as replication competent proviral DNA, with high risk for reactivation upon ART cessation, remains. Curative strategies to eradicate the infected cells or viral genome without further treatment are vital. Here, we develop and test the ability of the CRISPR-Cas9 gene editing method for elimination of the SIV viral genome in rhesus macaques.

**Methods:** We employed AAV-9 as a vector to deliver CRISPR-Cas9 designed to target sequences spanning the LTR and Gag genes and permanently inactivating proviral DNA by excising intervening DNA fragments. Adult Chinese rhesus macaques (n=8) were i.v. infected with SIVmac239. At 8 weeks post infection, animals were treated daily with a drug regimen of tenofovir, emtricitabine and dolutegravir (5.1/50/2.5mg/kg daily by s.q.). *Ex vivo* gene editing was performed in PBMCs by AAV9-CRISPR-Cas9 transduction, PCR amplification and Sanger sequencing of the amplicons to assess the potency and precision of viral DNA elimination. In a proof of concept *in vivo* study, 4 animals, 3 were given an i.v. infusion of AAV-9-CRISPR-Cas9 (10<sup>13</sup> GC/kg), and after three weeks, animals were necropsied, blood and tissues were harvested for virological and gene excision evaluations.

**Results:** In all SIV-infected animals, *ex vivo* excision of viral DNA was confirmed by the detection of distinct DNA fragments of 464bp and 358bp resulting from the removal of intervening DNA sequences between 5'LTR to Gag and 3'LTR to Gag, respectively. *In vivo*, both 5'LTR to Gag and 3'LTR to Gag excision were confirmed in blood and various tissues, including brain and testes, of animals that received a AAV-9-CRISPR-Cas9 infusion. All Sanger sequencing results confirmed the breakpoint of the viral DNA. Delivery was confirmed by the presence of Cas9 and expression of both gRNAs.

**Conclusions:** We demonstrated, for the first time, high specificity and efficacy of the CRISPR technology for targeting SIV proviral LTR and Gag regions, which led to both *ex vivo* and *in vivo* editing of SIV DNA. These observations support the potential use of CRISPR-Cas9 technology as a curative strategy that warrants further investigation.

**Funding:** This work was supported by NIH R01 NS104016.

### **Sandra Dross, PhD ORAL ABSTRACT**

*Post-Doctoral Research Fellow, Department of Microbiology, University of Washington*

## **Immunogenicity and efficacy of a therapeutic conserved elements (CE) DNA vaccine in combination with $\alpha$ PD1 therapy, latency reversal, and CCR5 gene-edited CD4+ T cells in SHIV-infected rhesus macaques**

S Dross<sup>1,2</sup>, MA O'Connor<sup>1,2</sup>, HC Tunggal<sup>1,2</sup>, TB Lewis<sup>1</sup>, JM Osborn<sup>1</sup>, JS Li<sup>2</sup>, CW Peterson<sup>3</sup>, JT Fuller<sup>1,2</sup>, KR Jerome<sup>3</sup>, HP Kiem<sup>3</sup>, GN Pavlakis<sup>4</sup>, BK Felber<sup>5</sup>, JI Mullins<sup>2</sup>, DH Fuller<sup>1,2</sup>

<sup>1</sup>University of Washington National Primate Research Center, Seattle, WA, US; <sup>2</sup>Department of Microbiology, University of Washington, Seattle, WA, US; <sup>3</sup>Fred Hutchinson Cancer Research Center, Seattle, WA, US; <sup>4</sup>Human Retrovirus Section, Vaccine Branch, Center for Cancer Research, National Cancer Institute, Bethesda, MD, US <sup>5</sup>Human Retrovirus Pathogenesis Section, Vaccine Branch, Center for Cancer Research, National Cancer Institute, Frederick, MD, US

**Background:** To achieve HIV cure, combinatorial immunotherapy is likely necessary. We treated SHIV-infected, cART-suppressed rhesus macaques with therapeutic DNA vaccination expressing viral conserved elements(CE),  $\alpha$ PD1 to reverse immune exhaustion, TLR7-agonist GS-986 to reverse viral latency, and CCR5-gene-disrupted CD4+ T cells. We hypothesized these carefully timed combinatorial immunotherapies would enhance immunogenicity and efficacy of CE vaccination to reduce or eliminate virus in SHIV-infected cART-treated macaques.

**Materials and Methods:** Three groups (N=8/group) of SHIV-1157ipd3N4-infected macaques began cART 7 weeks post-infection (wpi). At 34 wpi, the 1st group began receiving CE DNA vaccinations expressing SIV Gag and HIV Env CE (5 doses with full-length genes included in 3rd-4th doses) with CCR5-gene-disruption prior to cART-withdrawal (CE/ $\Delta$ CCR5). The 2nd group received vaccinations and CCR5-gene-disruption combined with  $\alpha$ PD1 before the 1st, 3rd and 5th vaccinations and 10 weekly doses of GS-986 post-4th vaccination (CE/ $\alpha$ PD1/GS986/ $\Delta$ CCR5). The 3rd group received cART alone (controls). T-cell CE responses were measured in the blood (PBMC) and mesenteric lymph nodes (MLN) by intracellular staining before immunotherapy and 2 weeks after vaccinations 4, 5 and cART-withdrawal (28, 56, 75, and 81wpi).

**Results:** Vaccine responses peaked at 56wpi and waned by 75wpi despite the 5th vaccination. CE/ $\alpha$ PD1/GS986/ $\Delta$ CCR5 animals' T-cell responses were elevated 56wpi in PBMC but not MLN, suggesting that  $\alpha$ PD1 may enhance vaccine responsiveness but not permeate mucosal tissues. GS986 induced one viral blip in 1/8 animals. CD4 %CCR5 expression increased post-CCR5-gene-disruption in groups 1&2, suggesting infusion may have paradoxically increased targets for infection. During analytical treatment interruption (ATI) time-to-viral rebound and viral loads were similar in all groups. Polyfunctional T-cell CE responses in the MLN during ATI correlated inversely with ATI viral burden suggesting mucosal CE T-cell responses are important to viral control.

**Conclusions:** CE DNA vaccination of SHIV-infected, cART-suppressed macaques increased CE T-cell responses with  $\alpha$ PD1 improving PBMC, but not MLN, responses. These mucosal polyfunctional CE responses were associated with viral control during ATI. GS986 and CCR5-gene-disruption did not enhance immune responses or viral control. Our collective findings imply optimization of our immunotherapies is needed to protect CD4s, reverse latency and mucosal exhaustion, and enhance CE-specific T-cell polyfunctionality, trafficking and durability.

**Pamela Skinner, PhD INVITED SPEAKER**

*Professor, Microbiology, Immunology, and Cancer Biology, Department of Veterinary and Biomedical Sciences, University of Minnesota*

**CAR/CXCR5 T cell immunotherapy in SIV infected art suppressed rhesus macaques**

Mary S Pampusch<sup>1</sup>, Jhomary Alegria-Berrocal<sup>1</sup>, Hadia M Abdelaal<sup>1</sup>, Geoffery Hart<sup>2</sup>, Eva G Rakasz<sup>3</sup>, Elizabeth Connick<sup>4</sup>, Edward A Berger<sup>5</sup> and Pamela J Skinner<sup>1</sup>

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HIV and SIV replication is concentrated within lymphoid follicles during chronic infection, where low levels of virus-specific CTL permit ongoing viral replication. Elevated levels of SIV-specific CTL in B cell follicles are linked to decreased levels of viral replication in follicles and decreased plasma viral loads. These findings provide the rationale for development of a strategy for targeting follicular viral replication using T cells co-expressing an antiviral chimeric antigen receptor (rhesus CD4-MBL-CAR) and the follicular homing chemokine receptor CXCR5. We hypothesize that CAR/CXCR5 T cells can target virally infected cells in follicles, and lead to sustained remission of SIV/HIV. To begin to test this hypothesis, we engineered CAR/CXCR5 T cells and used them in a 10-animal study in which CAR/CXCR5 transduced T cells were labeled with the live cell stain CTV and infused into rhesus macaques. In our initial study, CAR/CXCR5 cells ( $0.35 \times 10^8$  cells/kg) were infused into an SIV-infected rhesus macaque and tissues were evaluated 2 days later. In a study using six SIV-infected ART-suppressed rhesus macaques, CAR/CXCR5 cells ( $0.75-2 \times 10^8$  cells/kg) were infused, animals were released from ART, and monitored for 2 months. Three untreated animals were included as controls. Cells were evaluated by flow cytometry and trans-well migration assays prior to infusion. The localization and abundance of CTV-labeled cells was examined in tissues after infusion. *In vitro*, transduced and expanded CAR/CXCR5 T cells maintained populations of central memory T cells which migrated to the CXCR5 ligand CXCL13 and suppressed viral replication. *In vivo*, CTV labeled cells were detected in multiple tissues at 2 days post-infusion. Abundant CTV-labeled cells were detected both in follicular and extrafollicular areas of lymph nodes and showed evidence of *in vivo* expansion. Using RNAScope, CAR/CXCR5 cells were specifically detected in the lymph node follicles of treated animals. Preliminary findings show that in four of six treated animals, the viral load was reduced relative to the control animals, and also showed significant reductions in viral loads with increased doses of cells. These findings support further study of CD4-MBL-CAR/CXCR5 T cell immunotherapy as a strategy to achieve sustained remission of HIV infections.

### Hadia M. Abdelaal, PhD ORAL ABSTRACT

Post-Doctoral Research Fellow, Department of Veterinary and Biomedical Science, University of Minnesota

## Location, abundance and persistence of CAR/CXCR5 transduced T cells within lymphoid tissues of SIV- infected rhesus macaques.

H Abdelaal<sup>1</sup>, MS Pampusch<sup>1</sup>, EA Berger<sup>2</sup>, PJ Skinner<sup>1</sup>

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During chronic HIV-1 and SIV infection, virus replication is concentrated within B cell follicles of secondary lymphoid tissue, while virus-specific CTL are largely excluded from these sites. This suggests that the inability of virus-specific CTL to fully suppress virus replication may be due to their deficiency in B cell follicles. High levels of follicular SIV-specific CTL inversely correlate with levels of follicular viral replication and with plasma viral loads. These findings support development of a functional cure to target the follicular reservoir of viral replication using T cells co-expressing the B cell follicle homing molecule, (CXCR5) and an SIV-specific chimeric antigen receptor (CD4-MBL-CAR). We hypothesize that CAR/CXCR5 T cells can home to B cell follicles, interact with and kill virus-infected cells resulting in better control of viremia. To test these hypotheses, we engineered autologous T cells to express CAR/CXCR5 T cells and infused them into six ART-suppressed SIV-infected rhesus macaques at the day of ART interruption. Animals were monitored for 2 to 3 months. Three untreated ART-suppressed SIV-infected animals served as controls. We used RNAScope in situ hybridization combined with immunohistochemistry on paraffin-embedded lymphoid tissue sections to determine the location, abundance, and persistence of the CAR/CXCR5 T cells. Furthermore, we determined the location and abundance of SIV-infected cells. In situ, CAR/CXCR5 T cells were located primarily in the B cell follicles of spleen and lymph nodes of the treated animals. Preliminary results show evidence of *in vivo* expansion of CAR/CXCR5 T cells and direct interaction between CAR/CXCR5 T cells and virus-infected cells within B cell follicles at 2 days post-treatment (DPT). CAR/CXCR5 T cells were most abundant at 2 and 6 DPT and low levels detected at 28 DPT. Four of the six treated animals showed better control of viremia relative to the control group and the controlling animals showed the highest level of CAR/CXCR5 T cells in lymph node at 14 DPT. These results demonstrate successful homing of the CAR/CXCR5 T cells to the B cell follicle and direct interaction with virally infected cells *in vivo*. These findings support the CAR/CXCR5 immunotherapy as a functional cure for HIV/SIV infections.

**Michael Farzan, PhD ORAL ABSTRACT**

*Professor and Co-Chair, Department of Immunology and Microbiology, Scripps Research Institute, Florida Campus*

## Approaching long-term SHIV-AD8 remission with AAV-expressed eCD4-Ig

M Farzan<sup>1</sup>, MR Gardner<sup>1</sup>, ME Davis-Gardner<sup>1</sup>, CH Fellingner<sup>1</sup>, I Fetzter<sup>1</sup>, J Weber<sup>1</sup>

<sup>1</sup>Department of Immunology and Microbiology, Scripps Research

**Background:** eCD4-Ig is an exceptionally broad HIV-1 entry inhibitor that uniquely neutralizes all of the 270 HIV-1, HIV-2 and SIV isolate it has been tested against, in every case with IC80 values < 10 µg/ml. eCD4-Ig's breadth and potency derives from the fact that it closely and simultaneously mimics the HIV-1 receptor CD4 and the HIV-1 coreceptor. Consistent with this breadth, eCD4-Ig is much harder to escape than broadly neutralizing antibodies (bNABs). To date full escape has not been observed and viruses which partially escape eCD4-Ig in cell culture and *in vivo* pay clear fitness costs. Adeno-associated virus (AAV)-expressed eCD4-Ig functions as an effective vaccine alternative, and protects rhesus macaques from repeated high-dose viral challenges with both SHIV-AD8 and SIVmac239. Unlike bNABs and other multispecific antibody-like inhibitors, eCD4-Ig markedly improves the endogenous ADCC activity of patient sera. It does so by altering the conformation of HIV-1 Env, allowing otherwise dormant V3 and CD4i antibodies to bind Env.

**Results:** Six SHIV-AD8-infected rhesus macaques were placed on combined anti-retroviral therapy (ART) 12 weeks after infection and inoculated with AAV-eCD4-Ig 42 to 50 weeks post-infection. ART was subsequently lifted and viral loads and eCD4-Ig concentrations were monitored for another year. We observed that relatively low concentrations of AAV-expressed eCD4-Ig (3-19 µg/ml) prevent viral rebound of an established SHIV-AD8 infection after ART cessation all six macaques, establishing a sustained state of virologic remission in most animals for at least a year.

**Conclusion:** Macaques "functionally cured" in this manner provide an ideal platform to monitor the impact of latency-reversing agents on the reservoir of latently infected cells, and to determine if an entry inhibitor with potent ADCC activity can itself change the rate of reservoir decay. Stable HIV-1 remissions may also be appealing and useful to humans, for example limiting transmission from non-compliant individuals or those without access to ART, enabling long-term drug holidays, and providing a backstop for an imperfect sterilizing cure. Efforts to increase the robustness and consistency of these functional cures will be described.

## SESSION 4 SPEAKERS

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### **Laurie Sylla, MHSA, BSW INVITED SPEAKER**

*Community Advisory Board Member, defeatHIV and NIH Martin Delaney Collaboratory*

### **You want me to what? Results of a qualitative study exploring acceptability of cell and gene therapy for hiv cure among a purposive sample of individuals receiving care at an HIV clinic in Seattle, WA**

To have an impact on the HIV epidemic, promising cell and gene therapy interventions that could lead to an HIV cure will need to be studied in and eventually adopted by people living with HIV (PLWH). Acceptability to PLWH will be paramount to successful trial enrollment and eventual uptake, should an efficacious strategy be identified. We conducted four focus groups with 19 individuals receiving care at a Seattle-based HIV clinic to elicit their thoughts on cell and gene therapy as a means of curing HIV and about participating in related trials. Our sample was predominantly Black (16/19) and evenly split M/F, with one person who identified as transgender. Participants had not heard of cell and gene therapy, had negative and fearful reactions to it, expressed overall unwillingness to subject themselves to perceived risks associated with cell and gene therapy studies, as well as distrust of research in general and the government specifically, and questioned the need for such an extreme intervention when they had effective, easy to tolerate treatment. Participants were skeptical about cure and especially hesitant about potential analytical treatment interruption. They perceived themselves to be in good health and voiced trust in their health care providers. Although this study sample was quite limited, results point to the need for targeted community education, HIV care provider engagement, and incorporation of social science research into pre-clinical research efforts. These findings also indicate the challenges that will need to be overcome to recruit individuals for trials when they are satisfied with their treatment and their health. Recommendations for future directions will be made.



### **Catherine M. Bollard, MD INVITED SPEAKER**

*Director, Center for Cancer and Immunology Research; Director, Program for Cell Enhancement and Technologies for Immunotherapy, Division of Blood and Marrow Transplantation, Children's National Health System*

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

## **Antigen-specific T cell therapies as a cure strategy for HIV after transplant**

Requirements for effective and long-lasting immunity against HIV include the infusion of HIV-specific T cell clones or polyclonal CTLs, and the genetic modification of T cells with artificial T cell receptors (TCRs) and chimeric antigen receptors (CARs). Clinical trials have previously focused on isolating CD8+ T cells that show strong IFN- $\gamma$  and cytotoxicity responses to HIV. Examples include T cells specific for HLA-A2-restricted epitopes in gp120, p17, p24, Gag, Pol or Nef. However, the lack of success using HIV-specific CD8+ T cell clones may have been due to antigen escape [25] low *in vivo* levels of HIV epitopes recognized by the T cell clones, or lack of CD4+ T cell help. To circumvent the problems of single epitope-specific T cells, polyclonal HIV-specific T cells expanded against multiple HIV antigens have been developed from seropositive and virus naïve donors. Multiepitope-specific T cells recognizing Gag, Nef, and Pol can suppress *In vitro* HIV replication. These polyclonal HIV-specific T cells include CD4+ T cells, which can improve *in vivo* persistence and can be expanded against HIV peptides irrespective of the patient's HLA type, thereby broadening their therapeutic applicability. Autologous multiepitope-specific T cells are now in two ongoing clinical trials and are opening up the possibility of antiviral T cell therapy in HIV+ individuals with hematologic malignancies necessitating autologous or allogeneic transplant.

## SESSION 5 SPEAKERS

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

*Associate Professor of Microbiology, Department of Microbiology and Center for Cellular Immunotherapies, Perelman School of Medicine, University of Pennsylvania*

### **Designing T cells for HIV Cure Studies**

J Riley<sup>1</sup>, J Jadlowsky<sup>1</sup>, R Leibman<sup>1</sup>, C Maldini<sup>1</sup>, M Richardson<sup>1</sup>, J Hoxie<sup>1</sup>, P Tebas<sup>1</sup>

<sup>1</sup>Department of Microbiology and Center for Cellular Immunotherapies, Perelman School of Medicine, University of Pennsylvania,

Although T cells play an important role in controlling virus replication, they are themselves targets of HIV-mediated destruction. Direct genetic manipulation of T cells for adoptive cellular therapies could facilitate a functional cure by generating HIV-1-resistant cells, re-directing HIV-1-specific immune responses, or a combination of the two strategies. In contrast to a vaccine approach, which relies on the production and priming of HIV-1-specific lymphocytes within a patient's own body, adoptive T cell therapy provides an opportunity to customize the therapeutic T cells prior to administration. However, at the present time it is unclear how to best engineer T cells so that sustained control over HIV-1 replication can be achieved in the absence of antiretrovirals. This talk focuses on efforts to date to engineer T cells to fight HIV infection and how data from early Phase I clinical trials is providing the basis and rationale for future trials aimed to cure HIV infection.

**Christopher Peterson, PhD INVITED SPEAKER**

Staff Scientist, Fred Hutchinson Cancer Research Center

## HIV-specific chimeric antigen receptor T cells expand and persist following antigen boost in infected, suppressed nonhuman primates

C Peterson<sup>1,2</sup>, B Rust<sup>1</sup>, L Colonna<sup>1</sup>, K Brandenstein<sup>1</sup>, N Poole<sup>1</sup>, W Obenza<sup>1</sup>, C Maldini<sup>3</sup>, G Ellis<sup>3</sup>, L Kean<sup>4</sup>, J Riley<sup>3</sup>, HP Kiem<sup>1,2</sup>

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

<sup>3</sup>University of Pennsylvania, Philadelphia, PA, USA <sup>4</sup>Harvard Medical School, Boston, MA, USA

**Background:** An effective strategy to achieve antiretroviral therapy (ART)-free remission in HIV<sup>+</sup> individuals is likely to require active and passive approaches. Here, an optimized CD4-based Chimeric Antigen Receptor molecule (CD4CAR) was delivered to CCR5-edited nonhuman primate (NHP) T cells, which were infused into animals infected with simian/human immunodeficiency virus (SHIV) and suppressed long-term on ART. The goal of this study was to model a highly promising cell-based approach to enable stable virus remission, ahead of upcoming clinical trials.

**Methods:** Rhesus macaques (n = 4) were infected with SHIV containing an HIV envelope, and suppressed by ART for at least 1 year prior to intervention. Autologous T cells were first edited with NHP CCR5 CRISPR ribonucleoproteins, then modified with covalent-pseudotyped, SIV-based lentiviral vectors expressing HIV/SHIV-specific CD4CAR. CAR T-cell products were infused without a conditioning regimen. To boost the persistence of these cells *in vivo*, transplanted animals received a single dose of cell-associated viral envelope antigen. Flow- and PCR-based assays were used to characterize the T-cell infusion product and the persistence of these cells in blood and tissues.

**Results:** Adjustment of the manufacturing protocol to augment the ratio of CD4 to CD8 CAR T cells increased the persistence of CAR<sup>+</sup> CD4 lineages *in vivo*. CCR5-edited CD4CAR T-cell products derived from pre-infection cell sources were more efficiently modified relative to cells from post-infection, ART-suppressed sources. Infusion of cell-based antigen was well-tolerated and led to significant increases in the percentage of CAR<sup>+</sup> T cells in peripheral blood.

**Conclusions:** Relative to CARs directed against hematological malignancies, previous studies in patients and NHPs demonstrated low-level persistence of virus-specific CAR T cells *in vivo*. To our knowledge, ours is the first study to boost virus-specific CAR T cells in infected, suppressed hosts. This exciting finding led us to interrupt ART; these animals are currently under close monitoring for multiple parameters including viral rebound kinetics and *in vivo* expansion of CAR<sup>+</sup> cells. Due to the lack of a cytotoxic conditioning regimen prior to cell infusion, the safety profile of our approach is highly favorable, reinforcing the promise of virus-specific CAR-based approaches for viral reservoir reduction in HIV<sup>+</sup> individuals.

### **Kim Anthony-Gonda, PhD ORAL ABSTRACT**

*R&D Manager, Infectious Diseases, Lentigen Technology*

## **Novel multi-specific anti-HIV duoCAR-T cells display broad antiviral activity and potent elimination of HIV-infected cells *in vitro* and *in vivo***

K. Anthony-Gonda<sup>5</sup>, A Bardhi<sup>1</sup>, A Ray<sup>1</sup>, N Flerin<sup>1</sup>, W Chen<sup>2</sup>, C Ochsenbauer<sup>3</sup>, JC Kappes<sup>4</sup>, W Krueger<sup>5</sup>, D Schneider<sup>5</sup>, R Orentas<sup>5</sup>, DS Dimitrov<sup>6</sup>, H Goldstein<sup>1</sup>, B Dropulic<sup>5</sup>

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Adoptive immunotherapy using chimeric antigen receptor gene-modified T cells (CAR-T) has made significant contributions to the treatment of certain B-cell malignancies. Such treatment modalities also show promise for the development of a single treatment for HIV/AIDS and obviating the need for long-term anti-retroviral drug therapy. We hypothesized that HIV-1-based lentiviral vectors encoding chimeric antigen receptors (CAR) targeting multiple highly conserved sites on the HIV-1 envelope glycoprotein (Env) using a two-molecule CAR architecture (termed duoCAR) would significantly improve CAR potency, breadth, and resistance to HIV infection. To assess CAR-T cell functionality, we adapted a previously described neutralization assay that utilizes replication-competent infectious molecular clones (IMC) of HIV encoding different env genes and a Renilla luciferase reporter (Env-IMC-LucR) to monitor the inhibitory activity of different anti-HIV CAR vectors. We show that transduction with lentiviral vectors encoding multi-specific anti-HIV duoCARs conferred primary T cells with the capacity to potently suppress HIV infection (up to 99%) in contrast to single-molecule CAR-T cells (monoCAR) while simultaneously protecting them from genetically diverse Env-IMC-LucR viruses *in vitro*. Furthermore, the genetically modified CAR-T cells also potently suppressed broadly neutralizing antibody (bNAb)-resistant Env-IMC-LucR strains, including a VRC01/3BNC117-resistant virus. Lastly using a humanized NOD/SCID/IL-2Rγ<sup>-/-</sup> model of splenic HIV-1 infection (hu-spl-PBMC-NSG), we show that anti-HIV duoCAR-T cell therapy significantly suppressed HIV infection, prevented CD4<sup>+</sup> T cell depletion, and abated HIV persistence. We conclude that multi-specific duoCAR-T cells are superior to conventional monoCAR-T cells and are highly efficacious against broad and bNAb-resistant Env-IMC-LucR viruses *in vitro* and *in vivo*, respectively. When combined with strategies that target the latent reservoir, the anti-HIV duoCAR may offer a path towards functionally curing HIV.

**Mayra Carrillo, PhD ORAL ABSTRACT**

Post-Doctoral Research Fellow, Department of Medicine-Hematology and Oncology,  
University of California Los Angeles

## Comparing adoptive T cell versus hematopoietic stem cell (HSC)-based HIV-specific CAR-T cell therapy approaches *in vivo*

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<sup>1</sup>Department of Medicine-Hematology and Oncology, UCLA

**Background:** Chimeric antigen receptor (CAR) design against HIV has been a popular area of focus to develop a functional cure. We are utilizing a CD4-based CAR fused with second generation costimulatory domains for targeting and killing HIV infected cells. One of the major limitations of CAR T cell therapy is the lack of *in vivo* persistence, which results in minimal clinical efficacy. In our studies using the humanized BLT mouse model, we compared two CAR-based gene therapy approaches: one using a classical adoptive T cell approach and one using a stem cell-based approach. We are attempting to determine the optimal strategy that would provide sustained antiviral responses and promote long-term persistence of anti-HIV CAR T cells in hopes of eradicating the virus.

**Methods:** In our adoptive T cell approach, we stimulated spleen-derived T cells from humanized mice and transduced with our CD4-based CAR vectors. We attempted to optimize this strategy through the improvement of cell handling techniques and culturing conditions to generate persistent memory CAR+ T cells. CAR T cell treatment was applied to infected humanized BLT mice. In our stem-cell based approach, we modified CD34+ hemopoietic stem cells with our CD4-based CAR vectors to generate humanized BLT mice reconstituted with stem-cell derived CAR T cells. Following reconstitution, the animals were infected with HIV. In both cases, the antiviral effects, cellular persistence and cellular function were monitored and compared.

**Results:** Our results show that adoptive T cell-based CAR therapy had limited *in vivo* persistence and expansion, even after 2 CAR modified T cell infusions and co-administration of cytokines. This led to minimal control of plasma viral load. In contrast, in our stem cell-based CAR T cell approach, we observed sustained CAR-expressing T cell expansion following HIV infection. In addition, stem cell derived CAR T cells had a significant, sustained impact on viral loads.

**Conclusion:** Our results strongly suggest that a stem cell-based CAR T cell approach may be more optimal to generate long term persistence and antiviral responses against HIV infection when compared to a peripheral adoptive CAR T cell approach.

### Eytan Herzig, PhD **ORAL ABSTRACT**

Post-Doctoral Research Fellow, Gladstone Institutes, University of California San Francisco

## Using convertibleCAR-T cells programmed with broadly neutralizing antibodies to attack the latent HIV reservoir

E Herzig<sup>3</sup>, K Kim<sup>1</sup>, SG Deeks<sup>2</sup>, LS Weinberger<sup>3</sup>, WC Greene<sup>3</sup>

<sup>1</sup>Xyphos Biosciences, Inc, <sup>2</sup>University of California San Francisco, <sup>3</sup>Gladstone Institutes / UCSF

One potential approach to achieving a sustained viral remission or functional HIV cure is to first reduce the size of the reservoir and then engineer an immune response that can effectively control the shrunken reservoir in the absence of ART. We have constructed fusion proteins, called MicAbodies, comprised of full-length broadly-neutralizing HIV antibodies coupled to a mutated form of ULPB2, a member of the MIC family (ligands for NKG2D receptor). These MicAbodies, selectively engage a variant of NKG2D (iNKG2D) that has been engineered as a chimeric antigen receptor (CAR) and expressed on CD8 T cells (convertibleCAR-T Cells). Together, the HIV-specific MicAbody and the convertibleCAR-T cells can detect and eliminate HIV infected cells. *In vivo* proof of concept of the platform's killing capabilities in NSG mice explored convertibleCAR-T efficacy against Raji tumors and demonstrated rituximab-based MicAbody as well as convertibleCAR-T dose-dependent control of tumor mass. In HIV-infected primary cells, the cytotoxic effector function of these cells is activated when a MicAbody simultaneously engages both the HIV Env target and iNKG2D-CAR on convertibleCAR-T CD8 T cells. We have found that four different MicAbodies (3BNC60, 3BNC117, PGT121 and 10-1074), can specifically kill HIV-infected blood, tonsil or spleen cells in a dose-dependent manner with convertibleCAR-T cells and HIV specific MicAbodies. This specific killing did not occur when a non-HIV MicAbody or the parental un-transduced CD8 T cells were present. Furthermore, a mix of these four MicAbodies was effective in reducing the reactivable latent reservoir in stimulated cultures of CD4 T cells from HIV-infected patients on suppressive ART *ex vivo*. These results suggest that reactivated reservoir cells can be targeted and killed by convertibleCAR-T cells armed with MicAbodies.

**Aude Chapuis, MD INVITED SPEAKER**

*Assistant Member, Program in Immunology, Clinical Research Division,  
Fred Hutchinson Cancer Research Center*

**Optimizing T cell receptor gene therapy for cancer**

My lab is translating T cell receptor (TCR)-engineered T-cell therapies for patients with solid and liquid tumors, working to understand relevant immune escape mechanisms and developing strategies to circumvent obstacles to clinical efficacy. To advance optimally safe and effective TCRs, we devised a system to identify the highest affinity TCRs from the pooled peripheral repertoires of human leukocyte antigen (HLA)-matched donors, from which autoreactive clones had been removed by thymic selection.

In the solid-tumor realm, we developed adoptive T cell therapy for patients with Merkel cell carcinoma (MCC), a virus-driven cancer that is an ideal model to test TCR-based treatments targeting non-self epitopes. We treated patients using autologous Merkel cell polyomavirus (MCPy)-specific CD8+ T cell lines and an immune-checkpoint inhibitor. Dramatic tumor reductions were associated with dense infiltrates of activated T cells in two patients, but relapses occurred 22 and 18 months later. Single cell RNA sequencing (scRNAseq) identified dynamic transcriptional suppression of the targeted HLAs suggesting strong and specific immunologic pressure of therapeutic T cells. To address HLA downregulation as a tumor escape mechanism, we have initiated a trial utilizing a high-affinity HLA A\*0201-restricted TCR to engineered autologous T cells thus conferring a high-affinity TCR to all patients. As soon as safety is confirmed with this TCR alone, T cells carrying other MCPy-targeting TCR(s) with alternate HLA restrictions will be combined.

Overall, revealing the *in vivo* escape mechanisms that undermine the efficacy of transgenic T-cell therapy in patients can inform significant improvements in the design of TCR-engineered immunotherapies.

## SESSION 6 SPEAKERS

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

*Member, Vaccine and Infectious Disease Division, Fred Hutchinson Cancer Research Center, Professor and Head, Virology Division, Department of Laboratory Medicine, University of Washington*

### *In vivo* gene editing for herpes simplex virus cure

After initial infection at mucosa, herpes simplex virus (HSV) establishes lifelong latency in neurons of the peripheral nervous system, which represents the source of recurrent disease. Current antiviral therapies reduce symptoms and viral shedding, but do not cure the infection. In contrast, gene editing offers the possibility to lethally mutate or even eliminate latent viral genomes. We performed gene editing of HSV, using adeno-associated virus (AAV)-delivered meganucleases, to treat latent HSV infection in a well-established mouse model. We observe that AAV-delivered meganucleases, but not CRISPR/Cas9, mediated highly efficient gene editing of HSV, eliminating over 90% of latent virus from superior cervical ganglia. Single-cell RNA sequencing demonstrated that both HSV and individual AAV serotypes were non-randomly distributed among neuronal subsets in ganglia, implying that improved delivery to all neuronal subsets may lead to even more complete elimination of HSV. The levels of HSV elimination observed in these studies, if translated to humans, would likely significantly reduce HSV reactivation, shedding, and lesions. Further optimization of meganuclease delivery and activity is likely possible, and may offer a pathway to a cure for HSV infection.



**Holly Peay, PhD INVITED SPEAKER***Senior Researcher – RTI International***Participant experiences with remission trials: data from a social/behavioral study**H Peay<sup>1</sup>, T Jupimai<sup>2</sup>, S Rennie<sup>3</sup>, J Cadigan<sup>3</sup>, S Isaacson<sup>3</sup>, N Ormsby<sup>3</sup>, K Kuczynski<sup>3</sup>, G Henderson<sup>3</sup><sup>1</sup>RTI International, <sup>2</sup>Chulalongkorn University, <sup>3</sup>University of North Carolina at Chapel Hill And the Thai Red Cross Research Centre/SEARCH

Our social/behavioral study is nested into the SEARCH HIV remission (“cure”) trials in Thailand. We assess decision making and decision satisfaction of remission trial joiners and decliners. There have been four remission trials, three of which tested an experimental agent with ART treatment interruption (ATI) and one included only ATI. All but one participant across all four trials experienced viral rebound.

The study includes three mixed-method data collection points for joiners and two for decliners. The first is focused on decision making process and influences, trial-related hopes/expectations, the meaning of cure, and decision satisfaction. The second, for joiners only, focused on trial experiences and decision satisfaction. The final data collection point included reaction to trial outcome, trial experiences (joiners only), perceived benefits and harms, and decision satisfaction.

We obtained data from 54 joiners and 20 decliners. The large majority are male and MSM. The chance to experience ATI was perceived as a clear benefit of trial participation, even by most decliners. They valued the opportunity to test their bodies in a monitored environment. Both joiners and decliners also appreciated the opportunity to contribute to science. Most who joined were optimistic about personal benefits. They demonstrated awareness of trial-related risks but trusted in staff’s close monitoring. They reported having sufficient information and making an informed choice.

During the trial, joiners described that ATI increased their feelings of normalcy. For some, ATI was associated with more worry than expected. Joiners reported moderately high trial burden. Several became identifiable on standard HIV antibody tests, which caused distress related to potential social harm. Overall trial and decision satisfaction remained high for almost all joiners, even after rebound.

Some challenges identified include: 1) ongoing relationships with the trial team that may influence decisions and in some cases lead to perceived pressure to participate; 2) hoped-for personal benefits are unlikely; 3) “altruistic” motivations are appealing on the surface, but deeper concerns arise related to internalized stigma and perceived obligation to “atone”; 4) the personal experience of viral rebound may result in challenges to self-concept. Ongoing work includes community engagement and recommendation development for investigators.

### **Olivier Humbert, PhD INVITED SPEAKER**

*Staff Scientist, Fred Hutchinson Cancer Research Center*

## Stable and therapeutically relevant engraftment of a CRISPR-Cas9-edited HSC-enriched population in nonhuman primates

Olivier Humbert<sup>1</sup>, Stefan Radtke<sup>1</sup>, Hans-Peter Kiem<sup>1</sup>

<sup>1</sup>Clinical Research Division, Fred Hutch Cancer Research Center

Genome engineering of hematopoietic stem and progenitor cells (HSPCs) has the potential to cure genetic and infectious diseases. For an effective therapy, these nuclease-based approaches must achieve high editing performance while maintaining the multilineage engraftment capacity of HSPCs. Toward this goal, we previously reported the robust engraftment of CRISPR/Cas9-edited CD34<sup>+</sup> HSPCs in the nonhuman primate (NHP) model to recapitulate naturally occurring mutations identified in individuals with hereditary persistence of fetal hemoglobin (HbF). Remarkably, we achieved comparable results by restricting CRISPR/Cas9 treatment to the highly enriched stem cell phenotype CD34<sup>+</sup>CD90<sup>+</sup>CD45RA<sup>+</sup>, which reduced the number of cells to be edited by 10-fold.

Here, we provide a long-term assessment of engraftment in these animals, which have now been monitored for over 2 years post-transplantation. Editing frequency in peripheral blood stabilized at up to 26% and resulted in the production of up to 20% HbF-expressing erythrocytes. Safety of our approach was demonstrated by stabilization of all blood cell counts, which remained within normal range during the entire course of the study, and by the absence of off-target activity. Taken together, these data showed robust engraftment of CRISPR/Cas9-edited HSPCs in a large animal model of autologous transplantation and validated the use of the stem cell-enriched CD34<sup>+</sup>CD90<sup>+</sup>CD45RA<sup>+</sup> phenotype. This novel and clinically translatable approach is highly relevant for HIV research where hematopoiesis can be engineered to protect from virus infection.

**Hildegard Büning, PhD INVITED SPEAKER***Professor, Institute of Experimental Hematology, Hannover Medical School***Tailoring adeno-associated viruses (AAV) for gene therapy**Hildegard Büning<sup>1-4</sup>

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Adeno-associated viral (AAV) vectors are the most widely used delivery system for *in vivo* gene therapy. Already three market approvals for AAV vector-based therapies for hitherto untreatable monogenetic disorders have been granted and more are expected in the near future. However, so far high vector doses are required to achieve transgene expression at therapeutically relevant levels. AAV's broad tropism whereby considerable amounts of vector particles are sequestered in off-target tissues even if vectors are applied locally and pre-existing immunity are further challenges that need to be addressed.

Since the native tropism as well as pre-existing immunity is mainly defined by the viral capsid, capsid engineering is exploited to overcome these drawbacks and to optimize the AAV vector system for clinical use. Using AAV serotype 2 (AAV-2) as basis, we have developed strategies to re-directed AAV's tropism towards pre-defined target cells and/or to overcome pre- and/or post entry barriers of cell transduction including the presence of neutralizing antibodies.

Equally important for tailoring this promising vector system is a better understanding of the AAV-host interaction. We are in particular interested in AAV's interaction with the liver as this organ is not only one of the main targets in gene therapy, but also unique in a number of features including immune responses towards pathogens or vectors. We thereby identified autophagy as biomarker determining the outcome of liver-directed gene therapy with AAV vectors. Intrigued by this dependency, we performed proof-of-concept studies of drug-induced autophagy in mice and non-human primates. The impressive increase in transduction efficiency indicate that this newly identified cell response towards AAV can be exploited to enhance the efficacy of the AAV vector system at least for liver-directed gene therapy.

### **Guangping Gao, PhD INVITED SPEAKER**

*Co-Director, Li Weibo Institute for Rare Diseases Research; Director, Horae Gene Therapy Center and Viral Vector Core; Professor of Microbiology and Physiological Systems; Penelope Booth Rockwell Professor in Biomedical Research, University of Massachusetts Medical School; President, American Society of Gene and Cell Therapy (ASGCT)*

## Posttranscriptional regulation to diminish transgene immunity in genetic delivery of rAAV-vectored antibody – strategy and mechanism

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Vectorizing antiviral broad neutralizing antibody is an attractive strategy for prophylactic and therapeutic applications in preventing and treating infectious diseases. Among the current variety of vectors available for *in vivo* gene delivery of vectored antibodies, recombinant adeno-associated virus (rAAV) stands out for its high efficiency, stability, and low immunotoxicity profiles. rAAV can efficiently target muscle tissues in particular, holding the promise to serve as “biofactories” for sustained secretion of antiviral reagents. Nevertheless, *in vivo* delivery of rAAV-vectored antibody is often limited by host immune responses against the transgene product, resulting in formation of anti-drug-antibody and loss of antiviral effect. Thus, development of strategies to prevent anti-transgene immunity is crucial.

The employment of endogenous microRNA (miRNA)-mediated regulation to detarget transgene expression from antigen presenting cells (APCs) has shown promise for reducing immunogenicity. However, the mechanisms underlying miRNA-mediated modulation of anti-transgene immunity by APC detargeting are not fully understood. Using the highly immunogenic ovalbumin (OVA) protein as a proxy for foreign antigens such as antiviral antibody, we revealed that the mechanism for APC-specific attenuation of transgene immunity by miRNA-mediated regulation involves circumventing cell-mediated immunity via repressing co-stimulatory signals in dendritic cells and inhibiting cytotoxic CD8<sup>+</sup> T lymphocytes (CTLs). Humoral immunity is found to be less of a contributing factor once the CTL response is overcome and levels of secreted transgene product are elevated in the circulation.

Our findings demonstrate that post-transcriptional regulation through miRNA binding in APCs is a robust strategy for overcoming transgene immunity towards secreted proteins, thereby potentially improving the efficacy and safety of prophylactic and therapeutic antiviral gene delivery.

**Koki Morizono, MD, PhD INVITED SPEAKER**

*Adjunct Professor, Division of Hematology and Oncology, Department of Medicine, David Geffen School of Medicine, University of California, Los Angeles*

*UCLA AIDS Institute, David Geffen School of Medicine, University of California, Los Angeles*

**Anti-HIV antibody and B-cell receptor expression by targeted transduction of B-cells *in vivo***

Neutralizing antibodies against HIV-1 are very potent for suppressing HIV-1 replication. However, the serum concentrations of the antibodies must be kept at therapeutic levels for long periods of time to eliminate HIV-1-infected cells, because persistently infected cells reside in deep tissues. Therefore, multiple administrations of the antibodies are required, which would likely be expensive and labor-intensive. Gene therapy vectors can be used to produce therapeutic antibodies. However, transgene expression by any gene therapy vector, including lentiviral vectors, can induce immune reactions against the transgenes, thereby decreasing the therapeutic effects of transgene products and elimination of the transduced cells. Therefore, it is important to express transgenes in the cell types that can develop tolerance for transgene products. B-cells physiologically express wide varieties of valuable antibody regions generated by recombination and mutations of genes, and are known to induce tolerance for the valuable regions. We have developed lentiviral vectors that can selectively transduce splenic B-cells after systemic administration. By exploiting this B-cell-specific transduction by our lentiviral vectors, we are attempting to express anti-HIV-1 antibodies and antibody-like molecules specifically in B-cells to develop tolerance for the transgene products. Using this highly B-cell-specific transgene expression system, we will also express both secretory and membrane-anchored forms of antibodies (and antibody-like molecules) in B-cells to generate an anti-HIV-1 B-cell receptor (BCR) on B-cells. We anticipate that anti-HIV-1 BCR elicits signals upon binding to the HIV-1 envelope protein that induce differentiation and growth of the transduced cells. This antibody-based therapy using B-cell targeted lentiviral transduction will be applicable not only for HIV-1 infection, but also for other types of infectious diseases.

## SESSION 8 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* gene editing of human T cells for HIV therapy

HIV gene therapy typically involves reinfusion of autologous hematopoietic stem and T cells gene-modified for HIV resistance. However, poor post-infusion engraftment of gene-modified cells remains a major hurdle for ART-free remission necessitating myeloablative conditioning regimens. *In vivo* gene therapy, which does not involve ex-vivo cell manipulations, may address this issue. That said, effective transgene delivery to human hematopoietic cells *in vivo* is a formidable challenge in the gene therapy field.

We have developed a lentiviral vector surface-decorated with an antibody to human CD7 to allow targeted and effective transduction human T cells and monocytes. Importantly, as a key step toward clinical application, we have generated CD7-tropic lentiviral virus-like particles (VLPs) carrying CRISPR-SpCas9 ribonucleoprotein complexes (RNPs) in the absence of DNA encoding SpCas9 and sgRNA, which allows rapid clearance of the nuclease components in target cells. We demonstrate that intravenous treatment of R5-HIV-positive, ART suppressed humanized mice with this CD7-tropic lentiviral VLPs encapsulating Cas9/RNP targeting CCR5, the coreceptor for HIV-1, results in editing of the CCR5 gene, abrogating surface CCR5 expression in transduced T cells. These CCR5-edited CD4 T cells selectively expand after ART interruption, leading to stabilization of peripheral blood CD4 T cell levels, control of plasma viral loads, and eventually, ART-free remission.

The approach we report here therefore represents an important advance to the traditional use of lentiviral vectors in gene therapy in that it obviates the need for isolation and ex-vivo transduction of hematopoietic cells. Further, we also attain impressive transduction of naïve/resting human T cells, which may address the issue of the poor *in vivo* gene marking. We expect our work to be a significant advance towards achieving ART-free remission and of compelling interest to the fields of clinical gene-therapy for a functional cure for HIV-1.

**André Lieber, MD, PhD INVITED SPEAKER**

Professor, Medical Genetics, Department of Medicine, University of Washington

***In vivo* HSC gene therapy using HDAd5/35++ vectors**

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We developed an *in vivo* hematopoietic stem cell (HSC) transduction approach that involves G-CSF/AMD3100-triggered mobilization of HSCs from the bone marrow into the peripheral blood stream and the intravenous injection of an integrating, helper-dependent, capsid-modified adenovirus (HDAd5/35++) vector system. HDAd5/35++ vectors target human CD46, a receptor that is abundantly expressed on HSCs. HSCs, transduced in the periphery, return to the bone marrow (shown in CD46 transgenic mice, humanized mice and pigtail macaques). Transgene integration is achieved either (in a random pattern) using a hyperactive Sleeping Beauty transposase (SB100x) or targeted to a safe locus (AAVS1) via HDR. HDAd5/35++ vectors can also be used for short-term expression of CRISPR/Cas9 or Base Editors. Without a disease-related proliferative stimulus, low-dose and transient treatment with O<sup>6</sup>BG/BCNU (*in vivo* selection<sup>TM</sup>) is required to achieve efficient (50-100%) transgene marking in peripheral blood cells. We employed our approach in mouse models for *i*) hemoglobinopathies: We achieved near complete correction in a mouse model of thalassemia by SB100x-mediated gamma-globin gene addition. The combination of SB100x-mediated gamma gene addition and CRISPR/Cas9-mediated re-activation of endogenous gamma globin expression resulted in higher gamma-globin levels in peripheral red blood cells than the individual approaches resulting in a cure of murine sickle cell disease. *ii*) Cancer: We demonstrated that prophylactic *in vivo* HSC gene therapy with an immune checkpoint inhibitor reverses tumor growth in syngeneic mouse tumor models. *iii*) hemophilia A: Using a beta-globin LCR mediating erythroid-specific expression of non-erythroid proteins, we demonstrated stable supraphysiological plasma levels of a bioengineered human factor VIII version (ET3) without negative effects on hematopoiesis. High plasma anti-ET3 antibody titers did not compromise the therapeutic effect of our approach, i.e. the phenotyping correction of bleeding after tail clipping in hemophilia A mice. Theoretically, erythroid cells derived from *in vivo* transduced HSCs can be used as a sustained factory for high-level production of virus inhibitors. Efficacy and safety studies in non-human primates are ongoing. Current efforts are also focused on establishing a vector system that is not neutralized by pre-existing serum antibodies against adenovirus serotype 5-derived vectors.

### **Leah Sabin, PhD INVITED SPEAKER**

*Staff Scientist, Regeneron Pharmaceuticals, Inc.*

## Engineering AAV vectors for antibody-mediated retargeting to specific cell types *In vitro* and *in vivo*

L Sabin<sup>1</sup>, C Wang<sup>1</sup>, K Le<sup>1</sup>, M Leon<sup>1</sup>, D Velez<sup>1</sup>, M Beyman<sup>1</sup>, M Golabek<sup>1</sup>, R Sattler<sup>1</sup>, C Schoenherr<sup>1</sup>, A Economides<sup>1</sup>, C Kyratsous<sup>1</sup>

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Adeno-associated virus (AAV) is currently one of the leading viral vectors used in gene therapy to treat human diseases. AAV has a favorable safety profile and the vector provides long-term, stable transgene expression. Although AAV has many advantages as a gene therapy vector, one of the drawbacks to its use in systemic gene delivery is the relatively broad tropism of the virus. For many potential gene therapy applications, limiting infection to specific tissues or cell types would be advantageous. We are therefore interested in developing a cell type-specific AAV vector. Our goal is to rationally engineer the virus to target specific cell types by genetically abolishing the natural tropism of the virus, then redirecting the virus to target cells using monoclonal antibodies.

To accomplish this, we have taken two complementary antibody-based approaches. The first uses a modular protein tagging system to covalently couple antibodies to surface-exposed residues on the viral capsid. The second approach utilizes bispecific antibodies; one arm of the antibody binds to an epitope tag inserted into the viral capsid, while the other mediates binding to cell-surface proteins. We have successfully used both of these approaches to retarget AAV2 to specific cell types *In vitro*, using the cellular target of the retargeting antibodies to drive the specificity of infection, and have demonstrated efficient transduction of a variety of cell types in culture using antibodies that recognize several different cellular targets. To test our retargeting systems *in vivo*, we injected our retargeted viruses systemically into transgenic mice engineered to express the human protein recognized by the retargeting antibody. We found that both retargeting modalities were able to specifically transduce the intended target tissue in transgenic mice expressing the human protein, while the same virus showed very little off-target transduction in control animals. Although we initially optimized our retargeting systems for AAV2, we have now successfully applied both techniques to additional AAV serotypes, and believe our systems can be capable of retargeting any AAV serotype. Currently, our efforts focus on applying these AAV retargeting approaches *in vivo* to non-human primates and additional cell types and tissues in mice.



**Els Verhoeyen, PhD INVITED SPEAKER**

Research Director, Ecole Normale Supérieure de Lyon, Université Côte d'Azur, Nice, France

## Novel lentiviral pseudotypes for gene therapies and cancer therapies and 'nanoblades' for efficient gene editing in T and B cells and blood stem cells.

T-cell therapies would strongly benefit from gene transfer into long-lived persisting naïve T cells or T-cell progenitors. Here we demonstrated that baboon envelope glycoprotein pseudotyped lentiviral vectors (BaEV-LVs) outperformed by far other LV-pseudotypes for transduction of naïve adult and fetal IL-7-stimulated T cells. Remarkably, BaEV-LVs transduced T-cell progenitors, generated by culture of CD34<sup>+</sup>-cells on Delta-like ligand-4 (DL-4). T-cell lineage reconstitution was accelerated in NOD/SCID $\gamma$ C<sup>-/-</sup> recipients after T-cell progenitor injection as compared to hematopoietic stem cell (HSC) transplantation. Furthermore,  $\gamma$ C-encoding BaEV-LVs very efficiently transduced DL-4 generated T-cell precursors from a patient with X-linked severe combined immunodeficiency (SCID-X1), which fully rescued T-cell development *In vitro*. Ultimately, the co-injection of LV-corrected T-cell progenitors and HSCs might accelerate T-cell reconstitution in immunodeficient patients.

Chimeric antigen receptor (CAR) T cells brought substantial benefit to patients with B-cell malignancies. Here, we provide evidence that human CD19-CAR T cells can be generated directly *in vivo* using the lentiviral vector CD8-LV specifically targeting human CD8<sup>+</sup> cells. Further, upon injection of CD8-LV into mice transplanted with human CD34<sup>+</sup> cells, induction of CAR-T cell differentiation and CD19<sup>+</sup> B cell depletion was observed in 7 out of 10 treated animals. Notably, three mice showed elevated levels of human cytokines in plasma, indicative of a cytokine release syndrome. Our data demonstrate the feasibility of *in vivo* reprogramming of human CD8<sup>+</sup> CAR T cells active against CD19<sup>+</sup> cells.

Programmable nucleases delivery into target cells can be challenging, specially into primary cells. A new technology was designed called "Nanoblades", a tool to deliver a genomic cleaving agent into cells (Mangeot et al. 2019). We developed MLV- or HIV-derived virus like particle (VLP), in which the viral structural protein Gag has been fused to the Cas9, which are thus loaded with Cas9 protein together with guide RNAs. To assure efficient Nanoblades delivery of Cas9/sgRNA cargo into human T, B and HSCs we pseudotyped them with baboon retroviral, measles virus or/and VSV-G envelopes. We obtained efficient gene editing in T, B and HSCs. In addition, we induced in HSCs expression cassette insertion in a specific genomic locus by combining nanoblades with AAV6 vectors carrying a donor cassette.

### Hsu-Yu Chen **ORAL ABSTRACT**

Graduate Student, Department of Molecular Microbiology and Immunology, University of Southern California

## Development of novel CD4-targeted vectors to disrupt the latent reservoir

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<sup>1</sup>Department of Molecular Microbiology and Immunology, Keck School of Medicine, University of Southern California

The reservoir of latently infected cells is a major barrier to HIV cure. Gene editing tools such as CRISPR/Cas9 have been suggested as a strategy that could specifically disrupt latent genomes, but methods to deliver them directly to latently infected cells *in vivo* will be required. Resting CD4+ memory T cells are a major cellular reservoir of latent HIV. To target these cells specifically, we are exploring the incorporation of different CD4 binding ligands into the envelope proteins of the paramyxoviruses measles virus (MeV) and Nipah virus (NiV). The resulting chimeric proteins can be used to pseudotype lentiviral vectors expressing HIV-specific CRISPR/Cas9. This system is possible because paramyxovirus entry uses dual envelope proteins that control cell attachment and fusion separately, allowing us to manipulate target binding without affecting the subsequent fusion function. We are using targeting ligands based on scFvs, DARPins, and the human fibronectin domain III (FN3) fragment. FN3 ligands have the advantage of being a smaller size, which reduces the size of libraries that need to be constructed and screened to select an optimized binder, while selected FN3 candidates maintain a similar target binding affinity and specificity as scFvs or DARPins. In addition, the lack of disulfide bonds in the FN3 structure should facilitate incorporation into chimeric proteins.

We are using CD4 binding FN3s as a targeting ligand in a chimeric NiV G protein, co-expressed with the NiV F protein in pseudotyped lentiviral vectors. Using Western blot, we showed successful incorporation of FN3-G into vector particles and specific transduction of CD4+ T cells with a GFP reporter vector in a population of resting PBMC *in vitro*. Following *in vivo* administration of the vectors into humanized mice (NSG mice engrafted with CD34+ HSC), we also observed specific CD4+ T cell transduction in blood, lymph node, spleen, and bone marrow. Further analysis of the spleen samples showed the ability of the vector to transduce resting and memory CD4 T cell subsets, which is an important consideration for delivery to the latent reservoir. Current experiments are delivering CRISPR/Cas9 nucleases to disrupt HIV genomes.

**Daniel Anderson, PhD INVITED SPEAKER**

*Associate Professor, Chemical Engineering, Institute for Medical Engineering and Science, Massachusetts Institute of Technology*

### Nucleic acid delivery systems for RNA therapy and gene editing

High throughput, combinatorial approaches have revolutionized small molecule drug discovery. Here we describe our work on high throughput methods for developing and characterizing RNA delivery and gene editing systems. Libraries of degradable polymers and lipid-like materials have been synthesized, formulated and screened for their ability to delivery RNA, both in vitro and in vivo. A number of delivery formulations have been developed with in vivo efficacy, and show potential therapeutic application for the treatment of genetic disease, viral infection, and cancer.

### **Drew Weissman, MD, PhD** INVITED SPEAKER

*Professor of Medicine, Immunology, Cell and Biology, Perelman School of Medicine, University of Pennsylvania*

## Targeting of LNPs to deliver nucleoside-modified mRNA therapeutics

Our laboratory developed nucleoside-modified mRNA as a means to deliver therapeutic proteins. We have used modified mRNA to deliver therapeutic hormones, cytokines, genetically deficient proteins, gene editing systems, and vaccines. Despite the huge potential of nucleoside-modified mRNA to deliver therapeutic proteins, the current main deficiency in its advancement to therapeutic use is *in vivo* delivery. The current optimal delivery system for RNA-based therapeutics are lipid nanoparticles (LNPs), which are in an FDA approved drug for siRNA delivery to treat Transthyretin amyloidosis. LNPs bind apoE and preferentially target liver cells. We have developed a new method to functionalize LNPs and specifically target the delivery of encapsulated nucleoside-modified mRNA to multiple cell types and tissues including; lung, heart, brain, CD4+ cells, T cells, and bone marrow stem cells. LNPs are functionalized with mAbs, or pieces of antibodies, FAbs, scFvs, and others, or ligands for cell surface proteins. This results in the specific delivery to the targeted cell or tissue with expression of the mRNA. Resting T cells *in vivo* can be gene edited at 80% efficiency. We believe this novel functionalization of delivery particles currently in clinical use offers an efficient method to target specific cell types and tissues for genetic modification or therapeutic protein expression. It is being used to target HIV latently infected cells, CCR5 in immune and bone marrow stem cells, to deliver CAR T's to T and NK cells, and to modify hepatocytes to express broadly neutralizing mAbs as an approach to a gene therapy-based cure for HIV.

**Christopher Jewell, PhD INVITED SPEAKER**

*Associate Professor, Associate Chair of Research, Fischell Department of Bioengineering, University of Maryland; Research Biologist, United States Department of Veterans Affairs*

## Harnessing engineered materials and nanotechnology to study and control immune function

C. M. Jewell<sup>1-4</sup>

<sup>1</sup>Fischell Department of Bioengineering, University of Maryland, College Park, MD. <sup>2</sup>Robert E. Fischell Institute for Biomedical Devices, College Park, MD. <sup>3</sup>United States Department of Veterans Affairs, VA Maryland Health Care System, Baltimore, MD. <sup>4</sup>Department of Microbiology and Immunology, University of Maryland Medical School, Baltimore, MD

Our research combines immunology, biomaterials, and nanotechnology to study fundamental immune processes, and exploits the unique capabilities of these technologies to tailor immune response to disease. In one example, I will discuss polymer scaffolds we are using to locally control the function of lymph nodes - key tissues that coordinate immune signaling and differentiation. This strategy involves injection of polymer depots that are too large to drain from lymph nodes. Instead, these carriers slowly degrade, releasing nucleic acids, antigens, adjuvants, or other cues that condition the local lymph node microenvironment. Immune cells locally conditioned in lymph nodes using this strategy then migrate to the periphery, generating antigen-specific, systemic function. This local programming thus provides a route for precision generation of strong, tailored responses by engineering the lymph node microenvironment. We have used this idea to promote pro-immune function to fight infection, or conversely, to induce immune tolerance to combat autoimmunity. In a second area, I will present the lab's efforts to build nanostructures entirely from immune signals using self-assembly, including nucleic acids. This rational design approach allows activation of programmable combinations and levels of the immune pathways triggered during encounter with an antigen. By this strategy, useful features of biomaterials can be mimicked - such as cargo protection and delivery of multiple immune cues to the same cells, but in a simpler manner because no carrier is required. Additionally, because the particles are assembled entirely from immune signals, the cargo loading level is 100%, concentrating signals and driving strong immune responses. This approach enables tunable control over the relative levels that combinations of immune pathways can be activated, ultimately allowing different responses to be generated against the same antigen. Using engineered materials for precision control of immune signaling could improve the efficacy of vaccines for infectious disease, and enhance the efficiency of vaccine translation.

Lymph nodes are key tissues that coordinate immune function. In this talk I will present some of the new ways we are using engineered materials to control the local microenvironment of lymph nodes for therapeutic vaccines aimed at cancer and autoimmunity. These ideas will be introduced through our recent studies in transgenic co-cultures, animal models of disease, and samples from human patients.

### Jing Wen, PhD **INVITED SPEAKER**

Scientist, Department of Microbiology, Immunology, and Molecular Genetics, David Geffen School of Medicine, University of California, Los Angeles; UCLA AIDS Institute

## Efficient ablation of HIV-1 provirus by CRISPR/Cas9 ribonucleoprotein delivered by polymer nanocapsules

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Eliminating HIV-1 reservoirs is crucial step toward achieving a “functional cure” and bringing the global HIV-1 epidemic to an end. Gene therapy targeting two primary HIV-1 reservoir cells, T cells and macrophages, can mutagenize HIV-1 provirus such that replication and reactivation from latency are aborted without need for lifetime therapy.

Recently, the CRISPR/Cas9 system has been used *In vitro* and *in vivo* to eliminate HIV-1 proviruses. Despite promising results *In vitro*, nucleic acids and proteins are rapidly lost from circulation; additionally, current *In vitro* delivery vehicles are not capable of CRISPR/Cas9 delivery that impacts HIV-1 reservoirs *in vivo*. AAV vectors are suited for *in vivo* application, but efficacy is limited by both immunogenicity and a broad target cell spectrum; the risk of non-specific delivery raises safety concerns in clinical applications.

We have developed a nanotechnology platform whereby anti-HIV-1 macromolecules are encapsulated within a thin polymer shell to shield cargo from the environment and confer high- efficiency delivery capabilities. We previously engineered two different anti-HIV-1 nanocapsules—one designed to deliver an shRNA expression cassette for the CCR5 gene and another to release ricin toxin A chain in response to HIV-1 protease activity—which protect cells from HIV-1 infection by downregulating CCR5 expression and specifically killing HIV-1-infected cells upon activation, respectively. Moreover, by adjusting surface properties, we optimized nanocapsules for extracellular delivery of antibodies *in vivo*—enabling greater penetration to lymph nodes and central nervous system sites with minimal immunogenicity. By conjugating targeting ligands, nanocapsules achieve specific ligand-targeted delivery *in vivo* for appreciable therapeutic effect.

Here, we extend our nanocapsule approach to eliminate the HIV-1 proviral gene by delivering CRISPR/Cas9 ribonucleoprotein (RNP) to the target HIV-1 LTR TAR region. By transducing with CRISPR/Cas9 RNP nanocapsules, 80% of reporter gene expression from lentiviral vector was ablated in primary human macrophages. We further demonstrated 60% elimination of HIV-1 provirus in quiescent latently infected primary T cells with the same CRISPR/Cas9 nanocapsule. Our nanocapsule delivery of gene-editing components presents a promising approach to overcoming the challenges of delivery in primary cells and provides basis for gene therapy against HIV-1 in mainstream clinical practice.

### **Ross Wilson, PhD INVITED SPEAKER**

*Project Scientist, Principal Investigator, University of California Berkeley, Innovate Genomics Institute*

## **Engineering genome editing enzymes for *in vivo* delivery to T cells**

HIV infection is now medically manageable, but antiretroviral therapy has serious side effects, is costly, and requires lifelong daily administration. Thus millions of current and future people would benefit from development of a true cure for HIV. Such a cure must be inexpensive, safe, effective, and accessible even for those in the developing world. Rapid advances in genome editing raise hopes that it could provide a definitive therapeutic approach to curing HIV by deleting host genes that are required for HIV infection. To make this a realistic and scalable therapeutic approach, new technology is needed to perform targeted gene manipulation in immune cells *in vivo*. This would ideally be performed by sending non-viral genome editing enzymes (i.e. CRISPR-Cas9), to the cells most in need of protection against HIV. Towards this end, we are working to engineer a new method of *in vivo* enzyme delivery that is specifically targeted toward T cells, rendering them resistant to HIV and thus providing a path to a cure.

### Sarah Slack **ORAL ABSTRACT**

*Undergraduate Researcher, Department of Bioengineering, University of Washington*

## Hybrid nanocarriers for lymphatic CD4+ T cell activation and HIV-1 latency reversal

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**Background:** The latent reservoir for HIV-1 is sustained by the longevity and proliferative capacity of resting CD4 T cells containing HIV in a highly suppressed state, rendering the infection invisible to the immune system. Complex mechanisms combine to create an extremely slow decay rate of the latent cell pool despite suppressive combination antiretroviral therapy. Strategies to accelerate this decay are being explored but none have yet been proven effective in reducing the reservoir *in vivo*. A synergistic approach will likely be needed to deplete the reservoir and establish a cure. We hypothesize that delivery of latency reversing agents (LRA) using nanocarriers (NCs) will improve drug solubility and safety, provide sustained drug release, and simultaneously deliver multiple drugs to reservoir tissues and cells.

**Methods:** We developed hybrid nanocarriers to incorporate physicochemically diverse LRAs and target reservoirs in lymphatic CD4+ T cells. Down-selection of LRA combinations were evaluated in J-Lat reporter cells and validated in CD4 T cells from virologically suppressed patients. Targeting was tested in PBMCs from NHPs. Targeting and toxicity were also evaluated in mice following size optimization for increased passive drainage to lymph nodes. CD4-targeted NCs separately formulated with ingenol-3-angelate and JQ1 were administered subcutaneously at two doses to maximize biodistribution to reservoir tissues and cells in NHPs. Drug concentrations in blood, gut and lymph node compartments were quantified and animals were monitored throughout the study for signs of toxicity.

**Results:** Optimized nanocarriers were used for identification of an LRA combination displaying synergistic latency reversal and low toxicity *In vitro* in model and patient cells. Long-term and specific activation of CD4 T cells in NHP PBMCs and in mouse lymph nodes were observed, with significant reduction in toxicity compared to free LRA delivery. NHP studies showed targeted NCs were less toxic compared to equivalently dosed unformulated (free) drug and improved delivery to reservoir tissues and cells.

**Conclusion:** This nanocarrier platform targets CD4 T cells, successfully inducing latency reactivation in HIV reservoirs, and it is currently being tested in an NHP model for reactivation of latent simian immunodeficiency virus. The platform may be extended to deliver other HIV cure agents.



**Pooja Bhardwaj, PhD**

Post-Doctoral Research Fellow, Vitalant Research Institute

**Surface engineering of extracellular vesicles to target HIV persistence**

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**Background:** Although antiretroviral therapy has dramatically reduced the mortality of HIV infection, treated individuals still experience immune dysregulation and chronic inflammation, driving interest in alternative therapies. Extracellular vesicles (EVs) have shown promise as engineerable therapeutic agents for a broad range of diseases. We aimed to engineer EVs with the capacity to block HIV infection and selectively target infected cells.

**Methods:** EVs were isolated from 1 ml of healthy donor plasma using polymer-based precipitation and column purification. Nanoparticle tracking analysis was used to determine the size of particles. 200 µg protein equivalents were decorated with single-chain variable fragment (scFv)-C1C2 fusion proteins with complementarity determining regions targeting the HIV envelope protein. Decorated EVs were incubated for two hours with a GFP-reporter HIV strain at 1:1, 2:1, and 4:1 ratios. Jurkat E6.1 cells and primary human CD4+ T cells were infected via spinoculation. GFP-reporter virus was incubated with no EVs, undecorated EVs, or anti-PD-1 scFv-decorated EVs as negative controls. Surface-engineered EVs and HIV particles were fluorescently labeled using ExoGlow-Protein EV labeling dye and CellBrite cytoplasmic membrane dye, respectively. Incubation reactions were visualized in two colors simultaneously and analyzed by single-particle tracking using a Nanoimager S (ONI) equipped with NimOS software.

**Results:** Engineered anti-HIV scFv-decorated EVs significantly inhibited HIV infection in Jurkat E6.1 cells with respect to all negative controls in a dose-dependent manner (n=3; p<0.05, paired t-test) and potently inhibited infection in primary human CD4+ T cells (n=5 donors; p<0.05, paired t-test), suppressing up to 98% of infection in the absence of toxicity. Nanoimaging revealed that HIV-targeting EVs clustered and moved in tandem with virions, in contrast to non-targeting and PD-1-targeting EV controls which tracked independently of virions.

**Conclusions:** Engineered EVs selectively bind to the HIV envelope and potently suppress infection *ex vivo*. Beyond viral suppression, designer EVs may accelerate the clearance of the HIV reservoir by delivering cytotoxic cargo specifically to infected cells expressing envelope at the cell surface (e.g. following administration of HIV latency reversal agents to promote envelope expression). Sustainable *in vivo* delivery may be achieved by infusion of cells engineered *ex vivo* to constitutively secrete cargo-loaded, HIV envelope-targeting EVs.

**Cheriko Boone, MSW, MPH**

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**Barriers to HIV cure research participation among persons living with and without HIV**

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**Background:** Functional and sterilizing HIV cures are on the horizon, with early trials of cure approaches underway. Current research on attitudes about HIV cure includes persons living with HIV (PLWH) and has not included HIV-uninfected (HIV-) persons, limiting our understanding of other communities who may influence the willingness of PLWH to enroll in trials. We sought to characterize knowledge, attitudes, and perceptions about HIV cure research among persons living with and without HIV in order to guide recruitment strategies.

**Methods:** Data were collected from an anonymous sample of adults between 9/2017 and 7/2018. Correlates of barriers to study participation were examined using logistic regression. Factor analysis was used to construct a 7-item scale of HIV cure pessimism; and linear regression was used to assess predictors of HIV cure pessimism, stratified by HIV status.

**Results:** Of 185 participants, the majority identified as male (55%), >30 years (69%), and white (63%); 27% were PLWH. Of the 135 HIV- respondents, 33% had a friend or family member living with HIV and 19% considered themselves at risk for HIV. While the perception of what an HIV cure meant and willingness to participate in invasive or high risk cure trials differed by HIV status, there were no differences ( $p < 0.05$ ) in motivations to participate. HIV- respondents were more likely ( $p < 0.05$ ) to perceive cost and lack of trust in healthcare providers, and PLWH were more likely to perceive risks of the treatment and lack of community education as barriers to cure research participation. After adjusting for confounding variables, HIV- persons identifying as Black/Asian/Latinx had higher HIV cure pessimism than their counterparts ( $p < 0.05$ ). Among PLWH, women had higher HIV cure pessimism than men ( $p < 0.05$ ).

**Conclusions:** Understanding barriers to HIV cure research participation is critical as we develop novel curative therapies. Knowledge of barriers and facilitators to cure research is also essential to preemptively preparing for the social and behavioral implications of eventual dissemination of curative therapies. We found salient differences between persons living with and without HIV that must be considered when developing strategies for recruitment and community engagement around HIV cure-related research.

**Mohamed Bouzidi, PhD**

Post-Doctoral Research Fellow, Vitalant Research Institute

**Efficient antiviral genome editing in primary and brain-derived myeloid cells using CRISPR/Cas9 ribonucleoprotein complexes**

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**Background:** Although HIV eradication efforts have mainly focused on latently-infected CD4+ T cells, myeloid cells harbor provirus during antiretroviral therapy, contributing to inflammation and HIV-associated neurocognitive disorder. CRISPR/Cas9 excision of HIV provirus in the myeloid compartment has not been reported as yet. In this study, we investigated the capacity of CRISPR/Cas9 ribonucleoprotein complexes (Cas9-RNPs) to 1) excise HIV provirus, and 2) knock out key host dependency factors in primary monocyte-derived macrophages (MDM) and brain-derived microglia to render them refractory to HIV infection.

**Methods:** Cas9-RNPs were produced *In vitro* by incubating purified *Streptococcus pyogenes* Cas9 protein and guide RNAs (gRNAs) targeting the HIV LTR promoter and CCR5 and CXCR4 coreceptor genes at 37C for 15 minutes. To generate MDM, PBMCs were isolated from fresh blood (two healthy donors) using Ficoll-Paque gradient separation and were subsequently plated in 75 cc flasks. Non-adherent cells were aspirated, and adherent cells were stimulated using 50 ng/ml M-CSF. After five days, Cas9-RNPs were delivered into MDM using the Lonza 4D Nucleofector. Cells were then seeded into six-well plates with 50 ng/ml M-CSF, and were harvested three days later. Genome editing was additionally evaluated in C20 cells (microglial cell line), and HC69.5 cells (C20 cells infected with a GFP reporter HIV construct). Flow cytometry was used to measure viability and HIV LTR activity, and genomic DNA was extracted, amplified, Sanger sequenced, and analyzed using Inference of CRISPR Edits (ICE).

**Results:** In primary MDM, percentage of indels observed in CCR5 and CXCR4 loci ranged from 50% to 90%, while viability ranged from 80% to 90%. In C20 and HC69.5 cells, indels in CCR5 and CXCR4 loci ranged from 50% to 70%, while viability was ~70%. 82% of HC69.5 cells exhibited indels in the HIV LTR, associated with 87% reduction in the frequency of cells expressing GFP. No editing was observed across negative controls (Cas9-RNPs with scrambled gRNA).

**Conclusion:** Highly-efficient antiviral genome editing in myeloid cells can be achieved using Cas9-RNPs, with minimal effects on viability. Our data warrant development of *in vivo* Cas9-RNP delivery methods including nanoparticles and engineered exosomes to target the myeloid compartment in HIV-infected individuals.

**Noah Cassidy**

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**Optimization of a cross-subtype multiplex ddPCR assay for reservoir quantification in HIV-1 infected African cohorts**

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Despite uninterrupted antiretroviral therapy (ART), replication-competent proviral HIV DNA persists in long-lived cells and is the major barrier to a cure. Research on HIV reservoirs will guide future cure strategies and includes populations in sub-Saharan Africa with a high prevalence of non-B subtype HIV. Assays are needed that accurately quantify the replication competent reservoir in HIV subtypes A, C and D, the most common subtypes in Africa. We developed a multiplex droplet digital PCR (ddPCR) assay for subtype B HIV that can estimate the number of full-length intact proviruses, allowing accurate quantification of replication-competent reservoir size. We developed 2 complementary multiplex ddPCRs - each of which target 3 HIV genomic regions, with one region identical between the two ddPCRs for cross validation (5 target regions total). Here we optimized this assay to work across subtypes. We analyzed sequence diversity across the primer and probe binding sites of our subtype B ddPCRs using alignments of >900 HIV sequences of subtypes A, C and D from the Los Alamos National Library database. For nucleotide positions with <90% sequence conservation, we altered our primers via sequence changes and use of degenerate bases to capture the diversity within subtypes A, C, and D. To verify that the adapted primers capture cross-subtype diversity, we tested cellular DNA samples isolated from short-term culture of PBMCs from 13 infected individuals that included subtypes (based on pol) A (n=5), C(n=3), and D (n=5). In all 13 samples, our adapted primers amplified all 5 target regions. In 11 (85%) of the 13 cases, quantification across the 5 target regions were within an average of 1.3-fold (range 1.0-3.5 fold) of each other, suggesting accurate quantification across the HIV genome. In 2 (15%) of 13 cases (1 each of subtypes A and C), diversity in the primer binding site resulted in a -9-fold reduction in quantification in 1 of the 5 target regions, suggesting further primer optimization of that region is necessary. We plan to use this cross-subtype assay to study correlates of HIV reservoir size and decay in HIV-infected Kenyan infants during long-term ART.

**Leonard Chavez, PhD***Staff Scientist, Vitalant Research Institute***Sequence entropy-guided CRISPR/Cas9 excision of HIV using a multi-targeting lentivirus**LR Chavez<sup>1</sup>, KA Raymond<sup>1</sup>, S Desai<sup>1</sup>, BTM Korber<sup>2</sup>, SK Pillai<sup>1</sup><sup>1</sup>Vitalant Research Institute, San Francisco, CA, USA. <sup>2</sup>Los Alamos National Laboratory, Los Alamos, NM, USA

**Background:** CRISPR/Cas9 gene editing technology offers a promising cure strategy for HIV/AIDS. Despite its therapeutic potential, several obstacles remain: 1) gRNA design must account for circulating strain diversity within and across HIV-infected individuals; 2) multiple sites must be targeted simultaneously to guard against viral escape mutants; 3) off-target editing of the host genome must be eliminated; and 4) in vivo delivery must efficiently transport Cas9 and multiple gRNAs to target cells.

**Methods:** To overcome these obstacles, we adapted an HIV vaccine immunogen design algorithm to identify highly-conserved gRNA targets across the HIV genome. We targeted these sites, simultaneously, by implementing several gRNA multiplexing strategies within a single lentiviral vector that encodes the enhanced specificity SaCas9 nuclease (eSaCas9), which reduces off-target editing. Editing of HIV proviral target sites was measured using Tracking of Indels by Decomposition (TIDE) analysis. To evaluate effects of observed gene editing on the infectivity of reactivated latent virus, we engineered the J-Lat with Env provided in Trans (J-Lat-EnTr) cell line which restores HIV envelope expression in the well-characterized J-Lat latency model, allowing for production of infectious virions. Finally, to edit HIV-infected primary CD4+ T cells, we coupled the expression of eSaCas9 from our lentiviral vector to that of the HIV provirus.

**Results:** We identified six gRNAs that were highly conserved across the entire HIV-1 M-group, and allowed for efficient editing of the HIV provirus within the gag, primer binding site (PBS), and 5' LTR regions. Editing across sites had no effect on transcriptional reactivation from latency, but reduced the production and infectivity of progeny virus upon latency reversal. Lastly, coupling the expression of eSaCas9 to that of the HIV provirus via a minimal LTR promoter, allowed for editing of infected primary CD4+ T cells.

**Conclusions:** These observations suggest that the use of a lentiviral vector encoding eSaCas9 and multiple gRNAs targeting conserved sites of the HIV genome will be an effective strategy to excise provirus in vivo, as a stand-alone therapeutic, or in combination with latency reversal agents in a "shock and knock" eradication approach.

### Adam Dingens, PhD

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## An antigenic atlas of HIV-1 escape from broadly neutralizing antibodies to guide the development of antibody-based immunotherapies

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**Background:** Anti-HIV broadly neutralizing antibodies (bnAbs) are promising immunotherapeutics for cure strategies. The efficacy of bnAb-based therapies depends in part on how readily the virus can escape neutralization. While structural studies can define contacts between bnAbs and Env, only functional studies can define mutations that confer escape. However, gaining a complete view of antibody escape has been limited by functional studies that rely on one-at-a-time mutagenesis.

**Methods:** We used mutational antigenic profiling to quantify how all possible single amino-acid mutations to BG505 Env affect neutralization of HIV by nine bnAbs targeting five epitopes, focusing on bnAbs under development as immunotherapeutics.

**Results:** For most bnAbs, mutations at only a small fraction of structurally defined contact sites mediated escape, and most escape occurred at sites that are near but do not directly contact the antibody. These data distinguish each antibody's functionally defined epitope from their structurally defined epitopes. These data also helped interpret viral evolution in bnAb immunotherapy clinical trials—in vivo escape mutations occurred in the functional epitope, including at sites that'd previously been overlooked because they were outside the structural epitope.

Additionally, we used this data to quantify the ease of viral escape for each bnAb, which we show is distinct from antibody breadth. We suggest that both measures may be useful for assessing the potential efficacy of bnAb-based therapies.

Further, viral escape from a pool of two bnAbs (3BNC117 and 10-1074) was similar to the modeled combination of the bnAbs' independent action, suggesting these antigenic maps for individual bnAbs may be useful for modeling viral escape from combinations of bnAbs. Further, there were no mutations that escaped both antibodies, agreeing with results from recent clinical trials that administered this bnAb combination. Lastly, we unbiasedly quantified the strain-specificity in antibody escape by profiling escape from two bnAbs across multiple viral strains.

**Conclusion:** Our mutation-level antigenic atlas provides a comprehensive dataset for understanding viral immune escape. These data can help refine bnAb-based immunotherapies by helping to select which bnAb (or pool of which bnAbs) is most difficult to escape and in evaluating viral escape during bnAb clinical trials.

**Karsten Eichholz, PhD**

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## Development of a CAR and SHIV RNA FISH assay to quantitate spatial distribution of chimeric antigen receptor T cells and SHIV in tissue

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**Background:** Chimeric antigen receptors T cell-based immunotherapies offer many advantages over endogenous TCRs, including the ability to gene protect them from HIV infection and insert genes to traffic to the germinal center. Anti-CD19 CAR T cells have shown impressive clinical success to treat hematologic cancers and revived interest as an effective therapy for HIV. However, the decentralized distribution of the viral reservoir cells and the paucity of reactivated cells likely hamper the direct translation of the CAR approaches to treat HIV. To study CAR T cell trafficking in tissues we developed a multiplex CAR RNA FISH assay that can be adopted to phenotype CAR T cells, cancer cells and HIV-infected cells and combined it with a deep learning random reaction seed (dIRRS) algorithm for image analysis.

**Methods:** CAR RNA probes were validated on CAR T cell lines in vitro and on tissue section from a CD19+ BE2 neuroblastoma model after anti-CD19 CAR T cell infusion. These were combined with IHC for CD19 and RNA FISH for CD4, CD8, IFN $\gamma$  and other gene targets. SHIV RNA probes were validated on lymph node tissue sections from SHIV1157ipd3N4-infected pigtail macaques.

**Results:** We detected several  $10^4$  CD4 and CD8 CAR T cells/tumor tissue section of which only a small minority expressed high levels of IFN $\gamma$  mRNA and these were located in close contact with the residual CD19t-expressing tumor cells. We also detect  $\sim 10^5$  CAR- CD19- cells/tumor tissue section. A multiplex RNA FISH staining against tumor marker huCD171 indicated that these CAR- CD19- cells are CD19-tumor cells. We also used this assay to quantify SHIV1157ipd3N4-infected cells in macaque lymph nodes.

**Conclusion:** The development of this dIRRS-assisted RNA FISH-based tool set will allow us to study the interaction of anti-cancer and HIV CAR T cells with their respective target cells in tissues and thus, help to better understand CAR T cell-target cell interactions. The rational design of our CAR RNA FISH detection system and the algorithm can be easily adopted to other gene and cell therapy approaches in HIV and cancer.

### Qi Guo, PhD

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## HIV-1 inhibition by efficient engraftment of dual anti-HIV-1 gene modified human hematopoietic stem/progenitor cells by *in vivo* selection in humanized BLT mice

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**Background:** Hematopoietic stem/progenitor cell (HSPC) based gene therapy holds great promise for achieving an HIV-1 cure. However, it requires sufficiently high hematopoietic reconstitution with anti-HIV-1 gene modified HSPC. We developed an *in vivo* selection strategy using a clinically available prodrug, 6-thioguanine (6TG), to positively select hypoxanthine-guanine phosphoribosyltransferase (HPRT) knockdown HSPC to improve the engraftment of anti-HIV-1 gene modified cells. We hypothesize that 6TG-mediated *in vivo* selection of HPRT knockdown anti-HIV-1 gene modified HSPC can achieve a sufficiently high level of engraftment for efficient HIV-1 inhibition in humanized bone marrow/liver/thymus (huBLT) mice.

**Methods:** We developed an *in vivo* 6TG selectable lentiviral vector that co-expresses short hairpin RNAs (shRNA) for HPRT and CCR5 knockdown and over-expresses C46, an HIV-1 fusion inhibitor, to protect cells from HIV-1 infection. Human fetal liver-derived CD34+ HSPC were transduced with the lentiviral vector *ex vivo* and transplanted into huBLT mice. Mice were treated with 6TG (5mg/kg) once a week for 8 weeks for *in vivo* selection of HPRT knockdown anti-HIV-1 gene modified cells. The level of anti-HIV-1 gene modified human cells was monitored by flow cytometry and digital PCR in peripheral blood and tissues (bone marrow, thymus, and spleen). To investigate HIV-1 inhibition, mice were challenged with HIV-1 and viral load was monitored in peripheral blood and tissues.

**Results:** A high level of anti-HIV-1 gene modified human cell reconstitution was achieved in 6TG-treated huBLT mice (as high as 80% at 25 weeks post HSPC transplant). The high vector DNA copy numbers per human cell were achieved at average 1.3 copies/cell. The average viral load per milliliter plasma in the anti-HIV-1 gene modified huBLT mouse group ( $4\pm 2$  HIV-1 RNA copies/ $\mu$ L) was over 200-fold lower than the control EGFP group ( $842\pm 473$  HIV-1 RNA copies/ $\mu$ L).

**Conclusion:** Our results demonstrate that 6TG-mediated *in vivo* selection of HPRT knockdown anti-HIV-1 gene modified HSPC can achieve a sufficiently high level of anti-HIV-1 gene modified cells to inhibit HIV-1 viral load over 200-fold in huBLT mice.



**Jason Jang**

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## A thermo sensitive/replication restricted sendai virus mediated CRISPR/CAS delivery for efficient CCR5 editing in hematopoietic stem cells.

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**Background:** The ease and versatility of the CRISPR/Cas9-mediated gene editing technology has spurred an immense interest in using it to edit the CCR5 gene for anti HIV human hematopoietic stem/progenitor cells (HSPCs) based gene therapy. However, a highly efficient CRISPR/Cas9 delivery system to human HSPCs is critical to achieve HIV cure.

**Methods:** To maximize the safety and the levels of CCR5 gene modification in HSPCs, we recently developed a novel thermo sensitive/replication restricted Sendai virus vector for highly efficient CRISPR-Cas9 mediated editing of the CCR5 gene (ts rSeV-Cas9-CCR5) in human CD34+ HSPCs with minimal off-target effects.

**Results:** To improve safety, we generated a ts rSeV-Cas9-CCR5 by introducing known ts mutations in the SeV P and L genes that comprise the viral polymerase complex. These mutations potently and stably restrict SeV replication at 37°C, whilst maintaining efficient infection and replication at permissive temperatures up to 34°C. Unconcentrated titer of the ts rSeV-Cas9-CCR5 vector remained at 107-108 IU/mL, similar to the wild-type vector. Ts rSeV-Cas9-CCR5 consistently transduces human G-CSF mobilized peripheral blood derived and fetal liver derived CD34+HSPCs with high efficiencies (~90%) at a multiplicity of infection of 10 and transduction time of 1 hour, including the CD34+/CD38-/CD90+ (Thy1+)/CD49fhigh subpopulation capable of hematopoietic reconstitution by a single cell in a humanized NSG mouse. Remarkably, ts rSeV-Cas9-CCR5 also edits at unprecedented frequencies (>80%) in the absence of any selection. Ts rSeV-Cas9-CCR5 vector transduced CD34+ HSPCs differentiate into various lineage-specific colony forming units. Importantly, CCR5 genes were edited biallelic indels in 60-90% of CFUs.

**Conclusion:** Our current study demonstrated unprecedented efficiency (~80%) of CCR5 gene editing in human CD34+HSPCs by a thermo sensitive/replication restricted SeV CRISPR/Cas9 delivery system. SeV is non-pathogenic in humans, has an established safety record, and has been extensively studied and modified for gene therapy applications. Introducing ts mutations into SeV vectors will likely enhance the safety of ts rSeV-Cas9-CCR5 vector clinical applications requiring highly efficient gene editing in HSPCs for HIV cure.

**Elizabeth Kenkel**

*Research Scientist, Fred Hutchinson Cancer Research Center*

**Rapamycin treatment enables intramuscular gene transfer following subcutaneous delivery of AAV6 in AAV seropositive rhesus macaques**

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Adeno-associated virus (AAV) vectors are a potential gene delivery platform for anti-HIV therapeutics due to their strong safety record, ability to be produced at high titer, and low immunogenicity. Local administration of AAV, such as AAV1 to muscle, has been used to deliver soluble eCD4-Ig to block SHIV infection and AAV6 has the potential to transduce CD4+ T cells based on its in vitro tropism. Unfortunately, sequestration in non-target organs following systemic delivery, and pre-existing immunity, hinder AAV6's in vivo performance. To overcome these challenges, we investigated if rapamycin immunosuppression could facilitate efficient gene delivery via AAV6 or AAV6 retargeted to CD4 (AAV6-CD4) delivered by intravenous (IV) or subcutaneous (SQ) injection in AAV6 seropositive rhesus macaques. Animals were treated with rapamycin for 7 or 15 days pre- to 14 days post-AAV administration and received one of the following treatments: PBS, AAV6 alone, or an equal dose of AAV6 + AAV6-CD4. Vector biodistribution and gene transfer were monitored longitudinally. Both injection routes resulted in detectable AAV6 and AAV6-CD4 vector genomes in PBMCs and most organs up to 28 days post administration, with the highest levels of vector genomes seen in liver, spleen, lymph nodes and muscle. This suggests that CD4 retargeting did not prevent vector sequestration. AAV6-CD4 transgene expression was not detected despite presence of vector genomes, but SQ injection of AAV6 mediated efficient gene expression in muscle with low-level gene expression also seen in spleen, lymph nodes, and livers with both injection routes. Both vectors were also well tolerated. Liver enzymes were elevated in 3 of 4 treated animals, but not until two weeks after rapamycin treatment ended. The animal that received the shortest duration of rapamycin treatment was the only animal with extensive muscle inflammation at necropsy and detectable T cell responses against transgene and AAV6 capsid at any time point. Our data suggest that rapamycin suppresses pre-existing anti-AAV immunity and that muscle is a potential target tissue for AAV6-based anti-HIV therapeutics.

**Colby Maldini**

Graduate Student, Department of Microbiology, University of Pennsylvania

**Engineering HIV-specific CD4+ CAR T cells to suppress HIV-1 replication**

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CD4+ T cells maintain critical roles in orchestrating immunity against pathogens. However, during HIV-1 infection, virus-specific CD4+ T cells are the primary targets for HIV-mediated depletion which results in the profound loss of CD4+ T cell-help and collapse of antiviral immunity. We sought to determine if the infusion of HIV-specific CAR-modified CD4+ T cells restore canonical CD4+ T cell-help, and whether CD4+ CAR T cells independently exert non-canonical cytolytic activity to suppress HIV-1 replication. Here, we demonstrate that CD4+ CAR T cells durably control virus infection *In vitro*, where the magnitude of suppression was influenced by the specific intracellular signaling domains (ICDs) used to generate the CAR. Importantly, some ICDs, such as the CD28-based CAR, enabled CD4+ T cells to suppress HIV-1 replication at comparable levels to CD8+ T cells. The ability of CD4+ CAR T cells to inhibit HIV-1 replication was likely due to cytolytic and non-cytolytic effector functions. We found that CD4+ CAR T cells directly lysed HIV-infected cells in a manner that was partially dependent on granule-mediated killing, and retained the ability to produce beta-chemokines after stimulation. Next, we compared the ability of CD4+ CAR T cells expressing different ICDs to impact HIV-1 infection in humanized mice. CAR T cells demonstrated robust expansion and persistence *in vivo*, and delayed HIV-mediated CD4+ T cell depletion. Interestingly, ICDs that conferred greater antiviral activity *In vitro* did not predict the outcome of CAR T cell therapy *in vivo*. However, it is likely that a defined mixture of CD4+ and CD8+ CAR T cells will comprise the ideal therapeutic product. To that end, we found that CD4+ CAR T cells expressing different ICDs synergized with CD8+ CAR T cells to suppress virus replication *In vitro*. Taken together, we have highlighted the importance of an underappreciated role for CD4+ T cells to directly inhibit HIV-1 replication and demonstrated that ICDs tune the magnitude, breadth and quality of the antiviral CD4+ CAR T cell response. These findings support further investigation into which combinations of CD4+ and CD8+ CAR T cell populations are necessary to engender a functional HIV-1 cure.

### Megan O'Connor, PhD

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## A DNA prime/protein boost prophylactic vaccine elicits robust humoral responses and reduces viremia in SHIV-162P3 infected pigtail macaques

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**Background:** The goal of a prophylactic vaccine against HIV/AIDS is to either prevent infection or to control infection once established. Here we utilize a DNA prime/protein boost vaccine strategy in a nonhuman primate (NHP) model to understand the mechanisms underlying vaccine-induced protective immunity. We analyzed viral-host interactions to determine if innate, adaptive, or inflammatory factors present before and/or after SHIV infection predict vaccine efficacy and/or distinguish NHPs that successfully control virus.

**Methods:** Pigtail macaques were mock immunized or immunized with a 2 dose DNA prime and 2 dose DNA+protein boost regimen. The DNA vaccine, delivered by particle mediated epidermal delivery (PMED), expressed SIVmac239 Gag p55, HIV-1 SF162 gp140, and BG505SOSIP and was co-delivered with an IL-12 DNA adjuvant. The protein vaccine consisted of trimeric SOSIP gp140 (BG505) and gp140 (SF162) proteins and was formulated in alum and delivered intramuscularly. Animals were challenged intrarectally with a single high dose of SHIV-162P3. Immunogenicity and efficacy of the vaccine was evaluated in blood and tissues by ELISA, serum neutralization assay in TZM-bl cells, and intracellular cytokine staining. Innate immune responses and inflammatory responses were measured by flow cytometry and bioplex immune assay, respectively.

**Results:** Sixty percent of animals receiving the DNA+protein vaccine (6/10), versus none of control animals (0/6), had reduced viremia (<1,000 copies/ml) by 7 weeks post-SHIV infection (wpi). Binding antibody against disrupted SIV virions and HIV-1 gp120 and neutralizing antibody (nAb) responses to the SHIV SF162P4, but not SHIV SF162P3, were generated in all vaccinated animals prior to challenge. Higher pre-challenge antibody responses against SIV virions ( $p < 0.001$ ) and HIV-1 gp120 ( $p = 0.021$ ) were associated with post-SHIV viral control 4-7 wpi. Reduced SHIV burden was not associated with measured innate and inflammatory responses 2 wpi.

**Conclusions:** A DNA prime/protein boost regimen induced anti-SHIV antibody responses that correlated with improved viral control in vaccinated animals and challenged with a R5, Tier 2 SHIV, a result that may suggest a role for non-neutralizing antibodies in viral control. Defining the immune correlates of vaccine success or failure in this study will provide a guide for rationale design of prophylactic and therapeutic HIV vaccines.

**Rebecca Olson, MS**

Graduate Student, Department of Immunology, University of Washington

**HIV latent cells have acquired resistance to cell-intrinsic innate immunity**

RM Olson<sup>1</sup>, M Gale Jr<sup>1</sup>

<sup>1</sup>Department of Immunology, University of Washington

The persistence of latent cells that silently harbor HIV proviruses despite antiretroviral therapy is a major barrier to an HIV cure. HIV infection triggers cell-intrinsic immune defenses which can block viral replication in acutely infected cells yet remain silent through latent infection. These defenses include cytoplasmic receptors that sense viral RNA and trigger type 1 interferon production and signaling, ultimately activating transcription of hundreds of antiviral genes. We hypothesized that latent cells have acquired resistance to these cell-intrinsic innate responses and interferon signaling. Using a variety of *In vitro* models of HIV latency, we evaluated the efficacy and function of type 1 interferon signaling in latent cells. We found that established HIV latent cell lines have functional viral RNA sensing pathways that drive type 1 interferon production. However, in comparison to naive cells, latent cells respond poorly to interferon stimulation and fail to upregulate select antiviral interferon-stimulated genes (ISGs). RNA sequencing analysis of latent vs uninfected cells stimulated with IFN revealed a network of dysregulated ISGs that may be important for restricting latency. Using an *In vitro* HIV infection model, we discovered that IFN treatment after infection blocks the transition to latency, while IFN signaling blockade promotes the latent phenotype. These findings suggest that reservoir cells have intrinsic defects in interferon signaling, which may be acquired during initial infection and/or actively maintained throughout the transition to latency. Our work reveals a previously undescribed role for type 1 interferon in regulating latency, which may be exploited to design curative therapies aimed at eradicating the reservoir.

**Mary Pampusch, PhD**

*Researcher, Department of Veterinary and Biomedical Sciences, University of Minnesota*

**Pilot study of CAR/CXCR5 T cell immunotherapy in SIV infected art suppressed rhesus macaques**

MS Pampusch<sup>1</sup>, JA Berrocal<sup>1</sup>, HM Abdelaal<sup>1</sup>, G Hart<sup>2</sup>, EG Rakasz<sup>3</sup>, E Connick<sup>4</sup>, EA Berger<sup>5</sup>, PJ Skinner<sup>1</sup>

<sup>1</sup>Veterinary and Biomedical Sciences, University of Minnesota. <sup>2</sup>Department of Medicine, University of Minnesota. <sup>3</sup>WNPRC, University of Wisconsin. <sup>4</sup>Division of Infectious Diseases, University of Arizona. <sup>5</sup>Laboratory of Viral Diseases, NIAD, NIH

HIV and SIV replication is concentrated within lymphoid follicles during chronic infection, where low levels of virus-specific CTL permit ongoing viral replication. Elevated levels of SIV-specific CTL in B cell follicles are linked to decreased levels of viral replication in follicles and decreased plasma viral loads. These findings provide the rationale for development of a strategy for targeting follicular viral replication using T cells co-expressing an antiviral chimeric antigen receptor (rhesus CD4-MBL-CAR) and the follicular homing chemokine receptor CXCR5. We hypothesize that CAR/CXCR5 T cells can target and kill virally infected cells in follicles, and lead to long-term durable remission of SIV or HIV. To begin to test this hypothesis, we engineered CAR/CXCR5 T cells and used them in a 10-animal study in which CAR/CXCR5 transduced T cells were labeled with the live cell stain CTV and infused into rhesus macaques. In a preliminary study, CAR/CXCR5 cells ( $0.35 \times 10^8$  cells/kg body weight) were infused into an SIV-infected rhesus macaque and tissues were evaluated 2 days later. In a pilot study using six SIV-infected ART-suppressed rhesus macaques, CAR/CXCR5 cells ( $0.75\text{-}2 \times 10^8$  cells/kg) were infused, animals were released from ART and monitored for 2 to 3 months. Three untreated animals were included as controls. Cells were evaluated by flow cytometry and trans-well migration assays prior to infusion. The localization and abundance of CTV-labeled cells was examined in tissues after infusion. In vitro, transduced and expanded CAR/CXCR5 T cells maintained populations of central memory T cells which migrated to the CXCR5 ligand CXCL13 and suppressed viral replication. In vivo, CTV labeled cells were detected in multiple tissues at 2 days post-infusion. Abundant CTV-labeled cells were detected both in follicular and extrafollicular areas of lymph nodes and showed evidence of in vivo expansion. Using RNAScope, CAR/CXCR5 cells were specifically detected in the lymph node follicles of treated animals. Preliminary findings show that in four of six treated animals, the viral load was reduced relative to the control animals. These findings support further study of CD4-MBL-CAR/CXCR5 T cell immunotherapy as a strategy to provide long-term durable remission of HIV and SIV infections.

## Hillary Tunggal

Graduate Student, Department of Microbiology, University of Washington

### Immunogenicity and efficacy of a therapeutic SIV DNA vaccine co-formulated with a novel adjuvant cocktail

HC Tunggal<sup>1</sup>, PV Munson<sup>1</sup>, MA O'Connor<sup>1</sup>, D Bratt<sup>1</sup>, N Hajari<sup>1</sup>, K Bagley<sup>2</sup>, DH Fuller<sup>1</sup>

<sup>1</sup>University of Washington, Seattle, WA, USA; Washington National Primate Research Center, Seattle, WA, USA <sup>2</sup>Profectus Biosciences, Baltimore, MD, USA

**Background:** We previously showed that a therapeutic SIV DNA vaccine, adjuvanted with E. coli heat labile enterotoxin (LT), achieved durable viral control in 50% of SIV-infected macaques. Here, we evaluated whether a novel combination of potent adjuvants that includes LT and additional immunomodulators could further improve vaccine immunogenicity and efficacy.

**Materials & Methods:** Rhesus macaques were infected intravenously with SIV $\Delta$ B670, put on cART 6 weeks post-infection (wpi), and starting at 32 wpi, received 5 DNA immunizations spaced 4 weeks apart. One group received an SIV multi-antigen DNA vaccine (MAG) expressing SIV Gag, Pol, Env and Nef, adjuvanted with LT (MAG+LT; N=5). A second group received the MAG vaccine formulated with an adjuvant/immunomodulator cocktail (AC) consisting of soluble CD80, soluble PD-1, IL-12, IL-33, RALDH2, and the A1 subunit of LT (LTA1) (MAG+AC; N=5). The control group received empty plasmid DNA (Controls; N=4). Viremia was measured throughout the study by RT-PCR. SIV-specific T-cell responses and antibody titers were quantified in blood and tissues by ELISpot, intracellular cytokine staining, and ELISA.

**Results:** All 14 animals exhibited robust peak viremia at 2 wpi (median  $10^7$  viral RNA copies/mL of plasma), but virus was not fully suppressed during cART. Both vaccine groups exhibited boosting of SIV-specific T-cell responses and antibody titers post-vaccination. There was no difference in IgG titers between vaccine groups; however the MAG+AC group demonstrated a trend towards higher Gag-specific T-cell responses post-vaccination. After halting cART, virus rebounded to near pre-cART levels in most animals within three weeks. However, 3/5 MAG+AC animals sustained viremia below  $10^3$  viral RNA copies/mL of plasma after stopping cART, compared to 1/5 MAG+LT animals and 1/4 controls. Animals exhibiting the most suppression of virus during cART developed stronger SIV-specific T-cell responses post-vaccination, which corresponded with lower viremia post-cART.

**Conclusions:** Incomplete suppression of virus on cART resulted in reduced therapeutic vaccine immunogenicity and efficacy. Despite this, a novel combination of adjuvants showed promise in improving vaccine efficacy. These results suggest that novel combinations of adjuvants may improve immunogenicity and efficacy of therapeutic HIV DNA vaccines and demonstrate the importance of effective cART in maximizing potency of immunotherapeutic regimens.



### defeatHIV – Cell and Gene Therapy for HIV Cure

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

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

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

- **George Washington University**  
**BELIEVE:** Bench to Bed Enhanced 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 (**I4C**)
- **University of North Carolina, Chapel Hill**  
Collaboratory of AIDS Researchers for Eradication (**CARE**)

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





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Fred Hutchinson Cancer Research Center was established in 1975 and is one of the world's leading cancer research institutes. Its interdisciplinary teams of scientists conduct research throughout the world to advance the prevention, early detection and treatment of cancer and other diseases. Fred Hutch's mission is the elimination of cancer and related diseases as causes of human suffering and death. Fred Hutch researchers pioneered bone-marrow transplantation for leukemia and other blood diseases. This research has cured thousands of patients worldwide and has boosted survival rates for certain forms of leukemia from zero to as high as 85 percent. Recognizing that infectious agents contribute to up to a quarter of the world's cancers, Fred Hutch researchers also study infectious diseases, including HIV- and AIDS-related malignancies. Fred Hutch's internationally acclaimed scientists include three Nobel Laureates, a MacArthur fellow, members of the National Academy of Sciences, members of the Institute of Medicine, members of the American Academy of Arts and Sciences, members of the American Association for the Advancement of Science and current and former Howard Hughes Medical Institute investigators.

Fred Hutch occupies spectacular, modern facilities on the 15-acre Robert W. Day Campus. The campus overlooks South Lake Union, Seattle's downtown lakefront neighborhood, which is emerging as Seattle's hub for life sciences research organizations. Campus labs and offices occupy about 1.5 million square feet, supporting more than 2,700 faculty and staff members who are working to eliminate cancer, HIV and other related diseases.

Fred Hutch is consistently among the top NCI-funded academic and research institutes and is ranked first in National Institutes of Health funding among all U.S. independent research institutions.

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

## HOST ORGANIZATIONS

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The mission of the Curative Therapies for HIV (Cure) Scientific Working Group is to accelerate work toward a cure for HIV, fostering new ideas and promoting new technologies. To achieve our mission the Cure SWG continues to bring together UW/Fred Hutch CFAR affiliated researchers, unaffiliated external HIV researchers and community members in order to develop critical local expertise and enhance areas of local strength. Collaboration is essential to our mission, and by working with researchers and community members of broad experience and from diverse backgrounds, we hope to serve as an internationally-recognized center for the study of curative therapies for HIV.

[depts.washington.edu/cfar](https://depts.washington.edu/cfar)

# UW Medicine

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## LABORATORY MEDICINE

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### VIROLOGY

The University of Washington Virology Division is one of twelve divisions that comprise the Department of Laboratory Medicine in the University's School of Medicine. The Virology Division's eleven 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, as well as post-doctoral trainees within the Virology Division. UW Medicine teaching programs are consistently ranked among the best in the country in rankings by U.S. News & World Report.

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 cutting edge laboratory tests, creating new vaccines, inventing and patenting new technology, and elucidating basic cellular processes in health and disease. The Division's faculty are internationally recognized for their clinical and basic science research.

**[uwvirology.org](http://uwvirology.org)**

## Thursday, August 22 from 7-10pm

Please join us as we set sail on an Argosy Cruise adventure around scenic Lake Union and Lake Washington on the Lady Mary. Dinner and drinks will be provided. Round trip shuttles will run from the Marriott Residence Inn Hotel to South Lake Union.



### Location and Transportation:

The boarding location for our Argosy Cruise at South Lake Union on the dock at the Museum of History and Industry (MOHAI). After the poster session, attendees will board the round-trip shuttle from the Marriott Residence Inn Seattle to the MOHAI boat launch.

### Dinner and Reception:

The 3-hour charter experience will take meeting participants through Lake Union, Portage Bay, the Montlake Cut, and past the University of Washington onto Lake Washington—the largest naturally occurring lake in the State of Washington.

During this dinner cruise of Lake Washington you'll learn the history of the area and cruise past magnificent lakeside properties as you relax and enjoy one of the world's truly beautiful urban lakes. The Lady Mary will depart from the MOHAI dock at precisely 7:00pm and will arrive at 10:00pm.

### Schedule:

**5:00pm:** Poster Session begins

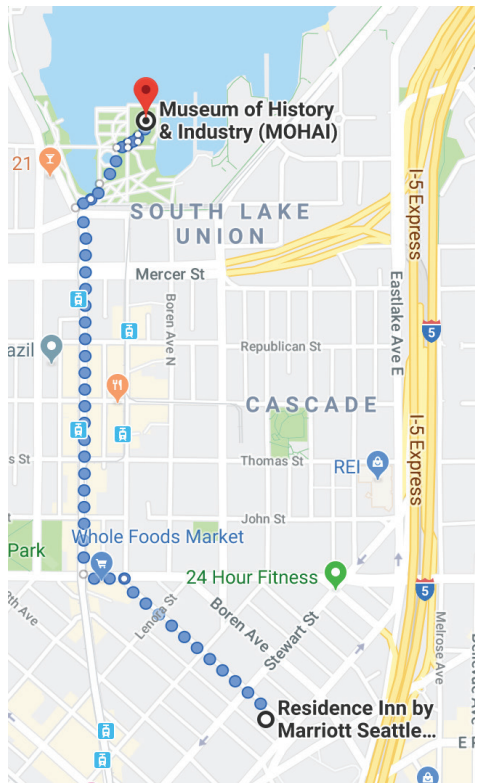
**6:20pm:** Poster Session ends

**6:30pm:** Attendees may start boarding the shuttle outside the Marriott Residence Inn

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

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

**10:15pm:** Return shuttle service to the Marriott Residence Inn



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## amfAR

### 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 advocacy. Since 1985, amfAR has invested nearly \$550 million in its programs and has awarded more than 3,300 grants to research teams worldwide.

Today amfAR's research focus is on the development of a cure for HIV. Our initiatives include the amfAR Institute for HIV Cure Research, a five-year \$20 million grant to the University of California, San Francisco and IciStem, a European research consortium created and funded by amfAR that is following a cohort of stem cell transplant recipients including the London and Düsseldorf patients.

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



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Wednesday, August 21st 7pm  
Central Branch of the Seattle Public Library  
1000 4th Ave, Seattle, WA

Two HIV activists who took part in an early gene therapy trial to cure or control HIV and the scientist who led that research will be talking about their experiences which helped usher in a new scientific era at “Medical Angels”, a free community forum led by people living with HIV



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## 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.

**Danielle Campbelle**

*DARE*

**David Palm**

*CARE-UNC*

**William (Bill) Freshwater**

*BEAT-HIV Wistar Institute & UPENN*

**Richard Strange**

*BELIEVE - George Washington University*

**Helena Maki**

*defeatHIV*

**Mark Wagner**

*I4C*



[cgt4hivcure2019.org](http://cgt4hivcure2019.org)

